Long Title: Evaluation of Automated Microvascular Flow Analysis Software AVA 4.0: A Validation Study

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ABSTRACT: 339/350 words

BACKGROUND:
Real-time automated analysis of videos of the microvasculature is an essential step in the development of research protocols and clinical algorithms that incorporate point-of-care microvascular analysis. Validation studies on the software packages developed to perform these analyses have reported low agreement with the current referent standard semi-automated analysis method. In response to the call for validation studies of available automated analysis software by the European Society of Intensive Care Medicine, we report the first human validation study of AVA 4.0.

METHODS:
Two retrospective perioperative datasets of human microcirculation videos (P1 and P2) and one prospective healthy volunteer dataset (V1) were used. Video quality was assessed using the Microcirculation Image Quality Selection (MIQS) score. Videos were initially analysed with (1) AVA software 3.2 by two experienced users through a semi-manual method, followed by an analysis with (2) AVA automated software 4.0 for perfused vessel density (PVD), total vessel density (TVD), and proportion of perfused vessels (PPV). Bland-Altman analysis and intraclass correlation coefficients (ICC) were used to measure agreement between the two methods. Each method′s ability to discriminate between microcirculatory states before and after induction of general anesthesia was assessed using paired t-tests.

RESULTS:
Fifty-two videos from P1, 128 videos from P2 and 26 videos from V1 met inclusion criteria for analysis. Correlational analysis and Bland Altman analysis revealed poor agreement and no correlation between AVA 4.0 and AVA 3.2. Increasing video length did not improve agreement. Automated analysis consistently underestimated measures of vessel density. Following the induction of anesthesia, TVD and PVD measured using AVA 3.2 increased significantly for P1 and P2 ($p < 0.05$). However, these changes could not be replicated with the data generated by AVA 4.0.

CONCLUSIONS: AVA 4.0 is not a suitable tool for research or clinical purposes at this time. Future validation studies of automated microvascular flow analysis software should aim to measure the new software′s agreement with the referent standard, its ability to discriminate between clinical states and the quality thresholds at which its performance becomes unacceptable.

KEYWORDS: microcirculation, sublingual, human, automated analysis, AVA, validation

BACKGROUND

Advances in microscopic imaging technology and digital video analysis have led to the discovery of hemodynamic incoherence: a cardiovascular state characterized by disparity between macrocirculation variables, such as blood pressure and cardiac output, and microcirculation variables, such as perfused vessel density.[1] A series of investigations of the human sublingual microcirculation in various clinical states have identified a set of microvascular phenotypes that are associated with loss of hemodynamic coherence and adverse clinical outcomes: increased heterogeneity, reduced capillary density, reduced microvascular flow, and tissue edema.[2-10] Real-time access to microvascular variables would allow clinicians to monitor hemodynamic coherence, effectively expanding the vital signs available to inform clinical decision-making and expedite treatment delivery. Automated video analysis is an essential step in the development of point-of-care microvascular assessment. The current referent method for analyzing videos of microcirculation entails video acquisition at the bedside with a handheld vital microscope (HVM), followed by offline semi-automated video analysis (AVA 3.2 software, MicroVision Medical, Amsterdam, The Netherlands). This process, while it has shown to have excellent intra-observer reliability (ICC =0.89) and good to moderate inter-observer reliability (k = 0.48-0.66), is limited in its applicability to guide real-time treatment decisions of critically ill adults.[11,12] Data acquisition from these videos requires an experienced user to segment the videos into still images where the vessels are then individually traced prior to evaluating the quality of the microcirculation. In addition to this already lengthy and laborious process, the 2018 consensus guidelines from the European Society of Intensive Care medicine for microcirculation image

analysis also requires a detailed assessment of video quality using the Microcirculation Image Quality Score (MIQS).[13] Acquired videos are scored on an ordinal scale of 0 (optimal quality), 1 (acceptable quality), and 10 (unacceptable quality), ranking videos across six domains. This manual process has limited feasibility in the dynamic critical care setting. Thus, an equally reliable and precise automated software that integrates video quality evaluation is imperative. Following the call for automated software, Microvision Medical created AVA 4.0 (MicroVision Medical BV, Amsterdam, The Netherlands), a fully automated software developed to assist in real-time microcirculatory data analysis. This software package has already been used to acquire and analyze microvascular data that has been peer-reviewed and published.[14-16]

Prior to clinical use, automated microcirculation analysis software must be validated against the existing referent standard (manual analysis) to confirm the accuracy, precision and reliability of the generated data.[17] Performance of a different automated microvascular analysis software CCTools (Braedius Medical, Huizen, The Netherlands) has recently been compared to the gold standard method in three human studies [14,18,19]. The results of these studies demonstrated that the software was not accurate enough for clinical use. To date, only one validation study has been published using AVA 4.0.[20] Arnemann and colleagues published a validation study using the conjunctival microvasculature of sheep undergoing a hemorrhagic shock protocol. A notable strength of this validation study was the use of a physiological state transition (i.e. hemorrhagic shock) to investigate each method's ability to discriminate between distinct microvascular states. The authors reported poor agreement between AVA 4.0 and AVA 3.2 for all variables examined. They also reported that AVA 4.0 was unable to

discriminate states of hemorrhagic shock, whereas the referent AVA 3.2 did so reliably. The authors also highlighted two important limitations of their study. First, they used a non-human model; and second, they acquired data at the conjunctival mucosa. Most human studies to date have used data acquired from the sublingual microcirculation, Arnemann et al's findings, though compelling, have limited generalizability to the human clinical context.[20]

The aim of this study was to extend Arnemann's preliminary findings and investigate the agreement between AVA 4.0 and the referent semi-automated AVA 3.2 method in the analysis of human sublingual microcirculation videos.

We hypothesized that the data generated by AVA 4.0 would exhibit moderate agreement with that of AVA 3.2. We also hypothesized that long high quality videos would exhibit higher agreement than shorter videos. Lastly, we hypothesized that both methods would reveal significant increases in TVD and PVD following induction of general anesthesia, a robust finding that has previously been reported.[21-23]

METHODS

Study Design

Three datasets of human sublingual microcirculation were considered in this validation study. Two of which were previously acquired from patients undergoing cardiac (P1), or general (P2) surgery. Data from the P1 has previously been published.[24] The third dataset was prospectively acquired from three healthy volunteer participants. Volunteer participants did not receive any anesthesia or undergo any surgical procedure (V1).

Outcome Variables

The variables of interest in the software comparison were perfused vessel density (PVD), total vessel density (TVD), and proportion of perfused vessels (PPV).

Procedures

Measurements

Microscan (MicroVision Medical BV, Amsterdam, The Netherlands), a commercially available sidestream darkfield imaging microscope was used to capture all videos of the microcirculation. For the prospective dataset (V1), sublingual microcirculation was measured at ten different time points for each volunteer. Video quality was evaluated using the MIQS. Two experienced operators (CG and MB) independently analyzed each of the videos for quality, and videos with a score of 10 on any of the six dimensions of MIQS were excluded. Disagreements were resolved by discussion and if consensus was not reached, the video was excluded. Instead of using discrete categories and cut-offs for video duration as described in the original MIQS, the exact number of frames was recorded for each video. Furthermore, multiple segments of varying duration for each volunteer video were generated, to allow for quantitative analysis of the effects of video duration on the results of automated video analysis.

Validity of AVA 4.0

AVA 4.0 validation was carried out in two steps: 1) Measurement of agreement between AVA 3.2 and AVA 4.0 analyses on all three datasets, 2) The ability for both AVA 3.2 manual analysis and AVA 4.0 automated analysis to discriminate between two

established microcirculatory states: pre- and post-induction of general anesthesia. Datasets P1 and P2 had previously been analyzed using AVA 3.2. The appropriate calibration measures for the retrospective datasets were obtained from the original AVA 3.2 analysis reports and were entered into AVA 4.0 settings prior to automated analysis.

V1 was analyzed with AVA 3.2 using the aforementioned validated referent methodology. All three datasets were then analyzed using the automated software, AVA 4.0. The measurements from both the manual and automated software packages for PVD, TVD, PPV were recorded for all datasets.

Statistical Analysis

Statistical analyses were completed using R statistical analysis software[25] or Microsoft Excel (2019). Significance level was set *a priori* at $p = 0.05$. Raw data and analysis scripts are available upon reasonable request.

All variables from AVA 3.2 and AVA 4.0 were evaluated using histograms and Kolomogorov-Smirnov tests were used to confirm normal distributions. Homoscedasticity was tested using F-tests, with the null hypothesis that variance did not differ between comparison groups (e.g. comparing PVD before and after induction of general anesthesia in dataset P1). For groups meeting normality and homoscedasticity assumptions, paired t-tests were used to compare microvascular variables preceding and immediately following induction of general anesthesia in the P1 and P2 datasets.

To compare the level of agreement between AVA 4.0 and 3.2, the intraclass correlation coefficient (ICC) was calculated using a two-way analysis of variance (ANOVA). The ICC for each variable, TVD, PVD, and PPD were calculated separately.

All ICC values are reported along with the 95% confidence intervals. ICC values below 0.40 are considered as "poor", between 0.40 and 0.59 as "fair", between 0.60 and 0.74 as "good" and greater than 0.74 as "excellent". [26]

Additionally, the method proposed by Bland and Altman was used to assess the agreement between the two methods of analysis.[27] For each microcirculatory variable, a Bland-Altman plot shows the difference between measurements taken by AVA 3.2 and AVA 4.0 for each video versus the average of the measurements taken by the two methods, along with limits of agreement. The agreement between the two methods is summarized by calculating the mean difference between the two methods along with 95% confidence interval. The limits of agreement (LOA) further extend the confidence interval for the mean difference to account for sampling error.

RESULTS

A total of 457 videos were evaluated for inclusion. Fifty-two videos (29%) from P1, 128 videos (64%) from P2, and 19 videos (65%) from V1 met the MIQS standards for inclusion in the analysis. The most common reason for video exclusion was the presence of pressure artifacts.

In all three samples, PPV measurements from AVA 4.0 showed significant deviation from the normal distribution, whereas normality assumptions were met for TVD and PVD. In the V1 and P2 samples, differences in PPV measurements from AVA 3.2 and AVA 4.0 also deviated from the normal distribution. A normal distribution was never yielded, despite several transformations being performed. Therefore, results for PPV should be interpreted with caution. Pooling the three samples resulted in major deviation from the normal distribution assumption for all variables. As such, analyses were not

reproduced in the pooled sample.

The comparison of the two analysis methods evaluated by ICC revealed a poor agreement for all microcirculatory variables (Table 1).

Table 1. Intraclass correlation coefficient between AVA 3.2 and AVA 4.0

Variable	**Data set**	**n**	**ICC [95% CI]**	**Agreement**
TVD	Volunteer	19	0.03 [-0.05, 0.19]	Poor
	Patients 1	52	0.04 [-0.06, 0.18]	Poor
	Patients 2	128	0.03 [-0.04, 0.13]	Poor
PVD	Volunteer	19	0.05 [-0.06, 0.24]	Poor
	Patients 1	52	0.06 [-0.06, 0.22]	Poor
	Patients 2	128	0.07 [-0.06, 0.23]	Poor
PPV	Volunteer	19	0.32 [-0.15, 0.67]	Poor
	Patients 1	52	0.02 [-0.09, 0.16]	Poor
	Patients 2	128	-0.02 [-0.17, 0.14]	Poor

The Bland-Altman plots (Figures 1-3) and summary statistics (Table 2) showed significant biases and non-systematic high variability in all measurements and samples. The limits of agreement were wide for all variables.

Table 2. Bland-Altman analysis between AVA 3 and AVA 4

Variable	**Data set**	**n**	**Mean bias [95% CI]**	**LOA**
TVD	Volunteer	19	9.69 [7.88, 11.5]	1.83 – 17.5
	Patients 1	52	-42.6 [-50.3, -34.9]	-98.0 – 12.8
	Patients 2	128	13.0 [11.9, 14.2]	0.04 – 26.0
PVD	Volunteer	19	9.00 [7.10, 10.9]	0.73 – 17.3
	Patients 1	52	-48.7 [-56.1, -41.4]	-101.8 – 4.32
	Patients 2	128	10.5 [9.27, 11.7]	-3.07 – 24.0

PPV	Volunteer	19	2.77 [-3.86, 9.40]	-26.1 – 31.7
	Patients 1	52	-11.7 [-14.4, -9.00]	-31.3 – 7.88
	Patients 2	128	-6.77 [-10.0, -3.51]	-43.6 – 30.0

Analyzing the same video multiple times with AVA 4.0 did not alter the computed microvascular variables. However, changing the video duration did significantly alter the results. Unexpectedly, video duration positively correlated with differences between AVA 3.2 and 4.0 for both TVD ($r = 0.17$, $p < 0.05$) and PVD ($r = 0.21$, $p < 0.05$), albeit with low coefficients of correlation. In other words, as we increased the duration of videos, results generated by automated analysis tended to diverge more from the referent standard. Examination of the raw data revealed many seemingly random outliers generated by AVA 4.0 at various video durations, from the very short (< 10 frames) to the very long (> 500 frames).

As expected, TVD and PVD measured using AVA 3.2 significantly increased following the induction of general anesthesia, in both P1 (TVD, $p = 0.05$; PVD, $p = 0.009$) and P2 (TVD, $p = 0.01$; PVD, $p = 0.03$). However, these changes were not observed when using the measurements generated by AVA 4.0 for P1 (TVD, $p = 0.89$; PVD, $p = 0.89$) or P2 (TVD, $p = 0.40$; PVD, $p = 0.22$). In each case, assumptions of homoscedasticity and normality were satisfied as described in the methods section.

DISCUSSION

The microcirculation is emerging as an important system to assess and monitor patients hemodynamic and metabolic states at their bedside. However, the clinical value of this assessment hinges on timely analysis and generation of reliable data. Novel

automated analysis software provides the opportunity for real-time analysis of the microcirculation. Pursuant to the request for validation studies of automated software in the second consensus guidelines on the assessment of sublingual microcirculation [17] we herein present the first validation study of AVA 4.0 using human sublingual microcirculation.

Bland Altman and correlational analysis applied to all three datasets (P1, P2, V1) revealed that the microvascular data generated by AVA 4.0 does not accurately reflect data generated using the referent method (Figures 2-4). Increasing video length did not improve agreement. Furthermore, AVA 4.0 was unable to discriminate between different microvascular states that have previously been characterized (i.e. pre- vs. post-induction of general anesthesia), whereas data generated using the referent method did replicate the findings that TVD and PVD increase following induction of general anesthesia (Figures 5-6).[21-23]

These results are consistent with a previous validation study of AVA 4.0 conducted with videos of the conjunctival microcirculation of sheep subjected to experimentally-induced hemorrhagic shock.[20] Our study expands their findings to the human microcirculation and reaffirms the conclusion that AVA 4.0 cannot be used to accurately assess the human microcirculation. This conclusion has important implications. First, it calls into question the findings of previously published articles using AVA 4.0 and other non-validated software to analyse microvascular data in humans.[14-16,20] Investigators who used the software to analyze data and did not generate significant results should also reconsider the validity of their methods and re-analyze their data using manual analysis.

Furthermore, future implementations of automated microcirculation analysis software should be validated prior to undertaking clinical studies.

Our study has limitations worth considering. First, large portions of the videos in our retrospective datasets are shorter than 90 frames, the recommended cut-off included in the MIQS. It is worth noting that the perioperative datasets used in this validation study were recorded prior to the MIQS being published in 2013.[13] To explore whether video duration had a significant impact on the performance of AVA 4.0, we prospectively recorded long, high quality videos in healthy volunteers and analyzed video segments of different lengths. We hypothesized that longer videos would be associated with smaller differences between AVA 3.2 and AVA 4.0. Our data suggests that increasing video duration does *not* increase agreement between AVA 3.2 and AVA 4.0. As the MIQS score pertains to the semi-automated software, modifications may need to be made to the scoring scheme with respect to future implementations of automated analysis software.[13]

Hardware limitations must also be considered: accurate detection of red blood cell (RBC) velocities is significantly limited when using a low image acquisition rate (i.e. 25 Hz) relative to the velocity of the RBCs, which causes blurring within vessels. A simulation study suggested that increasing image acquisition rate from 25 Hz to 100Hz can significantly improve automated detection of perfused vessels.[28] Additionally, third generation IDF cameras have been reported to detect 20-30% more capillaries than the SDF microscope used in this study.[29]

A notable strength of our study is the inclusion of data acquired from patients in a clinical setting as well as data acquired from healthy volunteers in a controlled setting. By including retrospective clinical datasets and prospective research datasets, which vary

in image quality and video duration, we were able to evaluate if failure of the automated software was purely due to video quality. Our results demonstrate however, fundamental limitations of the software itself, independent of the quality or duration of videos.

In addition to using traditional measures of statistical correlation and agreement, we also tested the software′s ability to discriminate between clinical states. Considering that automated analysis eliminates human bias during analysis, future iterations of these software packages may one day outperform semi-automated analysis. Therefore, validation studies of automated analysis software should seek to replicate clinical findings in human patients as well as discriminate between known microvascular states in addition to computing measures of correlation and agreement. These research end-points will be critical to facilitate the transition from semi-automated to fully automated microvascular analysis.

CONCLUSION

We found little to no correlation or agreement between the microcirculation variables computed by AVA 4.0 and the referent AVA 3.2 using two large video datasets acquired in clinical settings, and one prospective video dataset in healthy volunteers. Furthermore, AVA 4.0 was unable to discriminate preoperative from anesthetized microcirculatory states. We conclude that AVA 4.0 is not ready to be implemented in research or clinical protocols, and that studies reporting results using this software should be critically re-evaluated.

Abbreviations
PVD: perfused vessel density; TVD: total vessel density; PPV: proportion of perfused vessels; ICC: intraclass correlation coefficient; HVM: handheld vital microscope; ANOVA: analysis of variance; MIQS: microcirculation image quality selection

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Author's contributions
Conception and design: CG, MK and MB

Data acquisition: CG, MK, SST, DB and MB

Data analysis: CG and GS

Data interpretation: CG, MK, EC, and MB

Manuscript drafting: CG and EC

Critical revision of the manuscript for important intellectual content: CG, MK, SST, EC,

GS, DB and MB

Availability of data and materials

Raw data and analysis scripts are available upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics Approval

In each case, data was acquired following approval by the Montreal Neurological

Institute and Hospital′s Research Ethics Board and obtaining informed consent. The

results of the P2 dataset have previously been published elsewhere[24]

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1. Hemodynamic coherence: Its meaning in perioperative and intensive care medicine, Best Practice and Research: Clinical Anaesthesiology(2016).
2. De Backer D, Creteur J, Preiser J-C, Dubois M-J, Vincent J-L. Microvascular Blood Flow Is Altered in Patients with Sepsis. In. *American Journal of Respiratory and Critical Care Medicine.* Vol 1662002:98-104.
3. Top APC, Ince C, De Meij N, Van Dijk M, Tibboel D. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. In. *Critical Care Medicine.* Vol 39: Lippincott Williams and Wilkins; 2011:8-13.
4. Edul VSK, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. In. *Critical Care Medicine.* Vol 402012:1443-1448.
5. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent-microcirculatory alterations are associated with organ failure and death in patients with septic shock. In. *Critical Care Medicine.* Vol 322004:1825-1831.
6. Tachon G, Harrois A, Tanaka S, et al. Microcirculatory alterations in traumatic hemorrhagic shock. In. *Critical Care Medicine.* Vol 42: Lippincott Williams and Wilkins; 2014:1433-1441.
7. Koning NJ, Vonk ABA, van Barneveld LJ, et al. Pulsatile flow during cardiopulmonary bypass preserves postoperative microcirculatory perfusion irrespective of systemic hemodynamics. In. *Journal of Applied Physiology.* Vol 1122012:1727-1734.
8. O'Neil MP, Fleming JC, Badhwar A, Guo LR. Pulsatile versus nonpulsatile flow during cardiopulmonary bypass: Microcirculatory and systemic effects. In. *Annals of Thoracic Surgery.* Vol 942012:2046-2053.
9. Atasever B, Boer C, Goedhart P, et al. Distinct alterations in sublingual microcirculatory blood flow and hemoglobin oxygenation in on-pump and off-pump coronary artery bypass graft surgery. In. *Journal of Cardiothoracic and Vascular Anesthesia.* Vol 252011:784-790.
10. Hemodynamic coherence and the rationale for monitoring the microcirculation, Critical Care(2015).
11. Petersen SM, Greisen G, Hyttel-Sorensen S, Hahn GH. Sidestream dark field images of the microcirculation: intra-observer reliability and correlation between two semi-quantitative methods for determining flow. In. *BMC Medical Imaging.* Vol 142014:14.
12. Sardinha J, Mackinnon S, Lehmann C. Rapid clinical assessment of the sublingual microcirculation - Visual scoring using microVAS in comparison to standard semi-automated analysis. In. *Clinical Hemorheology and Microcirculation.* Vol 72: IOS Press; 2019:229-238.
13. Massey MJ, LaRochelle E, Najarro G, et al. The microcirculation image quality score: Development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. In. *Journal of Critical Care.* Vol 282013:913-917.

14. Sharawy N, Mukhtar A, Islam S, et al. Preliminary clinical evaluation of automated analysis of the sublingual microcirculation in the assessment of patients with septic shock: Comparison of automated versus semi-automated software. In. *Clinical Hemorheology and Microcirculation.* Vol 67: IOS Press; 2017:489-498.
15. Machin DR, Gates PE, Vink H, Frech TM, Donato AJ. Automated measurement of microvascular function reveals dysfunction in systemic sclerosis: A cross-sectional study. In. *Journal of Rheumatology.* Vol 44: Journal of Rheumatology; 2017:1603-1611.
16. Helmerhorst HJF, de Wilde RBP, Lee DH, et al. Hemodynamic effects of short-term hyperoxia after coronary artery bypass grafting. In. *Annals of Intensive Care.* Vol 7: Springer Verlag; 2017.
17. Ince C, Boerma EC, Cecconi M, et al. Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine. In. *Intensive Care Medicine.* Vol 44: Springer Verlag; 2018:281-299.
18. Carsetti A, Aya HD, Pierantozzi S, et al. Ability and efficiency of an automatic analysis software to measure microvascular parameters. In. *Journal of Clinical Monitoring and Computing.* Vol 31: Springer Netherlands; 2017:669-676.
19. Hilty MP, Pichler J, Ergin B, et al. Assessment of endothelial cell function and physiological microcirculatory reserve by video microscopy using a topical acetylcholine and nitroglycerin challenge. In. *Intensive Care Medicine Experimental.* Vol 5: Springer Nature; 2017.
20. Arnemann P-H, Hessler M, Kampmeier T, et al. Comparison of an automatic analysis and a manual analysis of conjunctival microcirculation in a sheep model of haemorrhagic shock. In. *Intensive care medicine experimental.* Vol 42016:37.
21. Turek Z, Sykora R, Matejovic M, Cerny V. Anesthesia and the microcirculation. In. *Seminars in cardiothoracic and vascular anesthesia.* Vol 132009:249-258.
22. Bansch P, Flisberg P, Bentzer P. Changes in the sublingual microcirculation during major abdominal surgery and post-operative morbidity. In. *Acta Anaesthesiologica Scandinavica.* Vol 58: Acta Anaesthesiol Scand; 2014:89-97.
23. Drullinsky D, Noiseux N, Bracco D. 398 Anesthesia Induction Improves Sublingual Micro-Circulation. In. *Canadian Journal of Cardiology.* Vol 28: Elsevier BV; 2012:S248-S249.
24. Bienz M, Drullinsky D, Stevens L-M, Bracco D, Noiseux N. Microcirculatory response during on-pump versus off-pump coronary artery bypass graft surgery. In. *Perfusion.* Vol 31: SAGE Publications Ltd; 2016:207-215.
25. Wickham HR. A language and environment for statistical computing. In. Vienna, Austria2012.
26. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. In. *Journal of Chiropractic Medicine.* Vol 15: Elsevier USA; 2016:155-163.

27. Martin Bland J, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet.* Vol 3271986:307-310.
28. Bezemer R, Dobbe JG, Bartels SA, et al. Rapid automatic assessment of microvascular density in sidestream dark field images. In. *Medical and Biological Engineering and Computing.* Vol 49: Springer Verlag; 2011:1269-1278.
29. Aykut G, Veenstra G, Scorcella C, Ince C, Boerma C. Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation. In. *Intensive Care Medicine Experimental.* Vol 3: Springer Science and Business Media LLC; 2015:4.