Responses to the comments of Reviewer 1

We thank the reviewer for the helpful comments.

Jumar and coworkers report increased retinal capillary rarefaction in patients with untreated mild-moderate hypertension. The study is well described and well reported. I nonetheless have several reservations and suggestions:

1. In the Introduction, it is not clear whether the authors believe rarefaction is a cause or result of hypertension.

Answer:

The authors believe that hypertension causes capillary rarefaction. Capillary rarefaction may indicate end-organ damage in the microvascular bed. Other microvascular changes caused by hypertension have been shown to predict cardiovascular events.1 Capillary rarefaction has the advantage of noninvasive assessment and might be a promising parameter to assess end-organ damage in patients with hypertension and maybe also helps to categorize patients with elevated risk for cardiovascular events in the future. An additional explaining paragraph has been added to the introduction.
2. The relevance of the Introduction sentence "Hypertension-associated over activity of the rennin angiotensin system is known to alter the cerebral blood supply and contribute to increased susceptibility to stroke and dementia" is not clear.

Answer:

We thank the reviewer for this helpful comment and changed the paragraph to the following:

Hypertension is known to be associated with an over activity of the renin angiotensin system. This over activity has been shown to cause vasoconstriction also in the cerebral blood supply, which was associated with an increased susceptibility to stroke and dementia.2 Measurement of cerebral arterioles and capillaries in humans is difficult and invasive. There is rising evidence that retinal vessels may mirror the cerebral and systemic microcirculation.3

3. Please define "stage 1 or 2 hypertension" in terms of BP cutoffs. Also, please explain the term "trough mean" (trough mean sitting systolic BP…).

Answer:

Patients had to present with the following BP values: systolic BP 140-179 mmHg and diastolic BP 90-109 mmHg. Office BP was measured three times in a quite sitting position according to guidelines. The mean value of these three measurements was calculated and defined as the trough mean sitting BP. This information has been added to the method section.

4. Results of 24 h ABPM are described but the methodology is not mentioned at all.

Answer:

An additional explaining sentence has been added to the method section. 24h ambulatory BP was measured with the Mobil-O-Graph I.E.M. System., for which good validity and reliability has been demonstrated.4, 5

5. Results, 1st paragraph, OD is mentioned twice with opposing statements.

Answer:

We apologize for this error. OD was significantly smaller in hypertensive patients compared to the healthy control group (p = 0.038). This has now been corrected.
6. In multiple regression models, serum creatinine values should be probably log transformed if distribution is skewed. Also check cholesterol distribution.

Answer:

We thank the reviewer for this important point. In addition to Kolmogorov-Smirnov test Levene-test has been performed to check homogeneity of variance, which has now been also mentioned in the section statistical methods. For each variable showing Levene p values ≤ 0.05 data were considered as unbalanced and therefore not normal distributed. Non-parametric analysis was performed in these cases using Mann-Whitney-U-Test. Levene Test showed p = 0.829 for creatinine and p = 0.597 for cholesterol, therefore data have been considered as balanced. Kolmogorov-Smirnov test showed p = 0.51 for cholesterol and p = 0.49 for creatinine. Therefore cholesterol and creatinine have been considered as normally distributed.

7. Discussion: "endo-organ" should be "end-organ".

Answer:

We thank the reviewer and corrected the error.

8. Please discuss the applicability of the method in routine clinical practice - are the needed devices routinely available?

Answer:

A new paragraph discussing the applicability of the method in routine clinical practice has been added to the discussion section.

9. Table 1 - healthy control group SBP distribution 127 ± 18 mmHg suggest that some are hypertensive - please verify and exclude hypertensive subjects (same for 24 h DBP).

Answer:

We thank the reviewer for drawing our attention at this error. BP values have been checked with the primary data and corrected. No hypertensive subjects are included in the healthy control group.
10. The fit lines in Figures 4A and 4B appear incorrect - please verify and provide the raw data as supplement.

Answer:

We thank the reviewer for drawing our attention at this error. The fit lines in Figures 4A and 4B have been corrected and the raw data is now provided in table 3.


Responses to the comments of Reviewer 2

We thank the reviewer for the helpful comments.

This is a well-written and designed manuscript to access capillary rarefaction of retinal vessels between hypertensive (n=134) and normotensive (n=55) subjects. This is an ancillary study to use patients who had participated in four randomized clinical trials that has been already completed. Here are my comments and critiques for this study:

1. The subjects come from four different clinical trials. So authors need to explain the similarities of patients’ characteristics among four clinical trials. Written explanation on exclusion/inclusion criteria and basic characteristics or constructing a table regarding basic characteristics for four clinical trials might be beneficial for readers to ensure homogeneity of your study population.

Answer:

We thank the reviewer for this helpful comment. A new paragraph explaining the similarities of patients’ characteristics among the four clinical trials has been added to the method section.

2. The study population is a sub-population selected/chosen from four different clinical trials. Thus, it might be informative to provide characteristics of patients from the study population and from whole four clinical trials in order to avoid selection bias issue.

Answer:

We thank the reviewer for drawing our attention at this very important point. We stated in the method section that all healthy individuals form the control group in the two clinical trials mentioned (www.clinicaltrials.gov NCT00152698, NCT01319344) have been included in our analysis. In the hypertensive group 134 out of 158 patients participating in two trials (www.clinicaltrials.gov NCT01318395, NCT00627952) fulfilled pixel criteria for inclusion in our analysis. A new supplemental table has been created comparing characteristics of the clinical trial hypertensive population with our hypertensive group.

3. This is an unbalanced study design between two groups and it’s interesting for control group to show smaller size compared to hypertensive group. Authors need to explain why the study is unbalanced and the control group has smaller size.

Answer:
This is a very important point. Our control group is of smaller size compared to the hypertensive group, which is a clear limitation of the study. As our patients have been recruited a center specialized in hypertension care, clinical trials including hypertensive patients are more frequent than those including healthy individuals. We are currently working on this issue and definitely want to increase our healthy study population. As this is a pilot study intended to show first differences in capillary rarefaction between hypertensive and healthy individuals we believe the unbalanced study design might be acceptable. We carefully analyzed the balance of statistical data distribution using Levene Test. Unbalanced data, such as BMI and sex have been compared using Mann-Whitney-U-Test. A paragraph mentioning this clear limitation of our study has been added to the limitation section.

4. It's appropriate to check normality assumption for the data using Kolmogorov-Smirnov test, but authors also need to check homogeneity between two groups because the study seems to be highly-unbalanced to apply independent t-tests.

Answer:

We thank the author for this important comment. As we described in the section statistical methods, normally distributed data were compared by unpaired student t-test. For not normal distributed parameters Mann-Whitney-U-Test was used. In addition to Kolmogrov-Smirnov test Levene-test has been performed to check homogeneity of variance. For each variable showing Levene p values ≤ 0.05 data were considered as unbalanced and therefore not normal distributed. Non-parametric analysis was performed in these cases as described above. This process has now been described in more detail in the section statistical methods.

5. Authors accessed retinal capillary density and flow measurements (ICD, CapA-, RCF) based on four different model options (non-adjusted, model 1, model 2, and model 3). But it's hard to read and understand texts and table 1 and 2. So you need to rewrite the results for better understanding purpose. In addition to this, authors also need to explain implication of comparison (coefficients and p-values) of the measurements among four analysis types.

Answer:

The result section has been rewritten and implication of comparison among four analysis types has been explained in advance in the second paragraph of the result section.
6. The reader might be confusing about what is new for this study. Authors might need to explain it in discussion section.

Answer:

A new paragraph and several additional sentences explaining the novelty of findings in our study has been added to the discussion section.

7. Minors/others: Line 36: delete OD

Answer: We thank the reviewer, the error has been corrected.