Corneal nerve and endothelial cell damage in patients with transient ischemic attack and minor ischemic stroke

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Abstract

Objective
To determine if corneal confocal microscopy can identify corneal nerve and endothelial cell abnormalities and may be useful in the prognostication of patients with transient ischemic attack [1] or minor ischemic stroke (IS).

Methods
Thirty-six patients admitted with TIA (n = 14) or minor IS (n = 22) underwent transcranial Doppler evaluation and corneal confocal microscopy and were compared with 18 healthy controls.

Results
Corneal nerve fiber density (P = 0.002), branch density (P = 0.004) and fiber length (P = 0.004) were significantly lower in patients with TIA or minor IS compared to controls, with no difference between patients with TIA and minor IS. Endothelial cell density (P = 0.003) was lower and endothelial cell area (P = 0.003) and perimeter (P = 0.006) were significantly higher in patients with TIA or minor IS compared to controls, with no difference between patients with TIA and minor IS. There were no differences in corneal nerve or endothelial cell morphology between patients with and without abnormal cerebrovascular reactivity. HbA1c was independently associated with CNFL, and endothelial cell polymegathism and pleomorphism were associated with both HbA1c and total cholesterol.

Conclusion
Corneal confocal microscopy identifies corneal nerve fiber loss and endothelial cell abnormalities in patients with TIA and minor IS and independent associations with HbA1c and cholesterol.
Introduction
Stroke is associated with high fatality rates and major disability in survivors [2]. Transient Ischemic Attack [1] and minor ischemic stroke (IS) share similar pathophysiology to stroke [3]. Although, the ABCD2 score has been used to prognosticate the risk of subsequent stroke [4], a meta-analysis showed that it does not reliably discriminate patients at low or high risk of recurrent stroke [5]. Similarly, neuroimaging may enhance the prognostic ability following TIA and minor stroke. However, recent analyses of patients with TIA or minor IS show that white matter lesions are associated with disability at 90 days, but not with stroke progression or stroke recurrence [6], and micro bleeds predict neither 90-day outcome or recurrence [7].

Cerebral auto-regulation assures hemodynamic integrity of the cerebral circulation [8] and maintains cerebral blood flow (CBF) [9]. In addition to arterial blood pressure, intracranial pressure and cerebral venous pressure may affect auto regulation and CBF [10]. Whilst impaired cerebral auto regulation is associated with poor functional and prognostic outcomes in patients with ischemic stroke [9], only a third of patients with acute ischemic stroke have impaired cerebral auto regulation and it does not relate to stroke type or severity [11].

Corneal confocal microscopy (CCM) is a noninvasive ophthalmic imaging technique, which allows rapid, high-resolution imaging of the cornea. We have pioneered this technique to identify axonal loss in patient with diabetes [12], impaired glucose tolerance [13, 14] and other peripheral neuropathies [15]. CCM can also detect corneal nerve loss in Parkinson’s disease [16], amyotrophic lateral sclerosis [17] and multiple sclerosis [18]. Recently, in patients with major ischemic stroke we have shown a significant reduction in corneal nerves [19] and abnormalities in endothelial cells [20].

Hypothesis
We hypothesize that patients with TIA and minor IS will have evidence of corneal nerve and endothelial cell abnormalities which will aid in prognostication of patients with TIA and minor stroke.

Methods
Forty patients with TIA or minor IS, aged between 18-80-year-old and able to provide consent were enrolled in the study. The diagnosis of TIA or minor ischemic stroke was confirmed clinically and radiologically by neurologists and neuroradiologists using AHA criteria [21]. Patients with craniocerebral trauma, hypertensive encephalopathy, brain tumor, atrial fibrillation or taking anticoagulants were excluded. Three patients were excluded as they were found to be TIA mimics and one had cerebral venous sinus thrombosis. Thirty-six patients underwent Transcranial Doppler Ultrasound (TCD) and Corneal Confocal Microscopy (CCM). Ethical approvals were obtained from the Institutional Review Boards of Hamad General Hospital and Weill Cornell Medicine in Qatar.

Corneal confocal microscopy
All patients underwent CCM (Heidelberg Retinal Tomograph III Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany). To perform the CCM examination, local anesthetic (0.4% benoxinate hydrochloride; Chauvin Pharmaceuticals, Chefaro, United Kingdom) was used to anesthetize both eyes, and Viscoatears (Carbomer 980, 0.2%, Novartis, United Kingdom) was used as the coupling agent between the cornea and the CCM [22]. The examiners captured central sub-basal nerve plexus images using the section mode (Fig 1). On the basis of depth, contrast, focus, and position, 6 images per patient were selected [23].
Corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL) and corneal nerve fiber tortuosity (CNFT) were analysed manually using CCMetrics (M. A. Dabbah, ISBE, University of Manchester, Manchester, United Kingdom) [12] and the investigator was blinded to the diagnosis. Corneal endothelial cell density, area, perimeter and degree of polymegathism (cell size variability) and pleomorphism (cell shape variability) were quantified using automated CEAS software [24].

**Transcranial doppler ultrasound**

Blood flow in the right and left middle cerebral arteries [25] was measured using a trans-temporal approach. Basal and peak flow velocities and cerebrovascular reactivity to hypercapnia was measured by the Breath-Holding Index (BHI) [26].

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\frac{Mean\ MCAV - Mean\ MCAV\ baseline}{Mean\ MCAV\ at\ baseline} \times \frac{100}{Seconds\ of\ breath\ holding} \geq 0.69
\]

**Statistical analysis**

Statistical analyses were performed using IBM SPSS Statistics software Version 25. Normality of the data was assessed using the Shapiro-Wilk test and by visual inspection of the histogram and a normal Q-Q plot. Data are expressed as mean and SD for the normally distributed variables and as median and range for the skewed variables. Inferential analyses were conducted for the corneal nerve and endothelial cell outcomes using both parametric (T-test and ANOVA) and non-parametric (Mann-Whitney U and Kruskal–Wallis) tests, with Bonferroni adjustment. To investigate the association between risk factors for corneal nerve and
Results

Clinical and metabolic characteristics

The clinical and metabolic characteristics are summarized in Table 1. Thirty-six patients with TIA (n = 14) and minor IS (n = 22) were compared with 18 age-matched healthy controls without diabetes, hypertension or previous TIA/stroke. Of the 36 patients with TIA and IS, based on HbA1c and history, 13 had no diabetes; 9 had pre-diabetes and 14 had Type 2 diabetes. There was no significant difference in age (P = 0.241), HbA1c (P = 0.243), total cholesterol (P = 0.092); LDL-C (P = 0.309); HDL-C (P = 0.105); TG (P = 0.192) or body mass index (P = 0.195) between control subjects and patients with TIA or minor IS. Systolic blood

Table 1. Clinical, metabolic and CCM parameters in patients with TIA, minor IS and healthy controls.

| Clinical characteristics | Control   | TIA       | Minor IS  | P-Value |
|--------------------------|-----------|-----------|-----------|---------|
| Age (years)              | 43.39 ± 13.73 | 47.36 ± 8.71 | 48.84 ± 8.77 | 0.241   |
| NIHSS                    | N/A       | 1 ± 1     | 2 ± 2     | 0.001†  |
| Mean BHI                 | N/A       | 0.52 ± 0.57 | 0.47 ± 0.55 | 0.787   |
| HbA1c (%)                | 5.6 ± 0.30 | 6.0 ± 1.10 | 7.0 ± 2.70 | 0.243   |
| Total cholesterol (mmol/L) | 3.95 ± 1.93 | 4.32 ± 1.02 | 5.13 ± 1.40 | 0.092   |
| LDL-C (mmol/L)           | 2.96 ± 1.07 | 2.51 ± 0.93 | 3.12 ± 1.23 | 0.309   |
| HDL-C (mmol/L)           | 1.12 ± 0.21 | 0.91 ± 0.21 | 0.97 ± 0.23 | 0.105   |
| TG (mmol/L)              | 1.2 ± 0.70 | 2.1 ± 1.50 | 2.3 ± 1.80 | 0.192   |
| SBP (mmHg)               | 120.9 ± 12.40 | 147.57 ± 24.83† | 143.45 ± 23.02† | 0.012†  |
| DBP (mmHg)               | 75.1 ± 7.29 | 85.0 ± 14.81 | 88.23 ± 13.90 | 0.040   |
| BMI (kg/m²)              | 25.97 ± 1.84 | 26.46 ± 2.35 | 28.34 ± 4.61 | 0.195   |
| CCM                      |           |           |           |         |
| CNFD (fibers/mm²)        | 38.18 ± 7.85 | 30.12 ± 8.32† | 28.86 ± 8.05† | 0.002†  |
| CNBD (branches/mm²)      | 69.79; 87.50 | 44.79; 119.79 | 37.5; 108.33† | 0.004†  |
| CNFL (mm/mm²)            | 21.55 ± 4.19 | 16.80 ± 5.07† | 16.41 ± 5.20† | 0.004†  |
| CNFT (TC)                | 0.04; 0.10 | 0.03; 0.07 | 0.03; 0.09 | 0.186   |
| ECD (cells/mm²)          | 3633 ± 176.00 | 3411 ± 408.00 | 3366 ± 229.00† | 0.003†  |
| ECA (μm²)                | 222 ± 11.00 | 240 ± 30.00° | 241 ± 18.00° | 0.003°  |
| ECP (μm)                 | 53.0 ± 1.00 | 55.0 ± 4.00° | 55.0 ± 2.00° | 0.006°  |
| EC polymegathism (%)     | 52.0 ± 5.00 | 51.0 ± 3.00 | 52.0 ± 5.00 | 0.825   |
| EC pleomorphism (%)      | 34.0 ± 5.00 | 34.0 ± 4.00 | 35.0 ± 6.00 | 0.894   |

All results were expressed as mean ± SD, except CNBD and CNFT expressed as median; range. TIA: Transient ischemic attack; IS: Ischemic Stroke; LDL-C: Low-density lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; TG: Triglycerides; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; CNFD: Corneal nerve fiber density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length; CNFT: Corneal nerve fiber tortuosity; ECD: Endothelial cell density; ECA: Endothelial cell area; ECP: Endothelial cell perimeter; EC: Endothelial cell. NIHSS and mean BHI were not assessed for the control group.

* Statistically significant differences between groups using ANOVA.
‡ Statistically significant difference between groups using Kruskal-Wallis test.
†Post hoc results differ significantly from the control group after adjustment for multiple comparisons using Bonferroni correction (P<0.02).

https://doi.org/10.1371/journal.pone.0213319.t001

endothelial cell parameters, Pearson and Spearman correlation were performed as appropriate. Multiple linear regression analysis was conducted to evaluate the independent association between corneal nerve and endothelial cell parameters and their covariates. Significance level was set at α = 0.05. Prism 6 (version 6.0g; Graphpad software Inc, CA) was used to plot the charts.
pressure (SBP) was significantly higher ($P = 0.012$) in patients with TIA or minor IS compared to controls.

**Corneal confocal microscopy**

CNFD ($P = 0.002$), CNBD ($P = 0.004$) and CNFL ($P = 0.004$) were significantly lower in patients with TIA or minor IS compared to controls, with no difference between patients with TIA or minor IS (Table 1, Figs 2 and 3). Endothelial cell density ($P = 0.003$) was lower and endothelial cell area ($P = 0.003$) and perimeter ($P = 0.006$) were significantly higher with no difference in the degree of polymegathism ($P = 0.825$) and pleomorphism ($P = 0.894$) between patients with TIA or minor IS compared to controls and no difference between patients with

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Fig 2. Corneal nerve fiber parameters in control subjects and patients with TIA and minor IS. (A) CNFD: Corneal nerve fiber density; (B) CNBD: Corneal nerve branch density, (C) CNFL: Corneal nerve fiber length; Data are expressed as mean ± SD. TIA: Transient Ischemic Attack; IS: Ischemic stroke.

https://doi.org/10.1371/journal.pone.0213319.g002
TIA or minor IS (Table 1, Figs 4 and 5). There was no difference in CNFD ($P = 0.77$), CNBD ($P = 0.08$), CNFL ($P = 0.45$), endothelial cell density ($P = 0.44$), endothelial cell area ($P = 0.41$), perimeter ($P = 0.42$), polymegathism ($P = 0.95$), and pleomorphism ($P = 0.90$), between participants without diabetes, pre-diabetes and diabetes.

Cerebrovascular reactivity

64% of patients with TIA ($n = 14$) and 68% with minor IS ($n = 22$) had abnormal BHI. Comparing patients with normal and abnormal BHI there was no significant difference in: CNFD

Fig 3. Images of corneal sub-basal nerve plexus. Control subject (A), patient with TIA (B) and a patient with minor IS (C).

https://doi.org/10.1371/journal.pone.0213319.g003
(30.52 ± 7.10; 28.80 ± 8.49; P = 0.58); CNBD (33.85 and 64.58; 41.93 and 127.08; P = 0.36); CNFL (16.71 ± 4.11; 16.49 ± 5.51; P = 0.91); CNFT (0.03 and 0.07; P = 0.93); ECD (3399.84 ± 344.58; 3371.92 ± 274.36; P = 0.80); ECA (239.58 ± 24.89; 241.02 ± 21.19; P = 0.86); ECP (55.23 ± 2.78; 55.3 ± 2.56; P = 0.94); percentage with polymegathism (52.49 ± 4.68; 51.32 ± 4.14; P = 0.47) and pleomorphism (35.03 ± 7.01; 34.14 ± 4.90; P = 0.67).

**Correlation.** NIHSS at presentation correlated with CNFD ($r = 0.364$, $P = 0.031$) and CNFL ($r = 0.345$, $P = 0.046$). There was no correlation between corneal nerve and endothelial cell parameters and BHI or age.

**Multiple linear regression.** There were independent associations between some corneal nerve and endothelial cell parameters with age, HbA$_{1c}$ and total cholesterol (Table 2). There was a significant association between HbA$_{1c}$ with CNFL ($B = -0.768$, $P = 0.04$) and endothelial...
cell pleomorphism ($B = -1.261, P = 0.001$). Total cholesterol was associated with endothelial cell polymegathism ($B = -1.628, P = 0.016$) and pleomorphism ($B = 2.637, P = 0.001$). Age was significantly associated with endothelial cell pleomorphism ($B = 0.412, P < 0.001$). BHI was not associated with CNFL ($B = -0.137, P = 0.217$); CNFD ($B = 2.1, P = 0.456$); endothelial cell density ($B = -124.545, P = 0.306$); endothelial cell area ($B = 9.607, P = 0.295$); endothelial cell perimeter ($B = 1.309, P = 0.228$), polymegathism ($B = 1.758, P = 0.272$) or pleomorphism ($B = -1.829, P = 0.321$). CNBD and CNFT were skewed, therefore they were not included in the multiple regression analysis.

Fig 5. Images of corneal endothelium. Control subject (A), patient with TIA (B) and a patient with minor IS (C).

https://doi.org/10.1371/journal.pone.0213319.g005
Table 2. Independent risk factors for altered corneal nerve and endothelial cell parameters in patients with TIA and minor IS.

| Parameter                  | B      | 95% CI     | SE    | P-Value |
|----------------------------|--------|------------|-------|---------|
| **CNFD (fibers/mm²)**      |        |            |       |         |
| Age (years)                | -0.199 | (-0.551–0.153) | 0.1795 | 0.268   |
| Mean BHI                   | 2.1    | (-3.42–7.619)  | 2.8162 | 0.456   |
| HbA1c (%)                  | -1.038 | (-2.222–0.147) | 0.6044 | 0.086   |
| Total cholesterol (mmol/L) | 1.514  | (-0.908–3.936) | 1.2339 | 0.22    |
| TG (mmol/L)                | -1.315 | (-2.914–0.284) | 0.8157 | 0.107   |
| **CNFL (mm/mm²)**          |        |            |       |         |
| Age (years)                | -0.137 | (-0.36–0.08)  | 0.1113 | 0.217   |
| Mean BHI                   | 0.61   | (-2.81–4.03)  | 1.746  | 0.727   |
| HbA1c (%)                  | -0.768 | (-1.50 – 0.03) | 0.3747 | 0.04    |
| Total cholesterol (mmol/L) | 0.681  | (-0.82–2.18)  | 0.7662 | 0.374   |
| TG (mmol/L)                | -0.558 | (-1.55–0.43)  | 0.5057 | 0.270   |
| **ECD (cells/mm²)**        |        |            |       |         |
| Age (years)                | -9.881 | (-25.172–5.41) | 7.8018 | 0.205   |
| Mean BHI                   | -124.545 | (-363.039–113.949) | 121.6829 | 0.306 |
| HbA1c (%)                  | 14.899 | (-33.596–63.395) | 24.7431 | 0.547   |
| Total cholesterol (mmol/L) | -13.324 | (-113.824–87.175) | 51.2761 | 0.795   |
| TG (mmol/L)                | 14.665  | (-51.387–80.717) | 33.7005 | 0.663   |
| **ECA (μm²)**              |        |            |       |         |
| Age (years)                | 0.704  | (-0.45–1.858)  | 0.5888 | 0.232   |
| Mean BHI                   | 9.607  | (-8.392–27.606) | 9.1834 | 0.295   |
| HbA1c (%)                  | -0.879  | (-4.539–2.781) | 1.8673 | 0.638   |
| Total cholesterol (mmol/L) | 1.223  | (-6.361–8.808)  | 3.8698 | 0.752   |
| TG (mmol/L)                | -0.758  | (-5.743–4.227)  | 2.5434 | 0.766   |
| **ECP (μm)**               |        |            |       |         |
| Age (years)                | 0.051  | (-0.086–0.187)  | 0.0696 | 0.468   |
| Mean BHI                   | 1.309  | (-0.818–3.436)  | 1.085  | 0.228   |
| HbA1c (%)                  | 0.002  | (-0.431–0.434)  | 0.2206 | 0.994   |
| Total cholesterol (mmol/L) | 0.01   | (-0.886–0.906)  | 0.4572 | 0.982   |
| TG (mmol/L)                | -0.108  | (-0.697–0.481)  | 0.3005 | 0.72    |
| **EC Polymegathism (%)**   |        |            |       |         |
| Age (years)                | -0.198 | (-0.399–0.003)  | 0.1027 | 0.054   |
| Mean BHI                   | 1.758  | (-1.381–4.896)  | 1.6012 | 0.272   |
| HbA1c (%)                  | 0.637  | (-0.001–1.275)  | 0.3256 | 0.050   |
| Total cholesterol (mmol/L) | -1.628 | (-2.951–0.306)  | 0.6747 | 0.016   |
| TG (mmol/L)                | 0.628  | (-0.241–1.497)  | 0.4435 | 0.157   |
| **EC Pleomorphism (%)**    |        |            |       |         |
| Age (years)                | 0.412  | (0.18–0.643)   | 0.118  | 0.001   |
| Mean BHI                   | -1.829 | (-5.437–1.78)   | 1.841  | 0.321   |
| HbA1c (%)                  | -1.261 | (-1.995 – 0.527) | 0.3743 | 0.001   |
| Total cholesterol (mmol/L) | 2.637  | (1.116–4.157)   | 0.7758 | 0.001   |
| TG (mmol/L)                | -0.321 | (-1.32–0.679)   | 0.5099 | 0.529   |

BHI: Breath holding index; HbA1c: Glycated hemoglobin; TG: Triglycerides; CNFL: Corneal nerve fiber length; CNFD: Corneal nerve fiber density; ECD: Endothelial cell density; ECA: Endothelial cell area; ECP: Endothelial cell perimeter; EC: Endothelial cells.

https://doi.org/10.1371/journal.pone.0213319.t002
**Discussion and conclusions**

This is the first study to demonstrate corneal nerve and endothelial cell pathology in patients with TIA or minor IS, extending our previous findings in patients with major stroke [19, 20]. Diabetes, hypertension, smoking, dyslipidemia [27–29], obesity [25] and metabolic syndrome [30] are known risk factors for stroke and are linked to cerebral white matter lesions and silent lacunar brain infarcts [31], but have limited prognostic value for recurrent stroke in patients with TIA and minor IS [4]. Impaired cerebral reactivity has been associated with the risk of subsequent stroke in patients with TIA [32, 33], and smoking, hypertension, diabetes and cholesterol are related to altered CBF in patients with TIA and minor stroke [31, 34]. Endothelial dysfunction is involved in the pathophysiology of TIA [35] and lacunar stroke [36] and has been implicated in the development of silent lacunar infarcts and white matter lesions [37]. It may also act as an independent predictor for a recurrent ischemic event [38, 39].

The corneal endothelium has traditionally been thought to play a role in primarily regulating the passage of nutrients and metabolic waste to and from the cornea [40], however, it also shows thrombogenic potential after exposure to extracellular matrix and collagen [41]. We have previously demonstrated a reduction in corneal endothelial cell density in patients with diabetes [42, 43]. We have also recently developed an automated image analysis system to quantify corneal endothelial cell morphology and shown reduced corneal endothelial cell density and hypertrophy in patients with diabetes [24]. Given that we found corneal nerve and endothelial cell abnormalities in patients with TIA and minor stroke, we assessed for associations with cerebrovascular reactivity and risk factors for stroke. We show no difference in corneal endothelial cell and nerve morphology between patients with and without abnormal cerebrovascular reactivity, suggesting alternate mechanisms driving these two abnormalities in patients with cerebrovascular disease.

Contrary to our previous studies in subjects with impaired glucose tolerance and diabetes (12, 13), we failed to demonstrate a difference in corneal nerve and endothelial cell pathology between participants without diabetes, pre-diabetes and diabetes. This was despite an association between endothelial cell polymegathism and pleomorphism with total cholesterol and HbA1c and between CNFL and HbA1c. Indeed, we have previously shown a loss of corneal nerves in subjects with impaired glucose tolerance (IGT) and type 2 diabetes with a major stroke compared to controls, but no difference between participants with IGT and T2DM, despite an association between corneal nerve morphology with HbA1c and triglycerides [19]. We can only attribute this lack of difference to an as yet unidentified confounding bias in this population with cerebrovascular disease, the relatively small cohort size and the influence of concurrent medication. CNFL and CNFD correlated directly with the severity of stroke at presentation, arguing that alterations in corneal nerve morphology are not related to the acute event. Age correlated with CNFL, which agrees with a number of previous studies [44, 45].

A limitation of this study is the small sample size of younger, predominantly South Asian patients, which might limit the generalizability of our study findings. However, this is the first study to show an abnormality in corneal nerves and endothelial cells in patients with TIA and minor stroke, and extend our recent findings in patients with major stroke. There is a need for larger, longitudinal studies to assess the prognostic value of corneal nerve and endothelial cell imaging in relation to recurrent TIA or stroke.

**Supporting information**

**S1 Dataset.**

(XLSX)
Acknowledgments
This research was facilitated by the research division at WCM-Q, Ultrasound department, and Neurology Services at HMC, Qatar.

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References
1. Deeb A, Al Qahtani N, Attia S, Al Suwaidi H, Nagelkerke N. Does Reducing Basal Insulin During Ramadan Fasting by Children and Adolescents with Type 1 Diabetes Decrease the Risk of Symptomatic Hypoglycemia? Diabetes Technol Ther. 2016; 18(9):539–42. Epub 2016/08/09. https://doi.org/10.1089/ dia.2016.0197 PMID: 27500913.
2. Benamer HT, Grosset D. Stroke in Arab countries: a systematic literature review. J Neurol Sci. 2009; 284(1–2):18–23. Epub 2009/05/12. https://doi.org/10.1016/j.jns.2009.04.029 PMID: 19428027.
3. Duca A, Jagoda A. Transient Ischemic Attacks: Advances in Diagnosis and Management in the Emergency Department. Emerg Med Clin North Am. 2016; 34(4):811–35. Epub 2016/10/16. https://doi.org/10.1016/j.emc.2016.06.007 PMID: 27741990.
4. Yaghi S, Willey JZ, Khatri P. Minor ischemic stroke: Triaging, disposition, and outcome. Neurol Clin Pract. 2016; 6(2):157–63. Epub 2016/04/23. https://doi.org/10.1212/CPJ.0000000000000234 PMID: 27104067; PubMed Central PMCID: PMCPMC4828677.
5. Wardlaw JM, Brazzelli M, Chappell FM, Miranda H, Shuler K, Sandercock PA, et al. ABCD2 score and secondary stroke prevention: meta-analysis and effect per 1,000 patients triaged. Neurology. 2015; 85(4):373–80. Epub 2015/07/03. https://doi.org/10.1212/WNL.0000000000001780 PMID: 26136519; PubMed Central PMCID: PMCPMC4520819.
6. Zerna C, Yu AYX, Modi J, Patel SK, Coulter JI, Smith EE, et al. Association of White Matter Hyperintensities With Short-Term Outcomes in Patients With Minor Cerebrovascular Events. Stroke. 2018; 49(4):919–23. Epub 2018/03/16. https://doi.org/10.1161/STROKEAHA.117.017429 PMID: 29540612.
7. Zerna C, Modi J, Bilston L, Shoaamantes A, Coutts SB, Smith EE. Cerebral Microbleeds and Cortical Superficial Siderosis in Patients Presenting With Minor Cerebrovascular Events. Stroke. 2016; 47(9):2236–41. Epub 2016/08/11. https://doi.org/10.1161/STROKEAHA.116.013418 PMID: 27507863.
8. Reinhard M, Roth M, Muller T, Guschlbauer B, Timmer J, Czosnyka M, et al. Effect of carotid endarterectomy or stenting on impairment of dynamic cerebral autoregulation. Stroke. 2004; 35(6):1381–7. Epub 2004/04/17. https://doi.org/10.1161/01.STR.0000127533.46914.31 PMID: 15087557.
9. Aoi MC, Hu K, Lo MT, Selim M, Olufsen MS, Novak V. Impaired cerebral autoregulation is associated with brain atrophy and worse functional status in chronic ischemic stroke. PloS One. 2012; 7(10):e46794. Epub 2012/10/17. https://doi.org/10.1371/journal.pone.0046794 PMID: 23071639; PubMed Central PMCID: PMCPMC3468603.
10. Gao E, Young WL, Pile-Spellman J, Ornstein E, Ma Q. Mathematical considerations for modeling cerebral blood flow autoregulation to systemic arterial pressure. Am J Physiol. 1998; 274(3 Pt 2):H1023–31. Epub 1998/04/08. PMID: 9530217.

11. Llwyd O, Sainet AS, Panerai RB, Lam MY, Saed NP, Brodie F, et al. Cerebral Haemodynamics following Acute Ischaemic Stroke: Effects of Stroke Severity and Stroke Subtype. Cerebrovasc Dis Extra. 2018; 8(2):8–9. Epub 2018/07/12. https://doi.org/10.1159/000487514 PMID: 29996123.

12. Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, et al. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. Invest Ophthalmol Vis Sci. 2014; 55(4):2071–8. Epub 2014/02/27. https://doi.org/10.1167/ iovs.13-13787 PMID: 24569580; PubMed Central PMCID: PMCPMC3979234.

13. Asghar O, Petropoulos IN, Alam U, Jones W, Jeziorska M, Marshall A, et al. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. Diabetes Care. 2014; 37(9):2643–6. Epub 2014/06/28. https://doi.org/10.2337/dc14-0279 PMID: 24969581; PubMed Central PMCID: PMCPMC410158.

14. Tavakoli M, Marshall A, Pitcaethly R, Fadavi H, Gow D, Roberts ME, et al. Corneal confocal microscopy: a novel means to detect nerve fibre damage in idiopathic small fibre neuropathy. Exp Neurol. 2010; 223(1):245–50. Epub 2009/09/15. https://doi.org/10.1016/j.expne urol.2009.08.033 PMID: 19748505; PubMed Central PMCID: PMC2938826.

15. Ferdoussi M, Azmi S, Petropoulos IN, Fadavi H, Ponirakis G, Marshall A, et al. Corneal Confocal Microscopy Detects Small Fibre Neuropathy in Patients with Upper Gastrointestinal Cancer and Nerve Regeneration in Chemotherapy Induced Peripheral Neuropathy. PLoS One. 2015; 10(10):e0193994. Epub 2015/10/03. https://doi.org/10.1371/journal.pone.0193994 PMID: 26430773; PubMed Central PMCID: PMCPMC4592260.

16. Kass-Illyia L, Javed S, Gosdal D, Kobylycki C, Marshall A, Petropoulos IN, et al. Small fiber neuropathy in Parkinson’s disease: A clinical, pathological and corneal confocal microscopy study. Parkinsonism Relat Disord. 2015; 21(12):1454–60. Epub 2015/11/19. https://doi.org/10.1016/j.parkreldis.2015.10.019 PMID: 26578039; PubMed Central PMCID: PMC4671992.

17. Ferrari G, Grisan E, Scarpa F, Fazio R, Comola M, Quattrini A, et al. Corneal confocal microscopy reveals trigeminal small sensory fiber neuropathy in amyotrophic lateral sclerosis. Front Aging Neurosci. 2014; 6:278. Epub 2014/11/02. https://doi.org/10.3389/fnagi.2014.00278 PMID: 25360111; PubMed Central PMCID: PMCPMC4199282.

18. Mikolajczak J, Zimmermann H, Kheirkhah A, Kadas EM, Oberwahrenbrock T, Muller R, et al. Patients with multiple sclerosis demonstrate reduced subbasal corneal nerve fibre density. Mult Scler. 2017; 23(14):1847–53. Epub 2016/11/05. https://doi.org/10.1177/1352458516677590 PMID: 27811337; PubMed Central PMCID: PMC5513781.

19. Khan A, Akhtar N, Kamran S, Ponirakis G, Petropoulos IN, Tunio NA, et al. Corneal Confocal Microscopy Detects Corneal Nerve Damage in Patients Admitted With Acute Ischemic Stroke. Stroke. 2017; 48(11):3012–6. Epub 2017/10/12. https://doi.org/10.1161/STROKEAHA.117.018289 PMID: 29018135.

20. Khan A KS, Akhtar N, Ponirakis G, Al-Muhammad H, Petropoulos IN, et al. Corneal Confocal Microscopy detects a Reduction in Corneal Endothelial Cells and Nerve Fibres in Patients with Acute Ischemic Stroke. Sci Rep 2018 8(1):17333 . https://doi.org/10.1038/s41598-018-35298-3 PMID: 30476334.

21. Powers JW, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2018; 49(3):e46–e110. Epub 2018/01/26. https://doi.org/10.1161/STR.0000000000000158 PMID: 29367334.

22. Tavakoli M, Malik RA. Corneal confocal microscopy: a novel non-invasive technique to quantify small fibre pathology in peripheral neuropathies. J Vis Exp. 2011;(47). Epub 2011/01/21. https://doi.org/10.3791/2194 PMID: 21248693; PubMed Central PMCID: PMC3182640.

23. Vagenas D, Pritchard N, Edwards K, Shahidi AM, Sampson GP, Russell AW, et al. Optimal image sample size for corneal nerve morphometry. Optom Vis Sci. 2012; 89(5):812–7. Epub 2012/03/13. https://doi.org/10.1097/OPX.0b013e31824ee8c8 PMID: 22427024.

24. Al-Fahdawi S, Qahwaji R, Al-Waisy AS, Ipson S, Ferdoussi M, Malik RA, et al. A fully automated cell segmentation and morphometric parameter system for quantifying corneal endothelial cell morphology. Comput Methods Programs Biomed. 2018; 160:11–23. Epub 2018/05/08. https://doi.org/10.1016/j .cmpb.2018.03.015 PMID: 29728238.

25. Mitchell AB, Cole JW, McArdle PF, Cheng YC, Ryan KA, Sparks MJ, et al. Obesity increases risk of ischemic stroke in young adults. Stroke. 2015; 46(6):1690–2. Epub 2015/05/07. https://doi.org/10.1161/ STROKEAHA.115.008940 PMID: 25944320; PubMed Central PMCID: PMCPMC4581373.

26. Silvestrini M, Troisi E, Matteis M, Cupini LM, Caltagirone C. Transcranial Doppler assessment of cerebrovascular reactivity in symptomatic and asymptomatic severe carotid stenosis. Stroke. 1996; 27(11):1970–3. Epub 1996/11/01. PMID: 8898800.
27. Baird TA, Parsons MW, Barber PA, Butcher KS, Desmond PM, Tress BM, et al. The influence of diabetes mellitus and hyperglycaemia on stroke incidence and outcome. J Clin Neurosci. 2002; 9(6):618–26. Epub 2003/02/27. PMID: 12604269.

28. Kiyohara Y, Ueda K, Fujishima M. Smoking and cardiovascular disease in the general population in Japan. J Hypertens Suppl. 1990; 8(5):S9–15. Epub 1990/09/01. PMID: 2286856.

29. Shuaib A. Alteration of blood pressure regulation and cerebrovascular disorders in the elderly. Cerebrovasc Brain Metab Rev. 1992; 4(4):329–45. Epub 1992/01/01. PMID: 1486018.

30. Li X, Li X, Lin H, Fu X, Lin W, Li M, et al. Metabolic syndrome and stroke: A meta-analysis of prospective cohort studies. J Clin Neurosci. 2017; 40:34–8. Epub 2017/03/08. https://doi.org/10.1016/j.jocn.2017.01.018 PMID: 28268148.

31. Wijnhoud AD, Koudstaal PJ, Dippel DW. Relationships of transcranial blood flow Doppler parameters with major vascular risk factors: TCD study in patients with a recent TIA or nonsissing ischemic stroke. J Clin Ultrasound. 2006; 34(2):70–6. Epub 2006/03/21. https://doi.org/10.1002/jcu.20193 PMID: 16547983.

32. Silvestrini M, Vernieri F, Pasqualett P, Matteis M, Passarelli F, Troisi E, et al. Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. JAMA. 2000; 283(16):2122–7. Epub 2000/05/03. PMID: 10791504.

33. Markus H, Cullinane M. Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. Brain. 2001; 124(Pt 3):457–67. Epub 2001/02/27. PMID: 1122446.

34. Sacco RL, Kargman DE, Gu Q, Zamanillo MC. Race-ethnicity and determinants of intracranial atherosclerotic cerebral infarction. The Northern Manhattan Stroke Study. Stroke. 1995; 26(1):14–20. Epub 1995/01/01. PMID: 7839388.

35. Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, et al. The vascular bed of the human heart: a systematic review. Cardiovasc Ultrasound. 2010; 8:46. Epub 2010/10/20. https://doi.org/10.1186/1476-7120-8-46 PMID: 20956212; PubMed Central PMCID: PMCPM1713036.

36. Stevenson SF, Doublay FN, Shuler K, Wardlaw JM. A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls. Stroke. 2010; 41(6):e434–42. Epub 2010/04/07. https://doi.org/10.1161/STROKEAHA.110.569855 PMID: 20395619.

37. Knothenerus II, Ten Cate H, Lodder J, Kessels R, van Oostenbrugge RJ. Endothelial dysfunction in lacunar stroke: a systematic review. Cerebrovasc Dis. 2009; 27(5):519–26. Epub 2009/04/18. https://doi.org/10.1159/000212672 PMID: 19372654.

38. Santos-Garcia D, Blanco M, Serena J, Rodriguez-Yanez M, Leira R, Castillo J. Impaired brachial flow-mediated dilation is a predictor of a new-onset vascular event after stroke. Cerebrovasc Dis. 2011; 32(2):155–62. Epub 2011/07/23. https://doi.org/10.1159/000328651 PMID: 21778713.

39. Beer CD, Potter K, Blacker D, Arnolda L, Hankey GJ, Puddey IB. Systemic vascular function, measured with forearm flow mediated dilation, in acute and stable cerebrovascular disease: a case-control study. Cardiovasc Ultrasound. 2010; 8:46. Epub 2010/10/20. https://doi.org/10.1186/1476-7120-8-46 PMID: 20956212; PubMed Central PMCID: PMCPM1713036.

40. He Z, Forest F, Gain P, Rageade D, Bernard A, Acquart S, et al. 3D map of the human corneal subbasal nerve plexus: a longitudinal study. Invest Ophthalmol Vis Sci. 2016; 57(3):853–8. Epub 2016/03/05. https://doi.org/10.1167/iovs.15-18735 PMID: 26943147.

41. Sage H, Pritzl P, Bornstein P. Secretory phenotypes of endothelial cells in culture: comparison of aortic, venous, capillary, and corneal endothelium. Arteriosclerosis. 1981; 1(6):427–42. Epub 1981/11/01. PMID: 7347207.

42. Bitirgen G, Ozkagnici A, Malik RA, Kerimoglu H. Corneal nerve fibre damage proceeds diabetic retinopathy in patients with type 2 diabetes mellitus. Diabet Med. 2014; 31(4):431–8. Epub 2013/10/15. https://doi.org/10.1111/dme.12324 PMID: 24117485.

43. Szalai E, Deak E, Modis L Jr., Nemeth G, Berta A, Nagy A, et al. Early Corneal Cellular and Nerve Fiber Pathology in Young Patients With Type 1 Diabetes Mellitus Identified Using Corneal Confocal Microscopy, Invest Ophthalmol Vis Sci. 2016; 57(3):853–8. Epub 2016/03/05. https://doi.org/10.1167/iovs.15-18735 PMID: 26943147.

44. Dehghani C, Pritchard N, Edwards K, Vagenas D, Russell AW, Malik RA, et al. Morphometric stability of the corneal subbasal nerve plexus in healthy individuals: a 3-year longitudinal study using corneal confocal microscopy. Invest Ophthalmol Vis Sci. 2014; 55(5):3195–9. Epub 2014/04/26. https://doi.org/10.1167/iovs.14-13959 PMID: 24764058.

45. Tavakoli M, Ferdousi M, Petropoulos IN, Morris J, Pritchard N, Zhivov A, et al. Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. Diabetes Care. 2015; 38(5):838–43. Epub 2015/01/31. https://doi.org/10.2337/dc14-2311 PMID: 25633665; PubMed Central PMCID: PMCPM4407754.