Predictors of Development and Progression of Retinopathy in Patients with Type 2 Diabetes: Importance of Blood Pressure Parameters

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Diabetic retinopathy (DR) is a chronic microvascular complication associated with a worse prognosis. We aimed to evaluate the predictors of development/progression of DR in a cohort of 544 high-risk patients with type 2 diabetes who had annual ophthalmologic examinations over a median follow-up of 6 years. Ambulatory blood pressure (BP) monitoring and aortic stiffness by carotid-femoral pulse wave velocity were performed. Multivariate Cox survival analysis examined the independent predictors of development or progression of DR. During follow-up, 156 patients either newly-developed or worsened DR. Patients who developed/progressed DR had longer diabetes duration, higher ambulatory and clinic BP levels, higher aortic stiffness, and poorer glycemic control than patients who did not develop/progressed DR. After adjustments for baseline retinopathy prevalence, age and sex, a longer diabetes duration (p < 0.001), higher baseline ambulatory BPs (p = 0.013, for 24-hour diastolic BP), and higher mean cumulative exposure of HbA1c (p < 0.001), clinic diastolic BP (p < 0.001) and LDL-cholesterol (p = 0.05) during follow-up were the independent predictors of development/progression of DR. BP parameters were only predictors of DR development. In conclusion, a longer diabetes duration, poorer glycemic and lipid control, and higher BPs were the main predictors of development/progression of DR. Mean cumulative clinic diastolic BP was the strongest BP-related predictor.

Diabetic retinopathy (DR) is an important cause of visual impairment and blindness among patients with diabetes. Further, visual impairment as a result of diabetic retinopathy has a significant negative impact on the patient's quality of life and their ability to successfully manage their disease. The risk of DR is mainly attributable to HbA1c and diabetes duration. Many studies reported that better glycemic control reduces retinopathy progression. However, DR can develop despite intensive glucose control, supporting that other risk factors are involved in the pathophysiology of DR. In addition, in patients with type 1 diabetes, metabolic control as measured by HbA1c and disease duration account for only 11% of the risk of retinopathy, leaving 89% to other factors. These data suggest that besides chronic hyperglycemia and diabetes duration, other metabolic factors, such as dyslipidemia, high blood pressure (BP), and chronic inflammation may contribute to overall risk of DR progression.

Otherwise, the influence of BP in development/progression of DR is more controversial. A prospective study reported that systolic BP (SBP) reduction may improve DR and diastolic BP (DBP) increase may worsen DR. A recent meta-analysis of randomized controlled trials (RCTs), in which both type 1 and type 2 diabetic participants were included, demonstrated a beneficial effect of BP reduction in retinopathy development, but not in its progression. And, up to now, the prognostic importance of ambulatory blood pressures for DR development/progression has not been investigated yet. A previous cross-sectional analysis from the Rio de Janeiro Type 2 Diabetes cohort, examining the associations between clinic and ambulatory blood pressure parameters and the presence of microvascular complications, reported that, except for DR and advanced nephropathy, ambulatory BPs are better predictors of DR development/progression.

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correlates of chronic complications than clinic BPs. Moreover, although ambulatory blood pressures have been demonstrated to be better predictors of total mortality and of cardiovascular morbidity and mortality than clinic BPs in type 2 diabetes and in several other clinical conditions, no study investigated its importance as a predictor of development/progression of DR, or compared its prognostic value with that from clinic BPs.

Aortic stiffness is a proposed index of accumulated vascular risk factors burden, and it has been associated with the presence of DR. We have previously demonstrated that increased aortic stiffness predicted the future development of diabetic peripheral neuropathy in type 2 diabetes. Considering that microvascular diabetic complications have some common determinants, it is important to investigate whether aortic stiffness is also a prognostic marker of DR development or progression.

Therefore, we aimed to investigate the independent predictors of DR development or progression in a cohort of high-risk patients with type 2 diabetes, with special attention to the prognostic importance of ambulatory blood pressures in relation to clinic BPs, and to assess if aortic stiffness, assessed by its gold-standard method the carotid-femoral pulse wave velocity, provides additional prognostic information to DR development/progression.

**Results**

**Baseline characteristics and incidence of development or progression of DR during follow-up.** Six hundred and forty-six patients were evaluated at baseline. Of them, 54 patients (8%) had proliferative retinopathy, 12 (1.8%) had glaucoma, and 13 patients (2%) had dense cataract and were excluded for the current analysis. During the first year of follow-up 13 patients died and 10 patients had no ophthalmologic examination, totaling 544 patients with at least two annual ophthalmologic re-evaluations (median number of ophthalmologic examinations was 5 per patient). At baseline, 144 patients (26.5%) had non-proliferative retinopathy (101 mild, 29 moderate and 14 severe). After a median follow-up of 5.8 years (range 1 to 11 years), 156 patients (28.7%) either developed or progressed DR: 77 patients (19.3% of those without DR) newly-developed DR, while 79 patients (54.9% of those with non-proliferative DR) worsened it. Table 1 outlines the characteristics of all patients and of those with and without development/progression of DR. Patients who developed/progressed DR had longer diabetes duration, used more frequently insulin, and had greater prevalences of other microvascular complications than patients who did not develop/progressed DR. Patients who developed/progressed DR had higher clinic BPs during follow-up, higher baseline ambulatory BPs, higher aortic stiffness, and poorer glycemic and lipid control than patients who did not develop/progressed DR.

**Independent predictors of development or progression of DR.** Table 2 presents the results of the multivariate Cox regression analysis for the independent predictors of the composite endpoint of development or progression of DR and of the separate endpoints. Longer diabetes duration and higher HbA1c levels measured at any time interval during follow-up were the strongest predictors of development/progression of DR. At baseline, ambulatory BPs, but not clinic ones, were independent predictors of retinopathy development or progression. During follow-up, clinic DBPs were the main BP-related predictors of retinopathy development or progression; and in the cumulative exposure model, mean HbA1c and clinic DBP were equivalent predictors of development/progression of DR. Higher LDL-cholesterol levels at baseline (with borderline significance) and in the cumulative exposure model were independent predictors of retinopathy development/progression. Regarding the separate endpoints, BP parameters were mainly predictors of new DR development, but not of DR progression (except for a borderline association with 2nd-year DBP), whereas HbA1c levels were strong predictors of both endpoints.
| Characteristics                              | All patients (n = 544) | Patients with development/progression of retinopathy (n = 156) | Patients without development/progression of retinopathy (n = 388) | p-value |
|---------------------------------------------|------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------|
| Age (years)                                 | 60.2 (9.5)             | 59.4 (9.0)                                                    | 60.5 (9.6)                                                    | 0.21    |
| Male gender (%)                             | 37.1                   | 32.7                                                         | 38.9                                                         | 0.20    |
| BMI (kg/m²)                                 | 29.7 (5.3)             | 29.6 (4.6)                                                   | 29.8 (5.6)                                                   | 0.80    |
| Smoking, current/past (%)                   | 45.2                   | 45.5                                                         | 45.1                                                         | 0.99    |
| Physical activity (%)                       | 24.1                   | 27.6                                                         | 22.7                                                         | 0.27    |
| Diabetes duration (years)                   | 7 (3–15)               | 11 (7–18)                                                   | 5 (2–11)                                                     | <0.001  |
| Chronic diabetic complications (%)          |                        |                                                              |                                                              |         |
| Cerebrovascular disease (%)                 | 8.3                    | 10.3                                                        | 7.5                                                          | 0.30    |
| Coronary artery disease (%)                 | 14.7                   | 13.5                                                        | 15.2                                                         | 0.69    |
| Peripheral arterial disease (%)             | 15.5                   | 17.9                                                        | 14.5                                                         | 0.36    |
| Retinopathy                                 | 26.5                   | 50.6                                                        | 16.8                                                         | <0.001  |
| Nephropathy                                 | 28.8                   | 43.6                                                        | 22.9                                                         | <0.001  |
| Peripheral neuropathy                       | 27.0                   | 39.7                                                        | 21.9                                                         | <0.001  |
| Cardiovascular autonomic neuropathy         | 22.2                   | 24.4                                                        | 21.2                                                         | 0.45    |
| Diabetes treatment (%)                      |                        |                                                              |                                                              |         |
| Metformin                                   | 88.1                   | 87.8                                                        | 88.1                                                         | 0.99    |
| Sulfonylureas                                | 45.8                   | 42.3                                                        | 47.2                                                         | 0.34    |
| Insulin                                     | 46.1                   | 67.3                                                        | 37.6                                                         | <0.001  |
| Dyslipidemia (%)                            | 86.6                   | 91.0                                                        | 84.8                                                         | 0.070   |
| Statins use (%)                             | 76.3                   | 81.4                                                        | 74.2                                                         | 0.094   |
| Arterial hypertension (%)                   | 85.5                   | 87.8                                                        | 84.5                                                         | 0.35    |
| Number of anti-hypertensive drugs           | 3 (1–3)                | 3 (1–3)                                                     | 3 (1–3)                                                      | 0.87    |
| ACE inhibitors/AR blockers (%)              | 92.4                   | 92.3                                                        | 92.5                                                         | 0.99    |
| Diuretics (%)                               | 66.1                   | 65.8                                                        | 66.2                                                         | 0.99    |
| Calcium channel blockers (%)                | 28.4                   | 31.6                                                        | 31.0                                                         | 0.92    |
| Beta-blockers (%)                           | 48.7                   | 43.9                                                        | 50.8                                                         | 0.15    |
| Clinic blood pressures (mmHg)               |                        |                                                              |                                                              |         |
| Baseline SBP                                | 146 (24)               | 147 (23)                                                     | 146 (24)                                                     | 0.44    |
| Baseline DBP                                | 84 (13)                | 85 (14)                                                     | 84 (13)                                                      | 0.45    |
| Mean first-year SBP                         | 140 (19)               | 141 (20)                                                    | 139 (19)                                                     | 0.18    |
| Mean first-year DBP                         | 79 (11)                | 80 (10)                                                     | 78 (11)                                                      | 0.12    |
| Mean second-year SBP                        | 140 (18)               | 142 (19)                                                    | 139 (18)                                                     | 0.033   |
| Mean second-year DBP                        | 78 (11)                | 79 (10)                                                     | 77 (11)                                                      | 0.025   |
| Mean cumulative SBP                         | 139 (16)               | 142 (16)                                                    | 138 (16)                                                     | 0.005   |
| Mean cumulative DBP                         | 77 (9)                 | 79 (10)                                                     | 77 (9)                                                       | 0.001   |
| Ambulatory blood pressures (mmHg)           |                        |                                                              |                                                              |         |
| 24-hour SBP                                 | 128 (15)               | 131 (16)                                                    | 127 (14)                                                     | 0.003   |
| 24-hour DBP                                 | 74 (10)                | 75 (11)                                                     | 73 (9)                                                       | 0.023   |
| Daytime SBP                                 | 130 (15)               | 132 (16)                                                    | 129 (14)                                                     | 0.017   |
| Daytime DBP                                 | 75 (10)                | 77 (12)                                                     | 74 (9)                                                       | 0.029   |
| Nighttime SBP                               | 120 (18)               | 124 (20)                                                    | 118 (16)                                                     | 0.001   |
| Nighttime DBP                               | 68 (11)                | 70 (11)                                                     | 67 (10)                                                      | 0.002   |
| Nocturnal SBP fall (%)                      | 9.7 (11.4)             | 7.9 (12.6)                                                  | 10.5 (10.7)                                                  | 0.029   |
| Normal SBP dipping pattern (%)              | 56.1                   | 52.3                                                        | 57.7                                                         | 0.29    |
| Nocturnal DBP fall (%)                      | 9.0 (9.6)              | 7.8 (9.8)                                                   | 9.5 (9.5)                                                    | 0.062   |
| Normal DBP dipping pattern (%)              | 47.0                   | 41.9                                                        | 49.2                                                         | 0.15    |
| Laboratory variables                        |                        |                                                              |                                                              |         |
| Fasting glucose (mmol/L)                    | 8.9 (3.7)              | 9.6 (4.5)                                                   | 8.6 (3.3)                                                    | 0.011   |
| Baseline HbA₁c (%)                          | 8.0 (1.9)              | 8.7 (2.0)                                                   | 7.7 (1.7)                                                    | <0.001  |
| Mean first-year HbA₁c (%)                   | 7.6 (1.5)              | 8.2 (1.7)                                                   | 7.4 (1.3)                                                    | <0.001  |
| Mean second-year HbA₁c (%)                  | 7.7 (1.5)              | 8.5 (1.7)                                                   | 7.4 (1.3)                                                    | <0.001  |
| Mean cumulative HbA₁c (%)                   | 7.7 (1.3)              | 8.3 (1.5)                                                   | 7.4 (1.2)                                                    | <0.001  |
| Triglycerides (mmol/L)                      | 1.6 (1.1–2.4)          | 1.6 (1.1–2.6)                                               | 1.6 (1.1–2.4)                                                | 0.80    |
| HDL-cholesterol (mmol/L)                    | 1.09 (0.30)            | 1.09 (0.32)                                                 | 1.09 (0.29)                                                  | 0.91    |
| Baseline LDL-cholesterol (mmol/L)           | 3.02 (0.99)            | 3.14 (1.04)                                                 | 2.97 (0.96)                                                  | 0.063   |

Continued
Table 1. Characteristics of diabetic patients divided according to development or progression of retinopathy during follow-up. Values are proportions, and means (standard deviations) or medians (interquartile range).

| Characteristics                                      | All patients (n = 544) | Patients with development/progression of retinopathy (n = 156) | Patients without development/progression of retinopathy (n = 388) | p-value |
|------------------------------------------------------|------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|---------|
| Mean first-year LDL-cholesterol (mmol/L)             | 2.79 (0.85)            | 2.97 (0.89)                                                     | 2.72 (0.82)                                                     | 0.002   |
| Mean second-year LDL-cholesterol (mmol/L)           | 2.66 (0.79)            | 2.80 (0.80)                                                     | 2.60 (0.78)                                                     | 0.007   |
| Mean cumulative LDL-cholesterol (mmol/L)            | 2.62 (0.69)            | 2.77 (0.70)                                                     | 2.56 (0.68)                                                     | 0.001   |
| Glomerular filtration rate (ml/min/1.73m²)          | 90 (28)                | 88 (27)                                                        | 92 (31)                                                        | 0.16    |
| Albuminuria (mg/24h)                                | 13 (7–38)              | 18 (8–84)                                                       | 11 (6–23)                                                       | <0.001  |
| Aortic stiffness (cf-PWV, m/s)                       | 9.2 (2.0)              | 9.7 (1.8)                                                       | 9.1 (2.1)                                                       | 0.010   |
| Increased aortic stiffness (cf-PWV>10 m/s, %)        | 21.4                   | 27.8                                                            | 18.7                                                            | 0.025   |

Increased aortic stiffness during the first-year of follow-up was a borderline significant predictor of new DR development, but not of DR progression. Kaplan-Meier analyses showed that a mean cumulative HbA1c exposure ≥7.0%, DBP ≥85 mmHg and LDL-cholesterol ≥2.59 mmol/L (100 mg/dL) were all associated with higher risks of developing/worsening DR (Fig. 1). This was also evidenced for a baseline ambulatory 24-hour SBP ≥130 mmHg, a 24-hour DBP ≥80 mmHg and an increased aortic stiffness (cf-PWV >10 m/s) (Fig. 2).

Table 3 presents the predictive strength of several BP parameters for the composite endpoint of DR development/progression and for the separate endpoints, adjusted for the other independent predictors. As a whole, clinic DBP was a stronger predictor than clinic SBP during follow-up, whereas at baseline ambulatory BPs were superior to clinic BPs. Moreover, BPs were only associated with new development of DR, but not with its progression. The strongest BP-related predictor of new DR development was mean cumulative clinic DBP during follow-up, where a 1-SD increment was associated with a 53% higher risk of DR development. On baseline ABPM, daytime and nighttime BPs were roughly equivalent in their predictive values, and the nocturnal BP fall provided no additional predictive information at all.

**Discussion**

This prospective study with a 6-year median follow-up of a relatively large sample of high cardiovascular risk patients with type 2 diabetes has three main novel findings. First, it demonstrated that at study entry ambulatory BPs were better predictors for new DR development than clinic BPs. Second, that during follow-up mean cumulative clinic DBP was the strongest BP-related predictor of DR development. Third, that aortic stiffness is not independently associated with development/progression of DR, different from what we observed for diabetic peripheral neuropathy, where an increased aortic stiffness was a predictor for its development and progression. Additionally, we demonstrated the prognostic importance of higher LDL-cholesterol levels during follow-up for development/progression of DR, and confirmed the pivotal roles of poor glycemic control and diabetes duration on installation and worsening of DR. Overall, these results suggest that, in high-risk type 2 diabetic patients, DBP may have a greater influence on development of DR; and that ambulatory blood pressure monitoring should be performed routinely in the management of these patients, not only because it allows better cardiovascular risk stratification, but also because it adds prognostic information regarding increased risk of DR development.

Remarkable studies have shown that more intensive glycemic and BP control reduced the onset and progression of DR in patients with type 1 and type 2 diabetes. However, more recent RCTs reported controversial results in type 2 diabetes, particularly regarding BP control, with some studies showing benefit to DR prevention, while others showed no advantage at all, or only small benefit for some specific ophthalmologic lesions. A recent meta-analysis supported a benefit of a more intensive blood pressure control intervention with respect to 4- to 5-year incidence of diabetic retinopathy, but not to its progression. In this context, epidemiological cohort studies are still important. Some recent cohort studies reported clinic BPs as predictors of development or progression of DR, whereas other cohorts failed in demonstrating it. Indeed, we did not find any predictive power of baseline clinic BPs for future development/progression of DR, but stronger predictive capacity of mean clinic BPs during on-treatment follow-up. Furthermore, we confirmed that on-treatment clinic BPs may be more important predictors to newly-development (incidence) of DR than to DR progression, as previously suggested. Otherwise, as far as we know, except for a recent small study in type 1 diabetic patients, this is the first study to evaluate ambulatory BPs as predictors of DR development/progression. We demonstrated that baseline ambulatory BPs are stronger predictors than clinic BPs to development/progression of DR. This finding is not unexpected, since ABPM provides several BP measurements (in general 40 to 60) during day and night, while clinic BPs were usually measured only twice during clinical attendance. Moreover, ambulatory BPs have been largely shown as superior to clinic BPs for cardiovascular risk stratification. Overall, our findings support the increasing use of ABPM into clinical diabetes management.
were also receiving simvastatin, whereas the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, a randomized study trial using fenofibrate, showed a reduction in DR progression. The Steno-2 study parameter consistently found to be associated with DR incidence or progression. Nonetheless, there is more evidence that links serum levels of total and LDL-cholesterol, and triglycerides to the presence of hard exudates, since retinal exudates are often due to leakage of lipids from abnormal retinal capillaries, which is supported by our results. Most importantly, some recent RCTs suggested that treatment of dyslipidemia might prevent development of DR.

Aside from well-established biochemical pathways related to chronic hyperglycemia, no other biochemical path has been conclusively demonstrated to be important for development/progression of DR. In DR, there is endothelial cell injury, loss of pericytes and break-down of blood-retinal barrier, mainly due to chronic hyperglycemia. Such alterations of microvasculature lead to dysregulation of retinal perfusion, hence making eyes with DR more prone to hyperperfusion damage from hypertension. Although the exact mechanism for the pathogenesis of hypertensive damage in eyes with DR is not ascertained, it has been hypothesized that an augment in BP may be beneficial in preventing the development and progression of DR by reducing the damage to endothelial cells, blood vessels and surrounding tissues from hyperperfusion. Otherwise, the differential influence of systolic and diastolic BP on DR development is still debatable, with some longitudinal studies showing superiority of systolic BP over diastolic BP, while other studies showing preponderance of DBP. DBP reflects more peripheral vascular resistance, hence small-resistance arterial function, while SBP reflects mainly central large-artery haemodynamics. In the present study, DBPs were stronger predictors of DR development than SBPs; hence, we may speculate that, in terms of physiopathological mechanisms of DR development, small-resistance arteries alterations, with endothelial dysfunction favoring vasoconstricting over vasodilating properties, might be more important than large-artery dysfunction. This is reinforced by the small predictive importance of aortic stiffness, which also measures large-artery function.

Opposite to glycemic control, the role of lipids in the pathogenesis of DR is less clear. There is no single lipid parameter consistently found to be associated with DR incidence or progression. Nonetheless, there is more evidence that links serum levels of total and LDL-cholesterol, and triglycerides to the presence of hard exudates, since retinal exudates are often due to leakage of lipids from abnormal retinal capillaries, which is supported by our results. Most importantly, some recent RCTs suggested that treatment of dyslipidemia might prevent development of DR. The ACCORD eye study reported a beneficial effect of fenofibrate therapy in patients who were also receiving simvastatin, whereas the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, a randomized study trial using fenofibrate, showed a reduction in DR progression.
also showed a significant reduction in the progression of DR in patients treated with statins and/or fibrates in the intensive therapy in relation to standard therapy. More recently, the VADT study reported a possible interaction between intensive glycemic control and improved lipid parameters during follow-up, where a reduction in progression of DR was only observed in patients under intensive glycemic control who also improved lipid control. Taking into account the above investigations, lipid-lowering medications may have an additive effect to yield better control of DR than only strict glycemic and BP control and laser treatment.

Aortic stiffness is regarded as a marker of cumulative risk factors burden on vasculature, however, increased aortic stiffness was not demonstrated to be an independent predictor of future development/progression of DR, different from what we had previously demonstrated for peripheral diabetic neuropathy in this same population. This might suggest that, although both have common determinants, there may be differences in their physiopathological mechanisms. Although higher HbA1c and blood pressure levels were common predictors of both peripheral neuropathy and DR development/progression; diabetes duration was strongly associated with DR development/progression, but not with neuropathy progression. Indeed, diabetes duration was associated with increased aortic stiffness in our cohort, and it was its inclusion into the multivariable models that mostly attenuated the prognostic importance of aortic stiffness for DR development/progression. Even though, a small, marginally significant, effect of increasing aortic stiffness on DR incidence was still demonstrated here.

There are limitations of this study that warrant discussion. First, the accuracy of diabetic retinopathy grading based on clinical ophthalmologic examination without fundus photographs. In the present study, all exams were performed by the same experienced retinal specialist, and the patients with new findings on fundoscopic examination were re-evaluated in three months to confirm the findings of fundoscopy. However, it was neither compared with the accuracy of grading based on the gold standard seven-field stereo fundus photographs, nor it was possible to assess a second observer's grading. Nonetheless, possible random missing or incorrect diagnosis of DR incidence/progression would actually underestimate the statistical significance towards the null hypothesis for the associations between risk factors and DR development/progression. Second, this study enrolled middle-aged to elderly high-risk individuals with long-standing type 2 diabetes treated on a tertiary-care center. Therefore, our findings may not be generalized to younger patients with recent-onset diabetes or treated at primary-care. Finally, it was an observational cohort study; hence, neither cause-and-effect relationships, nor physiopathological mechanisms, can be inferred, but only speculated.

In conclusion, this study demonstrated that longer diabetes duration, poorer glycemic and lipid control, and higher BPs were the main predictors of development/progression of DR. Mean cumulative clinic diastolic BP during follow-up was the strongest BP-related predictor, although at baseline ambulatory BPs were superior to clinic BPs as predictors of new DR development. These findings support the importance of performing ambulatory BP monitoring in type 2 diabetes management, not only to refine cardiovascular risk stratification, but also to predict future onset and progression of DR. Future prospective studies with intensive multifactorial management, including optimal metabolic and cardiovascular risk factors control, and with longer follow-ups may demonstrate if it is capable of preventing or delaying development/progression of DR in patients with type 2 diabetes.
Table 3. Predictive value of several clinic and ambulatory blood pressure parameters measured at different time-intervals during follow-up for future development and/or progression of diabetic retinopathy (composite and separate endpoints). Models were adjusted for age, sex, diabetes duration, presence of retinopathy (for analyses of the composite development/progression of retinopathy), nephropathy and peripheral neuropathy at baseline, and their respective HbA1c and LDL-cholesterol levels. Abbreviations: HR, hazard ratio; CI, confidence interval; SD, standard deviation.

| Blood pressure parameters                  | Composite endpoint: retinopathy development/progression (n = 544 patients, 156 endpoints) | Separate endpoint: retinopathy progression (n = 144 patients, 79 endpoints) | Separate endpoint: retinopathy development (n = 400 patients, 77 endpoints) |
|--------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Systolic blood pressures (1-SD increment)  |                                                                                     |                                                                          |                                                                          |
| Baseline clinic                            | HR 0.92 0.78, 1.09 p-value 0.32                                                     | HR 0.83 0.65, 1.07 p-value 0.16                                           | HR 1.02 0.80, 1.28 p-value 0.90                                           |
| Mean first-year clinic                      | HR 0.96 0.81, 1.14 p-value 0.64                                                     | HR 0.87 0.69, 1.14 p-value 0.35                                           | HR 1.01 0.79, 1.30 p-value 0.92                                           |
| Mean second-year clinic                     | HR 1.17 0.99, 1.38 p-value 0.070                                                    | HR 1.13 0.87, 1.46 p-value 0.35                                           | HR 1.20 0.96, 1.50 p-value 0.11                                           |
| Mean cumulative clinic                      | HR 1.11 0.94, 1.31 p-value 0.21                                                    | HR 1.02 0.78, 1.33 p-value 0.87                                           | HR 1.20 0.96, 1.48 p-value 0.10                                           |
| Ambulatory 24-h                             | HR 1.19 1.01, 1.40 p-value 0.037                                                    | HR 0.98 0.73, 1.31 p-value 0.88                                           | HR 1.27 1.05, 1.54 p-value 0.013                                           |
| Ambulatory daytime                          | HR 1.16 0.99, 1.36 p-value 0.073                                                    | HR 0.94 0.71, 1.24 p-value 0.67                                           | HR 1.27 1.04, 1.53 p-value 0.017                                           |
| Ambulatory nighttime                        | HR 1.22 1.04, 1.43 p-value 0.015                                                    | HR 1.10 0.84, 1.45 p-value 0.48                                           | HR 1.28 1.05, 1.55 p-value 0.016                                           |
| Nocturnal fall                              | HR 0.90 0.78, 1.05 p-value 0.18                                                     | HR 0.85 0.69, 1.06 p-value 0.15                                           | HR 0.94 0.76, 1.17 p-value 0.60                                           |
| Diastolic blood pressures (1-SD increment)  |                                                                                     |                                                                          |                                                                          |
| Baseline clinic                             | HR 1.01 0.86, 1.18 p-value 0.94                                                    | HR 0.90 0.70, 1.16 p-value 0.41                                           | HR 1.07 0.86, 1.32 p-value 0.57                                           |
| Mean first-year clinic                      | HR 1.14 0.95, 1.37 p-value 0.15                                                    | HR 0.96 0.70, 1.32 p-value 0.81                                           | HR 1.25 1.00, 1.56 p-value 0.050                                           |
| Mean second-year clinic                     | HR 1.27 1.06, 1.51 p-value 0.010                                                   | HR 1.23 0.93, 1.62 p-value 0.15                                           | HR 1.28 1.01, 1.63 p-value 0.039                                           |
| Mean cumulative clinic                      | HR 1.36 1.14, 1.61 p-value 0.001                                                   | HR 1.13 0.86, 1.50 p-value 0.38                                           | HR 1.53 1.24, 1.90 p-value <0.001                                          |
| Ambulatory 24-h                             | HR 1.22 1.03, 1.46 p-value 0.024                                                    | HR 0.98 0.74, 1.30 p-value 0.89                                           | HR 1.37 1.10, 1.71 p-value 0.005                                           |
| Ambulatory daytime                          | HR 1.24 1.05, 1.48 p-value 0.012                                                   | HR 0.97 0.74, 1.27 p-value 0.84                                           | HR 1.41 1.15, 1.75 p-value 0.003                                           |
| Ambulatory nighttime                        | HR 1.27 1.07, 1.51 p-value 0.008                                                   | HR 1.11 0.83, 1.47 p-value 0.48                                           | HR 1.35 1.09, 1.69 p-value 0.006                                           |
| Nocturnal fall                              | HR 0.93 0.79,1.10 p-value 0.42                                                     | HR 0.85 0.66, 1.09 p-value 0.20                                           | HR 0.99 0.79, 1.26 p-value 0.96                                           |

Methods

Patients and baseline procedures. This was a prospective study, nested within the Rio de Janeiro Type 2 Diabetes Cohort Study, with 646 patients with type 2 diabetes enrolled between August 2004 and December 2008 and re-evaluated annually for DR until December 2015 in the diabetes outpatient clinic of our tertiary-care University Hospital. All participants gave written informed consent, the local Ethics Committee (School of Medicine and University Hospital Joined Committee of Research Ethics) had previously approved the study protocol (approval number: 124/04), and all the methods were performed in accordance with the Declaration of Helsinki. The characteristics of this cohort, the baseline procedures and the diagnostic definitions have been detailed elsewhere9, 12, 13, 41. In brief, inclusion criteria were all adult type 2 diabetic individual up to 80 years old with either any microvascular (retinopathy, nephropathy or neuropathy) or macrovascular (coronary, cerebrovascular or peripheral artery disease) complication, or with at least two other modifiable cardiovascular risk factors (hypertension, dyslipidemia or smoking). Exclusion criteria were morbid obesity (body mass index ≥40 kg/m²), advanced renal failure (serum creatinine >180 μmol/L or estimated glomerular filtration rate <30 ml/min/1.73 m²) or the presence of any serious concomitant disease limiting life expectancy. For this sub-study, those patients who at baseline had glaucoma, dense cataract, or proliferative diabetic retinopathy were also excluded, as well as those who did not have at least two annual follow-up ophthalmologic examinations. All were submitted to a standard baseline protocol that included a thorough clinical examination, a laboratory evaluation, 24-hour ambulatory BP monitoring (ABPM) and aortic stiffness assessment by carotid-femoral pulse wave velocity (cf-PWV). Diagnostic criteria for diabetic chronic complications were detailed previously9, 12, 13, 41. Briefly, coronary heart disease was diagnosed by clinical, electrocardiographic criteria, or by positive ischemic stress tests. Cerebrovascular disease was diagnosed by history and physical examination, and peripheral arterial disease by an ankle-brachial index <0.9. The diagnosis of nephropathy needed at least two albuminurias >30 mg/24 h or proteinurias ≥0.5 g/24 h or confirmed reduction of glomerular filtration rate (<60 ml/min/1.73 m², or serum creatinine >130 μmol/L). Peripheral neuropathy was ascertained by clinical examination (knee and ankle reflex activities, feet sensation with the Semmes-Weinstein monofilament, vibration with a 128-Hz tuning fork, pin-prick and temperature sensations and neuropathic symptoms were evaluated by a standard validated questionnaire13. Clinic BP was measured three times using a digital oscillometric BP monitor (HEM-907XL, Omron Healthcare, Kyoto, Japan) with a suitable sized cuff on two occasions two weeks apart at study entry. The first measure of each visit was discarded and BP considered was the mean between the last two readings of each visit. Arterial hypertension was diagnosed if mean SBP ≥140 mmHg or DBP ≥90 mmHg or if anti-hypertensive drugs had been prescribed. ABPM was recorded during 24-hour at study entry using Mobil-O-Graph, version 12 equipment (Dynamapa, Cardios LTDA., São Paulo, Brazil). A reading was taken every 15 minutes throughout the day and every 30 minutes at night. Nighttime period was ascertained for each individual patient from registered diaries. Patients were instructed to keep their routine activities during this day. Parameters evaluated were 24-hour, daytime and nighttime BPs and the nocturnal BP fall (calculated as the percentage decrease in...
nighttime BP in relation to daytime levels). Normal dipping pattern was defined as a nocturnal BP fall ≥10%\(^{10}\). Laboratory evaluation included fasting glycemia, glycated hemoglobin, serum creatinine and lipids. Albuminuria and proteinuria were evaluated in two non-consecutive sterile 24-hour urine collections. Aortic stiffness was evaluated by cf-PWV measurement during the first-year of follow-up, using the foot-to-foot velocity method with the Complior equipment and software (Artech-Medical, Paris, France), as previously described\(^{12,41}\). Direct carotid-femoral distance was corrected by a 0.8 factor and a cf-PWV > 10 m/s was considered as increased aortic stiffness, as recommended\(^{40}\). The patients were followed-up regularly at least 3–4 times a year and hence all had at least 2 to 4 annual HbA\(_{1c}\), serum lipids and clinic BP measurements.

**Assessment of development and progression of DR.** Presence and severity of DR was determined at baseline and annually by a single retinal specialist. Following the International Clinical Diabetic Retinopathy and Diabetic Macular Edema Disease scales\(^{42}\), severity of DR was categorized into 5 stages: no retinopathy, mild non-proliferative retinopathy, moderate non-proliferative retinopathy, severe non-proliferative retinopathy and proliferative retinopathy. When there were inter-eye differences in DR severity, the eye with the severest DR was considered for DR classification. Incidence of DR was defined as having no DR signs in both eyes at baseline and having mild to severe non-proliferative DR or proliferative DR in either of the eyes at any annual examination. Progression of DR was defined as having mild non-proliferative DR at baseline and having severe non-proliferative DR, or proliferative DR or laser photocoagulation at any subsequent annual ophthalmologic examination; or as having moderate/severe non-proliferative DR at baseline and proliferative DR at any subsequent examination. All incident new DR and worsening DR cases were confirmed on a second ophthalmologic examination at least 3 months apart.

**Statistical analysis.** Continuous data were described as means (SD) or as medians (interquartile range). The primary endpoint was the development or progression of DR, according to the criteria detailed before. Patients with and without DR development/progression were compared by unpaired t test (for continuous normal variables), Mann-Whitney test (for continuous asymmetric variables) and by \(\chi^2\) test (for categorical variables). The independent predictors of the primary endpoint occurrence were examined by Kaplan-Meier estimation of its cumulative incidence during follow-up (with the predictors dichotomized at clinic meaningful cut-off values and compared by log-rank tests), and by multivariate Cox regression models. Candidate variables to enter the multivariate Cox analyses, based on biological plausibility, were the following: age, sex, body mass index (BMI), smoking status, physical activity, diabetic and anti-hypertensive treatment (number and classes of drugs), diabetes duration, macrovascular (coronary, cerebrovascular and peripheral arterial disease) and microvascular (retinopathy, nephropathy and neuropathy) diabetic complications at baseline, aortic stiffness, clinic and ambulatory BPs, HbA\(_{1c}\), HDL- and LDL-cholesterol. Four models were fitted according to the time-interval the covariates were evaluated: a baseline model, a first-year, a second-year and a cumulative exposure model. In the first and second-years models, BMI, clinic BPs, HbA\(_{1c}\), and lipid mean levels were updated to the values recorded during the first and second years of follow-up; and in the cumulative exposure model the mean values of these covariates were calculated from the baseline until censoring or endpoint occurrence. Ambulatory BPs were entered only into the baseline model, whereas aortic stiffness was entered into the first-year model. Regardless of their significance, age, sex and presence of DR, nephropathy and peripheral neuropathy at baseline were forced into all models. Separate analyses for new DR development and DR progression were also performed. A forward selection procedure was used to select the independent predictors with a p-value < 0.10 as the criterion to enter and to remain into the models. To assess the relative prognostic importance of each BP parameter, clinic and ambulatory BP parameters were evaluated in separate Cox models, adjusted for the same covariates of the original predictive model. Cox regression results were presented as hazard ratios (HRs) with their 95% confidence intervals (CIs); and to allow comparisons among covariates, HRs were calculated for standardized increments of 1-SD for HbA\(_{1c}\), BPs and lipids. Statistics were performed with SPSS version 19.0 (SPSS Inc, Chicago, IL, USA), and 2-tailed p-value < 0.05 was considered significant.

**Data availability statement.** The Rio de Janeiro Type 2 Diabetes Cohort Study is an ongoing study, and its dataset is not publicly available due to individual privacy of the participants. However, it may be available from the corresponding author on reasonable request.

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Author Contributions

C.R.L.C., N.C.L. and G.F.S. conceived and designed the study, followed-up the patients and obtained the data. E.D. performed all ophthalmologic examinations. C.R.L.C. drafted the manuscript. G.F.S. analyzed the data and
is the guarantor. All authors helped interpret the results and reviewed the manuscript. G.F.S. had full access to all of the data and takes responsibility for the integrity of the data and the accuracy of data analysis.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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