BMJ Open Diabetes Research & Care

Chronic complications in patients with newly diagnosed type 2 diabetes: prevalence and related metabolic and clinical features: the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) 9

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To cite: Bonora E, Trombetta M, Dauriz M, *et al*. Chronic complications in patients with newly diagnosed type 2 diabetes: prevalence and related metabolic and clinical features: the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS) 9. *BMJ Open Diab Res Care* 2020;**8**:e001549. doi:10.1136/bmjdrc-2020-001549

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Received 9 May 2020
Revised 13 July 2020
Accepted 15 July 2020

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ABSTRACT

Introduction We explored the presence of chronic complications in subjects with newly diagnosed type 2 diabetes referred to the Verona Diabetes Clinic. Metabolic (insulin secretion and sensitivity) and clinical features associated with complications were also investigated.

Research design and methods The comprehensive assessment of microvascular and macrovascular complications included detailed medical history, resting ECG, ultrasonography of carotid and lower limb arteries, quantitative neurological evaluation, cardiovascular autonomic tests, ophthalmoscopy, kidney function tests. Insulin sensitivity and beta-cell function were assessed by state-of-the-art techniques (insulin clamp and mathematical modeling of glucose/C-peptide curves during oral glucose tolerance test).

Results We examined 806 patients (median age years, two-thirds males), of whom prior clinical cardiovascular disease (CVD) was revealed in 11.2% and preclinical CVD in 7.7%. Somatic neuropathy was found in 21.2% and cardiovascular autonomic neuropathy in 18.6%. Retinopathy was observed in 4.9% (background 4.2%, proliferative 0.7%). Chronic kidney disease (estimated glomerular filtration rate <60 mL/min/1.73 m^2) was found in 8.8% and excessive albuminuria in 13.2% (microalbuminuria 11.9%, macroalbuminuria 1.3%). Isolated microvascular disease occurred in 30.8%, isolated macrovascular disease in 9.3%, a combination of both in 9.1%, any complication in 49.2% and no complications in 50.8%.

Gender, age, body mass index, smoking, hemoglobin A1c and/or hypertension were independently associated with one or more complications. Insulin resistance and beta-cell dysfunction were associated with macrovascular but not microvascular disease.

Conclusions Despite a generally earlier diagnosis for an increased awareness of the disease, as many as ~50% of patients with newly diagnosed type 2 diabetes had clinical or preclinical manifestations of microvascular and/or macrovascular disease. Insulin resistance might play an independent role in macrovascular disease.

Trial registration number NCT01526720.

Significance of this study

What is already known about this subject?

- Type 2 diabetes mellitus (T2DM) can remain undiagnosed for years before being detected.
- Unrecognized and untreated hyperglycemia could result in organ damage and contribute to classic macrovascular and microvascular complications.

What are the new findings?

- When carefully searched, chronic complications of diabetes are revealed in as many as 50% of patients with newly diagnosed T2DM.
- Microvascular, mainly neurological, are more prevalent than macrovascular complications.
- State-of-the-art assessed insulin resistance and beta-cell dysfunction are independently associated with macrovascular complications.

How might these results change the focus of research or clinical practice?

- More effective strategies to anticipate T2DM diagnosis seem to be warranted.
- A more detailed and comprehensive search for organ/system damage should be implemented as soon as the diagnosis of T2DM is established.

INTRODUCTION

The stages of type 2 diabetes mellitus (T2DM) include a period in which the disease is undiagnosed. In the 90s, the time elapsing before

diagnosis was estimated to be up to 10 years.[1] Hyperglycemia can generate functional and structural damages which might yield macrovascular and/or microvascular complications during this more or less long period of undetected disease. In fact, a number of studies reported that a sizable proportion of subjects with newly diagnosed T2DM already have chronic complications.[2–16] Nowadays, at least in Western countries, T2DM is probably diagnosed at an earlier stage[17] and this might have reduced the prevalence of complications at time of diagnosis. However, an updated information on the prevalence of chronic complications when T2DM is first diagnosed is rather scant and generally incomplete. Most studies, in fact, did not focus on all potential complications and none of them carefully evaluated subclinical vascular disease. Yet, very few studies have explored the association of complications with main pathogenic defects of T2DM, that is, insulin resistance and beta-cell dysfunction.

The aim of the present study was to assess the prevalence and associated clinical and metabolic features of all traditional chronic complications of T2DM in a large cohort of newly diagnosed patients referred to the Diabetes Clinic of Verona in the last years.

SUBJECTS AND METHODS

Study population

The Verona Newly Diagnosed Type 2 Diabetes Study is an ongoing study on genetics, pathophysiology and clinics of patients with newly diagnosed T2DM.[18–23] As of January 1, 2002, all patients with T2DM referred to the Diabetes Clinic embedded into the Division of Endocrinology, Diabetes and Metabolic Diseases of the University and Hospital Trust of Verona and whose disease was diagnosed in the past 6 months were offered to participate in this study. Recruitment was ended on December 31, 2015 and a follow-up was then planned and is ongoing. All participants signed an informed consent form. The clinical evidence on which the diagnosis of T2DM had been made was reviewed at the recruitment and the diagnosis was confirmed according to standard criteria. The large majority of patients were drug-naïve (~95%) or, if already treated with antidiabetic drugs (~5%), underwent a treatment washout of at least 1 week before metabolic tests were performed. Exclusion criteria were age >75 years, non-Italian ancestry, current insulin treatment, presence of anti- glutamic acid decarboxylase antibodies and history of malignancies or any condition severely impairing liver and/or kidney function. In this paper, we report data collected from 806 patients. Not all of them accepted to undergo the proposed complete assessment but most tests were carried out in >85% of patients. Cardiovascular autonomic tests were performed in 68% of the cohort.

Clinical data

Weight and height were measured and body mass index (BMI) calculated by dividing weight in kilograms by the square of height in meters. Waist circumference (to the nearest 0.5 cm) was measured with a plastic tape meter at the level of the umbilicus. Blood pressure was measured with a standard mercury manometer on the right arm when sitting. Hypertension was diagnosed when systolic blood pressure was ≥140 mm Hg and/or diastolic blood pressure was ≥90 mm Hg and/or antihypertensive drugs were used. A confirmed history of myocardial infarction, angina, coronary revascularization, stroke, transitory ischemic attack, carotid revascularization, non-traumatic amputation, gangrene and/or lower limb revascularization was considered a valid proxy for prior clinical cardiovascular disease (CVD). A resting 12-lead ECG was performed (CardioDirect 12 unit; Metasoft 3.9 software) and interpreted according to Minnesota coding system.[24] In particular, ECG abnormalities were categorized as 'definite', 'probable' or 'possible coronary heart disease' and only 'definite' ECG abnormalities were used for diagnosing myocardial ischemia. Ultrasonography scanning of common and internal carotid arteries was performed as previously described (Esaote Wall Track System, Esaote S.p.A., Genova, Italy) and a cut-off of 40% was used to define a significant arterial stenosis.[22] Ultrasonography scanning of lower limb arteries was performed and any detected stenosis or moderate-to-severe reduction of blood flow at proximal and/or distal level was considered as a marker of peripheral artery disease. Presence of diabetic retinopathy (DR) was detected by indirect ophthalmoscopy after pupillary dilation by a single expert ophthalmologist. DR was categorized into background and proliferative. Distal symmetric polyneuropathy (DSPN) was looked for by assessing ankle reflex, touch sensation by Semmes-Weinstein monofilament and vibration perception threshold by biothesiometer. A dichotomous approach (yes/no) was used to categorize it. Cardiovascular autonomic neuropathy (CAN) was searched and diagnosed as previously described.[23]

Laboratory testing and metabolic studies

Venous blood was drawn in the morning after an overnight fast in all patients. Plasma glucose and serum creatinine and lipids were assayed by standard laboratory procedures. Hypercholesterolemia was arbitrarily defined when statins were used and/or low-density lipoprotein (LDL) cholesterol was above the current recommended target of <70 mg/dL (<1.8 mmol/L). Hemoglobin A1c (HbA1c) was measured with a high performance liquid chromatography method, standardized according to IFCC. In case of discrepancy between the three tests (fasting plasma glucose, 2-hour plasma glucose, HbA1c), the one documenting diabetes (value above the diagnostic cut-off) was used for diagnosis according to standard criteria.[25] Glomerular filtration rate (GFR) was estimated from the four-variable Modification of Diet in Renal Disease study equation.[26] Chronic kidney disease (CKD) was established when estimated glomerular filtration rate was <60 mL/min/1.73 m^2. Urinary albumin excretion rate was measured from a

24-hour urine sample by an immunonephelometric method on at least two occasions. Microalbuminuria and macroalbuminuria were defined as urinary excretion of 30–300 and >300 mg/day, respectively. Subjects underwent a euglycemic hyperinsulinemic clamp and a 75 g oral glucose tolerance test (OGTT) with frequent and prolonged sampling (up to 4–5 hours) for assessment of beta-cell function which was reconstructed by mathematical modeling, as previously described.[20 21]

Statistical analysis

Statistical analyses were carried out with standard techniques (χ^2 test, multiple logistic regression analysis). Skewed variables were logarithmically transformed to improve normality before analyses were performed. Data are presented as median and IQR or as percentage of total.

RESULTS

Table 1 reports main clinical features of subjects under study. Two-thirds of them were males. Median age was 60 years. Most patients were overweight or had obesity. Average fasting and 2-hour OGTT plasma glucose levels were mildly elevated and the same holds true for HbA1c. A number of subjects were diagnosed by OGTT or HbA1c rather than fasting plasma glucose. More than half of the subjects were treated with antihypertensive medications and one-fifth with statins. Blood pressure was generally well controlled but LDL cholesterol was above current target in most subjects.

Data on prior clinical CVD were available in all subjects. Ultrasonography of carotid artery or lower limb arteries in 89% and 88%, respectively. Neurological assessment was available in all subjects and cardiovascular autonomic tests in 68%. Fundus oculi was examined by ophthalmoscopy in 88% of subjects. Subjects undergoing insulin clamp and OGTT were 96% and 93%, respectively.

Table 2 summarizes the prevalence of various chronic complications. A prior clinical cardiovascular event was revealed in >10% of subjects. Another ~8% had preclinical manifestations of CVD (ischemic ECG and/or plaques into carotid or lower limbs arteries). CKD was found in ~9% and albuminuria in ~13% (mostly microalbuminuria). DSPN was observed in ~21% and CAN in ~19%. Retinopathy was observed in ~5% of subjects (proliferative in less than one out of five).

In subjects who had a complete staging of organ/system damage (heart, arteries, kidney, eye, nerves) (n=614, 76%), microvascular disease occurred in 30.8%, macrovascular disease in 9.3%, both in 9.1% and either in 49.2%. As a consequence, 50.8% had no detectable complications. The inclusion of CAN into this analysis reduced the number of subjects (n=438, 54%) but did not substantially change these proportions (eg, at least one complication was found in 50.2%).

We performed multivariate logistic regression analyses in which single chronic complications were the dependent variables and gender, age, smoking, BMI, HbA1c, hypertension and hypercholesterolemia were the independent variables (table 3). In these analyses, CVD was associated with male gender, age, smoking and hypertension, CKD was associated with female gender and age, microalbuminuria-macroalbuminuria was associated with male gender, smoking and HbA1c, DR was not significantly associated with any variable in the model, DSPN was associated with BMI and smoking, CAN was associated with BMI, smoking and hypertension. The replacement in the analyses of hypercholesterolemia with LDL cholesterol and hypertension with systolic blood pressure did not change the results. When carotid stenosis or lower limb atherosclerosis were treated as dependent variables, the former was significantly associated with age and smoking, and the latter with male gender, age, hypertension and hypercholesterolemia (data not shown). The

Table 1 Main clinical features of subjects under study

Variable	Median (IQR) or percentage
Males (%)	68.0
Age (years)	60 (52–66)
Body mass index (kg/m^2)	29.3 (26.6–32.9)
Waist circumference (cm)	100 (94–109)
HbA1c (mmol/mol) (%)	49 (44–56) 6.6 (6.2–7.3)
Fasting plasma glucose (mmol/L)	7.0 (6.2–7.9)
2-hour OGTT plasma glucose (mmol/L)	12.9 (10.4–16.0)
LDL cholesterol (mmol/L)	2.99 (2.41–3.58)
HDL cholesterol (mmol/L)	1.14 (0.96–1.34)
Triglycerides (mmol/L)	1.39 (1.03–2.0)
Statins (%)	20.5
Hypercholesterolemia (%)	96.5
Systolic blood pressure (mm Hg)	134 (120–145)
Diastolic blood pressure (mm Hg)	80 (80–90)
Antihypertensive drugs (%)	56.8
Hypertension (%)	76.7
eGFR (mL/min/1.73 m^2)	81.7 (70.8–94.1)
Antiplatelet or anticoagulant drugs (%)	16.6
Smokers (current/prior) (%)	50.5
Insulin sensitivity (µmol/min/m^2 BSA)	605 (380–874)
Beta-cell function—derivative control (pmol/m^2 BSA/(mmol/L/min)	444 (51–945)
Beta-cell function—proportional control (pmol/m^2 BSA)/(mmol/L/min)	47 (25–76)

BSA, body surface area; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test.

Table 2 Prevalence of chronic complications of diabetes

Complication	Prevalence (%)
Overall cardiovascular disease	18.9
Prior clinical cardiovascular disease	11.2
Ischemic ECG	5.6
Carotid stenosis >40%	6.0
Lower limb stenosis (any)	6.6
Chronic kidney disease (eGFR <60 mL/min/1.73 m^2)	8.8
Albuminuria (micro or macro)	13.2
Microalbuminuria	11.9
Macroalbuminuria	1.3
Distal symmetric polyneuropathy	21.2
Cardiovascular autonomic neuropathy	18.6
Retinopathy of any type	4.9
Background retinopathy	4.2
Proliferative retinopathy	0.7

eGFR, estimated glomerular filtration rate.

inclusion of triglycerides and HDL cholesterol in the models yielded similar results with triglycerides being positively associated with CKD (OR 2.53, 95% CI 1.29 to 4.98, p=0.007) and HDL cholesterol being negatively associated with albuminuria (OR 0.25, 95% CI 0.09 to 0.65; p=0.005). The replacement of BMI with waist circumference showed that the latter, as for BMI, was a predictor of CAN (OR 1.02, 95% CI 1.00 to 1.04, p=0.036; per each cm of waist) but not of other complications.

We have also run multivariate analyses where microvascular (pooled) or macrovascular complications were the dependent variables and insulin sensitivity and beta-cell function parameters (derivative or, alternatively, proportional control) were included in the models as independent variables. In these analyses, both insulin sensitivity and beta-cell function (derivative control) were negatively associated with macrovascular disease (table 4). No association of microvascular disease with these metabolic variables was found. This finding was confirmed when CAN was excluded or when single microvascular complications were treated as dependent variables (data not shown). When BMI was replaced by waist circumference and triglycerides and HDL cholesterol were included in the model data were substantially confirmed, although the association of insulin sensitivity with macrovascular disease lost its statistical significance. In this analysis, waist circumference, as for BMI, was a negative predictor of macrovascular disease (OR 0.98, 95% CI 0.96 to 1.00, p=0.043; per each cm of waist) and the latter was not significantly associated with triglycerides or HDL cholesterol.

DISCUSSION

We observed that approximately 50% of subjects in this cohort with newly diagnosed T2DM had target organ/system damage if the latter was searched in-depth with

Table 3 Independent predictors of single chronic complications in multivariate analyses

	CVD	CKD	U-Alb	DR	DSPN	CAN
Gender (male vs female)	2.98 (1.60 to 5.53) **p=0.001**	0.26 (0.14 to 0.48) **p<0.001**	2.04 (1.11 to 3.77) **p=0.022**	1.23 (0.53 to 2.84) p=0.625	0.75 (0.50 to 1.12) p=0.162	0.89 (0.52 to 1.51) p=0.656
Age (per year)	1.06 (1.03 to 1.09) **p<0.001**	1.07 (1.03 to 1.11) **p<0.001**	1.00 (0.97 to 1.02) p=0.899	1.04 (1.00 to 1.09) p=0.072	1.01 (0.99 to 1.03) p=0.623	0.99 (0.97 to 1.02) p=0.536
BMI (per unit)	0.99 (0.94 to 1.04) p=0.623	1.04 (0.99 to 1.10) p=0.132	1.03 (0.98 to 1.07) p=0.236	1.02 (0.95 to 1.10) p=0.539	1.03 (1.00 to 1.07) p=0.067	1.05 (1.01 to 1.10) **p=0.017**
Smoking (past/current vs never)	1.71 (1.06 to 2.76) **p=0.028**	0.99 (0.54 to 1.82) p=0.968	1.84 (1.12 to 3.02) **p=0.016**	0.89 (0.41 to 1.89) p=0.753	1.55 (1.06 to 2.28) **p=0.023**	1.88 (1.16 to 3.07) **p=0.011**
HbA1c (per log unit)	1.88 (0.45 to 7.87) p=0.388	0.16 (0.02 to 1.56) p=0.115	6.46 (1.64 to 25.46) **p=0.008**	4.60 (0.51 to 41.36) p=0.173	1.42 (0.44 to 4.59) p=0.560	1.58 (0.34 to 7.30) p=0.556
Hypertension (yes vs no)	2.17 (1.12 to 4.21) **p=0.022**	1.29 (0.57 to 2.94) p=0.542	1.35 (0.74 to 2.46) p=0.322	0.70 (0.29 to 1.66) p=0.416	1.30 (0.82 to 2.07) p=0.262	2.04 (1.12 to 3.73) **p=0.021**
Hypercholesterolemia (yes vs no)	1.65 (0.47 to 5.82) p=0.440	1.14 (0.25 to 5.27) p=0.868	1.05 (0.30 to 3.71) p=0.937	0.56 (0.13 to 2.54) p=0.455	0.77 (0.32 to 1.89) p=0.573	0.98 (0.31 to 3.02) p=0.948

ORs and 95% CIs are reported. Significant p values are in bold.
BMI, body mass index; CAN, cardiovascular autonomic neuropathy; CKD, chronic kidney disease; CVD, cardiovascular disease; DR, diabetic retinopathy; DSPN, distal symmetric polyneuropathy; HbA1c, hemoglobin A1c; U-Alb, microalbuminuria or macroalbuminuria.

Table 4 Independent predictors of macrovascular and pooled microvascular complications in multivariate analyses including insulin sensitivity and beta-cell function

	Macrovascular	Microvascular
Gender (male vs female)	2.15 (1.26 to 3.68) **p=0.005**	0.81 (0.50 to 1.32) p=0.399
Age (per year)	1.07 (1.04 to 1.10) **p<0.001**	1.01 (0.99 to 1.03) p=0.497
BMI (per 1 unit)	0.94 (0.89 to 0.99) **p=0.012**	1.04 (1.00 to 1.09) p=0.070
Smoking (past/current vs never)	2.38 (1.52 to 3.73) **p<0.001**	1.70 (1.10 to 2.62) **p=0.016**
HbA1c (per log unit)	1.17 (0.28 to 4.79) p=0.830	2.17 (0.53 to 8.79) p=0.284
Hypertension (yes vs no)	2.93 (1.57 to 5.48) **p=0.001**	1.50 (0.90 to 2.46) p=0.121
Hypercholesterolemia (yes vs no)	1.17 (0.40 to 3.48) p=0.775	0.96 (0.36 to 2.54) p=0.927
Insulin sensitivity (per log unit)	0.70 (0.50 to 0.99) **p=0.043**	1.01 (0.69 to 1.46) p=0.974
Beta-cell function (*derivative control*) (per log unit)	0.92 (0.86 to 0.99) **p=0.023**	1.03 (0.95 to 1.11) p=0.474

Macrovascular=prior CVD and/or ischemic ECG and/or carotid stenosis and/or lower limb stenosis. Microvascular=CKD and/or microalbuminuria-macroalbuminuria and/or DR and/or DSPN and/or CAN. ORs and 95% CIs are reported. Significant p values are in bold.
BMI, body mass index; CAN, cardiovascular autonomic neuropathy; CKD, chronic kidney disease; CVD, cardiovascular disease; DR, diabetic retinopathy; DSPN, distal symmetric polyneuropathy; HbA1c, hemoglobin A1c.

several techniques, including ultrasonography scanning of carotid and lower limb arteries, comprehensive neurological evaluation and ophthalmoscopy. Focusing on microvascular complications (eye, kidney, nerves), test abnormalities compatible with neuropathy were more common than those documenting retinopathy or nephropathy. This observation is noteworthy as neuropathy is often a neglected microvascular complication of diabetes because the eye and the kidney generally receive more attention than nerves. Overall, as many as 40% of subjects had microvascular disease, with a proportion twofold higher than macrovascular disease. Noteworthy, as many as 10% had both microvascular and macrovascular damage and as many as ~50% had either.

We have observed that gender, age, BMI, smoking, HbA1c and hypertension were variably associated with specific microvascular complications and gender, age, smoking and hypertension were associated with macrovascular complications. Interestingly, male gender was associated with CVD whereas female gender was associated with CKD. A classic risk factor such as hypercholesterolemia was not associated with macrovascular complications and hypertension was not associated with CKD. The cross-sectional setting of the study is the likely explanation. Yet, almost all subjects were on statins or had cholesterol levels above the cut-off of 70 mg/dL and the majority of hypertensive subjects were on treatment.

Insulin sensitivity and insulin secretion were negatively associated with macrovascular complications. In previous longitudinal studies, we found that insulin resistance was a predictor of CVD in T2DM and in the general population.[27 28] In a study conducted several years ago in the UK, Roy Chowdhury *et al*[29] observed an association between impaired insulin secretion and retinopathy but no association of this complication with insulin sensitivity. However, they used different and surrogate methods (eg, Homeostasis Model Assessment) to assess insulin secretion and sensitivity. Martinell *et al*[11] observed an inverse association between insulin secretion and retinopathy and no association with insulin resistance. However, they have used surrogate methods to assess these metabolic functions. We were unable to observed any association of insulin secretion with microvascular disease.

In this study, we explored virtually all classic sites of chronic complications (heart, arteries, eye, kidney, nerves) and this is at variance with most previous studies, some of which are also quite dated. In these studies, conducted in the last 40 years in large cohorts of patients with newly diagnosed T2DM recruited in Western countries, the prevalence of complications was quite variable, most likely for substantial differences in the methods of their detection.[2–16] Prevalence of retinopathy ranged from 1%[4] to 21%.[2] Prevalence of DSPN ranged from 3%[13] to 42%.[16] Prevalence of microalbuminuria/macroalbuminuria ranged from 7%[3] to 20%.[10] CKD was observed in 3%[2 13] up to 21%.[9] In these studies, prior cardiovascular events were often presented separately: myocardial infarction ranged from 5%[13] to 11%,[12] stroke ranged from 2%[10] to 5%,[8] peripheral vascular disease ranged from 2%[4 14] to 40%.[8] In none of these studies, carotid or lower limb ultrasonography were used to detect plaques and only one of them explored CAN, finding a prevalence of 4%.[16] Therefore, we feel that our study is more comprehensive than those previous studies.

Multivariate analyses were run only in few of the above referenced studies. Looker *et al*[5] found associations of retinopathy with male gender, HbA1c, BMI and blood pressure. An association of retinopathy with male gender, HbA1c and blood pressure was observed also by Kostev and Rathmann.[6] Kostev *et al*[8] have reported that DSPN was associated with male gender and age. Interestingly, we found an association between smoking and neuropathy (both DSPN and CAN). This finding is consistent with recent data from others[30 31] and could be attributed to the damage exerted by smoking on vasa nervorum as well as its direct detrimental effects on nerve structure and function. The latter includes an increased oxidative stress, with reactive oxygen species and Advanced Glycosilation End-Products as mediators, leading to mitochondrial dysfunction, inflammation, DNA damage and apoptosis.[32 33] The lack of significant associations of HbA1c with some of the complications (eg, neuropathy

or retinopathy) is reasonably due to the cross-sectional design of the study.

As far as we know, this is the only study exploring in the same cohort all major complications of diabetes and relating them to classic risk factors and to major pathogenic determinants of T2DM (ie, insulin resistance and beta-cell dysfunction). We feel that our data are important as they point out to what extent the diabetes milieu can deteriorate health status of subjects before diagnosis even in the presence of mild-to-moderate hyperglycemia and how often chronic complications might be detected at time of diabetes diagnosis if they are carefully and comprehensively searched. This happens despite the increased awareness for diabetes occurred in the last 20–30 years. Yet, a role of insulin resistance in macrovascular disease emerged independently of classic risk factors, thus consolidating previous findings in subjects with and without diabetes.[27 29]

Strengths of the study are: large number of subjects; no selection of patients (only those older than 75 years were not examined); exclusion of patients with Latent Autoimmune Diabetes of Adults; lack of any interference by antihyperglycemic drugs; assessment of all major organs/systems suffering from chronic hyperglycemia, including autonomic nervous system; investigation of carotid and lower limb arteries and not solely of prior CVD clinical events; measurement of insulin sensitivity and insulin secretion with state-of-the-art techniques.

Limits of the study are: inclusion of Caucasian subjects only; lack of investigation of CHD with dynamic techniques (eg, stress ECG or stress echocardiography).

In conclusion, despite a generally earlier diagnosis of T2DM occurring in the last two decades as compared with previous decades for an increased awareness of the disease, as many as ~50% of newly diagnosed patients have clinical or preclinical manifestations of microvascular and/or macrovascular disease. Our findings might promote an additional effort to further anticipate T2DM diagnosis by tracing undetected cases. Yet, our study might translate into a stronger commitment for staging organ/system damage in T2DM as soon as the diagnosis is established.

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Acknowledgements Dr Stefano Casati who performed ophthalmoscopy in all subjects is gratefully acknowledged. The superb assistance of the nurses of the Division of Endocrinology, Diabetes and Metabolism of the Hospital Trust of Verona in performing metabolic studies is greatly acknowledged.

Contributors EB, MT and RCB designed the protocol and planned statistical analyses. All authors collected data and contributed to their interpretation and discussion. FM and MZ performed laboratory work. MLB modeled data of insulin secretion. LS made data entry and statistical analyses. EB wrote the manuscript, MT and RCB edited it and all authors reviewed it.

Funding The study was supported by grants from the Italian Ministry of the Education, University and Research (PRIN 2009WYP4AS to EB; PRIN 2015373Z39_002 to EB; PRIN 2015373Z39_004 to RCB; PRIN 2010098WFZ2 to RCB), the University of Verona (scientific achievement-based grants to EB, MT, RCB), the University of Parma (scientific achievement-based grants to RCB), the Foundation of the European Association for the Study of Diabetes (EFSD/Novartis grant to RCB), the Foundation of the Italian Diabetes Society (research grant to MT).

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval The protocol was approved by the local Ethics Committee of the Azienda Ospedaliera Universitaria Integrata di Verona (No. 955).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article. Data are filed at the Endocrinology, Diabetes and Metabolism Division of the Department of Medicine, University of Verona.

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