REVIEW ARTICLE

WILEY

C-peptide determination in the diagnosis of type of diabetes and its management: A clinical perspective

Ernesto Maddaloni MD[1] | Geremia B. Bolli MD[2] | Brian M. Frier MD[3] | Randie R. Little PhD[4] | Richard D. Leslie MD[5] | Paolo Pozzilli MD[5,6] | Raffaela Buzzetti MD[1]

[1]Experimental Medicine Department, Sapienza University of Rome, Rome, Italy

[2]Department of Medicine and Surgery, Section of Endocrinology and Metabolism, University of Perugia, Perugia, Italy

[3]The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland, UK

[4]Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, Missouri, USA

[5]Centre for Immunobiology, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK

[6]Department of Medicine, Unit of Endocrinology and Diabetes, Campus Bio-Medico University of Rome, Rome, Italy

Correspondence
Paolo Pozzilli, MD, Department of Medicine, Unit of Endocrinology and Diabetes, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128, Rome, Italy.
Email: p.pozzilli@unicampus.it

Abstract

Impaired beta-cell function is a recognized cornerstone of diabetes pathophysiology. Estimates of insulin secretory capacity are useful to inform clinical practice, helping to classify types of diabetes, complication risk stratification and to guide treatment decisions. Because C-peptide secretion mirrors beta-cell function, it has emerged as a valuable clinical biomarker, mainly in autoimmune diabetes and especially in adult-onset diabetes. Nonetheless, the lack of robust evidence about the clinical utility of C-peptide measurement in type 2 diabetes, where insulin resistance is a major confounder, limits its use in such cases. Furthermore, problems remain in the standardization of the assay for C-peptide, raising concerns about comparability of measurements between different laboratories. To approach the heterogeneity and complexity of diabetes, reliable, simple and inexpensive clinical markers are required that can inform clinicians about probable pathophysiology and disease progression, and so enable personalization of management and therapy. This review summarizes the current evidence base about the potential value of C-peptide in the management of the two most prevalent forms of diabetes (type 2 diabetes and autoimmune diabetes) to address how its measurement may assist daily clinical practice and to highlight current limitations and areas of uncertainties to be covered by future research.

KEYWORDS
C-peptide, insulin, insulin deficiency, insulin secretion, pancreatic beta cell

1 | INTRODUCTION

The discovery by Steiner of proinsulin, which is composed of insulin and a connecting peptide (C-peptide), led to the measurement of C-peptide, as distinct from insulin, by Heding and Rubenstein.[1-3] For several years, the measurement of C-peptide in blood and urine has been used as a biomarker of pancreatic beta-cell function because it is secreted in equimolar amounts with insulin (Figure 1)[4] and, unlike insulin, is not extracted by the liver.[5] The assay shows no interference from concomitant insulin therapy. Measurement of C-peptide can be used to assess endogenous insulin secretory capacity, thereby paralleling the extent of residual beta-cell secretion in any form of
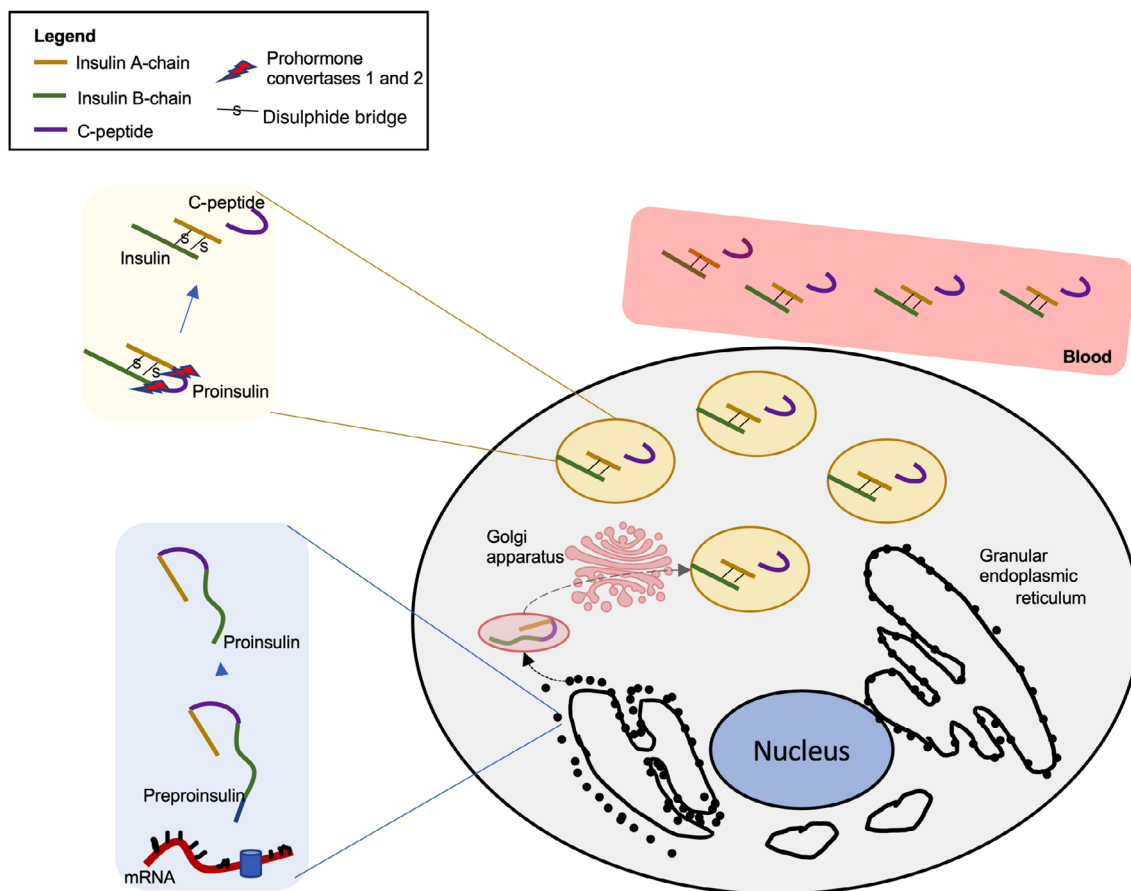
FIGURE 1 Schematic representation of C-peptide production and secretion in equimolar amounts with insulin. C-peptide is a 31 amino acid peptide (molecular weight $\sim$ 3000 g/mol) derived from the cleavage of proinsulin in insulin. Briefly, preproinsulin is synthesized in the granular endoplasmic reticulum, where it is cleaved by microsomal enzymes in proinsulin, which consists of a single chain of 86 amino acids including the A and B insulin chains, the C-peptide and two dipeptide linkages of basic amino acids. Proinsulin is transported by small transfer vesicles to the Golgi apparatus, where it is packed into clathrin-coated secretory granules together with prohormone convertases 1 and 2. These enzymes are responsible for cutting proinsulin at the dipeptide linkages, whereas a carboxypeptidase E removes the pairs of basic amino acids, finally resulting in the 51 amino acid insulin molecule and in the 31 amino acid connecting peptide (C-peptide) residue. C-peptide is stored in the secretory granules of pancreatic beta cells and then secreted in the bloodstream in equimolar amounts with insulin.[5] In contrast to insulin, C-peptide has a negligible extraction by the liver and has a constant renal peripheral clearance, which approximates the glomerular filtration rate.[130] However, the urinary excretion of C-peptide is comparatively low, suggesting that most of the C-peptide extracted by the kidney is metabolized by renal tissues, with only a small fraction excreted in the urine[130,131]

diabetes. Nevertheless, until recently, C-peptide has seldom been used in the clinical setting. In the current article the clinical relevance of C-peptide is reviewed, with an emphasis on the renewal of interest in its measurement and how it can assist the clinician in the management of patients with type 2 diabetes or autoimmune diabetes, the most prevalent forms of diabetes, while at the same time also highlighting its limitations and the current areas of uncertainties.

2 | SEARCH STRATEGY AND SELECTION CRITERIA

References for this review were identified through searches of PubMed for articles published up to November 2021, by use of the following keywords alone or in combination: 'C-peptide', 'proinsulin', 'insulin', 'beta-cell', 'type 1 diabetes', 'autoimmune diabetes', 'type

2 diabetes', 'complications', 'retinopathy', 'nephropathy' and 'cardio-vascular'. Only articles published in English were included. Articles were also identified through searches in the authors' personal files. Articles resulting from these searches and relevant references cited in those articles were reviewed. All authors contributed with interactive discussion to the selection of references among all identified articles by highlighting strengths and limitations.

3 | C-PEPTIDE MEASUREMENT

3.1 | Standardization of C-peptide measurement

Assessment of endogenous insulin secretion by measuring plasma C-peptide is widely accepted. However, considerable variation currently exists between different assay methods of C-peptide, giving variable

results, despite their common traceability to the original World Health Organization standard (International Reference Reagent (IRR) 84/510). Standardization of C-peptide results would facilitate comparison of data from different research studies and among results from clinical laboratories at different sites, which are using different assay methods. Harmonization can be achieved by calibration of all measurement procedures so that they are traceable to the same reference system. In principle, as C-peptide is a well-defined chemical entity, its concentration can be measured in SI units, with a traceability chain linking patient results from routine laboratory procedures, via commutable secondary reference materials (in serum) and reference measurement procedures to a primary reference material (pure substance) value that is assigned from the mass fraction of C-peptide in the material. This traceability would allow clinical results to be compared across measurement systems, location and time, and is essential for patient care and research translation. However, at the current time, C-peptide results from routine methods are not adequately standardized. Even although the standardization process has been approved and standardization materials are available, manufacturers have still not adjusted their calibration.

Efforts to harmonize C-peptide results have been pursued by several scientific societies. Although initial lack of communication among these organizations slowed the standardization process, subsequent efforts have led to a better coordinated plan.[6]

Reference methods have been established in the United States and Japan[7,8] and certified reference materials[9] can insure the comparability of results between these reference methods allowing for assignment of values to secondary reference materials. It has been shown that recalibration by manufacturers using these matrix-appropriate secondary reference materials (frozen serum samples with values assigned by the reference method) greatly improves the comparability of results among methods.[6,10,11] Mean between-method coefficient of variation was 19.1% for manufacturers' usual calibration and 7.5% after recalibration with secondary reference materials. These materials are currently available for use by manufacturers. A traceability scheme has been accepted along with the next steps for implementation and the American Diabetes Association (ADA) has advised manufacturers to initiate this process. Interest from manufacturers in moving forward with standardization could come from specific clinical recommendations from well-recognized clinical organizations with high promotion potentials (e.g. ADA, European Association for the Study of Diabetes [EASD], among others) that would increase awareness of the clinical utility of C-peptide testing.

3.2 | Fasting, random or stimulated C-peptide?

Samples for the measurement of C-peptide in the blood may be collected in the fasting or non-fasting (so-called 'random C-peptide') state, or after a stimulation test. Differences in cut-off values, interpretation and clinical or research convenience between the tests[12] primarily derive from the physiological differences between fasting, random or stimulated C-peptide.

TABLE 1 Factors influencing C-peptide concentration independently from beta-cell reservoir

Factor	Effect on C-peptide
Blood glucose concentrations	• Low blood glucose may result in low C-peptide concentrations • When blood glucose >7.8 mmol/L (140 mg/dl), C-peptide concentrations should be interpreted as stimulated values
Incretins	• Impairments in incretin physiology may result in impaired beta-cell response to meals • Time from last meal and meal composition may influence C-peptide values because of an incretin effect on beta cells
Insulin resistance	• Higher C-peptide values
Renal function	• C-peptide clearance is lower in people with reduced glomerular filtration rate
Lack of standardization	• C-peptide values from different laboratories may not similarly reflect beta-cell reservoir

3.2.1 | Fasting and random C-peptide

Fasting C-peptide is the expression of steady state, that is, static response of beta cell to ambient (arterial) plasma glucose concentration, whereas random (non-fasting) C-peptide is primarily affected by the incretin effect and elevation in plasma glucose following ingestion of the previous meal. A study conducted in children with type 1 diabetes showed that the C-peptide concentrations, whether measured fasting or 90 minutes after (90-CP) a mixed meal tolerance test (MMTT), were strongly related to the gold standard, namely, the C-peptide area under the curve (AUC) post-MMTT.[13] However, the 90-CP showed higher sensitivity and specificity than fasting C-peptide in identifying people with a peak C-peptide of 0.2 nmol/L or higher, which is a marker of clinically meaningful beta-cell reserve because it is associated with fewer complications and less severe hypoglycaemia.[14] Compared with stimulation tests, which are the current gold standard in the research setting, fasting and random measurements are easier to use in clinical practice because they are less time-consuming to collect, less invasive and better tolerated by patients. While neither of the two tests has definitely been proven to be superior to the other, interpretation of random C-peptide values is dependent on the time from the last meal, as well as the composition of that meal. Conversely, results obtained in the fasting state are less affected by confounders and are easier to standardize. However, because hypoglycaemia may reduce beta-cell secretion, low blood glucose values should be excluded before drawing the blood sample for the measurement of fasting C-peptide. These observations gave rise to the compelling suggestion that absolute C-peptide values should be interpreted alongside concomitant blood glucose concentrations, while considering the time and composition of the previous meal, and checking renal function to ensure that renal C-peptide clearance is normal (Table 1).

3.2.2 | Stimulation tests

The use of formal stimulation tests is currently proposed to be the gold standard for the primary outcome in the clinical investigation of insulin secretion.[15] In addition to the quantitative assessment of residual C-peptide, stimulation tests also allow an evaluation of the dynamics of beta-cell response to provocative stimuli. However, because of their complexity and cost, studies generally have not included large numbers of participants, and stimulation tests are not routinely used in clinical practice. The glucagon stimulation test (GST), the MMTT and the oral glucose tolerance test (OGTT) have extensive evidence supporting their validity for the estimation of the residual insulin secretory capacity in people with autoimmune diabetes,[16] should be conducted after 12 hours of fasting, and results should be interpreted while considering all the factors listed in Table 1. However, stimulated C-peptide largely depends on the nature of the stimulus used (injected glucagon or consumption of a meal or of glucose). Indeed, crucial differences exist in their execution, physiological mechanisms of response and interpretation. C-peptide is either measured up to 6 minutes after the intravenous administration of glucagon 1 mg (GST), or at regular intervals up to 120 minutes after the oral ingestion of either a mixed meal (MMTT) or 75 g of glucose (OGTT). Glucagon stimulates insulin secretion acutely and pharmacologically, independently of glucose and incretin hormones, so produces a short-lived response. Conversely, the glucose load and the mixed meal stimulate insulin secretion physiologically, both in a glucose-dependent and a glucose-independent manner, producing a sustained response of insulin over time. A recent study in healthy volunteers has shown that intravenous glucagon elicits a faster rise in C-peptide concentration than the MMTT.[17] The same study also showed that the GST is independent of the incretin axis, unlike the MMTT.[17] Indeed, incretins are gut hormones responsible for the amplification of insulin secretion after an oral glucose challenge compared with an intravenous administration of glucose, a phenomenon called the 'incretin effect'.[18] Incretins are secreted by endocrine cells of the intestinal epithelium in a dose-response relationship with the intestinal glucose load.[19] Beta cells are sensitive to incretins by increasing insulin secretion when levels of incretins rise.[20] Overall, this coupled mechanism enables healthy individuals to increase insulin secretion progressively as amounts of ingested glucose increase. Therefore, although more time-consuming, the MMTT may better mimic the actual pancreatic response to ingestion of a meal during everyday life and has been shown to be more reproducible and better tolerated than the GST.[21]

The ability to estimate C-peptide on very small volumes of blood has allowed its estimation on dried blood spots; importantly, multiple sampling at home throughout the day provides estimates for C-peptide that correlate strongly with levels following a MMTT.[22] For the purpose of standardization between studies, the MMTT is currently the recommended test to use in therapeutic trials in type 1 diabetes.[23] Nonetheless, relevant differences in beta-cell secretory capacity might be observed between individuals in response to an everyday meal or to a stimulation test. Although no comparative data with MMTT exist, measurement of serum C-peptide 2-3 hours after the usual meal consumed every day, should in theory reflect insulin secretory capacity in everyday life better than the MMTT. This is because the everyday meal is solid, whereas the MMTT is liquid, and therefore exerts different effects on gastric emptying (affecting the rate of entry of oral glucose into circulation) and also influences the incretin effect.[24] Furthermore, the composition of meals also significantly influences the C-peptide response. Meals high in protein, and plant-based meals, have been shown to induce higher C-peptide AUC compared with meals high in monounsaturated fat and compared with energy- and macronutrient-matched meat-based meals, respectively, despite similar blood glucose responses.[25,26] Ideally, according to these authors, standardization of a solid mixed meal should be the favoured physiological approach for research and clinical purposes in the future. However, it is difficult to standardize a solid meal because even the same food might result in different beta-cell stimulation on different occasions. For example, the bread, even in the same amount and nominal composition, differs considerably across geographical areas and may result in variable stimulation of beta-cell secretion on different occasions. On balance, perhaps the less physiological liquid meal offers better chances for standardized use in different centres and over different times.

4 | C-PEPTIDE IN THE DIAGNOSIS AND MANAGEMENT OF DIABETES TYPES

Several studies have been conducted in the last 5 years to elucidate the clinical role of C-peptide in the management of diabetes types (Table 2). In this section their major findings are summarized in the context of previous knowledge and issues not yet resolved.

4.1 | C-peptide and autoimmune diabetes

Autoimmune diabetes is characterized by insulin deficiency established because of the autoimmune destruction of pancreatic beta cells. This condition has heterogeneous clinical manifestations, encompassing cases of rapid and severe loss of endogenous insulin secretory capacity that requires insulin therapy at the time of diabetes diagnosis, and cases of mild insulin deficiency characterized by slow progression towards an insulin-dependent state and is most often diagnosed in adults.[27] According to the most recent international classifications, we will refer to the former as classical type 1 diabetes, and to the latter as latent autoimmune diabetes in adults (LADA), which is included under the rubric of type 1 diabetes, but retains its own identity.[28]

4.1.1 | C-peptide in the natural history of classical type 1 diabetes

In contrast to people with type 2 diabetes, those with type 1 diabetes typically have a plasma C-peptide that is consistently below the lower

TABLE 2 Recent advancements in knowledge about C-peptide in clinical diabetes

Study	Diabetes type	C-peptide levels (nmol/L) of clinical interest		Interpretation
Jacobsen et al.[34]	T1D (stage 1)	Index 60* <1.0	→	Reduced risk (77%) of T1D among children with multiple pancreatic aAb
Evans-Molina et al.[35]	T1D (stage 1)	N/A		Compared with aAb negative youths, those with detectable pancreatic aAb have lower C-peptide levels already ≥5 y before T1D onset Among progressors, fasting C-peptide increases and early C-peptide response to OGTT decreases as the onset of T1D approaches
Willemsen et al.[22]	T1D	N/A		C-peptide measurement in dried blood spots is feasible to monitor beta-cell function slopes at home
Rickels et al.[43]	T1D	>0.40 (after MMTT)	→	Higher time in range
Zenz et al.[44]	T1D	≥0.05 (fasting)	→	Higher glucagon and endogenous glucose production in response to hypoglycaemia
Gibb et al.[45]	T1D	>0.01 (random)	→	Lower time below range
Marren et al.[46]	T1D (>5 y)	>0.02 (after MMTT)	→	Lower rate of self-reported hypoglycaemia
Gubitosi-Klug et al.[47]	T1D	>0.03 (after MMTT)	→	Lower risk of severe hypoglycaemia
Thivolet et al.[48]	T1D	>0.03 (after MMTT)	→	No association with glucagon response to MMTT
Jeyam et al.[50]	T1D	>0.20 (random)		Lower insulin requirement, HbA1c, DKA and hypoglycaemia risk. The association with hypoglycaemia episodes was linear down to C-peptide levels of 0.003 nmol/L
Foteinopoulou et al.[118]	T1D	≥0.20 (random)	→	Consider further evaluations to eventually reclassify diabetes type
Buzzetti et al.[91]	LADA	<0.30	→	Identify people requiring insulin therapy
		≥0.30 and ≤0.70	→	Identify people who might benefit from a flexible therapeutic approach and from regular C-peptide measurements over time
		>0.70	→	Identify people who can be treated according to the T2D guidelines and who should repeat C-peptide measurement if glycaemic control deteriorates
Wod et al.[89]	Adult-onset newly diagnosed diabetes	0.30 (fasting)	→	Stratify people with adult-onset diabetes for different risk metabolic profiles independently from GADA and age at onset
Sokooti et al.[97]	T2D	N/A		Fasting C-peptide improves the FOS risk score for the estimation of T2D risk in the general population (the higher the C-peptide, the higher the risk) Sensitivity analyses showed C-peptide was an independent predictor only among people without hypertension
Tuccinardi et al.[104]	T2D (insulin-treated)	0.36 (fasting)	→	Cut-off with 45% sensitivity and 81% specificity for identifying people with T2D on basal-bolus treatment among people with T2D on insulin treatment
Landgraf et al.[113]	T2D	≤0.40 (fasting)	→	Worse HbA1c values and higher rate of hypoglycaemic episodes (including severe) after starting basal insulin, despite lower insulin dose (IU/kg), compared with people with higher C-peptide values

TABLE 2 (Continued)

Study	Diabetes type	C-peptide levels (nmol/L) of clinical interest		Interpretation
Hope et al.[114]	T2D (insulin-treated)	<0.20 (random)	→	High hypoglycaemic risk, including risk of severe hypoglycaemias

Note. This table summarizes the main findings of studies published within the last 5 y about the clinical implications of C-peptide measurement for the management of autoimmune and type 2 diabetes.

Abbreviations: aAb, autoantibodies; DKA, diabetic ketoacidosis; FOS, Framingham offspring; GADA, glutamic acid decarboxylase antibodies; IU, international units; LADA, latent autoimmune diabetes in adults; MMTT, mixed-meal tolerance test; N/A, not appropriate; OGTT, oral glucose tolerance test; T1D, type 1 diabetes; T2D, type 2 diabetes.

*Index 60 is a composite measure of fasting C-peptide, 60 min glucose and 60 min C-peptide ([0.3695 × (log10[fasting C-peptide])] + [0.0165 × 60 min glucose] − [0.3644× 60 min C-peptide]).

limit of the normal range as determined from healthy non-diabetic controls matched for body weight (i.e. usually below 0.2-0.4 nmol/L). These low C-peptide values are consistent with the clinical diagnosis, where advanced or severe insulin deficiency has followed the loss of most pancreatic beta cells. However, in people who develop diabetes in adolescence and adulthood, given that the reduction in C-peptide is a continuum, a very low value does not have high predictive sensitivity for severe insulin deficiency and insulin dependence within 3 years of diagnosis.[29,30] The loss of beta cells, however, starts well before the onset of the disease. Stimulated C-peptide levels gradually decline from 30 months before the diagnosis of diabetes in progressors,[31] and are preserved in non-progressors.[32]

The Diabetes Prevention Trial-Type 1 highlighted that the evaluation of C-peptide dynamics in response to oral challenges is crucial in the study of type 1 diabetes, specifically showing that a reduced early response can differentiate at-risk children who will progress to develop type 1 diabetes from those who will not progress.[33] Index 60 is a composite estimate, which, when higher than 1.0, describes a lower C-peptide response to a meal challenge than what can be expected based on the fasting C-peptide values and on the 60-minute postchallenge blood glucose values. Index 60 has high predictive value for progression to type 1 diabetes in those with multiple diabetes-associated autoantibodies.[34] Similarly, the Type 1 Diabetes TrialNet Study Group showed that autoantibody-positive youths progressing towards overt type 1 diabetes in 5 years or longer have a lower fasting C-peptide and a lower early C-peptide response to an OGTT than their autoantibody-negative peers.[35] These changes are contemporaneous with the premature loss of the first phase insulin response that is observed during the preclinical phase of type 1 diabetes.[36,37] However, compared with healthy non-diabetic controls, adolescents in stage 1 of type 1 diabetes (i.e. normoglycaemic but with detectable pancreatic autoantibodies) also suffer from a reduction in the static second phase component of the beta-cell response to an oral glucose load, which may reflect an impairment of the translocation and maturation of insulin granules.[38]

After the clinical onset of type 1 diabetes, some individuals rapidly lose endogenous insulin secretory capacity within a few months, while it is partially retained in others, at least in the initial years after the disease onset. While low C-peptide invariably predicts insulin deficiency, which at mealtimes provokes marked postprandial hyperglycaemia, a low, but still measurable C-peptide indicates the persistence of residual endogenous insulin secretion, which restrains hepatic glucose production and controls fasting plasma glucose (FPG).

4.1.2 | Clinical outlook in classical type 1 diabetes

Heterogeneity of C-peptide levels exists before, and at the time of clinical diagnosis of type 1 diabetes. The assessment of C-peptide during the prodromic phase of type 1 diabetes (i.e. after seroconversion but before clinical onset) has been proposed as a marker to predict progression towards the disease in at-risk children.[32] After clinical onset, measurement of C-peptide may be helpful to confirm insulin deficiency in patients with signs of diabetes-related autoimmunity, without having a clear phenotype for type 1 diabetes, or who have a strong family history of diabetes suggesting monogenic diabetes.[12] In this regard, it is estimated that about 15%-40% of people with type 1 diabetes are no longer lean, having become overweight or frankly obese, and there is concern that the percentage of people with type 1 diabetes who become overweight is increasing globally.[39] Some people with obesity and type 1 diabetes develop characteristics of the metabolic syndrome and require high doses of insulin.[40,41] This phenotype has also been named 'double diabetes' because the phenotype mimics both type 1 diabetes and type 2 diabetes.[42]

Total loss of endogenous insulin secretion is a quite different condition when compared with even minimal maintenance of residual secretion, which translates into important clinical differences in type 1 diabetes regarding glycaemic control, metabolic status and risk of late vascular complications. Unmeasurable C-peptide or its concentrations of less than 0.05-0.10 nmol/L (note the detection level can vary according to different assays), indicates nearly total endogenous insulin deficiency, and the need for exogenous insulin replacement. Total insulin deficiency is associated with a greater risk of diabetic ketoacidosis (DKA), greater difficulty in maintaining HbA1c at less than 7.0% (<53 mmol/mol) because of high glucose variability, as well as a higher risk of hypoglycaemia. On the other hand, C-peptide concentrations above 0.10-0.20 nmol/L translate into less difficult glycaemic control, lower glycaemic variability and a lower risk of hypoglycaemia. In this regard, Rickels et al. recently showed that higher C-peptide (defined as peak MMTT C-peptide >0.40 nmol/L) is associated with more time

in range and lower mean blood glucose.[43] In the same study, people with higher C-peptide also had a greater glucagon response than people with very low C-peptide (i.e. those with peak MMTT C-peptide <0.007 nmol/L).[43] This finding is consistent with another study showing significantly higher glucagon concentrations during hyperinsulinaemic stepwise hypoglycaemic clamps among people with type 1 diabetes with detectable (≥0.05 nmol/L) compared with undetectable C-peptide.[44] As a result, people with type 1 diabetes and preserved C-peptide experience fewer hypoglycaemia events than their counterparts with undetectable C-peptide.[14,45-47] Notably, however, residual insulin micro-secretion (defined as peak C-peptide levels >0.03 nmol/L after a MMTT) did not influence peak glucagon levels after a MMTT, suggesting that the few remaining functioning beta cells may be unable to exert an efficient paracrine action to halt the inappropriate glucagon secretion in response to meals observed in people with type 1 diabetes.[48,49] A recent, large study of more than 6000 people with type 1 diabetes with unsatisfactory glycaemic control (HbA1c 8.0%-8.5%, 63-68 mmol/mol) over an average period of 5.2 years, has shown that fasting C-peptide of more than 0.20 nmol/L, compared with fasting C-peptide of less than 0.005 nmol/L, is associated with lower insulin requirement, lower HbA1c and a reduced risk of DKA and hypoglycaemia.[50] Of note, in terms of hypoglycaemia risk, a continuous relationship with hypoglycaemic episodes down to the limit of C-peptide detection (0.003 nmol/L) was found.[50] These interesting observations may result from the buffering activity of a minimally maintained endogenous insulin secretion on glucose homeostasis,[46] emphasizing the importance of therapeutic efforts to minimize loss of endogenous insulin secretion over time in type 1 diabetes. Despite the documented benefits of residual C-peptide in the type 1 diabetes population, detectable C-peptide was not associated with significant benefits in pregnant women with type 1 diabetes in terms of pregnancy outcomes.[51]

4.1.3 | Areas of uncertainties in classical type 1 diabetes

In the natural history of type 1 diabetes, less efficient insulin processing in the early stages of the disease has been suggested by several studies, especially in children aged younger than 7 years at diagnosis, in whom an increase of proinsulin/C-peptide ratio precedes the onset of hyperglycaemia.[52-54] However, an increase in basal proinsulin concentrations has also been described in non-diabetic twins of people with insulin-dependent diabetes.[55] In this regard, a potential role for the proinsulin/C-peptide ratio as a biomarker of progression towards overt disease has been proposed, but longitudinal studies are needed to confirm this hypothesis. Also, in contrast to stimulated C-peptide, fasting C-peptide has been shown to remain stable[31] or even to increase in progressors during the prodromic phase of type 1 diabetes in a small group of children.[56] However, these observations await confirmation.

The factors involved in the differential loss of endogenous insulin secretion over time observed among people with type 1 diabetes are multiple and certainly include differential degrees of glucotoxicity to the beta cell, delayed diagnosis and age at onset,[29,30,57] but most factors remain unknown.

A better risk–benefit ratio associated with the use of sodium-glucose co-transporter-2 (SGLT2) inhibitors among people with type 1 diabetes has been suggested among people with a body mass index (BMI) of 27 kg/m^2 or higher,[58,59] although a recent meta-analysis has also suggested that the risk of DKA increases with excessive insulin dose reduction, higher BMI and higher insulin resistance.[60] DKA might occur among users of SGLT2 inhibitors when there is an imbalance between the amount of insulin required to halt lipolysis and the amount of available (endogenous or exogenous) insulin. Whether the measurement of C-peptide may improve DKA risk assessment in people with type 1 diabetes who are using SGLT2 inhibitors, remains to be proven.

C-peptide levels were also able to individuate subgroups of people with type 1 diabetes responding better to liraglutide treatment in a post hoc analysis of the ADJUNCT ONE trial.[61] This is in line with a subsequent retrospective analysis conducted in 11 people with type 1 diabetes and detectable C-peptide who benefitted from glucagon-like peptide-1 receptor agonist (GLP-1 RA) therapy in terms of glycaemic control, weight and insulin dose reduction.[62] Although this might suggest an additional clinical role for C-peptide to facilitate the introduction of new therapies as add-on to insulin in type 1 diabetes, further studies should be performed to confirm this intriguing hypothesis.

4.1.4 | C-peptide in the natural history of adult-onset autoimmune diabetes

Different age at onset of autoimmune diabetes is often associated with different clinical, demographic and immunogenetic features,[63-67] consistent with a so-called glutamic acid decarboxylase (GAD)-histocompatibility antigen (HLA) DR3 immune genotype of the disease.[68,69] The prevalence of detectable C-peptide varied from 19% in people diagnosed before the age of 15 years and diabetes duration greater than 15 years, to 92% in those with onset after the age of 35 years and diabetes duration of less than 5 years.[70] A genetic risk score for type 1 diabetes based on the HLA DR3 and DQ8-DR4 serotypes was strongly associated with early age at onset and inversely associated with C-peptide persistence, the latter having a heritability of 26%.[70] Furthermore, a proportion of cases are affected by LADA, not requiring insulin at diagnosis.[71-73] Consequently, people with adult-onset autoimmune diabetes usually have a variable amount of preserved beta-cell function when diagnosed with diabetes.[70,74,75]

Fasting C-peptide at LADA onset may even be higher than in matched healthy non-diabetic controls, suggesting that insulin resistance plays a role in the pathogenesis of this form of diabetes.[76] On the other hand, in these individuals, fasting C-peptide can be lower compared with people with type 2 diabetes, and severe impairment of acute insulin secretion after glucose-arginine stimulation has been shown in a proportion of patients with LADA compared with both

non-diabetic controls and people with type 2 diabetes.[77] This evidence strongly shows the central role of a marked insulin secretory defect in the pathogenesis of LADA. Nonetheless, a wide heterogeneity in the natural history of beta-cell function in adult-onset autoimmune diabetes is recognized,[64,78-81] with beta-cell reserve differing among patients with this form of diabetes, mainly because of differences in the severity of the autoimmune process, as well as the probable presence of patients with type 2 diabetes with false positive GADA (false positive GADA may occur if assays with low specificity are used and if GADA are measured in populations with low pretest risk of autoimmune diabetes[82]). Indeed, people who present with high autoantibody levels, multiple diabetes autoantibodies and/or high type 1 diabetes gene risk scores, exhibit a more severe beta-cell dysfunction at disease onset and a more rapid decline towards insulin dependency.[79,83-86] In particular, a biphasic pattern of C-peptide loss has been shown in Chinese people with LADA, with a rapid decline in fasting C-peptide levels during the first 5 years after diabetes diagnosis in those who have high GADA levels, while about 30% of people experience slower progression to beta-cell failure during the first 8 years of diabetes.[79] In this regard, adult-onset autoimmune diabetes patients with low GADA levels may have similar C-peptide levels and a similar rate of changes in C-peptide as those with type 2 diabetes.[87] On the other hand, detailed studies of one cohort of LADA patients found that the difference in C-peptide could be ascribed to their lower BMI.[88]

4.1.5 | Clinical outlook in adult-onset autoimmune diabetes

Overall, both C-peptide and diabetes autoantibodies, more than age at onset, define groups of patients with diabetes with clinically relevant differences in glycaemic control and cardiometabolic risk.[89] Therefore, the measurement of C-peptide at regular intervals in adult-onset autoimmune diabetes is helpful to monitor individual disease progression and to stratify the risk of metabolic decompensation. Such an approach is important given the consistently poor diabetes control in such cases compared with type 2 diabetes, which is strictly linked to the development of microvascular complications.[90] An international expert panel has highlighted the relevance of measuring C-peptide in LADA to guide clinical decisions.[91] Briefly, the panel concluded in favour of a personalized treatment approach in LADA, identifying three broad categories of random C-peptide levels, strictly linked to therapeutic decisions, yet conscious of the graded effect of C-peptide levels. While a multiple-insulin regimen is recommended for people with a random C-peptide of less than 0.30 nmol/L, a more flexible approach may be used in those with random C-peptide concentrations of 0.30-0.70 nmol/L, who might be treated with insulin in combination with other therapies to prevent vascular complications, and who may benefit from a regular follow-up of beta-cell reserve by monitoring C-peptide levels at least every 6 months. Note that the slightly higher C-peptide at less than 0.30 nmol/L, accepting that it is a random value and is in adults, is not dissimilar to less than

0.20 nmol/L, which has been defined elsewhere as the threshold for absolute insulin dependence.[92] Finally, the expert panel advise treating people with LADA who have random C-peptide levels of more than 0.70 nmol/L, according to the ADA/EASD algorithm,[93] and to repeat the C-peptide measurement if glycaemic control deteriorates. More recently, a perspective article from a panel of experts gathered by the Juvenile Diabetes Research Foundation, while confirming the cut-off for this therapeutic decision in adult-onset autoimmune diabetes as described above, also recommended considering the measurement of C-peptide in adults more than 3 years after diabetes onset if clinical features suggest a possible diagnosis of autoimmune diabetes.[94]

4.1.6 | Areas of uncertainties in adult-onset autoimmune diabetes

Although the increased awareness about the clinical and pathophysiological heterogeneity of adult-onset autoimmune diabetes has led to flexible therapeutic algorithms being proposed, based on the evaluation of beta-cell function, the suggested C-peptide cut-offs are in part arbitrary because of the graded effect of C-peptide, which makes it difficult to differentiate distinct categories. Therefore, the longitudinal evaluation of C-peptide changes over time might be more appropriate to drive clinical decisions than distinct boundaries defined on cross-sectional measurements, just as identifying those at risk of progression to early insulin therapy is more important than trying to classify cases into type 1 diabetes or type 2 diabetes. Longitudinal studies are needed to support this hypothesis. Furthermore, just as in type 2 diabetes, insulin resistance is often present in people with adult-onset autoimmune diabetes, affecting the value of C-peptide as a marker of beta-cell competence.

4.2 | C-peptide and type 2 diabetes

4.2.1 | C-peptide in the natural history of type 2 diabetes

The origin of hyperglycaemia in type 2 diabetes is a variable combination of pancreatic beta-cell dysfunction along with hepatic and peripheral insulin resistance. However, the progression of hyperglycaemia is mainly caused by a continuing deterioration of beta-cell function, albeit at variable rates of progression.[95] A prodromic hyperinsulinaemic phase in response to the increased insulin resistance precedes the progressive decline of beta-cell function observed later in the natural history of type 2 diabetes.[96] In this prediabetic stage, higher C-peptide levels are associated with an increased risk of progression towards overt type 2 diabetes, with a stronger relationship than insulin levels.[97,98] Nonetheless, a dysfunctional beta cell is already present before the onset of hyperglycaemia, as confirmed by the high absolute proinsulin levels and by the high proinsulin/C-peptide or proinsulin/insulin ratios observed among people with prediabetes.[99-101] Of

note, the use of C-peptide instead of insulin as denominator for the calculation of proinsulin ratios may be a better indicator of distressed beta cells and better predict the progression towards type 2 diabetes because it is not affected by the impaired hepatic clearance of insulin.[102,103] After the onset of type 2 diabetes, plasma C-peptide progressively declines. Despite this, it may still be detectable for more than 20 years, even in people who have been converted to insulin therapy, whether they are using basal insulin alone or a basal-bolus regimen.[104]

4.2.2 | Clinical outlook

To date, the clinical use of C-peptide to address clinical decision-making in people with type 2 diabetes is hampered by the lack of firm and convincing evidence that shows a clear benefit of C-peptide measurement in terms of risk assessment and response to therapy. C-peptide response after stimulation (meal or glucagon) has been suggested as a marker to predict response to liraglutide in small, Japanese studies.[105,106] A larger observational prospective study has also suggested that measurement of C-peptide can help to predict the response to therapy with a GLP-1 RA among insulin-treated patients, who showed a 0.4% lower reduction in HbA1c after 6 months of GLP-1 RA therapy for each 1 nmol/L lower fasting C-peptide. Nonetheless, in the same study, C-peptide levels were not associated with response to therapy in non-insulin–treated patients.[107] In addition, post hoc analyses of pooled data from the SUSTAIN 1-3, the AWARD 1, 3 and 5, and the GetGoal-M, -P and -S trials, which excluded people on insulin therapy, showed no association between baseline beta-cell function and response to semaglutide, dulaglutide and lixisenatide, respectively.[108-110]

A few studies also suggest C-peptide may help in understanding the heterogeneity of type 2 diabetes, and potentially provide useful guidance for the safe initiation and titration of basal insulin. In particular, C-peptide has been used instead of insulin for the calculation of the homeostasis model assessment (HOMA2)-B to distinguish people who have severe insulin-deficient type 2 diabetes (SIDD).[111] This group of people had the highest type 2 diabetes genetic risk score, the poorest metabolic control over time, more rapid progression towards sustained insulin use and a higher risk of diabetic retinopathy compared with other clusters of adult-onset diabetes.[111,112]

C-peptide has recently been shown to have a putative role in predicting the response to treatment with basal insulin and risk of hypoglycaemia.[113] The relationship between fasting C-peptide and clinical outcomes was examined in 2165 insulin-naïve people with type 2 diabetes in whom basal insulin glargine (Gla-100) was to be initiated. The patients were stratified according to their fasting C-peptide (≤0.40; >0.40-1.20; >1.20-2.00; >2.00 nmol/L). Before commencing Gla-100, low C-peptide levels were associated with longer diabetes duration, lower BMI and higher FPG. HbA1c reduction post–Gla-100 was similar in all four groups after 24 weeks of treatment, but the insulin dose was lowest in the group with the lowest fasting C-peptide (≤0.40 nmol/L) and highest in the group with the highest fasting C-peptide

(>1.20 nmol/L).[113] The overall incidence and event rates of nocturnal and severe hypoglycaemia were higher at week 24 in the groups that had lower fasting C-peptide levels. A low fasting C-peptide also identified the most insulin-deficient, insulin-sensitive subgroup/phenotype with an enhanced risk of severe hypoglycaemia, suggesting the need for more careful titration of basal insulin, and a need for cautious prandial insulin replacement to lower HbA1c, while mindful of the risk for hypoglycaemia. In another study, continuous glucose monitoring was used to determine whether measurement of random non-fasting C-peptide could assess hypoglycaemia risk in insulin-treated type 2 diabetes.[114] Low random C-peptide (<0.2 nmol/L) was associated with greater glucose variability and a higher risk of hypoglycaemia when compared with matched insulin-treated patients with type 2 diabetes in whom beta-cell function was preserved (random C-peptide >0.6 nmol/L). The measurement of random C-peptide can therefore either identify patients with insulin-treated type 2 diabetes who are at increased risk of experiencing hypoglycaemia or alert the clinician to cases who might have other forms of diabetes, including type 1 diabetes, ketosis-prone diabetes or latent autoimmune diabetes.

4.2.3 | Areas of uncertainties

As outlined above, the evaluation of beta-cell competence in people living with type 2 diabetes treated with insulin might help in predicting the extent of response to alternative therapies, including those that require the presence of endogenous insulin secretion to be efficacious, such as GLP-1 RAs. Conversely, C-peptide may have a role in clinical management to better inform when insulin replacement therapy (basal, prandial or both) would be appropriate. In particular, C-peptide is inversely related both to glucose variability and to the magnitude of glucose excursion after meals,[12] so that estimates of C-peptide might be useful in determining whether prandial insulins should be considered, or when it might possibly be withdrawn in favour of alternative approaches such as a GLP-1 RA and/or SGLT2 inhibitor.[115,116] Large, prospective randomized clinical studies are needed to evaluate whether a clear cut-off of C-peptide can be established in this regard, towards a precision medicine approach.

Overall, most of the potential clinical applications for C-peptide in type 2 diabetes are only hypothetical and not yet supported by firm evidence. It is important to note that the sole use of C-peptide for the estimation of beta-cell function in type 2 diabetes may be hampered by concomitant insulin resistance. Clinicians should also consider that some antidiabetes drugs, such as incretin therapies, could theoretically affect the clinical interpretation of C-peptide concentrations. In this regard, it has been shown recently that low-dose gliclazide may augment the classical incretin effect and increase late phase insulin secretion after an oral glucose challenge.[117] New research should be encouraged to clarify whether, to what extent, and which ongoing antidiabetes therapy affect C-peptide concentrations, and the best strategies to mitigate this possible confounder. In addition, whether longitudinal monitoring of C-peptide in people with non-SIDD type 2 diabetes helps in identifying those progressing over the years to

more severe dysfunction of beta cells and ultimately insulin deficiency, requiring initiation or intensification of insulin replacement,[95] remains to be shown.

4.3 | C-peptide to differentiate type 2 diabetes from autoimmune diabetes?

A joint ADA/EASD consensus report on the management of type 1 diabetes in adults[92] recommended the evaluation of C-peptide beyond 3 years after diabetes diagnosis in autoantibody-negative adults receiving insulin treatment, with C-peptide values of less than 0.20 nmol/L suggesting a diagnosis of type 1 diabetes and values of more than 0.60 nmol/L indicating type 2 diabetes. The consensus also highlighted that C-peptide concentrations of 0.20-0.60 nmol/L should be interpreted with caution, as no absolute value in this range is clearly discriminatory between autoimmune or monogenic diabetes, but can also be observed in people with insulin-treated type 2 diabetes. However, guidelines for management 3 years postdiagnosis are of limited clinical value when faced with a recently diagnosed case of uncertain pathogenesis and natural history. To emphasize these uncertainties, another review considered C-peptide estimates within 3 years postdiagnosis and used minimally different values, suggesting that insulin treatment be offered to individuals with random C-peptide values of less than 0.30 nmol/L.[94]

In another recent study, the measurement of random C-peptide in people with a clinical diagnosis of type 1 diabetes for 3 years or more, followed by a further evaluation if levels were 0.2-0.9 nmol/L, allowed reclassification of 6.8% of the tested cohort into different diabetes types, mostly type 2 diabetes. Of note, most of the reclassifications occurred among people with adult-onset diabetes. In the same study, a cost implication analysis suggested that measurement of C-peptide was cost-effective in this setting.[118]

It should, however, be noted that the use of fasting or random C-peptide to define type 1 diabetes is fraught with difficulty because levels merge from type 1 diabetes into type 2 diabetes, along with the lack of a biomarker to define type 2 diabetes. Taken in isolation, serum C-peptide may not distinguish one from the other. For example, a multivariable clinical diagnostic model that integrates clinical features, diabetes-associated autoantibodies and a type 1 diabetes Gene Risk Score, had 100% specificity, but only 41% sensitivity to identify adult-onset type 1 diabetes when that was defined by progression to insulin treatment within 3 years and not by fasting C-peptide levels of less than 0.2 nmol/L.[119] More than half of the cases with type 1 diabetes would be missed if C-peptide levels alone were considered in adult-onset autoimmune diabetes. Such a gradation extends to the distinction between adult-onset and childhood-onset type 1 diabetes.[70] If type 1 diabetes is assumed to be represented by a C-peptide level of less than 0.20 nmol/L, then many cases diagnosed in adult age would not be eligible for treatment with an insulin pump, given that 25% of people with classical type 1 diabetes have higher C-peptide levels when tested 6-9 years postdiagnosis.[30] When random C-peptide is high at 3 years postdiagnosis, this usually is consistent with a diagnosis of type 2 diabetes, but it should be noted that the mean fasting C-peptide in classical type 1 diabetes in adults at 5 years postdiagnosis was 0.17 nmol/L (standard deviation 0.33).[29] By using only C-peptide to differentiate between autoimmune diabetes and type 2 diabetes, without measuring diabetes-associated autoantibodies, sensitivity is lost at the expense of a gain in specificity.

5 | C-PEPTIDE AND VASCULAR COMPLICATIONS OF DIABETES

5.1 | C-peptide and microvascular complications

C-peptide may also act as a biomarker to assist with risk stratification of diabetes complications. The Diabetes Control and Complications Trial (DCCT) showed that patients with type 1 diabetes experience better outcomes in the presence of preserved C-peptide early in the course of the disease.[120] More specifically, it is suggested that among people with duration of type 1 diabetes of 1-5 years, a 50% higher value of stimulated C-peptide (0.10-0.15 nmol/L) is associated with a 25% reduction in the risk of sustained retinopathy.[121] Similarly, data from the Scottish Diabetes Research Network Type 1 Bioresource showed that people with type 1 diabetes and a random (non-fasting) C-peptide of 0.20 nmol/L or higher are 50% less probable to develop retinopathy than people with a random C-peptide of less than 0.005 nmol/L, after adjustment for age and diabetes duration.[50] The form of the relationship between C-peptide and risk of incident retinopathy was approximately linear near to the lower limit of detection of the assay (0.003 nmol/L), with no evidence of a threshold effect. This observation has subsequently been shown in people with type 2 diabetes, who exhibited a lower incidence of retinopathy in association with a higher fasting C-peptide, after adjustment for multiple confounders.[122-124] Furthermore, Ahlqvist and colleagues showed that those two clusters of adult-onset diabetes characterized by insulin deficiency, representing about one-quarter of all adult-onset cases, had a higher risk of diabetic retinopathy.[111] In the DCCT, C-peptide levels among people with duration of type 1 diabetes of 1-5 years were also related to a reduced risk of nephropathy, albeit the number of renal events was low.[121] Bo and colleagues confirmed a 73% lower risk of nephropathy and a 61% lower risk of neuropathy in patients with type 2 diabetes in the highest tertile of C-peptide levels.[122] Similarly, in a cross-sectional study conducted in people with type 2 diabetes, Kim et al. reported that patients with lower C-peptide concentrations were about 1.5 times more probable to be affected by nephropathy or by neuropathy.[123] However, more recent data from the DCCT/Epidemiology of Diabetes Interventions and Complications cohort failed to show a significant association between microvascular complications and C-peptide response to MMTT, evaluated after an average diabetes duration of 35 years.[47]

5.2 | C-peptide and macrovascular complications

In contrast to microvascular disease, the association between C-peptide and macrovascular complications of diabetes is controversial,

TABLE 3 Clinical usefulness of the assessment of plasma C-peptide levels in people with diabetes

Setting	Potential clinical role	Areas of uncertainties
Uncertain diagnosis in long-standing insulin-treated diabetes	• To recognize type 1 diabetes some years after onset, when autoantibody measurement may result in false negative	• C-peptide values 0.20-0.60 nmol/L are not discriminatory • Correct timings of first C-peptide measurement and of subsequent retesting are still arbitrary
Type 1 diabetes	• During preclinical phase, to assess the risk and rapidity of progression towards diabetes onset • During the honeymoon period, to assess the rate of beta-cell function decline, for a prompt intervention when absolute insulin deficiency occurs • To confirm insulin deficiency in patients without a definite type 1 diabetes phenotype	• Lack of standardization of C-peptide measurement
Type 2 diabetes	• To identify subgroups of people with type 2 diabetes within the severe insulin-deficient cluster • To predict the response to treatment with basal insulin and the related hypoglycaemia risk • To individualize people on insulin therapy less probable to respond to a GLP-1 RA	• C-peptide as a marker of beta-cell competence is affected by the presence of insulin resistance • Often overestimates beta-cell competence because of concomitant hyperglycaemia • C-peptide levels could theoretically be affected by ongoing antidiabetes therapies • Whether C-peptide may help in identifying patients requiring insulin therapy and those who may safely withdraw from ongoing insulin therapy is yet to be proven • A role for C-peptide in assessing the risk of euglycaemic ketoacidosis in candidates for SGLT2 inhibitor therapy has to be proven
Adult-onset autoimmune diabetes not on insulin therapy	• To predict disease progression • To guide the decision about the right timing to start insulin therapy and use of different glucose-lowering agents	• Suggested C-peptide cut-offs are in part arbitrary because of the graded effect of C-peptide • Insulin resistance if often present in adult-onset autoimmune diabetes, affecting the value of C-peptide as a marker of beta-cell competence
Diabetes complications	• To aid hypoglycaemia and complications risk stratification	• Lack of definitive data showing direct tissue effects

Abbreviations: GLP-1 RA, glucagon-like peptide-1 receptor agonist; SGLT2, sodium-glucose co-transporter-2.

apparently differing between type 1 diabetes and type 2 diabetes. Studies conducted in type 1 diabetes suggest that preserved C-peptide is associated with better metabolic control and, in turn, with fewer cardiovascular events. On the other hand, factors associated with beta-cell dysfunction (such as the presence of diabetes-associated autoantibodies, the number of detectable autoantibodies and their blood levels) do not help to stratify the risk of cardiovascular events in people with adult-onset diabetes.[125] Furthermore, most of the studies conducted in type 2 diabetes suggest that higher C-peptide levels are associated with cardiovascular events and increased mortality.[126,127] These C-peptide data should be interpreted with caution but point to a probable distinction between type 1 diabetes and type 2 diabetes. While, in type 1 diabetes, C-peptide is a marker of insulin production, in type 2 diabetes the interpretation of C-peptide levels is difficult in view of the co-existing insulin resistance. Indeed, the opposite associations described between C-peptide and diabetes complications in the two different types of diabetes may be an epi-phenomenon of the better metabolic control observed in people with type 1 diabetes who have preservation of C-peptide, and vice versa, of the difficulty in achieving metabolic control in individuals with type 2 diabetes who have markedly elevated levels of C-peptide because of greater insulin resistance.

Some evidence has raised the possibility that C-peptide may exert direct effects on the inflammatory and vascular cells involved in the pathogenesis of complications, although at present these observations are unconfirmed.[128,129]

6 | CONCLUSIONS

Overall, given its comparatively low cost, accuracy of measurement and the range of potential applications, measurement of C-peptide in clinical practice may be a valuable and cost-effective tool to select and tailor diabetes management in different clinical settings. Nevertheless, although C-peptide measurement has been incorporated into diagnostic and therapeutic algorithms of overt autoimmune diabetes, data on its utility in the prediction of type 1 diabetes and in the clinical management of type 2 diabetes in its different stages are limited, and do not yet support a widespread use of C-peptide measurement in these situations.

Several uncertain aspects need to be fully addressed in future studies before the evaluation of C-peptide can be efficaciously implemented in specific contexts, such as in people with predominant insulin resistance (Table 3). While C-peptide measurement is useful to disentangle uncertain diagnoses and in the longitudinal follow-up of

certain populations, some issues related to its clinical application are still unresolved. These include the standardization of C-peptide determination, consensus on the interpretation of C-peptide values on the basis of the concomitant blood glucose concentration, and the uniform nature of a solid mixed meal and its use instead of liquid mixed meals. Finally, it is desirable that randomized clinical trials are performed to clarify how C-peptide may be used as a biomarker to identify clusters, and stratify the individual response to different diabetes treatments.

AUTHOR CONTRIBUTIONS

All the authors contributed to data search and discussion, manuscript writing and editing. The corresponding author is the guarantor.

CONFLICT OF INTEREST

The authors declare no conflicts of interests related to this manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14785.

DATA AVAILABILITY STATEMENT

Data sharing not applicable - no new data generated.

ORCID

Ernesto Maddaloni https://orcid.org/0000-0003-3844-9463
Geremia B. Bolli https://orcid.org/0000-0003-4966-4003
Paolo Pozzilli https://orcid.org/0000-0001-5090-636X
Raffaela Buzzetti https://orcid.org/0000-0003-1490-6041

REFERENCES

1. Heding LG. Radioimmunological determination of human C-peptide in serum. *Diabetologia*. 1975;11(6):541-548.
2. Ludvigsson J, Lise G. Heding, 1936–2008. *Diabetologia*. 2009;52(10): 2245-2246.
3. Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH. Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest*. 1975;55(6):1278-1283.
4. Steele C, Hagopian WA, Gitelman S, et al. Insulin secretion in type 1 diabetes. *Diabetes*. 2004;53:426-433.
5. Masharani U, German MS. Pancreatic hormones and diabetes mellitus. *Greenspan's Basic & Clinical Endocrinology*. 9th ed. China: McGraw Hill Lange; 2011:573-655.
6. Little RR, Wielgosz RI, Josephs R, et al. Implementing a reference measurement system for C-peptide: successes and lessons learned. *Clin Chem*. 2017;63(9):1447-1456.
7. Stoyanov AV, Connolly S, Rohlfing CL, Rogatsky E, Stein D, Little RR. Human C-peptide quantitation by LC-MS isotope-dilution assay in serum or urine samples. *J Chromatogr Sep Tech*. 2013;4(3):172.
8. Kinumi T, Ichikawa R, Arimoto H, Takatsu A. Traceable amino acid analyses of proteins and peptides by isotope-dilution mass spectrometry using precolumn derivatization reagent. *Anal Sci*. 2010; 26(9):1007-1010.
9. Kinumi T, Goto M, Eyama S, Kato M, Kasama T, Takatsu A. Development of SI-traceable C-peptide certified reference material NMIJ CRM 6901-a using isotope-dilution mass spectrometry-based amino acid analyses. *Anal Bioanal Chem*. 2012;404(1): 13-21.
10. Little RR, Kinumi T, Connolly S, Kabytaev K. Implementing a reference measurement system for C-peptide: an addendum. *Clin Chem*. 2017;63(12):1904-1905.
11. Rohlfing C, Tennill A, Stein D, Rogatsky E, Little R. *Manufacturer Standardization of C-Peptide Assays to an Isotope-Dilution Mass Spectrometry Candidate Reference Method*. Washington, DC: AACC; 2011.
12. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. *Diabet Med*. 2013;30(7): 803-817.
13. Besser REJ, Shields BM, Casas R, Hattersley AT, Ludvigsson J. Lessons from the mixed-meal tolerance test: use of 90-minute and fasting C-peptide in pediatric diabetes. *Diabetes Care*. 2013;36(2): 195-201.
14. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. The diabetes control and complications trial research group. *Ann Intern Med*. 1998;128(7):517-523.
15. Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function. ADA workshop report. *Diabetes*. 2004;53(2004): 250-264.
16. Pozzilli P, Maddaloni E, Buzzetti R. Combination immunotherapies for type 1 diabetes mellitus. *Nat Rev Endocrinol*. 2015;11(5): 289-297.
17. Guglielmi C, Del Toro R, Lauria A, et al. Effect of GLP-1 and GIP on C-peptide secretion after glucagon or mixed meal tests: significance in assessing B-cell function in diabetes. *Diabetes Metab Res Rev*. 2017;33(6):e2899.
18. Holst JJ, Gasbjerg LS, Rosenkilde MM. The role of Incretins on insulin function and glucose homeostasis. *Endocrinology*. 2021;162(7): 1-10.
19. Bagger JI, Knop FK, Lund A, Vestergaard H, Holst JJ, Vilsbøll T. Impaired regulation of the incretin effect in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2011;96(3):737-745.
20. Thorens B, Porret A, Bühler L, Deng SP, Morel P, Widmann C. Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor. *Diabetes*. 1993;42(11):1678-1682.
21. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care*. 2008;31(10):1966-1971.
22. Willemsen RH, Burling K, Barker P, et al. Frequent monitoring of C-peptide levels in newly diagnosed type 1 subjects using dried blood spots collected at home. *J Clin Endocrinol Metab*. 2018;103(9):3350-3358.
23. Greenbaum CJ, Harrison LC. Guidelines for intervention trials in subjects with newly diagnosed type 1 diabetes. *Diabetes*. 2003;52(5): 1059-1065.
24. Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M. Gastric emptying in diabetes: clinical significance and treatment. *Diabet Med*. 2002;19(3):177-194.
25. Shah M, Franklin B, Adams-Huet B, et al. Effect of meal composition on postprandial glucagon-like peptide-1, insulin, glucagon, C-peptide, and glucose responses in overweight/obese subjects. *Eur J Nutr*. 2017;56(3):1053-1062.
26. Kahleova H, Tura A, Klementova M, et al. A plant-based meal stimulates incretin and insulin secretion more than an energy-and macronutrient-matched standard meal in type 2 diabetes: a randomized crossover study. *Nutrients*. 2019;11(3):486.
27. World Health Organization. *Classification of Diabetes Mellitus*. Geneva, Switzerland: WHO; 2019. https://www.diabetesatlas.org. Accessed April 5, 2022.

28. American Diabetes Association Professional Practice Committee. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2022. *Diabetes Care.* 2021;45(Suppl_1):S17-S38.

29. Barker A, Lauria A, Schloot N, et al. Age-dependent decline of β-cell function in type 1 diabetes after diagnosis: a multi-Centre longitudinal study. *Diabetes Obes Metab.* 2014;16(3):262-267.

30. Davis AK, DuBose SN, Haller MJ, et al. Prevalence of detectable C-peptide according to age at diagnosis and duration of type 1 diabetes. *Diabetes Care.* 2015;38:476-481.

31. Sosenko JM, Palmer JP, Greenbaum CJ, et al. Patterns of metabolic progression to type 1 diabetes in the diabetes prevention trial-type 1. *Diabetes Care.* 2006;29(3):643-649.

32. Schatz D, Cuthbertson D, Atkinson M, et al. Preservation of C-peptide secretion in subjects at high risk of developing type 1 diabetes mellitus - a new surrogate measure of non-progression? *Pediatr Diabetes.* 2004;5(2):72-79.

33. Sosenko JM, Palmer JP, Rafkin LE, et al. Trends of earlier and later responses of C-peptide to Oral glucose challenges with progression to type 1 diabetes in diabetes prevention trial-type 1 participants. *Diabetes Care.* 2010;33(3):620-625.

34. Jacobsen LM, Bocchino L, Evans-Molina C, et al. The risk of progression to type 1 diabetes is highly variable in individuals with multiple autoantibodies following screening. *Diabetologia.* 2020;63(3):588-596.

35. Evans-Molina C, Sims EK, DiMeglio LA, et al. β cell dysfunction exists more than 5 years before type 1 diabetes diagnosis. *JCI Insight.* 2018;3(15):e120877.

36. Sosenko JM, Skyler JS, Beam CA, et al. Acceleration of the loss of the first-phase insulin response during the progression to type 1 diabetes in diabetes prevention trial-type 1 participants. *Diabetes.* 2013;62(12):4179-4183.

37. Koskinen MK, Helminen O, Matomäki J, et al. Reduced β-cell function in early preclinical type 1 diabetes. *Eur J Endocrinol.* 2016;174(3):251-259.

38. Galderisi A, Moran A, Evans-Molina C, et al. Early impairment of insulin sensitivity, β-cell responsiveness, and insulin clearance in youth with stage 1 type 1 diabetes. *J Clin Endocrinol Metab.* 2021;106:2660-2669.

39. Buzzetti R, Zampetti S, Pozzilli P. Impact of obesity on the increasing incidence of type 1 diabetes. *Diabetes Obes Metab.* 2020;22(7):1009-1013.

40. Corbin KD, Driscoll KA, Pratley RE, Smith SR, Maahs DM, Mayer-Davis EJ. Obesity in type 1 diabetes: pathophysiology, clinical impact, and mechanisms. *Endocr Rev.* 2018;39(5):629-663.

41. Codner E. Type 1 diabetes, obesity and PCOS: Is type 1 stepping into the shoes of type 2 diabetes? *Clin Endocrinol.* 2020;95:265-266.

42. Pozzilli P, Buzzetti R. A new expression of diabetes: double diabetes. *Trends Endocrinol Metab.* 2007;18(2):52-57.

43. Rickels MR, Evans-Molina C, Bahnson HT, et al. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. *J Clin Invest.* 2020;130(4):1850-1862.

44. Zenz S, Mader JK, Regittnig W, et al. Impact of C-peptide status on the response of glucagon and endogenous glucose production to induced hypoglycemia in T1DM. *J Clin Endocrinol Metab.* 2018;103(4):1408-1417.

45. Gibb FW, McKnight JA, Clarke C, Strachan MWJJ. Preserved C-peptide secretion is associated with fewer low-glucose events and lower glucose variability on flash glucose monitoring in adults with type 1 diabetes. *Diabetologia.* 2020;63(5):906-914.

46. Marren SM, Hammersley S, McDonald TJ, et al. Persistent C-peptide is associated with reduced hypoglycaemia but not HbA1c in adults with longstanding type 1 diabetes: evidence for lack of intensive treatment in UK clinical practice? *Diabet Med.* 2019;36(9):1092-1099.

47. Gubitosi-Klug RA, Braffett BH, Hitt S, et al. Residual β cell function in long-term type 1 diabetes associates with reduced incidence of hypoglycemia. *J Clin Invest.* 2021;131(3):e143011.

48. Thivolet C, Marchand L, Chikh K. Inappropriate glucagon and GLP-1 secretion in individuals with long-standing type 1 diabetes: effects of residual C-peptide. *Diabetologia.* 2019;62(4):593-597.

49. Hare KJ, Vilsbøll T, Holst JJ, Knop FK. Inappropriate glucagon response after oral compared with isoglycemic intravenous glucose administration in patients with type 1 diabetes. *Am J Physiol Endocrinol Metab.* 2010;298(4):E832-E837.

50. Jeyam A, Colhoun H, McGurnaghan S, et al. Clinical impact of residual C-peptide secretion in type 1 diabetes on glycemia and microvascular complications. *Diabetes Care.* 2021;44(2):390-398.

51. Meek CL, Oram RA, McDonald TJ, Feig DS, Hattersley AT, Murphy HR. Reappearance of C-peptide during the third trimester of pregnancy in type 1 diabetes: pancreatic regeneration or fetal hyperinsulinism? *Diabetes Care.* 2021;44(8):1826-1834.

52. Truyen I, De Pauw P, Jørgensen PN, et al. Proinsulin levels and the proinsulin:c-peptide ratio complement autoantibody measurement for predicting type 1 diabetes. *Diabetologia.* 2005;48(11):2322-2329.

53. Sims EK, Chaudhry Z, Watkins R, et al. Elevations in the fasting serum proinsulin–to–C-peptide ratio precede the onset of type 1 diabetes. *Diabetes Care.* 2016;39(9):1519-1526.

54. Triolo TM, Pyle L, Seligova S, et al. Proinsulin:C-peptide ratio trajectories over time in relatives at increased risk of progression to type 1 diabetes. *J Transl Autoimmun.* 2021;4:100089.

55. Heaton DA, Millward BA, Gray P, et al. Evidence of beta cell dysfunction which does not lead on to diabetes: a study of identical twins of insulin dependent diabetics. *BMJ.* 1987;294(6565):145-146.

56. Åkerman L, Ludvigsson J, Swartling U, Casas R. Characteristics of the pre-diabetic period in children with high risk of type 1 diabetes recruited from the general Swedish population-the ABIS study. *Diabetes Metab Res Rev.* 2017;33(6):e2900.

57. Lauria A, Barker A, Schloot N, et al. BMI is an important driver of β-cell loss in type 1 diabetes upon diagnosis in 10 to 18-year-old children. *Eur J Endocrinol.* 2015;172(2):107-113.

58. Mathieu C, Dandona P, Birkenfeld AL, et al. Benefit/risk profile of dapagliflozin 5 mg in the DEPICT-1 and -2 trials in individuals with type 1 diabetes and body mass index ≥27 kg/m2. *Diabetes Obes Metab.* 2020;22(11):2151-2160.

59. Danne T, Edelman S, Frias JP, et al. Efficacy and safety of adding sotagliflozin, a dual sodium-glucose co-transporter (SGLT)1 and SGLT2 inhibitor, to optimized insulin therapy in adults with type 1 diabetes and baseline body mass index ≥ 27 kg/m^2. *Diabetes Obes Metab.* 2021;23(3):854-860.

60. Musso G, Sircana A, Saba F, Cassader M, Gambino R. Assessing the risk of ketoacidosis due to sodium-glucose cotransporter (SGLT)-2 inhibitors in patients with type 1 diabetes: a meta-analysis and meta-regression. *PLoS Med.* 2020;17(12):e1003461.

61. Mathieu C, Zinman B, Hemmingsson JU, et al. Efficacy and safety of liraglutide added to insulin treatment in type 1 diabetes: the ADJUNCT ONE treat-to-target randomized trial. *Diabetes Care.* 2016;39(10):1702-1710.

62. Kuhadiya ND, Prohaska B, Ghanim H, Dandona P. Addition of glucagon-like peptide-1 receptor agonist therapy to insulin in C-peptide-positive patients with type 1 diabetes. *Diabetes Obes Metab.* 2019;21(4):1054-1057.

63. Leslie RD, Palmer J, Schloot NC, Lernmark A. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. *Diabetologia.* 2016;59(1):13-20.

64. Buzzetti R, Zampetti S, Maddaloni E. Adult-onset autoimmune diabetes: current knowledge and implications for management. *Nat Rev Endocrinol.* 2017;13(11):674-686.

65. Redondo MJ, Hagopian WA, Oram R, et al. The clinical consequences of heterogeneity within and between different diabetes types. *Diabetologia.* 2020;63(10):2040-2048.

66. Zampetti S, Spoletini M, Petrone A, et al. Association of TCF7L2 gene variants with low GAD autoantibody titre in LADA subjects (NIRAD study 5). *Diabet Med.* 2010;27(6):701-704.

67. Petrone A, Suraci C, Capizzi M, et al. The protein tyrosine phosphatase nonreceptor 22 (PTPN22) is associated with high GAD antibody titer in latent autoimmune diabetes in adults: non insulin requiring autoimmune diabetes (NIRAD) study 3. *Diabetes Care.* 2008;31(3):534-538.

68. Battaglia M, Ahmed S, Anderson MS, et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. *Diabetes Care.* 2020;43(1):5-12.

69. Zhou Z, Xiang Y, Ji L, et al. Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): a nationwide, multicenter, clinic-based cross-sectional study. *Diabetes.* 2013;62(2):543-550.

70. McKeigue PM, Spiliopoulou A, McGurnaghan S, et al. Persistent C-peptide secretion in type 1 diabetes and its relationship to the genetic architecture of diabetes. *BMC Med.* 2019;17(1):165.

71. Pozzilli P, Di Mario U. Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. *Diabetes Care.* 2001;24(8):1460-1467.

72. Park Y, Hong S, Park L, et al. LADA prevalence estimation and insulin dependency during follow-up. *Diabetes Metab Res Rev.* 2011;27(8):975-979.

73. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: a disease with increasing heterogeneity. *Lancet.* 2014;383:1084-1094.

74. Hernandez M, Mollo A, Marsal J, et al. Insulin secretion in patients with latent autoimmune diabetes (LADA): half way between type 1 and type 2 diabetes: action LADA 9. *BMC Endocr Disord.* 2015;15(1):1.

75. Maddaloni E, Moretti C, Mignogna C, Buzzetti R. Adult-onset autoimmune diabetes in 2020: an update. *Maturitas.* 2020;137:37-44.

76. Seissler J. Latent (slowly progressing) autoimmune diabetes in adults. *Curr Diab Rep.* 2008;8(2):94-100.

77. Carlsson Å, Sundkvist G, Groop L, Tuomi T. Insulin and glucagon secretion in patients with slowly progressing autoimmune diabetes (LADA) 1. *J Clin Endocrinol Metab.* 2000;85(1):76-80.

78. Pieralice S, Zampetti S, Maddaloni E, Buzzetti R. "H" for heterogeneity in the algorithm for type 2 diabetes management. *Curr Diab Rep.* 2020;20(5):14.

79. Li X, Chen Y, Xie Y, et al. Decline pattern of Beta-cell function in adult-onset latent autoimmune diabetes: an 8-year prospective study. *J Clin Endocrinol Metab.* 2020;105(7):2331-2340.

80. Maddaloni E, Lessan N, Al Tikriti A, Buzzetti R, Pozzilli P, Barakat MT. Latent autoimmune diabetes in adults in The United Arab Emirates: clinical features and factors related to insulin-requirement. *PLoS One.* 2015;10(8):e0131837.

81. Zampetti S, Capizzi M, Spoletini M, et al. GADA titer-related risk for organ-specific autoimmunity in LADA subjects subdivided according to gender (NIRAD study 6). *J Clin Endocrinol Metab.* 2012;97(10):3759-3765.

82. Jones AG, McDonald TJ, Shields BM, Hagopian W, Hattersley AT. Latent autoimmune diabetes of adults (LADA) is likely to represent a mixed population of autoimmune (type 1) and nonautoimmune (type 2) diabetes. *Diabetes Care.* 2021;44(6):1243-1251.

83. Lohmann T, Kellner K, Verlohren H-J, et al. Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA). *Diabetologia.* 2001;44(8):1005-1010.

84. Turner R, Stratton I, Horton V, et al. UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet.* 1997;350(9087):1288-1293.

85. Zampetti S, Campagna G, Tiberti C, et al. High GADA titer increases the risk of insulin requirement in LADA patients: a 7-year follow-up (NIRAD study 7). *Eur J Endocrinol.* 2014;171(6):697-704.

86. Grubb AL, McDonald TJ, Rutters F, et al. A type 1 diabetes genetic risk score can identify patients with GAD65 autoantibody–positive type 2 diabetes who rapidly progress to insulin therapy. *Diabetes Care.* 2019;42(2):208-214.

87. Liu L, Li X, Xiang Y, et al. Latent autoimmune diabetes in adults with low-titer GAD antibodies: similar disease progression with type 2 diabetes. *Diabetes Care.* 2015;38(1):16-21.

88. Juhl CB, Bradley U, Holst JJ, Leslie RD, Yderstraede KB, Hunter S. Similar weight-adjusted insulin secretion and insulin sensitivity in short-duration late autoimmune diabetes of adulthood (LADA) and type 2 diabetes: action LADA 8. *Diabet Med.* 2014;31(8):941-945.

89. Wod M, Yderstræde KB, Halekoh U, Beck-Nielsen H, Højlund K. Metabolic risk profiles in diabetes stratified according to age at onset, islet autoimmunity and fasting C-peptide. *Diabetes Res Clin Pract.* 2017;134:62-71.

90. Maddaloni E, Coleman RL, Agbaje O, Buzzetti R, Holman RR. Time-varying risk of microvascular complications in latent autoimmune diabetes of adulthood compared with type 2 diabetes in adults: a post-hoc analysis of the UKprospective diabetes study 30-year follow-up data (UKPDS 86). *Lancet Diabetes Endocrinol.* 2020;8(3):206-215.

91. Buzzetti R, Tuomi T, Mauricio D, et al. Management of latent autoimmune diabetes in adults: a consensus statement from an international expert panel. *Diabetes.* 2020;69(10):2037-2047.

92. Holt RIG, DeVries JH, Hess-Fischl A, et al. The Management of Type 1 diabetes in adults. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care.* 2021;44(11):2589-2625.

93. Buse JB, Wexler DJ, Tsapas A, et al. 2019 update to: Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care.* 2020;43(2):487-493.

94. Leslie RD, Evans-Molina C, Freund-Brown J, et al. Adult-onset type 1 diabetes: current understanding and challenges. *Diabetes Care.* 2021;44(11):2449-2456.

95. UKPDS Group. UK prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. prospective diabetes study group. *Diabetes.* 1995;44(11):1249-1258.

96. Martin BC, Warram JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet.* 1992;340(8825):925-929.

97. Sokooti S, Kieneker LM, de Borst MH, et al. Plasma C-peptide and risk of developing type 2 diabetes in the general population. *J Clin Med.* 2020;9(9):3001.

98. Kim J-D, Kang SJ, Lee MK, et al. C-peptide-based index is more related to incident type 2 diabetes in non-diabetic subjects than insulin-based index. *Endocrinol Metab.* 2016;31(2):320-327.

99. Mykkänen L, Haffner SM, Kuusisto J, Pyörälä K, Hales CN, Laakso M. Serum proinsulin levels are disproportionately increased in elderly prediabetic subjects. *Diabetologia.* 1995;38(10):1176-1182.

100. Schulze MB, Solomon CG, Rifai N, et al. Hyperproinsulinaemia and risk of type 2 diabetes mellitus in women. *Diabet Med.* 2005;22(9):1178-1184.

101. Zethelius B, Hales CN, Lithell HO, Berne C. Insulin resistance, impaired early insulin response, and insulin propeptides as predictors of the development of type 2 diabetes: a population-based, 7-year follow-up study in 70-year-old men. *Diabetes Care.* 2004;27(6):1433-1438.

102. Vauhkonen I, Niskanen L, Mykkanen L, Haffner S, Uusitupa M, Laakso M. Hyperproinsulinemia is not a characteristic feature in the offspring of patients with different phenotypes of type II diabetes. *Eur J Endocrinol*. 2000;143(2):251-260.

103. Loopstra-Masters RC, Haffner SM, Lorenzo C, Wagenknecht LE, Hanley AJ. Proinsulin-to-C-peptide ratio versus proinsulin-to-insulin ratio in the prediction of incident diabetes: the insulin resistance atherosclerosis study (IRAS). *Diabetologia*. 2011;54(12):3047-3054.

104. Dario T, Riccardo G, Silvia P, et al. The utility of assessing C-peptide in patients with insulin-treated type 2 diabetes: a cross-sectional study. *Acta Diabetol*. 2020;58:411-417.

105. Iwao T, Sakai K, Sata M. Postprandial serum C-peptide is a useful parameter in the prediction of successful switching to liraglutide monotherapy from complex insulin therapy in Japanese patients with type 2 diabetes. *J Diabetes Complications*. 2013;27(1):87-91.

106. Takabe M, Matsuda T, Hirota Y, et al. C-peptide response to gluca-gon challenge is correlated with improvement of early insulin secre-tion by liraglutide treatment. *Diabetes Res Clin Pract*. 2012;98(3):e32-e35.

107. Jones AG, McDonald TJ, Shields BM, et al. Markers of β-cell failure predict poor glycemic response to GLP-1 receptor agonist therapy in type 2 diabetes. *Diabetes Care*. 2016;39:250-257.

108. Aroda VR, Capehorn MS, Chaykin L, et al. Impact of baseline charac-teristics and beta-cell function on the efficacy and safety of subcu-taneous once-weekly semaglutide: a patient-level, pooled analysis of the SUSTAIN 1-5 trials. *Diabetes Obes Metab*. 2020;22(3):303-314.

109. Mathieu C, Del Prato S, Botros FT, et al. Effect of once weekly dula-glutide by baseline beta-cell function in people with type 2 diabetes in the AWARD programme. *Diabetes Obes Metab*. 2018;20(8):2023-2028.

110. Bonadonna RC, Blonde L, Antsiferov M, et al. Lixisenatide as add-on treatment among patients with different β-cell function levels as assessed by HOMA-β index. *Diabetes Metab Res Rev*. 2017;33(6):e2897.

111. Ahlqvist E, Storm P, Käräjämäki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol*. 2018;6(5):361-369.

112. Mansour Aly D, Dwivedi OP, Prasad RB, et al. Genome-wide associ-ation analyses highlight etiological differences underlying newly defined subtypes of diabetes. *Nat Genet*. 2021;53(11):1534-1542.

113. Landgraf W, Owens DR, Frier BM, Zhang M, Bolli GB. Fasting C-peptide, a biomarker for hypoglycaemia risk in insulin-naïve people with type 2 diabetes initiating basal insulin glargine 100 U/mL. *Dia-betes Obes Metab*. 2019;22:315-323.

114. Hope S V, Knight BA, Shields BM, et al. Random non-fasting C-peptide testing can identify patients with insulin-treated type 2 dia-betes at high risk of hypoglycaemia. *Diabetologia*. 2018;61(1):66-74.

115. Rosenstock J, Nino A, Soffer J, et al. Impact of a weekly glucagon-like peptide 1 receptor agonist, albiglutide, on glycemic control and on reducing prandial insulin use in type 2 diabetes inadequately con-trolled on multiple insulin therapy: a randomized trial. *Diabetes Care*. 2020;43(10):2509-2518.

116. Bolli GB, Porcellati F, Meier JJ. Switching from insulin bolus treat-ment to GLP-1 RAs added to continued basal insulin in people with type 2 diabetes on basal-bolus insulin. *Diabetes Care*. 2020;43(10):2333-2335.

117. Cordiner RLM, Mari A, Tura A, Pearson ER. The impact of low-dose gliclazide on the incretin effect and indices of beta-cell function. *J Clin Endocrinol Metab*. 2021;106(7):2036-2046.

118. Foteinopoulou E, Clarke CAL, Pattenden RJ, et al. Impact of routine clinic measurement of serum C-peptide in people with a clinician-diagnosis of type 1 diabetes. *Diabet Med*. 2021;38(7):e14449.

119. Lynam A, McDonald T, Hill A, et al. Development and validation of multivariable clinical diagnostic models to identify type 1 diabetes requiring rapid insulin therapy in adults aged 18–50 years. *BMJ Open*. 2019;9(9):e031586.

120. Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care*. 2003;26(3):832-836.

121. Lachin JM, McGee P, Palmer JP, DCCT/EDIC Research Group. Impact of C-peptide preservation on metabolic and clinical out-comes in the diabetes control and complications trial. *Diabetes*. 2014;63(2):739-748.

122. Bo S, Gentile L, Castiglione A, et al. C-peptide and the risk for inci-dent complications and mortality in type 2 diabetic patients: a retro-spective cohort study after a 14-year follow-up. *Eur J Endocrinol*. 2012;167(2):173-180.

123. Kim B-Y, Jung C-H, Mok J-O, Kang S-K, Kim C-H. Association between serum C-peptide levels and chronic microvascular compli-cations in Korean type 2 diabetic patients. *Acta Diabetol*. 2012;49(1):9-15.

124. Chung JO, Cho DH, Chung DJ, Chung MY. Relationship between serum C-peptide level and diabetic retinopathy according to esti-mated glomerular filtration rate in patients with type 2 diabetes. *J Diabetes Complications*. 2015;29(3):350-355.

125. Maddaloni E, Coleman RL, Pozzilli P, Holman RR. Long-term risk of cardiovascular disease in individuals with latent autoimmune diabe-tes in adults (UKPDS 85). *Diabetes Obes Metab*. 2019;21(9):2115-2122.

126. Marx N, Silbernagel G, Brandenburg V, et al. C-peptide levels are associated with mortality and cardiovascular mortality in patients undergoing angiography: the LURIC study. *Diabetes Care*. 2013;36(3):708-714.

127. Cardellini M, Farcomeni A, Ballanti M, et al. C-peptide: a predictor of cardiovascular mortality in subjects with established atherosclerotic disease. *Diabetes Vasc Dis Res*. 2017;14(5):395-399.

128. Vasic D, Walcher D. C-peptide: a new mediator of atherosclerosis in diabetes. *Mediators Inflamm*. 2012;2012:1-8.

129. Wahren J. C-peptide and the pathophysiology of microvascular complications of diabetes. *J Intern Med*. 2017;281(1):3-6.

130. Zavaroni I, Deferrari G, Lugari R, et al. Renal metabolism of C-peptide in man. *J Clin Endocrinol Metab*. 1987;65(3):494-498.

131. Henriksen JH, Tronier B, Bülow JB. Kinetics of circulating endoge-nous insulin, C-peptide, and proinsulin in fasting nondiabetic man. *Metabolism*. 1987;36(5):463-468.