Defining criteria for the introduction of liraglutide using the glucagon stimulation test in patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: To define a set of criteria using indices of β-cell function, including results from the glucagon stimulation test, for liraglutide introduction in patients with type 2 diabetes.

Materials and Methods: In the present retrospective cohort study, patients were included in our analysis if their β-cell function had been evaluated with a glucagon stimulation test and a 24-h urinary C-peptide (U-CPR) excretion test before switching from insulin therapy to liraglutide monotherapy. The efficacy of liraglutide was determined by the extent to which glycemic control was achieved or if glycated hemoglobin levels were maintained at <7.0% after liraglutide monotherapy for 24 weeks.

Results: Liraglutide was effective in 36 of 77 patients. In the liraglutide-effective cases, the following parameters were higher: fasting C-peptide (CPR0) levels, C-peptide levels 6 min after glucagon stimulation (CPR6), the C-peptide index (CPI; CPR0 × 100/fasting plasma glucose) and stimulated C-peptide index (S-CPI; CPR6 × 100/plasma glucose 6 min after glucagon stimulation). U-CPR did not differ between liraglutide-effective and liraglutide-ineffective cases. Using receiver operating characteristic analysis adjusted for baseline characteristics, the independent cut-off value for effective liraglutide introduction was 0.72 for CPI and 1.92 for S-CPI.

Conclusions: Evaluation of β-cell function using the glucagon stimulation test is useful for determining the efficacy of liraglutide introduction in patients with type 2 diabetes. (J Diabetes Invest, doi: 10.1111/jdi.12082, 2013)

KEY WORDS: Glucagon stimulation test, Liraglutide, Type 2 diabetes

INTRODUCTION

Despite the advances in treatment options for patients with type 2 diabetes, achieving optimal glycemic control without hypoglycemia and bodyweight (BW) gain remains difficult[1,2]. A new class of incretin-based antidiabetic agents, the glucagon-like peptide-1 (GLP-1) receptor agonists, are now available for the treatment of type 2 diabetes. In view of the low risk of hypoglycemia and lesser effect on BW with GLP-1 receptor agonist therapy[3–5], they are increasingly being used in the clinical setting as an alternative treatment for patients with type 2 diabetes. GLP-1 receptor agonists enhance glucose-dependent insulin secretion from pancreatic β-cells[6]. We know that the preservation of β-cell function is required for the effective treatment of patients with type 2 diabetes. As a surrogate of exact β-cell function, the glucagon stimulation test has been used widely because the C-peptide level 6 min after 1 mg i.v. glucagon stimulation has been shown to correspond to the maximal C-peptide level after a standard meal[7]. However, the criteria for effective introduction of the GLP-1 receptor agonist liraglutide based on β-cell function have not yet been clarified. Thus, we aimed to define criteria for liraglutide introduction using the glucagon stimulation test in patients with type 2 diabetes.

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MATERIALS AND METHODS

Protocol

The present retrospective cohort study analyzed the medical records of Japanese patients with type 2 diabetes at Chigasaki Municipal Hospital who were switched from insulin therapy to liraglutide monotherapy between June 2010 and August 2012. We excluded patients with the following conditions from the present study: anti-glutamic acid decarboxylase antibodies, severe nephropathy with eGFR <30 mL/min/1.73 m^2, liver cirrhosis or malignancy. Liraglutide treatment was started at a dosage of 0.3 mg/day and titrated up to 0.9 mg/day (maximum allowable dosage in Japan) in increments of 0.3 mg per week. Patients were included in our analysis if their β-cell function had been evaluated with a glucagon stimulation test and a 24-h urinary C-peptide (U-CPR) excretion test before switching to liraglutide monotherapy. In the glucagon stimulation test, stimulation was carried out by i.v. injection of 1 mg of glucagon (Glucagon G Novo; Novo Nordisk, Bagsvaerd, Denmark). The levels of fasting plasma glucose (FPG), plasma glucose 6 min

after glucagon stimulation (Glu6), fasting serum C-peptide (CPR0) and serum C-peptide 6 min after glucagon stimulation (CPR6) were measured. Serum C-peptide levels were measured by chemiluminescence immunoassay kit (Siemens Healthcare Diagnostics, Eschborn, Germany). C-peptide index (CPI) was calculated as CPR0 (ng/mL) × 100/FPG (mg/dL). Stimulated C-peptide index (S-CPI) was calculated as CPR6 (ng/mL) × 100/Glu6 (mg/dL). The homeostasis model assessment of insulin resistance (HOMA2-IR) was calculated using the HOMA Calculator version 2.2.2[8]. Glycated hemoglobin (HbA_{1c}), FPG and 2-h postprandial glucose (PPG2h) levels (self-monitored), as well BW at baseline, and 12 and 24 weeks after liraglutide introduction were also measured. According to the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) position statement on management of hyperglycemia in type 2 diabetes[9], lowering HbA_{1c} to <7.0% in most patients is recommended to reduce the incidence of microvascular disease. In the present study, we focused on maintaining an adequate glucose level even after switching to liraglutide monotherapy from insulin therapy. Thus, efficacy of liraglutide determined the extent of glycemic control achieved or if HbA_{1c} levels were maintained at <7.0% after liraglutide monotherapy for 24 weeks. Cases with HbA_{1c} levels ≥7.0% at 24 weeks or those requiring liraglutide discontinuation because of sustained hyperglycemia (defined as FPG >200 mg/dL over more than two continuous measurements) were defined as ineffective cases. We carried out efficacy analysis using a modified last-observation-carried-forward (mLOCF) approach, as assessed by BW and the levels of HbA_{1c}, FPG and PPG2h levels. The last valid observation between baseline and 12 weeks from initiation of therapy was carried forward for the missing 12 weeks of measurements; in addition, the last valid observation between 12 and 24 weeks was carried forward for the missing 24 weeks of measurements. HbA_{1c} data were collected as Japan Diabetes Society (JDS) values, and then converted to National Glycohemoglobin Standardization Program (NGSP) values by the following conversion formula: HbA_{1c} (NGSP; %) = 1.02 × HbA_{1c} (JDS; %) + 0.25%[10]. The estimated glomerular filtration rate was calculated using the Japan Nephrology Society equation[11]. The present study was carried out according to the Declaration of Helsinki and was approved by the institutional review board of Chigasaki Municipal Hospital, Kanagawa, Japan.

Statistical Analysis

Student's *t*-test was used to compare continuous variables, and the chi-test was used to compare the proportion of liraglutide-effective and liraglutide-ineffective cases. We used repeated measures analysis of variance (RM-ANOVA) and *post-hoc* Tukey's Honestly Significant Difference (HSD) test to evaluate the effects of therapy based on BW and the levels of HbA_{1c}, FPG and PPG2h. Values are expressed as mean ± standard deviation or absolute values. To determine the contribution of each of the indices of β-cell function to the effectiveness of liraglutide, univariate and multivariate logistic regression analyses were used. A receiver operating analysis was used to define the cut-off values indicative of successful liraglutide introduction. A two-sided *P*-value of <0.05 was considered statistically significant. Statistical analyses were carried out with JMP 10 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 77 patients were included in our analysis. Liraglutide treatment was discontinued in 18 patients because of sustained hyperglycemia. A total of 17 patients discontinued liraglutide before 12 weeks, and one patient discontinued liraglutide between 12 and 24 weeks. Cases of severe hypoglycemia or diabetic ketoacidosis were not observed. A total of 59 patients completed liraglutide monotherapy for 24 weeks. The mean age was 62.2 ± 11.1 years, the mean duration of type 2 diabetes was 10.3 ± 7.7 years, and the mean body mass index (BMI) at baseline was 25.3 ± 5.1 kg/m^2. Baseline HbA_{1c} levels were 7.5 ± 1.7%. Liraglutide was effective in 36 of 77 patients. The clinical characteristics affecting the effectiveness of liraglutide are shown in Table 1. Patient age, BW, BMI and waist circumference were not significantly different between liraglutide-effective and liraglutide-ineffective cases. Indices of β-cell function, CPR0, CPR6, CPI and S-CPI, were higher in liraglutide-effective cases. U-CPR and HOMA2-IR results were not significantly different between liraglutide-effective and liraglutide-ineffective cases. The duration of type 2 diabetes was shorter in liraglutide-effective cases. Baseline HbA_{1c} levels and insulin dosages were higher in liraglutide-ineffective cases. The time-course of treatment is shown in liraglutide-effective and liraglutide-ineffective cases, respectively (Table 2). HbA_{1c} was decreased in liraglutide-effective cases (6.8 ± 1.3% to 6.1 ± 0.4%, $P = 0.005$), but was not changed in liraglutide-ineffective cases. FPG was not changed in liraglutide-effective cases, but was increased in liraglutide-ineffective cases (126.1 ± 41.6 to 160.9 ± 40.2 mg/dL, $P = 0.02$). PPG2h was decreased in liraglutide-effective cases (165.3 ± 56.8 to 135.9 ± 24.3 mg/dL, $P = 0.007$), but was not changed in liraglutide-ineffective cases. BW was decreased in liraglutide-effective cases (65.9 ± 16.0 to 64.1 ± 16.0 kg, $P = 0.01$), but was not changed in liraglutide-ineffective cases. However, in patients who could continue to use liraglutide over 12 weeks, BW decreased in both liraglutide-effective ($n = 36$) and liraglutide-ineffective cases ($n = 24$), and the decrease was not significantly different between these cases (−1.7 ± 3.9 kg vs −3.0 ± 3.9 kg, respectively; $P = 0.23$). Univariate logistic regression analysis showed CPR0, CPR6, CPI and S-CPI were significant predictors of effectiveness of liraglutide (Table 3). Multiple logistic regression analysis showed CPI and S-CPI were independent predictors of the effectiveness of liraglutide after adjustments were made for age, sex, BMI, diabetes duration and baseline HbA_{1c} levels (CPI: odds ratio (OR) 9.60; 95% CI 2.25–60.8; S-CPI: OR 3.38; 95% CI 1.48–9.30). CPR0 and CPR6 were not independent predictors of the effectiveness of liraglutide after adjustments were made (Table 3). Using a receiver operating characteristic (ROC) analysis, the cut-off value for effective liraglutide introduction was 0.72 for CPI

Table 1 | Baseline characteristics of patients according to the effectiveness of liraglutide

	Total	Effective	Ineffective	*P*-value
Sex, male/female (*n*)	46/31	21/15	25/16	NS
Age (years)	62.2 ± 11.1	62.0 ± 11.8	62.4 ± 10.5	NS
Bodyweight (kg)	66.1 ± 15.0	65.9 ± 16.0	66.3 ± 14.2	NS
BMI (kg/m^2)	25.3 ± 5.1	25.0 ± 5.4	25.6 ± 4.8	NS
Waist circumference (cm)	89.5 ± 12.6	88.5 ± 11.8	90.5 ± 13.4	NS
Duration of diabetes (years)	10.3 ± 7.7	7.7 ± 7.7	12.6 ± 7.0	0.005
HbA_{1c} (%)	7.5 ± 1.7	6.8 ± 1.3	8.2 ± 1.8	<0.001
FPG (mg/dL)	117.4 ± 37.3	107.5 ± 29.3	126.1 ± 41.6	0.03
PPG2 h (mg/dL)	181.8 ± 64.5	165.3 ± 56.8	195.1 ± 68.0	NS
Glu6 (mg/dL)	149.8 ± 46.8	141.4 ± 47.5	157.1 ± 44.9	NS
CPR0 (ng/mL)	1.06 ± 0.75	1.27 ± 0.86	0.87 ± 0.59	0.02
CPR6 (ng/mL)	2.56 ± 2.22	3.35 ± 2.86	1.87 ± 1.10	0.003
CPI	0.92 ± 0.59	1.18 ± 0.67	0.70 ± 0.39	<0.001
S-CPI	1.77 ± 1.57	2.41 ± 2.03	1.21 ± 0.61	<0.001
U-CPR (μg/day)	60.1 ± 45.5	63.0 ± 44.5	57.6 ± 46.7	NS
HOMA2-IR	2.57 ± 2.02	3.00 ± 2.27	2.20 ± 1.71	NS
Insulin dosage (units/day)	26.7 ± 26.4	17.3 ± 9.9	35.0 ± 33.1	0.003
Insulin regimen				
Basal-bolus therapy (*n*)	40	20	20	NS
Premixed insulin (*n*)	23	9	14	
Basal insulin only (*n*)	14	7	7	
eGFR (mL/min/1.73 m^2)	67.8 ± 17.8	65.9 ± 18.6	69.6 ± 17.1	NS

Data are expressed as number or mean ± standard deviation. *P*-values were determined using the unpaired *t*-test or χ^2-test between liraglutide-effective and liraglutide-ineffective cases. BMI, body mass index; CPI, C-peptide index; CPR0, fasting C-peptide; CPR6, C-peptide 6 min after glucagon stimulation; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; Glu6, plasma glucose 6 min after glucagon stimulation; HbA_{1c}, glycated hemoglobin; HOMA2-IR, homeostasis model assessment of insulin resistance; NS, not significant; PPG2 h, 2-h postprandial glucose; S-CPI, stimulated C-peptide index; U-CPR, urine C-peptide.

Table 2 | Time-course of treatment (modified last observation carried forward analysis)

	Baseline (*n* = 77)	12 weeks (*n* = 77)	24 weeks (*n* = 60)	RM-ANOVA *P*-value
HbA_{1c} (%)				
Effective	6.8 ± 1.3	6.0 ± 0.4*	6.1 ± 0.4*	<0.001
Ineffective	8.2 ± 1.8	8.4 ± 1.8	8.3 ± 1.1	NS
FPG (mg/dL)				
Effective	107.5 ± 29.3	108.3 ± 20.1	107.7 ± 26.1	NS
Ineffective	126.1 ± 41.6	181.2 ± 59.8*	160.9 ± 40.2*	0.02
PPG2 h (mg/dL)				
Effective	165.3 ± 56.8	133.2 ± 24.2*	135.9 ± 24.3*	0.002
Ineffective	195.1 ± 68.0	210.7 ± 71.4	180.7 ± 42.9	NS
Bodyweight (kg)				
Effective	65.9 ± 16.0	64.1 ± 15.5*	64.1 ± 16.0*	0.01
Ineffective	66.3 ± 14.2	64.1 ± 14.1	66.2 ± 15.3	NS

Data are expressed as mean ± standard deviation. *$P < 0.05$ by Tukey's HSD test versus baseline. FPG, fasting plasma glucose; NS, not significant; PPG2 h, 2-h postprandial glucose on self-monitoring of blood glucose, RM-ANOVA, repeated measures analysis of variance.

Table 3 | Univariate and multivariate logistic regression analysis of indices of β-cell function and successful liraglutide introduction

	OR (95% CI) univariate	*P*-value	OR (95% CI) multivariate	*P*-value
CPR0	2.35 (1.17–5.58)	0.03	2.41 (0.96–6.88)	NS
CPR6	1.68 (1.20–2.59)	<0.001	1.54 (1.02–2.54)	NS
CPI	7.63 (2.50–30.45)	0.001	9.60 (2.25–60.8)	0.006
S-CPI	3.42 (1.79–7.61)	<0.001	3.38 (1.48–9.30)	0.002
U-CPR	1.00 (0.99–1.01)	NS	1.01 (0.99–1.02)	NS

Multivariate logistic regression analyses were adjusted for age, sex, body mass index, duration of diabetes and baseline glycated hemoglobin levels. Odds ratio (OR) for each variable expressed per one unit change in indices of β-cell function. CI, confidence interval; CPI, C-peptide index; CPR0, fasting C-peptide; CPR6, C-peptide 6 min after glucagon stimulation; NS, not significant; S-CPI, stimulated C-peptide index; U-CPR, urine C-peptide.

and 1.92 for S-CPI (Table 4). The cut-off values of S-CPI showed a slightly higher area under the ROC curve than that of CPI, but the difference was not statistically significant ($P = 0.85$; Figure 1).

DISCUSSION

The present study showed that the preservation of β-cell function was required for effective liraglutide introduction in patients with type 2 diabetes. Two indices of β-cell function, CPI and S-CPI, were useful for predicting the effectiveness of liraglutide after adjustments were made for age, sex, BMI,

Table 4 | Cut-off values of indices of β-cell function for predicting effective liraglutide introduction in patients with type 2 diabetes

	Cut-off value	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV	LR(+)	LR(–)
CPI	0.72	0.75 (0.62–0.84)	0.81	0.61	0.64	0.78	2.06	0.32
S-CPI	1.92	0.76 (0.63–0.85)	0.53	0.88	0.79	0.68	4.33	0.54

AUC, area under the receiver operating characteristic curve; CI, confidence interval; CPI, C-peptide index; LR(+), positive likelihood ratio; LR(–), negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; S-CPI, stimulated C-peptide index.

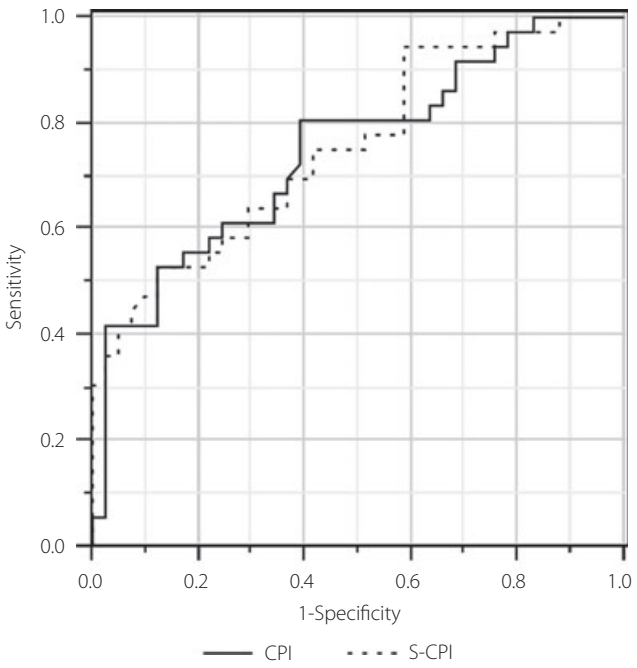

Figure 1 | The receiver operating characteristic curve analysis of indices of β-cell function for predicting effective liraglutide introduction in patients with type 2 diabetes. The stimulated C-peptide index showed a slightly higher area under the curve of 0.76 compared with a C-peptide index of 0.75, but the difference was not statistically significant ($P = 0.85$).

duration of diabetes and baseline HbA_{1c} levels. S-CPI presented the best cut-off value for predicting the effectiveness of liraglutide by having higher area under the receiver operating curve, specificity and positive predictive value. CPI showed high sensitivity for predicting liraglutide effectiveness, but its specificity was low. Thus, we recommend CPI as a screening test and S-CPI for definitive testing of the potential for effective liraglutide introduction. These cut-off values for the prediction of liraglutide effectiveness would enable safe individualization of liraglutide therapy in patients with type 2 diabetes. Liraglutide produces a glucose-lowering effect, mainly through glucose-dependent stimulation of insulin secretion from pancreatic β-cells and glucagon suppression. Decrease in β-cell function is often observed in Asian patients with type 2 diabetes, including Japanese patients[12]. Thus, the pre-evaluation of β-cell function before changing a patient's therapy provides valuable clues for choosing the appropriate option for patients with type 2 diabetes. We chose the glucagon stimulation test to assess β-cell function, because it is widely used to assess endogenous insulin secretion for clinical purposes, provides good intrasubject reproducibility and can be easily carried out in the clinical setting[13]. In addition, the glucagon stimulation test has the advantage of a much faster action on β-cells than the 75-g oral glucose tolerance test (6 min vs 120 min), allowing a marked reduction in the duration to assess β-cell function. We used CPI and S-CPI as indices of β-cell function, because the C-peptide-to-glucose ratio is predictive of preserved β-cell area[14], and recent studies have reported that a decrease in the C-peptide-to-glucose ratio indicated patients with type 2 diabetes who required insulin therapy[15]. Because type 2 diabetes is a progressive disease, β-cell volume[16] and function[17,18] decrease with disease duration. In the present study, the univariate regression model showed that both CPI and S-CPI were inversely associated with the duration of type 2 diabetes (CPI: $r = -0.02$, $P = 0.03$, S-CPI: $r = -0.06$, $P = 0.01$). Therefore, both CPI and S-CPI might be indicators representative of residual β-cell mass, and might vary consistently with the duration of type 2 diabetes. We also assessed the correlation between FPG levels and duration of type 2 diabetes. In univariate regression model analysis, FPG showed a positive trend with duration, but this trend was not statistically significant ($r = 0.20$, $P = 0.08$). Shorter duration of type 2 diabetes was also a good predictor of effective liraglutide introduction (OR 0.91; 95% CI 0.85–0.98). In the present study, insulin resistance assessed by HOMA2-IR (OR 1.25; 95% CI 0.98–1.70), BMI (OR 0.98; 95% CI 0.89–1.07) and waist circumference (OR 0.99; 95% CI 0.95–1.02) were not predictive factors for effective liraglutide introduction. This suggests that effective liraglutide treatment depends not on insulin resistance, but on the insulin-secreting function of β-cells. The present study showed that in patients, who could continue to use liraglutide over 12 weeks, BW decreased in both liraglutide-effective and liraglutide-ineffective cases, but the decrease did not show any statistically significant differences between these cases. This suggests that the BW reduction effect of liraglutide was independent of glycemic control. There were some limitations in the present study. First, because the present study was carried out as a retrospective cohort study, the duration of insulin therapy before liraglutide introduction was not controlled. However, because baseline HbA_{1c} and FPG levels were well controlled with insulin therapy before liraglutide introduction, the presence

of glucose toxicity and transient β-cell dysfunction were not suspected. Second, as the maximum allowable liraglutide dose in Japan is 0.9 mg, the cut-off value of CPI and S-CPI might be lower at a higher maximum liraglutide dose. However, our criteria provide effective liraglutide action even at low doses. Third, in our analyses, the baseline BMI values in the present study participants were relatively low compared with those in type 2 diabetes patients in Western countries. However, because the effectiveness of liraglutide did not depend on BMI or insulin resistance, our criteria for liraglutide introduction could be valuable for patients with higher BMI and insulin resistance. Previous reports have shown the effectiveness of liraglutide on the basis of β-cell function assessment by glucose tolerance tests; however, these data were obtained from smaller groups of subjects and for a shorter duration of treatment[19]. To the best of our knowledge, criteria for liraglutide introduction for a duration of 24 weeks using the glucagon stimulation test in patients with type 2 diabetes has not been reported yet. Our data provide effective individualization of liraglutide therapy. If patients were selected before liraglutide introduction on the basis of our criteria, after switching to liraglutide monotherapy from insulin therapy, HbA_{1c} level would decrease by $-0.6 \pm 1.3\%$ ($P = 0.009$) from baseline without severe hyperglycemia and BW gain. In conclusion, we recommend using CPI = 0.72 as a screening test, and S-CPI = 1.92 as a definitive test for safe and effective liraglutide introduction in patients with type 2 diabetes.

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We do not have any potential conflicts of interest.

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