

Impaired insulin secretion predicting unstable glycemic variability and time below range in type 2 diabetes patients regardless of glycated hemoglobin or diabetes treatment

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Keywords

Glucose variability, Hypoglycemia, Insulin secretion

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ABSTRACT

Aims/Introduction: To identify the coefficient of variation (CV) threshold for unstable glucose variability (GV) and hypoglycemia, and to characterize a patient population with unstable GV and hypoglycemia.

Materials and Methods: This was an observational study that enrolled 284 Japanese outpatients with type 2 diabetes who underwent continuous glucose monitoring. The C-peptide index (CPI = [(fasting serum C-peptide) / (plasma glucose)] × 100) was used as a marker of endogenous insulin secretion. The CV threshold between stable and unstable GV was defined as the upper limit of the CV distribution in the subgroup of patients who did not receive insulin nor insulin secretagogues (relatively stable GV subgroup, $n = 104$). The optimal CV range corresponding to time below target range $\geq 4\%$ was determined for all patients using receiver operating characteristic curve analysis. Various characteristics of patients with unstable GV and hypoglycemia were extracted using multivariate logistic regression analysis.

Results: The upper limit of the CV in the relatively stable GV subgroup was 40. The optimal CV range corresponding to time below target range $\geq 4\%$ was also defined as CV ≥ 40 (area under the curve 0.85) for all patients. The CPI was an independent risk for CV ≥ 40 (odds ratio 0.17, 95% confidence interval 0.04–0.50, $P < 0.01$). The optimal cut-off point for CPI to predict a CV cut-off value of 40 was equivalent to 0.81 (area under the curve 0.80).

Conclusions: A CV of 40 discriminates unstable GV and hypoglycemia from stable GV in Japanese outpatients with type 2 diabetes. Impaired insulin secretion might affect the stability of GV.

INTRODUCTION

Poor glycemic control with unstable glucose variability (GV) leads to the onset and progression of diabetes-related microvascular and macrovascular complications^{1,2}. Hypoglycemia is associated with a mortality risk in diabetes patients complicated by acute coronary events³. Glycemic control has been assessed in a number of ways other than glycated hemoglobin (HbA1c),

which is one of the most established markers for assessing the months-long changes in average glucose. Continuous glucose monitoring (CGM) tracks dynamic glucose levels of interstitial fluid in subcutaneous fatty tissue throughout the day. CGM allows measurement of the amplitude and the timing of GV, shown as the risk of hypoglycemia and hyperglycemia, on the different timescales from HbA1c levels^{4,5,6}. Numerous CGM-based metrics and their thresholds for GV were introduced with the expansion of CGM into clinical practice^{6,7,8}.

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International consensus recommends a coefficient of variation (CV) as the primary measure of GV⁹. Stable GVs are defined as a CV <36%, and CV ≥36% for unstable GVs¹⁰. However, this threshold was established based on the study in Western outpatients with diabetes. It remains possible that the thresholds for CGM-based metrics of GV in Japanese outpatients with type 2 diabetes differ from the international consensus. In addition, patient characteristics that might affect unstable GV and hypoglycemia are currently unknown.

A decline in endogenous insulin secretion has been observed over many decades in diabetes patients¹¹. Because impaired insulin secretion plays a key role in the development of type 2 diabetes, there remains a possibility that impaired insulin secretion is associated with poor glycemic control and unstable GV. In addition, insulin secretion capacity in Japanese patients with type 2 diabetes is, in general, less than that in Western patients¹². We have hypothesized that the assessment of GV in Japanese patients with type 2 diabetes is different from that in Western patients due to the extensive impaired insulin secretion in Asian patients.

The present study aimed to identify the CV threshold for unstable GV and hypoglycemia, and to characterize a patient population with unstable GV and hypoglycemia in Japanese outpatients with type 2 diabetes using CGM.

METHODS

Study population and design

The present study was an observational study. The data were prospectively analyzed. Japanese outpatients with type 2 diabetes were recruited from April 2018 to September 2019 at the following four medical institutions: Hokkaido University Hospital (Sapporo, Japan), NTT Sapporo Medical Center (Sapporo, Japan), Kushiro Red Cross Hospital (Kushiro, Japan) and Tomakomai City Hospital (Tomakomai, Japan). In this study, patients, aged ≥20 years were eligible if they consented to undergo ambulatory CGM regardless of HbA1c levels, sex, duration of diabetes or complications from diabetes. Patients with the following conditions were excluded: (i) type 1 diabetes; (ii) in hospital within the past 3 months; (iii) diabetic ketosis/coma; (iv) serious infection; (v) pre- and post-operation; (vi) trauma within the past 6 months; (vii) receiving steroid therapy; (viii) having difficulty with dietary intake; or (ix) pregnant or lactating. A total of 284 patients were enrolled into this study. Patients provided CGM data, fasting blood samples and clinical information (age, sex, anthropometric measurements, duration of diabetes in years, treatment regimen and medical history).

The study was registered with the University Hospital Medical Information Network Center registration number UMIN000029993. The study protocol was approved by the institutional review board at Hokkaido University Hospital Clinical Research and Medical Innovation Center (017-0147), and it was carried out in accordance with the Declaration of Helsinki. Signed informed consent was obtained from all the patients.

Biochemical analyses and data collection

For this study, blood samples were collected after an overnight fast to measure levels of plasma glucose, C-peptide, HbA1c and estimated glomerular filtration rate (eGFR). These parameters were measured using standard techniques. C-peptide index (CPI) was calculated using the following formula: $100 \times \text{fasting C-peptide (ng/mL)}/\text{plasma glucose (mg/dL)}$. It was used to represent endogenous insulin secretion¹³. The weight and height of the patients were measured using a calibrated scale. The body mass index was calculated as the weight (in kg) divided by height (in m²). Other data including age, sex, diabetes medications and medical history were collected using a questionnaire administered by the attending physicians.

CGM

All patients underwent ambulatory CGM for 14 consecutive days using the same technology from 2018 to 2019 (i.e., FreeStyle Libre Pro sensor; Abbott Diabetes Care, Alameda, CA, USA). We excluded data on the first and last days of wearing the device from the analysis due to the possible CGM system inaccuracy when it was attached and detached¹⁴. We analyzed CGM data for all other available periods. Various metrics for GV were calculated using GlyCulator2 software (Medical University of Lodz, Lodz, Poland)¹⁵. The following parameters were analyzed as the metrics for GV: standard deviation (SD; SD around the mean glucose value), CV ($100 \times [\text{SD of glucose}] / [\text{mean glucose}]$), mean amplitude of glycemic excursions (MAGE)¹⁶ and mean glucose. We also calculated the three key CGM measurements, including the percentage of readings and time per day within the target glucose range (3.9–10.0 mmol/L), time below target glucose range (TBR; <3.9 mmol/L) and time above target glucose range (>10.0 mmol/L), which international consensus recommended¹⁷.

Statistical analysis

To identify the CV threshold for separating stable from unstable GV, we extracted three subgroups. Among the 284 patients, 17 patients were treated with diet and/or insulin sensitizers (i.e., metformin and/or glitazones) alone. They were selected as a subgroup with stable GV, called the SGV subgroup, as previously reported¹⁰. In fact, these patients have a very low or no risk of hypoglycemic episodes¹⁸. Besides the SGV subgroup, 104 patients without insulin secretagogues (i.e., insulin and/or sulfonylurea or glinides) were selected to serve as a subgroup for relatively stable GV, called the RSGV subgroup. Patients who were excluded from the RSGV subgroup were classified as the unstable GV subgroup, after which biochemical and anthropometric characteristics were compared among the three subgroups using one-way analysis of variance, the χ^2 -test or the Kruskal–Wallis test, as appropriate. Because patients in the SGV and RSGV subgroups were used as references for stable GV, as aforementioned, the upper limit of CV distribution in both SGV and RSGV subgroups was called the CV threshold

between stable and unstable GV. We also determined the optimal cut-off point for CV corresponding to TBR $\geq 4\%$, according to the international consensus recommended as a key CGM measure of hypoglycemia¹⁷, for all patients using receiver operating characteristic (ROC) curve analysis. The log likelihood of the logistic regression analysis was carried out to examine the effects of CGM measures. Furthermore, we characterized a patient population with unstable GV and hypoglycemia after all the patients were divided into two additional groups based on whether their CVs were above or below the CV cut-off value. We used the Mann–Whitney *U*-test to compare means for continuous variables (such as age), and Fisher's exact test to compare proportions for categorical variables (such as sex) between the groups. The results are shown as the median (interquartile range) for positively skewed variables. Significant variables with $P < 0.05$ in the univariate analysis were included in multivariate logistic regression analysis. ROC curve analysis was used again to define the CPI cut-off value that corresponded to the CV cut-off value that showed unstable GV and hypoglycemia. All tests were two-sided, and $P < 0.05$ was considered to represent statistical significance. Statistical analyses were carried out using JMP Pro 14.0.0 (SAS Inc., Cary, NC, USA).

RESULTS

Participant characteristics

Among the 311 patients enrolled in the present study, 15 were excluded because of unexpected disruptions in the CGM. Two patients were excluded, as they withdrew consent before undergoing CGM, and four due to the treatment interruption before analyzing the CGM data. Because of skin irritation under the sensor, one patient prematurely discontinued CGM. One patient who had missing blood samples and another patient who stopped CGM for unrelated inpatient treatment were excluded. Three patients who started unrelated steroid therapy before analyzing CGM data were excluded. The remaining 284 patients (123 women) were considered to be eligible and were included in the subsequent analyses (Figure 1).

Distribution of CV for glucose

The 284 patients were divided into the following three subgroups: SGV ($n = 17$), RSGV ($n = 104$) and unstable GV ($n = 180$). The biochemical and anthropometric characteristics of the full analytical group and each subgroup are shown in Table 1. Age, body mass index, duration of diabetes, and values for HbA1c, CPI and eGFR differed between the three subgroups. CV, SD and MAGE, and key CGM measurements, such as TBR, time per day within the target glucose range and time above target glucose range, also differed between the three subgroups. No significant differences in CV and TBR values were found between the four medical institutions (data not shown). Figure 2 shows the CV distributions overall, and in the SGV and RSGV subgroups. The upper limit of the CV distribution was found to be 40 in both subgroups, and there was no statistically significant difference for CV ($P = 0.81$). As recommended by the EP28-A3C/Clinical and Laboratory Standards Institute¹⁹, we recalculated these data using the 97.5th percentile, which yielded values of 39.1 for CV distribution for SGV and 39.2 for RSGV. These values were very similar to the CV threshold of 40 for both SGV and RSGV. A CV cut-off value of 40 was used as a reference threshold to separate stable from unstable GV.

Relationship between glucose variability and hypoglycemia

We constructed a ROC curve, the area under the ROC curve (AUC) and the 95% confidence interval (CI) to evaluate the effects of CV, SD, MAGE and HbA1c on TBR $\geq 4\%$ as a marker of hypoglycemia. The ROC curve for all patients showed that CV had the best performance for AUC (0.85, 95% CI 0.76–0.91; Figure 3), and the optimal cut-off point of CV in predicting hypoglycemia was 40.0 (sensitivity of 40% and specificity of 99%). The optimal cut-off point was 67.5 mg/dL (AUC 0.60, 95% CI 0.49–0.69) for SD, 169.8 (AUC 0.61, 95% CI 0.50–0.70) for MAGE and 44 mmol/mol (AUC 0.64, 95% CI 0.55–0.73) for HbA1c (Figure 3). The log likelihood of the logistic regression analysis is shown in Table S1. The optimal

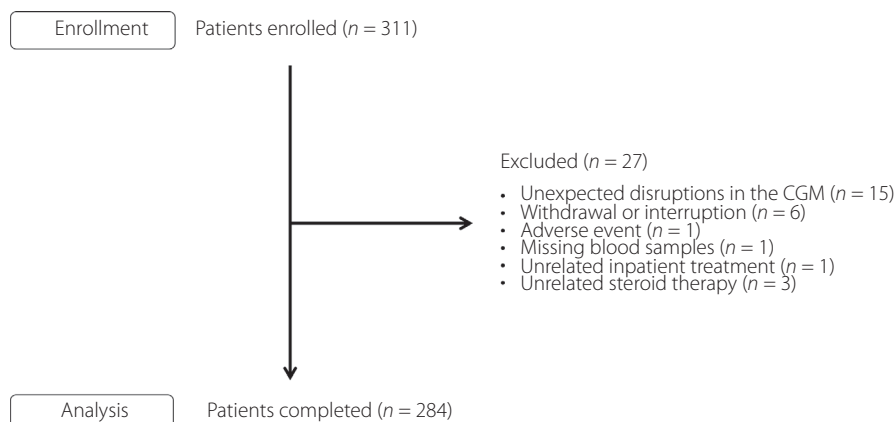


Figure 1 | Flow chart of patients throughout the study. CGM, continuous glucose monitoring.

Table 1 | Patient characteristics overall and in the stable glucose variability, relatively glucose variability and unstable glucose variability subgroup

	Total patients	SGV subgroup	RSGV subgroup	UGV subgroup	P-value
<i>n</i>	284	17	104	180	
Age (years)	68.0 (59.0–76.0)	61.0 (51.0–72.5)	67.0 (56.3–72.8)	69.0 (62.0–78.0)	<0.05
No. women, <i>n</i> (%)	123 (43.3)	11 (64.7)	51 (49.0)	72 (40.0)	0.11
BMI (kg/m ²)	25.0 (22.6–27.9)	22.8 (20.7–23.6)	25.8 (22.6–28.4)	24.7 (22.5–27.6)	<0.05
Diabetes duration (years)	14 (8–22)	4 (1–10)	8 (4–14)	17 (12–24)	<0.05
Diabetes treatment, <i>n</i> (%)					
Any insulin sensitizers	193 (68.0)	11 (64.7)	73 (70.2)	120 (66.7)	0.75
Any insulin secretagogues	180 (63.4)	0 (0)	0 (0)	180 (100.0)	<0.05
FPG (mg/dL)	137.0 (119.3–157.5)	151.0 (118.5–164.0)	142.0 (120.0–156.0)	134.0 (119.0–159.5)	0.73
HbA1c (%)	7.1 (6.7–7.8)	6.8 (6.3–7.2)	7.1 (6.5–7.6)	7.2 (6.8–7.8)	<0.05
HbA1c (mmol/mol)	54 (50–61)	51 (45–55)	54 (48–60)	55 (51–61)	<0.05
CPI (ng/mL per mg/dL)	1.2 (0.9–1.8)	1.0 (0.7–1.3)	1.4 (1.0–2.0)	1.1 (0.7–1.5)	<0.05
eGFR	66.0 (53.3–79.5)	75.0 (65.1–82.0)	69.3 (59.1–82.0)	63.3 (47.5–76.1)	<0.05
24-h mean Glucose (mg/dL)	146.2 (129.0–166.3)	139.2 (125.5–153.0)	142.2 (129.0–163.5)	147.8 (129.4–167.5)	0.24
CV	27.8 (23.7–32.5)	27.1 (21.1–30.0)	25.2 (21.9–28.2)	29.6 (25.4–34.9)	<0.05
SD (mg/dL)	40.3 (33.2–51.4)	34.9 (27.6–47.3)	36.3 (30.4–43.6)	44.0 (35.4–54.1)	<0.05
MAGE	105.4 (87.5–134.0)	87.7 (71.3–117.3)	92.9 (76.8–113.1)	114.1 (93.6–141.2)	<0.05
TBR (%)	0.1 (0–2.1)	0 (0–1.9)	0 (0–0.4)	0.6 (0–3.0)	<0.05
TBR (minutes)	1.2 (0–29.8)	0 (0–27.2)	0 (0–5.2)	8.1 (0–43.5)	<0.05
TBR ≥ 4%, <i>n</i> (%)	44 (15.7)	1 (5.9)	7 (8.1)	37 (20.6)	<0.05
TIR (%)	76.9 (63.7–87.4)	87.8 (75.8–92.4)	83.0 (69.3–90.8)	73.5 (60.0–85.0)	<0.05
TAR (%)	20.2 (10.6–33.8)	12.2 (6.9–23.0)	16.0 (8.5–30.6)	23.7 (12.3–35.0)	<0.05

Values are expressed as the median (interquartile range), or number (%) of patients in each category. BMI, body mass index; CPI, C-peptide index; CV, coefficient of variation; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; MAGE, mean amplitude of glycemic excursions; RSGV, the relatively stable glucose variability subgroup that treated without insulin secretagogues; SD, standard deviation; SGV, the stable glucose variability subgroup that was treated with diet and/or insulin sensitizers alone; TAR, percentage of time above target glucose range; TBR, percentage of time below target glucose range; TIR, percentage of time within target glucose range; UGV, the unstable glucose variability subgroup that excluded the relatively stable glucose variability subgroup from all cases.

CV range corresponding to TBR ≥4% was defined as CV ≥40, which was consistent with the upper limit of the CV distribution of those without insulin secretagogues.

Predictive markers for instability of GV

All patients were divided into two other groups using a CV cut-off value of 40 to characterize the patient population with

unstable GV and hypoglycemia (Table 2). TBR, SD and MAGE in the CV ≥40 group were significantly higher compared with those in CV <40 group. Time per day within the target glucose range in the CV ≥40 group was significantly lower compared with the CV <40 group. However, there were no significant differences in HbA1c (55 vs 54, *P* = 0.63) and time above target glucose range (25.3 vs 19.8, *P* = 0.29). The patients'

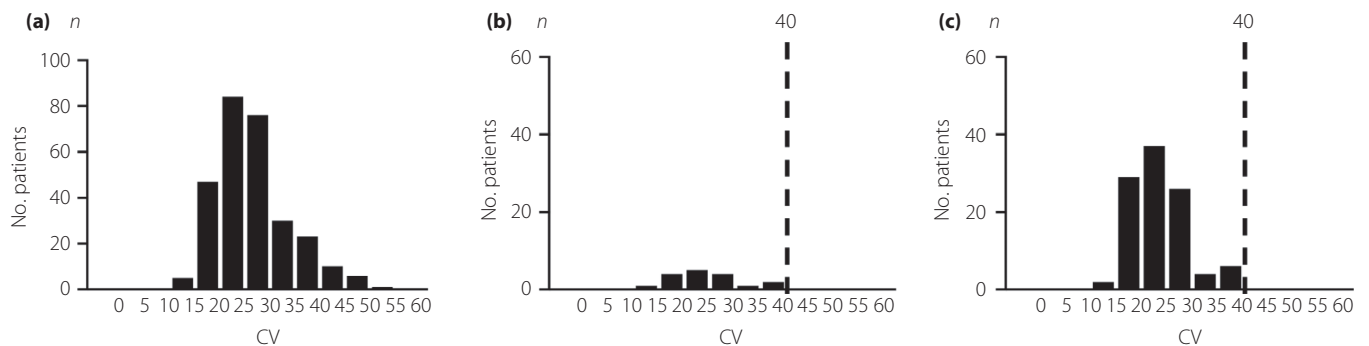


Figure 2 | Coefficient of variation (CV) distributions for glucose (a) overall (284 patients), in (b) the stable glucose variation (SGV) subgroup (diet and/or insulin sensitizers alone, 17 patients), and in (c) the relatively SGV (RSGV) subgroup (no insulin secretagogues, 104 patients). The upper limit of the CV distribution in both SGV and RSGV subgroups was 40.

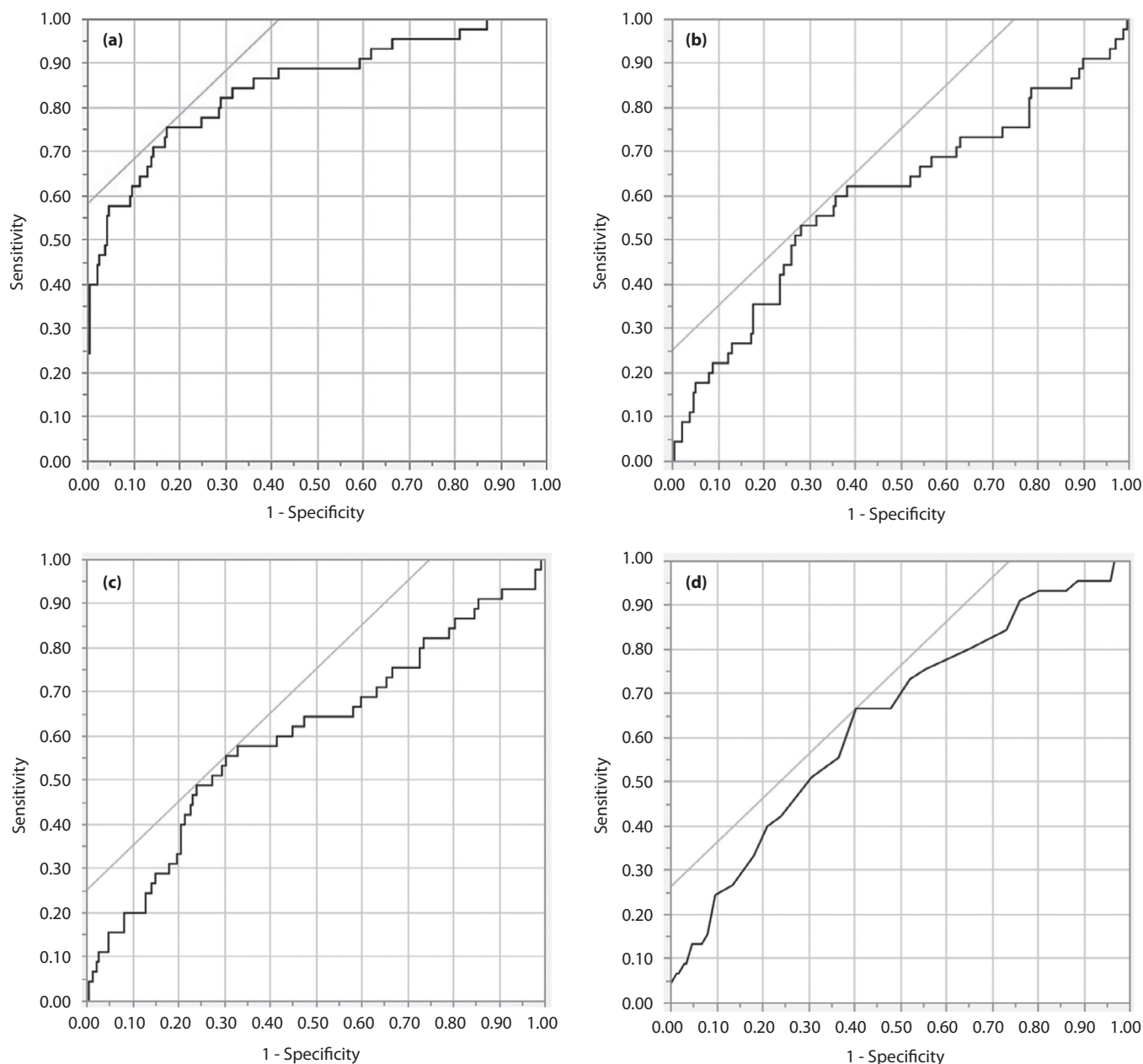


Figure 3 | Comparison of the area under the curve (AUC) for glucose variability metrics in predicting the percentage of time below target glucose range $\geq 4\%$ in continuous glucose monitoring. Receiver operating characteristic curves for predicting the percentage of time below target glucose range $\geq 4\%$ in (a) the coefficient of variation, (b) the standard deviation, (c) the mean amplitude of glycemic excursions and (d) glycated hemoglobin. Coefficient of variation, the cut-off point was 40.0 (area under the curve [AUC] 0.85, 95% confidence interval [CI] 0.76–0.91). Standard deviation, the cut-off point was 67.5 mg/dL (AUC 0.60, 95% CI 0.49–0.69). The mean amplitude of glycemic excursions, the cut-off point was 169.8 (AUC 0.61, 95% CI 0.50–0.70). Glycated hemoglobin, the cut-off point was 44 mmol/mol (AUC 0.64, 95% CI 0.55–0.73).

background in the CV ≥ 40 group showed that age was significantly greater and duration of diabetes was significantly longer compared with those in the CV < 40 group. Body mass index was significantly lower, and the rate of insulin use in the CV ≥ 40 group was significantly higher compared with the CV < 40 group. Fasting blood glucose, CPI and eGFR levels in

the CV ≥ 40 group were significantly lower compared with those in the CV < 40 group. There were no indications of multicollinearity between these variables (Tables S2;S3). Among these items correlated with CV, CPI was an independent predictive marker for CV elevation based on the multivariate logistic regression analysis (odds ratio [OR] 0.17, 95% CI 0.04–0.50,

Table 2 | Patient characteristics between the coefficient of variation ≥ 40 group and coefficient of variation < 40 group

	CV ≥ 40 group	CV < 40 group	P-value
<i>n</i>	20	264	
Age (years)	77.5 (69.5–85.0)	67.0 (59.0–75.0)	< 0.05
No. women, <i>n</i> (%)	9 (45.0)	114 (43.2)	1.00
BMI (kg/m ²)	23.2 (20.3–24.5)	25.2 (22.8–28.3)	< 0.05
Diabetes duration (years)	21 (14–24)	14 (7–21)	< 0.05
Diabetes treatment, <i>n</i> (%)			
Any insulin	18 (90.0)	102 (38.6)	< 0.05
Any sulfonylurea or glinides	7 (35.0)	100 (37.9)	1.00
Any incretin-based drugs	13 (65.0)	221 (83.7)	0.06
Any insulin sensitizers	11 (55.0)	182 (68.9)	0.22
FPG (mg/dL)	123.0 (102.0–145.0)	137.0 (120.2–158.8)	< 0.05
HbA1c (%)	7.2 (6.8–7.9)	7.1 (6.7–7.7)	0.63
HbA1c (mmol/mol)	55 (51, 63)	54 (50, 61)	0.63
CPI (ng/mL per mg/dL)	0.5 (0.2–1.0)	1.2 (0.9–1.8)	< 0.05
eGFR	55.4 (40.4–70.2)	66.0 (54.9–79.9)	< 0.05
24-h mean glucose (mg/dL)	136.9 (119.4–147.8)	147.0 (129.6–166.9)	0.07
CV	44.5 (43.3–49.1)	27.4 (23.3–31.5)	< 0.05
SD (mg/dL)	65.3 (53.5–72.2)	39.2 (32.8–48.9)	< 0.05
MAGE	163.3 (136.8–186.4)	103.1 (84.8–129.1)	< 0.05
TBR (%)	14.0 (7.9–20.5)	0 (0–1.1)	< 0.05
TIR (%)	61.9 (51.9–65.6)	78.5 (65.4–88.0)	< 0.05
TAR (%)	25.3 (15.2–29.1)	19.8 (9.7–33.9)	0.29

Values are expressed as the median (interquartile range) or number (%) of patients in each category. Mann–Whitney *U*-test or Fisher's exact test was used to compare each parameter in the coefficient of variation (CV) ≥ 40 group and CV < 40 group. BMI, body mass index; CPI, C-peptide index; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; MAGE, mean amplitude of glycemic excursions; SD, standard deviation; TAR, percentage of time above target glucose range; TBR, percentage of time below target glucose range; TIR, percentage of time within target glucose range.

Table 3 | Clinical factors of unstable glucose variability including hypoglycemia analyzed by multivariate logistic regression analysis

	Odds ratio	95% CI	P-value
Age (years)	0.99	0.94–1.05	0.83
BMI (kg/m ²)	0.88	0.72–1.05	0.17
Diabetes duration (years)	1.00	0.93–1.06	0.97
Insulin treatment	7.20	1.44–36.11	< 0.05
FPG (mg/dL)	0.99	0.97–1.01	0.39
CPI (ng/mL per mg/dL)	0.17	0.04–0.50	< 0.05
eGFR	0.96	0.92–0.99	< 0.05

95% CI, 95% confidence interval; BMI, body mass index; CPI, C-peptide index; eGFR: estimated glomerular filtration rate; FPG, fasting plasma glucose.

$P < 0.01$). Insulin use and eGFR were also predictive markers for unstable GV in addition to CPI (OR 7.20, 95% CI 1.44–36.11, $P < 0.05$; OR 0.96, 95% CI 0.92–0.99, $P < 0.05$, respectively; Table 3).

The ROC curve showed that the optimal cut-off point of CPI to predict a CV cut-off value of 40 was 0.81 (AUC 0.80, sensitivity 65%, specificity 80%; Figure 4).

Furthermore, patients who received insulin were extracted from the overall study cohort, and the same analyses were

carried out (Tables S4;S5; Figure S1). The results in this subgroup were similar to those of the whole study cohort. Thus, CPI is an independent predictive marker of high CV, and the optimal cut-off point for CPI for predicting a CV of 40 is 0.81 (AUC 0.77, sensitivity 72%, specificity 65%).

DISCUSSION

The present study investigated the CV threshold for unstable GV and TBR $\geq 4\%$ in Japanese outpatients with type 2 diabetes using ambulatory CGM. The results showed that the CV cut-off point for stable GV in Japanese outpatients with type 2 diabetes was 40, this value being higher than the 36 recommended by international consensus⁹. Impaired insulin secretion, which is characteristic of Japanese type 2 diabetes, might be responsible for these results regardless of HbA1c or diabetes treatment.

Previous studies showed that CV was the best GV marker to identify patients with an increased risk of clinically significant hypoglycemia, among patients with type 1 or type 2 diabetes^{9,20}. To assess the validity of CV in Japanese outpatients with type 2 diabetes, we investigated the association between CV and GV in Japanese outpatients with type 2 diabetes regardless of their glycemic control status using a previously reported method¹⁰. Although HbA1c and mean glucose levels

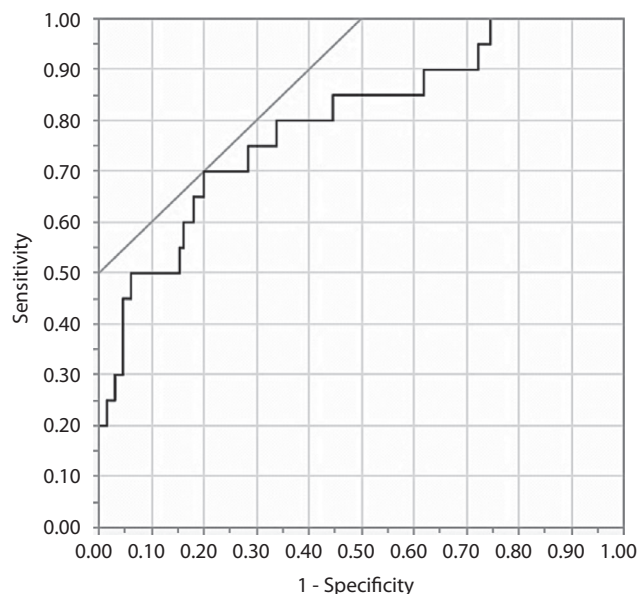


Figure 4 | Receiver operating characteristic curve analysis for identifying the cut-off value of the C-peptide index for predicting a coefficient of variation ≥ 40 in continuous glucose monitoring (area under the curve 0.80).

of patients in the present study were comparable to those of the previous study¹⁰. The upper limit of CV of 40 for stable GV in the present study was unexpectedly higher than the 36 recommended by international consensus⁹. We considered that there is limited evidence to support the use of CV 36 as a threshold in Japanese patients.

In the present study, the CV was more predictive of unstable GV and hypoglycemia compared with SD, MAGE and HbA1c. As shown in previous studies, both SD and MAGE had a bias toward hyperglycemia, because these markers were absolute measures^{4,21}. It has previously been reported that HbA1c does not reflect GV and hypoglycemia⁴.

The CV cut-off point corresponding to the threshold for TBR is currently unknown, although previous studies have shown that increasing the TBR is associated with increases in CV^{22,23}. The present data suggested that the optimal range of the CV corresponding to TBR $\geq 4\%$ was defined as CV ≥ 40 in Japanese outpatients with type 2 diabetes including hypoglycemia unawareness. This result was exactly consistent with the upper limit of the CV distribution. The present results suggested that unstable GV in Japanese outpatients with type 2 diabetes is associated with hypoglycemia.

We hypothesized that impaired insulin secretion is associated with unstable GV and hypoglycemia. To support the hypothesis, we showed that a decreased CPI was significantly associated with unstable GV when stratifying patients into two subgroups based on a CV of 40. In addition, CPI was an independent predictive marker for unstable GV based on multivariate logistic regression analysis.

However, insulin use and a low eGFR were other predictive markers for unstable GV in addition to CPI. Patients treated with insulin are known to have a higher risk of insulin-induced hypoglycemia²⁴. Chronic kidney disease is a risk for hypoglycemia because of altered drug metabolism, malnutrition and impaired renal glucose release²⁵. These findings are in agreement with the present results.

The relationship between impaired insulin secretion and unstable GV was examined in a previous study, but it was carried out only in patients without renal dysfunction²¹. The present multicenter study included patients with moderate renal dysfunction who had undergone CGM for longer days than the previous study. To the best of our knowledge, the present study is the first to show that a decreased CPI was related to both unstable GV and hypoglycemia. A CPI threshold to predict unstable GV and hypoglycemia was 0.81. A CPI of 0.8 was established as the threshold that determines the insulin need for Japanese patients with type 2 diabetes²⁶. Patients with undetectable C-peptide were at the greatest risk for unstable GV and hypoglycemia²⁷, because these patients have impaired counter-regulation in response to hypoglycemia²⁸. The present results suggested that even patients with a slight impairment of insulin secretion have a risk of unstable GV and hypoglycemia.

A CPI of 0.81 could help to identify patients who are at a higher risk of hypoglycemia without using CGM, because the CPI is easy to test using a simple fasting blood examination. This cut-off point would promote medical staff to check hypoglycemia unawareness by using CGM in daily clinical practice.

The present study had several limitations. First, the 284 patients in this study might not reflect the general population. However, we included patients regardless of glycemic control, and patients' heterogeneity in this study represented the Japanese people's general diabetes characteristics²⁹. This patient group might provide useful information about the association between patient characteristics and GV. Second, the findings in this study, including the identified thresholds, need to be validated in a prospective, randomly selected population. Third, the reliability of the CGM measurement quality, particularly in the hypoglycemic range, might be a limitation. However, we minimized these inaccuracies by excluding data from the first and last days of wearing the device. Fourth, the sensitivity of CV to TBR is relatively low, the likelihood ratio being 40. Our finding that a CV cut-off value of 40 predicts hypoglycemia with a high specificity means that this CV cut-off value will help to identify patients who are definitely at higher risk of hypoglycemia. Increasing the sensitivity of CV to TBR would necessitate combining CV with other patient characteristics. A future challenge will be to examine the multiple clinical factors associated with TBR.

In conclusion, a CV of 40 estimated by ambulatory CGM distinguishes GV between stable and unstable, including hypoglycemia, in Japanese outpatients with type 2 diabetes. CPI would help to identify patients at higher risk of hypoglycemia. Impaired insulin secretion, which is a characteristic of Japanese

patients with type 2 diabetes, might be associated with the instability of GV, including hypoglycemia, regardless of HbA1c or diabetes treatment.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1 | Receiver operating characteristic curve in C-peptide index (CPI) to predict coefficient of variation (CV) ≥ 40 in continuous glucose monitoring (CGM) in patients receiving insulin (area under the curve 0.77).

Table S1 | Log likelihood of the logistic regression analysis in continuous glucose monitoring measures.

Table S2 | Correlations in the variables associated with unstable glucose variability including hypoglycemia (Spearman's correlation).

Table S3 | The calculated variance inflation factors.

Table S4 | Characteristics of patients who received insulin between coefficient of variation ≥ 40 group and coefficient of variation < 40 group.

Table S5 | Clinical factors of unstable glucose variability including hypoglycemia in patients who received insulin analyzed by multivariate logistic regression analysis.