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The Logistic Regression and ROC Analysis of Group-based Screening for Predicting Diabetes Incidence in Four Years

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In diabetes screening with hemoglobin A1c in lieu of plasma glucose, the optimum cut-off point for predicting the incidence of diabetes mellitus in the four-year period was examined. In addition, considerations were given on items in the screening and questionnaire aside from hemoglobin A1c, which would be useful in predicting diabetes aside from hemoglobin A1c. The optimum cut-off point of hemoglobin A1c to predict diabetes, based on receiver operating characteristic curve, was 5.3 percent (sensitivity, 84.2%; specificity, 92.1%). Based on the logistic regression analysis, useful items (other than hemoglobin A1c) were alanine aminotransferase and γ-glutamyl transpeptidase. A combined application of hemoglobin A1c with alanine aminotransferase and γ-glutamyl transpeptidase for predicting the incidence of diabetes in the four-year period resulted in the sensitivity of 86.8% and the specificity of 96.3%. When the combined application was compared with the sole use of hemoglobin A1c at 5.3%, the combined use was superior to the latter in terms of both sensitivity and specificity, resulting in the reduction of false positives by more than 50%.

The number of diabetic patients is on the rise throughout the world. According to the report by the World Health Organization (WHO) in 1998, the number of people suffering from diabetes mellitus in 1995 was estimated to be about 135 million, whereas this number is expected to reach 300 million by the year 2025 (6). As the dietary habits of Japanese people have become westernized, the type 2 diabetes has increased in recent years. For instance, a study conducted in 2002 on the status of diabetes revealed that there were 7.4 million people suspected to have the disease, while the figure would reach 16.2 million inclusive of those who have a possibility of having or developing the disease (9). There is no doubt that diabetes mellitus in the future would be an enormous threat to the health of humankind, and the need on the global scale to initiate preventive efforts is imminent.

For diagnosing diabetes, the American Diabetes Association (ADA) recommends the use of fasting plasma glucose (FPG), because of its simplicity and economical aspect (2). The Japan Diabetes Association (JDS) recommends FPG and the additional usage of oral glucose tolerance test (OGTT) (1). Hemoglobin A1c (HbA1c) is influenced by the hemoglobin concentration in the blood; this makes the co-relationship between HbA1c and FPG frail (5).

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Furthermore, despite the production of standardized tests and the improvement in HbA1c's accuracy today (14), controlling its accuracy in the past was delicate and the overlaps between the diabetic and the non-diabetic based on the test results were substantial (7). These demerits are the reasons that the sole use of HbA1c is considered unsuitable for diagnosis.

On the other hand, HbA1c has the advantage over FPG and OGTT of not necessitating fasting and multiple blood draws. These aspects reduce the burden on the individuals who go through medical screening. The Industrial Safety and Health Law for workers allows the usage of HbA1c as a substitute for the blood glucose in screening, on the grounds that there would be many cases of blood glucose measurements without fasting (10). As a result, in group screening, recent years have seen an increase in the use of HbA1c in lieu of blood glucose.

In this study, we have conducted a four-year retrospective cohort study, involving 2,659 non-diabetic subjects who went through group screening. We herein report our considerations not only on the optimum cut-off point for HbA1c, which would successfully infer the future incidence of diabetes, but also on the data obtained from the screening as well as the questionnaire which would be useful in predicting the incidence.

MATERIALS AND METHODS

Subjects: Of the people who took group screening tests conducted by Hyogo Health Service Association during the period between April 1998 and December 2003, 2,818 people who had had their HbA1c and blood glucose measured every year became the subjects of this study. Of the subjects, we excluded 74 subjects who either had a history of diabetes or were in treatment for diabetes. In addition, 16 subjects with the hemoglobin of 9.9g/dl or less, 41 subjects with the HbA1c of 3.7% or less, or 6.5% or more, and 28 subjects with the FPG of 126mg/dl or more at the time of the registration were excluded from the study. Even though the casual plasma glucose of 200mg/dl or more was another criterion for exclusion, no one was in this category. Finally, in total, 2,659 subjects remained. The follow-up period was 4.1 ± 0.3 years (mean ± SD).

Methods of Measurements: For HbA1c measurement, a high performance liquid chromatography (HPLC), which has been standardized according to the JDS criteria and is capable of removing unstable molecules, was utilized. Blood glucose was measured through peak acceleration method for oxygen consumption based on a GOD immobilized O2 electrode. Biochemical measurements were carried out using Hitachi, 7170, while the hematological measurements were done with the use of STKS, Beckman Coulter.

Methods of Analyses: We conducted a logistic regression analysis to find items (other than HbA1c) in the screening that would be useful in predicting the subsequent onset of diabetes mellitus. The tested variables were as follows: age, sex, current drinking, current smoking, family history of diabetes, systolic blood pressure, diastolic blood pressure, body mass index (BMI), HbA1c, asparate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), total cholesterol, triglyceride, HDL cholesterol, uric acid, erythrocyte, hematocrit, hemoglobin, and leukocyte. For statistical analyses, Statistical Package for Social Sciences (SPSS) Version 12.0 was utilized. The steps of the logistic regression analysis, utilizing block entry of variables, are specified below.

1. For explanatory variables, each variable from the screening was selected individually and was entered as one of the independent variables in conjunction with age and HbA1c.

2. Using stepwise selection, subsequently, all variables found significant in the first step were entered together with age and HbA1c, so as to find statistically significant variables for predicting diabetes that are also independent of other variables.
3. The variables that were found statistically significant in step 2 were simultaneously used as independent variables, and only the ones that showed statistical significance were determined as useful in predicting the incidence of diabetes mellitus.
4. Using only the variables found to be useful in step 3, the function of logistic regression was calculated.

In each step, the dependent variable was the incidence of diabetes during the follow-up period. Utilizing the function of logistic regression, probability plot (PP) value was sought for estimating the incidence of diabetes among the entire subjects during the follow-up period.

Using the results of the above, we constructed receiver-operating characteristic (ROC) curves and calculated the sensitivities and specificities of HbA1c as well as PP-value, while also taking into account any difference from the results that would have been obtained if the Gold Standard by JDS had been applied. In order to compare the two, the areas under curves (AUCs) and 95% confidence interval (CI) were calculated. In addition, we sought optimum cut-off point for predicting diabetes during the follow-up period. The level of significance (p-value) was set at 0.05. The optimum cut-off point was defined as the closest point on the ROC curve to the point (0, 1) i.e., false positive rate of zero and sensitivity of 100%. In order to find out the distribution of HbA1c values at the time of registration and the corresponding PP-values, a scatter-plot was constructed. In addition, we categorized the subjects into two groups - one group of subjects who developed diabetes during the follow-up period, and the other group of subjects who did not - and examined the frequency distribution of the two groups.

RESULTS

During the follow-up period, averaging 4.1 years, a total of 38 subjects (1.4 %) developed diabetes. Their baseline characteristics are given in Table 1.

Logistic regression analysis: Corresponding with 1 through 4 of the methods of analyses in the above, the following results 1 - 4 were obtained.

1. Of the ones used at the time of registration, the useful screening items for predicting the incidence of diabetes that are independent of age and HbA1c were current drinking, current smoking, AST, ALT, γ-GTP, triglyceride, HDL cholesterol, hematocrit, hemoglobin, systolic blood pressure, and BMI.
2. Of the items that were found significant in the first step, the variables that were once again determined significant when entered together with age and HbA1c in the second step were HbA1c, ALT, γ-GTP, and HDL cholesterol.
3. When the four items found significant in the second step were entered together, the significance probability of HDL cholesterol was found to be 0.054. Since its p-value exceeded 0.05, we deleted this variable from the variables of significance. As a result, the variables that remained significant in this step were HbA1c, ALT, and γ-GTP.

4. The result of the logistic regression analysis with the utilization of the three remaining variables in step 3 is shown in Table 2.

The functional formula for predicting the incidence of diabetes, i.e. PP-value, came out as follows:

$$PP = 1 / (1 + 1/ \exp (HbA1c \times 5.175 + ALT \times 0.013 + \gamma\text{-GTP} \times 0.006 - 31.698)).$$

TABLE 1. Clinical characteristics of subjects at baseline

	Mean		SD
Age (years)	42.2	±	11.2
Plasma glucose (mg/dl)	95	±	12
HbA1c (%)	4.8	±	0.3
Systolic blood pressure (mmHg)	119	±	15
Diastolic blood pressure (mmHg)	74	±	11
BMI (kg/m^2)	22.8	±	3.2
AST (IU/l)	22	±	9
ALT (IU/l)	25	±	20
γ-GTP (IU/l)	41	±	48
Total cholesterol (mg/dl)	198	±	34
Triglyceride (mg/dl)	113	±	90
HDL cholesterol (mg/dl)	63	±	16
Uric acid (mg/dl)	5.4	±	1.5
Erythrocyte (×10^4/μl)	454	±	43
Hematocrit (%)	42.2	±	4.1
Hemoglobin (g/dl)	14.5	±	1.4
Leukocyte (×10^2/μl)	60	±	16
Fasting (yes/no)	2,042 / 617		
Sex (male/female)	1,720 / 939		
Family history of diabetes (yes/no)	324 / 2,335		
Current smoking (yes/no/unknown)	1,001 / 1,624 / 34		
Currsent drinking (yes/no/unknown)	1,767 / 862 / 30		

There were 2,659 subjects. A follow-up period was 4.1 ± 0.3 years.

TABLE 2. Logistic regression analysis

	Coefficient (β)	p-value	Odds Ratio	95% CI
HbA1c	5.175	< 0.001*	176.769	(62.174 , 502.575)
ALT	0.013	0.001*	1.013	(1.005 , 1.021)
γ- GTP	0.006	0.016*	1.006	(1.001 , 1.010)
Constant	-31.698	< 0.001*		

A logistic regression analysis was conducted in order to find items, other than HbA1c, that would be useful in predicting the incidence of diabetes. The table shows the end results of the analysis.
*P < 0.05

ROC curves and the AUCs for HbA1c and PP: In Figure 1, ROC curves for HbA1c and the estimated incidence, PP, are displayed. The AUCs were 0.933 for HbA1c (95% CI; 0.885 - 0.981) and 0.945 (0.904 - 0.987) for PP (Table 3). There is overlap between two AUCs of 95% CIs, and no statistically significant difference was seen. The optimum cut-off points were 5.3% for HbA1c, and 0.0435 for PP.

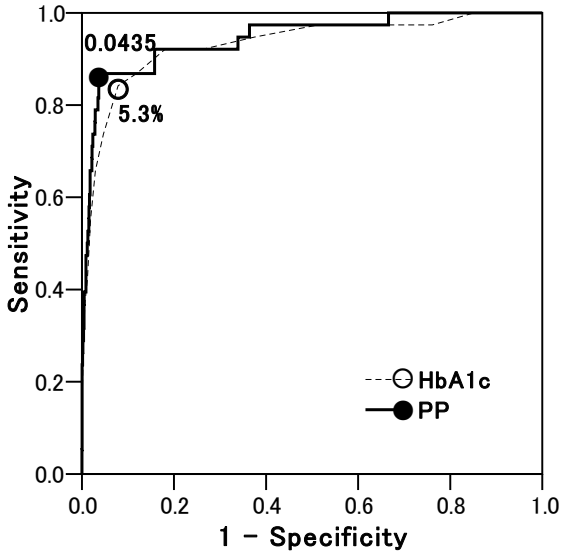

Figure 1. ROC curves of HbA1c and PP-value screening for predicting the incidence of diabetes. The optimum cut-off point was defined as the closest point on the ROC curve to the point (X, Y) = (0, 1), where X = 1 - specificity and Y = sensitivity. The points o and ● denote optimum cut-off points for HbA1c and PP-value, respectively. The PP (probability plot) is a value for estimating the incidence of diabetes among the entire subjects during the follow-up period through the use of the logistic regression function calculated from HbA1c, ALT, and γ-GTP.

TABLE 3. Area under the ROC curve (AUC)

	AUC	95% CI
HbA1c	0.933	(0.885 , 0.981)
PP	0.945	(0.904 , 0.987)

When ROC curves were constructed for Figure 1, the areas under the curves (AUCs), as well as 95% CI, were calculated.

Sensitivities and specificities of HbA1c and PP: The sensitivities as well as specificities of the following cut-off points for diabetes incidence are shown in Table 4: 5.3% in HbA1c, 5.9% in HbA1c (JDS standard), and 0.0435 in PP. Using 5.3% in HbA1c, the number of false negative cases would be only 6 out of 38 subjects who develop diabetes, although the number of false positive cases would reach 206. On the other hand, using the standard value of JDS would result in 28 false negative cases (73.7%) of the total of 38 subjects who developed diabetes during the follow-up period, although its number of false positive cases was only 8. Using PP, it was found that it maintained the number of false negatives as low as 5, while it could also reduce the number of false positive cases to 97 subjects.

TABLE 4. The number of subjects screened by different test criteria

Cut-off point		Number of true positives	Number of false positives	Number of false negatives	Number of true negatives	Sensitivity	Specificity
HbA1c	5.3%	32	206	6	2,415	84.2%	92.1%
	5.9%	10	8	28	2,613	26.3%	99.7%
PP	0.0435	33	97	5	2,524	86.8%	96.3%

Sensitivities and specificities of HbA1c at 5.3%, 5.9% (JDS standard), and PP at 0.0435 as cut-off points for predicting the incidence of diabetes during the follow-up period are shown.

Distribution of PP-values: The frequency distribution for the PP is shown in Table 5, as well as the scatter-plot for the PP-values in Figure 2. According to Table 5, among the subjects who developed diabetes, 5 subjects had PP-values of less than 0.0435; the counterpart figure for those who did not develop the disease was 2,524 subjects. In other words, there were 2,529 subjects in total who had PP-values lower than 0.0435, and this constituted 95.1% of the entire subject group. As Figure 2 shows, PP-values in the range of 5.5 - 6.0% in HbA1c exhibit a sharp increase.

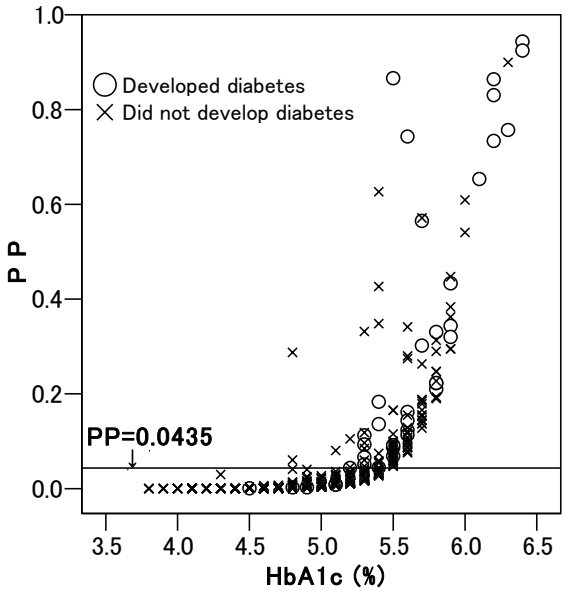

Figure 2. Distribution of HbA1c values at the time of the registration and their corresponding PP-values. Each ○ represents a subject who developed diabetes, while each × shows a non-diabetic subject.

TABLE 5. Frequency distribution of PP-values

PP-values	Developed diabetes		Did not develop diabetes	
	(n)	Accumulation rate	(n)	Accumulation rate
0 - 0.0435	5	13.2 %	2,524	96.3 %
0.0435 - 0.1	8	34.2 %	47	98.1 %
0.1 - 0.2	7	52.6 %	27	99.1 %
0.2 - 0.3	3	60.5 %	10	99.5 %
0.3 - 0.4	4	71.1 %	6	99.7 %
0.4 - 0.5	1	73.7 %	2	99.8 %
0.5 - 0.6	1	76.3 %	2	99.9 %
0.6 - 0.7	1	78.9 %	2	100.0 %
0.7 - 0.8	3	86.8 %	0	100.0 %
0.8 - 0.9	3	94.7 %	1	100.0 %
0.9 - 1.0	2	100.0 %	0	100.0 %
Total	38		2,621	

The distribution of each PP-value is shown, for categorizing the subjects into two groups – those who developed diabetes and those who did not - during the follow-up period.

DISCUSSION

The result of our study indicated the optimum cut-off point for HbA1c as 5.3%, which is lower than the currently existing JDS's cut-off point of 5.9%. Nevertheless, the difference requires careful considerations. The JDS standard has been calculated using the distribution of HbA1c values of healthy individuals (12, 13), and is intended to be used to screen currently-existing diabetes among the subjects. The usefulness of the JDS standard in clinical settings for that particular purpose has been acknowledged by many researchers. In contrast, the value calculated in this study utilizes the current distribution of HbA1c values in order to predict the incidence of diabetes over the four-year period. Moreover, the calculation employed by JDS uses the mean value (±1.96SD), and its analytical method differs from that of this study as described subsequently. In other words, the purposes, as well as analytical methods, differ between the two studies, and, hence, they are distinctive indicators, which cannot easily be compared.

Furthermore, the difference between the characteristics of the two subject groups might have contributed to the differences in cut-off points. Of the 725 JDS subjects, males constituted 380 (52.4%), whereas females comprised 345 (47.6%). The mean age was 53.1±13.5 years old. On the other hand, the subjects in this study totaled 2,659, of whom 1,720 (64.7%) were men and 939 (35.3%) were women. The mean age of the subjects was 42.2±11.2 years old. It can be observed that the subjects in this study, in overall, were younger than the JDS subjects by more than 10 years, and, in addition, a higher percentage of the subjects were composed of male subjects.

Hence, as diabetes progresses longitudinally, one's HbA1c value tends to be higher. Given this tendency, it would be possible to interpret that the distribution of HbA1c values in

this study is intended to be used among subjects of prediabetic conditions, whereas that of JDS is to screen currently-existing diabetes.

When the subjects of this study at the time of the registration were randomly selected and subsequently matched by age and sex with those of the JDS study, the mean HbA1c value± 1.96SD turned out to be 4.2 - 5.6 %, which resembled JDS values (N=690; 377 male subjects (54.6%); 313 female subjects (45.4%); mean age=50.8±12.0).

In this retrospective study, we constructed the ROC curves, while the optimum cut-off point was defined as the closest point on the ROC curves to the point at (X, Y) = (0, 1). There is a possibility that the presence or absence of information on cohort groups caused the difference in cut-off points, either 5.3% or 5.9%. By means of ROC curves in cohort studies, Gomyo et al. reported that the optimum cut-off points of HbA1c in screening for impaired glucose tolerance (IGT) and for IGT plus diabetes mellitus were 5.3% (3). Our estimate for the optimum cut-off point to predict the incidence of diabetes in the four-year period is also 5.3% and approximates the value indicated by Gomyo et al. If we had applied the JDS's cut-off point of 5.9% to predicting the incidence of diabetes mellitus in the four-year period, the sensitivity would have been 26.3%, a remarkably low value posing a serious problem in the screening. In this scenario, 73.7% of the subjects who would develop diabetes would have been considered healthy and no advice on improving health-related lifestyles would have been offered to them. From a perspective of preventive medicine, this would in no way be a favorable situation, and may indicate a need for reconsidering the JDS's cut-off point of 5.9% from multiple angles in order to maintain the validity of screening.

Aside from HbA1c, the useful items in screening for predicting the incidence of diabetes were ALT and γ-GTP, both of which are indicators for liver functions. The liver plays a significant role in homeostasis of blood glucose through intake and release of glucose. In consequence, it is known that liver dysfunctions are, with a high probability, concomitant with IGT. The relationship between diabetes mellitus and liver-related factors has already been discussed in some studies (8, 11). In this study, we utilized ALT, γ-GTP, and HbA1c as well as PP-value obtained from the logistic regression analysis to estimate predicted incidence. An ROC curve depicting PP-value was constructed. From the curve, 0.0435, the optimum cut-off point of PP-value, was estimated. One of the reasons for this cut-off point to be so low is that the majority (96.3% or 2,524 subjects) of the non-diabetic group composed of 2,621 subjects during the follow-up period had a PP-value of less than 0.0435. Comparing this cut-off point with the sole use of HbA1c at 5.3% (sensitivity 84.2%, specificity 92.1%), it was observed that not only the sensitivity improved but also the false positive cases decreased from 206 to 97, a reduction of more than 50%. Since sensitivity and specificity are in a trade-off relationship, it is impossible to simultaneously raise both through shifting the optimum cut-off point in HbA1c alone. Nevertheless, the use of HbA1c with other items in the screening, such as ALT and γ-GTP, will enhance both sensitivity and specificity. This is significant in terms of improving the validity of screening.

Macrovascular diseases, associated with diabetes, tend to occur before microangiopathy. In effect, there is a report that suggests the tendency for macrovascular diseases to occur preceding the diabetic status as defined by the blood glucose (4). Even though lowering the cut-off point would increase the number of false positive cases, prioritizing only specificity would result in a substantial number of false negative cases, particularly in Japan, wherein diabetes is escalating. Given the limited resources for medicine and health care, the cut-off point needs to be set, while giving considerations on reduction of cost,

maintaining/improving QOL, and prevention of the disease. In this study, however, the longitudinal economic aspects as well as facets related to QOL are not investigated, and these areas call for future studies.

From the above, it was suggested that the use of HbA1c alone in predicting the incidence of diabetes during a four-year period requires a cut-off point of 5.3%. In addition, it was confirmed that using other screening items such as ALT and γ-GTP would raise sensitivity as well as specificity, and could assist preventive efforts on diabetes mellitus.

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