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Original Articles

Improvement in Japanese Clinical Laboratory Measurements of Total Cholesterol and HDL-cholesterol by the US Cholesterol Reference Method Laboratory Network

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Background: Accurate and precise measurements of total cholesterol (TC) and HDL-cholesterol (HDL-C) are necessary for effective diagnosis and treatment of lipid disorders. We studied the impact of TC certification and HDL-C evaluation in Japanese clinical laboratories to standardize their measurements.

Methods: We selected 78 laboratories participated at least twice for TC and 46 laboratories participated twice for HDL-C in the standardization protocols developed by the Cholesterol Reference Method Laboratory Network (CRMLN). We compared the initial and subsequent results using the performance guidelines established by US National Cholesterol Education Program (NCEP).

Results: For TC, mean percentage bias of all participants was −0.93% and −0.49% for the initial and second rounds, respectively. Mean within-sample CV was 0.72% and 0.69% for the initial and second rounds, respectively. For HDL-C, mean percentage bias of all participants was −1.86% and −0.06% for the initial and second events, respectively. Mean among-run CV was 1.56% and 1.58% for the initial and second events, respectively.

Conclusions: TC accuracy in the second round than the initial round tended to improvement although statistically not significant, however in the five years follow-up, mean absolute percentage bias was reduced over time. HDL-C accuracy was statistically improved in the second event than the initial event. The precision for both TC and HDL-C did not change. This study shows CRMLN protocols contribute effectively to improvement of TC and HDL-C performance. ***J Atheroscler Thromb, 2003; 10: 145–153.***

Key words: Total cholesterol, HDL-cholesterol, Accuracy, Precision

Introduction

Research for epidemiological studies and clinical trials have demonstrated that high TC and/or high LDL-cholesterol (LDL-C) are an important risk factor for coronary heart disease (CHD) (1–5) and that low HDL-C is an independent predictor of risk for CHD (6–8). According to the recent studies LDL-lowering therapy robustly reduces risk for CHD (9–13).

Guidelines for diagnosis and treatment of lipid disorders were issued in three reports from the US NCEP Adult Treatment Panel (ATP) in 1988 for ATP-I, in 1993 for ATP-II and in 2001 for ATP-III (14). The European Atherosclerosis Society (EAS) issued similar guideline in 1998 (15). The Japan Atherosclerosis Society (JAS) issued the first guideline in 1997 and updated it in 2002 (16). To identify individuals at risk for CHD, the NCEP recommends initial classification in ATP-III using the medical decision points

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of 5.17 and 6.21 mmol/L (200 and 240 mg/dL) for TC and of 1.03 mmol/L (40 mg/dL) for HDL-C, and the JAS recommends initial classification in 2002 revision using the medical cut points of 5.69 mmol/L (220 mg/dL) for TC and of 1.03 mmol/L (40 mg/dL) for HDL-C. Any recent guideline emphasizes the importance for measurement of LDL-C rather than TC.

Accurate, reproducible, and comparable measurements of TC and HDL-C are needed for effective application of the guidelines. The NCEP recommendations indicate that the performance goals for TC are accuracy, expressed as percentage bias versus the accuracy base, of ≤ ± 3% and imprecision, expressed as CV, of ≤ 3% (17). The performance goals for HDL-C are accuracy, expressed as percentage bias versus the accuracy base, of ≤±5% and imprecision, expressed as CV, of ≤ 4% at HDL-C concentrations > 1.09 mmol/L (42 mg/dL) and as standard deviation ≤ 0.044 mmol/L (1.7 mg/dL) at HDL-C ≤ 1.09 mmol/L (42 mg/dL) (18, 19).

In 1990, the CDC established the CRMLN (20,21) to improve lipid and lipoprotein measurements by providing traceability to the accuracy bases for these analytes. In addition to US-based laboratories, CDC, in its role as a World Health Organization Collaborating Center for Reference and Research in Blood Lipids, extended the CRMLN to include selected international laboratories. The Osaka Medical Center for Health Science and Promotion (OMC) has been a member of the CRMLN since July 1992. The CRMLN developed a process by which manufacturers and clinical laboratories can establish traceability to the US National Reference System for Cholesterol (NRS/CHOL), as recommended by NCEP. In 1995, this process was extended to manufacturers producing products used to measure HDL-C (22).

Traceability for TC in a clinical laboratory is verified by comparing the field method with the Abell, Levy, Brodie and Kendall (AK) reference method for TC (23, 24) in a CRMLN laboratory. The comparison is performed based on the CRMLN's Certification Protocol for Clinical Laboratories (25). For HDL-C, the CRMLN has not yet established a specific protocol for certifying clinical laboratories. We therefore applied the CRMLN protocol for certifying manufacturers to Japanese clinical laboratories. The protocol for manufacturers involves a comparison between the field method and the designated comparison method (DCM) for HDL-C using a minimum of 40 to 50 fresh human specimens (26).

The impact of standardization in clinical laboratories has not been well documented. We report here the results for the effectiveness of the CRMLN's TC and HDL-C protocols toward improvement of Japanese clinical laboratory measurements. They participated in the certification process on a voluntary basis. At the same time, to know the effectiveness of long-term standardization, we also report the results based on 5 years of follow-up comparisons for TC.

Materials and Methods

Reference methods

The AK reference method (23, 24) consists of saponification of a 0.5-mL serum sample with alcoholic potassium hydroxide, extraction with hexane, evaporation of an aliquot of the extract, development of color with Liebermann-Burchard reagent at 620 nm, and calibration by the NIST SRM 911b pure cholesterol material. HDL-C DCM employs direct precipitation of the apo-B-containing lipoproteins with dextran sulfate of 50 kDa with magnesium, followed by measurement using the reference method for TC (22).

Comparison protocol for TC

Those laboratories standardizing TC methods followed the CRMLN's certification protocol for clinical laboratories. The protocol required the laboratory to collect a set of six fresh individual serum specimens, however they could combine serum up to two individual donors to obtain the necessary volume or concentrations. The following guidelines for collection were provided to the laboratories: 1) Collect two samples in each of three concentration regions: 2.59–5.17 mmol/L (100–200 mg/dL), 5.17–6.21 mmol/L (200–240 mg/dL), and > 6.21 mmol/L (> 240 mg/dL); 2) make sure the range of concentration between the lowest and the highest is at least 2.59 mmol/L (100 mg/dL); and 3) make sure at least 0.52 mmol/L (20 mg/dL) difference exist between the concentrations of samples in each of the three regions.

All participants used commercially prepared enzymatic reagents and human serum-based calibrators. The assay principle of all reagents is the cholesterol ester hydrolase-cholesterol oxidase-peroxidase chromogenic method. Specimens were analyzed in duplicate on three separate days for a total of six replicate measurements per sample. After these measurements were completed, frozen aliquots were shipped on dry ice by overnight express delivery to OMC.

Selection of laboratories for TC

Of the 291 Japanese clinical laboratories, 78 laboratories were selected for this study because they participated two or more times. Some of these laboratories were involved in an epidemiological study for the Japan Public Health Center-based prospective Study on cancer and cardiovascular diseases (JPHC Study) (27) and a clinical trial for the Pravastatin Anti-atherosclerosis Trial in the Elderly (PATE) (12, 28).

Initially, we compared the results of clinical laboratories that participated twice, and subsequently, we compared the results of laboratories that participated more

than twice during a 5-year period. During the 5 years of the study, 10 standardization rounds were conducted. Of the 78 laboratories that participated in the first two rounds, the number declined over 5 years so that only 9 of the original laboratories remained in the 10th round.

Data analysis for TC

The analysis spreadsheet calculated average percentage bias, average absolute percentage bias, average within-sample CV, within- and between-method outliers, and linear regression statistics. The regression statistics were used to calculate the bias at the medical decision points of 5.17 and 6.21 mmol/L (200 and 240 mg/dL). Laboratories meeting the following criteria were qualified to receive "Certificate of Traceability": average absolute percentage bias ≤ 3%, percentage bias at 5.17 and 6.21 mmol/L ≤ 3%, CV ≤ 3%, correlation coefficient (r^2) ≥ 0.975, and no within- or between-method outliers. The certificate is valid for 6 months.

Comparison protocol for HDL-C

Those laboratories standardizing HDL-C methods followed the CRMLN's evaluation protocol for manufacturers (26). The sample comparison is based upon the US National Committee for Clinical Laboratory Standards protocol "Method comparison and bias estimation using patient samples; approved guideline" (29). The protocol requires analysis of a minimum of 40–50 fresh patient specimens. Samples were selected with the range of HDL-C concentrations, 0.52–1.81 mmol/L (20–70 mg/dL). To achieve this goal, a minimum of five samples were collected in each of the following concentration regions: 0.52–0.75 mmol/L (20–29 mg/dL), 0.78–1.01 mmol/L (30–39 mg/dL), 1.03–1.27 mmol/L (40–49 mg/dL), 1.29–1.53 mmol/L (50–59 mg/dL), and 1.55–1.78 mmol/L (60–69 mg/dL). The remaining samples, a minimum of 15, were spread over the entire concentration range. All samples had triglyceride concentration < 2.26 mmol/L (200 mg/dL).

All participants used commercially prepared reagent kits and human serum-based calibrators. All of these methods are the "direct" methods. Not all laboratories used the same kit, but the products used were from three Japanese manufacturers: Kyowa Medex Co., Ltd., Tokyo (30); Daiichi Pure Chemicals Co., Ltd., Tokyo (31); and Wako Pure Chemical Industries, Ltd. , Osaka (32).

The fresh-frozen serum samples were prepared at OMC from fasting donors including patients and volunteers. Serum was dispensed into separate vials and frozen at – 60°C or below within 8 hours after separation. The frozen samples after check of lipoprotein electrophoresis were shipped to each participant on dry ice by overnight express delivery within 3 days of sample collection.

Clinical laboratories analyzed each sample in duplicate in one run. The total number of samples was divided among five analytical runs. Between analytical runs the samples were stored at – 60°C or below. Each laboratory analyzed its own quality control (QC) sample with HDL-C concentration of 0.78 to 1.55 mmol/L (30 to 60 mg/dL). Each laboratory used either a commercial QC product or prepared its own sample from pooled human serum. Single measurements from 20 recent analytical runs, including the runs where comparison samples were analyzed, were used to estimate among-run CV.

Selection of laboratories for HDL-C

Of the 200 Japanese clinical laboratories, 46 laboratories were selected for this study because they participated twice.

Data analysis for HDL-C

The analysis spreadsheet calculated average percentage bias, average absolute percentage bias, average within-sample within-run CV, within- and between-method outliers, and linear regression statistics. The regression statistics were used to calculate the bias at the medical decision points of 0.91 and 1.55 mmol/L (35 and 60 mg/dL). Among-run CV was also calculated from the single QC measurements with the field method. All laboratories obtained "Document of Comparison" stating that the specific analytical system had been compared with the DCM for HDL-C and listing the specific statistical parameters observed for the system. The document was valid for 2 years. For this study, the laboratories meeting the following criteria were considered to be standardized: average percentage bias ≤ 5%, percentage bias at the medical decision points of 0.91 and 1.55 mmol/L ≤ 5%, among-run CV ≤ 4%, r^2 ≥ 0.975, no more than one within-method outlier, and no between-method outliers.

Statistics

For every survey and each laboratory (e.g., first round versus second round), we calculated mean percentage bias, mean absolute percentage bias, and CV. To compare overall group mean biases and CVs on the initial and second rounds, we used the studentís *t*-test. We also calculated a *t*-statistic and *p*-value for each laboratory separately and evaluated these 78 *p*-values (33). A significance level of $\alpha = 0.05$ was used throughout this study.

In addition, for TC, we tested for reduction of bias over 10 surveys. To do this, we performed a linear weighed regression for each laboratory where the number of surveys (first to 10th) in which a laboratory participated was the independent variable and the percentage bias was the dependent variable. Weights corresponded to the inverse variances of the percentage biases obtained for each survey. Cases where the intercept is negative and the slope is positive, or vice versa, generally indicate improvement in the bias for the laboratory as more surveys are completed. We

plotted the intercepts versus the slopes for all 78 laboratories in a scatter plot. This procedure has the advantage of using all available data, rather than comparing only the first to the last surveys, for example.

Results

Standardization of TC in clinical laboratories

The average time between the first and second rounds for the 78 participating laboratories was 13 months; the median time difference was 7 months.

The performance of 78 clinical laboratories participating in the first and second rounds is presented in Table 1. Seventy-one (91.0%) and 72 (92.3%) laboratories met the performance criteria and received "Certificate of Traceability" in the first and second rounds, respectively. Overall, the pass rate in the second round did not improve significantly from the initial round.

The mean percentage bias for all laboratories improved by 0.44% between the first and second rounds, which was not statistically significant at the 95% level, but it suggests an actual difference. The mean absolute percentage bias improved by 0.20%, which did not reach statistically significance too. The mean average within-sample CV for the group of laboratories did not change.

In another evaluation of the data, we calculated the mean difference for each laboratory and formed a *t*-value for each laboratory on the basis of five degrees of freedom (from six sample means). Thus, 78 *p*-values are obtained. The median *p*-value for 78 laboratories was 0.024. Sixty-three of 78 (80.8%) were < 0.05 and statistically significant at the 95% probability level. Eighty percent of the laboratories showed significant improvement in their bias.

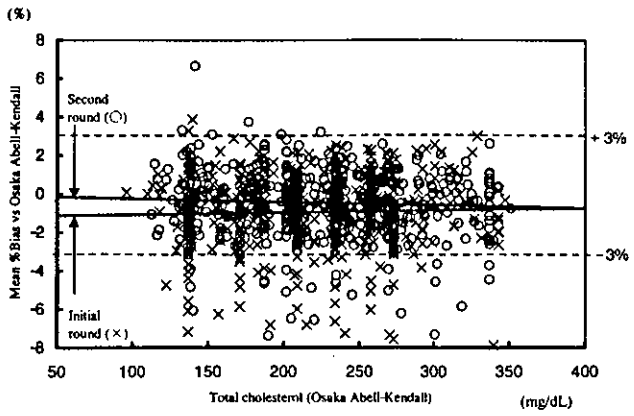

Fig. 1. Mean % Bias Plots for TC. Bias plots of 78 Japanese clinical laboratories that participated in the first and second TC certification round. Mean percentage bias is plotted versus the TC concentration (mg/dL) as determined by the AK reference method at Osaka. Horizontal dotted lines mark the NCEP bias guidelines at ± 3%. Data for all participants is presented together in this one plot. The regression line was $y = 0.0010 \times - 1.1715$ ($n = 78$, $r = 0.0284$) for the initial round (×) and $y = -0.0018 \times - 0.0626$ ($n = 78$, $r = -0.0538$) for the second round (○).

The bias of all participants in the initial and second rounds is shown in Fig. 1. This plot includes the bias of all individual samples analyzed by all 78 participants. Fewer samples had bias greater than ± 3% in the second round.

Table 1. Performance of participants in total cholesterol certification program

Round & *p*-value	Participants		Accuracy% bias		PrecisionCV
			Mean % bias (Mean ± SD)	Mean absolute % bias (Mean ± SD)	Mean within-sample CV (Mean ± SD)
Initial	All Labs	78	− 0.93 ± 1.77%	1.61 ± 1.27%	0.72 ± 0.33%
	Certified[a]	71	− 0.56 ± 1.31%	1.30 ± 0.72%	0.70 ± 0.34%
Second	All Labs	78	− 0.49 ± 1.75%	1.41 ± 1.20%	0.69 ± 0.30%
	Certifieda	72	− 0.24 ± 1.22%	1.14 ± 0.60%	0.69 ± 0.30%
p-value[b]	All Labs	78	0.11	0.31	0.65
	Certified		0.13	0.15	0.79

[a]: Only laboratories that met CRMLN performance criteria: Average percentage bias < = ± 3%, percentage bias at 5.17 and 6.21 mmol/L < = ± 3%, CV < = 3%, correlation coefficient (r^2) < = 0.975, and no within- or between-method outiers.
[b]: *p*-value for comparison of initial and second rounds

Table 2. Number of participants failing to meet specific CRMLN performance criteria for total cholesterol

Round	Accuracy	Precision	Outliers	
	Mean absolute% bias± 3%	Mean within -sample CV< = 3%	Within-method outliers	Between-method outliers
Initial	7	0	1	0
Second	5	0	1	0

In the first round of standardization, a total of seven laboratories failed to meet the criteria to obtain "Certificate of Traceablity". In the second round, a total of five laboratories failed. Table 2 summarizes the reasons that laboratories did not pass certification for both rounds of the protocol. No laboratories failed certification because of imprecision. The most common reason for failure was inaccuracy. Some laboratories failed on multiple criteria. Only one laboratory failed both rounds; this laboratory failed the first time because of inaccuracy and the second time because of within-method outliers. In a third round, this laboratory met all of the criteria.

Table 3 shows the results of 10 rounds of the TC certification protocol with the 78 laboratories that began with the initial standardization. The number of laboratories that remained in the program for all 10 rounds decreased over the 5 years of the study. However, the pass rate increased by the sixth round. Although the mean percentage bias did not appear to change significantly, the mean absolute percentage bias was reduced over time. A weighed regression of the mean absolute bias over the course of the 10 rounds (mean absolute percentage bias versus the number of surveys) had a slope of -0.050, an intercept of 1.340, and a p-value of 0.0008. This shows that the mean absolute percentage bias has been reduced significantly over the 10 surveys for these laboratories.

Standardization of HDL-C in clinical laboratories

The average period between the first and second events in the 46 participants was 20 months.

The initial and second performances of HDL-C by all participants is presented in Table 4. As a group, these laboratories' performance improved between the first and second events. For mean percentage bias and mean absolute percentage bias, differences between the initial and second events were statistically significant (p = 0.003 and p = 0.00002, respectively). Differences in the mean among-run CV between the first and second events were not statistically significant (p = 0.88). The percentage bias of all participants is shown in Fig. 2A and Fig. 2B for the first and second events, respectively.

Table 3. Trends in total cholesterol performance of certified laboratories

Round	Participants # (remaining labs (%))	Pass rate (%)	Accuracy % bias		Precision CV
			Mean % bias (Mean ± SD)	Mean absolute % bias (Mean ± SD)	Mean within-sample CV (Mean ± SD)
1	78	91.00%	−0.56 ± 1.31%	1.30 ± 0.72%	0.70 ± 0.34%
2	78 (100.0%)	92.30%	−0.24 ± 1.22%	1.14 ± 0.60%	0.69 ± 0.30%
3	47 (60.3%)	100.00%	0.00 ± 1.28%	1.20 ± 0.59%	0.63 ± 0.30%
4	36 (46.2%)	94.40%	−0.22 ± 1.25%	1.09 ± 0.69%	0.67 ± 0.34%
5	34 (43.6%)	91.20%	0.07 ± 1.34%	1.23 ± 0.60%	0.61 ± 0.27%
6	29 (37.2%)	100.00%	−0.11 ± 1.15%	1.04 ± 0.54%	0.64 ± 0.25%
7	26 (33.3%)	100.00%	−0.02 ± 1.27%	1.12 ± 0.65%	0.63 ± 0.27%
8	22 (28.2%)	95.50%	−0.28 ± 0.89%	0.90 ± 0.43%	0.67 ± 0.23%
9	18 (23.1%)	100.00%	−0.26 ± 1.05%	0.93 ± 0.59%	0.61 ± 0.35%
10	9 (11.5%)	100.00%	−0.29 ± 0.78%	0.66 ± 0.51%	0.68 ± 0.29%

Table 4. Performance of participants in HDL-cholesterol evaluation program

Event and p-value	Accuracy% bias		PrecisionCV
	Mean % bias(Mean ± SD)	Mean absolute % bias(Mean ± SD)	Among-run CV(Mean ± SD)
Initial	−1.86 ± 3.01%	4.22 ± 1.52%	1.56 ± 0.76%
Second	−0.06 ± 2.71%	2.88 ± 1.29%	1.58 ± 0.77%
p-value[a]	0.003	0.00002	0.88

[a]: p-value for comparison of initial and second events.

Table 5. Number of participants failing to meet specific CRMLN performance criteria for HDL-cholesterol

Event	Accuracy	Precision	Outliers	
	Absolute% bias± 5%	RunCV< = 4%	Within-method outliers	Between-method outliers
Initial	16	0	2	0
Second	2	1	3	0

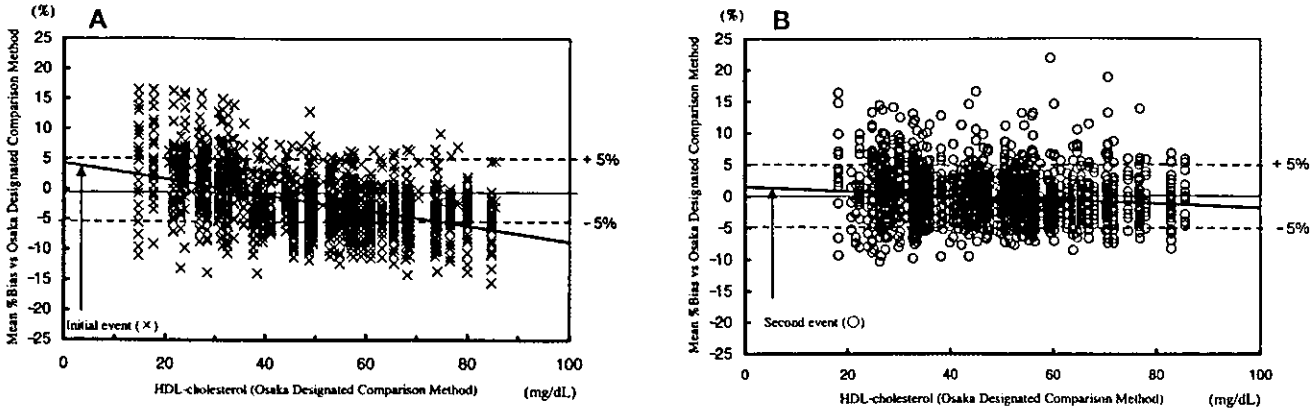

Fig. 2. Mean % Bias Plots for HDL-C. Bias plots of 46 Japanese clinical laboratories that participated in two evaluations of HDL-C performance. Mean percentage bias is plotted versus the HDL-C concentration (mg/dL) as determined by the designated comparison method at Osaka. Horizontal dotted lines mark the NCEP bias guidelines at ± 5%. Data for all participants is presented together in each plot. Figure 2 A: The regression line was $y = -0.1295x + 4.2998$ ($n = 46$, $r = -0.4549$) for the initial event(×). Figure 2 B: The regression line was $y = -0.0324x + 1.4207$ ($n = 46$, $r = -0.1380$) for the second event(○).

Table 5 summarizes the problems that some of these laboratories had in meeting the performance criteria. Precision was not a common problem. However, a number of laboratories had accuracy problems, particularly in the first event. The accuracy problems improved considerably in the second event of evaluations.

Discussion

Achieving accuracy within a laboratory and comparability between laboratories requires traceability to a defined common accuracy base. The most practical approach to achieve traceability is to ensure that manufacturers properly calibrate diagnostic products. This has been the primary focus of the CRMLN since its inception. Another approach to achieve comparability is to require that all clinical laboratories use the same method; however, this is neither practical or possible.

In Japan, no homogeneous systems exist where instrument, calibrators, and reagents are marketed by a single manufacturer. More commonly, heterogeneous systems are used where an instrument is purchased from one manufacturer, and calibrators and reagents are purchased from another manufacturer. A laboratory using a heterogeneous analytical system must assume primary responsibility for documenting performance and establishing traceability to the accuracy base. For this reason, many Japanese laboratories have chosen to participate in the CRMLN traceability programs. This offered us a unique opportunity to evaluate the impact of TC standardization and HDL-C evaluation by comparison of the initial and subsequent performances by clinical laboratories using all heterogeneous analytical systems.

Japanese manufacturers set accurate values on calibrators in three ways: 1) by performing a fresh sample comparison with OMC; this approach has been used for both TC and HDL-C; 2) by sending the calibrator to OMC for value-assignment; this approach has been used for HDL-C; and 3) OMC certifying enzymatic methods through the manufacturerís certification protocol; the manufacturers then use the enzymatic methods to assign calibrator values in-house.

For TC, accuracy by the certified laboratories tended to improvement in the second standardization compared to the initial standardization, however the improvement was not statistically significant. Although the mean bias for all laboratories was reduced by half between the initial and second performances, the difference is not statistically significant because of the relatively high variation between laboratories for this parameter. The *p*-value (0.11) observed for the *t*-test of the mean performance for the entire group of laboratories was not significant at the 95% level, but suggested a real difference. Although bias still exists, the smaller bias than in the initial standardization indicates that the laboratories improved their accuracy, namely the change in bias was in the desirable direction. The variability used to test this reflects among-laboratory variation that reduces the likelihood of finding a significant result.

The statistical tests to evaluate individual laboratoriesí performance between the first and second rounds confirm the suggestion of improvement in performance. This second approach avoids integrating the among-laboratory variability and is more powerful and confirms a trend for laboratories to improve between the first and second rounds.

The mean overall precision for this group of laboratories stayed very nearly the same between the initial and second performances. This is consistent with the fact that precision is not a problem with TC measurements. In fact, none of the laboratories failed to be certified because they did not meet the precision criterion.

When a laboratory failed to meet the criteria, OMC consulted with the laboratory to assist in determining the sources of and resolving the problem. The consultation would consist of a telephone call and/or a visit to OMC from laboratory personnel. If the source of the problem was determined to be with the calibrators or reagents, OMC consulted with the manufacturer to assist in resolving the problem. After the source of the problem was resolved, the laboratory had the opportunity to immediately participate in the certification protocol again. This consultation and certification procedure was followed until the laboratory could verify that it met the performance criteria.

Accuracy failure occurred in 11 laboratories during the first two rounds. The accuracy problem was resolved by changing the calibrator lot (two laboratories), changing of calibrator supply source (five laboratories), or stabilizing an unstable instrument (one laboratory). However, the cause in inaccuracy was not resolved in three laboratories.

The mean absolute percentage bias had a significant trend to lower values as laboratories continued participation in the certification program. We believe that this emphasizes the importance of regular participation with six months interval for TC over at least three years. We observed that, in general, laboratories that met the certification criteria in the first round continued to improve their bias the longer they participated in the program.

For HDL-C, accuracy was significantly improved in the second evaluation over the initial evaluation (34). Precision did not markedly improve. In the HDL-C evaluations, precision failure was resolved by maintenance of an unstable instrument. The accuracy problems that occurred in nine laboratories were resolved by reconstitution of the calibrator in five laboratories and a change of calibrator lot in three laboratories. The cause of inaccuracy was not resolved in one laboratory. Failure because of within-method outliers was resolved by readjustment of an unstable instrument in three laboratories and by a change in technologist in one laboratory. The cause of within-method outliers remained unresolved in one laboratory.

We understand that it will be desirable for using fresh-non-frozen samples for HDL-C measurement. However, in this study the fresh-frozen serum samples stocked at −60°C or below were used because first the HDL-C measurement should be divided among five analytical runs, namely five weeks as one run in a week and because second the reports are available for HDL-C can be determined accurately after storage at −70°C for up 1 month to 1 or 2 years (19,35). Any change in the samples was not found in the check of lipoprotein electrophoresis.

Commercial available kits for HDL-C measurement are developed based on various methodological principles (34). For this reasons the differences sometimes produce serious discrepancy among them for the patient samples that may have specific lipoprotein abnormality. This problem has not clearly been sorted and reported. Therefore, the manufacturers reagent kits should be furthermore focused to improvement for measurement of patient samples with lipid disorders.

In conclusion, accuracy for TC tended to improvement although not significantly, but for HDL-C improved between the initial and subsequent events. Precision was not significantly changed for either TC or HDL-C between the initial and subsequent rounds. Sustained participation in the TC certification program for 5 years demonstrated improved performance the longer a laboratory remained in the program, even while meeting the CRMLN performance criteria. We believe that continuous participation in the international standardization program from every clinical laboratory in Japan is a very essential part not only of the clinical or epidemiological study and practice for the risk management treatment but also of overseas publication of results in medical research involving Japanese peoples. The results of this study demonstrate that, at the outset of participation in the certification program, inaccuracy in TC and HDL-C testing was more of a problem than imprecision. CRMLN certification protocols will contribute effectively to improved accuracy for TC and HDL-C measurements.

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動脈硬化疫学研究における検査標準化

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KEY WORDS
●正確度と互換性
●精密度
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●Cholesterol Reference Method Laboratory Network (CRMLN)
Standardization and quality control of lipids in epidemiologic study of atherosclerosis
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はじめに

多人数を対象として検査を実施する疫学研究においては，一般に検査に従事する専門家や機関も複数となり，相互間のデータの比較性を保つために，検査の方法，判定基準などについての標準化が必要なことはよく理解されている。動脈硬化に関連する検査についても，WHOでは早くからこのことを取り上げており，心電図におけるミネソタコードによる判定などはその国際標準化の1例である。

筆者は1997年より国立循環器病センターよりの委託研究を受け「9指-3循環器疾患に対する新しい危険因子及びその評価方法に関する研究」および「12公-1動脈硬化性疾患の動向，スクリーニング法及び危険因子との関連に関する研究」の6年間にわたる共同研究のお世話を行った。その際，長年にわたり疫学共同研究を続けてきたフィールドの検査室で血清総コレステロール，HDLコレステロールの標準化を行うとともに，疫学的に新しい検査手技として，頸動脈超音波検査とインスリン抵抗性(マルチプルリスクファクター症候群に関連して)を取り上げて標準化の努力を行った。頸動脈超音波検査については，専門学会と協力して，その成果を「頸動脈エコーによる動脈硬化性病変のガイドライン(案)」[1]に示すことができた。一方，インスリン抵抗性に関しては，血糖の標準化はともかく，インスリン値の測定の標準化は十分には行いえず，動脈硬化疫学研究の今後に課題を残すこととなった。この班研究において，従来から疫学研究に従事されてきた方々は積極的に標準化の作業に応じられたが，一部の臨床の専門家からは標準化の意義は認めつつも，標準化への参加までは遠慮しておられるようであった。

J-LITなどわが国でも多くの臨床施

設が参加して，動脈硬化に関する大規模な治験が進行しつつある今日，治験に参加する機関は検査の標準化の重要性を改めて認識する必要があろう。

Ⅰ．臨床検査における精度管理とは

臨床検査における精度管理には，検査室内部で自ら評価を行う内部精度管理と第3者機関から評価を受ける外部精度管理の2つに大別される。内部精度管理の目標は主として測定値の精密さ(再現性)の維持・管理にあり，一方，外部精度管理は主として測定値の正確さにターゲットがある。検査室で発生する測定値の精密さや正確さをどのようにして客観的に評価するのか，その評価手段として各種の測定値の品質管理システムが考案されている。評価手段を検査室の技術達成レベル別に3分類すれば，第1段階として測定項目全般を対象とした測定精度のアウトラインを把握するための精度管理調査(サーベイ：Quality Control Survey)，第2段階として特定の検査項目を重点的に技術的な練度向上を目指した熟達度試験(Proficiency Testing Program)[2]，第3段階として長期間にわたる臨床試験や疫学研究を対象とした正確度と互換性を図るための標準化(Standardization)[3]に大別することができる。臨床試験や疫学研究を通じて抽出されたリスクファクターは最終的に論文化され，公表されるであろう。その時，仮に測定成績が正確性に欠け，施設間での比較可能性にも耐えられないものであるとすれば，長年の営々たる努力と研究にかけた経費は，一挙に吹き飛んでしまうことにもなりかねない。正にこの点が，動脈硬化の疫学研究における検査の標準化が必要とされるゆえんである。逆に，リスクファクターほどの意義はもたないけれども，診断や治療には必要となる検査項目まで第3レベルの標準化の対象とする必要性もないといえる。第1段階のサーベイで十分であろう。しばしば混同されるが，サーベイと標準化は，その意図する役割がそれぞれ異なる。欧米の学術雑誌にこれまで発表された心血管系疾患に関する研究成果の約70％が，研究の最初の段階から最終の段階までCDC(Centers for Disease Control and Prevention)の脂質標準化に応え，正確性と互換性に裏打ちされた成績をエビデンスとして解析する方法を採ることが一般化している。欧米諸国では，標準化が研究の基礎として重要であると認識されている証拠であろう。たとえば，LRC，MRFIT，CARE，WOSCOPSなど枚挙にいとまがない。一方，わが国の学術研究のいくつかは，標準化の必要性と重要性に必ずしも目覚めているとはいいがたい現状にある。たとえばわが国発の大規模な治験研究において標準化によるエビデンスが研究成果に示されていないことが，欧米の学術雑誌に掲載される機会を小さくしているのではないだろうか。

Ⅱ．世界的な標準化としての国際ネットワーク

日本臨床化学会標準品情報専門委員会の標準に関する用語によれば，標準化(Standardization)とは，「標準を設定し，これを活用する組織的行為」と定義する。コレステロールを例として説明する。米国におけるコレステロールの標準，すなわちコレステロールの正確さの基盤(Accuracy Base)となる基準分析法(Reference Method)は，商務省に所属するNIST (National Institute of Standards and Technology)が担当するIsotope Dilution/Gas Chromatography/Mass Spectrometry法[4]による絶対基準分析法(Definitive Method)と，保健社会福祉省に所属するCDCが担当するAbell-Kendall法[5]による実用基準分析法の2法が相互補完関係を保ちながら形成されている。この基盤は，実質的に世界標準であり，CDCはWHOの協力センター(WHO Collaborating Center for Reference and Research in Blood Lipids)としての承認を受けている。コレステロールの基盤で確定された正確性は，標準物質や血清などの被検物と標準化プログラムという媒体を通じて，①コレステロールの測定体系のうえで高位(基準分析法)の正確さを順次下位(比較対照法，日常分析法など)のものに合わせていく伝達性(Transferability)と，②測定体系のうえでより高い正確さに下位から次々と合わせていくトレーサビリティー(Traceability)というそれぞれ下行，上行の2つの経路を介してやり取りされる。組織的行為とは，伝達性とトレーサビリティーの組み込まれた標準化プログラムを現実に運用して，標準化を図る行為である。以上が欧米で用いられる標準化の概念である。わが国では標準化という用語がしばしば規格化や標準的測定法の策定などと混用される傾向が認められる。しかし，欧米における標準化は，厳密な条件を満たすものに限定して用いられている。標準化の目標が，第1に正確性，第2に国際的な互換性に力点が置かれていること

から，国際共同研究では1国内部での標準化では十分に対応できず，必然的に国際的な連携化へと進展して，最終的には国際ネットワークが形成される。現在，標準化が世界的なネットワークにまで発展したケースは3種類認められる。1つは国際臨床化学連合(IFCC)による酵素に関する国際ネットワーク，2つは同じく国際臨床化学連合(IFCC)によるグリコヘモグロビンA_{1c}に関する国際ネットワーク，3つはCDCによるコレステロールの国際ネットワークである。これらのネットワークの存在は，パリに本部を置く国際度量衡局(BIPM)とIFCCによって認められている[6]。

米国のCDCは，心血管疾患の危険因子とされる脂質の標準化を運用する国際的な拠点施設として世界的に知られている。CDCの実用基準分析法で確立された目標値は，国の基礎的な調査(例：米国ではNHAINES，日本では循環器疾患基礎調査や国民栄養調査)，疫学研究，臨床試験などで得られた測定値の信頼性を保証・比較するための規準として，40年以上も前から欧米諸国で広く受け入れられ，今日では世界的にも評価が定着している。CDCによる組織的行為は，コレステロールの正確度の基盤を源として，1958年に運用開始されたCDC-NHLBI脂質標準化プログラム[7]と1990年に運用開始されたCholesterol Reference Method Laboratory Network (CRMLN)脂質標準化プログラムの2つにより具体化されている。CDCの基準分析法は30年以上の技術的改良と世界的な批判に耐え，脂質標準化プログラムは優れて実践応用的である。標準化参加室に多くの負担をかけず，できる限り小さな作業で，可能な限り大きな成果を期す方法は，いかにもアメリカ的である。ここでは，国際標準化ネットワークのモデルともされるCRMLNの役割とその応用としての脂質標準化の現状についてふれる。

Ⅲ. Cholesterol Reference Method Laboratory Network(CRMLN)について

心血管疾患の診断・治療・予防を誤らないためには，脂質の測定値が，第1に正確であること(Accurate)，第2に精密であること(Stable and Reproducible)，第3に施設間で比較可能であること(Comparable)が基本要件となる。わが国で実施される多くのサーベイには，評価できる点が少なくない。しかし，外部精度管理の1つとしてのサーベイが，特に第1と第3の要件に関して，正面から応えているとはいえない。わが国の研究成果が世界で正当に評価されるためには，サーベイでは十分に対応しきれない。たとえば，日本医師会が年に1回全国的に実施する臨床検査精度管理調査は優れたサーベイの典型例であるが，この調査を通じてA評価を受けたとしても，それが国際社会でもA評価で通用するだろうかという問題を考えれば，答えは明らかである。国際社会で正当に評価されるためには，国際社会で承認されているシステムの下で標準化を受けることが必要である。標準化を実施するためには，原則が必要となる。その原則とは，①基準分析法の運用，②標準物質の存在，③標準化プログラムの運用，④新鮮血清の使用，⑤測定成績の解析ソフト，⑥測定精度の判定基準の確立，の6つである。標準化は，これらの6原則が有機的に機能して初めて，組織的行為が本来の機能を発揮する。なかでも，③の標準化プログラムと⑥の判定基準は，欧米における動脈硬化診療ガイドライン[8][9]を背景として構築されているだけに，欧米の成績との比較考察上便利である。

米国には30年以上も前から標準化が系統的に整備され，現実に機能し，今日着実に成果が上がっているシステムが存在する。その1つが，CDCを中心とする脂質の国際的なネットワークであるCRMLNである。CRMLNは，2003年6月現在，世界8ヵ国の計11の基準分析室で構成される国際組織に成長した(表1)。CRMLNでは，標準化は検量用標準血清と試薬を組み合わせて市販し，その製品に責任をもつ試薬メーカーを通じてエンドユーザーである臨床検査室に波及させるのがもっとも効果的と考える。欧米における標準化の重心は，メーカーにある。表2に示すように，総コレステロール，HDLコレステロール，LDLコレステロール，トリグリセライドの4項目ともに，メーカーを対象とした標準化プロトコル(Phase-3)は整備されているが，臨床検査室を対象としたプロトコルは，総コレステロール(Phase-1)に限られている。Phase-1の総コレステロールは，6濃度・3測定日分の凍結血清を試料とする標準化プログラムで，同じサンプルを臨床検査室と基準分析室の両者で測定して，臨床検査室の測定精度(正確度，精密度)を判定する。Phase-1では，凍結血清を準備するのは臨床検査室側にある点が特徴的である。臨床検査室を対象とした標準化の枠を拡大するために，大阪府立健康科学センター

は臨床検査室が対応しやすいタイプのHDLコレステロールとLDLコレステロールの標準化プログラム(Phase-2)を独自に開発して，全国的な要望に応えている。Phase-2では，6濃度・1測定日分の新鮮な非凍結の(原則)個人血清を検体とする標準化プログラムである。Phase-1とPhase-2の実施内容は，健康科学センターのホームページ(http://www.kenkoukagaku.jp)に詳しい。現在，CRMLN，および，健康科学センターによる標準化は，世界的な規模で公正・公平に実施に移され，その成果が公表されつつある[10)-13)]。CDC/CRMLNの判定基準を満たせば，Phase-1とPhase-3ではCDCから，また，Phase-2では健康科学センターから認証書が発行される。米国では，メーカーによる認証書の取得は，米国食品医薬品局(Food and Drug Administration；FDA)による製品販売認可条件の1つとされる。Phase-3による標準化の成果は，米国臨床化学会(American Association for Clinical Chemistry；AACC)のホームページ(http://www.aacc.org/standards/)，および，CDCのホームページ(http://www.cdc.gov/nceh/dls/crmln/crmln.htm)に公表されている。

図1と図2は，ある研究班における総コレステロール(Phase-1)とHDLコレステロール(Phase-2)の測定精度を，検査機関別に正確度と精密度に分けて表現した。図1の総コレステロールでは，この研究班に登録されている44施設の検査機関中の31施設(70.5%)が標準化状況にあることを示す。測定精度

表1．CRMLN参加基準分析室(2003年6月)

米国グループ
(1) State Laboratory of Hygiene, University of Wisconsin (WI)
(2) Northwest Lipid Reseach Laboratories, University of Washington (WA)
(3) Wadsworth Center for Laboratories and Research (NY)
(4) Pacific Biometrics Research Foundation (WA)

国際グループ(加盟順)
(5) Erasmus MC, University Medical Center Rotterdam (The Netherlands)
(6) Osaka Medical Center for Health Science and Promotion (Japan)
(7) Institute of Biochemistry, Glasgow Royal Infirmary (Great Britain)
(8) Canadian External Quality Assessment Laboratory (Canada)
(9) H.S. Raffaele (Italy)
(10) Fundacion Bioquimica Argentina (Argentina)
(11) Beijing Institute of Geriatrics (China)

表2．CDC/CRMLNの脂質標準化プロトコル

対象	Phase	標準化項目	実用基準法	第二次標準物質	標準化プロトコル	NCEPによる判定基準		
						正確度	精密度	総合誤差
メーカー	3	TC	Abell-Kendall法	CDC Frozen Pools NIST SRM909	総コレステロール用標準化プロトコル (April 1999)	±3%RV	CV≦3%	≦8.9%
メーカー	3	HDL	DCM	CDC Frozen Pools	HDLコレステロール用標準化プロトコル (June 1999)	±5%RV	CV≦4%	≦13%
メーカー	3	LDL	BQ法	CDC Frozen Pools	LDLコレステロール用標準化プロトコル (April 1999)	±4%RV	CV≦4%	≦12%
メーカー	3	TG	DCM	CDC Frozen Pools	トリグリセライド用標準化プロトコル (April 1999)	±5%RV	CV≦5%	≦15%
臨床検査室	1	TC	Abell-Kendall法	CDC Frozen Pools NIST SRM909	総コレステロール用標準化プロトコル (June 1994)	±3%RV	CV≦3%	≦8.9%

DCM：Designated Comparison Method(比較対照法)
NCEP：National Cholesterol Education Program (米国コレステロール教育プログラム)
RV：Reference Value(目標値)
BQ：Beta-Quantification
NIST：National Institute of Standards and Technology
SRM：Standard Reference Material(標準物質)

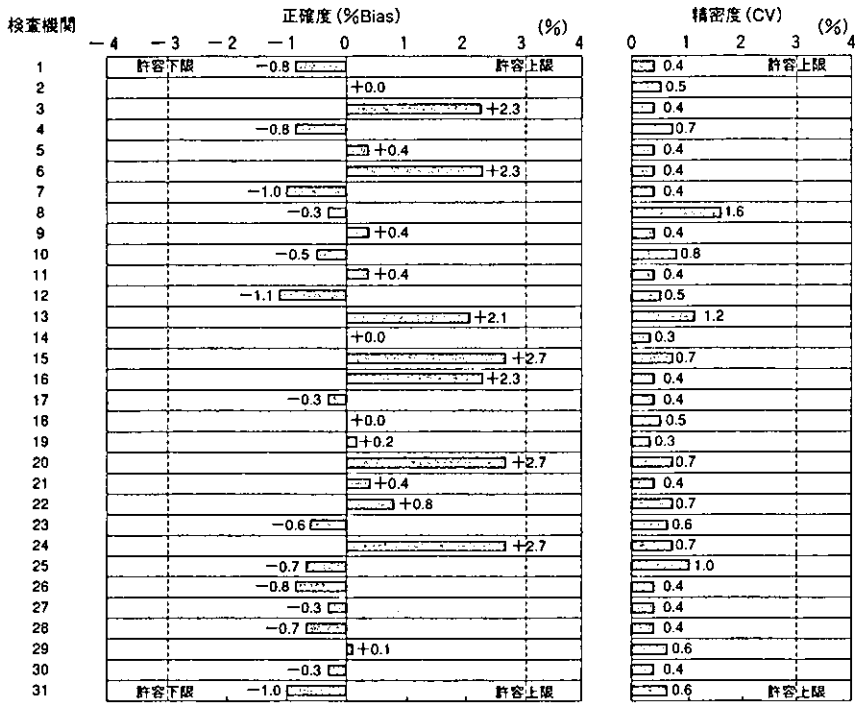

図1．1研究班における総コレステロールの測定精度

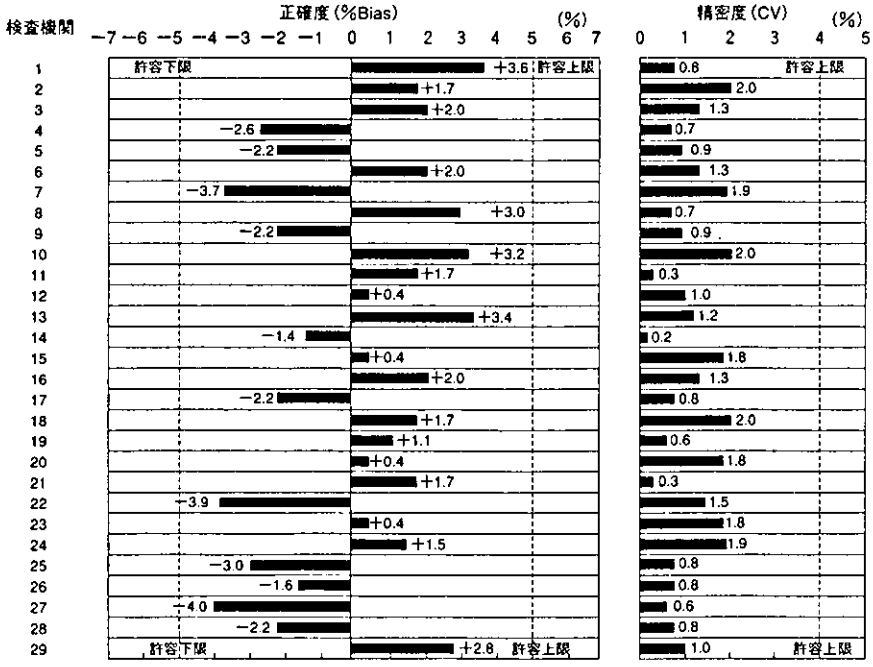

図2．1研究班におけるHDLコレステロールの測定精度

を構成する正確度を基準分析法の目標値に対する%Biasで，また，精密度を変動係数で表した場合，総コレステロールのAverage %Biasは＋0.34%(n＝31)，Average CVは0.57%(n＝31)であり，施設間における正確度の最大値と最小値の幅は3.9%に達することを明らかにしている。一方，HDLコレステロールでは，登録された44施設中のうち29施設(65.9%)が標準化を達成し，その測定精度は，Average %Biasが＋0.14%(n＝29)，Average CVは1.14%(n＝29)を示し，施設間における正確度の最大値と最小値の幅は7.6%に達することを示す。測定値の正確度も精密度もともに判定基準を満たすことから，この研究班の測定精度は十分であり，かつ互換性があると判断される。これが国際標準化の成果であり，論文化の際にその事実を記載すれば，欧米諸国の人たちも共通の理解ができる。

おわりに

大阪府立健康科学センターの脂質基準分析室は，前身である大阪府立成人病センター集検1部の時代からCDCおよびCRMLNと共同歩調を取りながら，世界中の試薬メーカーや臨床検査室を対象とした標準化に向けて一歩，一歩たゆまぬ努力を傾けてきた。脂質の標準化を通じて，わが国の臨床試験や疫学研究の成績が国際的に通用するよう少しでも寄与できれば幸いである。

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Independent predictors of recurrence of chronic subdural hematoma: results of multivariate analysis performed using a logistic regression model

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Object. The authors attempted to determine independent predictors that contribute to the recurrence of chronic subdural hematoma (CSDH).

Methods. A total of 105 consecutive patients who underwent surgery for CSDH were included in this study. Eleven patients underwent a repeated operation because the CSDH recurred. Univariate and multivariate analyses were performed to assess the relationships among various variables and CSDH recurrence. Finally, four variables were found to be independently associated with the recurrence of CSDH: 1) absence of a multiplicity of hematoma cavities on CT scans; 2) presence of a history of seizure; 3) width of the hematoma; and 4) absence of a history of diabetes mellitus (DM).

Conclusions. As previously reported, the width of the hematoma is related to the incidence of CSDH recurrence. In this study, the lack of a multiplicity of hematoma cavities was the favorite predictor of CSDH recurrence. In addition, histories of seizure and no past DM are closely related to the incidence of CSDH recurrence.

KEY WORDS • **chronic subdural hematoma** • **disease recurrence** • **multivariate analysis**

CHRONIC subdural hematoma is one of the most common types of intracranial hemorrhage, and often occurs in older patients.[2,6,7,9,14,19,26] Surgical treatment has been widely accepted as the most efficacious way to deal with CSDH;[16,24,25] however, some patients experience recurrence, at rates reported to range from 9.2 to 26.5%.[2,6,16,19,26] The clinical entity of CSDH has been established, but its clinical features and correlating factors are still controversial. Numerous factors potentially associated with the recurrence of CSDH have been reported in the literature.[2,4,6–9,13,14,16,19,26] We evaluated predictors associated with the recurrence of CSDH in 105 patients who underwent surgery for CSDH in our department.

Clinical Material and Methods

We studied 105 consecutive patients with CSDH (73 men and 32 women ranging in age from 40 to 97 years [median age 71.2 years]) who were admitted to the Department of Neurosurgery, Toyama Medical and Pharmaceutical University, where they underwent surgical procedures for the hematoma between June 1991 and April 2000. Three additional patients were excluded because their need for the craniotomy was due to the organization of the CSDH or because the hematoma was accompanied by another condition such as an arachnoid cyst or tumor.

The clinical data for these 105 patients with CSDH are summarized in Table 1. The initial surgical procedure included irrigation of the hematoma, which was performed using one burr hole in 103 patients and two burr holes in two patients. Subdural blood was evacuated and washed out with warm physiological saline solution. All patients underwent closed-system drainage, performed with the aid of a silicone tube, for 1 to 5 days (mean 1.7 days). Operations were performed with neuroleptanalgesia and local anesthesia in all patients.

The criterion for recurrence was an increase in hematoma thickness and a change in hematoma density on follow-up CT scans within 3 months postoperatively.[16] Reappearance of symptoms such as dementia, hemiparesis, and aphasia also indicated the recurrence of hematoma. In principle follow-up CT scanning was performed 1 day, 1 week, 1 month, 3 months, and 6 months postoperatively.

Variables considered in the analysis of factors associated with recurrence of CSDH included the following: patient sex and age; history of trauma; JCS[18] score on admission to the hospital (0–3 or > 3); symptomatic or nonsymptomatic hematoma; site of the hematoma (ipsilateral or bilateral);

Abbreviations used in this paper: CSDH = chronic subdural hematoma; CT = computerized tomography; DM = diabetes mellitus; JCS = Japan Coma Scale.

TABLE 1

*Demographic characteristics and clinical findings in 105 patients with CSDH**

Factor	No. of Patients (%)			
	RG	NRG	Total	p Value
no. of patients	11	94	105	
sex				
male	9 (81.8)	64 (68.1)	73	0.349
female	2 (18.2)	30 (31.9)	32	
age (yrs)†	71.4 ± 9.6	71.4 ± 10.5	71.4 ± 10	0.990
trauma				
present	9 (81.8)	69 (73.4)	78	0.546
absent	2 (18.2)	25 (26.6)	27	
initial JCS score				
0–3	9 (81.8)	90 (95.7)	99	0.060
>3	2 (18.2)	4 (4.2)	6	
symptoms				
present	11 (100)	93 (98.9)	104	0.731
absent	0 (0)	1 (1.1)	1	

* NRG = nonrecurrence group; RG = recurrence group.
† Values are expressed as means ± standard deviations.

layering of the hematoma; multiplicity of hematoma cavities; development of a hematoma capsule; presence of a midline shift on CT scans; interval between symptoms and surgery; presence of intracranial air 7 days after surgery; position of the drainage system; history of seizure, smoking, DM, cerebrovascular disease, heart disease, liver disease, or alcohol abuse; and the use of antiplatelet and anticoagulant drugs.

TABLE 2

Summary of initial CT and clinical findings, and perioperative findings in 105 patients with CSDH

Finding	No. of Patients (%)		p Value
	RG	NRG	
no. of patients	11	94	
hematoma site			
ipsilat	8 (72.7)	74 (78.7)	0.649
bilat	3 (27.3)	20 (21.3)	
layering of hematoma			
present	4 (36.4)	46 (48.9)	0.430
absent	7 (63.6)	48 (51.1)	
multiplicity of hematoma cavity			
present	0 (0)	27 (28.7)	0.039
absent	11 (100)	67 (71.3)	
development of capsule			
present	4 (36.4)	11 (11.7)	0.027
absent	7 (63.6)	83 (88.3)	
midline shift			
present	9 (81.8)	80 (85.1)	0.774
absent	2 (18.2)	14 (14.9)	
hematoma width (mm)*	23.4 ± 5.57	22.4 ± 5.58	0.568
interval from symptoms to surgery (days)*	18.3 ± 27.0	20.8 ± 26.9	0.770
intracranial air (7 days postop)			
present	11 (100)	77 (81.9)	0.123
absent	0 (0)	17 (18.1)	
position of drainage			
frontal	10 (90.9)	84 (89.4)	0.874
other	1 (9.1)	10 (10.6)	

* Values are expressed as means ± standard deviations.

TABLE 3

Summary of risk factors in 105 patients with CSDH

Risk Factor	No. of Patients (%)		p Value
	RG	NRG	
no. of patients	11	94	
medical history			
seizure			
yes	3 (27.3)	3 (3.2)	0.0011
no	8 (72.7)	91 (96.8)	
smoking			
yes	3 (27.3)	31 (33.0)	0.702
no	8 (72.7)	63 (67.0)	
hypertension			
yes	3 (27.3)	33 (35.1)	0.605
no	8 (72.7)	61 (64.9)	
DM			
yes	1 (9.1)	18 (19.1)	0.412
no	10 (90.9)	76 (80.9)	
cerebrovascular disease			
yes	4 (36.4)	18 (19.1)	0.184
no	7 (63.6)	76 (80.9)	
heart disease			
yes	1 (9.1)	11 (11.7)	0.797
no	10 (90.9)	83 (88.3)	
liver disease			
yes	1 (9.1)	6 (6.4)	0.733
no	10 (90.9)	88 (93.6)	
alcohol abuse			
yes	6 (54.5)	35 (37.2)	0.266
no	5 (45.5)	59 (62.8)	
antiplatelet or anticoagulation drugs			
yes	0 (0)	4 (4.3)	0.485
no	11 (100)	90 (95.7)	

The patient's medical history and initial JCS score on admission were collected from medical records written by the treating physicians. A history of seizure, hypertension, and DM, and the use of antiplatelet and anticoagulation drugs were determined and treatment was based on these findings until the diagnosis of CSDH could be obtained.

The width of the hematoma, a multiplicity of hematoma cavities, the site of the hematoma (unilateral or bilateral), the development of a hematoma capsule, the existence of intracranial air on CT scans 7 days postoperatively, the presence of the midline shift on CT scans on admission, and the presence of layering of the hematoma were evaluated independently by three observers who were blinded to the patients' medical charts and CT scans. "Layering of the hematoma" was defined as a hematoma containing two components of different densities with a clear boundary lying between them.[16] A "multiplicity of hematoma cavities" was defined as a hematoma with inhomogeneous contents and a high-density septum running between inner and outer membranes.[16]

We performed a univariate analysis to assess the relationships between each variable and the recurrence of CSDH by applying the Student t-test, the Mann–Whitney U-test, and the chi-square test. We also performed a multivariate statistical analysis of factors related to the recurrence of CSDH by using a logistic regression model. Variables in the final model were selected according to a stepwise method and

TABLE 4

*Univariate analysis of variables related to the recurrence of CSDH**

Variable	Unit	Univariate OR (95% CI)
multiplicity of hematoma cavities	yes	0.25 (0.01–1.39)
seizure	yes	11.40 (1.85–71.2)
width of hematoma	1 mm	1.03 (0.92–1.15)
DM	yes	0.42 (0.02–2.42)
site of hematoma	bilat	1.39 (0.28–5.31)
development of capsule	yes	4.31 (1.00–16.9)
initial JCS score	1 point	1.44 (0.90–2.32)
cerebrovascular disease	yes	2.41 (0.58–8.92)
intracranial air at 1 wk postop	yes	2.21 (0.38–41.9)

* CI = confidence interval; OR = odds ratio.

TABLE 5

Results of a multivariate analysis of variables related to the recurrence of CSDH

Variable	Unit	OR (95% CI)	p Value
multiplicity of hematoma cavities	yes	0.03 (0.001–0.49)	0.0096
seizure	yes	23.40 (1.83–775)	0.0146
width of hematoma	1 mm	1.22 (1.01–1.47)	0.029
DM	yes	0.06 (0.001–0.99)	0.049
site of hematoma	bilat	4.63 (0.66–37.3)	0.121
development of capsule	yes	4.16 (0.66–26.5)	0.125
initial JCS score	1 point	1.56 (0.82–2.98)	0.175
cerebrovascular disease	yes	2.91 (0.26–27.8)	0.356
intracranial air at 1 wk postop	yes	1.06 (0.10–31.5)	0.965

those deemed to have clinical importance were included. To determine the independent factors affecting recurrence of CSDH, odds ratios were evaluated after adjustment for other factors.

Results

Eleven patients (10.5%) experienced recurrence, whereas 94 patients (89.5%) did not. Demographic variables such as patient sex and age were found not to be related to the recurrence of CSDH on the univariate analysis (Table 1). No significant differences in the incidence of head injury, initial JSC score on admission, and symptoms on admission were observed between the two groups (Table 1). Among the initial CT scanning, clinical, and perioperative findings, absence of a multiplicity of hematoma cavities and development of a capsule were significantly associated with recurrence of CSDH (Table 2). Interobserver agreement on the quality of the method was estimated using the overall κ coefficient. The overall κ coefficient was defined as the mean of the simple or weighted κ coefficients between two observers. The indices of agreement were 0.86, 0.939, 0.584, 0.911, and 0.344 for layering of the hematoma, presence of intracranial air 7 days after surgery, multiplicity of hematoma cavities, presence of a midline shift, and the development of a hematoma capsule, respectively. For width of the hematoma, we estimated Spearman rank correlation coefficients. The correlation coefficients were very high (two of three correlation coefficients were > 0.93). When we evaluated relationships by performing a univariate analysis between the recurrence of CSDH and each risk factor, a history of seizure was found to occur surprisingly more frequently in the recurrence group than in the nonrecurrence group (Table 3).

After eliminating variables that were closely related to others, the following items were adopted as cofounders in the logistic regression model for the multivariate analysis: multiplicity of hematoma cavities (yes), history of seizure (yes), width of the hematoma (1 mm), DM (yes), site of the hematoma (bilateral), development of a capsule (yes), initial JCS score (1 point), history of cerebrovascular disease (yes), and intracranial air 7 days after surgery (yes). The odds ratios for these variables after univariate analysis are shown in Table 4. The analysis revealed that the absence of a multiplicity of hematoma cavities, presence of a history of seizure, width of the hematoma, and absence of a history of DM were significantly associated with recurrence of CSDH after adjustment for other cofactors (Table 5).

Discussion

In previous studies, many risk factors for recurrence of CSDH have been reported.[2,4,6–9,13,14,16,19,26] Advanced age, bleeding tendency, brain atrophy, alcohol abuse, and bilateral CSDHs have commonly been reported as risk factors for recurrence.[2,6,7,9,14,19,26] Risk factors, however, should change with advances in medical care and changes in environmental factors (for example, social and economic status, culture, and educational system). We therefore attempted to confirm the risk factors for recurrence of CSDH in our series by performing a multivariate analysis with the aid of a logistic regression model. Finally, the recurrence of CSDH was correlated with the following variables in the present study: 1) absence of a multiplicity of hematoma cavities; 2) presence of a history of seizure; 3) width of the hematoma; and 4) absence of a history of DM.

Multiplicity of Hematoma Cavities

In previous studies, multiplicity was positively correlated with the recurrence of CSDH.[6,24] In those studies, however, the authors defined "multiplicity" as multiple CSDHs, whereas we define multiplicity of hematoma cavities as the involvement of multiple cavities. It corresponds to the trabecular type of a hematoma described in a previous report.[16]

The pathogenesis of the formation and development of CSDH is still a matter of discussion. The findings of recent experimental studies have revealed that blood in the subdural space evokes an inflammatory reaction, resulting in deposition of fibrin, which is followed by the organization and formation of subdural neomembranes with ingrowth of neocapillaries.[24] Then, the plasminogen in the hematoma is transformed into plasmin by tissue plasminogen activator, which is extremely abundant in the outer membrane. Sequentially, both the breaking down of fibrin and fibrinogen and the production of an extraordinarily large amount of fibrin and fibrinogen degeneration products occur. These phenomena result in the liquefaction of blood clots, an increase in the permeability of capillary vessels, and interference with the mechanism, development, and progressive enlargement of the CSDH.[15] According to their description of hemostasis, Tanikawa and colleagues[24] believe that a he-

matoma with a large amount of intrahematomal membranes has a high rate of recurrence and that resection of the intrahematomal membrane, establishment of a connection with all other hematoma compartments, and evacuation and drainage of the hematoma fluid may result in promotion of subdural fluid reabsorption and, consequently, rebleeding and fibrinolysis can be prevented.

Irrigation with one burr hole, however, is usually sufficient to wash out the hematoma in multiple cavities. In most cases, multiplicity does not mean multiple closed cavities. We believe that all cavities are continuous with relatively wide routes of connection in many cases. If irrigation is performed completely, residual structures (intrahematomal membranes) should shrink and adhere to each other. El-Kadi, et al.,[6] demonstrated that the larger the CSDH, the lower its surface/volume ratio. The surface of the hematoma is the site at which both bleeding and reabsorption occur. Therefore, once certain critical ratios are passed, the hematoma will grow or decrease in size. Conversely, a CSDH with a multiplicity of hematoma cavities has a greater surface/volume ratio. Therefore, the finding that the presence of a multiplicity of hematoma cavities was a negative factor for the recurrence of CSDH is compatible and is coincident with the results of a previous report.[16] Nonetheless, completely closed cavities sometimes exist. In such cases, none of these isolated cavities should be left untreated.

History of Seizure

It is said that the common factors predisposing to the development of a CSDH are alcoholism, anticoagulation therapy and coagulopathies, seizure disorders, and cerebrospinal fluid shunts.[2,4,5,9,15,17,19,20] These factors are frequently associated with brain atrophy, decreased blood homeostasis, and head trauma. In this study, a history of seizure was one independent predictor of recurrence of CSDH; patient age was not found to be a predictor of recurrence. An additional factor other than brain atrophy, such as coagulopathy due to the direct effect of anticonvulsant agents[1,22] or an indirect effect by way of liver dysfunction,[3] may contribute to the recurrence of CSDH.

Width of the Hematoma

The width of a hematoma is usually determined at the level of its maximum thickness and has been reported to be correlated with patient age.[7] This result is attributed to the brain atrophy associated with aging, which provides the hematoma with space in which to grow. In the same fashion, we speculate that larger hematomas have a greater tendency to recur because postoperatively the subdural space is larger than that found after removal of a small lesion.

Diabetes Mellitus

Diabetes mellitus is a well-known risk factor for coronary heart disease and stroke.[5,10] Hyperglycemia is associated with arterial occlusive disease because it induces hyperviscosity of the blood and promotes atherosclerosis.[12] Platelet aggregation[21] and coagulation are activated,[11] and the osmotic pressure of the blood is increased in such a condition. Suzuki and associates[21] reported that osmotherapy performed using 20% mannitol is effective in stopping repeated bleeding of a CSDH. In the same way, the blood of patients with DM has a high osmotic pressure and increased platelet aggregation. The blood sugar level is elevated during the perioperative period and after every meal. This suggests that DM may play a role in decreasing the rebleeding tendency of a CSDH. Diabetes mellitus may contribute to attenuating the recurrence of CSDH.

The present study was a retrospective cohort study, and thus is potentially subject to sources of bias and variation. Nonetheless, the imaging data were collected prospectively by using serial CT scans. The sample size of the present study was not large, and further investigation is required to assess not only the independent predictors revealed in this study, but also those reported previously.

Conclusions

Absence of a multiplicity of hematoma cavities, presence of a history of seizure, width of the hematoma, and absence of a history of DM are independent predictors for the recurrence of CSDH.

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