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Detection of individual over-smoking using conventional laboratory tests

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Objective : The present study was designed to find useful markers for detecting the severity of smoking effects on the human body from conventional laboratory tests used in community health check-ups.
Methods : The subjects were 18,576 persons who visited Tokai University Hospital Health Check Clinic between January 5, 2000, and December 28, 2000. The data on their life-style information and laboratory tests were analyzed by multiple regression analysis and logistic analysis.
Results : White blood cell counts (WBC), the numbers of lymphocytes (Ly), hemoglobin concentration (Hb), mean corpuscular volume of red blood cells (MCV), and carcinoembriogenic antigen (CEA) were positively correlated with numbers of cigarettes consumed, while high density lipoprotein cholesterol (HDL-C) was negatively correlated, with statistically significant difference. Although the relationship between the grade of smoking habit and MCV, HDL-C and CEA was affected by alcohol intake, the WBC, MCV, HDL-C and CEA were shown to be the grading index for smoking effect regardless of drinking habit. Logistic analysis indicated that an accumulation of these marker abnormalities was not useful for differentiating smokers from non-smokers as compared with a single marker abnormality.
Conclusion : WBC, MCV, HDL-C and CEA can be the grading index for smoking effect. Abnormalities in one or some of those values observed in healthy smokers indicate some effects of smoking on their bodies.

Key words: smoking habit, health check, laboratory tests, detection of over-smoking

INTRODUCTION

Quitting smoking is necessary to prevent lifestyle-related diseases including various types of cancer, cardiovascular diseases and others, as has been known by large-scale studies (Peto R, *et al*., 1996 [26], Weiss W., 1997 [31], Kawane H., 1995 [21], Hirayama T., 1990 [12], Kannel WB., 1981 [20], Ball K, *et al*., 1974 [2], Dawber TR., 1980 [6], The Ministry of Health and Welfare., 1993 [30], Kadowaki T, *et al*., 2000 [19], Mino Y, *et al*., 2000 [23], Whitehead TP, *et al*., 1995 [32], Moskowitz WB, *et al*., 1999 [24]). Children should learn of the harmful health effects observed in smokers, and consider smoking as anti-social behavior. We reported a comparative study between Thailand and Japan on smoking habits from the socio-cultural aspects and analyzed the causes of difficulty of quitting smoking (Ito S, *et al*., 2004 [17]). The following two conclusions were obtained. The first, total smoking rate for both genders and all ages was higher in Thailand, but smoking rates among younger males and females were higher in Japan while older persons' smoking prevailed in Thailand. The second, the social strategies against smoking were stricter in Thailand (Ito S, *et al*., 2004 [17]). Next to primary prevention by education, we must develop an early detection method for the presence of smoking effects before tobacco-related diseases such as cardiovascular diseases, cancers, and others occur. Markers for detecting over-smoking should be considered just as γ-glutamyl transpeptidase (γGTP) is the indicator for alcohol overdrinking.

Our research group has investigated specific changes in conventional laboratory tests caused by smoking, and established over-smoking detection markers from conventional tests delivered in periodical health checks regulated by Japanese laws (Health Act in Community, and Health and Safety Act at Work). Our preliminary work was done for residents of Ebina City in Kanagawa Prefecture in order to reveal the smoking habits and their health conditions (Yamada M, *et al*., 1998 [33]). Heavy smokers showed increased white blood cell counts (WBC), increased levels of hemoglobin (Hb) and triglycerides (TG), and lower levels of total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) (Yamada M, *et al*., 1998 [33]). Itani *et al*. (1991) [16] reported that mean corpuscular volume of red blood cells (MCV), numbers of lymphocytes (Ly), urinary pH and carcinoembriogenic antigen (CEA) were useful indicators of the severity of smoking effects on the human body.

The present study was designed to clarify whether the above laboratory tests are markers indicating the severity of smoking effects on the human body, and whether these tests can identify a smoking condition.

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METHODS

Study Subjects

The subjects were 18,576 visitors to the Health Check Clinic of Tokai University Hospital between January 5, 2000, and December 28, 2000. All visitors were requested to provide information on past medical history of illness and to fill out questionnaires regarding drinking and smoking habits, diet, health condition, physical activity, sleeping time, and current drug use. The subjects underwent a physical examination, conventional laboratory tests including urinalysis, peripheral blood examination (red blood cell counts (RBC), Hb, MCV, WBC, Ly), clinical chemistry (total protein, albumin, albumin/globulin ratio, total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γGTP, TC, TG, HDL-C, blood sugar level (BS), creatinine, bound urea nitrogen (BUN), and oncogenic markers (CEA, alpha-fetoprotein), electrocardiogram, chest X-ray and gastrofluorography.

No subject had any health complaints, and no subject was undergoing therapy for tobacco-related diseases or cancer. All subjects signed the form indicating the purpose of the health check at University Hospital including health research. The ethics committee of both departments of Tokai University School of Medicine approved the study protocol in 2001.

Grading of Smoking Habits

The present study was designed to reveal the effect of smoking on the health body quantitatively so the association between the grade of smoking habit written in the questionnaires and the abnormal values of conventional laboratory tests was examined statistically.

The grading of smoking habits was designed as follows; 1: 0 cigarette (never smoked and ex-smokers), 2: less than 10 cigarettes per day, 3: 11 to 20 cigarettes per day, 4: more than 21 cigarettes per day.

Grading of Alcohol Drinking

According to the self-description by questionnaires the grading of alcohol drinking was divided as follows; 1: no drinking per week or 5 days of 22 gram and two days of no drinking or less than 3 days of 44 gram and other days of no drinking, 2: 22-gram intake every day or 4 to 5 days of 44 grams and other days no drinking, 3: 44-gram intake everyday or less than 3 days of 66 grams and other days of no drinking, 4: more than 4 days of 66 grams per week.

Statistical Analyses

A linear regression analysis between the values of laboratory tests and the magnitude of smoking was conducted. Qualitative values were expressed as the mean ± SD. Analysis of covariance (ANCOVA) was used for interaction effects of alcohol drinking on the relationship between the values of laboratory tests and the magnitude of smoking. If the regression coefficients were homogeneous across the presence of alcohol drinking by testing equality of slope, we calculated adjusted means of the values for laboratory tests with the presence of alcohol drinking as a covariate. To reveal the intensity of the modified effect of alcohol drinking on conventional laboratory tests affected by smoking habits, multiple regression analysis (backward elimination method) with the value of CEA as the independent variable and the other factors as dependent variables was conducted. Logistic regression analysis (backward elimination method) with the existence of an excess value of CEA as an independent variable was also performed.

To identify smokers in the whole subjects and identify excessive smokers in the smoking population, logistic regression analysis (backward elimination method) with the variable that shows the status of current smoking and with the variable that shows the status of excessive smoking as an independent variable was performed. The status of each value that shows an abnormal range for MCV, Hb, WBC, Ly, HDL-C, and CEA were treated as dependent variables in both models. The normal ranges of each value are indicated in Tables 4 and 5.

A level of $P < 0.05$ was considered to be significant. All analyses were performed using the computer program StatView 5.0 (SAS Institute, Cary, NC), except for the logistic regression analysis, which was performed with SPSS 11.0 (SPSS Inc, Tokyo, Japan).

RESULTS

Characteristics of the 18,576 Subjects

The numbers of each smoking grade by age and sex in the 18,576 subjects are shown in Table 1. The numbers of visitors in their twenties and thirties were smaller than those in their forties, fifties and sixties. In addition, smokers (of smoking grades 2 to 4) were seen in the forties to sixties more than in younger generations. As the purpose of the present study was to find the over-smoking markers from conventional laboratory tests, we focused on those in their forties to sixties of both genders. Since the effect of alcohol drinking on conventional laboratory tests is known, we examined the effect of smoking on human bodies modified by alcohol drinking. As the cardiovascular complications are usually observed at the people aged 40 years or older, we chose two age groups of 41 to 45 and 61 to 65 years old to compare the difference in the effect of smoking between these groups. And the numbers of subjects in both age groups were shown in Table 2.

Over-smoking Markers from Laboratory Tests

We compared the changes of laboratory data by smoking in 41-45-years group with those of 61-65-years group. The selected markers demonstrated a close association with the grades of smoking conditions by linear regression analysis (Figure 1, Table 3).

1. WBC: Increased WBCs were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of smoking 1 (never smokers and ex-smokers) shows $5453.8 \pm 1196.2/mm^3$, grade of smoking 2 (less than 10 cigarettes per day) $6034.2 \pm 1538.8/mm^3$, grade of smoking 3 (11 to 20 cigarettes per day) $6597.7 \pm 1715.1/mm^3$, and grade of smoking 4 (more than 21 cigarettes per day) $7409.9 \pm 2156.4/mm^3$. This increase of WBC by the smoking grade was statistically significant ($p < 0.001$). An association between the grade of smoking and WBC was also statistically significant in the remaining three groups.

Table 1 Number of subjects by grade of smoking, age and sex

Males

	≦25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66≦	total †
n	8	47	237	838	1552	2196	2727	2339	1007	930	11881
1*	4	13	114	427	785	1136	1502	1443	726	704	
2	0	7	29	75	117	165	173	150	58	82	
3	4	17	76	249	438	548	673	472	140	111	
4	0	10	18	87	212	347	379	274	83	33	

Females

	≦25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66≦	total
n	11	31	108	437	904	1383	1488	1146	630	554	6692
1	5	15	82	367	789	1206	1353	1064	590	532	
2	3	8	10	41	74	101	77	44	17	11	
3	2	4	10	28	35	68	51	32	15	11	
4	1	4	6	1	6	8	7	6	8	0	

† The total number of male subjects was 11,884, but 3 subjects were not included because they did not answer questions on smoking habit.

* The grade of smoking are as follows; 1: never smoked and ex-smoker, 2: less than 10 cigarettes per day, 3: 11 to 20 cigarettes per day, and 4: more than 21 cigarettes per day.

Table 2 Numbers of smokers with alcohol drinking in the age groups of 41-45 years old and 61-65 years old

Age Group (41-45) males

smoking \ alcohol	1**	2	3	4
1*	445	190	131	19
2	60	30	20	7
3	197	108	110	23
4	86	40	61	25

Age Group (41-45) females

smoking \ alcohol	1	2	3	4
1	717	58	13	1
2	62	10	2	0
3	22	8	3	2
4	4	2	0	0

Age Group (61-65) males †

smoking \ alcohol	1	2	3	4
1	413	174	119	19
2	31	17	10	0
3	61	32	41	6
4	33	13	27	10

Age Group (61-65) females

smoking \ alcohol	1	2	3	4
1	563	25	2	0
2	15	1	1	0
3	11	4	0	0
4	6	0	1	1

† The total numberd male subjects in age group(61-65) was 1,007, but 1 subject was not included because he did not answer the questions on drinking habit.

* The grade of smoking are the same as shown in Table 1.

**The grading of drinking habit as follows; 1: no drinking, 5 days of 22 g and two days of no drinking, or less than 3 days of 44 g and other days of no drinking, 2: 22 g intake every day, or 4 to 5 days of 44 g and other days of no drinking, 3: 44 g intake every day, or less than 3 days of 66 g and other days of no drinking, and 4: more than 4 days of 66 g per week.

Table 3 Linear regression analysis between the values of laboratory tests and grade of smoking

	Age	Regression coefficient	Intercept	P value	R^2
Males					
WBC	41-45	620.00	4816.8	<0.001	0.1870
	61-65	467.94	5303.4	<0.001	0.0923
Ly	41-45	134.07	1767.6	<0.001	0.0766
	61-65	125.56	1872.9	<0.001	0.0418
Hb	41-45	0.1229	14.868	<0.001	0.0211
	61-65	0.0742	14.816	0.037	0.0043
MCV	41-45	1.2078	89.129	<0.001	0.1137
	61-65	0.9680	91.900	<0.001	0.0542
HDL-C	41-45	−1.7958	60.449	<0.001	0.0213
	61-65	−1.4383	60.445	0.003	0.0087
CEA	41-45	0.7448	1.1225	<0.001	0.1979
	61-65	0.9241	1.7710	<0.001	0.1866
TC	41-45	−0.9528	201.36	0.183	0.0011
	61-65	−1.9563	207.33	0.046	0.0039
TG	41-45	8.5846	121.55	<0.001	0.0116
	61-65	9.4733	114.45	<0.001	0.0165
Females					
WBC	41-45	431.93	4698.0	<0.001	0.0280
	61-65	625.96	4722.4	<0.001	0.0517
Ly	41-45	95.542	1603.8	0.001	0.0118
	61-65	185.080	1784.4	<0.001	0.0253
Hb	41-45	0.1865	12.336	0.032	0.0051
	61-65	0.1661	13.135	0.040	0.0067
MCV	41-45	1.5138	86.344	<0.001	0.0139
	61-65	0.9869	90.421	0.001	0.0164
HDL-C	41-45	−3.0609	60.449	0.002	0.0106
	61-65	−2.7716	69.870	0.041	0.0067
CEA	41-45	0.8798	0.6581	<0.001	0.1386
	61-65	1.2908	1.1299	<0.001	0.1130
TC	41-45	−5.4578	200.91	0.005	0.0087
	61-65	−2.7716	221.12	0.989	
TG	41-45	4.5495	70.443	0.058	0.0040
	61-65	8.8421	91.996	0.030	0.0074

2. Ly: Increased numbers of Ly were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of smoking 1 shows 1900.6 ± 494.9/mm^3, grade of smoking 2, 2066.9 ± 475.7/mm^3; grade of smoking 3, 2158.9 ± 577.0/mm^3, and grade of smoking 4, 2313.1 ± 629.7/mm^3. This increase of Ly by the smoking grade was statistically significant ($p<0.001$). An association between the grade of smoking and Ly was also statistically significant in the remaining three groups.

3. MCV: Increased MCVs were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of smoking 1 shows 90.3 ± 3.6 fl, grade of smoking 2, 91.6 ± 4.3 fl; grade of smoking 3, 93.0 ± 3.8 fl, and grade of smoking 4, 93.6 ± 4.7 fl. This increase of MCV by the smoking grade was statistically significant ($p<0.001$). An association between the grade of smoking and MCV was also statistically significant in the remaining three groups.

4. Hb: Increased concentrations of Hb were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of smoking 1 shows 15.0 ± 0.9 g/dl, grade of smoking 2, 15.0 ± 1.0 g/dl; grade of smoking 3, 15.2 ± 1.0 g/dl, and grade of smoking 4, 15.4 ± 1.0 g/dl. This increase of Hb by the smoking grade was statistically significant ($p<0.001$). An association between the grade of smoking and Hb was also statistically

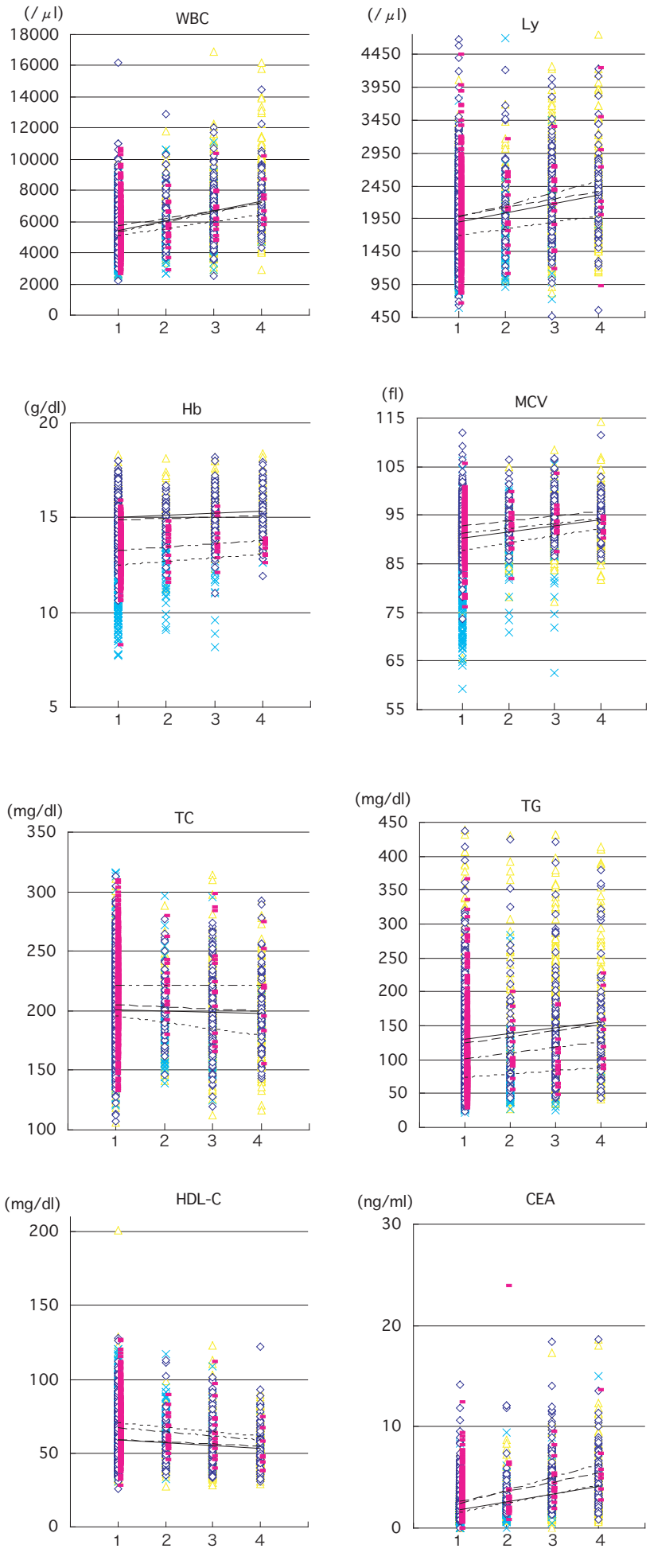

Fig. 1
The relations between the grade of smoking habits and laboratory test values.
Values of WBC (white blood cell counts), Ly (numbers of lymphocytes), Hb (hemoglobin concentration), MCV (mean corpuscular volume of red blood cells), TC (total cholesterol), TG (triglycerides), HDL-C (high density lipoprotein cholesterol) and CEA (carcinoembriogenic antigen) were compared among 4 groups with different smoking grades (1 to 4).
△——, 41 to 45-year-old males (n =1552);
×·····, 41 to 45-year-old females (n =904);
◇– – –, 61 to 65-year-old males (n =1007);
■–·–·–, 61 to 65-year-old females (n =630).

Table 4 Average laboratory data and grade of smoking in the age group of 41-45 years old

	Grade of smoking	Males			Females		
		n	mean	SEM	n	mean	SEM
WBC							
(4×10^3-8×10^3/μl)	1	785	5449.0	55.3	789	5142.0	46.7
	2	117	6033.6 [a.]	142.2	74	5310.8	152.4
	3	438	6601.3 [a.b.]	73.7	35	6239.7 [a.b.]	226.2
	4	212	7420.3 [a.b.c.]	106.6	6	6499.9 [a.]	535.3
Hb							
(males 13.5-17.5 females 11.5-15.5 g/dl)	1	785	15.0	0.0	789	12.5	0.0
	2	117	15.0	0.1	74	12.7	0.2
	3	438	15.2 [a.]	0.0	35	12.7	0.2
	4	212	15.4 [a.b.]	0.1	6	13.9	0.5
TC							
(140-220 mg/dl)	1	785	200.3	1.2	789	195.4	1.1
	2	117	199.0	3.0	74	190.8	3.5
	3	438	198.9	1.6	35	181.1	5.2
	4	212	197.3	2.3	6	194.7	12.2
TG							
(50-150 mg/dl)	1	785	130.7	3.3	789	75.2	1.3
	2	117	152.7	8.4	74	79.9	4.3
	3	438	144.2	4.4	35	76.3	6.4
	4	212	152.5 [a.]	6.3	6	107.6	15.1
HDL-C							
(40-100 mg/dl)	1	785	59.3	0.5	789	70.4	0.5
	2	117	55.2 [a.]	1.3	74	67.4	1.7
	3	438	54.5 [a.]	0.7	35	61.3 [a.]	2.6
	4	212	52.9 [a.]	1.0	6	58.3	6.1
MCV							
(84-99 fl)	1	785	90.4	0.1	–	–	–
	2	117	91.7 [a.]	0.4	–	–	–
	3	438	92.9 [a.b.]	0.2	–	–	–
	4	212	93.3 [a.b.]	0.3	–	–	–
Ly							
(800-3200/μl)	1	785	1896.4	19.3	789	1700.6	16.0
	2	117	2066.4 [a.]	49.7	74	1767.5	52.4
	3	438	2162.0 [a.]	25.7	35	1870.0	77.7
	4	212	2322.3 [a.b.c.]	37.2	6	2273.4 [a.b.]	183.9
urine pH							
(5.0-8.0)	1	785	6.2	0.0	789	6.3	0.0
	2	117	6.2	0.0	74	6.3	0.1
	3	438	6.2	0.0	35	6.4	0.1
	4	212	6.2	0.0	6	6.2	0.2
CEA							
(≦5.0 ng/ml)	1	785	1.9	0.1	789	1.6	0.0
	2	117	2.6 [a.]	0.2	74	2.1 [a.]	0.1
	3	438	3.3 [a.b.]	0.1	35	3.4 [a.b.]	0.2
	4	212	4.2 [a.b.c.]	0.1	6	4.8 [a.b.]	0.5

In the paracentheses are shown the normal ranges of each value in Tokai University Hospital.
[a.] $p<0.05$ vs. grade 1, [b.] $p<0.05$ vs. grade 2, [c.] $p<0.05$ vs. grade 3
SEM: standard error of means

significant in the remaining three groups.

5. TC: Decreased values of TC were observed according to the grade of smoking as shown in Figure 1. In the age group of 61- 65 years old (males), grade of smoking 1 shows 204.1 ± 31.3 mg/dl, grade of smoking 2, 130.9 ± 31.3 mg/dl; grade of smoking 3, 204.1 ± 31.5 g/dl, and grade of smoking 4, 204.5 ± 31.5 mg/dl. This decrease of TC by the smoking grade was statistically significant ($p = 0.046$). The age group of 41-45 years old (males), however, showed the tendency of decrease in TC values without significant difference according to the grade of smoking. For females, only the age group of 41-45 years old showed significant difference in their TC values, but the age group of 61-65 years old showed the same tendency without statistical significance.

6. TG: Increased values of TG were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of smoking 1 shows 129.1 ± 88.6 mg/dl, grade of smoking 2, 152.5

Table 5 Average of laboratory data and grade of smoking in the age group of 61-65 years old

	Grade of smoking	Males			Females		
		n	mean	SEM	n	mean	SEM
WBC							
(4×10^3-8×10^3/μl)	1	726	5752.6	54.9	590	5354.3	52.5
	2	58	6519.5 [a.]	193.7	17	5628.8	309.2
	3	140	6595.5 [a.]	125.1	15	6685.5 [a.]	330.5
	4	83	7331.6 [a.b.c.]	163.5	8	7359.3 [a.b.]	461.2
Hb							
(males 13.5-17.5 females 11.5-15.5 g/dl)	1	726	14.9	0.0	590	13.3	0.0
	2	58	14.8	0.1	17	13.6	0.2
	3	140	15.0	0.1	15	13.9	0.2
	4	83	15.2	1.0	8	13.3	0.3
TC							
(140-220 mg/dl)	1	726	205.3	1.2	590	221.0	1.3
	2	58	206.5	4.1	17	224.5	7.7
	3	140	201.0	2.7	15	224.7	8.2
	4	83	198.9	3.5	8	216.1	11.5
TG							
(50-150 mg/dl)	1	726	124.4	2.7	590	100.6	2
	2	58	136.7	9.7	17	117.3	11.7
	3	140	141.4	6.2	15	105.2	12.5
	4	83	148.3 [a.]	8.2	8	150.4 [a.]	17.5
HDL-C							
(40-100 mg/dl)	1	726	59.4	0.6	590	67.2	0.7
	2	58	58.5	2.0	17	63.8	3.8
	3	140	54.9 [a.]	1.3	15	64.7	4.1
	4	83	52.7 [a.]	1.7	8	46.3 [a.c.]	5.7
MCV							
(84-99 fl)	1	726	93.0	0.1	590	91.5	0.1
	2	58	94.4 [a.]	0.5	17	92.7	0.9
	3	140	94.8 [a.]	0.3	15	93.8	0.9
	4	83	94.6 [a.]	0.4	8	92.2	1.3
Ly							
(800-3200/μl)	1	726	1996.9	22.6	590	1969.5	22.5
	2	58	2167.1	79.7	17	2174.4	132.5
	3	140	2207.8 [a.]	51.5	15	2274.5	141.6
	4	83	2428.7 [a.]	67.3	8	2607.1 [a.]	197.6
urine pH							
(5.0-8.0)	1	726	6.4	0.0	590	6.4	0.0
	2	58	6.4	0.1	17	6.3	0.1
	3	140	6.3	0.1	15	6.4	0.2
	4	83	6.3	0.1	8	5.9	0.2
CEA							
(≦5.0 ng/ml)	1	–	–	–	590	2.4	0.0
	2	–	–	–	17	4.1 [a.]	0.4
	3	–	–	–	15	4.5 [a.]	0.4
	4	–	–	–	8	6.0 [a.b.]	0.6

In the paracentheses are shown the normal ranges of each value in Tokai University Hospital.
[a.] $p<0.05$ vs. grade 1, [b.] $p<0.05$ vs. grade 2, [c.] $p<0.05$ vs. grade 3

± 108.0 mg/dl; grade of smoking 3, 145.4 ± 86.3 g/dl, and grade of smoking 4, 156.0 ± 102.0 mg/dl. This increase of TG by the smoking grade was statistically significant ($p<0.001$). An association between the grade of smoking and TG was also statistically significant in the both genders of 61-65 years old.

7. HDL-C: Decreased values of HDL-C were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of smoking 1 shows 58.9 ± 14.6 mg/dl, grade of smoking 2, 55.1 ± 12.4 mg/dl; grade of smoking 3, 54.9 ± 14.0 mg/dl, and grade of smoking 4, 53.9 ± 12.9 mg/dl. This decrease of HDL-C by the smoking grade was statistically significant ($p<0.001$). An association between the grade of smoking and HDL-C was also statistically significant in the remaining three groups.

8. CEA: Increased values of CEA were observed according to the grade of smoking as shown in Figure 1. In the age group of 41-45 years old (males), grade of

Table 6 Multiple regression analysis (backward elimination) with CEA as the dependent variable

	B	SEM	β	t value	P value
Males (41-45 years old)					
Constant	0.943	0.803		1.174	0.241
RBC	−0.007	0.002	−0.132	−3.660	<0.001
Ly	−0.012	0.007	−0.044	−1.813	0.070
WBC	−0.000	0.000	0.158	5.837	<0.001
Hb	0.165	0.071	0.084	2.336	0.020
HDL-C	0.013	0.003	0.093	4.047	<0.001
smoking	0.605	0.043	0.362	13.934	<0.001
Males (61-65 years old)					
Constant	−5.140	2.863		−1.795	0.073
age	0.081	0.043	0.052	1.897	0.058
WBC	0.001	0.000	0.089	2.945	0.003
Hb	0.104	0.055	0.055	1.885	0.060
TC	−0.004	0.002	−0.065	−2.274	0.023
HDL-C	0.023	0.004	0.166	5.161	<0.001
GGTP	0.002	0.001	0.064	2.198	0.028
smoking	0.845	0.064	0.395	13.207	<0.001
alcohol	0.141	0.074	0.059	1.925	0.055
BMI	−0.048	0.026	−0.057	−1.875	0.061

B: non-standardized coefficient
β: standardized coefficient

Table 7 Logistic regression analysis (backward elimination) with CEA as the dependent variable

	Regression coefficient	SEM	χ^2 value	P value	OR (95%CI)
Males (41-45 years old)					
Constant	−11.160	2.094	28.403	<0.001	0.000
WBC	0.000	0.000	26.610	<0.001	1.000 (1.000-1.000)
TG	−0.040	0.001	10.435	0.001	0.996 (0.993-0.998)
smoking	0.749	0.099	57.229	<0.001	2.114 (1.742-2.567)
MCV	0.064	0.022	8.081	0.004	0.020 (1.020-1.114)
Males (61-65 years old)					
Constant	−3.828	1.223	7.804	0.002	0.022 (1.000-1.000)
WBC	0.000	0.000	4.333	0.037	1.000 (1.000-1.000)
HDL-C	0.019	0.007	8.358	0.004	1.019 (1.006-1.033)
smoking	0.736	0.089	68.616	<0.001	2.087 (1.754-2.484)
alcohol	0.226	0.107	4.455	0.035	1.254 (1.016-1.547)
BMI	−0.080	0.041	3.273	0.070	0.928 (0.856-1.006)

OR: odds ratio
CI: confidence interval

smoking 1 shows 1.9 ± 1.1 ng/dl, grade of smoking 2, 2.6 ± 1.5 ng/dl; grade of smoking 3, 3.3 ± 2.0 ng/dl, and grade of smoking 4, 4.1 ± 2.8 ng/dl. This increase of CEA by the smoking grade was statistically significant ($p < 0.001$). An association between the grade of smoking and CEA was also statistically significant in the remaining three groups.

Effect of Alcohol Drinking on Selected Markers

The adjusted means of the values for WBC, Ly, MCV, Hb, HDL-C, and CEA with the presence of alcohol drinking as covariates were obtained by ANCOVA. Table 4 (41-45 years old) and Table 5 (61-65 years old) show the mean values with SEM of the selected markers (WBC, Hb, TC, TG, HDL-C, MCV, Ly, urinary pH and CEA) by grades of smoking. These values exhibited significant correlation with the grades of smoking after correction by alcohol drinking. In these analyses, MCV in the age group of 41-45 years old and CEA in the age group of 61-65 years old were somewhat affected by alcohol drinking. WBC, Ly, and CEA among the 41-45 year-old group were statistical reliable markers, but TC, TG and HDL-C were also somewhat affected by alcohol drinking.

Multiple Regression Analysis and Logistic Regression Analysis for Selected Markers

The multiple regression analysis was performed using CEA, WBC, and HDL-C as dependent variables, and age, smoking, drinking, BMI, RBC, MCV, MCH, MCHC, Hb, WBC, Ly, NE, CEA, TC, TG, HDL-C, GPT, γGTP and CRP as dependent variable. The analysis using CEA as the independent variable showed that CEA was not affected by alcohol drinking in male groups (Table 6). In females, CEA was affected by

Table 8 Logistic analysis to differentiate smokers from non-smokers (forward selection)

	OR (95% Cl)	P value
Males (n =11873)		
Grade of drinking		
Grade of drinking 1*	Control	
Grade of drinking 2	2.76 (2.27-3.36)	<0.001
Grade of drinking 3	2.29 (1.87-2.81)	<0.001
Grade of drinking 4	1.51 (1.23-1.85)	<0.001
Unusual value in MCV	2.79 (2.35-3.32)	<0.001
Unusual value in WBC	4.79 (4.13-5.55)	<0.001
Unusual value in Ly	0.77 (0.70-0.86)	<0.001
Unusual value in HDL-C	2.48 (2.11-2.91)	<0.001
Unusual value in CEA	6.25 (5.44-7.17)	<0.001
Age	1.05 (1.05-1.06)	<0.001
BMI	1.05 (1.04-1.07)	<0.001
Females (n =6691)		
Grade of drinking		
Grade of drinking 1*	Control	
Grade of drinking 2	7.52 (3.33-16.96)	<0.001
Grade of drinking 3	2.67 (1.15-6.17)	0.022
Grade of drinking 4	1.04 (0.42-2.55)	0.941
Unusual value in MCV	2.73 (1.63-4.57)	<0.001
Unusual value in WBC	3.98 (1.92-8.28)	<0.001
Unusual value in Ly	3.40 (2.40-4.82)	<0.001
Unusual value in HDL-C	2.99 (1.62-5.50)	<0.001
Unusual value in CEA	10.39 (7.83-13.79)	<0.001
Age	1.06 (1.05-1.08)	<0.001
BMI	1.03 (1.00-1.06)	0.048

*The grades of drinking are the same as shown in Table 2.

Table 9 Logistic analysis to differentiate light smokers from heavy smokers (backward elimination)

	OR (95%Cl)	P value
Males (n =5025)		
Grade of drinking		
Grade of drinking 1*	Control	
Grade of drinking 2	2.44 (1.94-3.07)	<0.001
Grade of drinking 3	3.46 (2.69-4.44)	<0.001
Grade of drinking 4	2.12 (1.66-2.69)	<0.001
Unusual value in MCV	1.38 (1.13-1.69)	0.002
Unusual value in WBC	1.83 (1.57-2.13)	<0.001
Unusual value in Ly	0.83 (0.70-0.99)	0.037
Unusual value in CEA	1.81 (1.57-2.08)	<0.001
Females (n =689)		
Grade of drinking		
Grade of drinking 1	Control	
Grade of drinking 2	10.76 (3.59-32.29)	<0.001
Grade of drinking 3	9.68 (2.65-35.32)	0.001
Grade of drinking 4	8.73 (2.01-37.99)	0.004
Unusual value in CEA	3.78 (2.00-7.17)	<0.001

*The grades of drinking are the same as shown in Table 2.

alcohol drinking in the 61 to 65-year-old group, but not in the 41 to 45-year-old group (data not shown). Other markers were not affected by alcohol drinking in any age groups of both genders. Furthermore, logistic regression analysis using abnormality in CEA (higher than 5 ng/dl) as the dependent variable was performed. The presence of abnormality in CEA was significantly influenced by alcohol drinking in the 61 to 65-year old males, but not in the 41 to 45-year-old males (Table 7). The same result was obtained among the female groups (data not shown).

Logistic Analysis to Differentiate Smokers from Non-smokers

Logistic analysis to differentiate smokers from non-smokers revealed that the significant factors were high CEA readings, high WBC readings, alcohol drinking (44 grams alcohol intake 4 to 5 days a week, or 22 grams every day), high MCV readings, alcohol drinking (44 grams alcohol intake every day, or 66 grams

Table 10 Effects of accumulated number of abnormalities on differentiation of light smokers from heavy smokers

A : Accumulated number of abnormalities

	0	1	2	3	4	Total
light smokers	1666	1403	437	75	2	3583
heavy smokers	489	595	300	55	3	1442
Total	2155	1998	737	130	5	5025

B : Logistic analysis

	OR (95%CI)	Significant value
Grade of drinking		
Grade of drinking 1*	Control	
Grade of drinking 2	2.54 (2.03-3.18)	<0.001
Grade of drinking 3	3.44 (2.68-4.41)	<0.001
Grade of drinking 4	2.12 (1.67-2.69)	<0.001
Number of abnormalities		
No abnormalities	Control	
One abnormality	4.66 (0.75-28.91)	0.098
Two abnormalities	3.31 (0.53-20.50)	0.199
Three abnormalities	2.01 (0.30-12.48)	0.455
Four abnormalities	1.93 (0.30-12.32)	0.489

* The grades of drinking are the same as shown in Table 2. (n =5025)

3 days a week), alcohol drinking (66 grams alcohol intake for more than 4 days a week) for 11,873 men (Table 8). For 6,691 women, the same analysis indicated that the significant factors were high CEA readings, alcohol drinking (44 grams alcohol intake 4 to 5 days a week, or 22 grams every day), high WBC readings, low Ly readings, high MCV readings, alcohol drinking (44 grams alcohol intake everyday, or 66 grams 3 days a week) (Table 8).

Logistic Analysis to Differentiate Light Smokers from Heavy Smokers

Logistic analysis to differentiate light smokers (less than 20 cigarettes a day) from heavy smokers (more than 21 cigarettes a day) revealed that the significant factors were alcohol drinking (44 grams alcohol intake every day, or 66 grams 3 days a week), alcohol drinking (44 grams alcohol intake 4 to 5 days a week, or 22 grams every day), increase in WBC, increase in CEA, and increase in MCV for 5,025 men (Table 9). For 689 women, alcohol drinking (44 grams alcohol intake 4 to 5 days a week, or 22 grams every day), alcohol drinking (44 grams alcohol intake every day, or 66 grams 3 days a week), alcohol drinking (66 grams alcohol intake for more than 4 days a week), and increase in CEA were the significant factors differentiating light smokers from heavy smokers (Table 9).

However, logistic analysis to differentiate light smokers (less than 20 cigarettes a day) from heavy smokers (more than 21 cigarettes a day) by combination of those abnormalities showed no significance for the accumulation of abnormal values (Table 10).

Based on these analyses, MCV, WBC, HDL-C, and CEA were determined as markers for detecting the effects of smoking on human body. As shown in Table 10, if one of the abnormalities of these markers is seen in a healthy person, he/she appears to be a smoker with effects of smoking on his/her physical condition.

DISCUSSION

In order to detect smoking habit, measurement of serum nicotine and cotinine concentrations is usually used. However, the assay methods are not simple and also very expensive (Jacob, *et al.*, 1981 [18], Benowitz, *et al.*, 1989 [3], Perez-Stable EJ, *et al.*, 1995 [25]). If other smoking markers could be developed, individuals with these abnormalities could be considered or suspected to be affected by smoking, and physicians and paramedical staffs could use such smoking markers to prevent tobacco-related diseases. It is also important from a viewpoint that the response to smoking may be different in each individual, like γGTP values induced by drinking, and can not determined simply by the number of cigarette consumed. Smoking not only causes abnormalities that show up in conventional laboratory tests, but also reduces respiratory functions such as forced expiratory volume during 1 second (FEV_1), and forced vital capacity (Bolliger, *et al.*, 2002 [4]). Markers for detecting a smoker affected, however, should be selected from conventional laboratory tests because they are less expensive and easy to conduct.

In the present study, none of the laboratory data showed remarkable abnormalities in the smokers. In addition, such small differences may be caused by other factors including alcohol drinking, foods intake, sleep time and exercise, as well. However, since Figure 1 has clearly shown a dose-response relationship between the values of laboratory tests and the grades of smoking, we believe that the changes observed in the laboratory data represent the effects of smoking.

The effects of smoking on conventional laboratory tests have been reported from a number of laboratories (Chalmers DM, *et al.*, 1979 [5], IARC, 1986 [13], Iho S, *et al.*, 2003 [14], Suzuki N, *et al.*, 1999 [29], Garrison RJ, *et al.*, 1978 [9], Imamura H, *et al.*, 2002 [15]). They are composed of the abnormalities of four

major systems, that is, the erythrocyte system, leukocyte system, serum lipids, and CEA. The present study has revealed that some smokers exhibit abnormality in one of the above, and a few show accumulated numbers of abnormalities, as shown in the logistic analysis to discriminate smokers from non-smokers (Table 10).

1. Effect of Smoking on the Erythrocyte System

It has been speculated that smoking may cause increase in MCV and Hb because of a compensatory response to reduced oxygen-carrying capacity, caused by either the persistent presence of carboxyhemoglobin or chronic lung disease due to smoking (Chalmers DM, *et al.*, 1979 [5]). Recently, Yokoyama *et al.* (2003) [35] reported that inactive ALDH2 dramatically increased blood acetaldehyde levels after alcohol intake, and that alcohol-related red cell volume changes associated with inactive ALDH2 in Japanese men suggested the role of acetaldehyde in increasing MCV (Yokoyama M, *et al.*, 2003 [35]). Moreover, acetaldehyde, one of the major chemical constituents of tobacco smoke (IARC, 1986 [13]), may also cause increase in MCV. In the present study the same effect of smoking on the erythrocyte system was observed (Figure 1). Although ANCOVA revealed that the effect of alcohol was related somewhat to the grade of smoking, logistic analysis to differentiate smokers from non-smokers and that to differentiate light smokers from heavy smokers demonstrated the importance of MCV as a significant smoking marker. Hb, however, was not identified as a significant factor in the series of multiple regression analysis and three logistic analyses. If a healthy person does not show any abnormal laboratory data except increased MCV of, for example, over 100, his/her physical condition is considered to be affected by smoking.

2. Effect of Smoking on the Leukocyte System

WBC increases in smokers by grade of smoking. This finding has been reported previously, and decreasing subsets of WBCs have been investigated. Increased serum level of inflammatory cytokines observed in heavy smokers, such as RANTES (regulated upon activation, normal T cells expressed and secreted), affects the proliferation of neutrophils (Iho S, *et al.*, 2003 [14]), and nicotine has an apoptotic action to lymphocytes (Suzuki N, *et al.*, 1999 [29]). If a healthy person does not show any abnormal laboratory data except increased WBC of, for example, over 10,000/mm^3, he/she should be considered to be an affected smoker.

3. Effect of Smoking on Serum Lipid Levels

Decreased levels of HDL-C, LDL-C and total cholesterol, and increased level of TG have been observed in smokers (Garrison, *et al.*, 1978 [9]). Regarding the mechanism of the decreased HDL-C level observed in smokers, the smoke components have been suggested to influence enzymes that regulate lipoprotein metabolism such as lecithin, cholesterol acyltransferase (LCAT) or hepatic lipase (Richard, *et al.*, 1997 [27]). On the other hand, alcohol drinking induces the increase in serum level of HDL-C (Gaziano, *et al.*, 1993 [10], Minami, *et al.*, 2002 [22]). De Olivera e Silva *et al.* (2000) [7] reported that ethanol increased HDL-cholesterol by raising transport rates of the major HDL apolipoproteins, apo A-1 and apo A-II. Imamura *et al.* (2002) [15] investigated serum lipids in young women smokers and found that smokers had significantly lower level of mean HDL-C, HDL_2-C, total cholesterol, and LCAT activity than non-smokers. Smoking cessation or alcohol restriction induced reversal in HDL-C concentration (Minami, *et al.*, 2002 [22]). The present study, however, revealed that HDL-C is a promising marker to discriminate smokers from non-smokers. If a healthy person under 40 years of age does not show any abnormal laboratory data except decreased level of HDL-C of, for example, under 30 mg/dl, he/she should be considered to be an affected smoker.

4. Effect of Smoking on Serum Level of CEA

Since Gold and Freedman (1965) [11] found that CEA distributed in embryonic gastrointestinal tissues and cancer lesions of human, CEA has been used as a tumor marker. On the other hand, it has been shown that CEA is positive in smokers (Stevens, *et al.*, 1973 [28]; Alexander, *et al.*, 1976 [1]), and in 3% of male smokers (Fukuda, *et al.*, 1998 [8]), and that about 70% of the positive males are smokers (Fukuda, *et al.*, 1998 [8]). The mechanism of the increased serum level of CEA in smokers has not been clarified. If a healthy person does not show any abnormal laboratory data except increased level of CEA of, for example, over 6 ng/dl, he/she should be considered to be an affected smoker.

These markers also suggest the hazardous effects of smoking because decreased level of HDL-C is one of the risks for cardiovascular diseases. Increased WBCs suggest the inflammatory process after chronic exposure to smoking, and an increase in MCV suggests alcohol drinking and/or chronic exposure to smoking. The increased level of CEA has been reported to be a predicting marker for esophageal cancer (Yokoyama A, *et al.*, 2003 [34]), and even if the presence of cancer is not confirmed, it is an important marker for detecting an individual affected by smoking. A prospective study should be performed to determine whether the incidence of cardiovascular diseases and cancers is higher in the smokers with abnormal marker values than in others. It will eventually contribute to prevention and early diagnosis of those tobacco-related diseases.

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