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CONTENTS

Vol. 4 No. 1

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Original Article

- Contrast-enhanced Ultrasonography for Differential Diagnosis of Protruding Lesions of the Gallbladder
Hironao Miyoshi, Kazuo Inui, Satoshi Yamamoto, Hironao Matsuura, Yoshiaki Katano 3
- Outcomes of Elective Colonoscopy in Our Health Care Center
Sumiko Goto 8
- Prevalence of Atherosclerotic Lesions in Asymptomatic Japanese Subjects in a Health Check-up
Yuki Ohmoto-Sekine, Makiko Ishihara, Rieko Ishimura, Ritsuko Honda, Kazuhisa Amakawa,
Akiko Iwao, Kyoko Ogawa, Hiroshi Tsuji, Sugao Ishiwata, Minoru Ohno, Yasuji Arase 16
- Nonalcoholic Fatty Liver Disease is More Strongly Associated with Arteriosclerosis than is Abdominal Obesity
in Health Check-up Examinees
Minako Ito, Yasuhiko Mochimatsu 21
- Risk Factors for Cerebral Arteriosclerosis Detected by Magnetic Resonance Angiography in Brain Check-ups
Kazuhiro Ohwaki, Akira Tamura, Tomohiro Inoue, Isamu Saito 28
- New Low-cost Method for Detecting Abnormal Thyroid Function in Patients Making Use of a Set of Routine-tests:
Adding their Average Rates of Annual Time-series Variations Improves Diagnostic Accuracy
Sorama Aoki, Sono Nishizaka, Kenichi Sato, Kenji Hoshi, Junko Kawakami, Kouki Mori,
Yoshinori Nakagawa, Wataru Hida, Katsumi Yoshida 32
- Association between Leukocyte Count and Age, Body Mass Index, and Lifestyle-related Factors:
a Cross-sectional Study in Ningen Dock Examinees
Yoshiaki Hashimoto, Azusa Futamura 39

Notifications

- Acknowledgments 44
- The Regulations of ISND 45
- Instructions to Authors 50

Contrast-enhanced Ultrasonography for Differential Diagnosis of Protruding Lesions of the Gallbladder

Hironao Miyoshi, Kazuo Inui, Satoshi Yamamoto, Hironao Matsuura, Yoshiaki Katano

Abstract

Objective: We studied the usefulness of contrast-enhanced ultrasonography in assessment of protruding lesions of the gallbladder detected in ultrasonographic mass screening.

Patients and Methods: Twenty-eight patients were suspected to have gallbladder carcinoma based on screening examinations using non-contrast abdominal ultrasonography that detected protruding gallbladder lesions (pedunculated and at least 10 mm in diameter, 25; sessile, 3). We performed contrast-enhanced ultrasonography as a further examination in such patients, and diagnosed malignancy when the findings included high echogenicity, irregularly distributed flow of contrast medium, and a dendriform pattern, while low echogenicity and presence of microcystic structures suggested a benign lesion. We made the final diagnosis based on histopathologic examination of resected specimens or ultrasonographic findings after follow-up for at least 12 months. We determined the diagnostic performance of contrast ultrasonography and extent of surgical resection avoidance achieved with this method.

Results: Sensitivity of contrast ultrasonography in identifying neoplasms was 100% (4/4), specificity was 70.8% (17/24), and overall accuracy was 75.0% (21/28). Surgical intervention was avoided in 64.3% of patients (18/28).

Conclusion: Contrast ultrasonography represents a useful, minimally invasive method for evaluating protruding gallbladder lesions suspected to be cancer in abdominal ultrasonography.

Keywords gallbladder, cancer, cholesterol polyp, contrast ultrasonography

Polypoid lesions of the gallbladder are commonly detected in healthy persons undergoing mass screening. The reported prevalence of gallbladder polypoid lesions in Japan as detected by abdominal ultrasonography is 5.9%¹, compared with 4.3% for Denmark² and 6.9% for Taiwan³. Surgery is recommended for the treatment of pedunculated protruding gallbladder lesions of at least 10 mm in diameter as well as sessile protruding lesions, since lesions with such features are suggestive of gallbladder cancer or adenoma^{4,5}. However, non-neoplastic lesions such as cholesterol polyps, hyperplastic polyps, and localized adenomyomatosis of the gallbladder are also common among protruding lesions of this size.

Differentiating neoplastic from non-neoplastic lesions is very important. In the present study, we assessed the usefulness of contrast-enhanced ultrasonography (CEUS) in characterizing protruding lesions of the gallbladder initially detected in mass screening using ab-

dominal ultrasonography.

Patients and Methods

Our hospital performed conventional ultrasonography on 8,379 patients between 2009 and 2011. The subjects of this study were 28 patients from among them who were referred to our department for evaluation of protruding gallbladder lesions (25, pedunculated and at least 10 mm in diameter; 3, sessile) that were suspected to be carcinomas of the gallbladder when initially detected in ultrasonographic screening examination of the abdomen. The patients were 12 men and 16 women, ranging in age from 34 to 77 years (median, 53 years). The median size of the pedunculated lesions was 12.4 mm, and that of the sessile lesions 12.2 mm. Final diagnoses of the protruding lesions of the gallbladder were based on pathologic evaluation of surgically resected specimens or ultrasonographic findings obtained during a follow-up period of at least 12 months (median, 40

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months; **Table 1**). CEUS was performed by a gastroenterologist who had more than 14 years of experience in CEUS and was a Board Certified Fellow and Senior Fellow (Gastroenterology) of the Japan Society of Ultrasonics in Medicine.

Perflubutane (Sonazoid; Daiichi-Sankyo, Tokyo) was used as the contrast medium for ultrasonography. Ultrasonographic systems and settings used were SSA-790A (Toshiba Medical Systems, Tokyo) with a mechanical index of 0.1 to 0.3 and a frame rate of 10 to 15/second, or TUS-A 500 (Toshiba Medical Systems, Tokyo) with a mechanical index of 0.15 and a frame rate of 10 to 15/second. Scans were obtained over 30 seconds immediately after injection of contrast medium. In scanning using the TUS-A 500, micro-flow imaging was carried out, beginning 3 minutes after injection to ensure visualization. Because the setting conditions for CEUS vary according to the ultrasound device, it is necessary for them to be made by an engineer who knows its characteristics

well. CEUS should be performed under the direction of an expert when there is little experience.

In CEUS, lesions with high echogenicity, irregular flow distribution of contrast medium, or a dendriform pattern were considered likely to be neoplastic and possibly malignant based on our previous studies⁶⁻⁸. These lesions were assessed further by imaging modalities such as contrast computed tomography and endoscopic ultrasonography. On the other hand, lesions with either low echogenicity or microcystic structures in CEUS were considered benign (**Fig. 1**). Patients with such lesions were managed by periodic follow-up.

This study was conducted with the informed consent of each participant after the present investigators obtained approval from the Institutional Review Board of Fujita Health University.

We selected treatment based on CEUS findings and sometimes those of additional imaging procedures. The diagnostic performance of CEUS was assessed by com-

Table 1. Final Diagnosis of 28 Gallbladder Lesions

Macroscopic appearance	No. of lesions	Size (mm)	Median size (mm)	Final diagnosis	No. of lesions (resected cases)
Pedunculated	25	10~23	12.4	adenoma	2 (2)
		11~13	12.2	hyperplastic polyp	4 (4)
				cholesterol polyp	13 (2)
				adenomyomatosis	6 (6)
Sessile	3			adenocarcinoma (arising in adenoma)	2 (2)
				adenomyomatosis	1 (0)

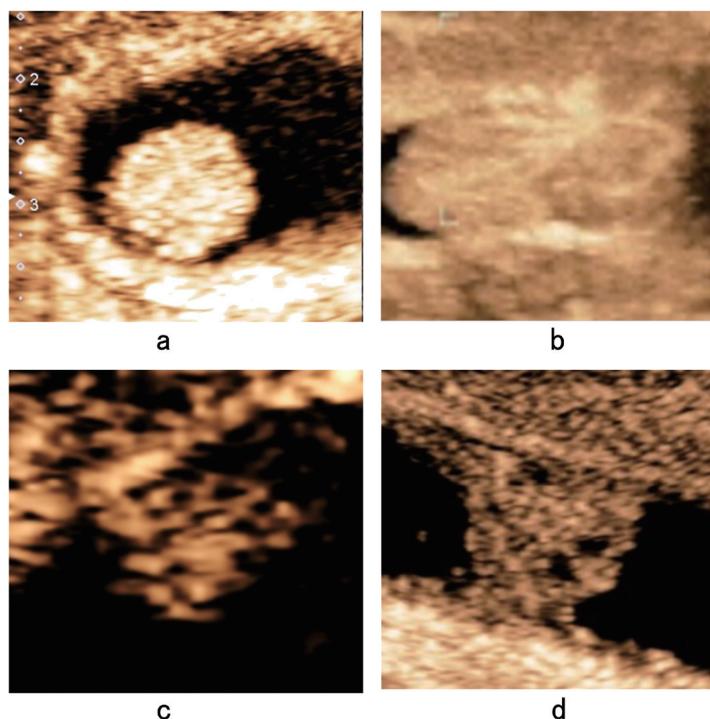


Fig. 1. Contrast Ultrasonographic Findings

Malignant findings include high echogenicity (a) and dendriform patterns (b). Benign findings include low echogenicity (c) and presence of microcystic structures (c, d).

parison of the sonographic diagnoses with either pathologic diagnoses of resection specimens or diagnoses obtained from follow-up over at least 12 months.

Results

Among the 25 protruding lesions that were pedunculated and measured at least 10 mm in diameter, 16 (64.0%) were diagnosed by contrast ultrasonography as benign and 9 (36.0%) as possibly malignant. Of the 3 sessile protruding lesions, 1 (33.3%) was diagnosed ultrasonographically as benign, and 2 (66.7%) were diagnosed as possibly malignant (**Table 2**).

Contrast ultrasonographic findings and final diagnoses were compared for the 28 protruding lesions. Lesions were diagnosed by CEUS as possibly malignant in 11 patients (39.3%), 10 of whom underwent surgical resection after other imaging procedures (see below). Pathologic evaluation of the resected specimens found that 2 were gallbladder carcinomas (20.0%), 2 were adenomas (20.0%), and 6 were non-neoplastic polyps (60.0%). One patient did not undergo surgery because the lesion suspected to be malignant in CEUS had no malignant findings in non-contrast computed tomography. Instead, the patient underwent follow-up examinations. Pathologically non-neoplastic polypoid lesions were hyperplastic polyps in 4 instances and cholesterol polyps in 2. Of the 17 patients with non-neoplastic lesions according to CEUS, 11 (64.7%) were diagnosed with polyps and 6 (35.3%) with adenomyomatosis. Surgical resection was not undertaken for any of these 17 patients.

The 17 patients with no malignancy findings plus the 1 patient whose suspicious-looking lesion no longer appeared malignant in computed tomography made 18 patients (64.3%) in whom surgical intervention was avoided (**Fig. 2**).

As for the diagnostic performance of CEUS for protruding lesions, sensitivity in detecting neoplasms was 100% (4/4), specificity was 70.8% (17/24), and overall diagnostic accuracy was 75.0% (21/28; **Table 3**).

No allergic reactions to contrast media or any other adverse outcomes of diagnostic procedures occurred in any patient in this study.

Discussion

Mihara *et al.*⁹ reported the detection rate for gallbladder cancer in mass screening of 1,306,947 persons as 0.01% (143 cases). Although abdominal ultrasonography is considered sufficient for early detection of gallbladder cancer, protruding lesions of the gallbladder include various types, such as adenocarcinoma, adenoma, hyperplasia, cholesterol polyp, inflammatory polyp, and adenomyomatosis. Okamoto *et al.*¹⁰ reported that gallbladder cancer was found in only 0.01% of 194,767 subjects examined, so almost all protruding gallbladder lesions detected by abdominal ultrasonography prove to be benign lesions, such as cholesterol polyps and adenomyomatosis of the gallbladder. Terzi *et al.*¹¹ reported that among 100 polypoid lesions of the gallbladder treated by surgical resection, there were 74 benign polypoid lesions, consisting of 39 cholesterol polyps, 20 adenomas, and 15 with adenomyomatous hyperplasia, and 26 malignant polypoid lesions. Thus, the majority of the surgically resected protruding lesions were benign. This means that preoperative differential diagnosis of cancer of the gallbladder is very important because even in the case of protruding lesions measuring 10–15 mm, the majority are found to be benign after surgical resection.

For the further evaluation of patients with abnormal abdominal ultrasonographic findings, it is recommended to perform endoscopic ultrasonography, computed tomography, magnetic resonance imaging, and/or magnetic resonance cholangiopancreatography. The reported diagnostic accuracy of endoscopic ultrasonography for protruding lesions of the gallbladder is 86.5% to 97.0%^{12,13}. In the case of non-contrast computed tomography, detection rates for gallbladder tumors (cancers, adenomas, and metastatic masses) and cholesterol polyps are 81% and 7%, respectively. For the diagnosis of gallbladder tumors, with a sensitivity of 88%, specificity of 87%, positive predictive value of 88%, negative predictive value of 87%, and the overall accuracy is 87%, the diagnostic performance of non-contrast and contrast computed tomography is high¹⁴. The accuracies of magnetic resonance imaging, helical computed tomography, and ultrasonography in the diagnosis of protruding gallbladder lesions were reported to be 93%, 75%, and 66%,

Table 2. Diagnosis of 28 Gallbladder Lesions by Contrast-enhanced Ultrasonography

Macroscopic appearance	No. of lesions	Benign findings	Malignant findings
Pedunculated	25	64.0% (16)	36.0% (9)
Sessile	3	33.3% (1)	66.7% (2)
Total	28	60.7% (17)	39.3% (11)

Table 3. Gallbladder Neoplasm Diagnosis (carcinoma, adenoma)

CEUS diagnosis	Final diagnosis	
	Neoplasm	Non neoplasm
Malignant findings	4	7
No malignant findings	0	17
Total	4	24

Sensitivity, 100% (4/4); specificity, 70.8% (17/24); accuracy, 75.0% (21/28) CEUS, contrast-enhanced ultrasonography

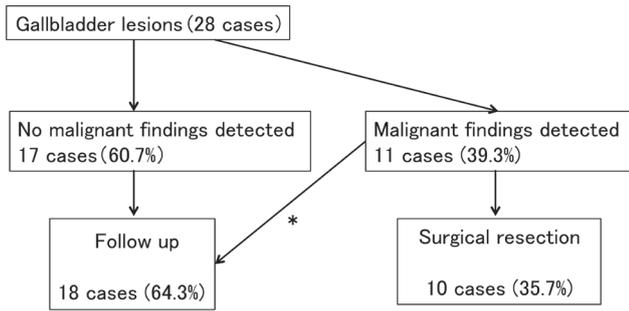


Fig. 2. Treatment Selection for Protruding Gallbladder Lesions According to Contrast Ultrasonographic Findings in the Present Study

* For one lesion there were no malignant finding in non-contrast computed tomography.

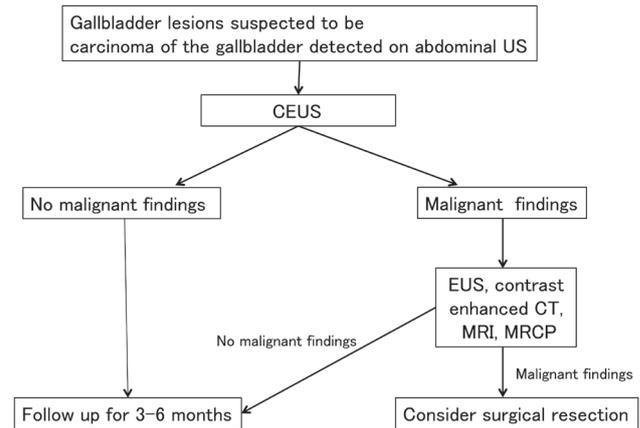


Fig. 3. Diagnostic Algorithm for Protruding Lesions of the Gallbladder

Malignant findings means more than 10mm in diameter and/or non-sessile protruding lesions.

US, ultrasonography; EUS, endoscopic ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; MRCP, magnetic resonance cholangiopancreatography.

respectively¹⁵. In dynamic magnetic resonance imaging, malignant protruding lesions of the gallbladder show early and sustained contrast enhancement, while benign lesions show early enhancement with subsequent wash-out¹⁶. Furthermore, the positive predictive value of high b-value diffusion-weighted magnetic resonance imaging was reported to be as high as 78% for cancer of the gallbladder and 22% for benign gallbladder diseases¹⁷.

While the above evaluations support the usefulness of endoscopic ultrasonography, computed tomography, and magnetic resonance imaging in the diagnosis of gallbladder diseases, these modalities all have drawbacks. Endoscopic ultrasonography is invasive, as it involves insertion of an endoscope. Computed tomography requires radiation exposure and sometimes administration of potentially allergenic contrast medium. Magnetic resonance imaging is problematic when magnetic metals or certain devices are present in the patient's body, or when the patient is claustrophobic. On the other hand, CEUS is not invasive. Zheng *et al.*¹⁸ reported that the sensitivity increased from 22.2 to 77.8% after CEUS in the staff radiologist, and from 22.2 to 66.7% in the resident radiologist. For the resident radiologist, the correctly characterized lesions were 57.8% before versus 70.7% after CEUS.

In recent years, CEUS has been used frequently for the diagnosis of gallbladder diseases. Numata *et al.*¹⁹ have found Galactose-palmitic acid mixt (Levovist) to be useful for diagnostic differentiation between benign polypoid lesions of the gallbladder and gallbladder cancer, based on its high specificity in detecting tortuosity of blood flow. Hattori *et al.*⁷ examined the echo pattern obtained by contrast ultrasonography within a polypoid

gallbladder lesion together with time-intensity curves (TIC) to obtain a more objective differential diagnosis; a linear pattern was highly specific for benign diseases, as was a branching pattern for carcinomas. Such echo patterns were reported to reflect histologic observations of greater vascular calibers and greater vascularity in gallbladder cancers than in non-neoplastic polyps and adenomyomatosis.

Reported true positive diagnosis rates of CEUS for protruding lesions of the gallbladder range from 75% to 96.3%, in which the findings of the present study are included. This rate range is comparable or slightly inferior to that attained with endoscopic ultrasonography, computed tomography, and magnetic resonance imaging. Importantly, though, we could avoid such additional examinations, as well as magnetic resonance cholangiopancreatography and surgery, for 64.3% of lesions by performing CEUS as the first diagnostic procedure following the initial abnormal ultrasonogram.

If no findings suggest malignancy, the patient is followed up for 6 months and if malignant findings are noted, further imaging is undertaken. If these procedures detect no malignant characteristics, the patient is followed up for an additional 3 to 6 months and if any finding suggestive of malignancy is noted, surgical resection is considered (Fig. 3).

Some issues concerning evaluation of image contrast effects in CEUS remain. Contrast effects vary with the imaging apparatus and the specific contrast agent used. Better standardization of contrast image evaluation is needed, as is further improvement of apparatus and contrast agents aiming toward more distinct demonstration of the microvasculature.

Conclusions

Our findings suggest that contrast ultrasonography can be the next diagnostic step in differential diagnosis of protruding gallbladder lesions detected by abdominal ultrasonography that are suspected to be cancer (10 or more millimeters, and/or sessile). This noninvasive contrast examination often eliminates the need for additional diagnostic procedures as well as surgical intervention.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interest.

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Outcomes of Elective Colonoscopy in Our Health Care Center

Sumiko Goto

Abstract

Objective: We documented outcomes after introducing elective total colonoscopy at our health care center, and investigated the utility of colonoscopy as a method of cancer screening.

Methods: Subjects were 55 patients who underwent elective colonoscopy at our center. Occult blood was detected using a fecal occult blood (FOB) immunoassay (2-day method). The carcinoembryonic antigen (CEA) level and lifestyle habits were used as health check-up data. We investigated a relationship between these results and the colonoscopy findings.

Results: Polyps were observed in 21 subjects. We found that out of the 55 subjects, 5 (9.1%) tested positive for FOB and 2 of them (40.0%) had polyps requiring follow-up observation and treatment. Among 44 subjects testing negative for FOB, 6 (13.6%) had polyps needing treatment and 4 of them were diagnosed with adenomas. Eight subjects negative for FOB had polyps that needed follow-up observation. Polyps requiring treatment and follow-up observation were detected in 14 (31.8%) of the 44 subjects negative for FOB. There was no relationship between the CEA levels and colonoscopy findings. In terms of lifestyle habits, subjects with significant lesions observed in colonoscopy usually had a smoking habit, frequent consumption of alcohol, and a tendency to exercise less.

Conclusion: The proportion of subjects with polyps requiring treatment who tested negative for FOB was high. Going forward, we believe that elective colonoscopy will be useful for the early detection of lesions and will be followed by decreasing colorectal cancer morbidity and mortality rates.

Keywords colonoscopy, polyp, fecal occult blood, cancer screening

According to the cancer statistic estimates published in 2015, the number of patients suffering from colorectal cancer was ranked first (estimated 135,800 patients) among all cancers, and the number of deaths due to colorectal cancer was ranked second after lung cancer (estimated 50,600 deaths), and these numbers showed a tendency towards an increase¹. Currently, fecal occult blood immunoassays (2-day method) are used to screen for colorectal cancer in Japan², and the usefulness of this method in reducing the colorectal cancer mortality rate was elucidated through case-control studies³⁻⁵. However, the diagnostic sensitivity of the fecal occult blood test for cancer differs depending on subjects, statistical methods and the number of tests, and sensitivity is reported to be between 60.0–100.0% in cases of advanced cancer and between 60.0–70.0% in early cancer⁶⁻⁸. When this method is used for cancer screening, some false negatives will be present. In our health care center, based on the high

level of interest in colorectal cancer, and the necessity of contributing to the detection of lesions that may have been missed in the fecal occult blood immunoassay because of false negatives, we introduced total colonoscopy as an elective colon cancer screening test. Colonoscopy is considered to be the test with the highest accuracy in the diagnosis of colon cancer. Although the utility of screening for colorectal cancer using colonoscopy has been verified experimentally during case-control studies and cohort studies⁹⁻¹¹, there is no evidence to support its role in the reduction of the colorectal cancer mortality rate². For this reason, in the present study, we tabulated the results of colonoscopy performed at our health care center that was not conducted as a secondary test after screening and have included a discussion of its utility.

Subjects and Methods

Subjects were 55 patients (49 men, 6 women, mean age 54.1 years) who underwent elective colonoscopy

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as part of screening tests at our health care center. Subjects who had a history of colon cancer, a polypectomy performed within the last 2 years, and subjective awareness of bloody stool were recommended to undergo colonoscopy at a department of gastroenterology in a hospital and excluded from this study. We used a questionnaire to inquire about the motivation for undergoing colonoscopy, and current subjective symptoms in the gastrointestinal system, including those affecting the stool and bowels. Fecal occult blood was tested by means of the immunoassay method (2-day method) and analyzed using the L-type IG Auto Hem method using a FOBIT Wako device (Wako Pure Chemical Industries, Ltd., Osaka). The cut-off value was set at 100 ng/mL. Two specimens were taken for fecal occult blood testing and the test was considered to be positive when at least one of these specimens tested positive. Serum CEA was used as the result of the health check-up, with normal values less than 5 ng/mL. The colonoscopy findings were evaluated by doctors who performed the colonoscopy and polyps required to have treatment or follow-up observation were defined as significant lesions. To determine the lifestyle habits of subjects, we used a medical questionnaire during the health check-up in which subjects were asked about frequency and volume of alcohol consumption, current smoking behavior, and regarding exercise, whether they exercised for 30 minutes more than two times per week, whether they walked more than an hour a day and about walking speed. These results were then compared with the colonoscopy findings. Statistical analysis of the relationship between the lifestyle habits and colonoscopy findings was performed by means of logistic regression.

This study was approved by the Ethical Committee of our hospital (Tokyo Takanawa Hospital, Tokyo, Japan), and all patients gave their written informed consent.

Results

Motivation for undergoing colonoscopy and subjective gastrointestinal symptoms

Twenty-seven of the subjects who underwent colonoscopy responded to the questionnaire, giving a response rate of 49.1%. The subjects selected responses from the predefined answers on the questionnaire (multiple selections possible) regarding their motivation for testing, and a free entry field was provided for reasons other than those given. In addition, if subjects selected "I was concerned about symptoms I noticed in my bowel or stool", we asked the subject to always describe the symptoms. The most common motivation for testing was "I thought I would get tested for the first time because I am now older than 40 years (or 50 years)" in 18 out of the 27 subjects (66.7%), followed by "I read about a recent increase in colorectal cancer in a newspaper or magazine" in 10 subjects (37.0%), "A friend or colleague was recently diagnosed with colorectal cancer" in 6 subjects (22.2%), "I have a relative who has suffered from cancer" in 4 subjects (14.8%) and "Several of my relatives have suffered from cancer" in 3 subjects (11.1%). These formed the majority of responses to the questionnaire. In terms of symptoms, the reported motivation was "I was concerned about symptoms I noticed in my bowel or stool" in 5 subjects (18.5%), "I was previously recommended to undergo detailed investigation as a result of fecal tests, but I did not go", "I have been previously diagnosed with a colon polyp but I have not undergone colonoscopy for years", and "I was worried about colorectal cancer because I have hemorrhoids" in 3 subjects each (11.1%) and "I have noticed blood in my stool" in 1 subject (3.7%) (**Table 1**). Examples of subjective symptoms related to defecation and the bowel are shown in **Table 2**, but no relationship between gastrointestinal subjective symptoms and colonoscopy screening results was observed.

Table 1. Motivation for Undergoing Colonoscopy Response rate; 49.1% (n=27)

Motivation	n	%
I thought I would get tested for the first time because I am now older than 40 years (or 50 years)	18	66.7
I read about a recent increase in colorectal cancer in the newspaper or magazine	10	37.0
A friend or colleague was recently diagnosed with colorectal cancer	6	22.2
I was concerned about symptoms I noticed in my bowel or stool	5	18.5
I have a relative who has suffered from cancer	4	14.8
I was previously recommended to undergo detailed investigation as a result of fecal tests, but I did not go	3	11.1
I have been previously diagnosed as colon polyp but I have not undergone colonoscopy for years	3	11.1
I was worried about colorectal cancer because I have hemorrhoids	3	11.1
Several of my relatives have suffered from cancer	3	11.1
I have noticed blood in my stool	1	3.7
Others	1	3.7

Table 2. Subjective Symptoms Related to Defecation and the Bowel (n=5)

Constipation
Repeated constipation and diarrhea
Chronic discomfort in stomach and colon
Fine stool
Feeling bloated in stomach
Frequent bowel movements
Swollen stomach due to gas
Bad smell of stool

Table 3. Overall Findings During Colonoscopy (n=55)

Finding	Total number of subjects	%
Hemorrhoid	23	31.5
Polyp	21	28.8
Diverticulae	13	17.8
Enteritis	1	1.4
No findings	15	20.5
Total*	73	100.0

* A cumulative total of subjects

Table 4. Overall Findings During Colonoscopy and Their Evaluation

Finding	Number of subjects	%	Evaluation
Polyp 5 mm ≤	8	14.5	Treatment required
Polyp 5 mm >	10	18.2	Follow-up observation required
Polyp 5 mm >	3	5.5	Observation not required
Others (Hemorrhoid, Enteritis, Diverticulae)	19	34.5	Observation not required
No findings	15	27.3	Normal
Total	55	100.0	

Overall findings during colonoscopy (total number of subjects)

The most common findings during colonoscopy among a cumulative number of 73 subjects were hemorrhoids in 23 subjects (31.5%) followed by polyps including anal polyps in 21 subjects (28.8%), diverticulae in 13 subjects (17.8%), and enteritis in 1 subject (1.4%). No abnormal findings were observed in 15 subjects (20.5%) and cancer was not detected in any subjects (Table 3).

Overall findings during colonoscopy and their evaluations

During colonoscopy, we detected polyps of greater than 5 mm that required treatment in 8 subjects (14.5%) and polyps of smaller than 5 mm that required follow-up observation in 10 subjects (18.2%). The evaluation that polyps do not require observation is made when polyps are considered to be hyperplastic polyps macroscopically or pathologically. Three subjects (5.5%) with polyps of smaller than 5 mm were evaluated as not requiring observation and other findings (hemorrhoids, enteritis, diverticulae) were noted in 19 subjects (34.5%). Subjects with polyps deemed to be significant lesions comprised 32.7% of the total, so such subjects were more common than those who had no abnormal findings at 27.3% (Table 4).

Comparison of fecal occult blood test results and colonoscopy findings

Among 55 subjects, fecal occult blood tests were positive in 5 (9.1%) and negative in 44 (80.0%), and 6 (10.9%) did not undergo testing. The breakdown of the total colonoscopy findings in those with positive fecal occult blood tests was as follows: 1 subject had a 15 mm

Table 5. Colonoscopy Findings in those with Positive Fecal Occult Blood Test (n=5)

Finding	Evaluation
1. Polyp; 15mm	Treatment required
2. Polyp; 4mm	Follow-up observation required
3. Polyp; 4mm	Observation not required
4. Enteritis	Observation not required
5. Hemorrhoid	Observation not required

polyp that required treatment, 2 had a 4 mm polyp, one of them requiring follow-up observation, and the other was diagnosed as a hyperplastic polyp by pathological examination, which did not require observation, and 1 subject each was diagnosed with enteritis and internal hemorrhoids, both of which did not require observation. At 5, although the total of subjects with positive fecal occult blood tests is a small number, significant lesions were detected in 2 of them (40.0%) (Table 5). Among the 44 subjects with negative fecal occult blood tests, 6 (13.6%) had polyps of greater than 5 mm, and treatment was required in all cases. Four out of these 6 subjects were diagnosed with adenoma either macroscopically or based on histopathology examinations. There were polyps of 2–4 mm in size in 8 subjects (18.2%), and all required follow-up observation. Histopathology examinations were not performed in any of these cases. Significant lesions requiring follow-up observation or treatment were detected in 14 (31.8%) out of the 44 subjects with negative fecal occult blood tests. Among the 6 subjects who did not undergo testing, 1 subject had a 6 mm polyp that required treatment

Table 6. Colonoscopy Findings in those with Negative Fecal Occult Blood Tests and in those without Fecal Occult Blood Tests

Subjects with negative fecal occult blood tests (n=44)	
Required treatment (n=6)	Required follow-up observation (n=8)
1. Polyp 18mm (adenoma)	1. Polyp 4mm
2. Polyp 18mm	2. Polyp 3mm
3. Polyp 8mm (adenoma)	3. Polyp 3mm
4. Polyp 8mm (adenoma)	4. Polyp 2mm
5. Polyp 6mm (adenoma)	5. Polyp 2mm
6. Polyp 5mm	6. Polyp 2mm
	7. Polyp 2mm
	8. Polyp 2mm
Subjects without fecal occult blood tests (n=6)	
Required treatment (n=1)	Required follow-up observation (n=1)
1. Polyp 6mm	1. Polyp 4mm

and 1 subject had a 4 mm polyp that required follow-up observation (Table 6).

Comparison of the CEA levels and colonoscopy findings

CEA levels were measured in 41 subjects. Only one subject had an abnormal CEA level (>5.0 ng/mL), of 6.3. Colonoscopy in this subject revealed diverticulae of the ascending colon and the fecal occult blood test was negative. The CEA levels in 6 out of the 8 subjects who had polyps of greater than 5 mm detected during colonoscopy and required treatment were 1.4–4.7 ng/mL, which were all normal. In the present study, there was no relationship between CEA levels and colonoscopy findings.

Relationship between lifestyle habits and colorectal polyps

Among the 53 subjects who were asked about lifestyle habits using the medical questionnaire, there were 'significant lesions' (polyps were observed during colonoscopy and either treatment or follow-up observation was required) in 17 subjects. They were designated as the significant lesions group (SL group). The other 36 subjects were designated as the no significant lesions group (no SL group). We then compared lifestyle habits between the two groups. In the SL group, 40.0% were active smokers, while 21.7% were non-smokers. Regarding alcohol consumption frequency, 47.1% of the SL group consumed alcohol daily, compared with 25.0% who did not. With regard to volume of alcohol consumption, we defined one unit of alcohol consumption as 350 mL of beer, 180 mL of Japanese sake, 2 glasses of wine, or a double shot of whisky. In the SL group, 16.7% consumed 3 or more units of alcohol daily, while 36.6% consumed less than 3 units of alcohol daily, which was against our prediction. Significant lesions therefore tended to be more common in smokers, those who consumed alcohol daily, and those who consumed less than 3 units of alcohol daily (Fig. 1). Regarding exercise habit, subjects who exercised for 30 minutes less

than twice a week had slightly more significant lesions compared with those who exercised more than twice a week (36.1% vs. 23.5%) and people walking at a normal to slow pace had more significant lesions compared with fast walkers (50.0% vs 30.6%). There was no relationship between the number of hours walked daily and the presence of significant lesions (Fig. 2).

Discussion

Fecal occult blood testing is easy, safe and affordable, and the immunoassay method in particular is not impaired by food or drug intake. It is therefore an extremely useful screening test. A reduction in the mortality rate in Japan due to fecal occult blood testing (immunoassay method) has already been verified by four case-control studies^{3-5,12}. Saito *et al.* performed a study³ in which they used only the immunoassay method for colon cancer screening during one year, and demonstrated a 60% reduction in mortality rates. According to the colorectal cancer screening guidelines, the grade of recommendation for fecal occult blood testing is level A when performed either as part of a treatment plan or electively. However, the diagnostic accuracy of fecal occult blood testing is reduced by the unavoidable false negatives that occur, and despite continual strategies to increase the rate of colorectal cancer screening by the Ministry of Health, Labor and Welfare, the current screening rate for both men and women is just 20%¹³. For this reason, we think that increasing the screening rate is an important challenge to tackle in order to achieve early detection of colorectal cancer to a greater extent.

While the diagnostic accuracy of colonoscopy is reported to be between 75–100%^{14,15}, it has several disadvantages that cannot be ignored, such as procedural accidents occurring during insertion, its attendant pain, and an insufficient number of doctors who can carry out colonoscopy. It has been reported that colonoscopy

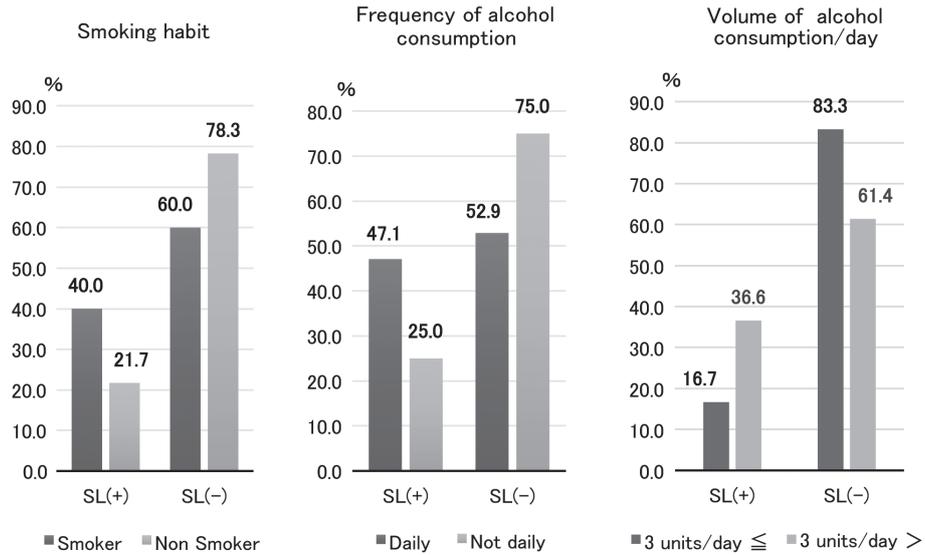


Fig.1. Relationship between Lifestyle Habits and Polyps-1

In the SL group, 40.0% were active smokers, while 21.7% were non-smokers. Regarding alcohol consumption frequency, 47.1% of the SL group consumed alcohol daily, compared with 25.0% who did not. With regard to volume of alcohol consumption, in the SL group, 16.7% consumed 3 or more units of alcohol daily, while 36.6% consumed less than 3 units of alcohol daily. Significant lesions therefore tended to be more common in smokers, those who consumed alcohol daily and those who consumed less than 3 units of alcohol daily. SL: significant lesion in colonoscopy

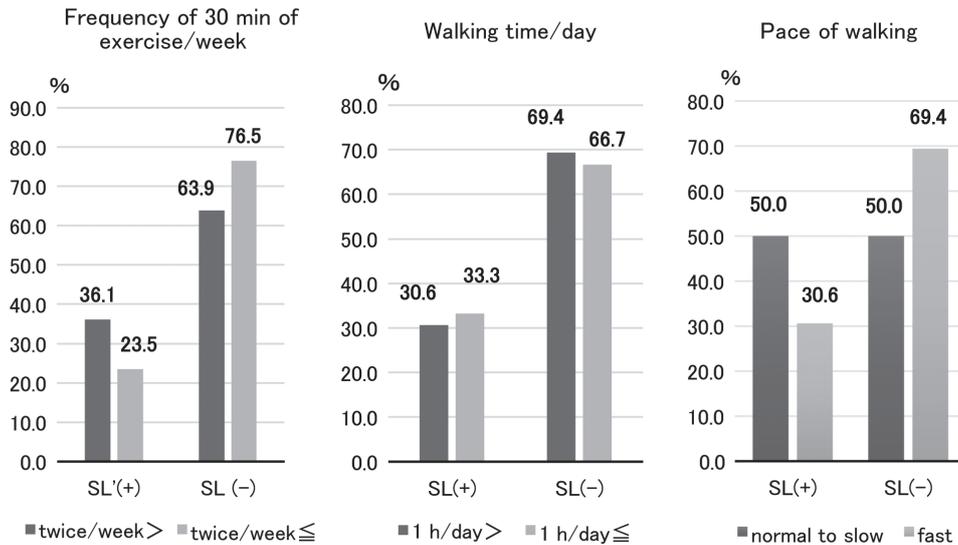


Fig.2. Relationship between Lifestyle Habits and Polyps-2

Subjects who exercised for 30 minutes less than twice a week had slightly more significant lesions compared with those who exercised more than twice a week (36.1% vs 23.5%) and walkers at a normal to slow pace had more significant lesions compared with fast walkers (50.0% vs 30.6%). There was no relationship between the number of hours walked daily and the presence of significant lesions. SL: significant lesion in colonoscopy

overall, the frequency of accidentally occurring symptoms was 0.012% and the death rate was 0.00082%¹⁶. The colorectal cancer screening guidelines therefore list the grade of recommendation for this examination as level C when performed as opportunistic screening,

and do not recommend it as part of population based screening².

At our health care center, we have encountered a number of patients who have tested negative for fecal occult blood during screening, but have subsequently

had colorectal polyps or colon cancer detected during colonoscopy screening and undergone resection or other operations. In the questionnaire regarding the motivation to undergo testing, several subjects reported undergoing colonoscopy because of news reports or cancer in friends or relatives, and we presume that concerns regarding colorectal cancer have increased. However, there was no relationship between subjective symptoms and significant lesions in subjects who reported requesting testing because of subjective symptoms, and we believe the chance of subjective symptoms contributing to early detection of colorectal cancer and adenomas is low.

Among the present 55 subjects, there were 15 (27.3%) who had no abnormalities, and no cases of colorectal cancer were observed. However, unexpectedly at 21, the total number of subjects who had polyps was almost the same as those with hemorrhoids at 23, and 5 mm or greater polyps were detected in 14.5% of all subjects and the rate of subjects with significant lesions was 32.7%, which were more common than in those with no abnormalities. Among the 5 subjects that tested positive for fecal occult blood, we detected polyps that required follow-up observation and treatment in 2 (40.0%), and we found that the rate of subjects undergoing detailed investigations after colorectal cancer screening was low at 60.0%. We believe that increasing this rate is an important challenge to meet in decreasing colorectal cancer morbidity and mortality rates.

Among the 44 cases that tested negative for fecal occult blood, 13.6% were found to require treatment during colonoscopy, and 18.2% required follow-up observation. These results are similar to those of some domestic and foreign studies^{6-8,17}, and shows that several false negatives occur during fecal occult blood testing. There were two subjects who tested negative for fecal occult blood and had polyps of 10 mm or greater in size. In addition, among the 6 subjects who tested negative for fecal occult blood and required treatment, there were 4 who were diagnosed with an adenoma either macroscopically or pathologically. The malignant conversion rate of adenomas is reported to be 0.46% for lesions of 5 mm or less in diameter and 28.2% for lesions of 10 mm or less in diameter¹⁸. We expect it is likely that, in addition to being able to achieve early detection and treatment of adenomas, elective colonoscopy will also be able to facilitate continued follow-up observation and therefore contribute to reducing the current mortality rate due to colorectal cancer. With regard to microscopic lesions, there have been cohort studies that reported changes at an extremely low rate during 2–3 years of follow-up observation, and studies indicating that there is no relationship between hyperplastic polyps of 5 mm or less in size that tend to occur

in the rectum and sigmoid colon and the incidence of adenomas¹⁹⁻²¹. In the present study, microscopic polyps were detected in 11 subjects. In these cases, this did not contribute to the early detection of cancer.

Going forward, it will be possible to create efficient colorectal cancer screening programs, and from the perspective of safety, we think that elective colonoscopy will be beneficial to both healthy patients and patients in whom microscopic lesions are detected.

Irrespective of whether there was hemorrhage from the lesion or not, factors that prevented the detection of blood in the stool during testing included inappropriate sampling and preservation methods, though we already know that superficial morphological characteristics and lesions in the right colon can easily be interpreted as false negatives²². In the present study, we did not perform a detailed investigation of the site and macroscopic classification of polyps, though the macroscopic classifications of polyps of greater than 10 mm in size in subjects with negative fecal occult blood tests were all 0 - Isp, and the sites were the rectum and transverse colon. It has not yet been established that colonoscopy reduces the colorectal cancer morbidity and mortality rate, but we expect meaningful results in this regard to be obtained from large-scale randomized controlled trials²³ that are currently underway.

Serum CEA levels were normal, even in the subjects with polyps of greater than 10 mm in size, and there was no relationship between CEA levels and polyp size. Also no significant lesions were found in 1 subject who had an abnormal CEA level so during the present study, we could not show that CEA was a useful marker for colonic lesions. Studies are currently investigating fecal genetic material and other biomarkers, but no evidence regarding the utility of either has yet been reported. Therefore, we can only select fecal occult blood as the method of screening at this point in time.

Regarding relationships between lifestyle habits and colorectal cancer, through cohort studies, research institutions both in Japan and abroad have largely reached a consensus regarding the fact that there is an established, or at least highly probable, relationship between colorectal cancer and the amount of physical activity, obesity, large volume alcohol consumption, smoking, and consumption of red and processed meat²⁴⁻²⁶. Although the number of subjects in the present study was small, we found that daily alcohol consumption^{25,27}, smoking^{26,27}, exercise frequency, and walking speed²⁸ might be all be related to the significant lesion occurrence rate noted in colonoscopy. It is very important to recommend colonoscopy screening proactively to patients who frequently consume alcohol, smoke and those who do not exercise daily.

Conclusion

In subjects who underwent colonoscopy in the present study, we found that the rate of significant lesions in subjects who were negative for fecal occult blood was high, and CEA and subjective symptoms were not considered effective for determining people to whom colonoscopy should be recommended. Proactively encouraging colorectal cancer screening with consideration given to safety could be useful for the early detection of lesions and may contribute to decreasing colorectal cancer morbidity and mortality rates.

Conflict of Interest

The author would like to declare that there was no conflict of interest involved in the preparation of this report.

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Prevalence of Atherosclerotic Lesions in Asymptomatic Japanese Subjects in a Health Check-up

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Abstract

Background: Subclinical atherosclerosis is a latent precursor of clinical cardiovascular disease (CVD). Several studies have examined the comorbidity of polyvascular disease (PVD) in patients with CVD, but the comorbidity of PVD in asymptomatic subjects has not been fully investigated.

Methods: Vascular screening was performed using MRA for the coronary and renal arteries and ultrasonography for the carotid artery. The calcium score for the coronary artery was analyzed by CT, and the ankle brachial index (ABI) was used for screening of peripheral artery stenosis. The prevalences of carotid, coronary, renal artery and peripheral artery diseases were analyzed in 655 subjects during a health check-up at our institution.

Results: Of the 665 subjects, 434 (65.3%) were males, the mean age was 60 years old, 55.2% had a history of hypertension, 61.7% had dyslipidemia, 13.2% had diabetes mellitus, and 41.2% were current or former smokers. A total of 63 lesions were detected in 58 subjects (8.7%), with 5 subjects (0.75%) having PVD: coronary and carotid lesions in 3 subjects, coronary and renal artery lesions in one, and carotid and renal artery lesions in one.

Conclusion: This is the first study on the prevalence of multiple atherosclerotic lesions in an asymptomatic Japanese population free from clinically apparent CVD. The present study revealed that 8.7% of asymptomatic subjects examined in a health check-up at our institution had multiple atherosclerotic lesions.

Keywords prevalence, cardiovascular disease, polyvascular disease, epidemiology

Atherosclerosis is a chronic, progressive disease with a long asymptomatic phase¹. Subclinical atherosclerosis is a latent precursor of clinical cardiovascular disease (CVD), including myocardial infarction (MI) and stroke². CVD remains the most common cause of death in the industrialized world, despite recent advances in treatment, and primary³ and secondary prevention⁴ have been shown to be most effective in combatting this epidemic. However, the design of appropriate prevention projects requires an understanding of the risk pattern profile of the population at risk.

A recent international cohort study (REduction of Atherothrombosis for Continued Health Registry; REACH) that included patients in Japan revealed that the prognosis of polyvascular disease (PVD) is poor regardless of ethnicity^{5,6}. Several studies have examined the comorbidity of PVD in patients with CVD, but the comorbidity of PVD in asymptomatic subjects has not

been fully investigated. Therefore, in this study, we evaluated the prevalences of carotid, coronary and renal artery, and peripheral artery diseases as well as risk factors, in asymptomatic subjects.

Methods

Study population

The basic inclusion criterion was asymptomatic subject who voluntarily underwent imaging including MRA and CT for vascular screening in a health check-up at Toranomon Hospital Health Management Center between October 2008 and March 2014. Vascular screening was performed using MRA for the coronary and renal arteries and ultrasonography for the carotid artery. The calcium score for the coronary artery was analyzed by CT, the ankle brachial index (ABI) was used in screening for peripheral artery stenosis, and laboratory biochemical data, including lipid and glucose profiles, were

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obtained.

Of 673 consecutive asymptomatic subjects, 8 were excluded due to a history of coronary artery disease ($n=2$), a history of cerebral infarction ($n=4$), or inability to undergo coronary MRA because of atrial fibrillation ($n=2$). Therefore, a total of 665 subjects were included in the analysis. Medical histories and current medications were obtained from questionnaires. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg without medication in the outpatient clinic in at least two separate measurements, or use of antihypertensive drugs. Diabetes mellitus was defined as fasting blood glucose ≥ 126 mg/dL or HbA1c $\geq 6.5\%$, or use of medication for diabetes. Dyslipidemia was defined as total cholesterol ≥ 240 mg/dL or low-density lipoprotein (LDL) cholesterol ≥ 140 mg/dL and/or triglyceride (TG) ≥ 150 mg/dL or high-density lipoprotein (HDL) cholesterol < 40 mg/dL, or use of lipid-lowering medication. Hyperuricemia was defined as uric acid ≥ 7.1 mg/dL. Obesity was defined as a body mass index (BMI) ≥ 25 kg/m², using the criterion in the WHO Asia-Pacific guidelines⁷. Metabolic syndrome was classified using the NCEP/ATP-III recommendations⁸. Smoking habits (never, former and current) were obtained from a self-completed questionnaire. This study was ap-

proved by the Institutional Review Board of our hospital. Informed consent was obtained from all subjects.

Definition of atherosclerosis disease

After screening using MRA, coronary artery disease was defined as $>75\%$ stenosis (left main trunk (LMT) $>50\%$ stenosis) on CT or coronary angiography, carotid artery disease was defined as $>50\%$ stenosis according to European Carotid Surgery Trial (ECST) criteria using ultrasonography, renal artery disease was defined as $>50\%$ stenosis on MRA, and peripheral artery disease (PAD) was defined as $>75\%$ stenosis on CT angiography after screening using ABI ≤ 0.9 .

Statistical analysis

Data are expressed as mean \pm SD for continuous variables, and as frequencies and percentages for categorical variables. The significance of differences in quantitative data was determined by the Mann-Whitney U-test. All analyses were performed using SPSS for Windows, ver. 13.0 (Chicago, IL) (IBM, USA), with $p < 0.05$ considered significant.

Results

The characteristics of the 665 subjects are shown in **Table 1**. Of the subjects, 434 (65.3%) were males, the mean age was 60 years old, 55.2% had a history of hypertension, 61.7% had dyslipidemia, 13.2% had diabetes mellitus, and 41.8% were current or former smokers. The prevalences of metabolic syndrome and low eGFR were 26.8% and 14.9%, respectively. A calcium score >100 was noted in 116 (17.4%) subjects.

Atherosclerotic disease was detected in 58 subjects (8.7%) (atherosclerotic group) and not found in 607 subjects (non-atherosclerotic group). A total of 63 lesions were detected in the 58 subjects (**Table 2**). PVD was detected in five of the 665 subjects (0.75%), including coronary and carotid lesions in 3, coronary and renal artery lesions in one, and carotid and renal artery lesions in one subject (**Fig. 1**). The subjects in the atherosclerotic group included more men and were older. The prevalences of diabetes mellitus, hyperuricemia, current and former smokers, metabolic syndrome and low estimated glomerular filtration rate (eGFR) (<60 mL/min/1.73 m²)

Table 1. Characteristics of Subjects

Item	Value*
Number of subjects	665
Age (years)	60.2 \pm 10.6
Male	434 (65.3)
Body mass index (kg/m ²)	23.4 \pm 3.2
Waist circumference (cm)	83.5 \pm 9.2
Systolic blood pressure (mmHg)	121.4 \pm 13.7
Diastolic blood pressure (mmHg)	77.6 \pm 9.1
Pulse wave velocity (cm/s)	1498 \pm 296
Ankle brachial index	1.16 \pm 0.06
Total cholesterol (mg/dL)	206.4 \pm 32.0
LDL cholesterol (mg/dL)	118.4 \pm 29.7
HDL cholesterol (mg/dL)	57.3 \pm 15.5
Triglyceride (mg/dL)	119.4 \pm 74.8
Fasting glucose (mg/dL)	103.9 \pm 19.9
HbA1c (%)	5.81 \pm 0.64
Creatinine (mg/dL)	0.8 \pm 0.3
eGFR (mL/min/1.73 m ²)	77.9 \pm 15.5
Uric acid (mg/dL)	5.9 \pm 1.3
Hypertension	367 (55.2)
Dyslipidemia	410 (61.7)
Diabetes mellitus	88 (13.2)
Smoking	
Never	387 (58.2)
Current + Former	278 (41.8)
CKD (eGFR < 60 mL/min/1.73 m ²)	99 (14.9)
Hyperuricemia (≥ 7.1 mg/dL)	144 (21.7)
Metabolic syndrome	178 (26.8)
Calcium score > 100	116 (17.4)

Data are expressed as number of cases (percent) or mean \pm SD.

Table 2. Vascular Lesions in Patient Population

Item	n (%)*
Total number of subjects	665 (100)
Subjects with vascular disease	58 (8.7)
Vascular lesions	
Coronary artery disease	39 (5.9)
Carotid artery disease	15 (2.3)
Renal artery disease	8 (1.2)
Peripheral artery disease	1 (0.2)

Data are expressed as number of cases (percent).

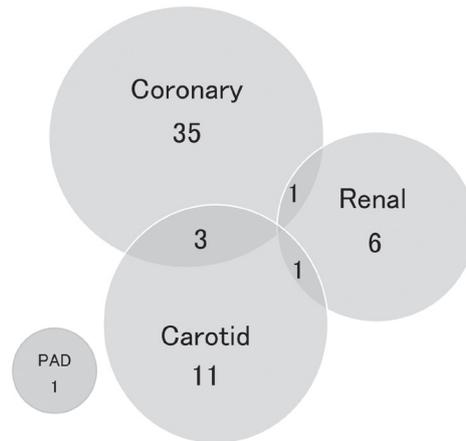


Fig. 1. Prevalences of Coronary, Carotid, Renal and Peripheral Artery Diseases

A total of 63 lesions were detected in 58 subjects, with two atherosclerotic lesions found in each of five subjects. Coronary and carotid lesions were found in three subjects, coronary and renal artery lesions were present in one subject, and carotid and renal artery lesions were found in one subject.

Table 3. Comparison of Atherosclerotic and Non-atherosclerotic Groups

Item	Atherosclerotic group n = 58	Non-atherosclerotic group n = 607	p value
Age (years)	66.6 ± 9.7	59.6 ± 10.4	0.018
Male	46 (79.3)	388 (63.9)	0.027
Body mass index (kg/m ²)	23.9 ± 3.0	21.6 ± 3.3	0.332
Waist circumference (cm)	85.4 ± 8.2	77.0 ± 9.2	0.038
Systolic blood pressure (mmHg)	122.6 ± 13.3	117.1 ± 14.2	0.096
Diastolic blood pressure (mmHg)	78.7 ± 8.7	73.6 ± 9.1	0.456
Pulse wave velocity (cm/s)	1696 ± 336	1480 ± 285	<0.001
Ankle brachial index	1.14 ± 0.09	1.16 ± 0.06	0.160
Total cholesterol (mg/dL)	204.2 ± 31.3	214.0 ± 33.3	0.936
LDL cholesterol (mg/dL)	117.0 ± 29.3	121.6 ± 30.5	0.489
HDL cholesterol (mg/dL)	54.2 ± 14.1	68.1 ± 15.6	0.012
Triglyceride (mg/dL)	128.9 ± 79.2	86.3 ± 42.8	0.541
Fasting glucose (mg/dL)	105.6 ± 19.8	103.9 ± 19.9	0.009
HbA1c (%)	6.28 ± 1.15	5.78 ± 0.66	0.001
Creatinine (mg/dL)	0.83 ± 0.20	0.79 ± 0.31	0.164
eGFR (mL/min/1.73m ²)	77.4 ± 15.2	80.0 ± 16.2	0.010
Uric acid (mg/dL)	6.2 ± 1.2	4.8 ± 1.0	0.019
Hypertension	39 (67.2)	328 (54.0)	0.054
Dyslipidemia	38 (65.5)	410 (67.5)	0.574
Diabetes mellitus	18 (31.0)	70 (11.5)	<0.001
Smoking			0.003
Never	23 (39.7)	364 (60.0)	
Current + Former	35 (60.3)	243 (40.0)	
CKD (eGFR <60mL/min/1.73m ²)	17 (29.3)	82 (13.5)	0.003
Hyperuricemia	21 (36.2)	123 (20.3)	0.007
Metabolic syndrome	25 (43.1)	153 (25.2)	0.004
Calcium score > 100	38 (65.5)	78 (12.9)	<0.001

Data are expressed as number of cases (percent) or mean ± SD.

were higher in the atherosclerotic group than in the non-atherosclerotic group. The calcium score was higher in the atherosclerotic group (Table 3).

Discussion

This is the first study on the prevalence of multiple atherosclerotic lesions in an asymptomatic Japanese population free from clinically apparent CVD. Previous studies^{9,10}

showed that the prevalence of multiple atherosclerotic lesions was higher in patients with coronary artery disease and cerebral infarction. In a study of comorbidities in coronary artery disease, Kawarada *et al.*⁹ reported prevalences of carotid stenosis and PAD of 13.7% and 15.3%, respectively; and Yamazaki *et al.* found that 14.0% of symptomatic patients among Japanese subjects in the REACH registry had lesions in multiple vascular beds¹⁰. In addition, a one year-after substudy of the REACH registry¹¹ showed that symptomatic PVD patients had a poor prognosis compared with those with a single atherosclerotic disease.

Three points of difference between the present study and the above previous studies are described in the following. First, the incidence of comorbidities in our patients was lower than that in symptomatic patients with CVD. This may be because our population was in an earlier stage of CVD compared to the symptomatic patients. Second, the coronary artery was the most common disease site among artery beds, which suggests that this artery may have factors that promote atherosclerosis. The influence of risk factors on each artery may differ because their structures and hemodynamics are different. For example, a recent study showed that the impact of hypertension as a CVD risk factor in the coronary artery differed from its impact in other arteries¹². From our results, screening for coronary artery disease, a common site of CVD in asymptomatic subjects, may be useful in modifying preventive strategies for PVD progression. Third, a lower prevalence (0.2%) of PAD was found in our study compared with the accepted rate of PAD of about 0.6–10% in the general population^{13–15}. This may be explained by differences in lifestyle between subjects in our study and those in other studies, although screening criteria were the same and the prevalences of risk factors, such as gender, diabetes, and smoking habits, were similar.

Based on the results of this study, vascular screening of a high risk population is reasonable because it would detect asymptomatic vascular lesions before intensive vascular interventions were needed. Thus, our subjects may be candidates for prophylactic interventions to prevent further development of CVD.

There are some limitations in this study. The subjects were those who voluntarily underwent vascular screening in a health check-up, and therefore, the results may not be applicable to the general population. Second, the study was cross-sectional. Longitudinal observation studies would be needed to ascertain the prevalence, risk factors and prognosis of high risk groups for CVD in the Japanese population.

Conclusion

This study showed that subclinical atherosclerotic

disease had developed to some extent in asymptomatic subjects who were free from clinical CVD, although the incidence was lower than that of a symptomatic CVD population. Prophylactic interventions may prevent further development of CVD in these subjects.

Conflict of Interest

The authors have no conflict of interest to declare.

Ethical approval

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Nonalcoholic Fatty Liver Disease is More Strongly Associated with Arteriosclerosis than is Abdominal Obesity in Health Check-up Examinees

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Abstract

Objective: Nonalcoholic fatty liver disease (NAFLD) is a condition in which fat accumulates in the liver, and it is considered the hepatic manifestation of metabolic syndrome (MetS). The diagnostic criteria for MetS in Japan require abdominal obesity (AO). Both NAFLD and AO are significant predictors of cardiovascular disease. The purpose of our study was to determine whether NAFLD or AO is more closely associated with arteriosclerosis based on the results of an analysis of carotid intima media thickness (IMT).

Methods: Between June 2011 and December 2013, 3,648 individuals undergoing a health check-up who had no history of liver disease underwent abdominal ultrasonography (US), and 752 subjects underwent carotid US. After excluding subjects with an alcohol consumption >20 g/day or a history of hypertension, diabetes, dyslipidemia and/or hyperuricemia, 368 subjects (202 males and 166 females, aged 29 to 87 years) remained. Using simple and multivariate analyses, the parameters associated with NAFLD, AO, and an increased maximum IMT were examined. Furthermore, we analyzed differences between subjects with NAFLD but not AO and those with AO but not NAFLD.

Results: Although an increased maximum IMT was independently associated with NAFLD, there was no association between an increased maximum IMT and AO. The group with NAFLD but not AO had a significantly higher proportion of subjects with an increased maximum IMT than the group with AO but not NAFLD, after adjusting for gender and age.

Conclusion: Our analysis suggests that NAFLD is more strongly related to the progression of arteriosclerosis than is AO.

Keywords nonalcoholic fatty liver disease (NAFLD), abdominal obesity (AO), arteriosclerosis, carotid intima media thickness (IMT)

The number of obese individuals has recently been increasing in Japan. Based on the results of the 2013 National Health and Nutrition Survey in Japan, the Ministry of Health, Labour, and Welfare reported that 28.6% of males and 20.3% of females were obese. Visceral adiposity is a significant independent predictor of insulin resistance, elevated blood pressure, and dyslipidemia, which are factors observed in metabolic syndrome (MetS). Furthermore, visceral adiposity is a source of free fatty acids, adipokines such as adiponectin, tumor necrosis factor- α (TNF- α) and plasminogen activator inhibitor type 1 (PAI-1)¹, and it leads to cardiovascular disease^{2–5}. MetS is a cluster of the most dangerous heart attack risk factors, such as diabetes, elevated fasting

plasma glucose, abdominal obesity (AO), dyslipidemia, and high blood pressure, and individuals with MetS are twice as likely to die from and three times as likely to have a heart attack or stroke compared with those without MetS⁶. However, the definition of MetS differs across various national guidelines and statements. The International Diabetes Federation published diagnostic criteria for MetS that require AO in 2005⁷, and in the same year, diagnostic criteria for MetS requiring AO were proposed in Japan.

Fatty liver is classified into two diseases based on its cause: alcoholic fatty liver and nonalcoholic fatty liver disease (NAFLD). Nonetheless, both of these diseases are conditions involving fat accumulation in the liver. In

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particular, NAFLD is considered the hepatic manifestation of MetS⁸. Eguchi *et al.*⁹ reported that the frequency of NAFLD was 29.7% in the general population, and that NAFLD is a common disease in Japan. Also, many investigators have shown that subjects with NAFLD have a higher carotid intima media thickness (IMT) than those without NAFLD^{8,10-13}. Some longitudinal studies have demonstrated that the incidence of cardiovascular disease is higher in subjects with NAFLD than in those without NAFLD¹⁴⁻¹⁷. Although both NAFLD and AO are conditions of excessive fat accumulation and are significant predictors of cardiovascular disease, no studies have evaluated which is more strongly related to arteriosclerosis. Non-invasive and safe assessment of IMT using carotid ultrasonography (US) is widely used as a surrogate marker of subclinical atherosclerosis^{12,18-19} and an increased IMT is directly associated with the incidence of cardiovascular disease¹⁸⁻¹⁹. The purpose of our study was to determine whether NAFLD or AO is more closely associated with arteriosclerosis based on the results of an analysis of IMT in individuals undergoing a health check-up.

Methods

This study was approved by the Ethics Committee of Yokohama City Minato Red Cross Hospital.

Subjects

Between June 2011 and December 2013, 3,648 individuals undergoing a health check-up (2,184 males and 1,464 females) who had no past history of liver disease underwent abdominal US. Of them, 752 (504 males and 248 females) also underwent carotid US on the day of the health check-up. After excluding subjects with an alcohol consumption > 20 g/day or a past history of hypertension, diabetes, dyslipidemia, and/or hyperuricemia, a cross-sectional observational study of the remaining 368 subjects (202 males and 166 females, aged 29 to 87 years) was conducted.

Measurements

The health check-up included recording the patient's medical history, prescribed medications, and lifestyle habits, such as alcohol use, exercise, snacking habits, and smoking habits; determining anthropometric variables; physical examination; and laboratory evaluation. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with an automated sphygmomanometer in a sitting position. The measurement of waist circumference (WC) was performed at the level of the umbilicus after normal expiration while standing. Venous blood samples were collected after an overnight fast of at least 12 hours. The samples were used for analysis of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), uric

acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (γ -GTP). Male and female subjects were diagnosed with AO if they had a WC \geq 85 cm or \geq 90 cm, respectively. High SBP was defined as SBP \geq 130 mmHg, high DBP as DBP \geq 85 mmHg, high LDL-C as LDL-C \geq 120 mg/dL, low HDL-C as HDL-C \leq 39 mg/dL, high TG as TG \geq 150 mg/dL, high FPG as FPG \geq 100 mg/dL, high HbA1c as HbA1c \geq 5.6 %, high UA as UA \geq 7.1 mg/dL, high AST as AST \geq 31 mg/dL, high ALT as ALT \geq 31 mg/dL, and high γ -GTP as γ -GTP \geq 51 mg/dL.

Assessment of fatty liver and carotid atherosclerosis

Abdominal US (XARIO XG SSA-680A, Toshiba Medical Systems, Japan) was performed by experienced technicians. The ultrasonographic photographs of each subject were reviewed by a gastroenterologist blinded to the individual data. Fatty liver was diagnosed on the basis of the 4 acknowledged findings: liver brightness, vascular blurring, hepatorenal contrast, and deep attenuation.

Carotid US (XARIO XG SSA-680A, Toshiba Medical Systems, Japan) was performed to assess evidence of atherosclerosis. The IMT was measured in the far wall of the common carotid artery, and increased maximum IMT was defined as a maximum IMT > 1.0 mm.

Comparisons between groups

Subjects were divided into two groups: those with NAFLD and those without NAFLD. Age, gender, lifestyle habits, and the presence of metabolic abnormalities, liver dysfunction, and increased maximum IMT were compared between the two groups. These parameters were also compared between subjects with and without AO.

According to the presence or absence of an increased maximum IMT, subjects were divided into two groups, and age, gender, lifestyle habits, and the presence of NAFLD, AO, metabolic disorders, and liver dysfunction were compared between the groups.

Furthermore, age, gender, lifestyle habits, and the presence of metabolic abnormalities, liver dysfunction, and increased maximum IMT were compared between subjects with NAFLD but not AO and those with AO but not NAFLD.

Statistical analysis

All analyses were performed using IBM SPSS Statistics 19 (IBM, USA). A probability value less than 0.05 was considered significant. Quantitative variables (expressed as the mean \pm standard deviation) were tested using unpaired *t* tests when normally distributed and Mann-Whitney's U test when non-normally distributed. Categorical variables (presented as absolute numbers and percentages) were compared using the chi-squared test. Furthermore, a stepwise logistic regression analysis was performed to determine which parameters were independently associated with NAFLD, AO, increased maximum IMT, or NAFLD without AO.

Results

The background characteristics of the subjects with and without NAFLD are presented in **Table 1**. Subjects with NAFLD had significantly higher frequencies of AO, high SBP, high LDL-C, low HDL-C, high TG, high FPG, high HbA 1c, high UA, high AST, high ALT, and high γ -GTP compared with those without NAFLD. An increased maximum IMT was observed in 66.3% of subjects with NAFLD and 53.5% of subjects without NAFLD ($p = 0.002$). **Table 2** shows the results of the multivariate analysis of risk factors for NAFLD. Increased maximum IMT was independently associated with NAFLD. Furthermore, AO, high TG, high FPG, high HbA 1c, high UA, and high ALT were also independently associated with NAFLD.

The background characteristics of subjects with and without AO are listed in **Table 3**. The proportions of all

metabolic abnormalities were significantly higher in subjects with AO than in those without AO. The proportions of subjects with increased maximum IMT were similar in the two groups. As **Table 4** shows, in the multivariate analysis, a significant association with AO was observed for male gender, NAFLD, high DBP, and high FPG. There was no association between AO and increased maximum IMT.

Table 5 shows that subjects with increased maximum IMT had a significantly higher frequency of NAFLD than those without, although the frequencies of subjects with AO were similar in the two subject groups. The frequencies of high SBP, high DBP, and high HbA 1c were significantly higher in subjects with increased maximum IMT than in those without. The results of multivariate analysis indicated that NAFLD, but not AO, was significantly associated with increased maximum IMT (**Table 6**).

Table 1. Background Characteristics of Subjects with and without NAFLD

	With NAFLD (n = 92)	Without NAFLD (n = 276)	Age-adjusted p value	Age-adjusted odds ratio
Age (years)	55.7±10.0	57.4±12.2	0.185	—
Gender (males/females)	67/25	135/141	<0.001**	2.789
AO (%)	55 (59.8%)	55 (19.9%)	<0.001**	5.895
High SBP (%)	24 (26.1%)	48 (17.4%)	0.032*	1.893
High DBP (%)	16 (17.4%)	29 (10.5%)	0.074	1.834
High LDL-C (%)	66 (71.7%)	140 (50.7%)	<0.001**	2.623
Low HDL-C (%)	10 (10.9%)	3 (1.1%)	<0.001**	10.792
High TG (%)	32 (34.8%)	23 (8.3%)	<0.001**	5.747
High FPG (%)	48 (52.2%)	50 (18.1%)	<0.001**	4.951
High HbA 1c (%)	77 (83.7%)	152 (55.1%)	<0.001**	5.040
High UA (%)	26 (28.3%)	16 (5.8%)	<0.001**	6.265
High AST (%)	21 (22.8%)	14 (5.1%)	<0.001**	5.412
High ALT (%)	37 (40.2%)	15 (5.4%)	<0.001**	11.995
High γ -GTP (%)	22 (23.9%)	20 (7.2%)	<0.001**	3.947
Increased maximum IMT (%)	61 (66.3%)	147 (53.3%)	0.002**	2.396
Without exercise habit (%)	51 (55.4%)	131 (47.5%)	0.257	0.990
With snacking habit (%)	21 (22.8%)	76 (27.5%)	0.330	0.759
With smoking habit (%)	24 (26.1%)	31 (11.2%)	0.001**	2.712

NAFLD: nonalcoholic fatty liver disease, AO: abdominal obesity, FPG: fasting plasma glucose, UA: uric acid
Age is shown as mean \pm standard deviation (SD).

* : $p < 0.05$, ** : $p < 0.01$

Table 2. Variables Associated with NAFLD in Multivariate Logistic Regression Analysis

	p value	odds ratio (95% CI)
AO	0.001**	2.992 (1.581–5.664)
High TG	0.001**	3.605 (1.650–7.879)
High FPG	0.021*	2.168 (1.123–4.185)
High HbA 1c	0.029*	2.220 (1.087–4.537)
High UA	0.002**	4.023 (1.664–9.725)
High ALT	<0.001**	8.195 (3.635–18.479)
Increased maximum IMT	0.046*	1.957 (1.013–3.783)
With smoking habit	0.023*	2.543 (1.141–5.669)

NAFLD: nonalcoholic fatty liver disease, AO: abdominal obesity, UA: uric acid

* : $p < 0.05$, ** : $p < 0.01$

Table 3. Background Characteristics of Subjects with and without AO

	With AO (n = 110)	Without AO (n = 258)	Age-adjusted p value	Age-adjusted odds ratio
Age (years)	55.5±10.7	57.6±12.0	0.110	—
Gender (males/females)	92/18	110/148	< 0.001**	6.897
NAFLD (%)	55 (50.0%)	37 (14.3%)	< 0.001**	5.896
High SBP (%)	28 (25.5%)	44 (17.1%)	0.022*	1.920
High DBP (%)	22 (20.0%)	23 (8.9%)	0.003**	2.642
High LDL-C (%)	72 (65.5%)	134 (51.9%)	0.009**	1.866
Low HDL-C (%)	10 (9.1%)	3 (1.2%)	0.002**	8.203
High TG (%)	30 (27.3%)	25 (9.7%)	< 0.001**	3.373
High FPG (%)	55 (50.0%)	43 (16.7%)	< 0.001**	5.043
High HbA 1 c (%)	76 (69.1%)	153 (59.3%)	0.029*	1.731
High UA (%)	24 (21.8%)	18 (7.0%)	< 0.001**	3.599
High AST (%)	16 (14.5%)	19 (7.4%)	0.045*	2.064
High ALT (%)	31 (28.2%)	21 (8.1%)	< 0.001**	4.243
High γ-GTP (%)	20 (18.2%)	22 (8.5%)	0.012*	2.320
Increased maximum IMT (%)	64 (58.2%)	144 (55.8%)	0.199	1.399
Without exercise habit (%)	55 (50.0%)	127 (49.2%)	0.885	0.967
With snacking habit (%)	25 (22.7%)	72 (27.9%)	0.252	0.735
With smoking habit (%)	21 (19.1%)	34 (13.2%)	0.195	1.488

AO: abdominal obesity, NAFLD: nonalcoholic fatty liver disease, UA: uric acid
Age is shown as mean ± standard deviation (SD). * : $p < 0.05$, ** : $p < 0.01$

Table 4. Variables Associated with AO in Multivariate Logistic Regression Analysis

	p value	odds ratio (95% CI)
Male gender	< 0.001**	5.162 (2.832–9.408)
NAFLD	< 0.001**	3.921 (2.204–6.974)
High DBP	0.049*	2.190 (1.002–4.788)
High FPG	0.001**	2.589 (1.467–4.570)

AO: abdominal obesity, NAFLD: nonalcoholic fatty liver disease. : $p < 0.05$, ** : $p < 0.01$

Table 5. Background Characteristics of Subjects with and without Increased Maximum IMT

	With increased maximum IMT (n = 208)	Without increased maximum IMT (n = 160)	Age-adjusted p value	Age-adjusted odds ratio
Age (years)	61.6±10.2	51.0±10.7	< 0.001**	—
Gender (males/females)	125/83	77/83	0.004**	2.040
NAFLD (%)	61 (29.3%)	31 (19.4%)	0.004**	2.242
AO (%)	64 (30.8%)	46 (28.8%)	0.231	1.364
High SBP (%)	56 (26.9%)	16 (10.0%)	0.012*	2.312
High DBP (%)	33 (15.9%)	12 (7.5%)	0.022*	2.435
High LDL-C (%)	129 (62.0%)	77 (48.1%)	0.080	1.522
Low HDL-C (%)	9 (4.3%)	4 (2.5%)	0.149	2.606
High TG (%)	30 (14.4%)	25 (15.6%)	0.535	1.225
High FPG (%)	62 (29.8%)	36 (22.5%)	0.074	1.620
High HbA 1 c (%)	147 (70.7%)	82 (51.3%)	0.043*	1.649
High UA (%)	24 (11.5%)	18 (11.3%)	0.303	1.476
High AST (%)	21 (10.1%)	14 (8.8%)	0.223	1.647
High ALT (%)	29 (13.9%)	23 (14.4%)	0.104	1.748
High γ-GTP (%)	27 (13.0%)	15 (9.4%)	0.071	1.990
Without exercise habit (%)	98 (47.1%)	84 (52.5%)	0.525	1.166
With snacking habit (%)	54 (26.0%)	43 (26.9%)	0.659	1.127
With smoking habit (%)	31 (14.9%)	24 (15.0%)	0.407	1.312

NAFLD: nonalcoholic fatty liver disease, AO: abdominal obesity, UA: uric acid
Age is shown as mean ± standard deviation (SD). * : $p < 0.05$, ** : $p < 0.01$

Table 6. Variables Associated with Increased Maximum IMT in Multivariate Logistic Regression Analysis

	<i>p</i> value	odds ratio (95% CI)
Age	< 0.001**	1.100 (1.074 – 1.127)
Male gender	0.024*	1.765 (1.076 – 2.896)
NAFLD	0.035*	1.847 (1.044 – 3.266)
High SBP	0.038*	1.997 (1.038 – 3.845)

NAFLD: nonalcoholic fatty liver disease. *: *p* < 0.05, **: *p* < 0.01

Table 7. Background Characteristics of Subjects with NAFLD but not AO and with AO but not NAFLD

	with NAFLD but not AO (<i>n</i> = 37)	with AO but not NAFLD (<i>n</i> = 55)	Gender and Age- adjusted <i>p</i> value	Gender and Age- adjusted odds ratio
Age (years)	58.1 ± 9.0	56.9 ± 10.9	0.578	—
Gender (males/females)	22/15	47/8	0.005**	4.193
High SBP (%)	8 (21.6%)	12 (21.8%)	0.674	1.260
High DBP (%)	3 (8.1%)	9 (16.4%)	0.510	0.615
High LDL-C (%)	28 (75.7%)	34 (61.8%)	0.278	1.721
Low HDL-C (%)	2 (5.4%)	2 (3.6%)	0.442	2.223
High TG (%)	11 (29.7%)	9 (16.4%)	0.066	2.743
High FPG (%)	17 (45.9%)	24 (43.6%)	0.338	1.583
High HbA 1c (%)	33 (89.2%)	32 (58.2%)	0.005**	5.878
High UA (%)	8 (21.6%)	6 (10.9%)	0.025*	4.071
High AST (%)	8 (21.6%)	3 (5.5%)	0.024*	5.500
High ALT (%)	10 (27.0%)	4 (7.3%)	0.005**	7.291
High γ -GTP (%)	9 (24.3%)	7 (12.7%)	0.078	2.856
Increased maximum IMT (%)	28 (75.7%)	31 (56.4%)	0.025*	3.652
Without exercise habit (%)	20 (54.1%)	24 (43.6%)	0.561	1.308
With snacking habit (%)	10 (27.0%)	14 (25.5%)	0.484	0.680
With smoking habit (%)	8 (21.6%)	5 (9.1%)	0.049*	3.554

NAFLD: nonalcoholic fatty liver disease, AO: abdominal obesity, UA: uric acid
Age is shown as mean ± standard deviation (SD). *: *p* < 0.05, **: *p* < 0.01

Table 8. Variables Associated with NAFLD but not AO in Multivariate Logistic Regression Analysis

	<i>p</i> value	odds ratio (95% CI)
Female gender	0.006**	4.825 (1.567–14.858)
High HbA 1c	0.005**	6.089 (1.741–21.287)
High ALT	0.012*	6.309 (1.510–26.360)

NAFLD: nonalcoholic fatty liver disease. *: *p* < 0.05, **: *p* < 0.01

The group of subjects with NAFLD but not AO had significantly higher proportions of high HbA 1c, high UA, high AST, high ALT, and increased maximum IMT than the group with AO but not NAFLD, after adjusting for gender and age (Table 7). Table 8 shows the results of the multivariate analysis of risk factors for subjects with NAFLD but not AO. In this analysis, female gender, high HbA 1c, and high ALT remained significant.

Discussion

Our finding that both NAFLD and AO are strongly related to metabolic disorders is consistent with previous research^{1,9–11,20–21}. Recent studies^{10–13} have shown that patients with NAFLD have significantly greater carotid IMT than those without NAFLD, which is consistent

with the results of the present study. Also, NAFLD was significantly associated with an increased maximum IMT even after adjusting for other parameters, whereas AO was not. Oike *et al.*²² found that AO diagnosed using WC was not significantly associated with atherosclerotic changes, which supports our results. Furthermore, in our study, the group of subjects with NAFLD but not AO had a significantly higher proportion of subjects with increased maximum IMT than the group of subjects with AO but not NAFLD, even after adjustment for gender and age. These results suggest that NAFLD is more closely related to the progression of arteriosclerosis than AO.

The histologic spectrum of NAFLD includes nonalcoholic fatty liver (NAFL), which involves predominantly macrovesicular steatosis, and nonalcoholic steatohepa-

titis (NASH), which can progress to cirrhosis and is considered a precursor of cryptogenic cirrhosis²³. Some cohort studies demonstrated that subjects with NASH had a higher incidence of cardiovascular disease and higher mortality from cardiovascular disease than those with NAFL, and the prognosis of NASH was poor^{11,13–14}. Targher *et al.*²¹ showed that IMT was significantly different between patients with NASH, patients with simple steatosis, and control subjects; the lowest levels were observed in control subjects, levels were intermediate in patients with steatosis, and highest in those with NASH. Moreover, the severity of histological features of NAFLD, such as steatosis, necroinflammation, and fibrosis, was strongly positively correlated with carotid IMT. Leach *et al.*²⁴ reported that the carotid IMT was significantly positively correlated with hepatic inflammation, which may be a link between NASH and cardiovascular disease. Therefore, as an increased IMT may indicate the possibility for progression to NASH and the development of atherosclerosis, it is crucial to promote preventive measures and early detection and intervention to improve the prognosis of NAFLD.

One interesting finding of the present study is that the group with NAFLD but not AO had a significantly higher proportion of subjects with an increased maximum IMT than the group with AO but not NAFLD. This suggests that intracellular fat accumulation in the liver is a greater risk factor for the progression of atherosclerosis than AO. Hiraoka *et al.*²⁵ reported that nonobese subjects with a normal WC and NAFLD had significantly more components of MetS and higher insulin resistance compared to nonobese subjects with a normal WC and no NAFLD. Our finding that high FPG and high HbA1c were significantly associated with the presence of NAFLD after adjusting for other parameters, but were not significantly associated with the presence of AO, suggests that NAFLD is more closely associated with hyperglycemia than AO. As the diagnostic criteria for MetS in Japan require AO, examinees without AO but with a fatty liver, who are at risk for atherosclerotic diseases, are not typically provided health guidance in this regard. Therefore, if AO is treated as a criterion similar to dyslipidemia, elevated blood pressure, and elevated FPG but not a requirement of MetS, and fatty liver diagnosed noninvasively by abdominal US is added to the criteria of MetS, this could more effectively promote early detection and prevention of atherosclerosis. However, mild fatty liver cannot be detected by US, and there may be measurement deviations among examiners. It is therefore desirable to consider whether fatty liver should be added to the list of criteria for MetS, while also taking into consideration cost-effectiveness and manpower. However, in all cases, abdominal US is recommended for healthy individuals without AO. If fatty liver is discovered, the patient should be advised

to undergo examinations that are useful for evaluating the progression of arteriosclerosis, such as carotid US, which could enable early detection and intervention.

Our study has several limitations. First, the diagnosis of NAFLD was based on ultrasound imaging but was not confirmed by liver biopsy, which is the best tool for diagnosing NAFLD. Second, the diagnosis of AO was made based on the measurement of WC, but not by computed tomography or magnetic resonance imaging. Third, our study population of individuals who had both abdominal and carotid US in their medical check-up might have some selection bias and not reflect the general population. Fourth, because the study design was cross-sectional, we can only speculate about the association between atherosclerosis and NAFLD. Prospective observational studies, especially those examining subjects with NAFLD but not AO and subjects with AO but not NAFLD, are therefore needed.

Conclusions

Our analysis suggests that NAFLD is more strongly related to the progression of arteriosclerosis than is AO. Because individuals who have NAFLD might also have progressive atherosclerosis, regardless of the presence of AO, they should receive a recommendation for examinations to evaluate the progression of arteriosclerosis, such as carotid US, to enable early detection and intervention for cardiovascular disease.

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Conflicts of Interest

The authors state that they have no conflicts of interest to disclose.

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Risk Factors for Cerebral Arteriosclerosis Detected by Magnetic Resonance Angiography in Brain Check-ups

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Abstract

Objective: Cerebral arteriosclerosis is a major public health problem because it is one of the main causes of ischemic stroke. A paucity of material is available on factors associated with cerebral arteriosclerosis detected by magnetic resonance angiography (MRA) in a healthy population. The aim of this study was to determine what factors were associated with cerebral arteriosclerosis detected by MRA in persons undergoing a brain check-up.

Methods: We retrospectively investigated 5,897 people aged 40 and over who visited a hospital for a brain check-up. We obtained data on body mass index (BMI), blood pressure (BP), total cholesterol, triglycerides, and fasting glucose (FG). We performed logistic regression analysis to examine associations between vascular risk factors and cerebral arteriosclerosis.

Results: Cerebral arteriosclerosis was observed in 326 subjects (5.5%). After adjustment for other risk factors, BMI (odds ratio [OR], 1.066; 95% confidence interval [CI], 1.024–1.110), systolic BP (OR, 1.019; 95%CI, 1.014–1.025), and FG (OR, 1.005; 95%CI, 1.003–1.008) were significantly associated with arteriosclerosis. Obesity (BMI ≥ 25 kg/m²; OR, 1.622; 95%CI, 1.258–2.090), hypertension (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg; OR, 1.911; 95%CI, 1.503–2.429), and impaired FG (FG ≥ 110 mg/dL; OR, 1.411; 95%CI, 1.111–1.791) were independent predictors. Factors related to dyslipidemia were not associated with arteriosclerosis.

Conclusions: Obesity, hypertension, and impaired FG were independently associated with a higher risk of arteriosclerosis detected by MRA in study participants undergoing brain check-ups, while dyslipidemia, which is a common risk factor of arteriosclerosis, was not.

Keywords cerebral arteriosclerosis, magnetic resonance angiography, risk factors

Stroke is a common cause of morbidity and mortality worldwide. Cerebral arteriosclerosis is a major public health problem as it is one of the main causes of ischemic stroke¹. Arteriosclerotic lesions progress silently for years until they become symptomatic. Identification of markers of latent cerebral arteriosclerosis could be useful in the setting of primary stroke prevention and may provide an opportunity for early preventive interventions^{2,3}. Although risk factors for cerebral arteriosclerosis have been reported, most of the studies were performed in symptomatic patients^{4–7}. Studies on risk factors for cerebral arteriosclerosis from its asymptomatic stage are limited.

Although conventional angiography is the accepted gold standard in diagnosing cerebral arteriosclerosis in patients with ischemic stroke, the invasive nature of the procedure and the risk of perioperative complications

hinder its expanded use. New non-invasive tests include transcranial Doppler ultrasonography, computed tomography angiography, and magnetic resonance angiography (MRA). MRA is a safe and reliable modality in studying intracranial circulation⁸. Although several studies have investigated risk factors for cerebral arteriosclerosis in asymptomatic subjects, it was assessed by transcranial Doppler ultrasonography in most of them^{9–12}. Thus, a paucity of material is available on factors associated with arteriosclerosis detected by MRA in healthy populations. In addition, few large population studies that have aimed to determine related vascular risk factors in asymptomatic subjects are available.

The aim of this study was to determine what factors were associated with cerebral arteriosclerosis detected by MRA in study participants undergoing a brain check-up.

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Methods

The internal review board of the Fuji Brain Institute and Hospital approved all of the procedures performed in this study. Informed consent was obtained from the participants. Between June 1994 and September 2007, 7,346 people aged 40 and over who visited Fuji Brain Institute and Hospital for a medical check-up of the brain were entered into this study.

Health examinations started at 8:30 a.m. and 12:30 p.m. The study subjects were weighed wearing underwear, a hospital gown, and no shoes. Height and weight were measured and the body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Blood pressure (BP) was measured with an automated sphygmomanometer on the right arm in the sitting position after a 5-min rest. Subjects with a start time of 8:30 a.m. had skipped breakfast and those with a start time of 12:30 p.m. had eaten a light meal in the morning and skipped lunch. Blood samples were drawn by venipuncture. Total cholesterol, triglycerides, and fasting glucose (FG) were measured for each individual in the hospital laboratory.

We excluded those who did not undergo a blood examination ($n = 1,193$) as well as 256 people who did not undergo MRA. In total, 5,897 subjects were included in the analysis.

Subjects were assessed for presence of any abnormal lesion on MRA as part of their health check-up. A standardized, validated definition was not used to identify arteriosclerosis in the brain check-up. Generally, it was identified as artery obstruction, artery stenosis, or flow gap abnormalities (discontinuity of blood flow column signal, with distal reconstruction)⁸. Several trained neu-

rosurgeons assessed MRA images for presence of arteriosclerosis.

We used the chi-squared test for categorical variables and *t*-test or Wilcoxon's rank sum test for continuous variables to compare subjects with and without arteriosclerosis on MRA. Each of the risk factors was categorized as follows: obesity as BMI ≥ 25 kg/m²; hypertension as systolic BP (SBP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg; hypercholesterolemia as total cholesterol levels ≥ 220 mg/dL; hypertriglyceridemia as triglyceride levels ≥ 150 mg/dL; impaired FG (IFG) as FG ≥ 110 mg/dL. The cut off points were based on the assessment categories of the Japan Society of Ningen Dock (category C or D) except for hypertriglyceridemia (category B or higher). We performed logistic regression to examine associations between risk factors and arteriosclerosis. A *p*-value < 0.05 was considered to indicate statistical significance. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC).

Results

The study group consisted of 63% men and 37% women. The age of study participants ranged from 40 to 89 years, with a mean of 55 years. The mean BMI, SBP, diastolic BP, and total cholesterol values were 23.4 kg/m² (missing data = 12), 125 mmHg, 76 mmHg (missing data = 1), and 203 mg/dL, respectively. The median triglycerides and FG were 132 mg/dL and 129 mg/dL, respectively.

Arteriosclerosis was observed in 326 participants (5.5%). As shown in **Table 1**, arteriosclerosis was significantly associated with older age (62 vs. 55 years; $p < 0.001$), and having a higher BMI (23.9 vs. 23.4 kg/m²; $p = 0.006$), SBP (137 vs. 124 mmHg; $p < 0.001$), and

Table 1. Characteristics of Participants With and Without Arteriosclerosis

	Participants with arteriosclerosis <i>n</i> = 326	Participants without arteriosclerosis <i>n</i> = 5571	<i>p</i>
Female, <i>n</i> (%)	116 (36)	2050 (37)	0.658
Age, years	62 ± 10	55 ± 8	< 0.001
BMI ^a , kg/m ²	23.9 ± 3.3	23.4 ± 2.9	0.006
Systolic blood pressure, mmHg	137 ± 23	124 ± 19	< 0.001
Diastolic blood pressure ^b , mmHg	77 ± 12	76 ± 12	0.092
Total cholesterol, mg/dL	202 ± 35	203 ± 37	0.843
Triglyceride, mg/dL	138 (95–199)	131 (90–194)	0.080
Fasting glucose, mg/dL	116 (100–150)	107 (93–128)	< 0.001
Obesity ^a , <i>n</i> (%)	113 (35)	1444 (26)	< 0.001
Hypertension, <i>n</i> (%)	145 (44)	1252 (22)	< 0.001
Hypercholesterolemia, <i>n</i> (%)	90 (28)	1663 (30)	0.389
Hypertriglyceridemia, <i>n</i> (%)	144 (44)	2294 (41)	0.286
Impaired fasting glucose, <i>n</i> (%)	194 (60)	2542 (46)	< 0.001

Values are the mean ± SD or the median (interquartile range) unless stated otherwise.

Obesity was defined as BMI ≥ 25 kg/m²; hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg; hypercholesterolemia was defined as total cholesterol levels ≥ 220 mg/dL; hypertriglyceridemia was defined as triglyceride ≥ 150 mg/dL; impaired fasting glucose was defined as fasting glucose ≥ 110 mg/dL.

^aMissing = 12, ^bMissing = 1.

Table 2. Odds Ratios of Arteriosclerosis

	Odds ratio	95% CI
Model 1		
Female	0.793	0.609 – 1.033
Age, per y	1.096	1.081 – 1.111
BMI, per kg/m ²	1.066	1.024 – 1.110
Systolic blood pressure, per mmHg	1.019	1.014 – 1.025
Total cholesterol, per mg/dL	1.001	0.999 – 1.001
Triglyceride, per mg/dL	1.000	0.999 – 1.001
Fasting glucose, per mg/dL	1.005	1.003 – 1.008
Model 2		
Female	0.826	0.639 – 1.068
Age, per y	1.100	1.085 – 1.115
Obesity	1.622	1.258 – 2.090
Hypertension	1.911	1.503 – 2.429
Hypercholesterolemia	0.929	0.709 – 1.217
Hypertriglyceridemia	1.071	0.837 – 1.391
Impaired fasting glucose	1.411	1.111 – 1.791

Obesity was defined as BMI ≥ 25 kg/m²; hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg; hypercholesterolemia was defined as total cholesterol levels ≥ 220 mg/dL; hypertriglyceridemia was defined as triglyceride ≥ 150 mg/dL; impaired fasting glucose was defined as fasting glucose ≥ 110 mg/dL.

FG (116 vs. 107 mg/dL; $p < 0.001$). When factors were assessed as categorical variables, obesity (35% vs. 26%; $p < 0.001$), hypertension (44% vs. 22%; $p < 0.001$), and IFG (60% vs. 46%; $p < 0.001$) were significantly associated with arteriosclerosis.

We also performed logistic regression model analysis to assess associations between risk factors and arteriosclerosis (**Table 2**). When using risk factors as continuous variables, arteriosclerosis was significantly associated with BMI (OR, 1.066; 95%CI, 1.024–1.110), SBP (OR, 1.019; 95%CI, 1.014–1.025), and FG (OR, 1.005; 95%CI, 1.003–1.008). When used as categorical variables, obesity (OR, 1.622; 95%CI, 1.258–2.090), hypertension (OR, 1.911; 95%CI, 1.503–2.429), and IFG (OR, 1.411; 95%CI, 1.111–1.791) were independent predictors after adjustment for other risk factors. Factors related to dyslipidemia, which are common risk factors for arteriosclerosis, were not associated with arteriosclerosis on MRA.

Discussion

In this study, we found that obesity, hypertension, and IFG were associated with a higher risk of arteriosclerosis, detected by MRA, in participants undergoing brain check-ups.

Our data were in agreement with previous studies, which reported that hypertension and diabetes mellitus (DM) were risk factors^{9,11–13}. Hypertension is an established risk factor for arteriosclerosis and hypercholesterolemia is a well-known risk factor for coronary arteriosclerosis. An important finding is that, in line with several previous studies^{10–12,14}, hypercholesterolemia

was not associated with cerebral arteriosclerosis in our study. As we did not obtain data on low-density lipoprotein cholesterol and high-density lipoprotein cholesterol, this may be partly due to using total cholesterol instead of low-density lipoprotein cholesterol. In addition, we did not obtain data on medications participants were taking. Earlier, we investigated the risk factors for small vessel disease detected by magnetic resonance imaging (MRI), such as lacunar infarctions, white matter hyperintensities, and microbleeds in the same population¹⁵. We found that hypercholesterolemia was significantly associated with a lower risk of small vessel disease. In addition to the difference in pathogenesis between large and small vessels, intracranial arteries might have a different pattern of response to deleterious vascular effects compared with other arterial beds.

Several studies have reported risk factors for arteriosclerosis detected by MRA in healthy people. In most of the studies, however, risk factors such as hypertension, dyslipidemia, and diabetes were analyzed as categorical variables^{13,14}. Limited data are available on the relationship between risk factors as actual measured values and arteriosclerosis detected by MRA. Since the present study was on a large population, relatively small impacts of risk factors could be detected.

This study has a number of limitations. First, the arteriosclerosis criteria were not specified clearly in advance. The presence or absence of arteriosclerosis may have been misclassified because our study lacks the detail and consistency possible when a study is conducted prospectively. The neurosurgeons assessing the films were not blinded to the participants' clinical data, such as BP

and laboratory results, because they also had to assess the results of those examinations for the purpose of the check-up. Ideally, they should have assessed the films independently of the participants' clinical data. Basically, however, determination of arteriosclerosis was made independently of risk factors. Second, we did not distinguish between participants with and without medication regarding BP and laboratory levels. Third, we cannot rule out the presence of potential confounding variables, including smoking and alcohol consumption, not accounted for in the analysis. However, it seems unlikely that such residual confounding effects would completely eliminate the observed associations. Other limitations were that we investigated a population who visited one hospital for health check-ups and the participants were all Japanese who might have tended to be more health-conscious. Our results should be interpreted cautiously in light of the aforementioned limitations.

In conclusion, obesity and hypertension were independently associated with a higher risk of arteriosclerosis detected by MRA in persons undergoing brain check-ups. In addition, IFG was an independent predictor of arteriosclerosis, while dyslipidemia, which is a common risk factor of arteriosclerosis, was not. Further studies are needed to clarify the relationship between vascular risk factors and cerebral arteriosclerosis in order to establish appropriate measures for preventing stroke.

Conflict of Interests

The authors declare no conflict of interest.

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New Low-cost Method for Detecting Abnormal Thyroid Function in Patients Making Use of a Set of Routine-tests: Adding their Average Rates of Annual Time-series Variations Improves Diagnostic Accuracy

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Abstract

Background: Patients with thyroid dysfunction (PTDs) are likely to go undiagnosed. Therefore, we developed a new, low-cost screening method using a set of six routine tests to identify PTDs. This new method can predict the probability of thyroid dysfunction at the time of the screening. We found a lot of new PTDs through our screening method in general health check-ups and determined that an 85% threshold for the predicted probability was sufficient, instead of the 60% probability used in the screening. However, there were about three times the number of false positives compared to the number of true positives, mainly due to the effects of individual differences and background diseases.

Objective: The aim of the present study was to develop a method considering time-series variations in routine tests in addition to the predicted probability, which had only been used previously, to improve predictive accuracy.

Methods: The present study included 155 subjects (15 true positives and 140 false positives) who were suspected of having thyrotoxicosis in our screening, whose thyroid hormones were measured, and they also had previous visit records. We calculated the average rate of annual time-series variations (RATV) in each routine test between previous and current visits for each subject, and then plotted RATV and predicted probabilities together in a scattergram.

Results: By adjusting both thresholds on the scattergram, the following optimized thresholds were obtained: 98% for predicted probability or 20% for RATV. This combination significantly decreased the number of false positives from 140 to eight without yielding any false negatives for Graves' disease.

Conclusions: The introduction of RATV markedly reduces the influence of individual differences and background diseases in routine tests.

Keywords thyroid dysfunction, routine tests, time series variation, pattern recognition methods

Patients with thyroid dysfunction (PTDs) are likely to go undiagnosed^{1–4}. Thyroid specialists have previously described difficulties in identifying patients with thyroid dysfunction based on physical findings alone and therefore, measurement of TSH levels is considered essential^{5,6}. However, because of the associated cost, TSH levels cannot be measured in all people who visit a clinic. Thus it is difficult to give all people a full health check-up including TSH testing in consideration of cost effectiveness and this issue remains a topic of debate^{7,8}.

Patients with overt thyroid dysfunction should soon undergo medical treatment such as with anti-thyroid drugs for Graves' hyperthyroidism or with thyroid hormone replacement drugs for hypothyroidism since uncontrolled overt thyroid dysfunction has a bad influence on the whole body. Therefore, early detection of such patients is very important.

Since some routine tests generally produce abnormal results in PTDs due to a surplus or lack of thyroid hormones, we attempted to create a low-cost prediction method for detecting PTDs incorporating data from a

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set of such routine tests. We previously analyzed data from routine tests in patients with confirmed thyroid diseases and normal control subjects using three types of pattern recognition methods (PRM)⁹⁻¹¹ in addition to medical statistics. We found that a prediction model using a set of three parameters (elevation in alkaline phosphatase (ALP) and decreases in serum creatinine (S-Cr) and total cholesterol (TC))¹²⁻¹⁴ or four parameters (previous three parameters in addition to elevation of heart rate (HR))¹⁵ allowed accurate screening for hyperthyroidism (thyrotoxicosis). We applied these prediction models to 4,355 Japanese people whose routine test data had already been measured in a general health check-up (known as “Ningen Dock”) at JR Sendai Hospital between July 2008 and December 2011, and identified 7 overt PTDs (2 patients with Graves’ disease, 2 with painless thyroiditis, and 3 with hypothyroidism) who were subsequently treated by thyroid specialists^{15,16}. None of the 7 PTDs had expressed concerns regarding their health. We also applied our prediction models to 8,831 examinees of the Ningen Dock at Tohoku Kosai Hospital, a larger institution than JR Sendai Hospital, between September 2011 and March 2013, and successfully identified 14 overt PTDs (8 patients with Graves’ disease, 2 with painless thyroiditis, and 4 with hypothyroidism)¹⁷. Although we successfully identified 21 PTDs who were previously undiagnosed, there were 218 false positives (91 at JR Sendai Hospital and 127 at Tohoku Kosai Hospital) in our screening, who were found to be normal after measuring serum levels of thyroid hormones (FT4) and TSH. Most of these false positives were due to the low threshold of the predicted probability (60%) for the set of screening parameters whose purpose was to prevent false negatives. An analysis of the screening results from the Ningen Dock at Tohoku Kosai Hospital revealed that setting a higher threshold value for the probability (85%), under which Graves’ diseases or overt hypothyroidisms would not be overlooked, decreased the number of false positives to 45¹⁷. Although this threshold level produced two false negatives, both had painless thyroiditis that did not require medical treatment and therefore overlooking them did not cause any clinical problems.

Most of the 45 false positives, who were still under suspicion even after adjusting the threshold level to 85%, had either poor or opposite to the pattern characteristic of thyroid dysfunctions time-series variations in each routine test, which suggested that these false positives arose from their individual differences in routine tests¹⁸⁻¹⁹ or under effects of background diseases.

In our previous study, we evaluated the usefulness of a time-series analysis¹⁵, but only a few cases of true positives and false positives were considered qualitatively. In the present study, we analyzed all cases of time-

series traceable patients with Graves’ hyperthyroidism and normal subjects (true positives and false positives in screening with 60% threshold) in order to evaluate time-series variations, and aimed to investigate quantitatively whether our model would improve screening ability or not. We only selected patients suspected of having thyrotoxicosis in our screening and whose previous visit for Ningen Dock had been within the last three years. After calculating average rates of annual time-series variations for each routine test between the current visit and the previous visit, we determined whether using it together with the predicted probability of thyrotoxicosis improved diagnostic accuracy or not.

This study was approved by the Tohoku Kosai Hospital Ethical Review Board and JR Sendai Hospital Ethical Review Board. All data were handled using linkable anonymization.

Subjects and Methods

Subjects

From among people who had undergone screening in the Ningen Docks of JR Sendai Hospital (between July 2008 and December 2014) and Tohoku Kosai Hospital (between September 2011 and March 2013) and whose previous visit had been within the last 3 years, we identified 15 true positives (8 at JR Sendai Hospital and 7 at Tohoku Kosai Hospital) and 140 false positives (104 at JR Sendai Hospital and 36 at Tohoku Kosai Hospital) for thyrotoxicosis, giving a total of 155 subjects for analysis in the present study (**Table 1**). In the screening, the threshold level of predicted probability was set to 60%, which was calculated based on our prediction model using a set of three routine tests (ALP, TC, and S-Cr) or a set of four routine tests (ALP, TC, S-Cr, and HR). The mean \pm SD intervals between visits were 383 ± 68 days for true positives and 379 ± 79 days for false positives, i.e. both were nearly equal to a year.

Table 2 gives details of 15 patients (true positives) with thyrotoxicosis identified by our screening. In this study, neither pregnant women nor women within one year after childbirth were present in the true positives. The clinical and laboratory data of each patient shown in the table are for the current visit. Additionally, for patients who consulted the Thyroid Outpatients Service of JR Sendai Hospital or Tohoku Kosai Hospital after our screening, the results of diagnosis are shown as Graves’ disease or painless thyroiditis.

Pattern recognition methods (PRM)

We used the PRM that we reported previously^{12-14,20} for the prediction models in our screening. The predicted probability for our screening was calculated using two types of PRMs. We first used Bayesian regularized neural networks (BRNN)¹⁰. This is a multi-layer neural network that was extended to include the Bayesian

Table 1 Means and SDs of Clinical Characteristics Stratified by True Positives and False Positives.

		Sex	n	Age (y.o)	FT4 (ng/dL)	TSH (μIU/mL)	ALP (IU/L)	S-Cr (mg/dL)	TC (mg/dL)	HR (/min)
True positives	Tohoku Kosai Hospital Ningen Dock	Female	6	50.17±7.71	3.01±0.74* ¹	<0.01	306.5±92.6	0.45±0.08	138.5±12.9	80.2±10.9
		Male	1	54	3.56* ¹	<0.01	376	0.65	148	70
	JR Sendai Hospital Ningen Dock	Female	4	48.5±12.3	3.38±1.38* ²	0.028±0.037	303.3±175.0	0.42±0.07	163.8±20.9	91.3±10.6* ³
		Male	4	50.5±2.9	3.18±1.51* ²	<0.005	386.0±114.7	0.66±0.11	157.3±30.3	83.8±11.5* ³
False positives	Tohoku Kosai Hospital Ningen Dock	Female	15	49.1±12.2	1.27±0.16* ¹	1.43±0.86	262.3±106.1	0.46±0.05	161.6±27.1	73.6±12.8
		Male	21	52.1±7.3	1.28±0.18* ¹	1.40±0.67	375.1±96.9	0.67±0.10	165.0±29.6	70.1±10.9
	JR Sendai Hospital Ningen Dock	Female	24	50.9±8.7	1.28±0.19* ²	1.69±0.86	294.9±211.0	0.49±0.08	175.5±26.3	82.1±12.8* ³
		Male	80	52.8±7.1	1.30±0.18* ²	1.76±1.78	302.3±71.9	0.68±0.11	170.0±24.9	75.2±14.5* ³

Values are the mean ± SD.

*¹: Reference range of FT4 in the Tohoku Kosai Hospital Ningen Dock was 0.70–1.48 [ng/dL]

*²: Reference range of FT4 in the JR Sendai Hospital Ningen Dock was 0.90–1.70 [ng/dL]

*³: Heart rate in JR Sendai Hospital was pulse rate measured by automated sphygmomanometer (sitting position), while heart rate in Tohoku Kosai Hospital was determined from ECG (recumbent position). We corrected the pulse rate to heart rate using a regression formula¹⁵ when calculating predictive probabilities.

Table 2. Clinical and Laboratory Features of 15 Time-series Traceable Subjects with Overt Thyrotoxicosis Identified in Our Screening, whose Previous Visit to Ningen Dock was within Three Years

	Subject No.	Sex	Age	Diagnosis	FT4 (ng/dL)	TSH (μIU/mL)	ALP (IU/L)	S-Cr (mg/dL)	TC (mg/dL)	HR (/min)
Tohoku Kosai Hospital Ningen Dock	K1	Female	41	Painless Thyroiditis	1.76* ¹	<0.01	216	0.47	164	81
	K2	Female	54	Graves' Disease	3.24* ¹	<0.01	154	0.37	131	89
	K3	Male	54	Graves' Disease	3.56* ¹	<0.01	376	0.65	148	70
	K4	Female	57	Graves' Disease	3.35* ¹	<0.01	396	0.57	134	81
	K5	Female	61	Graves' Disease	3.65* ¹	<0.01	375	0.33	128	58
	K6	Female	47	Graves' Disease	2.25* ¹	<0.01	392	0.45	146	92
	K7	Female	41	Graves' Disease	3.78* ¹	<0.01	306	0.53	128	80
JR Sendai Hospital Ningen Dock	JR1	Male	48	Painless Thyroiditis	1.80* ²	<0.005	198	0.56	184	102* ³
	JR2	Male	48	Graves' Disease	5.60* ²	<0.005	494	0.56	106	74* ³
	JR3	Female	35	Graves' Disease	5.35* ²	<0.005	468	0.33	162	85* ³
	JR4	Female	42	Thyrotoxicosis* ⁴	2.20* ²	0.008	135	0.44	171	92* ³
	JR5	Male	55	Thyrotoxicosis* ⁴	2.02* ²	<0.005	461	0.83	174	74* ³
	JR6	Female	49	Thyrotoxicosis* ⁴	1.99* ²	0.092	122	0.51	132	80* ³
	JR7	Male	51	Graves' Disease	3.28* ²	<0.005	391	0.67	165	85* ³
	JR8	Female	68	Graves' Disease	3.99* ²	<0.005	488	0.40	190	108* ³

*¹: Reference range of FT4 in Tohoku Kosai Hospital Ningen Dock was 0.70–1.48 [ng/dL]

*²: Reference range of FT4 in JR Sendai Hospital Ningen Dock was 0.90–1.70 [ng/dL]

*³: Heart rate in JR Sendai Hospital was pulse rate measured by automated sphygmomanometer (sitting position), while heart rate in Tohoku Kosai Hospital was determined from ECG (recumbent position). We corrected the pulse rate to heart rate using a regression formula¹⁵ when calculating predictive probabilities.

*⁴: No diagnosis was made for patients who did not consult the JR Sendai Hospital Thyroid Outpatient Service.

probability framework for treating model parameters in order to avoid defects such as overfitting, which are encountered in the conventional maximum likelihood approach. We used the Software for Flexible Bayesian Modeling package²¹ by Neal for the BRNN. We subsequently applied the support vector machine (SVM)¹¹ with the LIBSVM package²² and selected radial basis function kernels.

In the present study, we defined the predicted probability as a predictive rate, calculated with BRNN or SVM using a set of routine tests performed during the current visit to Ningen Dock.

Rates of annual time-series variations in routine tests

We calculated the time-series variation rates for each subject's routine test data between the previous visit and current visit to Ningen Dock. In the calculation, we di-

vided the interval between visits in days for each subject by 365 in order to normalize inconsistencies in numbers of days in intervals. Therefore, the rate of the annual time-series variation (RATV) was obtained by the following equation:

$$v_R = \frac{R_c - R_p}{R_p} \cdot \frac{1}{\frac{D_c - D_p}{365}}$$

,where v and R denote the RATV and the measured value of each routine test (ALP, TC, S-Cr, and HR), and D denotes the date of visit to Ningen Dock. Subscript c denotes current visit while p denotes previous visit to Ningen Dock. We summed the v_{RS} from each routine test and divided this by the number of routine tests (three

or four) to obtain the average $RATV\bar{v}_x$ for each subject. Furthermore, since v_{TC} and v_{S-Cr} moved in a negative direction when subjects had thyrotoxicosis, we changed their signs so that all v_{RS} had positive values before summing them.

Scattergram of average rate of annual time-series variations and predicted probabilities

A scattergram for the subjects was prepared by plotting the average RATV along the vertical axis (y) and the predicted probability of thyrotoxicosis along the horizontal axis (x). The predicted probability on the x-axis started from 60%, which was the low threshold set to avoid false negatives in our screening. A horizontal line was drawn on the scattergram at 0% on the y-axis, showing the average RATV and therefore, if subjects plotted above the horizontal line, it meant that their time-series variations were changing toward the pattern characteristic of thyrotoxicosis. On the other hand, if subjects plotted below the line, it meant that their time-series variations were changing in a direction opposite to the pattern characteristic of thyrotoxicosis. The threshold level was then adjusted to reduce the number of false positives under the condition of maintaining the present sensitivity for true positives. We compared the number of false positives obtained with the threshold adjustment using the predicted probability (x-axis) and average RATV (y-axis) separately and together. In order to adjust both thresholds simultaneously, we employed the Solver add-in (evolutionary algorithms) bundled with Microsoft Excel to minimize the number of false posi-

tives and produce no false negatives for Graves' diseases.

Since two predicted rates were calculated, one with a set of three routine tests and one with a set of four routine tests, the predicted probability higher than the threshold (over 60%) was adopted when plotting the predicted probability of a subject. If both predicted probabilities were higher than 60%, that for four routine tests was selected because we had confirmed that a set of four routine tests was more precise than a set of three in our previous study¹⁵. Similarly, the number of routine tests used to calculate the average rate of annual time-series variations was adopted in accordance with the predicted probability selected.

In addition, further before/after visit records were monitored for subjects who appeared to need a more detailed analysis according to the results of the scattergram. In this case, every routine test value was normalized using the reference range. Values were converted to percentages, with the median value being 0%, upper limit being 100%, and lower limit being -100%. We plotted time-series percentages in order to track variations in each subject.

Results

The plotted scattergram is shown in **Fig. 1**. Looking at the predicted probabilities on the x-axis of the graph, the ten true positives of Graves' disease, represented by the symbol ●, were predicted with high probabilities of more than 89%. In contrast, subjects with painless thyroiditis or undiagnosed thyrotoxicoses, represented by

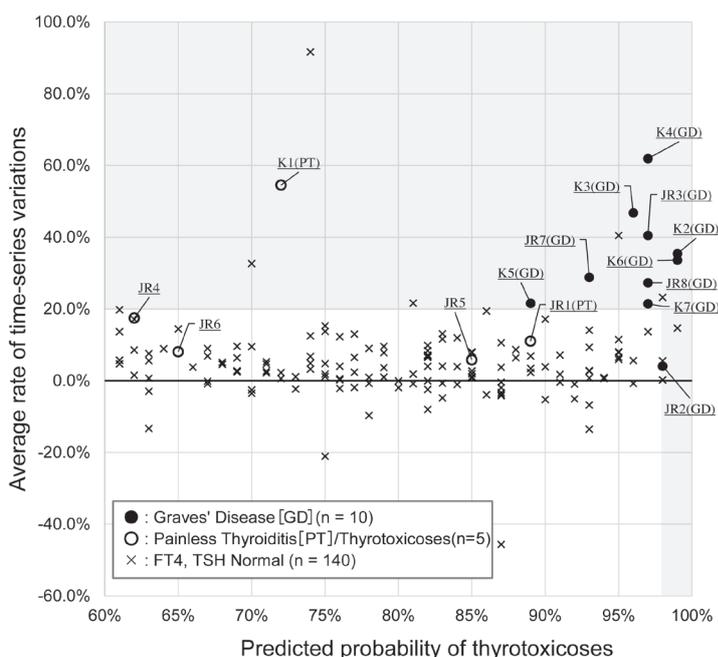


Fig. 1. Scattergram of the average rate of annual time-series variations in routine tests and the predicted probability of thyrotoxicosis calculated with BRNN or SVM using current visit routine test data. The shaded area denotes the region if an optimal combination of both thresholds (20% for the average RATV or 98% for the predicted probability) is applied.

the symbol ○, were predicted with lower probabilities than those with Graves' disease; whereas false positives with normal FT4 and TSH, represented by the symbol ×, were predicted with various probabilities. Therefore, adopting the threshold of 89% for predicted probabilities so as not to overlook patients with Graves' disease requiring treatment would reduce the number of false positives from 140 to 33.

The y-axis of the graph showed that nine out of the ten subjects with Graves' disease also had a high average RATV of more than 20%, whereas the few subjects with painless thyroiditis or thyrotoxicoses had a lower % RATV. Although the average RATV varied among the true positives, all time-series variations in the true positives were changing toward the pattern characteristic of thyrotoxicosis (above 0%), whether they were Graves' disease or not. In contrast, most subjects with normal FT4 and TSH (133 of 140) displayed mild variations in the rates within the range -20% to 20%. Therefore, only considering the average RATV, the use of a threshold level of 20% would significantly decrease the number of false positives, from 140 to five, although a subject with Graves' disease would become a false negative.

When we calculated both thresholds of the predicted probability (x-axis) and average RATV (y-axis) at the same time using Solver, we obtained the following thresholds: 98% for the predicted probability or 20% for the average RATV. This optimal combination of thresholds decreased the number of false positives from 140 to eight without producing any false negatives of Graves' disease. The number of false positives (FP) and false negatives of Graves' disease (FN) for each combination of thresholds are shown in **Table 3**. Since there are no subjects above the average RATV of 95%, numbers in the top row of the table (threshold value 95% for average RATV) denote changes in the numbers of FP and FN using only predicted probability as the threshold.

Accordingly, considering only the predicted probability, raising the threshold level to 90%, 95%, and 98% would produce one, two, and seven FN respectively. However, using the thresholds of the average RATV simultaneously under the "or" condition could prevent these FN.

Regarding subject JR8, who was diagnosed with Graves' disease (FT4: 3.99 ng/dL) in our screening in December, 2014, in the screening in 2013, JR8 was also found to have a high FT4 level (3.00 ng/dL). Although JR8 did not visit the Thyroid Outpatient Service of JR Sendai Hospital in spite of our encouragement in 2013, JR8 did take advantage of this service with our encouragement in 2014. Thus, in **Fig. 1**, the average RATV for JR8 was calculated under the influence of Graves' disease. In addition, the ALP, TC, S-Cr, and HR for JR8 in 2013 were 246 IU/L, 198 mg/dL, 0.41 mg/dL, and 99 /min, respectively. A comparison of these values with those measured in 2014, as shown in **Table 2**, revealed that TC, S-Cr, and HR changed only slightly whereas ALP increased significantly, to 488 IU/L from 246 IU/L, and therefore, the average RATV of JR8 was high (28%) and located in the upper-right area of **Fig. 1**.

Discussion

In the present study, we verified the usefulness of considering time-series variations quantitatively¹⁵, not qualitatively, by simultaneously plotting both the predicted probability and average RATV on a scattergram. Most subjects with Graves' disease were concentrated in the upper right area of the scattergram since they had a higher predicted probability and average RATV. Patients with painless thyroiditis or undiagnosed thyrotoxicoses had lower predicted probabilities and lower average RATVs, and were considered to have mild thyrotoxicosis based on thyroid hormone data (shown in **Table 2**).

When we compared predicted probability thresholds and average RATV independently, the latter markedly

Table 3. Number of False Positives (FP) and False Negatives of Graves' Diseases (FN) for Each Combination of Thresholds Under the "or" Condition

Threshold value for average rate of time-series variations	60%		80%		90%		95%		98%		100%	
	FN	FP	FN	FP								
95%	0	140	0	74	1	30	2	13	7	4	10	0
60%	0	140	0	75	1	31	2	14	6	5	9	1
40%	0	140	0	75	1	31	2	14	4	6	7	2
30%	0	140	0	76	1	32	2	15	4	7	5	3
25%	0	140	0	76	1	32	1	15	2	7	3	3
20%	0	140	0	76	0	33	0	16	0	8	1	5
15%	0	140	0	79	0	37	0	21	0	13	1	10
10%	0	140	0	85	0	47	0	32	0	26	1	24
5%	0	140	0	102	0	75	0	62	0	61	1	60
0%	0	140	0	128	0	112	0	106	0	105	0	105

decreased the number of false positives. This indicates that taking time-series variations into account is very useful in screening. Although one false negative (JR2) occurred when we used the threshold of the average RATV only, this subject had a high predictive probability of 98% and therefore, it is reasonable that both threshold levels, 98% for the predicted probability or 20% for the average RATV, were obtained separately by Excel Solver under the condition of avoiding false negatives of Graves' disease. Using this combination of threshold levels, the number of false positives was eight, with no false negatives for Graves' disease, and additionally a subject with painless thyroiditis (K1) was identified. However, considering the importance of preventing false negatives in screening, it may be desirable to have threshold levels with a more suitable margin than the former threshold level determined by Excel Solver, which only decreased false positives maximally. For example, as shown in **Table 3**, when we set the threshold of the predicted probability at 95% or that of the average RATV at 15%, this decreased the number of false positives to 21, which is one sixth of 140, and there were no false negatives of Graves' disease. As there were 2 false negatives when there were no thresholds for the average RATV, this confirmed that the evaluation of time-series variations using the average RATV not only decreased false positives but also prevented false negatives, who would potentially be patients with Graves' disease having lower predicted probabilities than can be found with this combination. In any case, suitable combinations of these thresholds may be set according to the needs of the facilities at which screening is performed.

In the case of JR2, the only true positive for Graves' disease who had a low average RATV (4%), every value of the four routine tests had already changed towards the pattern characteristic of thyrotoxicosis before that, in 2008, and appeared to become saturated, and therefore the calculated value of the average RATV between 2008 and 2009 was low. These results suggested that JR2 already had Graves' disease before 2005. In addition, comparing the values of HR before and after treatment, the median of the individual normal range for HR in JR2 may have been nearer the lower limit of the reference value (53 /min), similar to that of an athlete's heart, and this may reflect individual differences. The presence of a patient having undiagnosed thyrotoxicosis for a period of a few years, who had reached routine test data saturation, suggests the necessity and usefulness of our screening methods. Considering the possibility of saturation in routine test data based on this finding, screening only using the average RATV is insufficient for identifying patients with undiagnosed thyroid dysfunction, and therefore, the simultaneous use of RATV and predicted probability is considered essential. Furthermore, taking

elevations in ALP in thyrotoxicoses due to excess bone metabolism into account, the delay of one year in ALP recovery observed in JR2 may be interpreted as originating from a delay in normalization of bone metabolism²³. This was confirmed by time-series variations in JR8 who did not consult a doctor between 2013 and 2014 despite having a high FT4 level. This subject had only slight changes in TC, S-Cr, and HR between 2013 and 2014, whereas ALP was significantly increased. Therefore, not only previous visit routine data, but also other previous visit data, if measured, will be useful for identifying such patients with saturated routine tests.

Although individual differences are known to exist in routine tests, we were confident that their influence was largely reduced through the introduction of the average RATV. We were able to reduce the number of false positives from 140 to eight without adding any false negatives of Graves' diseases. The time-series variations were tracked easily in the Ningen Dock examinees because a large percentage of them were repeaters (approximately 70% were repeaters^{24,25}) and missing data are rare in Ningen Dock and therefore, our time-series analysis in the present screening may be realistic and useful. As we consider that the results obtained by including RATV in the analysis are generally applicable, it is expected that RATV will also be applied to other groups.

We identified the presence of thyrotoxicosis in this study but had previously found that another set of four parameters (elevations in lactate dehydrogenase (LDH), S-Cr, and TC and a lower red blood cell count (RBC)) allowed accurate screening for hypothyroidism²⁰ and therefore further studies are needed in order to confirm the usefulness of RATV in hypothyroidism.

It remains unclear whether there were false negatives in our screening because we did not measure serum levels of TSH and FT4 in all participants, only in those suspected of being PTDs with a probability of over 60% by our previous prediction model using only current visit data. Our new screening model using time-series variations should be better at avoiding false negatives than our previous screening model. It should also greatly reduce the number of false positives and improve the accuracy of our screening methods using a set of routine tests.

Disclosure of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Association between Leukocyte Count and Age, Body Mass Index, and Lifestyle-related Factors: a Cross-sectional Study in Ningen Dock Examinees

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Abstract

Background: The reference range for leukocyte count is wide, and examination of factors affecting leukocyte count is important.

Methods: We conducted a cross-sectional study of apparently healthy Ningen Dock examinees who underwent a health check-up in 2012. Information regarding current medication and lifestyle habits was obtained via a self-reported questionnaire.

Results: Overall, 5,853 men and 4,447 women were included in the study. Multiple regression analyses revealed that leukocyte count in men was most strongly associated with the level of smoking, followed by body mass index (BMI), level of alcohol consumption, and frequency of exercise. Leukocyte count in women was most strongly associated with BMI, followed by level of smoking, age and level of alcohol consumption. When comparing groups stratified by age after adjusting for confounding factors, the mean leukocyte count in women was highest in the youngest group (age < 40 years; 5,295/ μ L), gradually decreased as age advanced, and was lowest in those aged 50–59 years (4,638/ μ L). In contrast, the mean leukocyte count of men was constant (5,448–5,565/ μ L) across age groups. When comparing men and women in each age group, leukocyte count in women was lower in those aged \geq 50 years.

Conclusions: Leukocyte count was strongly associated with level of smoking and BMI in both men and women, but an association with age was observed only in women. We believe it is important to interpret leukocyte counts in Ningen Dock examinees, taking factors associated with leukocyte count into consideration.

Keywords leukocyte count, body mass index (BMI), smoking, age

Blood leukocyte counts are used as a marker of diagnosis and degree in infections, blood diseases and various other conditions. The reference range for leukocyte count is wide, ranging from approximately 3,500/ μ L to 9,500/ μ L. Recent studies have suggested that people with higher leukocyte counts within the reference range have a higher incidence or prevalence of lifestyle-related diseases such as diabetes, dyslipidemia, or hypertension^{1–5}. Leukocyte count is known to be affected by many factors, including smoking, body mass index (BMI), and alcohol consumption^{3,6–8}. Inconsistent results have been reported regarding a sex difference^{9–11}.

The aim of this study was to examine factors affecting leukocyte counts in apparently healthy Ningen Dock examinees.

Methods

This cross-sectional study was conducted in Ningen Dock examinees who underwent a health check-up in 2012. The following subjects were excluded: those receiving hospital treatment for diabetes, hypertension, dyslipidemia, hyperuricemia, liver diseases, or malignant tumors, those with a past history of cardiovascular or cerebrovascular disease, and those with incomplete questionnaires. Information regarding current medication, smoking habits, alcohol consumption, and exercise frequency was obtained by self-reported questionnaire. For the question regarding exercise, subjects chose one from the following four answers: almost no exercise, two-three times per month, one-two times per week, three-four times per week, and \geq five times per week. These exercise frequencies were converted to 0, 2.5, 6,

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14, and 24 times per month, respectively, for the purposes of the study. Alcohol intake was recorded as the average weekly frequency and average daily amount. Participants converted consumption of all types of alcohol to the number of bottles of Japanese rice-wine (sake) based on their ethanol content. The unit and alcohol content of Japanese sake were one go (180 mL) and approximately 15%, respectively. For practical reasons, it was assumed that a large can (500 mL) of beer, 100 mL of clear distilled liquor, 60 mL of spirits and 180 mL of wine were all equivalent to one go of Japanese sake.

Statistical analysis

Data were analyzed using Dr SPSS II for Windows (SPSS Inc., IL, USA). Multiple regression analysis was used to test possible associations of various factors with leukocyte counts. The Bonferroni test, Fisher's least significant difference test, or Student's *t*-test was used to assess significant differences between group means. Analysis of covariance was used to adjust the means of leukocyte counts for confounding factors.

Ethical consideration

General informed consent was obtained from the participants. The study was approved by the ethics committee of the Ageo Central General Hospital.

Results

Overall, 5,853 men and 4,447 women were included in the study. The average age of the men and women was approximately 51 years. BMI and levels of smoking and alcohol consumption were higher in men than in women (**Table 1**). The average leukocyte counts in men and women were 5,520/ μ L and 4,875/ μ L ($p < 0.001$), respectively. Multiple regression analysis revealed that leukocyte counts in men were most strongly associated with level of smoking, followed by BMI, level of alcohol consumption, and frequency of exercise (**Table 2**). Leukocyte counts in women were most strongly associated with BMI, followed by level of smoking, age, and level of alcohol consumption.

Mean leukocyte counts between groups stratified by level of smoking (**Table 3**) or BMI (**Table 4**) were also compared, after adjusting for confounding factors. Regarding the effect of smoking, leukocyte count in men was lowest in non-smokers and elevated as the level of smoking increased (**Table 3**). Leukocyte count in women also elevated as the level of smoking increased, but analyses could not be performed in women who smoked ≥ 30 cigarettes/day because of the low number of women in this category. Regarding the effect of BMI, leukocyte count in both men and women was lowest in

Table 1. Clinical Characteristics of Participants

	Men	Women	<i>p</i>
<i>n</i>	5853	4447	
Age (years)	50.5 (10.5)	51.0 (10.1)	*
BMI (kg/m ²)	23.6 (3.1)	21.9 (3.3)	***
Smoking (cigarettes/day)	6.0 (9.3)	1.2 (4.1)	***
Drinking (go/week)	5.5 (6.3)	1.5 (3.6)	***
Exercise (times/month)	5.2 (7.3)	5.7 (7.5)	***
Leukocytes (/ μ L)	5520 (1547)	4875 (1330)	***

Values are presented as mean (SD). *: $p < 0.05$, ***: $p < 0.001$

Table 2. Factors Associated with Leukocyte Counts Assessed by Multiple Regression Analysis

	Men		Women	
	T value	<i>p</i>	T value	<i>p</i>
Age (years)	-1.4		-10.3	***
BMI (kg/m ²)	15.2	***	19.2	***
Smoking (cigarettes/day)	31.2	***	13.5	***
Drinking (go/week)	-5.9	***	-2.3	*
Exercise (times/month)	-4.3	***	-1.3	

*, $p < 0.05$, ***: $p < 0.001$

Table 3. Leukocyte Counts of Participants Stratified by Level of Smoking, After Adjusting for Confounding Factors

Smoking (cigarettes/day)	0	1-9	10-19	20-29	30-39	40-
Men						
<i>n</i>	3793	198	801	869	139	53
Leukocytes (/ μ L)	5133 (23)	5366 (99)	5928 (49)	6559 (48)	6784 (119)	7286 (192)
<i>p</i>			***	***	***	***
Women						
<i>n</i>	4023	102	228	87	4	3
Leukocytes (/ μ L)	4800 (20)	5143 (123)	5516 (83)	6199 (134)	4952 (620)	7064 (716)
<i>p</i>		*	***	***		**

Confounding factors: age, BMI, amount of drinking, and frequency of exercise
Leukocyte counts (/ μ L) are presented as mean (SE).

*, $p < 0.05$, **, $p < 0.01$, ***: $p < 0.001$ vs. group of non-smokers

Table 4. Leukocyte Counts of Participants Stratified by BMI, After Adjusting for Confounding Factors

BMI (kg/m ²)	< 19	19.0–20.9	21.0–22.9	23.0–24.9	25.0–26.9	27.0–28.9	≥29
Men							
<i>n</i>	257	845	1583	1480	954	421	313
Leukocytes (/μL)	4974 (87)	5165 (48)	5406 (35)	5557 (36)	5733 (45)	5875 (68)	6203 (79)
<i>p</i>			***	***	***	***	***
Women							
<i>n</i>	768	1267	1112	635	328	179	158
Leukocytes (/μL)	4510 (45)	4658 (35)	4850 (37)	5080 (49)	5347 (69)	5472 (93)	6075 (99)
<i>p</i>			***	***	***	***	***

Confounding factors: age, amount of smoking, amount of drinking, and frequency of exercise
Leukocyte counts (/μL) are presented as mean (SE).

***: $p < 0.001$ vs. group of BMI < 19

Table 5. Leukocyte Counts of Participants Stratified by Age, After Adjusting for Confounding Factors

Age (years)	<40	40–49	50–59	60–69	≥70
Men					
	5524 (0.48)	5565 (0.31)	5505 (0.34)	5448 (0.45)	5509 (0.84)
<i>p</i>					
Women					
	5295 (0.53)	5032 (0.31)	4638 (0.34)	4672 (0.43)	4902 (0.94)
<i>p</i>		***	***	***	**

Confounding factors: BMI, amount of smoking, amount of drinking, and frequency of exercise
Leukocyte counts (/μL) are presented as mean (SE).

** $p < 0.01$, *** $p < 0.001$ vs. group of age < 40 years

Table 6. Comparison of Leukocyte Counts between Men and Women Stratified by Age, After Adjusting for Confounding Factors

Age (years)	<40	40–49	50–59	60–69	≥70
Men	5372 (0.48)	5414 (0.32)	5355 (0.33)	5177 (0.42)	5166 (0.79)
Women	5519 (0.61)	5365 (0.38)	4883 (0.38)	4781 (0.46)	4833 (1.05)
<i>p</i>			***	***	*

Confounding factors: BMI, amount of smoking, amount of drinking, and frequency of exercise
Leukocyte counts (/μL) are presented as mean (SE).

* $p < 0.05$, *** $p < 0.001$

the lowest BMI group (BMI < 19 kg/m²) and elevated as BMI increased (Table 4).

Next we examined an association between leukocyte counts and age after adjusting for confounding factors (Table 5). In women, leukocyte count was highest in those aged < 40 years. It decreased age-dependently and was lowest in women in the 50–59 and 60–69 age groups. In men, leukocyte counts were constant (5,448–5,565 /μL) across age groups. When leukocyte counts were compared between men and women in each age group, those in women were higher at age < 40 ($p < 0.073$) and lower at age ≥ 50 (Table 6). No difference was observed between men and women in the 40–49 age group. Because there was a large difference in the level of smoking between men and women (6.0 and 1.2 cigarettes/day, respectively) (Table 1), we conducted the analysis comparing men and women in different age groups only in non-smokers. The results were approximately the same as those in the subjects overall.

Discussion

Our study suggested that BMI and level of smoking were strongly associated with leukocyte counts in both men and women, but an association between age and leukocyte count was only observed in women. Previous studies have already shown that smokers have higher leukocyte counts^{6–8,12}, which decreases to around the level observed in non-smokers within 12 months after quitting smoking¹². However, leukocyte counts in past-smokers are still significantly higher than those in never-smokers¹². In the present study, non-smokers included both never- and past-smokers. Therefore, the association of leukocyte count with smoking is expected to be stronger if past-smokers were excluded. The association between leukocyte count and smoking was much stronger in men than in women. This could be because the number of heavy smokers was much lower among women than men.

BMI was positively associated with leukocyte counts

as reported previously^{1-3,7,8}. While the mechanism of an association between leukocyte count and smoking or obesity has not been clearly elucidated, one mechanism might involve low-grade subclinical chronic inflammation induced by obesity and smoking. Adipocytes are known to secrete pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor- α ¹³ and cigarette smoking acts on lung tissue and promotes leukocytosis. Another mechanism might involve increases in epinephrine and cortisol levels associated with obesity and smoking¹⁴, as epinephrine and cortisol are known to increase leukocyte count¹⁵.

In contrast to smoking and BMI, the association between leukocyte count and age was observed only in women. Leukocyte count in women was highest in the youngest age group and was significantly decreased in women in their fifties. In contrast, there was no age-dependent change in leukocyte count in men. These findings suggest that estrogen or progesterone levels, which are known to be highest during the mid-twenties to the early-thirties and gradually decrease as age advances, might affect leukocyte count in women. The effect of contraceptives on leukocyte count is interesting. Fisch and Freedman showed that women who were using oral contraceptives containing 75–150 μ g estrogen had higher leukocyte counts with increasing estrogen dose, with progesterone not playing a significant role in increasing leukocyte counts¹⁶. Bain and England showed that contraceptives containing low dose estrogen (30–50 μ g) did not affect leukocyte count¹¹. Furthermore, Aisien *et al.* showed that after use of the Norplant system (subdermal implants containing only progesterone), endogenous estrogen levels were maintained at near-basal levels and leukocyte counts significantly decreased from 5,552 / μ L to 4,400 / μ L¹⁷. These findings strongly suggest that the level of estrogen is related to leukocyte count. Tsilidis *et al.* showed that the concentration of estradiol but not testosterone was positively associated with leukocyte count even in men¹⁸. This supports our finding that there was no association between leukocyte count and age in men.

Our study also showed that there was an obvious sex difference in leukocyte count and that this difference depended on age. Leukocyte count in women was higher at a younger age, but lower at an older age. Bain and England reported that women had higher leukocyte counts than men¹¹. In their study, median ages of men and women were 24 and 25 years, respectively. Allan and Alexander showed that leukocyte counts in women aged 50–65 years were significantly lower than those in men in the corresponding age group⁹. Our results are consistent with these previous findings.

In this study, we could not exclude subjects with viral or bacterial infections, or those taking drugs affect-

ing leukocyte count such as adrenocorticosteroids and contraceptives. However, we suppose that such subjects did not affect the results because there were very few of them.

Conclusion

Higher leukocyte counts within the normal range are associated with a higher risk of coronary heart disease^{19,20} and its risk factors¹⁻⁵. Therefore, we believe that it is necessary to explain these risks and the factors associated with leukocyte count to Ningen Dock examinees who have a higher leukocyte count.

Conflict of Interest

The authors have no conflict of interest to declare.

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The Regulations of the International Society of Ningen Dock

Article 1

Name

The name of the association shall be the International Society of Ningen Dock.

Article 2

Office

The Society has its principal office in Japan Society of Ningen Dock.

Article 3

Aims

The Society, an organization of Japan Society of Ningen Dock for international operations, aims to contribute to global health promotion by enhancing the development of ningen dock as a medical check-up system.

Article 4

Tasks

The Society conducts the following tasks to achieve the aims described in the preceding section.

1. Holds congress (World Congress on Ningen Dock), board meetings, lectures, and committee meetings
2. Publishes journals and news magazines
3. Communicates and cooperates with related academic societies both in Japan and overseas
4. Promotes research activities in ningen dock and related fields
5. Does whatever is necessary to achieve the aims of the Society

Article 5

Membership

1. The Society consists of the following members

- 1) Regular member

A regular member shall be a member of the International Society of Ningen Dock who agrees to the aims of the Society, and has expert knowledge, techniques, or experience in the areas associated with the Society.

- 2) Supporting member

A supporting member shall be a person, a corporation, or a group that agrees to the aims of the Society, and supports its programs.

- 3) Honorary member

An honorary member shall be recommended, from those who have significantly contributed to the areas associated with the Society, by the executive board.

2. Those who want to apply for regular or supporting membership of the Society shall submit the prescribed application form with the membership fee.
3. The board meeting will process applications mentioned in the preceding section, and promptly notify the applicants of its decision.

Article 6

Officials

1. The Society shall appoint the following honorary advisors and officials.

Honorary advisor: Number not decided

Congress president: 1

President: 1

Vice president: 3 (from Japan : 2, overseas: 1)

Board members: up to 25 (from Japan : 15 or less, overseas : 10 or less)

Auditor: 2

Article 7

Honorary advisor

1. An honorary advisor shall be appointed by the president from those who have contributed to the development of the Society for a long period, and approved by the executive board.
2. Honorary advisors shall be eligible to attend the board meeting, and to express opinions; honorary advisors will not have voting rights.

Article 8

Congress president

1. The congress president shall be recommended by the executive board and appointed by the president.
2. The congress president shall represent the Society and host the World Congress on Ningen Dock as a scientific meeting.

Article 9

President

1. The president shall be selected by and from among board members and delegated by the president of Japan Society of Ningen Dock.
2. The president shall preside the Society.

Article 10

Vice president

1. The vice president shall be appointed, from among board members, by the president.
2. The vice president shall assist the president. In the case of accident, one of the vice presidents will be appointed by the president and will temporarily take over the duties.

Article 11

Board members

1. Board members from Japan shall be selected among candidates from regular members at Japan Society of Ningen Dock.
2. Overseas board members shall be selected at the recommendation of the executive board.
3. Board members execute duties for the Society under the orders from the president.
4. Board members, together with the president and the vice president, comprise the executive board.

Article 12

Board meeting

1. The president will call a board meeting on an as-needed basis, and serves as the chairman of the meeting.
2. The board meeting will pass resolutions on important matters of the Society.
3. The board meeting shall have the right to start proceedings if the majority of all the board members (including a letter of proxy) attend the meeting.
4. The board meeting shall pass resolutions with the majority votes of attendances.

Article 13

Auditor

Auditors shall audit accounts of the Society, and report to the board meeting.

Article 14

Commissioner

For the aims of successful programs of the Society, the president will set up committees and divisions through the resolutions of the executive board, and delegate the commissioners to regular members or other members of the Society.

Article 15

Accounting

1. The fiscal year for the Society starts on April 1 every year and ends on March 31 the following year.
2. Expenses required for the Society shall be covered by the following revenues.
 - 1) Membership fees
 - 2) Grants
 - 3) Donations
 - 4) Others

Article 16

Modification of rules

The rules of the Society can be amended by the resolution of the executive board.

Article 17

Miscellaneous provisions

Detailed regulations necessary for the enforcement of the rules of the Society are defined elsewhere by the president with the approval of the executive board.

Article 18

Additional clause

The Regulations of the International Society of Ningen Dock will come into effect on September 15, 2006.

Detailed Regulations of the International Society of Ningen Dock

Detailed regulations of the International Society of Ningen Dock are defined as follows:

(Detailed regulations on members)

Article 1

1. Members shall pay the following annual membership fee; honorary members will be exempt from membership fee.
 - 1) Regular member : 2,000 yen
 - 2) Supporting member : from one unit (unit: 20,000 yen)
2. Annual membership fee paid shall not be refunded for any reason.
3. Members with foreign citizenship shall pay a 3-year membership fee of 50 dollars.

Article 2

Members will be given priority in the following events :

- 1) Participation in scientific meetings hosted by the Society;
- 2) Contributions of articles to and receipt of the journal of the Society.

Article 3

Members shall lose their memberships in the event of the following:

- 1) Withdrawal from membership;
- 2) Adjudication of incompetence or quasi-incompetence;
- 3) Death or adjudication of disappearance, or dissolution of the group in the case of a member of a supporting group;
- 4) Delinquency in payment of membership fee for over three year.

Article 4

Those intending to withdraw from the Society must submit the notice of withdrawal in the prescribed form to be approved by the executive board.

Article 5

The Society can expel a member to whom either of the following would apply, with a resolution of the executive board:

- 1) Those who violate their duty as members of the Society;
- 2) Those who damage the honor of members of the Society or act against the aims of the Society.

Article 6

Those who satisfy Sections 1 and 2 of Article 5 of the Regulations of the International Society of Ningen Dock will be accepted as members of the Society.

(Detailed regulations on officials)

Article 7

1. The president will be selected from the board members of Japan Society of Ningen Dock.
2. In principle, the majority of board members from Japan will be selected from among the board members of Japan Society of Ningen Dock.

3. Overseas board members will essentially be selected from Asia, Pacific Rim, North America, or Europe.

Article 8

1. The term of the congress president will be from the end of the congress of which he/she is in charge to the next congress.
2. The term of board members will be six years (two terms of three years).

(Detailed regulations on congress and board meeting)

Article 9

Congress and board meeting will be held as follows :

- 1) The title of the congress will be World Congress on Ningen Dock.
- 2) In principle, the congress and the board meeting will be held once every three years; with the resolution of the executive board, however, the congress and the board meeting will be held as needed.
- 3) The congress and the board meeting will be held at the same time.
- 4) The name of the congress president and the location of the next congress will be announced.

Article 10

1. Those who want to take part in the congress shall pay the participation fee, which is defined separately.
2. Participation fee for the congress will be defined accordingly by the congress president.
3. Only regular members shall be allowed to present the results of their studies, except those who have been approved by the congress president.

(Enforcement of the detailed regulations)

Article 11

1. The detailed regulations will come into effect on September 15, 2006.

INSTRUCTIONS TO AUTHORS

Ningen Dock International

Official Journal of Japan Society of Ningen Dock

Ningen Dock is the official journal of Japan Society of Ningen Dock, in which original articles, case reports, and review articles in both Japanese and English are published. Ningen Dock accepts only manuscripts that are original work in the field of ningen dock and related areas not previously published or being considered for publication elsewhere, except as abstracts. The manuscripts published in Ningen Dock will appear on the website of our society.

If the manuscript concerns a clinical study, it must be in accordance with the Declaration of Helsinki of 1964 (subsequent revisions included). Therefore, for a manuscript whose content is epidemiological or clinical research, the approval of the facility's Institutional Review Board (IRB) or the Ethics Committee of Japanese Society of Ningen Dock must have been obtained for the study described. Also, in the text, it should be indicated that informed consent has been obtained from subjects. Additionally, for case reports, it should be stated that adequate care has been taken to ensure the privacy of the subject concerned.

Online submission system

Ningen Dock uses an online submission system called ScholarOne Manuscripts.

Please access <http://mc.manuscriptcentral.com/ningendock>

This site is only in Japanese at this time.

Preparation of manuscript

All manuscripts must be written in English with MS-Word, Excel, PowerPoint and/or a common graphic format. Authors who are not fluent in English must seek the assistance of a colleague who is a native English speaker and is familiar with the field of the manuscript.

The title, abstract, text, acknowledgments, references, tables, and figure legends should begin on separate sheets, with pages numbered, and be typed double-spaced using the 12-point font size in MS-Word.

Files for submission should be prepared in English in a Microsoft Word or other file format that may be uploaded to the online system.

Available formats for files to be uploaded: doc (docx), xls (xlsx) ppt (pptx), jpg, tiff, gif, ai, eps, psd File names must consist of alphanumeric characters and an extension.

Example file names: Manuscript.doc, Fig1.jpg, Table1.xls, etc.

Please indicate the version of Microsoft Office used in a cover letter accompanying the uploaded files.

All measurements should be expressed in SI units. Less common abbreviations should be spelled out at first usage and the abbreviated form used thereafter.

Title page

Titles should be concise and informative. Include the full names of authors, names and addresses of affiliations, and name and address of a corresponding author to whom proofs are to be sent, including a fax number, telephone number and e-mail address.

Abstract

The abstract should not exceed 250 words, and should be arranged under the following subheadings: Objective, Methods, Results, Conclusions, and have up to 4 keywords.

Types of articles

Original articles: An original article should not exceed 3,000 words, and should be arranged as follows: Abstract, Objective, Methods, Results, Discussion, (Conclusion), (Acknowledgments), and References.

Case reports: A case report should not exceed 2,000 words, and be arranged as follows: Abstract (which should be a brief summary of the content without headings), Introduction, Case report, Discussion, and References.

Review articles: Review articles should not exceed 4,000 words. Review articles are usually by invitation. However, articles submitted without an invitation may also be considered by the Editorial Board.

References

References should be numbered consecutively in order of appearance in the text and cited in the text using superscript numbers. For example, according to the study by Sasamori¹⁾. For journals, the names and initials of the first three authors, followed by "et al" if there are other coauthors, the complete title, abbreviated journal name according to Index Medicus, volume, beginning and end pages, and year should be included. For books, the names and initials of the first three authors, followed by "et al" if there are other coauthors, the complete title, book name, edition number, beginning and end pages, name and city of publisher, and year should be included. Examples of references are given below.

Journal: Ishizaka N, Ishizaka Y, Nagai R, et al: Association between white cell count and carotid arteriosclerosis in Japanese smokers. *Atherosclerosis* 2004; 175: 95-100.

Book: Kaplan NM: Measurement of blood pressure. In: Kaplan NM(ed), *Kaplan's Clinical Hypertension*. 7th ed., Lippincott William & Wilkins, Philadelphia, 2002, 25-55.

Tables

Tables should be cited in the text, and numbered sequentially with Arabic numerals. Each table should be given a number and a brief informative title, and should appear on a separate page. Explain in footnotes all abbreviations used.

Figures

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Abbreviations

1	1,5-AG	1,5-anhydroglucitol	61	hCG	human chorionic gonadotropin
2	17-OHCS	17 α -hydroxycorticosteroid	62	HCV	hepatitis C virus
3	95% CI	95% confidence interval	63	HDL-C	high-density lipoprotein cholesterol
4	α-GI	α -glucosidase inhibitor	64	HLA	histocompatibility [leucocyte] antigen
5	β_2-MG	β_2 -microglobulin	65	HPLC	high-performance liquid chromatography
6	γ-GTP	γ -glutamyl transpeptidase	66	Ht	hematocrit
7	A/G ratio	albumin-globulin ratio	67	ICD	International Classification of Disease
8	ABI	ankle-brachial index	68	ICU	intensive care unit
9	ACTH	adrenocorticotrophic hormone	69	IFG	impaired fasting glucose
10	ADL	activities of daily living	70	IGT	impaired glucose tolerance
11	AFP	α -fetoprotein	71	IMT	intima-media thickness
12	ALP	alkaline phosphatase	72	LAP	leucine aminopeptidase
13	ALT	alanine aminotransferase	73	LDH	lactate dehydrogenase
14	Apo (a)	apolipoprotein (a)	74	LDL-C	low-density lipoprotein cholesterol
15	APTT	activated partial thromboplastin time	75	Lp(a)	lipoprotein (a)
16	AST	aspartate aminotransferase	76	LPL	lipoprotein lipase
17	BMI	body-mass index	77	MCH	mean corpuscular hemoglobin
18	CA 125	carbohydrate antigen 125	78	MCHC	mean corpuscular hemoglobin concentration
19	CA 19-9	carbohydrate antigen 19-9	79	MCV	mean corpuscular volume
20	cAMP	cyclic adenosine 3', 5'-monophosphate	80	METs	metabolic equivalent
21	CAPD	continuous ambulatory peritoneal dialysis	81	MetS	metabolic syndrome
22	CBC	complete blood cell count	82	MMG	mammography
23	Ccr	creatinine clearance	83	MRA	magnetic resonance angiography
24	cDNA	complementary deoxyribonucleic acid	84	MRI	magnetic resonance imaging
25	CEA	carcinoembryonic antigen	85	mRNA	messenger RNA
26	cGMP	cyclic guanosine 3', 5'-monophosphate	86	MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
27	ChE	cholinesterase	87	MSW	medical social worker
28	CKD	chronic kidney disease	88	NMR	nuclear magnetic resonance
29	COI	conflict of interest	89	PET	positron emission tomography
30	COPD	chronic obstructive pulmonary disease	90	PSA	prostate-specific antigen
31	CK	creatinine kinase	91	PTH	parathyroid hormone
32	CRP	c-reactive protein	92	PWV	pulse wave velocity
33	CT	computed tomography	93	QOL	quality of life
34	CVA	cerebrovascular accident	94	RBC	red blood cell
35	D-Bil	direct bilirubin	95	RF	rheumatoid factor
36	DBP	diastolic blood pressure	96	RI	radioactive isotope
37	DNA	deoxyribonucleic acid	97	RIA	radioimmunoassay
38	DRG	diagnosis-related group	98	RNA	ribonucleic acid
39	dsDNA	double stranded deoxyribonucleic acid	99	SBP	systolic blood pressure
40	EBM	evidence-based medicine	100	SD	standard deviation
41	ECG	electrocardiogram	101	SEM	standard error of the mean
42	eGFR	estimated glomerular filtration rate	102	STD	sexually transmitted disease
43	EIA	enzyme immunoassay	103	T-Bil	total bilirubin
44	ELISA	enzyme-linked immunosorbent assay	104	T₃	triiodothyronine
45	EPO	erythropoietin	105	T₄	thyroxine
46	ESR	erythrocyte sedimentation rate	106	TC	total cholesterol
47	FBG	fasting blood glucose	107	TG	triglyceride
48	FDA	Food and Drug Administration	108	TIA	transient (cerebral) ischemic attack
49	FEV	forced expiratory volume	109	TIBC	total iron binding capacity
50	FEV₁	forced expiratory volume in one second	110	tPA	tissue plasminogen activator
51	FEV₁ %	forced expiratory volume % in one second	111	TPHA	<i>Treponema pallidum</i> hemagglutination assay
52	FPG	fasting plasma glucose	112	TSH	thyroid stimulating hormone
53	FSH	follicle stimulating hormone	113	TTT	thymol turbidity test
54	FT3	free triiodothyronine	114	UCG	ultrasonic echocardiography
55	FT4	free thyroxine	115	UIBC	unsaturated iron binding capacity
56	FVC	forced vital capacity	116	UN	urea nitrogen
57	GFR	glomerular filtration rate	117	VLDL	very-low-density lipoprotein
58	GH	growth hormone	118	WBC	white blood cell
59	Hb	hemoglobin	119	WHO	World Health Organization
60	HbA 1c	hemoglobin A1c	120	ZTT	zinc sulfate (turbidity) test

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