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Current Status of Laryngopharyngeal Cancer Detected by Screening Upper Endoscopy: Comparison with Esophageal Cancer

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Abstract

Objective: This study aimed to survey the current status of laryngopharyngeal cancer detected by endoscopic screening of the upper gastrointestinal tract.

Methods: We retrospectively collected data from those who had health checkups at our center from August 2012 to January 2022. Detection rates for laryngopharyngeal and esophageal cancer were calculated and clinical characteristics were compared between them.

Results: Of 43,526 cases (mean age 60.0 years), 52 cases (0.12%) required further investigation in the laryngopharyngeal region. The detection rates for pharyngeal and esophageal cancer were 0.018% and 0.090%, respectively, a 5-fold difference. Both diseases predominated in men (≥89%) in their late 60s, and there was no difference in smoking or drinking histories and degree of obesity. Among pharyngeal cancers, the most popular type was the protruding type with a size of 20 mm or less, but the proportion of lesions adequate for endoscopic treatment was significantly low (29% vs 74%, p=0.032). There was a trend towards a positive correlation between the detection rate of epithelial tumors in the laryngopharyngeal region including dysplasia and esophageal cancer by each endoscopist (r=0.601, p=0.066).

Conclusion: These results suggest that the identification and observation techniques used for early detection of esophageal cancer in high-risk individuals can also be applied to the detection of laryngopharyngeal cancer. In addition to considering the anatomical characteristics and risk of laryngopharyngeal cancer, establishing standards for the observation of this region in screening endoscopy is an urgent issue.

Keywords pharyngeal cancer, esophageal cancer, screening upper endoscopy, risk factor

According to the latest cancer statistics from the National Cancer Center, the number of patients with oropharyngeal, esophageal, and gastric cancer in 2019 was 28,782 (11,400 oral cavity, 12,271 pharynx and 5,111 laryngeal cancer), 26,382, and 124,319, respectively. Because gastric cancer is overwhelmingly common, the main purpose of examination of the upper gastrointestinal (UGI) tract during a medical examination in Japan is to detect gastric cancer. However, from 2000 to 2021, the number of deaths from laryngopharyngeal, esophageal, and gastric cancers ranged from 5,066 to 8,001, 10,256 to 10,958, and 50,650 to 41,824, respectively. In other words, while the number of deaths from gastric cancer is decreasing, the number of deaths from laryngopharyngeal cancer is increasing. Comparing the age-adjusted mortality rate (/100,000 people) in 2000 and 2021, esophageal cancer decreased from 3.9 to 2.4. This is thought to be due to advances in diagnosis and treatment, including endoscopic treatment techniques. On the other hand, the rate for oropharyngeal cancer has remained almost the same (from 1.9 to 1.8).

A large-scale survey report[1] summarizing 16,251 cases of pharyngeal cancer and 46,529 cases of esophageal cancer experienced in Japan reported that the number of cases (rate) detected in a health screening program was only 25 cases (0.15%) and 538 cases (1.16%), respectively. For both diseases, alcohol consumption and smoking are common risk factors[2,3], and image enhancement endoscopy such as narrow-band imaging (NBI) contributes to early cancer detection[4,5]. Although these statistics differ in the target population,

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there is about a 7.7-fold (1.16/0.15) difference in detection rates in screening UGI endoscopy. Furthermore, among carcinoma in situ (Tis), which is indicated for endoscopic treatment, the number (ratio) of oropharyngeal cancers and hypopharyngeal cancers found by screening endoscopy was 3 cases (1.3%) and 4 cases (0.6%), respectively, whereas approximately 70% were found during follow-up for other diseases. Improving the detection rate of early-stage cancer, which is treated by resection through the mouth using minimally invasive endoscopic treatment, is important not only for improving prognosis, but also for preserving function, such as speech and swallowing, and cosmetic aspects.

Most reports of laryngopharyngeal cancer detected by UGI endoscopy are limited to case reports[6,7]. Therefore, on the basis of our approximately 10 years' experience with UGI endoscopy at our center, we attempted to understand the current status of laryngopharyngeal cancer and esophageal cancer detection rates, as well as the clinical characteristics of the two diseases, including risk factors and endoscopic findings.

Methods

Subjects and endoscopic procedure

From August 1st, 2012 to January 31th, 2022, all subjects who underwent screening UGI endoscopy during a comprehensive health check-up at the center for preventive medicine at our hospital were included. All endoscopic examinations, including those on multiple visits, were included in the analysis. The endoscope system used was an Olympus EVIS LUCERA (CLV-260) until July 2019, and a Fujifilm LASEREO7000 thereafter, and image-enhanced observation (NBI or Linked Color Imaging and Blue Light Imaging, respectively) was available. The endoscope models used were mainly GIF-Q260 or EG-L600WR7, respectively, and magnified observation was not possible with any of the models. The most common insertion route was the oral route, and less than 0.1% were nasal. The use of sedatives and analgesics was mainly based on the patient's wishes. Flunitrazepam or midazolam was used as a sedative, and pethidine hydrochloride was used as an analgesic. Combinations and dosages of sedatives and/or analgesics were obtained by reference to past records.

Our endoscopists each had over 5 years of experience in endoscopy, and most were specialists certified by the Japan Gastroenterological Endoscopy Society. There is no standard manual for endoscopic observation of the laryngopharyngeal region, and it is up to the individual judgement of the endoscopist. Depending on the degree of the subject's gag reflex, images were taken in white light imaging and/or NBI mode, either during insertion or withdrawal of the scope. The esophagus was observed mainly under white light image during insertion and NBI mode during withdrawal. In principle, biopsies were not performed for intraoral lesions.

Analysis and statistics

The rate of close examinations required was calculated when each endoscopist judged that close examination and treatment by an otorhinolaryngologist was necessary due to findings in the oropharyngeal region. The results of detailed examination and treatment after the consultation were confirmed by clinical records, and the detection rate of oropharyngeal cancer and dysplastic lesions was calculated and compared with esophageal cancer cases observed at the same time. If a detailed examination was not performed, but tumor disappearance or shrinkage was confirmed by subsequent UGI endoscopy, it was judged not to be malignant. We also extracted endoscopic diagnoses including suspicion, treatment methods, and final diagnosis by pathological findings or otorhinolaryngologists. In addition, we compared patient background factors including age, gender, body mass index (BMI), smoking (Brinkman Index), and alcohol consumption, which are risk factors for laryngopharyngeal and esophageal cancer.

For statistical analysis, mean and standard deviation values were described for continuous variables. The *t*-test was performed for comparison of mean values, and the chi-square test for comparison of ratios. The relationship between variables was assessed using Pearson's correlation coefficient or Spearman's rank-correlation coefficient, if appropriate. SPSS version 24 was used for analysis, and statistical significance was set at the $p<0.05$ level. This research was approved by the Ethics Committee of Keio University School of Medicine (approval number: 20221001) and was conducted in compliance with the Personal Information Protection Law as an opt-out.

Results

Cases requiring close examination in the oropharyngeal and laryngeal regions

A total of 43,526 subjects [27,436 males (63.0%), 16,090 females (37.0%)] were examined over the nine and a half years, with an average age of 60.0 years old. Most subjects (92.9%) undertook endoscopic examination under sedation. **Table 1** shows that 52 cases (0.12%) required detailed examination in the laryngopharyngeal region. All eight patients with cancer or suspected cancer in the pharynx were finally diagnosed with cancer. On the other hand, of the 8 patients with brownish areas who were suspected of having dysplastic epithelium, 6 (75%) were finally diagnosed with dysplasia by an otorhinolaryngologist, and the remaining 2 were diagnosed with lymphoid follicles. The positive predictive value for epithelial tumors including dys-

Table 1. 52 Cases Requiring Detailed Examination or Treatment After Screening EGD

Endoscopic diagnosis (including suspicious lesion)				Final diagnosis by otorhinolaryngologists		Treatment
cancer	8	oropharynx	1	cancer	1	ELPS
		hypopharynx	7	cancer	1	ELPS
					1	TOVS
					5	CRT
dysplasia (BA)	8	oropharynx	2	dysplasia	2	follow-up
		hypopharynx	2	dysplasia	2	follow-up
			2	lymphoid follicle		
		larynx	2	dysplasia	2	follow-up
lymphoma	1	oropharynx	1	lymphoma (PTCL)	1	chemotherapy
papilloma	7	tongue	1	papilloma		
		oropharynx	1	lymphoid follicle		
			1	(no examination)		disappeared
		hypopharynx	1	papilloma		
			2	normal finding		
		larynx	1	papilloma		
polyp	14	tongue	1	papilloma		
			1	lymphoid follicle		diminished
		oropharynx	2	papilloma		
			1	normal finding		
		hypopharynx	1	lymphoid follicle		
			1	normal finding		
		larynx	1	cyst		
			1	granuloma		follow-up
			5	normal finding		
submucosal tumor	14	tongue	1	hemangioma		
		oropharynx	1	cyst		
			1	normal finding		
		hypopharynx	4	lymphoid follicle		
			2	cyst		
			1	normal finding		
		larynx	1	hemangioma		
			2	cyst		
			1	normal finding		

EGD, esophagogastroduodenoscopy; ELPS, endoscopic laryngo-pharyngeal surgery; TOVS, transoral videolaryngoscopic surgery; CRT, chemoradiation therapy; BA, brownish area; PTCL, peripheral T-cell lymphoma

plasia in laryngopharyngeal lesions was 88% (14/16). Multiple submucosal tumors up to 20 mm in size, mainly in the pharynx region, were diagnosed as peripheral T-cell lymphoma[8]. Regarding benign tumors, endoscopic diagnosis of papilloma or hemangioma based on characteristic findings remained almost the same at the final diagnosis (2 out of 3 cases diagnosed as papilloma by UGI endoscopy had no findings at the time of otorhinolaryngological examination; one case was lymphoid follicles). On the other hand, half of the 14 lesions diagnosed as polyps or suspected polyps were finally diagnosed as having no abnormal findings by otolaryngologists.

Cases of pharyngeal cancer

None of the eight patients with pharyngeal cancer had a history of cancer in the oral cavity or esophagus. Three patients received endoscopic treatment (one of whom had Tis cancer), four received chemoradiotherapy and one received chemotherapy alone. For the latter five cases, the interval between endoscopy at the time of diagnosis and the previous endoscopy was 12, 35, 39, 63, and 74 months, and all examinations were confirmed by chart review to have been performed under sedation, with few gagging reflexes. A case of T2N1M0 hypopharyngeal cancer is presented in **Fig. 1**. At the time of diagnosis, there was an elevated lesion measuring 20 mm from the right pyriform fossa to the posterior annulus (**Fig. 1A**), and non-magnifying close-in NBI observation revealed an irregular papillary surface structure, raising the strong suspicion of hypopharyngeal cancer (**Fig. 1B**). In the endoscopic photograph taken 12 months ago, rough mucosa is suspected at the same site (**Fig. 1C**), but the NBI photograph is dark and difficult to observe (**Fig. 1D**). Visual grading of the hypopharynx[9] is graded as Grade 1, where the posterior ring is barely visible and only part of the pyriform sinus is visible.

Comparison between pharyngeal cancer and esophageal cancer cases

Cases with pharyngeal cancer and esophageal cancer identified by examination were 8 (0.018%) and 39 (0.090%), respectively, a 5-fold difference. One case

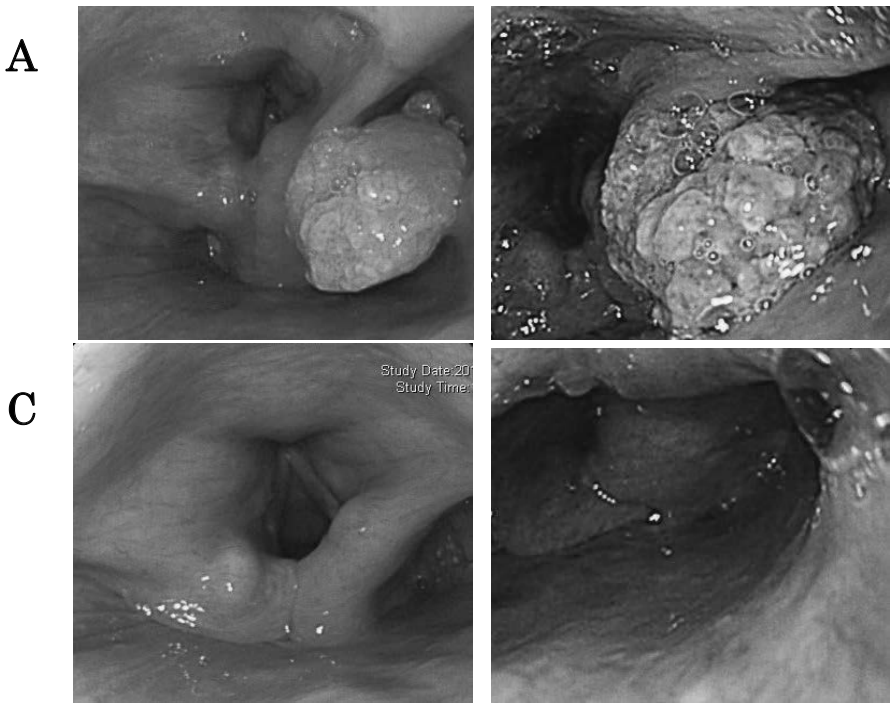

Fig. 1. A Case of Hypopharyngeal Cancer (T2N1M0)

At the time of diagnosis, there was a 20 mm-sized protruded lesion extending from the right pyriform sinus to the posterior part of the ring (A). Close-up NBI observation without magnification revealed dilated, tortuous atypical blood vessels and an irregular papillary surface structure, suggesting a hypopharyngeal cancer (B). In the endoscopic image taken 12 months ago, the visual grade of the hypopharynx[8] was equivalent to Grade 1, with the posterior part of the ring barely visible (C) and only a part of the piriform recess visible (D).

was a combination of pharyngeal cancer and esophageal cancer. After excluding this case, clinical characteristics were compared between the two groups (**Table 2**). The average age was in the late 60s, males were predominant (89% or more), and BMI was closely similar. As for alcohol consumption, the percentage of alcohol consumption exceeding the appropriate amount (20 g/day) was high (29%) in patients with esophageal cancer, but there was no significant difference overall, and no difference was observed in MCV. On the other hand, with regard to smoking, the Brinkman index was significantly higher in the esophageal cancer group. In pharyngeal cancer, the tumors were predominantly elevated (100% vs 21%, p=0.000) and mostly less than 20 mm in size, but the proportion of indications for endoscopic treatment was significantly lower (29% vs 74%, p=0.032).

Comparison of pharyngeal cancer and esophageal cancer detection rates by individual endoscopists

Fig. 2 shows the detection rate of individual endoscopists. There was no statistically significant difference between the detection rates of pharyngeal epithelial tumor and esophageal cancer, but there was a trend towards a positive correlation (r=0.601, p=0.066).

Discussion

Screening endoscopy for the UGI tract at our institution found 8 pharyngeal cancers and 6 dysplasias of the laryngopharyngeal region among 52 cases requiring close examination, with a positive predictive value of 88%. On the other hand, 39 cases of esophageal cancer were found, and a 5-fold divergence was observed between the detection rates of pharyngeal cancer and esophageal cancer. In addition to endoscopically diagnosable lesions such as papilloma, lesions endoscopically termed submucosal tumors and polyps were all benign diseases (lymphoid follicles, cysts, hemangiomas, and granulomas).

According to the nationwide cancer statistics in 2019, the ratio of patients with esophageal cancer to laryngopharyngeal cancer was almost 1.5. (=26,382/17,382). However, in a large-scale survey in Japan, there was an approximately 7.7-fold difference in screening detection rate between them, although the populations were different. In an observational study of 51,628 cases, which is the only report of cancer screening by upper endoscopy to date, no cancer was found among 123 cases (0.24%) requiring further investigation in the oropharyngeal region[10]. The reason for this is considered to be as follows. In UGI endoscopy, (1) there are many anatomical blind spots in the oral cavity, (2) detailed observation cannot be made because gagging reflexes are likely to occur, (3) the oral cavity

Table 2. Comparison of Clinical Characteristics of Pharyngeal Cancer and Esophageal Cancer

clinical characteristics	pharyngeal cancer (*n*=7)	esophageal cancer (*n*=38)	*p*
Age, years	68.4±14.5	67.6±9.1	0.847
Male	7 (100)	34 (89)	0.495
BMI, kg/m^2	23.7±2.6	23.2±3.3	0.687
Smoking history+	4 (57)	24 (63)	0.538
Smoking history (current)	3 (43)	5 (13)	0.094
Brinkman index	132±137	389±503	0.012
Drinking (0, <20, 20 g/d ≤)	1, 6, 0 (14, 86, 0)	2, 25, 11 (5, 66, 29)	0.213
MCV (fL)	96.3±3.4	96.7±6.1	0.851
Smoking and drinking	4 (57)	25 (66)	0.484
Neither smoking nor drinking	1 (14)	1 (3)	0.290
Type of tumor (protruded)	7 (100)	8 (21)	0.000
Tumor size (20 mm–)	2 (29)	10 (26)	0.613
Endoscopic treatment	2 (29)	28 (74)	0.032

BMI, body mass index; MCV, mean corpuscular volume.
The numbers including ± in the Table indicate the average value and standard deviation. The numbers in parentheses in the Table indicate percentages.

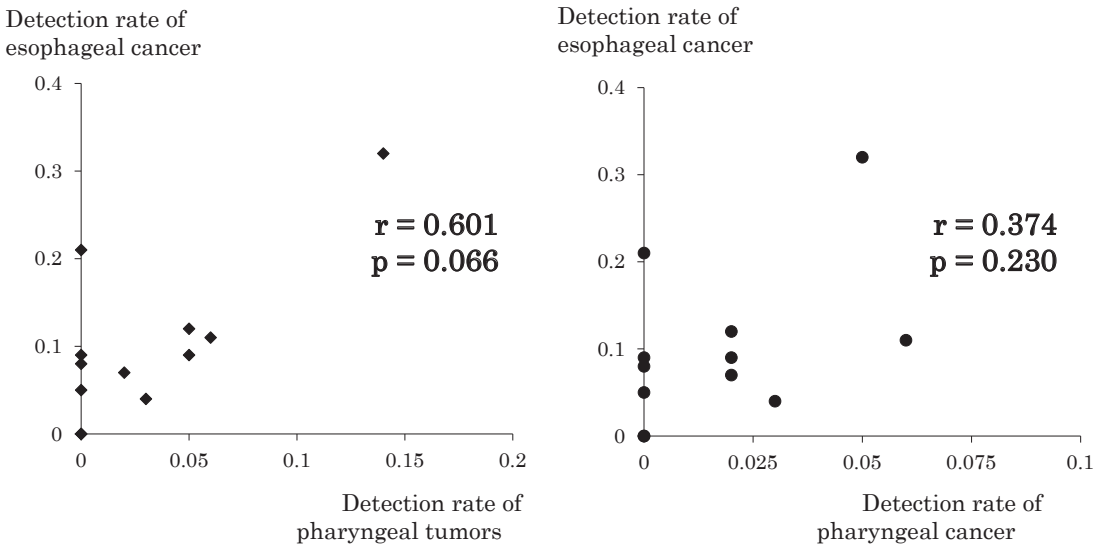

Fig. 2. Correlation Between Detection Rates of Laryngeal Tumor or Cancer, and Esophageal Cancer by Each Endoscopist

A total of 43,526 examinations included 14 pharyngeal epithelial tumors (8 cancers) and 39 cases of esophageal cancer. (Lt) Correlation between the detection rates of pharyngeal tumors and esophageal cancers. (Rt) Correlation between the detection rates of pharyngeal cancers and esophageal cancers.

observation method has not been yet standardized in screening examinations, and (4) no observation method consistent with cancer risk in the oropharyngeal region has been established. In cancer screening, it is necessary to determine the presence or absence of lesions or lesions that should be examined in detail, not only in the esophagus, stomach, and duodenum, but at least in the sites that can be observed through the insertion route. Regarding (1) and (3), Nakanishi *et al.*[5] reported that a high detection rate of 0.11% (10 cases/8,872 cases) had been achieved using the NBI and prescribed observation methods, even on exclusion of patients with a history of UGI cancer and patients with throat symptoms. There are also reports of an observation method using the Valsalva maneuver for the detection of hypopharyngeal cancer[11–13] and experience using Valsamouth®[14]. Murono *et al.*[9] used Valsamouth® to classify the observable regions of the hypopharynx (posterior cricoid, upper esophageal sphincter, etc.) into Grades 1 to 5, and reported that the Valsalva maneuver provided significantly greater visibility than the vocalization method. Hypopharyngeal cancer located at the right esophageal entrance, as shown in this case, is difficult to recognize, especially with normal observation methods. Regarding (2), Yamasaki *et al.*[15] reported that vocalization under the pre-administration of pethidine

alone broadened the observation range. Screening upper endoscopy is often performed under sedation at the request of the examinee, so it is difficult to obtain their cooperation, such as the Valsalva maneuver or vocalization. In addition, the main purpose is to discover gastric cancer, and the current situation is that sufficient time cannot be spent on observation of the laryngopharyngeal region. Alcohol consumption and smoking are recognized as common risk factors for hypopharyngeal, laryngeal, and esophageal cancers[2,3], and image-enhanced observation is used for early detection[4,5]. Additionally, a positive correlation trend was observed in the rates of detection of laryngopharyngeal epithelial tumors and esophageal cancer by endoscopists at our facility. Therefore, it is suggested that high-risk subject identification and observation techniques for early detection of esophageal cancer can also be applied to detect laryngopharyngeal cancer. Both pharyngeal and esophageal cancer patients whose cancers were detected at our institution were mostly men in their late 60s on average, 60% had a history of smoking, and 90% had hypopharyngeal cancer, mainly located in pyriform sinus. According to the cancer statistics in 2019, the incidence of pharyngeal cancer by age group exceeds 40 per 100,000 men in their 60s, and the crude (male/female) incidence by site for the nasopharynx, oropharynx, and hypopharynx including pyriform sinuses were 0.6 (0.9/0.4), 1.8 (3.0/0.7), and 4.0 (7.6/0.7), respectively. Thus, high-risk men aged over 60 years should be carefully observed with NBI for the hypopharynx, including the left and right pyriform, during insertion and/or withdrawal to increase the detection rate of this area. In particular, for patients at high cancer risk (history of esophageal cancer or multiple unstained Lugol's lesion, or smoking or drinking history), instruction on the Valsalva maneuver or vocalization prior to the examination, and observation with the patient's cooperation under pethidine premedication can contribute to improvement in early cancer detection, increasing indications for minimally invasive treatment. Furthermore, Nakamura *et al.*[16] reported that in half of the 20 laryngopharyngeal carcinomas whose natural history was observed, endoscopic findings worsened after 11 months. It is also necessary to consider recommending endoscopic examination at the otolaryngology department for high-risk subjects or when observation is difficult due to the gag reflex.

A limitation of this study is that it was an analysis of screening endoscopy at a single institution. Many of our elderly patients also visit our university hospital, and it is possible that they are generally at high risk of cancer. Additionally, there is no specification for laryngopharyngeal screening by upper endoscopy regarding who should be observed or how they should be observed, so endoscopists must make their own judgment. Since trans-nasal endoscopy is rarely performed, its usefulness has not been investigated. On the other hand, most doctors performing the procedure were gastrointestinal endoscopists certified by the Japan Gastroenterological Endoscopy Society, and they observed the oral region of more than 90% of subjects under sedation with relatively low gag reflexes. Therefore, the bias in this regard is considered to be low. Subjects requiring close examination could be examined at the otolaryngology department of our hospital, with the advantage that their clinical course, including treatment and prognosis, can be tracked. In addition, since the repeat examination rate was high, it was also possible to go back to examine previous endoscopic images.

Conclusion

According to recent nationwide cancer statistics, the ratio of patients with esophageal cancer to laryngopharyngeal cancer is almost 1.5, but there was a 5-fold discrepancy in detection rates of laryngopharyngeal and esophageal cancer by screening UGI endoscopy at our facility. Taking into consideration the anatomical characteristics and cancer risk in the laryngopharyngeal region, establishing a standard for observation of this region by screening UGI endoscopic examination is an urgent issue.

Acknowledgments

The authors thank the other endoscopists who performed endoscopy at our center.

Conflicts of Interest

KK is a member of Hills Joint Research Laboratory for Future Preventive Medicine and Wellness funded by Mori Building Co., Ltd. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. The other authors have no conflicts of interest to declare in regard to this research.

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Original Article

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Association Between Metabolic Syndrome and Mean Platelet Volume

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Abstract

Objective: Mean platelet volume, a measure of platelet size, could be a potentially simple approach for estimating platelet activity. The current study aimed to elucidate the relationship between mean platelet volume and metabolic syndrome, including an investigation of which component of metabolic syndrome was significantly associated with increased mean platelet volume.

Methods: We enrolled 910 individuals who underwent a complete medical check-up at Tokyo Women's Medical University between June 2018 and September 2022. The relationship between metabolic syndrome and mean platelet volume was analysed through contingency tables using multivariate logistic regression. Statistical significance was set at $p<0.05$.

Results: Metabolic syndrome, particularly the dyslipidemia and hypertension components, was significantly associated with increased mean platelet volume.

Conclusions: This study indicated that higher mean platelet volume is an independent risk factor for metabolic syndrome, which warrants extensive study, particularly given its potential for becoming a diagnostic tool during annual and general regular medical checkups, with low cost and wide availability.

Keywords medical check-up, mean platelet volume, metabolic syndrome

Mean platelet volume (MPV), a widely used marker of platelet size and activity, can be easily determined through routine automated hemograms and is routinely available at relatively low cost. Subjects with increased MPV have large platelets that are metabolically and enzymatically more active and have greater prothrombotic potential than normal platelets[1–5]. Elevated MPV has been associated with accelerated thrombopoiesis and increased risk for cardiovascular diseases[6–9]. In this regard, metabolic syndrome (MetS) is a group of cardiometabolic disorders comprising central obesity, dyslipidemia, elevated blood pressure and hyperglycemia[10,11]. Interestingly, research has suggested a potential relationship between MPV and MetS. In fact, insulin resistance, which plays a central role in the pathogenesis of MetS[12], has been associated with increased platelet activity[13,14]. Ding *et al.*[6] showed an association between higher MPV and MetS in patients with type 2 diabetes mellitus (DM). Tavil *et al.*[15] also showed that higher MPV was associated with MetS among patients with coronary artery disease. In contrast, Shah *et al.*[1] showed a relationship between higher MPV and diabetes but showed no relationship between higher MPV and MetS. The current study aimed to evaluate the relationship between increased MPV and MetS, including investigation of which component of MetS was significantly associated with increased MPV.

Methods

Participants and study design

This retrospective cohort study was carried out in accordance with the principles stipulated in the Declaration of Helsinki and was approved by the Ethics Committee of Tokyo Women's Medical University (approval date: March 11, 2024; approval number: 2023-0190). The study adhered to the Personal Information Protection Law by allowing participants to opt-out. This study cohort comprised individuals who underwent a complete medical check-up at Tokyo Women's Medical University (Japan) between June 2018 and September 2022. Specifically, we included those who underwent a periodic check-up at the Ningen Dock (a Japanese health check-up system).

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Data collection

The following clinical data were extracted from the periodic health check-up programme at the Ningen Dock: age, sex, physical characteristics (height, body weight and waist circumference), complete blood count, blood biochemistry results and urinalysis results. Details regarding participants' medical histories were obtained through medical interviews. Blood samples were taken from a peripheral vein after a 12-h overnight fast.

Definitions

MetS was defined according to the 2005 guidelines of the Evaluation Committee on Diagnostic Criteria for Metabolic Syndrome of Japan[16]. MetS diagnosis requires the presence of central obesity and at least two of the following three medical conditions: high blood pressure, including prior treatment for hypertension; dyslipidemia, including prior treatment for dyslipidemia; and impaired fasting glucose (IFG), including prior treatment for DM. Central obesity was defined as a waist circumference of ≥85 and ≥90 cm for men and women, respectively. Hypertension was defined as a systolic blood pressure (SBP) of ≥130 mmHg and/or diastolic blood pressure (DBP) of ≥85 mmHg. Dyslipidemia was defined as a serum triglyceride level of ≥150 mg/dL and/or a high-density lipoprotein cholesterol (HDL-C) level of <40 mg/dL. IFG was defined as a glucose level ≥110 mg/dL.

Insulin resistance was determined using the homeostasis model assessment-insulin resistance (HOMA-IR) score, which was calculated as follows: {[fasting glucose (mg/dL) × fasting insulin (μU/mL)]/405}. A score of ≥2.5 is considered the threshold for insulin resistance.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 29.0.1 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean (standard deviation) per group. Statistical difference was determined using two-sided Student's *t*-tests (for equal variance) or Welch's *t*-test (for unequal variance). Non-normally distributed variables were compared using the Mann–Whitney *U* test. Variables reported as proportions were compared using the chi-square test. Relationships between risk factors and MetS were examined using multivariate logistic regression analysis to determine the odds ratios (ORs). A p value <0.05 indicated statistical significance.

Results

Clinical characteristics of the study participants

Among the 910 participants included, 594 were men with a mean age of 67.3 (standard deviation 13.1) years and 316 were women with a mean age of 67.6 (standard deviation 12.5) years. If a participant underwent examination more than once, only the data on the first examination were used in this study.

Table 1 shows the clinical characteristics of the 294 and 616 participants with and without MetS, respectively. The MetS group was significantly older; had a significantly greater male-to-female ratio, waist circumference, and HOMA-IR score; had significantly higher SBP, DBP, MPV, triglyceride, uric acid (women only), fasting plasma glucose and hemoglobin A1c (HbA1c) levels; and had a significantly lower HDL-C level and estimated glomerular filtration rate (eGFR) than the non-MetS group.

Table 1. Clinical Data of Participants With or Without Metabolic Syndrome

Characteristics		Subjects with MetS	Subjects without MetS	*p* value
		Mean (SD) [Number] {% under treatment}		
Age		70.1 (11.5) [294]	66.1 (13.3) [616]	<0.001
Gender, men/women		[267/27]	[327/289]	<0.001
Systoric blood pressure (mmHg)		127.2 (15.3) [294] {66.3}	118.5 (16.2) [616] {26.1}	<0.001
Diastolic blood pressure (mmHg)		74.1 (12.6) [294] {66.3}	70.4 (11.4) [616] {26.1}	<0.001
MPV (fL)		10.0 (0.8) [294]	9.8 (0.8) [616]	0.002
HDL-C (mg/dL)	men	53.9 (13.2) [267] {67.0}	63.6 (16.9) [327] {24.8}	<0.001
	women	63.8 (15.3) [27] {66.7}	76.2 (16.9) [289] {33.2}	<0.001
Triglyceride (mg/dL)		165.3 (117.7) [294] {67.0}	96.6 (55.1) [616] {28.7}	<0.001
Uric acid (mg/dL)	men	6.1 (1.2) [267] {28.8}	6.0 (1.2) [327] {16.2}	0.205
	women	5.8 (1.4) [27] {7.4}	4.9 (1.0) [289] {0.0}	0.003
FPG (mg/dL)		121.0 (26.3) [294] {28.9}	102.8 (16.0) [616] {8.1}	<0.001
HbA1c (%)		6.3 (0.8) [294]	5.8 (0.5) [616]	<0.001
HOMA-IR		3.1 (2.0) [294]	1.5 (1.1) [616]	<0.001
eGFR		63.6 (16.9) [294]	68.1 (15.6) [616]	<0.001
Waist circumference (cm)	men	94.7 (7.0) [267]	85.8 (8.0) [327]	<0.001
	women	96.5 (5.7) [27]	79.3 (7.9) [289]	<0.001

MetS, metabolic syndrome; SD, standard deviation; MPV, mean platelet volume; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate

Association between MPV and MetS

To determine the association between MPV and MetS, a contingency table analysis was performed on participants with and without MetS via multivariate logistic regression analysis. Notably, we found that increased MPV was a significant risk factor for MetS (OR=1.3, $p<0.023$; **Table 2**). To investigate which component of MetS was significantly associated with increased MPV, a contingency table analysis was performed on participants with and without MetS who satisfied only one component (dyslipidemia, IFG or hypertension) via multivariate logistic regression analysis. Results showed that increased MPV was a significant risk factor for the dyslipidemia (OR=1.2, $p=0.043$; **Table 3**) and hypertension (OR=1.2, $p=0.038$; **Table 4**) components of MetS. MPV was not found to be a risk factor for the IFG component of MetS (data not shown).

Discussion

After investigating the relationship between MPV and MetS, the current study showed that increased MPV was a significant risk factor for MetS (**Table 2**). Obesity and visceral adiposity, which are pathological conditions indicative of MetS, induce insulin resistance and cause abnormal secretion of adipokines and cytokines, including leptin and interleukin, which trigger megakaryocytes to produce larger platelets[6,9,17].

Insulin resistance is one of the pathogenic mechanisms of MetS and is a common indicator of the risk of diabetes and MetS[18]. HOMA-IR, developed and validated by Matthews *et al.*[19], is a method for quantifying insulin resistance for clinical and research purposes in several populations based on fasting glucose and plasma insulin levels[20]. We also analysed the relationship between MPV and insulin resistance as measured by HOMA-IR. However, we did not find a significant association between increased MPV and HOMA-IR (data not shown).

We also investigated which component of MetS was significantly associated with MPV. We showed that increased MPV was a significant risk factor for the dyslipidemia and hypertension components of MetS (**Table 3** and **4**).

Wang *et al.* indicated that hypertriglyceridemia was associated with platelet hyperactivation[21]. A possible

Table 2. Multivariate Logistic Analysis of the Risk Factors for Metabolic Syndrome

Characteristics	Subjects with MetS (*n*=294)	Subjects without MetS (*n*=616)	Adjusted OR	95%CI	*p* value
	Number (%)				
Aging (over 60 years old)	225 (76.5)	409 (66.4)	2.2	1.55–3.27	<0.001
Men	267 (90.8)	327 (53.1)	8.9	5.62–13.98	<0.001
Women	27 (9.2)	289 (46.9)			
MPV			1.3	1.03–1.55	0.023
HOMA-IR≥2.5	135 (45.9)	71 (11.5)	6.2	4.29–9.05	<0.001

MetS, metabolic syndrome; OR, odds ratio; CI, confidence interval; MPV, mean platelet volume; HOMA-IR, homeostasis model assessment-insulin resistance

Table 3. Multivariate Logistic Analysis of Risk Factors for MetS Satisfying the Dyslipidemia Component

Characteristics	Subjects with MetS satisfying the dyslipidemia component (*n*=296)	Subjects without MetS satisfying the dyslipidemia component (*n*=614)	Adjusted OR	95%CI	*p* value
	Number (%)				
Aging (over 60 years old)	217 (73.3)	417 (67.9)	1.5	1.06–2.05	0.022
Men	267 (90.2)	327 (53.2)	8.2	5.43–12.50	<0.001
Women	29 (9.8)	287 (46.7)			
MPV			1.2	1.01–1.46	0.043

MetS, metabolic syndrome; OR, odds ratio; CI, confidence interval; MPV, mean platelet volume

Table 4. Multivariate Logistic Analysis of Risk Factors for MetS Satisfying the Hypertension Component

Characteristics	Subjects with MetS satisfying the hypertension component (*n*=317)	Subjects without MetS satisfying the hypertension component (*n*=593)	Adjusted OR	95%CI	*p* value
	Number (%)				
Aging (over 60 years old)	247 (77.9)	387 (65.3)	2.3	1.62–3.18	<0.001
Men	285 (89.9)	309 (52.1)	8.8	5.87–13.20	<0.001
Women	32 (10.1)	284 (47.9)			
MPV			1.2	1.01–1.47	0.038

MetS, metabolic syndrome; OR, odds ratio; CI, confidence interval; MPV, mean platelet volume

mechanism for this may be that hypertriglyceridemia activates platelets via an enhanced platelet response to adenosine diphosphate (ADP) and an increased resistance to the inhibitory effects of prostacyclin (PGI_2)[22,23]. Moreover, HDL-C and obesity have been consistently associated with markers of platelet activity[24–26].

Our findings also showed that increased MPV was a significant risk factor for the hypertension component of MetS (**Table 4**). Yagi *et al.*[27] showed that platelet aggregation ability increases with hypertension and also observed a decrease in platelet count and an increase in MPV as hypertension progresses. Furthermore, Saga *et al.* found that administering calcium channel blockers to hypertensive patients normalised blood pressure and decreased MPV[28], indicating the utility of MPV in clinical practice and health checkups.

MPV is a convenient and routinely available measure of platelet activation that can be utilised in inpatient and outpatient settings[2]. MPV can indicate rapid platelet activation and thrombotic risk given its correlation with platelet size, activation and increased aggregation[29,30]. Increased platelet size may signify high platelet turnover and younger platelets[31]. Larger and younger platelets are believed to increase physiologic activity and prothrombotic potential[3,32]. Currently, MPV is not examined during regular general health checkups performed based on the Ordinance on Industrial Safety and Health in Japan. Based on the findings of the current study, however, we believe that MPV should be extensively studied as a diagnostic tool during both annual medical and regular general health checkups considering its low cost, availability and utility as an indicator of not only thrombotic and inflammatory diseases but also MetS. Lifestyle improvements (diet and exercise therapy) are considered necessary to improve the health condition of patients with increased MPV.

Conclusions

This study indicated that higher MPV was an independent risk factor (marker) for MetS and should be extensively studied as a diagnostic tool during both annual medical and regular general health checkups owing to its low costs and availability.

Conflict of Interest

The authors have no conflict of interest to declare.

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Thrombocytosis: Data Analysis in Routine Health Check-up at Health Examination Facilities

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Abstract

Objective: To analyze the frequency of thrombocytosis diagnosed in general health examination institutions and the background of examinees to consider the direction of secondary screening.

Methods: We analyzed the complete blood count data collected at health examination foundation-related facilities from fiscal year 2016 to fiscal year 2020. Furthermore, we conducted detailed analysis including past medical history and current medical history for examinees in fiscal year 2020.

Results: The total number of platelet tests conducted and the number of cases meeting the criteria of the Japan Society of Ningen Dock and Preventive Medical Care Grade D (platelet count of 400,000/μL or higher) and the diagnostic criteria for essential thrombocythemia (ET) of the World Health Organization (platelet count of 450,000/μL or higher) at four health examination foundation-affiliated facilities from 2016 to 2020 were as follows:

· Fiscal year 2016: 43,964/805 (1.8%)/264 (0.60%)
· Fiscal year 2017: 50,171/1,095 (2.18%)/346 (0.69%)
· Fiscal year 2018: 54,854/1,222 (2.23%)/426 (0.78%)
· Fiscal year 2019: 59,160/1,464 (2.47%)/493 (0.83%)
· Fiscal year 2020: 62,420/1,678 (2.69%)/548 (0.88%)

Among the cohort of examinees undergoing total platelet tests, the number of individuals subjected to ET examination was 0.6–0.9% annually (approximately 500 individuals). The incidence of thrombocytosis was 1.5 to 2 times higher in females than in males, with a tendency towards microcytic anemia observed.

Conclusion: It was found that approximately 1% of all health examination attendees may be candidates for secondary examination for thrombocytosis. Currently, we are developing a comprehensive data analysis program that can comprehensively understand factors such as sustained hematopoietic increases and referral and attendance status for the same examinee.

Keywords thrombocytosis, health examination, essential thrombocythemia, secondary thrombocythemia

Thrombocytosis is observed in various diseases and conditions, but it is broadly classified into autonomous (neoplastic) thrombocytosis, represented by myeloproliferative neoplasms (MPNs), including essential thrombocythemia (ET); and secondary (reactive) thrombocytosis[1]. In either case, diagnosis based on clinical symptoms associated with increased platelets is unlikely, and is mostly presumed to be incidentally discovered during routine blood tests in health check-ups. The criteria for thrombocytosis, as determined by the Japan Society of Ningen Dock and Preventive Medical Care (JSNDPMC), designate a platelet count of 400,000/μL or higher as Grade D (requires detailed examination)[2–4], while the latest diagnostic criteria for ET proposed by the WHO in 2017 adopt a platelet count of 450,000/μL or higher[5]. In previous diagnostic criteria for ET, the threshold was a platelet count of 600,000/μL or higher[6]. However, due to reports of bleeding and thrombotic events occurring at lower platelet counts[7–9], the threshold was revised to

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450,000/μL or higher, exceeding the 95th percentile of normal platelet counts[10,11].

When considering ET, if the platelet count is 450,000/μL or higher, further detailed examination is warranted. Understanding the actual situation of thrombocytosis in health check-up and Ningen Dock examinees is crucial for health check facilities and institutions responsible for detailed examinations. Therefore, this report examines the actual status regarding thrombocytosis in general health check-up and Ningen Dock facilities by aggregating and analyzing the blood test data of examinees who underwent blood tests at health check facilities affiliated with our foundation.

Methods

Collection and implementation of blood test data for examinees

Blood test data for examinees who underwent blood tests at four facilities affiliated with our foundation (Niigata Kenshin Plaza, Higashi Niigata Kenshin Plaza, Nagaoka Health Service Center, Mobile Health checkup) from fiscal year 2016 (January to December) to fiscal year 2020 were collected and analyzed using a spreadsheet application (Excel® version). Blood tests after blood collection were contracted to SRL Corporation and BML Corporation. Analysis was done using the Mann–Whitney U test, and a p value <0.05 was considered to indicate statistical significance.

This clinical study was conducted with the approval of the Niigata Prefectural Health Foundation Ethics Review Board (HFNP-IRB) (Approval No. 2024-1). Regarding the protection of personal information and research participation consent, the facilities affiliated with our foundation publicly disclose the use of personal information for publication after strict anonymization for academic societies, etc., as well as in the privacy policy, and similar information is included in documents sent to individuals to obtain comprehensive consent. This clinical study adhered to this policy in its utilization of health examination data.

Results

Annual implementation status of blood tests

First, we analyzed the data from individuals undergoing routine health examinations at four facilities affiliated with our foundation. We found no significant differences in male-to-female ratio, age distribution, or occupations of examinee among these facilities. The examinees consisted of over 95% residents of Niigata Prefecture, with others including business travelers from other prefectures and manual workers from various Asian countries (specific names of other prefectures and Asian countries are unknown).

Among the four facilities, Niigata Kenshin Plaza and Higashi Niigata Kenshin Plaza primarily serve residents of Niigata City, Shibata City, Tainai City, Murakami City and surrounding areas (known as the Kaetsu region). Nagaoka Health Service Center serves residents of Nagaoka City, Sanjo City, Tsubame City, and surrounding areas (known as the Ken'o and Chuetsu regions), as well as residents of Joetsu City and surrounding areas (known as the Joetsu region), although individuals may also visit facilities distant from their residence more convenient to their workplace location. Mobile health checkups are conducted throughout all regions of Niigata Prefecture. The breakdown of the types of examinees by occupation was approximately 30% corporate employees, 20% manufacturing industry workers, 20% construction industry workers, 10% public servants, 10% agriculture, forestry, and fisheries workers, and approximately 10% service industry workers or workers in occupations whose details were unclear.

Table 1 summarizes the number of blood test implementations from fiscal year 2016 (January to December) to fiscal year 2020. The number of test items for hematological examination varies depending on the type of health examination (Ningen Dock, lifestyle disease screening, regular examinations A, B, C, etc.), with the number of tests decreasing in the order of red blood cell tests, white blood cell tests, and platelet tests. Specifically, in fiscal year 2016, there were

Table 1. Annual Number of Blood Tests Conducted by Fiscal Year

test items	gender	FY 2016	FY 2017	FY 2018	FY 2019	FY 2020
RBC	M+F	179439	186881	217587	227850	235605
	M	106806	112297	132577	137562	140087
	F	72633	74584	85010	90288	95518
WBC	M+F	108045	115238	125627	133530	140433
	M	67303	72440	78538	81918	85330
	F	40742	42798	47089	51612	55103
PLT	M+F	43964	50171	54854	59160	62420
	M	26698	29414	32144	34260	35934
	F	17266	20757	22710	24900	26486

test items: RBC=Red blood cell, WBC=White blood cell, PLT=Platelet
FY: fiscal year (January–December)
gender: M=male, F=female

179,439 instances of red blood cell testing, 108,045 instances of white blood cell testing, and 43,694 instances of platelet testing. The number of tests for each item increased annually, with 235,605 instances of red blood cell testing, 140,433 instances of white blood cell testing, and 62,420 instances of platelet testing in fiscal year 2020.

Trend in the number of thrombocytosis cases by fiscal year

The number of thrombocytosis cases among examinees who underwent platelet testing from fiscal year 2016 to fiscal year 2020 is shown in **Table 2**. The total number of platelet tests, the number (%) of cases with a platelet count of 400,000/μL or higher according to the JSNDPMC criteria (PLT-H: D (JSNDPMCC)), and the number (%) of cases with a platelet count of 450,000/μL or higher according to the WHO-ET diagnostic criteria (PLT-H (WETDC)) were as follows:

- Fiscal year 2016: 43,964/805 (1.80%)/264 (0.60%)
- Fiscal year 2017: 50,171/1,095 (2.18%)/346 (0.69%)
- Fiscal year 2018: 54,854/1,222 (2.23%)/426 (0.78%)
- Fiscal year 2019: 59,160/1,464 (2.47%)/493 (0.83%)
- Fiscal year 2020: 62,420/1,678 (2.69%)/548 (0.88%)

The number of cases targeted for ET differential diagnosis was approximately 1% annually (about 500 cases). Throughout each fiscal year, the number of PLT-H: D (JSNDPMCC) cases was approximately three times higher than the number of PLT-H (WETDC) cases. Within the PLT-H: D (JSNDPMCC) cohort, about 70% had platelet counts ranging from 400,000 to 449,000/μL (refer to the middle section of **Table 2**). In terms of gender ratio, the number of female cases was about 1.5 to 2 times higher than the number of male cases.

Background of examinees who underwent platelet testing in FY 2020

Next, we conducted a detailed analysis of the data for FY 2020, which had the highest number of examinees and the least data missing among FY 2016 to FY 2020. The background of all examinees who underwent platelet testing in FY 2020 was as follows: median age: 48 years (16–108), median platelet count: 259,000/μL (1.1–95) (with 4 cases having platelet counts in the 900,000/μL range), median hemoglobin (Hb)/hematocrit (Ht): 14.6 g/dL (4.6–22)/46.5% (18.0–76.8), and median white blood cell count: 5,300/μL (0.9–87,200) (**Table 3**). Although the median Hb of the entire platelet testing cohort was 14.6 g/dL, a tendency towards lower values was observed in the Hb median for the PLT-H: D (JSNDPMCC) cohort, at 13.4 g/dL, and in the PLT-H (WETDC) cohort, at 12.5 g/dL ($p<0.05$).

Hb and white blood cell count in examinees who underwent platelet testing in FY 2020

Results of Hb and white blood cell count tests for examinees who underwent platelet testing in FY 2020 are summarized in **Table 4**. In the platelet testing total cohort of 62,420, there were 390 cases (271 males, 119 females) judged as Hb-High: D (JSNDPMCC) (male: Hb>18/μL, female: Hb>16/μL), and 4,598 cases (4,479 males, 119 females) which met the WHO diagnostic criteria for polycythemia vera (male: Hb>16.5 g/dL or Ht>49%, female: Hb>16 g/dL or Ht>48%), referred to as Hb-H (WPVDC), with males predominating. In contrast, there were 1,916 cases (188 males, 1,728 females) judged as Hb-Low: D (JSNDPMCC) (male: Hb<12 g/dL, female: Hb<11 g/dL), with females overwhelmingly predominant. In the study of the PLT-H (WETDC) cohort of 548 cases (middle of **Table 4**), there were 5 cases of Hb-High: D (JSNDPMCC), 17 cases of Hb-H (WPVDC) (15 males, 2 females), but 191 cases of Hb-Low: D (JSNDPMCC) (11 males, 180 females), indicat-

Table 2. Annual Number of PLT Tests & PLT-H: D (JSNDC)[a], PLT-H (WETDC)[b] Conducted by Fiscal Year

Test Items	gender	FY 2016	FY 2017	FY 2018	FY 2019	FY 2020
PLT (total)	M+F	43964	50171	54854	59160	62420
	M	26698	29414	32144	34260	35934
	F	17266	20757	22710	24900	26486
PLT-H: D (JSNDPMCC) ≥40 × 10^4/μL (%*)	M+F	805 (1.80)	1095 (2.18)	1222 (2.23)	1464 (2.47)	1678 (2.69)
	M	299 (1.12)	359 (1.22)	450 (1.40)	568 (1.66)	587 (1.63)
	F	506 (2.93)	736 (3.55)	772 (3.40)	896 (3.60)	1091 (4.12)
40–44.9 × 10^4/μL (%**)	M+F	541 (67.20)	749 (68.40)	796 (65.14)	971 (66.33)	1130 (67.34)
	M	218 (72.90)	277 (77.16)	317 (70.44)	408 (71.83)	418 (71.21)
	F	323 (63.83)	472 (64.13)	479 (62.05)	563 (62.83)	712 (65.26)
PLT-H (WETDC) ≥45 × 10^4/μL (%***)	M+F	264 (0.60)	346 (0.69)	426 (0.78)	493 (0.83)	548 (0.88)
	M	81 (0.30)	82 (0.28)	133 (0.41)	160 (0.47)	169 (0.47)
	F	183 (1.06)	264 (1.27)	293 (1.29)	333 (1.34)	379 (1.43)

[a] PLT-H: D (JSNDPMCC): PLT high: grade D (Japan Society of Ningen Dock and Preventive Medical Care Criteria)
[b] PLT-H (WETDC): PLT-High: WHO Essential Thromobocythemia Diagnostic Criteria
%*: PLT-H: D (JSNDPMCC)/PLT (total) × 100
%**: PLT (40–44.9 × 10^4/μL)/PLT-H: D (JSNDPMCC) × 100
%***: PLT-H (WETDC)/PLT (total) × 100

Table 3. Summary of PLT Tests in FY 2020

	gender	No.tests	age			PLT[a]			Hb/Ht[b]			WBC[c]		
			median	min	max	median	min	max	median	min	max	median	min	max
PLT (total)	M+F	62420	48	16	108	25.9	1.1	95.0	14.6/46.5[d]	4.6/18.0	22.0/76.8	5.3	0.9	87.2
	M	35934	49	16	98	25.5	1.1	80.6	14.3/48.4	4.6/20.2	22.0/72.7	5.4	0.9	21.9
	F	26486	48	16	108	26.6	3.1	95.0	14.6/44.5	5.1/18.0	19.8/76.8	5.0	1.5	87.2
PLT-H: D (JSNDPMCC) ≥40	M+F	1678	46	19	93	42.9	40.0	95.0	13.4/42.0[e]	4.6/20.5	22.0/65.7	6.3	2.5	17.1
	M	603	48	20	90	42.1	40.0	91.5	15.3/43.2	4.6/22.5	22.0/65.7	6.8	2.8	17.1
	F	1075	45	19	93	43.2	40.0	95.0	12.3/41.5	4.9/20.5	16.6/56.6	6.0	2.5	16.6
40–44.9	M+F	1130	49	43	93	41.7	40.0	44.9	13.7/44.4	5.5/38.8	22.0/65.7	6.3	2.5	17.1
	M	434	51	43	80	41.9	40.0	44.9	15.6/45.5	8.5/38.9	22.0/65.7	6.8	2.8	17.1
	F	696	48	43	93	41.9	40.0	44.9	12.4/44.1	5.5/38.8	16.6/56.6	5.9	2.5	16.6
PLT-H (WETDC) ≥45	M+F	548	46	19	93	48.4	45.0	95.0	12.5/40.1[f]	4.6/20.5	20.0/62.2	6.4	2.6	16.0
	M	169	49	22	80	48.6	45.0	80.6	12.7/46.3	6.2/20.5	17.3/62.2	6.1	3.1	13.6
	F	379	46	19	93	48.3	45.0	95.0	12.6/37.3	4.6/22.0	20.0/53.5	6.5	2.6	16.0
45–49.9	M+F	344	49	19	77	46.2	45.0	49.9	12.9/40.7	4.6/20.5	18.3/55.2	6.3	2.6	15.3
	M	103	46	22	69	47.0	45.0	49.9	15.0/39.9	4.6/22.0	18.3/52.9	6.6	3.7	15.3
	F	241	46	19	77	46.8	45.0	49.9	11.6/41.1	4.9/20.5	16.3/55.2	6.2	2.6	13.0
50–59.9	M+F	168	46	23	72	52.9	50.0	59.9	12.0/36.6	5.3/23.2	20.0/62.2	6.6	3.8	16.0
	M	57	50	31	72	52.4	50.0	59.0	15.2/40.9	9.5/26.4	20.0/62.2	7.3	3.9	16.0
	F	111	44	23	66	53.4	50.0	59.9	10.3/37.1	5.3/23.2	16.4/55.1	6.1	3.8	11.9
60–69.9	M+F	24	45	34	93	62.5	60.1	69.8	10.4/36.2	6.2/27.3	16.1/51.6	6.8	2.6	13.3
	M	5	45	39	80	65.2	60.1	68.0	14.3/28.8	12.9/27.8	16.1/51.6	8.8	4.5	10.6
	F	19	45	34	93	63.6	60.1	69.8	9.2/36.3	6.2/27.3	12.6/48.4	7.2	6.2	12.6
70–79.9	M+F	7	48	38	60	75.2	71.7	79.7	13.8/41.1	6.2/27.3	15.1/49.1	7.8	5.3	14.2
	M	3	59	38	60	74.4	71.7	77.9	15.0/46.1	14.7/35.4	15.1/49.1	6.3	5.3	9.2
	F	4	48	42	51	75.2	74.8	79.7	9.1/41.1	6.2/27.3	13.8/44.8	10.3	4.0	14.2
80≥	M+F	5	48	45	55	90.7	80.6	95.0	13.8/36.9	8.0/27.3	14.7/44.3	7.0	5.5	13.6
	M	0	—	—	—	—	—	—	—	—	—	—	—	—
	F	5	47	45	55	90.7	80.6	95.0	13.8/36.9	8.0/27.3	14.7/44.3	7.0	5.5	13.6

[a] PLT: $\times 10^4/\mu L$
[b] Hb/Ht: Hb: g/dL, Ht: %
[c] WBC: $\times 10^3/\mu L$
p values were calculated by the Mann–Whitney U test
d vs e $p=0.0356$
d vs f $p=0.0269$

Table 4. PLT & Hb, WBC in FY 2020

	gender	No. tests	Hb-H: D (JSNDC)[a]	Hb-H (WPVDC)[b]	Hb-L: D (JSNDC)[c]
PLT (total)	M+F	62420	390	4598	1916
	M	35934	271	4479	188
	F	26486	119	119	1728
PLT-H (WETDC) ($\geq 45 \times 10^4/\mu L$)	M+F	548	5	17	191
	M	169	3	15	11[f]
	F	379	2	2	180[g]
WBC-H: D (JSNDPMCC)[d] ($>10 \times 10^3/\mu L$)	M+F	46	3	5	7
	M	20	2	4	2
	F	26	1	1	5
WBC-L: D (JSNDPMCC)[e] ($<3 \times 10^3/\mu L$)	M+F	3	0	0	3
	M	0	0	0	0
	F	3	0	0	3

[a] Hb-H: D (JSNDPMCC): Hb high: grade D (Japan Society of Ningen Dock and Preventive Medical Care Criteria)
[b] Hb-H (WPVDC): Hb high (WHO Polythythemia Vera Diagnostic Criteria)
[c] Hb-L: D (JSNDPMCC): Hb low: grade D (Japan Society of Ningen Dock and Preventive Medical Care Criteria)
[d] WBC-H: D (JSNDPMCC): WBC high: grade D (Japan Society of Ningen Dock and Preventive Medical Care Criteria)
[e] WBC-L: D (JSNDPMCC): WBC low: grade D (Japan Society of Ningen Dock and Preventive Medical Care Criteria)
f vs g $p=0.0083$

ing a large number of female examinees with anemia ($p<0.01$). Regarding the relationship with white blood cell count (bottom of **Table 4**), among the 548 cases, there were 46 cases meeting the criteria for WBC-High: D (JSNDPMCC) (WBC>10,000/μL). Among these, there were 3 cases of Hb-High: D (JSNDPMCC) and 5 cases of Hb-H (WPVDC), which were considered cases of so-called pancytosis.

Hb and MCV in the PLT-H (WETDC) cohort in FY 2020

In **Table 3**, PLT-H (WETDC) cases indicated a tendency towards anemia compared to the total cohort of examinees with platelet testing. The data for Hb and Mean Corpuscular Volume (MCV) are summarized in **Table 5**. The median MCV of the total cohort of plate-

let testing was 91.0 (55.3–126.6), while that of the PLT-H (WETDC) cohort was 88.6 (57–121.4). However, the median MCV of the Hb-Low: D (JSNDPMCC) cohort of 191 cases (11 males, 180 females) was 74.3 (57–109.6), with 174 cases having MCV<85, indicating microcytic anemia in 91% of cases, mostly female. Additionally, among cases with platelet counts above 450,000/μL, the number of cases not meeting the criteria for polycythemia or anemia (Hb Normal range, see bottom of **Table 5**) was 340 of 548 cases (males: 143 cases, females: 197 cases). The median MCV of these 340 cases was 92.3 (66.0–121.4), with 64 cases having MCV<85 (males: 6 cases, females: 56 cases).

Past and present medical history in the PLT-H (WETDC) cohort in FY 2020

We aggregated the past medical history and current medical status information of the examinees obtained from a questionnaire filled out during health check-ups (**Table 6**). Concerning past medical history, there were 325 examinees with no previous history, among which anemia was the most frequent current condition, in 87 cases, of which 85 were female. Among blood disorders, there were two cases of hereditary spherocytosis (HS) and ET in females; 11 cases of malignant tumors, of which 9 were in females; 13 cases of inflammatory diseases; and other lifestyle-related diseases. Regarding current medical status, there were 370 cases with no recorded information, with anemia in 21 cases, of which 20 were female); 4 cases of ET, all in females, with one case also having a recorded medical history); 5 cases of malignant tumor, of which 4 were in females; 13 cases of inflammatory diseases; and other lifestyle-related diseases.

Cases of ET and other hematologic disorders in the platelet testing cohort for the fiscal year 2020

In the platelet testing cohort for the fiscal year 2020, four cases of ET were identified by the self-reported medical history, including past and current conditions. All cases were female, aged between 42 and 55 years old, with platelet counts ranging from 482,000 to 915,000/μL (**Table 7**, top row). Among the four cases, one case (case 2) had been diagnosed with ET since 2017 (as indicated in the medical history). However, only one case (case 1) had visited the screening facility affiliated with our foundation in the fiscal year 2019, with a platelet count of 921,000/μL. In this analysis, it was unclear when these cases, excluding those diagnosed in 2017, were diagnosed with ET. Additionally, there were nine cases of leukemia (subtype unspecified), two cases of myelodysplastic syndrome (MDS) (subtype unspecified), and one case of paroxysmal nocturnal hemoglobinuria (PNH) identified, all with platelet counts below 400,000/μL (**Table 7**, bottom row).

Discussion

ET, which is a subtype of MPNs, is a chronic disease with a favorable prognosis. It is often discovered incidentally through health check-ups, as it typically presents no clinical symptoms[12–14]. However, despite its low frequency, there is a risk of ET progressing to other MPN subtypes, such as polycythemia vera (PV), primary myelofibrosis, or leukemia, leading to rapid disease progression[15]. Therefore, early diagnosis of ET and regular monitoring from the onset of symptoms are crucial.

In recent years, genetic abnormalities in the hematopoietic system in MPNs have been elucidated. Driver

Table 5. PLT & Hb, MCV in FY 2020

	gender	FY 2020	MCV[a] Median	MCV min	MCV max	MCV <85
PLT (total)	M+F	62420	91.0	55.3	126.6	
	M	35934	91.0	66.8	125.0	
	F	26486	88.0	55.3	126.6	
PLT-H (WETDC) (≥45 × 10^4/μL)	M+F	548	88.6	57.0	121.4	
≥45 × 10^4/μL	M	169	93.8	66.8	111.9	
≥45 × 10^4/μL	F	379	82.0	57.0	121.4	
Hb-H: D (JSNDPMCC)	M+F	5	96.9	87.0	107.1	
≥18.1 g/dL	M	3	95.7	87.0	96.9	
≥16.1 g/dL	F	2	—	97.5	107.1	
Hb-H (WPVDC)	M+F	17	94.3	87.0	107.1	
>16.5 g/dL (or Ht 49%)	M	15	93.0	91.9	103.0	
>16.0 g/dL (or Ht 48%)	F	2	—	97.5	107.1	
Hb-L: D (JSNDPMCC)	M+F	191	74.3	57.0	109.6	174
≤12.0 g/dL	M	11	79.4	66.8	103.7	8
≤11.0 g/dL	F	180	74.0	57.0	109.6	166
Hb: Normal range	M+F	340	92.3	66.0	121.4	64
12.1–16.5 g/dL	M	143	94.0	77.9	111.9	8
11.1–15.5 g/dL	F	197	90.7	66.0	121.4	56

[a] MCV: Mean Corpuscular Volume

Table 6. PLT-H (WETDC) & Past History, Present Illness in FY 2020

	gender		
	M+F	M	F
PLT-H (WETDC) (≥45 × 10^4/μL)	548	169	379
PH (Past History)			
not indicated	325	119	206
Anemia	87	2	85
Hematologic disease	2	0	2 (HS[a], ET[b])
Malignant tumor	11	2	9
gastric cancer		1	2
breast cancer			3
seminoma		1	
cervical cancer			2
ovarian cancer			2
Inflamatory disease	13	2	11
pneumonia		2	5
bronchitis			3
rheumatoid arthritis			2
meningitis			1
Gastro-intestinal disease	21	17	4
reflux esophagitis		8	1
gastric ulcer		3	2
ulcerative colitis		2	
gastic polyp		2	1
colon polyp		2	
Hypertension	20	10	10
Diabetes Mellitus	8	3	5
Dyslipidemia	13	8	5
Others	12	3	9
PI (Present Illness)			
not indicated	370	103	267
Anemia	21	1	20
Hematologic disease	4	0	4 (ET)
Malignant tumor	5	1	4
breast cencer			2
gastric MALToma		1	
cervical cancer			2
Inflamatory disease	13	5	8
rheumatoid arthritis		2	4
SLE			2
allergic dermatitis		3	2
Gastro-intestinal disease	4	2	2
ulcerative colitis		1	
crohn's disease		1	
reflux esophagitis			2
Hypertension	62	39	23
Diabetes Mellitus	26	14	12
Dyslipidemia	42	22	20
Others	17	2	15

[a] HS: Hereditay spherocytosis
[b] ET: Essential thrombocythemia

gene mutations that constitutively activate the JAK-STAT signaling pathway have been reported, with *JAK2 V617F* mutation detected in 50–60% of ET cases, *CALR* mutation in approximately 30%, and the *MPL* mutation, which is a receptor for thrombopoietin, in about 3%[5]. These mutations are not detected in secondary (reactive) thrombocytosis and are useful for differential diagnosis, enabling earlier confirmation of ET diagnosis.

According to the WHO diagnostic criteria for ET (2017), the major criteria include: 1. persistent platelet count ≥450,000/μL, 2. bone marrow biopsy findings, 3. exclusion of other blood disorders, including other MPN subtypes, and 4. presence of *JAK2 V617F*, *MPL*, or *CALR* mutations. Minor criteria include: 1. clonal proliferation of bone marrow megakaryocytes, and 2. exclusion of reactive thrombocytosis. A diagnosis of ET is confirmed if all four major criteria or the first three major criteria and either of the minor criteria are met[5].

Following confirmation of a persistent platelet count ≥450,000/μL, exclusion diagnosis of secondary thrombocytosis is crucial, including common iron-deficiency anemia, as reiterated in this analysis. While bone marrow biopsy is essential for definitive diagnosis when MPNs are suspected, it is not practical to perform bone marrow biopsies on all subjects. Detection of *JAK2 V617F*, *MPL*, or *CALR* mutations is effective as a secondary screening test that can be performed through blood sampling alone, given their presence in approximately 90% of ET cases.

In this study, approximately 1% (about 500 individuals) of the subjects who underwent platelet testing in four affiliated facilities in 2020 had platelet counts ≥450,000/μL.

Regarding WETDC, the criterion for platelet count is set at 450,000/μL or higher, which exceeds the 95th percentile of normal platelet counts. Therefore, platelet counts of 450,000 and above constitute 5% of the entire cohort, which is significantly higher compared to the results obtained this study (just under 1%). The cause of this discrepancy is currently unclear, but one possible reason is that the distribution of platelet counts differs between Europeans and Japanese, with Japanese possibly having lower counts. In this analysis as well, using the JNDPMCC (PLT≥400,000/μL, approximately 3% were classified as D grade, suggesting once again that Japanese tend to have lower platelet counts compared to Europeans. Of course, these results are based on limited data from four facilities affiliated with our health screening foundation, and it is unknown whether they apply to the entire Japanese cohort. To our knowledge, there are unfortunately no reports available regarding aggregated results of blood tests in the entire Japanese population for general health examinations. In the future, it is essential to conduct data analysis across Japan, including other blood tests, and if Japanese indeed have lower platelet counts compared to Europeans, it will be an important future task to consider whether the platelet count criterion of 450,000 adopted by WETDC is suitable for Japanese individuals. Nonetheless, this analysis illustrates the distribution of platelet counts among Japanese in general health examinations and provides valuable information for health screening facilities and

Table 7. Case Summary of ET & Other Hematological Diseases (OHD) in FY2020

	gender	age	PLT[a]	Hb[b]	MCV	WBC[c]	CRP[d]	Past History	Present Illness
case (ET)									
1	F	47	91.5	14	93.1	7.0	0.04	—	ET
2	F	55	90	11.9	83.0	7.7	0.03	ET (2017–)	ET, dyslipidemia
3	F	47	51.3	12	121.4	9.9	—	—	ET, dyslipidemia
4	F	42	48.2	12.8	81.8	5.6	—	ovarian cyst	ET, hypertensin
case (OHD)									
1	M	45	36.9	17.7	85.0	6.0	0.05	—	MDS[e]
2	F	48	24.7	13.4	—	21.9	—	leukemia	—
3	F	65	23.3	12.3	—	87.2	—	pneumonia	leukemia
4	M	38	22.3	11.8	92.0	5.1	0.16	—	leukemia
5	M	52	21.7	13.3	95.2	2.1	0.05	—	leukemia
6	F	68	21.3	12.3	98.0	4.3	0.27	—	leukemia
7	M	60	20.9	17.1	94.1	5.6	0.07	—	leukemia
8	M	54	13.8	14.6	98.0	14.3	0.06	—	leukemia
9	F	55	10.7	11.8	95.0	2.4	0.09	colon ca[f]., breast ca.	MDS
10	F	40	9.1	11.9	—	2.9	—	cervical ca.	PNH[g]
11	M	37	3.4	15.3	—	3.4	—	leukemia	—
12	F	51	3.1	7.3	—	2.4	—	leukemia	—

[a] PLT: $\times 10^4/\mu L$
[b] Hb: g/dL
[c] WBC: $\times 10^3/\mu L$
[d] CRP: mg/dL
[e] MDS: myelodysplastic syndrome
[f] ca.: cancer
[g] PNH: Paroxysmal Nocturnal Hemoglobinuria

institutions performing precise medical examinations.

In general, most cases of thrombocytosis are presumed to be secondary (reactive) thrombocytosis. It is estimated that a significant number of female patients with thrombocytosis have microcytic anemia (likely iron deficiency anemia) as the underlying cause.

However, among 548 cases, we found 340 cases where the platelet count exceeded 450,000/μL but did not meet the criteria for polycythemia or anemia (**Table 5**, lower section). Of these, 64 cases had MCV <85, suggesting that the remaining 276 cases might not be due to iron deficiency. Therefore, longitudinal data collection and analysis programs are deemed essential for analyzing the causes of platelet elevation in these cases. In the differential diagnosis of secondary thrombocytosis, it is crucial to accurately understand the patient's medical history, including past and present conditions. In this analysis, the current status of medical history as reported by the examinees was summarized (see **Table 6**). It was noted that a significant number of examinees had no documented medical history in either the past and present illness categories. Of course, it is highly likely that many examinees who do not actually have medical histories are being seen. However, during actual health check consultations, it is commonly experienced that examinees may have insufficient understanding of their medical history, including past and current conditions (for example, forgetting to mention ongoing treatments or mistakenly including them in past medical history).

Furthermore, among other blood disorders, nine cases of leukemia and two cases of MDS were identified, with no information available on these subtypes. Development of methods for detailed information collection is essential, including self-reported medical history and detailed current medical information. Currently, tablet-based questionnaire input for medical history has been initiated in our facilities, with expectations that this will allow more detailed and accurate capture of medical history.

In the fiscal year 2020, among approximately 62,420 individuals who underwent platelet testing, four cases of ET were identified. It is presumed that all four cases were not newly identified in the current health screening. Specifically, one among the four cases (case 1) had been first diagnosed with ET in 2017 (as documented in the medical history). Case 1 also attended our foundation's health screening facility in fiscal year 2019, with a platelet count of 921,000/μL at that time. In this analysis, excluding cases diagnosed in 2017, the exact timing of ET diagnosis for the remaining cases is unclear. Ongoing monitoring of these cases through detailed assessment of their medical histories and current conditions, as well as collaboration with other health screening institutions and treatment facilities, is considered essential. Furthermore, the annual incidence rate of new ET cases is reported as 0.2 to 2.3 individuals per 100,000 in Western populations[16,17]. Therefore, proactive registration in the Japanese Society of Hematology's Blood Disease Registration, led by the Academic and

Statistical Survey Committee, is crucial for understanding the incidence of new ET cases in Japan in the future[18].

Early detection of blood disorders, including MPNs other than ET, is also important. However, this study focused solely on the collection and analysis of annual subject data. Currently, a comprehensive data collection and analysis program is under development, which includes longitudinal blood test data, other hematological test data, medical history and treatment history before and after health check-ups, and referral status to medical institutions in real-time. Additionally, as evident from the analysis of ET cases in this study, it cannot be assumed that individuals undergoing health check-ups consistently visit the same facility. Therefore, data sharing and collaboration between different health check-up facilities are important challenges for the future.

Conclusion

This study provides insights into the prevalence and characteristics of thrombocytosis among examinees of health check-up and Ningen Dock facilities. The findings highlight the importance of routine platelet testing and further evaluation for individuals with elevated platelet counts, particularly in detecting underlying hematological disorders such as ET. Further research is warranted to explore the clinical significance and implications of thrombocytosis in the context of health screening and early disease detection.

Author's Disclosure of Conflicts of Interest

None specifically declared.

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Original Article

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Prediction of Underlying Heart Disease Using Electrocardiograms Obtained Before Development of Complete Left Bundle Branch Block: A Cross-sectional Study

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Abstract

Objective: To determine whether underlying heart disease can be predicted using electrocardiograms obtained before the development of complete left bundle branch block (CLBBB) which was incidentally detected during routine medical examination.

Methods: The primary endpoint was the association between abnormal electrocardiographic findings before the development of CLBBB and the prevalence of comorbid heart disease. The secondary endpoints were type of heart disease, age at development of CLBBB, and disease duration.

CLBBB was confirmed in 68 of 71,675 individuals (40,084 men and 31,591 women) who underwent electrocardiography from April 2021 to March 2023. Among them, 37 individuals who had available electrocardiograms before the development of CLBBB were included in this study.

Results: The median age was 64 (57–75) years, age at the development of CLBBB was 59 (53–71) years, and disease duration was 3 (0–6) years. Common electrocardiographic findings before the development of CLBBB were left ventricular hypertrophy (43.2%) and normal electrocardiograms (37.8%), followed by T-wave changes (10.8%). Structural heart disease was observed in 12 (32.4%) of the 37 patients. For patients with normal electrocardiograms, the prevalence of comorbid heart disease was 7.1%, which was lower than that for patients with abnormal findings (47.8%). Electrocardiograms before the development of CLBBB had a sensitivity of 91.6% and specificity of 52% for detecting comorbid heart disease. The probability of not having heart disease when the pre-CLBBB electrocardiogram was normal was 92.9%.

Conclusion: Underlying heart diseases are unlikely when electrocardiograms before the development of CLBBB are normal.

Keywords complete left bundle branch block, electrocardiogram, underlying heart disease

Complete left bundle branch block (CLBBB) is an electrocardiographic abnormality identified in 0.1%–0.8% of the general population[1] and may be accidentally detected on electrocardiography performed as part of routine medical examination. Most patients with CLBBB have underlying structural heart disease, leading to a generally poor prognosis. Thus, detailed examination is strongly recommended for patients with CLBBB even if they are asymptomatic[2,3]. The recommendation is repeated annually, imposing both mental and economic burdens on them. However, it has recently been reported that some patients with asymptomatic CLBBB do not have associated structural heart disease, and their prognosis is not necessarily poor[4,5]. These findings are consistent with the empirical knowledge of physicians involved in reading electrocardiograms obtained during routine medical examinations.

When we request the Department of Cardiovascular Medicine to perform a detailed examination for CLBBB detected during routine medical examination, we review previous electrocardiographic findings and attach them to a referral form if necessary. During this process, we often observe normal electrocardiograms.

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If structural heart disease often underlies CLBBB, electrocardiograms would be expected to show some changes before the development of CLBBB. However, electrocardiograms do not necessarily show evidence of ischemic changes and left ventricular hypertrophy, among others, before the development of CLBBB. Moreover, structural abnormalities are rarely detected during detailed examination in patients with CLBBB whose previous electrocardiograms are normal.

Although these findings are intriguing, there are few reports that have focused on electrocardiograms recorded before the onset of CLBBB. This scarcity is attributed to the uncommon practice of obtaining electrocardiograms from asymptomatic patients in hospitals. In contrast, electrocardiograms of healthy individuals are routinely obtained at medical checkup centers. The availability of these electrocardiograms allows the investigation of CLBBB electrocardiograms incidentally detected in asymptomatic patients.

Our study operates under the assumption that evaluating electrocardiograms from the year preceding initial CLBBB detection (referred to as electrocardiographic findings before CLBBB development) could offer insights into the presence or absence of underlying heart disease, thereby informing the prognosis associated with CLBBB. This study aimed to determine whether underlying heart disease can be predicted using such electrocardiograms obtained before the development of CLBBB which is incidentally detected during routine medical examination.

Materials and Methods

Patient population

We identified CLBBB patients among individuals who had undergone routine medical examinations, including electrocardiography, at two healthcare centers operated by the Kanagawa Prefecture Welfare Federation of Agricultural Cooperatives during the fiscal years 2021 and 2022 (April 1, 2021 to March 31, 2023). Their electrocardiograms before the development of CLBBB were reviewed. Patients with available electrocardiograms obtained prior to the development of CLBBB were included in this cross-sectional study.

The visitors to these centers include members of the Kanagawa Prefectural Union of Agricultural Cooperatives, workers in companies (businesses) and public agencies in Kanagawa Prefecture, and local residents eligible for specific health examinations.

This study was reviewed and approved by the Ethics Committee of Isehara Kyodo Hospital of the Kanagawa Prefecture Welfare Federation of Agricultural Cooperatives (receipt No. 151) and was performed in accordance with the principles of the Declaration of Helsinki. Considerable care was taken to ensure the confidentiality of study patients, including compliance with the document "Purposes of Using the Personal Information of Users" that is publicly available on the website of the Health and Welfare Center of the Kanagawa Prefecture Welfare Federation of Agricultural Cooperatives. On accessing visitor information, we assigned identification numbers to the records that were not associated with personal information. Informed consent was obtained in the form of an opt-out option provided on the website.

Methods

The primary endpoint was the association between abnormal electrocardiographic findings before the development of CLBBB and the prevalence of comorbid heart disease. Assessments were performed separately for men and women. The secondary endpoints were type of heart disease, age at development of CLBBB, and disease duration.

At both centers, electrocardiography was performed using Digital electrocardiographs ECG-2450 and 2550 (Nihon Kohden Corp., Tokyo, JAPAN) to record waveforms. The electrocardiogram analysis program ECAPS12C was used to analyze waveforms and perform automatic diagnosis. Physicians in charge of medical examinations reviewed the automatic diagnosis and performed primary reading. Subsequently, cardiovascular specialists performed secondary reading to confirm the diagnosis and assessment.

CLBBB was defined as a QRS duration of 0.12 seconds or longer; small R wave and deep broad S wave in lead V1–2; upward QRS complex and M-shaped R wave in leads I, aVL, and V5–6; and the absence of Q waves in leads I and V5–6.

The electrocardiogram-based diagnosis before the development of CLBBB was made with stored actual electrocardiographic waveforms. CLBBB was diagnosed according to the above criteria. Left ventricular hypertrophy was diagnosed using the criteria reported by Peguero *et al.* in 2017[6]. According to these criteria, left ventricular hypertrophy is diagnosed when the sum of the depth of the deepest S wave in the chest lead and the S wave in lead V4 is 23 mm or more for women and 28 mm or more for men.

Flat, negative, and biphasic T-waves were interpreted based on codes 5-1 to 5-5 of the Minnesota Code Classification System for Electrocardiographic Findings[7] and aggregated as T-wave changes. When T-wave changes coexisted with findings of left ventricular hypertrophy, left ventricular hypertrophy was diagnosed. Regarding atrioventricular conduction disturbance, first-degree atrioventricular block was defined as a PR interval of 0.21 or more seconds.

The presence and type of heart disease were determined based on medical history (including examina-

tion history), history of present illness (diagnosis), symptoms obtained through interview, auscultatory findings, chest radiographic findings, and electrocardiographic findings. Age at development was defined as age in the year when CLBBB was first detected during the routine medical examination. Disease duration was defined as the time to the date of the routine medical examinations in fiscal years 2021 or 2022. For patients who underwent routine medical examinations during both fiscal years, disease duration was the time to the date in fiscal year 2021.

Statistical analysis

All statistical analyses were performed using EZR version 1.55 (Jichi Medical University Hospital and Saitama Medical Center). EZR is statistical software with expanded functions of R and R commanders. For statistical processing, age is expressed as median and interquartile range, and the Mann–Whitney U test was used to test the significance of differences. Quantitative variables are expressed as total number and percentage, and were compared using Fisher's exact test. *p*-values less than 0.05 denoted statistically significant differences for all the tests.

Results

Profile of the patients

A total of 71,675 individuals (40,084 men and 31,591 women) underwent routine medical examinations, including electrocardiography, at two healthcare centers. Their mean age was 53.9 years (54.4 years for men and 53.4 years for women). Many of these examinees were regular visitors to these centers, with 78% of those who visited in fiscal year 2022 having also visited in fiscal year 2021.

Among these individuals, 68 patients were diagnosed with CLBBB. Electrocardiograms obtained before the development of CLBBB were available for 37 patients, including 21 men (57%). The median age of these 37 patients was 64 (57–75) years; median age at the development of CLBBB was 59 (53–71) years, and disease duration was 3 (0–6) years. These values did not differ by sex. Moreover, no sex-related differences were observed in the prevalence of hypertension, dyslipidemia, or diabetes (**Table 1**).

Electrocardiographic findings before the development of CLBBB

Common electrocardiographic findings before the development of CLBBB were left ventricular hypertrophy (43.2%) and normal electrocardiogram (37.8%), both of which accounted for the majority of the patients (**Table 2**, **Figs. 1** and **2**). In addition, T-wave changes and atrioventricular block (first-degree in 2 patients and second-degree Wenckebach-type in 1 patient) were observed.

The frequency of findings differed by sex. The most common finding in men was a normal electrocardiogram (52.4%). In contrast, left ventricular hypertrophy was the most common finding in women (50.0%). Furthermore, the proportion of women with normal electrocardiograms was lower than that in men, whereas the proportion of women with T-wave changes was higher than that in men.

Prevalence of underlying heart disease

We found that 12 (32.4%) of the 37 patients had heart disease (7 men and 5 women): 3 patients had heart failure, 3 had dilated cardiomyopathy, 2 had hypertensive heart disease, 2 had ischemic heart disease, 1 had hypertrophic cardiomyopathy, and 1 had aortic stenosis (**Table 3**). The prevalence and type of comorbid heart disease was comparable between men and wom-

Table 1. Patient Characteristics

	Total *n*=37	Male *n*=21	Female *n*=16	*p* value
Age (years)	64 (57–75)	64 (56–72)	65 (58.5–77)	0.462
Age of onset	59 (53–71)	58 (52–70)	61.5 (54.5–74.5)	0.374
Duration of the CLBBB (years)	3 (0–6)	4 (0–6)	2 (0–6.75)	0.852
Hypertension	25 (67.5%)	14 (66.7%)	11 (68.8%)	1
Hyperlipidemia	20 (54.0%)	12 (57.1%)	8 (50.0%)	0.746
Diabetes	8 (21.6%)	6 (28.6%)	2 (12.5%)	0.423

CLBBB, complete left bundle branch block
Continuous variables are expressed as median (range).

Table 2. Electrocardiographic Findings Before Development of CLBBB

	Total *n*=37	Male *n*=21	Female *n*=16	*p* value
Normal electrocardiogram	14 (37.8%)	11 (52.4%)	3 (18.8%)	0.0475
Left ventricular hypertrophy	16 (43.2%)	8 (38.1%)	8 (50.0%)	0.519
T wave change	4 (10.8%)	0 (0.0%)	4 (25.0%)	0.0276
Arterio-ventricular block	3 (8.1%)	2 (9.5%)	1 (6.3%)	1

CLBBB, complete left bundle branch block

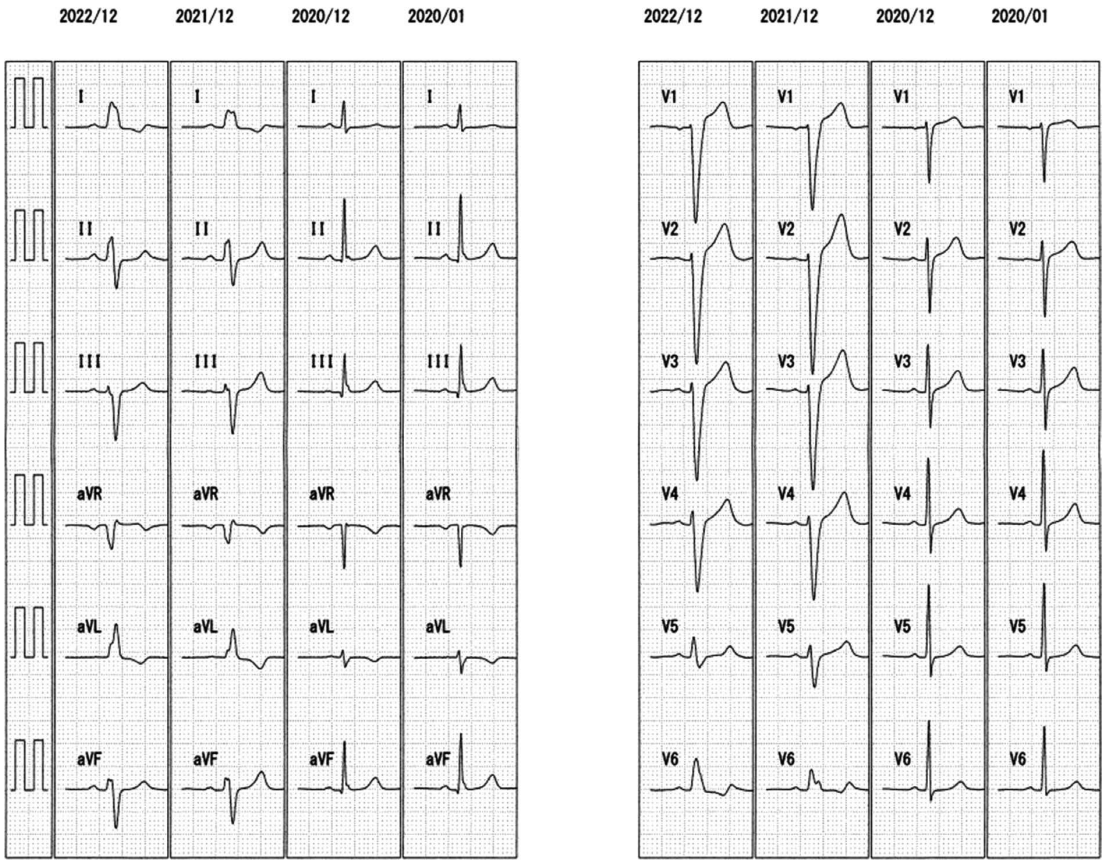

Fig. 1. Transition from Normal Electrocardiograms to CLBBB

A woman in her 60s exhibited normal electrocardiograms until December 2020. The patient did not present with any risk factors for coronary artery disease. CLBBB was subsequently detected in December 2021 without any reported cardiac events, and a detailed examination revealed no evidence of structural heart disease.
CLBBB, complete left bundle branch block

en. Cardiac events triggered the development of CLBBB in only 1 patient. This patient developed CLBBB after coronary artery bypass graft surgery. In this patient, the electrocardiogram obtained before the development of CLBBB indicated left ventricular hypertrophy.

The prevalence of comorbid heart disease among patients with normal electrocardiograms before the development of CLBBB was 7.1%, which was lower than that among patients with abnormal findings (47.8%) (**Table 4**).

Regarding the presence or absence of comorbid heart disease, an electrocardiogram obtained before the development of CLBBB had a sensitivity of 91.6% and a specificity of 52%. The probability of having heart disease (positive predictive value) was 47.8% when the electrocardiogram before the development of CLBBB indicated any findings, and the probability of not having a heart disease (negative predictive value) was 92.9% when the electrocardiogram was normal. The negative predictive value also exceeded 90% for both men and women when they were examined separately (90.9% for men and 100% for women).

Discussion

This study examined whether underlying heart disease can be predicted using electrocardiograms obtained before the development of CLBBB. Few studies have reported electrocardiographic findings before the development of CLBBB in the general population. Among reports presented in a review performed in 2007, only one study published in 1980 indicated that more than 50% of electrocardiograms before the development of CLBBB had findings within normal limits, whereas only a few electrocardiograms indicated left ventricular hypertrophy and other findings[1]. In that study, the most common electrocardiographic findings before the development of CLBBB were normal, which accounted for 54% of cases. Other common findings included left ventricular hypertrophy (14%) and ST/T-wave changes (14%)[8].

The results of our present study also showed that a normal electrocardiogram was associated with CLBBB in approximately 40% of patients. We confirmed that CLBBB detected in the general population includes cases that develop from patients in whom the electrocardiogram was previously normal. Understanding of

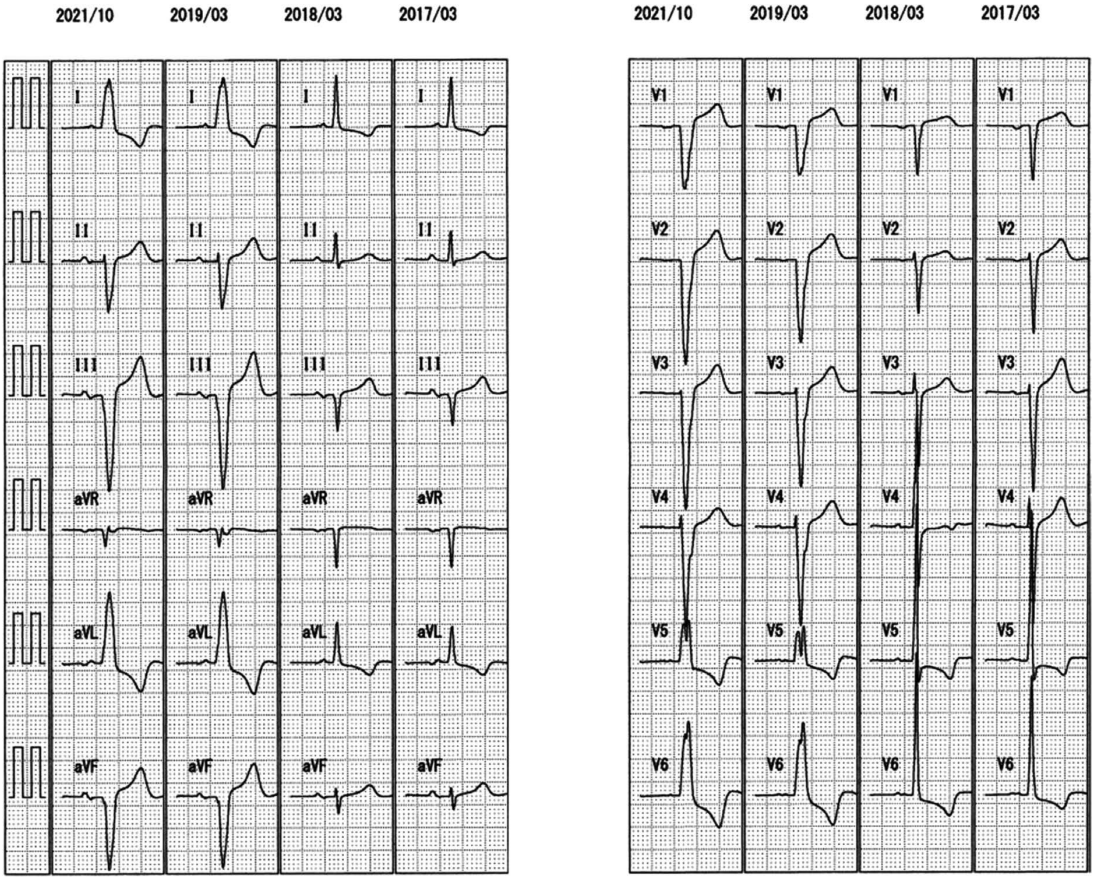

Fig. 2. Transition from an Electrocardiogram Indicating Left Ventricular Hypertrophy to CLBBB

A woman in her 80s displayed electrocardiograms indicating left ventricular hypertrophy until March 2018 (SV3+SV4 >23 mm). In March 2019, CLBBB was identified. Throughout this period, there were no reported cardiac events. The patient had a history of hypertension and underwent aortic valve replacement about 20 years ago.
CLBBB, complete left bundle branch block

Table 3. Number (%) of Patients with Underlying Heart Disease

	Total *n*=37	Male *n*=21	Female *n*=16	*p* value
Heart disease	12 (32.4%)	7 (33.3%)	5 (31.3%)	1
Congestive heart failure	3 (8.1%)	1 (4.8%)	2 (12.5%)	0.568
Dilated cardiomyopathy	3 (8.1%)	2 (9.5%)	1 (6.3%)	1
Hypertensive heart disease	2 (5.4%)	2 (9.5%)	0 (0.0%)	0.495
Ischemic heart disease	2 (5.4%)	2 (9.5%)	0 (0.0%)	0.495
Hypertrophic cardiomyopathy	1 (2.7%)	0 (0.0%)	1 (6.3%)	0.432
Aotic stenosis post-surgery	1 (2.7%)	0 (0.0%)	1 (6.3%)	0.432

Table 4. Prior Electrocardiographic Findings and the Number of Patients with Underlying Heart Disease

	Normal electrocardiogram		Abnormal electrocardiogram		*p* value
	n	Patients with underlying heart disease	*n*	Patients with underlying heart disease	
Total	14	1 (7.1%)	23	11 (47.8%)	0.013
Male	11	1 (9.1%)	10	6 (60.0%)	0.0237
Female	3	0 (0.0%)	13	5 (38.5%)	0.509

this finding to date has been poor.

The prevalence of left ventricular hypertrophy was 43.2%, which was higher than the previously reported prevalence (14%)[8]. This may be attributable to the use of the criteria proposed by Peguero *et al.* in 2017 for the electrocardiographic diagnosis of left ventricular

hypertrophy[6]. These criteria have comparable specificity but superior sensitivity for detecting left ventricular hypertrophy to those of the Socolow-Lyon voltage criteria, which are used for automatic electrocardiographic diagnosis. In recent years, they have increasingly been used in general clinical practice.

The electrocardiographic findings before the development of CLBBB differed between men and women. The prevalence of left ventricular hypertrophy was almost comparable for men and women; however, the proportion of men with a normal electrocardiogram was higher than that in women. In addition, T-wave changes were only observed in women. This may be attributable to the sex-related differences in electrocardiographic findings. A study involving a general population without underlying heart disease showed that the amplitude of the T-wave is lower for women than for men[9]. The prevalence of hypertension, dyslipidemia, or diabetes did not differ between men and women in the present study; therefore, these sex-related differences may explain why T-wave changes were only observed in women and why the proportion of patients with a normal electrocardiogram was lower among women than among men.

The prevalence of comorbid heart disease associated with CLBBB was 32%, and did not differ by sex. This prevalence was low, which contradicts the commonly accepted conventional theory that most cases of CLBBB are associated with heart disease[2]. This seemed to be attributable to the differences in study participants.

A previous study on CLBBB involving patients receiving inpatient or outpatient treatment at a medical institution reported that 90% of CLBBB cases had structural heart disease[10]. However, in studies of the general population, the prevalence of structural heart disease is not necessarily high. For instance, a study conducted on US Air Force personnel in 1975 found that 94% of LBBB cases were not associated with cardiovascular disease[11]. Similarly, in a study conducted on Japanese A-bomb victims, it was reported that underlying heart diseases at the development of LBBB were ischemic heart disease (22.7%) and cardiomyopathy/valvular heart disease (4.6%)[12].

Another study used the results of medical examinations of 14,540 individuals aged 40 years or older in Korea[13] and reported the presence of CLBBB in 14, of whom 5 had underlying heart disease. The prevalence of comorbid heart disease was 35.7%, which was comparable with that in the present study. Routine medical examinations target asymptomatic individuals who do not regularly visit medical institutions or individuals with mild conditions who are visiting medical institutions. CLBBB appeared to be less associated with underlying heart disease in this population.

Of the 14 patients with normal electrocardiograms before the development of CLBBB, only 1 (7.1%) had underlying ischemic heart disease. In contrast, 11 (47.8%) of the 23 patients with abnormal electrocardiographic findings before the development of CLBBB had comorbid heart disease. These results appear to be reasonable, in that if structural heart disease underlies CLBBB and abnormalities caused by the disease are involved in the development of conduction disturbance of the left bundle branch, electrocardiograms should show changes reflecting such abnormalities before the development of CLBBB. This is indicated by the results of the present study showing that abnormal electrocardiographic findings before the development of CLBBB were observed in 11 (91.7%) of 12 patients with CLBBB who were confirmed to have comorbid heart disease.

In the present study, electrocardiographic findings before the development of CLBBB were normal for 37.8% of patients with CLBBB, and the prevalence of comorbid heart disease was low. If electrocardiographic findings before the development of CLBBB are normal, the presence of comorbid heart disease can be ruled out with a probability of 92.9% (negative predictive value). This suggests that such CLBBB is not a functional abnormality of the specialized conducting system associated with structural heart disease, such as ischemia, cardiomyopathy, and myocarditis, but is caused by degeneration of the specialized conducting system.

This novel finding can be useful for follow-up in patients without any subjective symptoms and who are accidentally found to have CLBBB during routine medical examinations. When electrocardiograms before the development of CLBBB reveal the presence of problems, we should make repeated efforts to identify any underlying heart disease. On the other hand, when the electrocardiogram is normal, the probability of the presence of an underlying comorbid heart disease appears low. Thus, although an initial detailed examination for heart disease must be performed, repetition of burdensome examinations in subsequent years may be unnecessary. Clinicians should monitor patients through routine annual medical examinations, while paying attention to the development of atrioventricular block and symptoms and guiding patients on how to respond to the onset of symptoms such as syncope and dizziness.

Limitations

This study has some limitations. First, the presence or absence of heart disease in our study was based on medical history (including examination history), history of hospital visits (diagnosis), symptoms, auscultatory findings, chest radiographic findings, and elec-

trocardiographic findings. We targeted participants of medical checkups provided at healthcare centers with limited equipment but did not perform any detailed examinations, such as echocardiography, magnetic resonance imaging, or coronary angiography. Consequently, mild heart disease may have been overlooked. However, because our method can detect almost all types of heart disease that are treated in general clinical practice, the core findings of this study provide useful insights for clinical practice.

Second, this was a cross-sectional study, and we could not consider the prognosis of CLBBB. The probability of the presence of underlying heart disease seems low for patients with a normal electrocardiogram before the development of CLBBB, but we did not investigate whether this would lead to a decrease in the incidence of adverse events, including death. In addition, we revealed that CLBBB without associated heart disease is relatively common; however, we could not discuss the etiology of this condition. Longitudinal studies of CLBBB involving the general population are warranted to determine the prevalence and long-term prognosis of CLBBB without associated underlying heart disease and provide further elucidation of the etiology.

Finally, sample size of this study is not large enough. Because CLBBB is not a common electrocardiographic abnormality, we could not obtain a large number of participants who had available electrocardiograms before the development of CLBBB. However, the fact that CLBBB is not common also indicates that the findings of this study offer new, potentially useful information for this patient population. A multicenter study is required to further clarify our findings.

Conclusions

We examined electrocardiographic findings before the development of CLBBB which was accidentally detected during routine medical examination. There were two major findings: "normal electrocardiogram" and "left ventricular hypertrophy." For patients with CLBBB and a prior normal electrocardiogram, the prevalence of comorbid heart disease is low. The study findings indicate that electrocardiographic findings before the development of CLBBB are useful for determining whether CLBBB is associated with comorbid heart disease.

Conflict of Interest

The authors state that they have no Conflict of Interest (COI).

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Analysis of Risk Factors for Colorectal Adenoma

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Abstract

Objective: Metabolic syndrome (MetS) is a condition consisting of three components (hypertension, dyslipidemia, and impaired fasting glucose tolerance). Our previous study demonstrated that MetS is a significant risk factor for colorectal adenoma (CRA). The current study aimed to identify a more convenient surrogate marker of CRA. In particular, mean platelet volume (MPV), in addition to MetS, in the development of CRA was investigated.

Methods: This study enrolled 451 participants who visited Tokyo Women's Medical University for a complete medical checkup between June 2018 and October 2022. The risk factors of CRA were analyzed using contingency tables via a multivariate logistic regression analysis. A *p*-value of <0.05 was considered statistically significant.

Results: Increased MPV, excessive alcohol use, and aging were significant risk factors of CRA. Further, smoking was a statistically significant risk factor of ≥5-mm CRA. This study also showed that CRA ≥5 mm which was potentially eligible for endoscopic resection was slightly more frequent in participants in their 60s.

Conclusions: With consideration to its low cost and availability, increased MPV should be extensively evaluated as a convenient tool for determining the need to undergo CRA screening. In addition, individuals who have excessive alcohol use and who smoke should undergo assessments to rule out CRA as they age, especially those in their 60s.

Keywords medical checkup, colorectal adenoma (CRA), mean platelet volume (MPV)

Metabolic syndrome (MetS) is a condition which consists of three components (hypertension, dyslipidemia, and impaired fasting glucose tolerance). Based on a previous study[1], MetS is a significant risk factor for colorectal adenoma (CRA).

This study aimed to identify another convenient surrogate marker of CRA. Mean platelet volume (MPV) is easy to evaluate and examination cost is relatively low. Hence, it is widely used as a marker. In particular, MPV is used as a marker of platelet size and activity, and can be determined easily via routine automated hemograms. Individuals with increased MPV have large platelets that are metabolically and enzymatically more active and have greater prothrombotic potential than normal platelets[2–6]. An elevated MPV is correlated with accelerated thrombopoiesis and increased risk of cardiovascular diseases[7–10]. Further, MPV can be a diagnostic marker of hepatocellular carcinoma, pancreatic adenocarcinoma, and gastric cancer[11–13]. Li *et al.*[14] found that patients with colon cancer have an elevated MPV. Moreover, this factor is associated with the tumor–nodule–metastasis stage of colon cancer. Studies have revealed that MPV may be a valuable prognostic marker in patients with colorectal cancer (CRC)[15,16]. However, Włodarczyk *et al.*[17] reported that patients with rectal cancer have a significantly lower MPV than healthy individuals. CRA, a precursor lesion of CRC, is a benign glandular tumor of the colon and rectum[18,19]. Previously, the association between MPV and CRA was investigated to validate if it can be potentially utilized as a biomarker of CRC diagnosis.

The current study aimed to analyze the correlation between the development of CRA and MPV, regardless of the presence of MetS. Our aim was to provide an effective understanding of which patients should undergo colonoscopy for CRA diagnosis.

Methods

Study design

This retrospective cohort study was performed in accordance with the principles of the Declaration of Helsinki. It was approved by the Ethics Committee of

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Tokyo Women's Medical University (approval date: May 2, 2024; approval number: 2021–0209), and adhered to the Personal Information Protection Law as an opt-out. The study cohort comprised participants who visited the Department of Complete Medical Checkup at Tokyo Women's Medical University, Japan, between June 2018 and October 2022. The inclusion criteria were participants who underwent a periodic checkup and colonoscopy at the Ningen Dock, which is the Japanese health checkup system. Participants who had incomplete records were excluded from the study population. If a participant underwent examination more than once, only the data from the first examination were used in this study. The exclusion criteria were participants with inflammatory bowel disease (Crohn's disease and ulcerative colitis) and Caucasians (**Fig. 1**). This study included participants treated for hypertension, dyslipidemia, diabetes mellitus (DM), and hyperuricemia.

Services delivered at the Ningen Dock

The periodic health checkup program at the Ningen Dock is comprehensive. It includes the following assessments: physical examination (height, body weight, and waist circumference), complete blood count, blood biochemistry, urinalysis, electrocardiography, abdominal ultrasonography, upper gastrointestinal tract barium swallow or endoscopic examination, colonoscopy, visual acuity test, tonometry, fundic examination (retinal photography), and hearing assessment. Details of the participants' medical and family histories and consumption levels of alcohol and tobacco were obtained via an interview with a doctor. The colonoscopy findings were evaluated by about five to ten physicians who had >5 years of experience in endoscopy, most of whom were specialists certified by the Japan Gastroenterological Endoscopy Society. CRA was diagnosed via endoscopy. However, if the participants underwent endoscopic polyp removal, CRA was diagnosed via pathological examination. Excluding those who declined, all participants underwent colonoscopy as part of the medical checkup.

Risk factors

This analysis included several potential risk factors such as aging, sex, excessive alcohol use, smoking, family history of CRC, hyperuricemia, impaired renal function, metabolic dysfunction-associated fatty liver disease (MAFLD), and nonalcoholic fatty liver disease (NAFLD). Excessive alcohol use was defined as consumption of >30 g of ethanol/day in men and >20 g of ethanol/day in women. Smoking was defined as a Brinkman Index of ≥400. Hyperuricemia was defined as an uric acid level of >7 mg/dL and/or current drug treatment. Impaired renal function was defined as an estimated glomerular filtration rate of <60 mL/min/1.73 m^2. The proposed criteria for a positive MAFLD diagnosis were based on the presence of FL in addition to one of the following three criteria: overweight or obesity (body-mass index of ≥23 kg/m^2 in Asians), presence of type 2 DM, or evidence of metabolic dysregulation with lean/normal weight (defined as a body-mass index of <23 kg/m^2 in Asians). Metabolic dysregulation was defined as the presence of at least two of the following metabolic risk abnormalities: (a) waist circumference of ≥90 or 80 cm in Asian men and women, respectively, (b) blood pressure of ≥130/85 mmHg or a specific drug treatment, (c) plasma triglyceride levels of ≥150 mg/dL or a specific drug treatment, (d) plasma HDL-C levels of <40 mg/dL for men and <50 mg/dL for women or

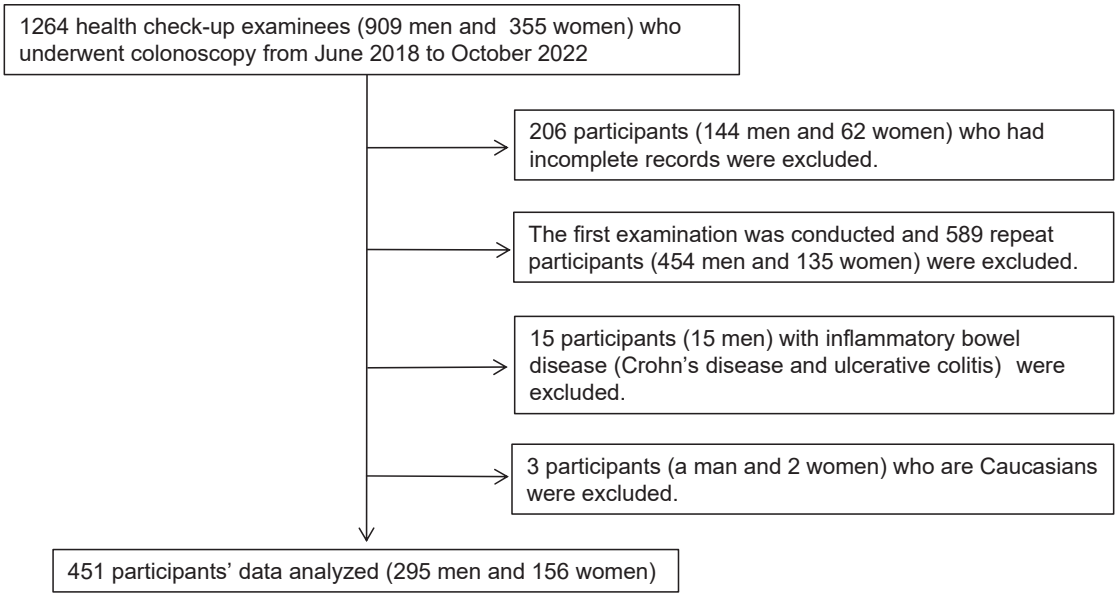

Fig. 1. Flow Chart of Participant Enrollment and Exclusion

a specific drug treatment, (e) prediabetes (i.e., fasting glucose levels of 100–125 mg/dL or a 2-h post-load glucose level of 140–199 mg/dL or a hemoglobin A1c level of 5.7%–6.4%), (f) Homeostasis model assessment-insulin resistance (HOMA-IR) score of ≥2.5, and (g) plasma high-sensitivity C-reactive protein level of >2 mg/L[20]. HOMA-IR score is calculated as follows: {[fasting glucose level (mg/dL)×fasting insulin level (μIU/mL)]/405}.

NAFLD was defined as FL in the absence of either hepatitis B or C virus infection and alcohol consumption (>30 g ethanol/day in men, >20 g ethanol/day in women)[21]. FL was defined as either high hepatorenal echo contrast, liver brightness, or deep attenuation on abdominal ultrasonography.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software version 29.0.1 (IBM Corp., Armonk, NY). Continuous variables were expressed as means (standard deviation) per group. The two-sided Student's *t*-test (for equal variance) or the Welch's *t*-test (for unequal variance) was used to assess statistically significant differences. The Mann–Whitney U test was utilized to evaluate variables with a non-normal distribution. The chi-square test was used to evaluate variables reported as proportions. Associations between the risk factors and colorectal adenomatous polyps (adenomas) were examined via multivariate logistic regression analysis and reported as odds ratios (ORs). A *p*-value of <0.05 was considered statistically significant. Moreover, explanatory variables (independent variables) that were included in the models were based on existing knowledge about the risk factors of CRA (age, sex, excessive alcohol use, smoking, and family history of CRC), MPV, hyperuricemia, impaired renal function, MAFLD, and NAFLD. The correlation coefficient of each variable considered in the models was <0.7. If the number of participants with an explanatory variable was 0, the variable was deleted.

Results

Study population

This study included 451 participants who underwent colonoscopy, namely 295 men with a mean age of 67.4 (standard deviation: 10.4) years and 156 women with a mean age of 68.6 (standard deviation: 9.7). CRA was detected in 202 (44.8%) participants. Overall, 46.8% of male participants and 41.0% of female participants had CRA. The cohort comprised all Asian individuals. The total number of participants, including those who did not undergo colonoscopy, was 771. Among them, 493 were men and 278 were women.

Fig. 2 shows the age- and sex-specific rates of CRA. The prevalence of CRA in all participants and in men was more likely to increase gradually with age. The prevalence of CRA in women was more likely to in-

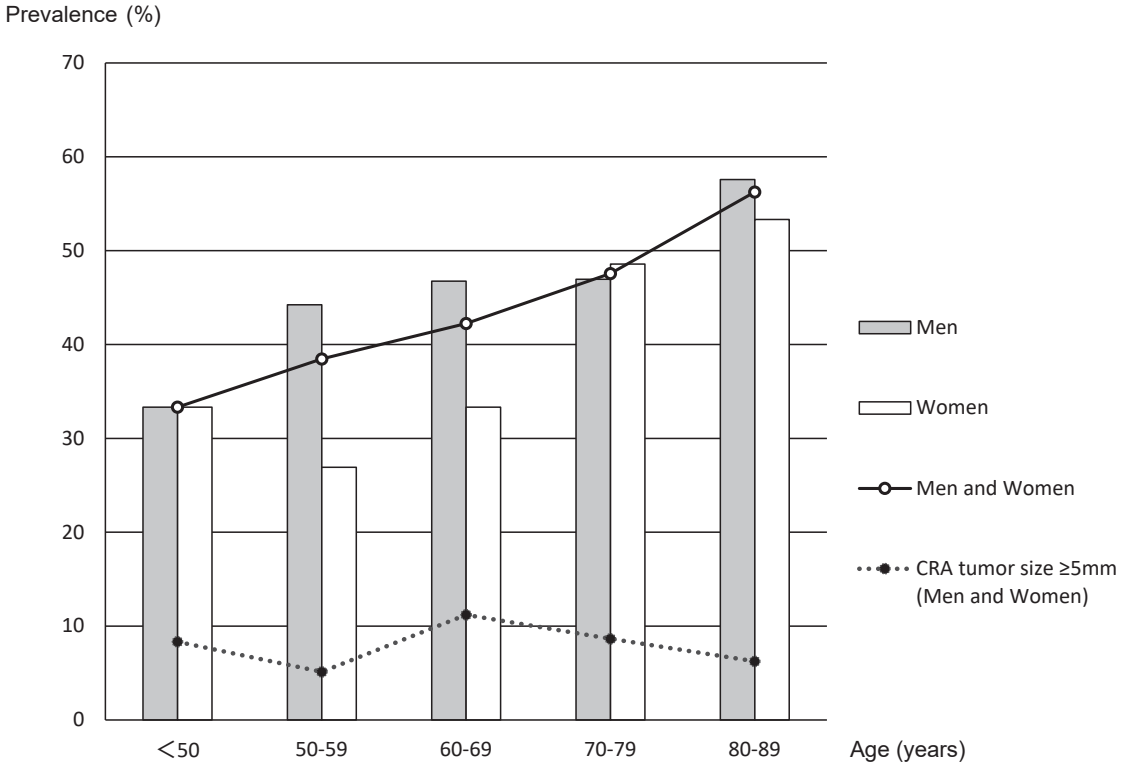

Fig. 2. Age- and Sex-specific Proportions of CRA and Age-specific Proportions of CRA Tumor Size ≥5 mm
CRA, colorectal adenoma

crease gradually after 50 years of age. Additionally, **Fig. 2** shows the age-specific rates of CRA tumor size ≥5 mm.

Clinical characteristics of participants and risk factors of CRA

Table 1 shows the clinical characteristics of 202 and 249 participants with and without CRA, respectively. The CRA group was significantly older and had significantly higher systolic blood pressure, triglyceride level and alcohol consumption than the group without CRA.

To investigate the risk factors significantly associated with CRA, a contingency table analysis using the multivariate logistic regression model was conducted between participants with CRA and those without. Results showed that excessive alcohol use (OR=1.9, p=0.019), increased MPV (OR=1.3, p=0.027) and aging (OR=1.03, p=0.008) were statistically significant risk factors of CRA (**Table 2**). Furthermore, the risk factors considered associated with ≥5-mm CRA were investigated. A contingency table analysis using the multivariate logistic regression model was conducted between participants with ≥5-mm CRA and those without ≥5-mm CRA. The results showed that smoking (OR=2.8, p=0.042) was a statistically significant risk factor of ≥5-mm CRA (**Table 3**).

Table 1. Clinical Data of the Participants With or Without CRA

Characteristics	Subjects with CRA	Subjects without CRA	*p* value
	Mean (SD) [Number]{% under treatment}		
Age	68.9 (9.9) [202]	67.0 (10.4) [249]	0.042
Gender, men/women	[138/64]	[157/92]	0.242
Systolic blood pressure (mmHg)	124.9 (16.2) [202]{47.0}	121.0 (15.7) [249]{34.9}	0.010
Diastolic blood pressure (mmHg)	72.8 (12.2) [202]{47.0}	71.6 (10.3) [249]{34.9}	0.250
Platelet (10^4/μL)	21.9 (5.2) [202]	21.8 (5.2) [249]	0.956
MPV (fL)	10.0 (0.9) [202]	9.8 (0.7) [249]	0.105
HDL-C (mg/dL)	64.6 (18.5) [202]{48.0}	67.8 (19.0) [249]{48.2}	0.099
Triglyceride (mg/dL)	128.3 (106.7) [202]{48.0}	110.6 (77.2) [249]{48.2}	0.022
Uric acid (mg/dL)	5.7 (1.3) [202]{14.9}	5.7 (1.2) [249]{15.7}	0.960
FPG (mg/dL)	110.2 (19.8) [202]{17.8}	109.4 (21.8) [249]{16.5}	0.277
HbA1c (%)	6.1 (0.7) [202]	6.0 (0.7) [249]	0.128
HOMA-IR	2.2 (1.9) [202]	1.9 (1.4) [249]	0.153
eGFR (mL/min/1.73 m^2)	67.4 (14.6) [202]	66.8 (14.3) [249]	0.670
Waist circumference (cm)	87.5 (9.5) [202]	86.8 (9.7) [249]	0.250
Nonoverlapping MAFLD (%)	14.9 [30/202]	10.8 [27/249]	0.203
Nonoverlapping NAFLD (%)	0.5 [1/202]	1.6 [4/249]	0.262
MAFLD and NAFLD (overlapping) (%)	24.8 [50/202]	27.3 [68/249]	0.539
alcohol consumption {ethanol(g)/day}	22.6 (26.3) [202]	15.9 (21.3) [249]	0.003
smoking (BI)	97.9 (333.1) [202]	80.3 (334.2) [249]	0.457
FH of CRC (%)	0.5 [1/202]	1.6 [4/249]	0.262

Results are presented as mean (standard deviation).
CRA, colorectal adenoma; SD, standard deviation; MPV, mean platelet volume; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment-insulin resistance; eGFR, estimated glomerular filtration rate; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, nonalcoholic fatty liver disease; BI, Brinkman Index; FH, family history; CRC, colorectal cancer

Table 2. Risk Factors of CRA Based on the Multivariate Logistic Analysis

Characteristics	Subjects with CRA (*n*=202)	Subjects without CRA (*n*=249)	Adjusted OR	95%CI	*p* value
	Number (%)				
Aging			1.03	1.01–1.05	0.008
Men	138 (68.3)	157 (63.1)	1.2	0.78–1.90	0.385
Women	64 (31.7)	92 (36.9)			
MPV			1.3	1.03–1.69	0.027
Hyperuricemia	52 (25.7)	65 (26.1)	0.9	0.53–1.40	0.550
eGFR<60 mL/min/1.73 m^2	60 (29.7)	80 (32.1)	0.8	0.52–1.26	0.355
Nonoverlapping MAFLD	30 (14.9)	27 (10.8)	0.9	0.42–1.74	0.667
Nonoverlapping NAFLD	1 (0.5)	4 (1.6)	0.5	0.05–4.55	0.532
excessive alcohol use	71 (35.1)	62 (24.9)	1.9	1.11–3.10	0.019
smoking (BI ≥400)	18 (8.9)	17 (6.8)	1.4	0.68–2.91	0.362
FH of CRC	1 (0.5)	4 (1.6)	0.3	0.03–2.97	0.308

CRA, colorectal adenoma; OR, odds ratio; CI, confidence interval; MPV, mean platelet volume; eGFR, estimated glomerular filtration rate; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, nonalcoholic fatty liver disease; BI, Brinkman Index; FH, family history; CRC, colorectal cancer

Table 3. Risk Factors for CRA of 5 mm or More in Size Based on Multivariate Logistic Analysis

Characteristics	Subjects with CRA ≥5 mm in size (*n*=38)	Subjects without CRA and Subjects with CRA <5 mm in size (*n*=413)	Adjusted OR	95%CI	*p* value
	Number (%)				
Aging			1.02	0.98–1.06	0.265
Men	24 (63.2)	271 (65.6)	0.7	0.31–1.54	0.370
Women	14 (36.8)	142 (34.4)			
MPV			1.2	0.80–1.78	0.383
Hyperuricemia	11 (28.9)	106 (25.7)	1.3	0.53–2.97	0.600
eGFR <60 mL/min/1.73 m^2	11 (28.9)	129 (31.2)	0.8	0.37–1.80	0.617
Nonoverlapping MAFLD	6 (15.8)	51 (12.3)	1.1	0.34–3.73	0.843
excessive alcohol use	13 (34.2)	120 (29.1)	1.3	0.52–3.08	0.602
smoking (BI ≥400)	6 (15.8)	29 (7.0)	2.8	1.04–7.63	0.042

CRA, colorectal adenoma; OR, odds ratio; CI, confidence interval; MPV, mean platelet volume; eGFR, estimated glomerular filtration rate; MAFLD, metabolic dysfunction-associated fatty liver disease; BI, Brinkman Index

Table 4. Clinicopathological Characteristics of CRA

Number of CRA	Number of subjects (%)
8	1 (0.5)
7	1 (0.5)
6	3 (1.5)
5	7 (3.5)
4	7 (3.5)
3	17 (8.4)
2	48 (23.8)
1	118 (58.4)
Tumor size (maximum dimention)	Number of subjects (%)
<5 mm	164 (81.2)
≥5 mm	38 (18.8)
Pathological diagnosis	Number of subjects (%)
Tubular adenoma	28 (87.5)
Sessile serrated adenoma/polyp	3 (9.4)
Carcinoma in adenoma	1 (3.1)

CRA, colorectal adenoma

Clinicopathological characteristics of CRA

Table 4 shows the clinicopathological characteristics of CRA. Approximately 58.4% of participants with CRA only had one CRA. The tumor size of 81.2% of participants with CRA was <5 mm. Furthermore, 87.5% of participants with CRA were diagnosed with tubular adenoma via pathological examination.

Discussion

This study revealed that increased MPV was a substantial risk factor of CRA (**Table 2**). Rizzo *et al.*[22] found that inflammation, which plays a role at different stages of the carcinogenesis process, was associated with increased risk of developing colon cancer. Patients with colon cancer have a significantly high level of proinflammatory cytokines such as interleukin-6[23]. Recent studies showed that interleukin-6 has a direct effect on megakaryocytes and causes platelet activation and aggregation[14]. MPV can be used as an index of activated platelets[24], and CRA is a precursor lesion for different types of colorectal cancers[18,19]. Hence, MPV is associated with CRA. The adjusted odds ratio of MPV, which is a risk factor of CRA, is 1.3, which is not markedly high. However, MPV is an extremely convenient and low-cost examination. Thus, it can be a useful surrogate marker of CRA.

CRA is a precursor of CRC[25]. Colorectal tumorigenesis in alcohol drinkers may be regulated by genetic factors[26–28]. In particular, alcohol intake can lead to folate deficiency in the colon and rectum, possibly owing to folate malabsorption. Furthermore, intestinal bacteria, which have a high alcohol dehydrogenase activity, could oxidize ethanol in the colorectum and produce a considerable level of acetaldehyde[29,30]. In addition, alcohol may affect DNA repair, suppress immune surveillance to tumors, alter bile acid composition, and induce the expression of liver cytochrome P-450 enzymes[31,32].

Moreover, this study showed that smoking was a substantial risk factor for ≥5-mm CRA. Toyomura *et al.*[33] revealed that cigarette smoking, not alcohol use, was likely to be strongly associated with large adenomas (diameter: ≥5 mm). Smoking is an important risk factor for developing CRC and colonic polyps[34]. The mechanism for this association is via oxidative stress and cellular DNA damage. In particular, carcinogens in smoke diffuse passively via the circulatory system into the colonic mucosa, where they interrupt cellular replication and hinder the DNA repair process. In addition, the carbon monoxide in cigarette smoke indirectly leads to DNA mutations and cellular hypoxia[35].

Age is the predominant nonmodifiable risk factor for the development of colon adenomas. A large body of research has shown that the prevalence of adenomas increases predictably with age, rising from 10% to 15% from individuals aged 50–55 years to those aged 70–75 years, which is the oldest age stratum[36–38]. In addition, based on this study, aging was one of the risk factors of CRA (**Table 2**). This study also showed that CRA ≥ 5 mm potentially eligible for endoscopic resection was slightly more frequent in participants in their 60s (**Fig. 2**). Colonoscopy would be recommended for older par-

ticipants, particularly those in their 60s.

Limitations

The limitations of this study include its retrospective design, conduct at a single institution and small sample size. Thus, further studies with a larger sample size and follow-up are required.

Conclusions

Smoking, excessive alcohol use, increased MPV, and aging were surrogate markers of CRA. Considering its low cost, availability, and use as an indicator of not only thrombotic and inflammatory diseases but also CRA, increased MPV should be extensively evaluated as a convenient tool for determining the need to undergo CRA screening. Furthermore, individuals who had excessive alcohol use and who smoke should undergo assessment to rule out CRA as they age, especially those in their 60s.

Conflict of Interest

The authors have no conflict of interest to declare.

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Original Article

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Quality and Yield Assessment of RNA Extracted from Fine-needle Aspiration Samples Obtained by Puncture of Surgically Resected Breast Cancer Specimens

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Abstract

Objective: Breast fine-needle aspiration cytology is minimally invasive and simple, and continues to be an essential diagnostic tool. This study serves as a preliminary investigation into the potential of using cytology specimens for RNA extraction with sufficient quality for subsequent gene expression analysis, with a focus on breast cancer samples obtained via puncture from surgical resection specimens.

Methods: The samples were obtained via puncture from surgical resection specimens of breast cancers from October 2016 to September 2018. We gathered 140 samples, including 86 from tumor areas and 54 from non-tumor areas. Specimens were punctured with an 18-G needle, transferred into tubes containing 1 mL of RNA extract solution kept on ice, and frozen for storage.

The samples were thawed on ice, and RNA extraction was conducted on ice to prevent RNA degradation due to temperature increase. RNA concentrations were identified using a UV spectrophotometer and a fluorescent RNA-binding dye. Additionally, RNA integrity number (RIN) and DV200 were calculated to assess RNA quality.

Results: A strong correlation was found between RNA concentrations identified by the two distinct methods. Additionally, a moderate correlation was observed between RIN and DV200. Multivariate linear analysis of RIN identified DV200 as an independent factor although it showed no association with RNA concentration or histopathological diagnosis.

Conclusions: Our findings demonstrated that maintaining strict temperature control during the RNA extraction process enables the extraction of high-quality RNA from specimens.

Keywords RNA Integrity Number (RIN), breast cancer, fine-needle aspiration cytology (FNA-C), DV200

The detection of ductal carcinoma in situ (DCIS), a noninvasive form of breast cancer, is on the rise due to widespread mammographic screening. However, early diagnosis of breast cancer, especially DCIS, remains challenging. We hypothesized that genetic diagnosis using breast fine-needle aspiration cytology (FNA-C) could be beneficial in this regard. FNA-C is a simple and minimally invasive procedure, rendering it a necessary diagnostic tool despite advances in other diagnostic technologies such as magnetic resonance imaging. However, cytosolic tests such as FNA-C are often qualitative and subjective. Disease-specific RNAs have been anticiapted as promising diagnostic biomarkers. Therefore, if RNA biomarkers of breast cancers, including DCIS, are successfully developed, they are expected to have substantial clinical relevance. In this regard, we previously identified a number of RNA biomarker candidates to diagnose DCIS by microarray analysis of samples obtained by puncture from surgical resection specimens[1].

Nevertheless, despite its potential benefits, RNA-based diagnosis has not gained widespread acceptance

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due to certain concerns. One such concern is RNA molecule instability, which sometimes causes the poor yield and quality of RNA extracted from specimens. In short, RNA is particularly unstable due to the presence of RNases. Keeping samples at a low temperature during the RNA extraction procedure is generally effective in minimizing RNA degradation[2]. Therefore, this study aimed to assess the possibility of extracting RNA of sufficient quality from breast cancer samples obtained by puncture from surgical resection specimens, with the goal of enabling RNA-based diagnosis.

Materials and Methods

Samples obtained by puncture from surgical resection specimens

This study included 63 patients who underwent breast cancer surgery from October 2016 to September 2018 at Nippon Medical School Musashikosugi Hospital. After resection, surgical specimens were punctured with an 18-G needle, placed in a tube containing 1 mL of RNAiso Plus (TaKaRa, Tokyo, Japan) on ice, and frozen for storage.

We obtained 140 samples, including 86 and 54 isolated from tumor areas and non-tumor areas, respectively. Histopathological diagnosis revealed that 10 patients had DCIS and 53 patients had invasive cancer.

This study was conducted following the ethical guidelines of the Declaration of Helsinki, and all patients provided informed consent. The Ethics Committee of Nippon Medical School Musashikosugi Hospital approved this study. All data were analyzed anonymously.

RNA extraction from the samples

RNA extraction was performed according to the acid guanidinium thiocyanate-phenol-chloroform method[3]. Briefly, total RNA was extracted from the samples using RNAiso Plus (Takara-Bio, Kusatsu, Japan) in accordance with the manufacturer's instructions, except that all steps were conducted on ice to prevent a temperature increase during the procedure.

Moreover, linear acrylamide (Thermo Fisher Scientific, Waltham, MA, USA) was included as a co-precipitant during the isopropyl alcohol precipitation step. Each RNA sample was resuspended in 10 μL of RNase/DNase-free distilled water.

Assessment of the quantity and quality of the samples

RNA concentrations were identified by UV spectrophotometry using a BioSpec-nano spectrophotometer device (SHIMADZU, Kyoto, Japan) and a QuantiFluor RNA system (Promega, Madison, WI, USA), which is an RNA-binding fluorescent dye that is advantageous in quantifying small amounts of RNA.

RNA quality assessment

The Agilent 2100 Bioanalyzer (Agilent, Santa Clara, USA) was used together with the RNA 6000 Nano Lab Chip kit (Agilent) for RNA quality analysis[4]. RNA integrity number (RIN) and DV200, a parameter defined as the percentage of RNA fragments with >200 nucleotides, were used to objectively assess the quality of RNA.

Statistical analysis

The Mann–Whitney U test and Spearman's rank correlation analysis were used for statistical comparison between groups. p-values of <0.05 were considered statistically significant. Multivariate linear regression analysis was performed to determine the variables associated with the RIN index. To evaluate multicollinearity among variables, we calculated the variance inflation factor (VIF) with the VIF cut-off for significant collinearity set at 2. EZR, a modified version of R software (version 1.53; R Foundation for Statistical Computing, Vienna, Austria) with additional biostatistical functions, was used for all statistical analyses[5].

Results

Tumor area/non-tumor area and variables analyzed

Table 1 summarizes a comparison of tumor and non-tumor areas regarding RNA concentration, RIN, and DV200. All characteristics of RNA concentration, RIN, and DV200 were higher in the tumor area than in the non-tumor area, with statistically significant differences between the two areas ($p<0.001$).

Univariate analysis of RIN in all samples

Table 2 presents the Spearman rank correlation coefficient results, which indicate no significant correlation between RIN and RNA concentration values measured by the two distinct methods. However, the results do

Table 1. Characteristics of Study Samples

Characteristic	All $n=140$	Tumor $n=86$	Non-tumor $n=54$	Mann–Whitney U-test (p-value)
RNA concentration				
BIO-SPEC [ng/μL]	372.5 (8.3–8645.6)	652.4 (15.0–8645.6)	283.5 (8.3–1552.1)	<0.001
QuantiFluor [ng/μL]	353.8 (6.7–221653.4)	587.3 (6.9–221653.4)	231.6 (6.7–1039.1)	<0.001
RIN	5.4 (1.1–9.5)	6.4 (2.1–9.5)	4.2 (1.1–8.0)	<0.001
DV200 (%)	68.0 (1–95)	71.5 (1–95)	61.5 (5–85)	<0.001

median (range)
RIN, RNA Integrity Number

show a correlation between RIN and DV200 (r=0.67, p<0.001).

Correlation between two RNA concentrations and between RIN and DV200

The correlation between RNA concentrations identified by the BioSpec-nano and QuantiFluor RNA systems revealed a correlation coefficient of 0.86 for all samples, which was notably high at 0.89 in tumor areas. As depicted in **Fig. 1**, the correlation between RIN and DV200 displayed a coefficient of 0.67 for all samples.

Multivariate linear analysis of RIN

Multivariate linear regression analysis was used to identify variables associated with RIN values in all samples. Bivariate correlation analyses of continuous variables (RNA concentrations determined by the QuantiFluor RNA system, DV200) revealed significant associations between RIN and DV200 (p<0.001), but no significant associations were observed between RIN and RNA concentrations (**Table 3**). The VIFs for RNA concentrations and DV200 were both 1.004. We used multivariate linear regression analysis to further identify variables associated with RIN values in the 86 tumor area samples. Bivariate correlation analysis of continuous (RNA concentration measured by QuantiFluor RNA system, DV200) and categorical variables (pathological diagnosis; invasive cancer/DCIS) revealed significant associations between RIN and DV200 (p<0.001) (**Table 4**). VIF for RNA concentration, DV200, and pathological diagnosis was 1.005, 1.004, and 1.005, respectively. All VIF values were <2, indicating no significant collinearity.

Table 2. Univariate Analysis of RIN in All Samples

Independent variable	Spearman's rank correlation coefficient (r, p value)
RNA concentration	
BioSpec-nano [ng/μL]	0.32, <0.001
QuantiFluor [ng/μL]	0.42, <0.001
DV200 [%]	0.67, <0.001

RIN, RNA Integrity Number

Discussion

Although RNA biomarkers are considered promising tools for pathologies that are difficult to diagnose using current modalities. However, RNA instability is a problem in some cases. Here, we found that this was also the case for breast cancer FNA-C samples, as we failed to extract RNA with sufficient quality by following a

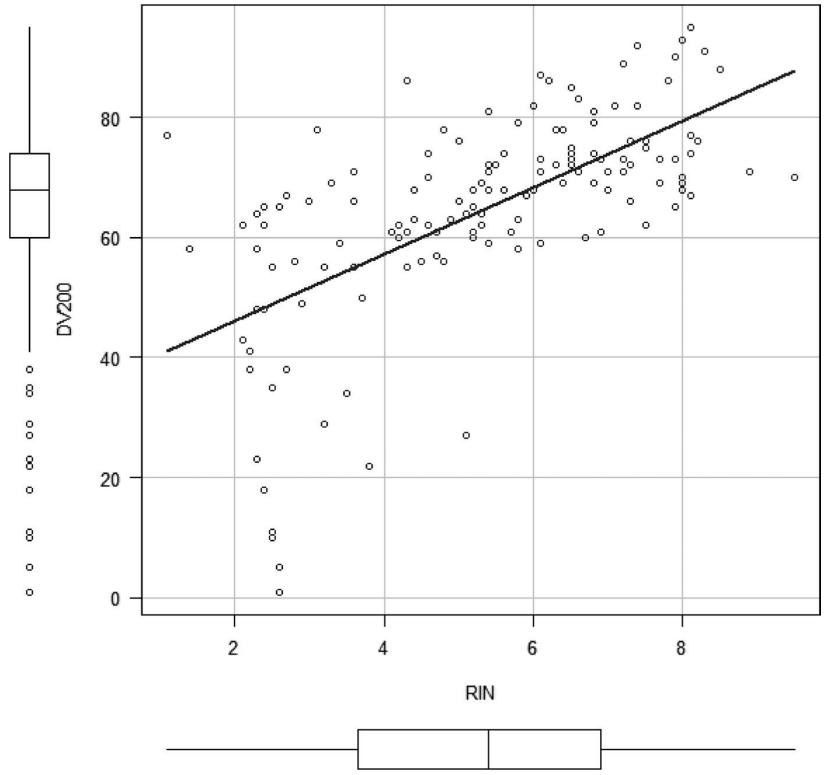

Fig. 1. Correlation Between RIN and DV200 in All Samples

The horizontal axis represents RIN and the vertical axis represents DV200. A boxplot is a standardized way of displaying the dataset based on a five-number summary: the minimum (0th percentile), the maximum (100th percentile), the sample median (50th percentile), and the first and third quartiles (25th or 75th percentile). Outliers are plotted as individual points beyond the whiskers on the boxplot.
RIN, RNA Integrity Number

Table 3. Multivariate Linear Analysis of RIN in All Samples

Independent variables	Estimated regression coefficicient	Standard error	*t* value	*p* value
(intercept)	0.73	0.50	1.45	0.15
RNA concentration QuantiFluor [ng/μL]	<0.001	<0.001	0.73	0.47
DV200 [%]	0.071	0.0075	9.48	<0.001

RIN, RNA Integrity Number

Table 4. Multivariate Linear Analysis of RIN in 86 Tumor Areas

Independent variables	Estimated regression coefficicient	Standard error	*t* value	*p* value
(intercept)	1.24	0.69	1.80	0.08
RNA concentration QuantiFluor [ng/μL]	<0.001	<0.001	0.52	0.60
DV200 [%]	0.073	0.0090	8.08	<0.001
pathological diagnosis invasive cancer/DCIS	−0.37	0.36	−1.03	0.31

DCIS, ductal carcinoma in situ; RIN, RNA Integrity Number

standard protocol for RNA extraction. In contrast, we achieved improved RNA quality by simply controlling temperature during sample handling, i.e., keeping the samples on ice to the maximum extent possible to avoid temperature increase.

This study revealed that the RIN and DV200 values of RNA from tumor areas were higher than those from non-tumor areas. This is mainly because non-tumor areas are very sparse with few cells compared to tumor areas; thus, they have more contaminants and are more susceptible to RNAase.

A strong correlation was found between RNA concentrations measured by two distinct methods. This result indicates that contamination of molecules affecting OD 260 and OD 280 values in the extracted RNA samples is negligible, and that RNA quantification by UV spectrometry, which is much simpler and cheaper than that using RNA-binding fluorescent dye, is accordingly sufficient. Additionally, a correlation was observed between RIN and DV200. Although DV200 is more strongly recommended as an indicator to evaluate the degree of RNA degradation[4], our results indicate that the two parameters may be equivalent for this purpose.

Conversely, no correlation was observed between RIN and RNA concentration (**Table 2**). Further, multivariate linear analysis of RIN revealed that DV200 was an independent factor but not related to RNA concentration (**Table 3**). This point is mentioned in the Application Note published by Agilent Technologies[6]. RINs are not sensitive to RNA concentration, consistent with our result.

Previously, we extracted RNA from DCIS specimens obtained by surgical specimen puncture immediately postoperatively[1]. Moreover, we performed RNA extraction from specimens of duct-washing cytology contemporaneously with mammary ductoscopy[7]. In these studies, we selected samples with sufficient RNA yield and quality for subsequent analyses, such as microarray and quantitative reverse transcription polymerase chain reaction (RT-PCR). However, the quality of some of these RNA samples was too low quality to allow analysis, such as microarray and quantitative RT-PCR analyses, which remained a concern.

Therefore, this study aimed to primarily answer the question, "How can the quality of RNA extracted from the FNA-C specimens of breast cancer be improved?" Our results indicate that keeping the sample temperature cool is effective in avoiding RNA degradation, and thereby increasing RNA quality. Based on the results of this paper, we have already carried out microarray analysis and identified some promising candidates of breast cancer biomarkers[1]. Notably, one of these candidates —MALINC1— has been evaluated as an early-stage breast cancer biomarker by another research group[8]. If tumor markers are established in the future, it will be possible to diagnose benign or malignant tumors at the fine-needle aspiration cytology stage in the outpatient clinic, which we believe could contribute to preventive medicine. This study was conducted using surgically resected specimens, but verification using actual FNA specimens is required in the future.

Limitations

Some study limitations should be considered when interpreting these results. First, the findings were based on a small sample size. Second, the puncture step from the surgical specimen, typically conducted at room temperature, may ideally need to be performed at a lower temperature, despite efforts to keep the samples on ice during the RNA extraction process.

Conclusion

By keeping the samples cooled on ice, we successfully extracted RNA of sufficient quality from breast cancer FNA-C samples.

Conflict of Interest

The authors have no conflicts of interest.

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Exploring the Potential for Oral Sulfate Solutions to Reduce the Pretreatment Burden of Colon Capsule Endoscopy: A Preliminary Small Population Study

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Abstract

Objective: Although colon capsule endoscopy (CCE) is a non-invasive method, the procedure requires a substantial dose of bowel cleansing agent, which can be burdensome. This preliminary study aimed to verify the feasibility and effectiveness of using a small, more palatable dose of cleansing agent for CCE.

Methods: The study involved 11 participants (six men and five women; mean age, 54.6±7.4 years) who underwent CCE at our facility between November 2022 and August 2023 and received oral sulfate solution as the bowel cleanser. The participants also completed a questionnaire survey. Four participants underwent CCE without the standard 1 h water drinking restriction before capsule ingestion.

Results: The survey results indicated that seven participants perceived the examination as either easy or relatively easy. However, two participants with non-restriction on water drinking reported prolonged procedure times (>10 h) and significant difficulty. Despite this, >50% of the participants considered the bowel cleanser dosage (687 mL on average) as manageable, with a palatable taste. Bowel cleansing was categorized as excellent or good in all cases. The mean procedure time was 7 h 44 min. Lesions were detected in 90.1% of the cases, with colonic polyps identified in 63.6%.

Conclusions: A reduced dose of bowel cleansing agents was effective in alleviating the pretreatment burden of CCE and in ensuring adequate cleansing for lesion detection. CCE utilizing reduced doses of bowel cleansing solution offers a safe and effective alternative for colorectal cancer screening, promising accurate diagnostic performance.

Keywords bowel cleansing, colon capsule endoscopy, colon cancer screening

Colorectal cancer is a leading cause of morbidity and mortality in Japan[1], highlighting the urgent need for effective screening strategies. Timely detection plays a crucial role in mitigating the effects of colorectal cancer, a disease with far-reaching consequences. Despite its significance, the consistently low rates of primary and secondary colorectal cancer screening present a formidable challenge in the battle against this disease. Addressing and improving these screening rates has become a pivotal task in the efforts to reduce cancer-related fatalities.

Presently, the fecal occult blood test (immunoassay) is the recommended primary screening method for colorectal cancer in Japan and is widely employed in cancer screening programs. However, data from the 2019 National Survey of Basic Living Conditions indicate a screening rate of only 44.5% for men and 38.5% for women, falling in both cases below the desired 50% threshold[2]. Colonoscopy is recommended for secondary screening; however, the results of a 2017 survey by the Japan Cancer Society demonstrated a secondary screening rate of 68.7%[3], suggesting that a substantial number of individuals with positive results for the fecal occult blood test did not undergo colonoscopy.

Reasons cited for avoiding colonoscopy include challenges associated with examination preparation, potential pain, discomfort, feelings of embarrassment, and personal perceptions that the examination was unnecessary, with bleeding attributed to hemorrhoids. Addressing these barriers and enhancing screening rates are essential to promoting early detection and paving the way for an effective approach to the prevention and

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management of colorectal cancer.

Colon capsule endoscopy (CCE) is a minimally invasive, less uncomfortable, and less socially awkward alternative for individuals requiring examination. Unlike conventional colonoscopy, which requires specialized endoscopists, CCE offers the advantage of being performed by nonspecialists, potentially addressing the challenge of securing sufficient endoscopists to improve screening rates for colorectal cancer[4,5]. Despite some drawbacks, such as the requirement for a significant amount of bowel cleansing agent, prolonged examination duration, high costs, and the inability to perform a biopsy, this approach holds promise as a potential alternative for secondary colorectal cancer screening.

Aims

This preliminary study was undertaken to scrutinize the acceptability of CCE using a recently launched oral sulfate solution, before performing a large-scale comparative study. In this study, we focused on mitigating some of the disadvantages of CCE. Specifically, we investigated the use of a bowel cleansing solution that could be administered in small doses and had a more palatable taste. We aimed to enhance the acceptability of CCE for examinees and minimize both the burden of the cleansing process and the overall examination time. Reducing the burden associated with CCE is likely to improve the convenience of the procedure for patients and contribute to promoting the widespread use of this innovative technology in colorectal cancer screening.

Methods

This prospective, single-center study was conducted at the Center for Preventive Medicine of The Jikei University Hospital, Tokyo, Japan. Our center mainly provides Ningen Dock, an annual medical check-up system involving a series of medical examinations over 1–2 days. This study was approved by the Ethics Committee of The Jikei University School of Medicine (approval number: 34-140 (11291); approval date: September 5, 2022) and was conducted in accordance with the 1964 Declaration of Helsinki. Written informed consent was obtained from each participant before enrollment.

Study participants

The study comprised 11 participants (six men and five women; mean age, 54.6±7.4 years) who presented for medical check-ups. The participants underwent CCE at our facility between November 2022 and August 2023. At our facility, CCE is offered as an optional examination during medical checkups, and all participants undergo CCE voluntarily. Three participants underwent a stool-occult blood test, including Ningen Dock, which yielded negative results. No patients exhibited symptoms of gastrointestinal disorders, nor did they have any other noticeable disease. One patient had previously undergone a colonoscopy, but none of them had specifically undergone CCE previously.

Colon Capsule Endoscopy (CCE)

As displayed in **Table 1**, a modified Japanese Association for Capsule Endoscopy (JACE) CCE regimen was performed[6]. An oral sulfate solution (Sulprep®; Fuji Pharma, Co., Ltd.) was utilized as the bowel cleansing agent. Examination image reading, a description of the findings, and acceptability to the participant (e.g., pain and burden status) were assessed. The degree of bowel cleansing was evaluated using a grading scale for the assessment of colon cleansing before CCE[7]. The CCE procedure time (defined as the time elapsed from the moment the patient started ingesting the bowel cleansing solution to the excretion of the capsule), the time elapsed from swallowing the capsule to its excretion, the stomach transit time (from the cardia to the pylorus of the stomach), the colon transit time (from the moment the capsule reached the cecum to its excretion), the duration of bowel cleansing (defined as the time elapsed from the moment the patient started ingesting the bowel cleansing solution to the swallowing of the capsule), the cleansing agent dosage, and the frequency

Table 1. Bowel Preparation Procedure

Day	Time	Procedure
-3	before sleep	Sennoside, 2 tablets (12 mg)
-2	before sleep	Sennoside, 2 tablets (12 mg)
-1	for all meals	Low residue diet
	before sleep	Sodium picosulfate (10 mL)+water (200 mL)
0 (Test day)	8:00	Gascon drop* (5 mL)+water (25 mL) OSS (120 mL)+water (250 mL) for 10 min each (repeated until completely cleaned; maximum of 8 courses)
	10:00 or later	1 h without drinking (seven participants)
	11:00 or later	Mosapride citrate hydrate (20 mg) The CCE capsule is swallowed
	The time the CCE capsule reaches the jejunum (12:00 or later)	Castor oil (30 mL)+magnesium citrate solution (600 mL) administered over 1 h (two courses) Magnesium citrate solution (600 mL) administered over 1 h

* Gascon drop: 20 mg/mL dimethylpolysiloxane
CCE: colon capsule endoscopy; OSS: oral sulfate solution (Sulprep®)

Table 2. The Questionnaire Administered to the Participants

Q1. How was your experience of undergoing CCE?
General
Specific
1. Bowel cleansing solution
Amount
Taste
Procedure
2. Duration
3. Booster
4. Swallowing CCE
5. Abdominal pain
6. Abdominal fullness
7. Fear
8. Shame
9. Hunger
10. Analgia
For those who had previously undergone colonoscopy
Q2. Did you find CCE more difficult than a colonoscopy?
Refer to questions 1–10 above
Q3. If you require further bowel testing in the future, would you prefer to undergo a CCE or a colonoscopy?
CCE/colonoscopy/I do not want to undergo another CCE

CCE: colon capsule endoscopy

Table 3. Colon Capsule Endoscopy Status

Evaluated item		Results
Completed		All
Incidental diseases		None
Procedure time	Average	7 h 44 min
	Minimum	6 h
	Maximum	12 h
Duration of bowel cleansing	Average	2 h 51 min
	Minimum	2 h
	Maximum	4 h
Bowel cleansing solution dosage	Average	687 mL
	Minimum	480 mL
	Maximum	960 mL
Number of boosters required	Average	2 courses (2 h)
	1 course	2 participants
	2 courses	6 participants
	3 courses	3 participants
Total dosage of bowel cleansing solution and booster	Average	1309 mL
	Minimum	630 mL
	Maximum	1860 mL

of booster usage were analyzed, along with the CCE findings. The CCE images were evaluated, and the corresponding diagnoses were made by two CCE-certified JACE specialists. In cases where a diagnosis differed between the two specialists, a final diagnosis was determined following discussion.

To further reduce the burden on the participants, the 1 h restriction on water drinking prior to capsule swallowing was lifted for four participants to determine whether the procedure time could be reduced. Restricting water intake before capsule ingestion is thought to enhance bowel cleansing by promoting defecation within 1 h.

Questionnaire survey

A questionnaire-based survey (**Table 2**) was administered using the questionnaire utilized in the ColoCam-J study by the JACE (https://the-jace.org/news/4403/). The questionnaire was employed to assess the ability of the participants to tolerate the procedure. Each question was rated on a 5-point scale, ranging from hard/none (1 point) to easy/too much (5 points).

Results

All patients completed the procedure without experiencing any serious complications. None of the participants had any incidental disease. **Table 3** demonstrates the details of the CCE procedure, including the procedure time, duration of colon cleansing, dosage of the colon-cleansing agent, and booster time. The average procedure time was 7 h 44 min, which was longer than the previously reported average procedure time (5–6 h)[8]. The longest CCE required 12 h, which was almost twice the average time. The average duration of colon cleansing was 2 h 51 min. This was longer than the standard duration (1–2 h) when using the oral colon cleansing agent MOVIPREP®, containing sodium, potassium, and ascorbic acid[9]. However, this duration was equivalent to that observed (2–4 h) when using another oral colon cleansing agent (NIFLEC®) containing sodium and potassium[10]. The average dosage of the colon cleansing agent was 687 mL, which was lower than that used with other agents (1–2 L)[9,10]. Six participants required two booster courses, suggesting that most people might need a two-course booster; however, important individual differences were observed. The average value of the total dosage of bowel cleansing solution and booster was 1,309 mL, which was lower than that previously reported at another facility[11]. Ohmiya *et al.* reported a median total dosage of bowel cleansing solution and booster of 1,600 mL, whereas the total dosage in our study was 81.8% of that value.

Cleansing results data are presented in **Table 4**. All participants achieved cleansing levels as excellent or good, indicating adequate effectiveness for endoscopic observation.

The CCE findings are presented in **Table 4**. Seven participants had colon polyps, representing a detection rate of 63.6%. The overall lesion detection rate was 90.1%, suggesting that most individuals may have had some lesion in their colon.

Contrary to expectations, the four participants for whom the 1 h restriction on water drinking before capsule swallowing was lifted demonstrated procedure times that were approximately 1.5 h longer than those for patients who complied with the restriction (**Table 5**). In particular, the transit time of the capsule through the colon was 3.5 h longer. In contrast, the time for bowel cleansing appeared to decrease to approximately 3.5 h;

Table 4. Colon Cleansing Results and CCE Findings

Evaluated items		Total (*n*=11)	Patients subjected to restriction on water drinking 1 h before capsule swallowing (*n*=7)	Patients not subjected to restriction on water drinking 1 h before capsule swallowing (*n*=4)
Degree of bowel cleansing	Excellent	8 participants	5 participants	3 participants
	Good	3 participants	2 participants	1 participants
	Fair	none	none	none
	Poor	none	none	none
Findings	Colon polyps	7 participants	4 participants	3 participants
	Angioectasia	2 participants	1 participants	1 participants
	Colon diverticula	1 participant	1 participant	none
	Colon SMT	1 participant	1 participant	none
	Non-specific redness	1 participant	none	1 participants
	Proportion of participants with significant findings	90.1%	85.7%	100.0%
	Proportion of participants with colon polyps	63.6%	57.1%	75.0%

CCE: colon capsule endoscopy; SMT: submucosal tumor

Table 5. Comparison of CCE Status Between Patients With and Without 1 h Water Restriction before Capsule Swallowing

		Patients subjected to restriction on water drinking for 1 h before capsule swallowing (*n*=7)	Patients not subjected to restriction on water drinking for 1 h before capsule swallowing (*n*=4)
Procedure time*	Average	7 h 18 min	8 h 48 min
	Minimum	6 h	6 h 30 min
	Maximam	9 h	12 h
Time elapsed from swallowing to excretion of the capsule	Average	2 h 30 min	6 h 8 min
	Minimum	1 h 47 min	4 h
	Maximam	3 h 9 min	8 h 52 min
Stomach transit time	Average	24 min	35 min
	Minimum	7 min	8 min
	Maximum	30 min	57 min
Colon transit time	Average	48 min	4 h 29 min
	Minimum	18 min	2 h 8 min
	Maximum	1 h 47 min	7 h 23 min
Bowel cleansing solution dosage	Average	702 mL	660 mL
	Minimum	480 mL	480 mL
	Maximum	960 mL	960 mL
Total dosage of bowel cleansing solution and booster	Average	1080 mL	1710 mL
	Minimum	630 mL	1260 mL
	Maximum	1260 mL	1860 mL

* Time elapsed from the moment the patient started ingesting the colon cleansing solution to the excretion of the capsule
CCE: colon capsule endoscopy

specifically, it averaged 1 h 20 mins for patients permitted to drink water before capsule ingestion compared to 4 h 48 min for those who were not so permitted. The average duration of stomach transit did not differ significantly between the two groups. However, the maximum stomach transit time was approximately twice as long in the group without water restriction compared with the group that complied with this restriction.

The dosage of the bowel cleansing solution was also comparable between participants who had restrictions on water consumption 1 h before capsule swallowing and those without restriction. However, the average total dosage of bowel cleansing solution and booster was approximately 1.6 times higher in the group not subjected to the restriction on water drinking compared to the group that was restricted.

The analysis of the survey results is displayed in **Table 6**. The results indicated that 63.6% of the study participants perceived the entire test to be either easy or considerably easy and that the overall test was acceptable. 54.5% of the participants responded that the dose of bowel cleansing solution was easy or considerably easy to ingest. Moreover, 54.5% of the participants responded that the flavor was palatable.

Participants who had previously undergone a colonoscopy indicated that he found the CCE procedure easier to undergo than a colonoscopy. He indicated that the quantity and taste of the bowel cleansing solution

Table 6. Analysis of Survey Results

Evaluated items		Answer	Number of participants		
			Total (*n*=11)	Patients subjected to restriction on water drinking for 1 h before capsule swallowing (*n*=7)	Patients not subjected to restriction on water drinking for 1 h before capsule swallowing (*n*=4)
General		Easy/considerably easy	7 (63.6%)	6 (85.7%)	1 (25.0%)
Colon cleansing solution	Amount	Easy/considerably easy	6 (54.5%)	5 (71.4%)	1 (25.0%)
	Flavour	Good/considerably good	6 (54.5%)	4 (57.1%)	2 (50.0%)
	Procedure	Easy/considerably easy	9 (81.8%)	6 (85.7%)	3 (75.0%)
Booster		Easy/considerably easy	6 (54.5%)	4 (57.1%)	2 (50.0%)
Duration		Easy/considerably easy	5 (45.5%)	5 (71.4%)	0
Swallowing capsule		Easy/considerably easy	9 (81.8%)	6 (85.7%)	3 (75.0%)
Symptom/Feeling	Abdominal pain	Nothing/mostly nothing	9 (81.8%)	6 (85.7%)	3 (75.0%)
	Abdominal fullness	Nothing/mostly nothing	7 (63.6%)	5 (71.4%)	2 (50.0%)
	Fear	Nothing/mostly nothing	11 (100.0%)	7 (100.0%)	4 (100.0%)
	Shame	Nothing/mostly nothing	7 (63.6%)	5 (71.4%)	2 (50.0%)
	Hunger	Nothing/mostly nothing	11 (100.0%)	7 (100.0%)	4 (100.0%)
	Analgia	Nothing/mostly nothing	4 (36.4%)	4 (57.1%)	0
Subjects who had previously undergone colonoscopy (*n*=1*)					
Comparison with colonoscopy		Considerably easy			
General		Acceptable			
Colon cleansing solution	Amount	Relatively difficult			
	Flavour	Relatively poor			
	Procedure	Relatively difficult			
Symptom/Feeling		None			
Which would you choose if you were to have a colonoscopy next time?					
CCE			7 (63.6%)	5 (71.4%)	2 (50.0%)
Colonoscopy			2 (18.2%)	1 (14.3%)	1 (25.0%)
I do not want to undergo another CCE			2 (18.2%)	1 (14.3%)	1 (25.0%)

* The participant belonged to the group that had been subjected to a 1 h restriction on water drinking prior to swallowing of the capsule
CCE: colon capsule endoscopy

utilized in colonoscopy were relatively non-acceptable, and the process involved in bowel cleansing was somewhat challenging. Furthermore, most participants (63.6%) expressed a preference to undergo CCE at their next colon examination.

In addition, a majority of the participants (85.7%) in the group that adhered to the water restriction found the CCE process to be easy, whereas only one participant (25%) in the non-restricted group provided the same response (**Table 6**). Two participants in the non-restricted group took 7 h 20 min and 8 h 52 min, respectively, from swallowing the capsule to its excretion, whereas the average time for the group subjected to water restrictions was 2–3 h. This may explain why participants in the non-restricted group perceived the CCE process as difficult.

Discussion

In this study, the administered amount of bowel cleanser was reduced to approximately 40–70% of the conventional dose[9,10], thereby decreasing the sense of burden. During the study period, 97 patients underwent a colonoscopy at our facility, and the average dosage of MOVIPREP® was approximately 1,054 mL. The dosage for Sulprep® was lower, at approximately 68% of that used for MOVIPREP®. However, the time required to use the bowel cleanser was >1.5 times longer than that required for conventional cleansing. Based on the questionnaire findings, while the oral sulfate solution did not reduce the procedure time, the patients deemed the procedure time acceptable.

To reduce the examination time, we tested a modified protocol in which the participants were not restricted from drinking water during the hour immediately before swallowing the capsule. This modified protocol was applied to help prevent dehydration resulting from the use of an enteric lavage solution. Contrary to our expectations, our results suggest that a 1 h restriction on water drinking is important to improving the cleaning of the colon and shortening the transit time of the capsule within the colon. These findings suggest that the 1 h restriction on water drinking before capsule swallowing might be important for effective cleansing of the colon. Further, our study findings indicated that the dosage of bowel cleansing solution and booster was higher in the group not subjected to the water restriction compared with the restricted group. This finding suggests that the restriction was necessary to allow the capsule to move through the digestive tract smoothly. However, owing to the limited number of study partici-

pants, reaching a definitive conclusion regarding this matter is not possible and further large-sample studies are needed for clarification.

The detection rate of colorectal polyps using colon capsule endoscopy has been reported to range from 24% to 74%, with variations among reports[12]. In this study, the detection rate of colon polyps was 63.6%, indicating that polyps could be detected in a high percentage of patients, leading to hospital visits for prompt treatment. Furthermore, the detection rate for colon polyps using colonoscopy has been previously reported to range between 41.2%[13] and 59.4%[14]. We deemed the sensitivity of CCE for detecting lesions in our study to be adequate.

Furthermore, CCE has been reported to have a sensitivity of 88% and a specificity of 82% for the detection of adenomas >6 mm, and of 92% and 95%, respectively, for the detection of adenomas >10 mm when colonoscopy is employed as the standard investigation for colorectal cancer screening[15]. Despite its high sensitivity and specificity in detecting colon cancers, the sensitivity of CCE for the detection of small adenomas may be low. Therefore, even if no adenoma is detected at a single examination, periodic capsule endoscopy, such as a repeat examination within 2–3 years, may prevent small adenomas from escaping detection.

Currently, CCE is indicated only when colonoscopy is not possible in clinical practice. However, CCE can be performed on request during voluntary colorectal cancer screening. Even those who are reluctant to undergo colonoscopy may decide to undergo colonoscopy if the CCE detects findings that are suspicious of colorectal cancer. This would contribute to an increase in the number of cases of colorectal cancer detected at an early stage.

For capsule endoscopy to play a role in colorectal cancer screening, it is necessary not only to improve the acceptability of the procedure among examinees, but also to make the costs associated with screening lower and to address issues concerning the shortage of manpower for image reading.

Limitations

A limitation of this study was its small study population. With such small participant numbers, we were unable to conduct a comparative study to include patients who had been administered conventional bowel cleansers at our institution. In addition, owing to the small sample size, the effect of individual differences in the results was too high to draw definitive conclusions. Further studies involving large patient numbers are needed to allow for appropriate statistical comparisons to reach more reliable conclusions.

Conclusions

The burden associated with the dose of bowel cleanser was minimized, which also reduced the overall burden on the examinees. Moreover, the degree of colonic cleansing was sufficient for lesion detection.

We consider CCE to be effective for colorectal cancer screening owing to its safety and efficacy in lesion detection. Furthermore, the technique has the potential to increase the proportion of patients referred to hospitals for further evaluation.

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Conflict of Interest

The authors declare no conflicts of interest associated with this manuscript.

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Using Machine Learning to Evaluate the Association between Atherosclerotic Risk Factors and Mean Platelet Volume

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Abstract

Objective: This study aimed to identify risk factors associated with mean platelet volume (MPV), focusing on arteriosclerotic factors and lifestyle-related diseases, in the general Japanese population using artificial intelligence (AI).

Methods: We enrolled 2,970 participants between June 6, 2018, and October 31, 2022. First, the risk factors associated with increased MPV were analyzed through machine learning using the AI software Prediction One. Subsequently, these factors were investigated and confirmed through contingency tables using multivariate logistic regression analysis.

Results: Machine learning indicated that the important predictors of increased MPV comprised nonalcoholic fatty liver disease (NAFLD) only (fatty liver with NAFLD but not metabolic dysfunction-associated fatty liver disease [MAFLD]), increased fibrosis-4 (FIB-4) index, alcohol consumption, and levels of fasting plasma glucose, triglycerides, estimated glomerular filtration rate, high-sensitivity C-reactive protein, high-density lipoprotein cholesterol, systolic blood pressure, and cholinesterase. Contingency tables using multivariate logistic regression analysis also revealed NAFLD only, increased FIB-4 index, and metabolic syndrome (particularly its dyslipidemia and impaired fasting glucose components) as risk factors for increased MPV.

Conclusions: Using AI, this study indicated that increased MPV was associated with atherosclerotic risk factors and lifestyle-related diseases. Machine learning may be an effective tool for analyzing multiple factors simultaneously, exploring their relationships, and identifying new biomarkers.

Keywords medical check-up, mean platelet volume, machine learning

Mean platelet volume (MPV) is a simple and inexpensive blood test indicator and is commonly used to measure platelet size and evaluate platelet activity[1]. Elevations in MPV values have been detected in patients with coronary artery disease[2–4] and have been associated with cardiovascular risk factors such as diabetes[5], hypertension[6], and dyslipidemia[7]. In our previous study[8], we demonstrated that metabolic syndrome (MetS), particularly the dyslipidemia and hypertension components, is significantly associated with increased MPV. Some studies have also shown a significant association between MPV and nonalcoholic fatty liver disease (NAFLD)[9–13]. Despite the known association of MPV with these diseases, only a few studies have comprehensively examined it using various data sources such as health check-up data. In this study, we aimed to reveal factors associated with MPV, focusing on arteriosclerotic factors and lifestyle-related diseases using various types of data, including health check-up data.

Conventional statistical analysis that is time- and cost-consuming typically requires the arbitrary selection of variables based on the findings of previous studies. Hence, a risk exists that potentially important variables may not be included in the statistical analysis[14].

In studies using conventional statistical methods, when dealing with mass data, multiple simultaneous analyses can be complicated, while biases may occur depending on the selection of factors. However, automated artificial intelligence (AI) software, such as Prediction One, can help develop beneficial models without the need for time-consuming variable optimization or arbitrary selection of variables[14]. Compared to logistic regression, there are fewer limitations on the number of variables used in the Prediction One framework, and AI can identify key variables that may have been overlooked in previously reported statistical models. Machine learning can aid in the identification of factors

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that contribute to predicted values without being affected by preconceived notions, enabling the analysis of large amounts of data in a short time. Accordingly, we used machine learning to analyze the factors affecting MPV to clarify its clinical significance.

Methods

Study design

This retrospective cohort study was performed in accordance with the principles stipulated in the Declaration of Helsinki and was approved by the Ethics Committee of Tokyo Women's Medical University (approval date: March 11, 2024; approval number: 2023-0190). The study adhered to the Personal Information Protection Law by allowing participants to opt out. The study cohort comprised individuals who underwent a complete medical check-up at Tokyo Women's Medical University (Japan) between June 6, 2018, and October 31, 2022. Specifically, participants who underwent abdominal ultrasonography as part of the "Ningen Dock" (a Japanese periodic health check-up system) were included in the study. The only exclusion criterion was the race of participants; individuals of non-Asian ethnicity were excluded (**Fig. 1**).

Services delivered as part of the Ningen Dock

The periodic Ningen Dock health check-up program is comprehensive and includes the following assessments: physical characteristics (height, body weight, and waist circumference), complete blood count, blood biochemistry, urinalysis, electrocardiogram, pulmonary function test, abdominal ultrasonography, upper gastrointestinal tract barium meal or endoscopic examination, colonoscopy, visual acuity testing, tonometry, fundic examination (retinal photography), and hearing assessment. Medical history and lifestyle factors related to alcohol and tobacco use were obtained through medical interviews.

Predictors

MPV was classified as follows: 1 – MPV <9.3 fL (decreased MPV); 2 – 9.3 fL ≤MPV <10.3 fL; and 3 – MPV ≥10.3 fL (increased MPV).

The factors for predicting increased MPV (MPV ≥10.3 fL) in our model were selected from the participants' data. Candidate factors for predicting increased MPV using machine learning are shown in **Table 1**.

Definitions

The fibrosis (FIB)-4 index[15] was calculated using the following formula: [age (years) × aspartate aminotransferase (AST)(U/L)]/[platelet count (10^9/L) × root alanine aminotransferase (ALT)(U/L)]. The FIB-4 index was classified as follows: 1 – FIB-4 index <1.3; 2 – 2.67 >FIB-4 index ≥1.3; 3 – 3.25 >FIB-4 index ≥2.67; and 4 – FIB-4 index ≥3.25.

The FIB-5 index[16] was calculated using the following formula: [albumin (g/L) × 0.3+platelet count (10^9/L) × 0.05] − [alkaline phosphatase (ALP)(U/L) × 0.014+AST to ALT ratio × 6+14].

NAFLD was defined as fatty liver (FL) in the absence of either hepatitis B or C virus infection and alcohol consumption (>30 g ethanol/d in men, >20 g ethanol/d in women)[17]. FL was defined as either high hepatorenal echo contrast, liver brightness, or deep attenuation on abdominal ultrasonography.

The diagnosis of metabolic dysfunction-associated fatty liver disease (MAFLD) was based on the presence of FL in addition to one of the following three criteria: overweight or obesity [body-mass index (BMI) of ≥23 kg/m^2; Asian population-specific], presence of type 2 diabetes mellitus (DM), or evidence of metabolic dysregulation with lean/normal weight (BMI of <23 kg/m^2; Asian population-specific)[18].

"NAFLD only" was defined as FL with NAFLD but not MAFLD, "MAFLD only" was defined as FL with MAFLD but not NAFLD, and "Overlapping NAFLD and MAFLD" was defined as FL with both NAFLD and MAFLD.

MetS was defined according to the 2005 guidelines of the Evaluation Committee on Diagnostic Criteria for

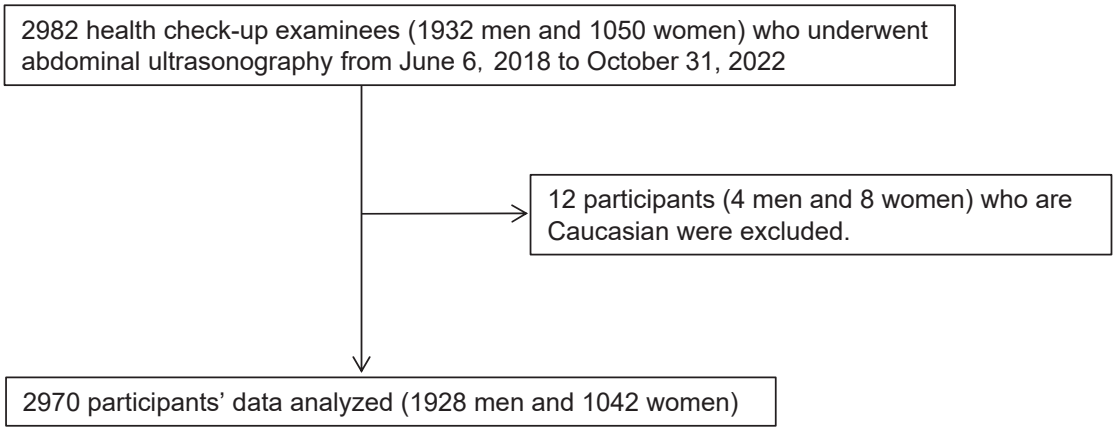

Fig. 1. Flow Chart of Participant Enrollment and Exclusion

Table 1. Candidate Factors for Predicting Increased MPV Using Machine Learning

Characteristics		
Age (years)	HOMA-IR	FIB-4 index
Sex, male/female	BUN (mg/dL)	FIB-5 index
SBP (mmHg)	Cr (mg/dL)	Urine protein
DBP (mmHg)	eGFR (mL/min/1.73 m^2)	BMI (kg/m^2)
Total protein (g/dL)	UA (mg/dL)	Waist circumference (cm)
Albumin (g/dL)	Serum sodium (mEq/L)	NAFLD only
hs-CRP (mg/dL)	Serum potassium (mEq/L)	MAFLD only
Total bilirubin (mg/dL)	Serum chloride (mEq/L)	Overlapping NAFLD and MAFLD
AST (U/L)	Serum calcium (mg/dL)	FL without NAFLD or MAFLD
ALT (U/L)	Serum phosphorus (mg/dL)	Percent vital capacity
LDH (U/L)	HDL-C (mg/dL)	Percentage of forced expiratory volume in 1 s
ALP (U/L)	LDL-C (mg/dL)	Fundus findings (Scheie classification)
γ-GTP (U/L)	TG (mg/dL)	Hearing ability
LAP (U/L)	Serum iron (μg/dL)	Alcohol consumption (g of ethanol/d)
ChE (U/L)	CEA (ng/mL)	Smoking (Brinkman Index)
Serum amylase (U/L)	CA19-9 (U/mL)	Patient identification card number
FPG (mg/dL)	AFP (ng/mL)	Health check-up date
HbA1c (%)	HS-PSA (ng/mL)	
Fasting insulin (μIU/mL)	CA125 (U/mL)	

MPV, mean platelet volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transferase; LAP, leucine aminopeptidase; ChE, cholinesterase; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment-insulin resistance; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate; UA, uric acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; AFP, alpha fetoprotein; HS-PSA, high-sensitivity prostate specific antigen; CA125, carbohydrate antigen 125; FIB-4 index, fibrosis-4 index; FIB-5 index, fibrosis-5 index; BMI, body-mass index; NAFLD, nonalcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease; FL, fatty liver

Metabolic Syndrome of Japan[19,20].

Insulin resistance was determined using the homeostasis model assessment-insulin resistance (HOMA-IR) score, which was calculated as follows: HOMA-IR score={[fasting glucose (mg/dL)×fasting insulin (μIU/mL)]/405}. A score of 2.5 was considered the threshold for insulin resistance.

Smoking was assessed using Brinkman Index (number of cigarettes smoked per day multiplied by number of years of smoking).

Risk factors

In our analysis, we examined several potential risk factors, including alcohol overuse and impaired renal function.

Alcohol overuse was defined as consumption of >30 g of ethanol per day for men and >20 g of ethanol per day for women.

Impaired renal function was defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2.

Statistical analyses

Continuous variable data are expressed as means (standard deviation) per group. Statistical differences were determined using the Mann–Whitney U test. Variables expressed as proportions were compared using the chi-square test.

Prediction models were built using Prediction One (https://predictionone.sony.biz; Sony Network Communications Inc., Tokyo, Japan), an ensemble learning model of neural networks, and gradient-boosted decision trees (a model that makes predictions based on the weighted average of the predictions of two models). Missing values were automatically calculated using common machine-learning techniques, such as a gradient-boosting tree. Neural networks and gradient-boosting trees have model settings (hyperparameters), but the settings are automatically adjusted (hyperparameter tuning) before use in an ensemble model. Both neural networks and gradient-boosting trees show high predictive performance for tabular data (table formatters), and prediction accuracy is further improved by using an ensemble model; Prediction One adopts this method. The area under the curve (AUC) of the model and strong variables with their weights were automatically calculated using the artificial neural network. To evaluate the AI-built prediction model, we performed internal validation of the AUC, accuracy, precision, recall, and F-value of the model.

Prediction One allows predictive analysis. Its basic functions include binary classification, multivalued classification, numerical prediction (regression), and time series prediction (regression), in addition to learning, evaluation, and prediction using machine learning. Moreover, Prediction One has independently developed a model selection function and data preprocessing function to achieve highly accurate predictions. We used the

desktop version of Prediction One, thus eliminating the need for data transfer to an external server. As long as our personal computers are managed properly, there is no risk of personal or confidential information being leaked. We chose Prediction One owing to its high accuracy and safe handling of information.

A prediction model was also developed using multivariate logistic regression analysis based on the results obtained using Prediction One. A multiple logistic regression analysis was performed to calculate the odds ratios (ORs) for quantifying the association of independent variables with increased MPV. Statistical analysis was performed using the IBM SPSS software (version 29.0.1; IBM Corporation, Armonk, NY, USA). A *p*-value <0.05 was considered statistically significant.

Results

Study population

We included 2,970 participants in this study. Of these, 1,928 were men, with a mean age of 69.4 (standard deviation: 12.2) years, whereas 1,042 were women, with a mean age of 69.8 (standard deviation: 11.6) years. **Table 2** shows the platelet size distribution. Increased MPV was detected in 828 (27.9%) participants. Overall, 28.3% of men and 27.1% of women had increased MPV.

Fig. 2 shows the prevalence of increased MPV across different age groups. Notably, increased MPV tended to be slightly more common among people in their 50s than in people of other age groups.

Table 2. Platelet Size Distribution

Characteristics	Number (%)
MPV<9.3 fL (decreased MPV)	646 (21.8)
9.3 fL≤MPV<10.3 fL	1496 (50.4)
MPV≥10.3 fL (increased MPV)	828 (27.9)

MPV, mean platelet volume

Clinical characteristics of the study participants

Table 3 shows the clinical characteristics of the participants with and without increased MPV. The increased MPV group (MPV ≥10.3 fL) had significantly higher levels of ALT, ALP, γ-glutamyl transferase, fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), blood urea nitrogen, and triglyceride (TG) as well as increased FIB-4 index, BMI, percentage of NAFLD only, and percentage of MAFLD only compared with those in the non-increased MPV group. However, the increased MPV group had significantly lower levels of platelets, high-sensitivity C-reactive protein (hs-CRP), and low-density lipoprotein cholesterol than the non-increased MPV group.

Predicting increased MPV

Tables 4 and **5** show the AI prediction model for increased MPV generated using Prediction One. **Table 4** indicates the values of parameters for the generated AI model. In the accuracy evaluation of the prediction model, the AUC was 86.02%. The accuracy, precision, and recall values were 81.48%, 64.00%, and 77.11%, respectively, when the F-value was 69.95%.

Table 5 presents a ranking of variables contributing to elevated MPV generated using Prediction One. Rankings are shown from first to tenth. The top 10 contributory factors for predicting increased MPV, excluding patient identification card number and health check-up date, were NAFLD only, FIB-4 index, alcohol consumption, and levels of FPG, TG, eGFR, hs-CRP, high-density lipoprotein cholesterol (HDL-C), systolic

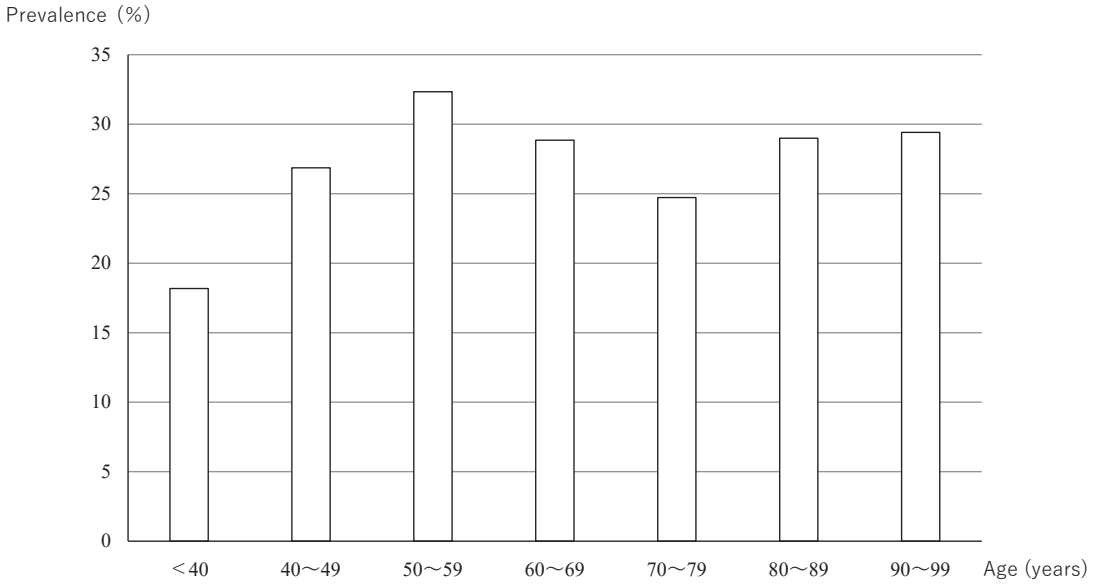

Fig. 2. Prevalence of Increased MPV Across Different Age Groups
MPV, mean platelet volume

Table 3. Clinical Data of Participants With or Without Increased MPV

Characteristic	Participants with increased MPV	Participants without increased MPV	*p*-value
	Mean (Standard deviation) [Number]		
Age (years)	69.2 (12.1) [828]	69.6 (12.0) [2142]	0.320
Sex, male/female	[546/282]	[1382/760]	0.466
SBP (mmHg)	122.8 (17.0) [827]	124.5 (16.6) [2141]	0.075
DBP (mmHg)	72.2 (12.4) [827]	72.5 (11.4) [2141]	0.452
Platelet (10^4/μL)	19.4(5.1) [828]	22.4 (5.1) [2142]	<0.001
hs-CRP (mg/L)	1.1 (3.2) [828]	1.2 (4.3) [2142]	0.018
Total protein (g/dL)	7.065 (0.4) [828]	7.062 (0.4) [2142]	0.753
Albumin (g/dL)	4.28 (0.3) [828]	4.26 (0.3) [2142]	0.086
Total bilirubin (mg/dL)	0.96 (0.3) [828]	0.95 (0.3) [2142]	0.667
AST (U/L)	24.2 (11.2) [828]	23.6 (12.0) [2142]	0.353
ALT (U/L)	23.0 (15.5) [828]	21.7 (13.9) [2142]	0.033
LDH (U/L)	178.1 (37.1) [828]	176.4 (33.9) [2142]	0.373
ALP (U/L)	164.9 (87.7) [828]	154.1 (100.3) [2142]	0.004
γ-GTP (U/L)	44.1 (63.9) [828]	38.6 (42.1) [2142]	0.017
LAP (U/L)	49.5 (12.3) [828]	48.6 (10.4) [2139]	0.477
ChE (U/L)	321.1 (73.2) [828]	321.0 (79.7) [2139]	0.368
Serum amylase (U/L)	81.0 (30.6) [828]	84.1 (49.0) [2142]	0.369
FPG (mg/dL)	112.1 (24.7) [828]	108.3 (18.8) [2142]	0.006
HbA1c (%)	6.1 (0.8) [828]	6.0 (0.6) [2142]	0.012
HOMA-IR	2.2 (2.2) [828]	2.0 (1.6) [2139]	0.117
BUN (mg/dL)	16.2 (5.7) [828]	15.3 (4.2) [2139]	<0.001
Cr (mg/dL)	0.897 (0.4) [828]	0.859 (0.3) [2142]	0.063
eGFR (mL/min/1.73 m^2)	64.4 (16.3) [828]	65.7 (15.6) [2142]	0.089
UA (mg/dL)	5.7 (1.3) [828]	5.6 (1.2) [2142]	0.058
HDL-C (mg/dL)	65.8 (19.8) [828]	66.7 (17.9) [2142]	0.053
LDL-C (mg/dL)	111.3 (28.8) [828]	112.8 (27.2) [2142]	0.042
TG (mg/dL)	117.2 (80.8) [828]	106.7 (70.8) [2142]	0.013
FIB-4 index	2.0 (1.1) [828]	1.8 (1.2) [2142]	<0.001
FIB-5 index	−10.5 (2.7) [828]	−10.6 (3.2) [2142]	0.790
BMI (kg/m^2)	23.6 (3.7) [824]	23.1 (3.3) [2133]	0.013
Waist circumference (cm)	87.4 (10.5) [824]	86.3 (9.5) [2135]	0.094
NAFLD only (%)	1.5% [12/821]	0.6% [12/2136]	0.015
MAFLD only (%)	12.1% [99/821]	9.2% [196/2136]	0.019
Alcohol consumption (g of ethanol/d)	16.9 (23.5) [812]	16.7 (23.0) [2120]	0.714
Smoking (Brinkman Index)	67.0 (247.3) [822]	88.0 (344.7) [2135]	0.679

Results are presented as mean (standard deviation). MPV, mean platelet volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transferase; LAP, leucine aminopeptidase; ChE, cholinesterase; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment-insulin resistance; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate; UA, uric acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; FIB-4 index, fibrosis-4 index; FIB-5 index, fibrosis-5 index; BMI, body-mass index; NAFLD, nonalcoholic fatty liver disease; MAFLD, metabolic dysfunction-associated fatty liver disease

Table 4. Values of Parameters in the Generated AI Model

	%
AUC	86.02
Accuracy	81.48
Precision	64.00
Recall	77.11
F-value	69.95

AI, artificial intelligence; AUC, area under the Receiver Operating Characteristic (ROC) curve

Table 5. The Top 10 Contributory Factors for Predicting Increased MPV Using Machine Learning

1st	NAFLD only
2nd	FIB-4 index
3rd	Alcohol consumption
4th	FPG
5th	TG
6th	eGFR
7th	hs-CRP
8th	HDL-C
9th	SBP
10th	ChE

MPV, mean platelet volume; NAFLD, nonalcoholic fatty liver disease; FIB-4 index, fibrosis-4 index; FPG, fasting plasma glucose; TG, triglyceride; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; ChE, cholinesterase

blood pressure (SBP), and cholinesterase.

Risk factors for elevated MPV

Based on the results of Prediction One, we developed a prediction model using multivariate logistic regression analysis. The risk factors of increased MPV were NAFLD only (OR=3.4, p=0.003), increased FIB-4 in-

Table 6. Multivariate Logistic Regression Analysis of the Risk Factors of Increased MPV (1)

Characteristic	Participants with increased MPV (*n*=804)	Participants without increased MPV (*n*=2091)	Adjusted OR	95%CI	*p*-value
	Number (%)				
Age			0.97	0.96–0.98	<0.001
Men	531 (66.0)	1359 (65.0)	0.9	0.71–1.04	0.11
Women	273 (34.0)	732 (35.0)			
NAFLD only	12 (1.5)	12 (0.6)	3.4	1.51–7.86	0.003
FIB-4 index			2.1	1.82–2.40	<0.001
Alcohol overuse	221 (27.5)	539 (25.8)	0.98	0.80–1.20	0.846
FPG ≥110 mg/dL	318 (39.6)	664 (31.8)	1.4	1.19–1.73	<0.001
TG ≥150 mg/dL	165 (20.5)	331 (15.8)	1.2	0.99–1.57	0.060
eGFR <60 mL/min/1.73 m^2	289 (35.9)	734 (35.1)	1.03	0.85–1.25	0.752
hs-CRP >0.2 mg/dL	77 (9.6)	193 (9.2)	1.01	0.75–1.35	0.948
HDL-C <40 mg/dL	48 (6.0)	62 (3.0)	1.8	1.20–2.77	0.005
SBP ≥130 mmHg	283 (35.2)	758 (36.3)	0.96	0.80–1.15	0.674
ChE <175 U/L	6 (0.7)	22 (1.1)	0.5	0.18–1.22	0.121

MPV, mean platelet volume; OR, odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; FIB-4 index, fibrosis-4 index; FPG, fasting plasma glucose; TG, triglyceride; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; ChE, cholinesterase

Table 7. Multivariate Logistic Regression Analysis of the Risk Factors of Increased MPV (2)

Characteristic	Participants with increased MPV (*n*=797)	Participants without increased MPV (*n*=2083)	Adjusted OR	95%CI	*p*-value
	Number (%)				
Age			0.97	0.96–0.98	<0.001
Men	523 (65.6)	1353 (65.0)	0.9	0.74–1.08	0.245
Women	274 (34.4)	730 (35.0)			
NAFLD only	12 (1.5)	12 (0.6)	3.2	1.41–7.40	0.005
FIB-4 index			2.1	1.83–2.41	<0.001
MetS	290 (36.4)	671 (32.2)	1.3	1.09–1.59	0.005
ChE <175 U/L	6 (0.8)	22 (1.1)	0.4	0.17–1.11	0.081
eGFR <60 mL/min/1.73 m^2	288 (36.1)	732 (35.1)	1.01	0.84–1.22	0.912
hs-CRP >0.2 mg/dL	74 (9.3)	192 (9.2)	1.03	0.77–1.38	0.830
Alcohol overuse	221 (27.7)	538 (25.8)	0.99	0.81–1.21	0.921

MPV, mean platelet volume; OR, odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; FIB-4 index, fibrosis-4 index; MetS, metabolic syndrome; ChE, cholinesterase; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein

Table 8. Multivariate Logistic Regression Analysis of the Risk Factors of Increased MPV (3)

Characteristic	Participants with increased MPV (*n*=797)	Participants without increased MPV (*n*=2083)	Adjusted OR	95%CI	*p*-value
	Number (%)				
Age			0.97	0.96–0.98	<0.001
Men	523 (65.6)	1353 (65.0)	0.9	0.70–1.04	0.122
Women	274 (34.4)	730 (35.0)			
NAFLD only	12 (1.5)	12 (0.6)	3.4	1.47–7.70	0.004
FIB-4 index			2.1	1.85–2.43	<0.001
One component of MetS (central obesity with hypertension)	307 (38.5)	757 (36.3)	0.9	0.73–1.16	0.477
One component of MetS (central obesity with dyslipidemia)	288 (36.1)	661 (31.7)	1.3	1.02–1.63	0.035
One component of MetS (central obesity with IFG)	231 (29.0)	490 (23.5)	1.3	1.04–1.65	0.020
ChE <175 U/L	6 (0.8)	22 (1.1)	0.4	0.17–1.13	0.088
eGFR <60 mL/min/1.73 m^2	288 (36.1)	732 (35.1)	1.009	0.83–1.22	0.923
hs-CRP >0.2 mg/dL	74 (9.3)	192 (9.2)	1.03	0.77–1.38	0.858
Alcohol overuse	221 (27.7)	538 (25.8)	0.995	0.82–1.21	0.957

MPV, mean platelet volume; OR, odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; FIB-4 index, fibrosis-4 index; MetS, metabolic syndrome; IFG, impaired fasting glucose; ChE, cholinesterase; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein

dex (OR=2.1, p<0.001), HDL-C <40 mg/dL (OR=1.8, p=0.005), and FPG ≥110 mg/dL (OR=1.4, p<0.001) (**Table 6**). As HDL-C <40 mg/dL and FPG ≥110 mg/dL are MetS components, we also investigated MetS in detail, including the factors involved. We accordingly identified MetS (OR=1.3, p=0.005) as a risk factor for

increased MPV (**Table 7**). Furthermore, central obesity coupled with dyslipidemia (MetS component) (OR=1.3; p=0.035) and central obesity coupled with impaired fasting glucose (IFG) (MetS component) (OR=1.3; p=0.020) were significantly associated with increased MPV (**Table 8**).

Discussion

In this study, less than 30% of the 2,970 participants had high MPV. Regarding the platelet size distribution, *in vitro*, Polanowska-Grabowska *et al.*[21] reported that small, medium, and large platelets accounted for 24%, 47%, and 29% of the total, respectively; the increased MPV rate in this study is therefore considered appropriate.

Among the factors contributing to increased MPV obtained in this analysis using AI, dyslipidemia (HDL-C <40 mg/dL), impaired glucose tolerance (FPG ≥110 mg/dL), and NAFLD are known risk factors for atherosclerosis. NAFLD is no longer considered a primary liver disease but rather an indicator of MetS, insulin resistance, and other lifestyle-related disorders (e.g., diabetes, dyslipidemia, or hypertension)[22–24] that are known risk factors of atherosclerosis.

NAFLD is characterized by an increase in the levels of various inflammatory mediators such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α)[25]. Platelets respond to these inflammatory mediators by altering their size, which is measured as MPV[26]. Another pathology observed in patients with NAFLD[27,28] is increased insulin resistance, which in turn causes an increase in MPV[4,5].

Using multivariate logistic regression analysis to investigate the risk factors of increased MPV based on the results of AI, this study showed that NAFLD, increased FIB-4 index, and MetS (especially the dyslipidemia and IFG components) were significantly associated with increased MPV (**Tables 7** and **8**). Obesity and visceral adiposity, the pathological conditions indicative of MetS, induce insulin resistance and cause abnormal secretion of adipokines and cytokines, including leptin and interleukin, which trigger megakaryocytes to produce larger platelets[29–31]. HDL-C and obesity are also associated with markers of platelet activity[32–34].

The FIB-4 index, which considers age, AST and ALT levels, and platelet count[35], is one of the most widely accepted liver fibrosis markers in patients with liver disease, such as those with hepatitis B[36] and C viral infections[37]. FIB-4 is reportedly an effective prognostic marker of death or rehospitalization in patients with heart failure[38–40]. Increased platelet activation and aggregation are closely related to cardiovascular complications[41]. This study revealed that an increased FIB-4 index was associated with increased MPV (**Tables 6**–**8**). Hence, increased MPV can serve as an indicator of not only thrombotic and inflammatory diseases but also arteriosclerotic conditions.

Different methods have been used for analyzing platelet activation, including optical aggregometry, platelet function analyzer (PFA-100), platelet reactivity test or platelet aggregate ratio, flow cytometry, and thromboxane B (2) generation[42]. All these tests present limitations owing to complex preanalytic factors, reduced specificity, and poor reproducibility. By contrast, MPV is a simple marker whose evaluation does not require advanced or expensive technology[3]. We believe that MPV should be extensively used as a diagnostic tool during annual medical and regular general health check-ups, considering its low cost, availability, and utility as an indicator of not only thrombotic and inflammatory diseases but also arteriosclerotic conditions.

Limitations

This study has certain limitations, such as its retrospective design and the involvement of a single institution.

In this study, 1,928 men and 1,042 women were examined; that is, the study included approximately 1.85 times more men than women. Additionally, women over the age of 50 years constituted approximately 94.0% of all women. Gender differences can affect platelet count and MPV, especially MPV in postmenopausal women. Several reports have shown sex-related differences in platelet count and MPV which seem to reflect hormonal profiles. Butkiewicz *et al.*[43] reported higher platelet counts and lower MPV levels in women due to menstrual blood loss. Bain[44] suggested a similar correlation; however, other studies reported no correlations and no significant differences in MPV between sexes[45]. In another study, the platelet count was lower in postmenopausal women than in young menstruating women. However, MPV values were similar in both groups[46,47]. Overall, various theories have been proposed regarding the influence of gender and menopause on MPV, and further studies with larger sample sizes and reduced gender and age differences, and a follow-up prospective design are warranted.

Conclusions

Using AI, this study showed that high MPV was associated with atherosclerotic risk factors and lifestyle-related diseases. The machine-learning method may be an effective tool for analyzing a large number of factors simultaneously, exploring their relationships, and identifying new biomarkers. In the future, prospective studies using AI are warranted to evaluate approaches for improving the lifestyle (diet and exercise therapy) of

patients with increased MPV to prevent the risk of atherosclerosis.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Short Report

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Association Between Changes in Serum Uric Acid Level and Plasma Glucose Control Based on 10 Years of Observation in Participants with Normal Glucose Tolerance But Without Obesity

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Abstract

Objective: Hyperinsulinemia increases serum uric acid levels by decreasing urinary uric acid excretion. Moreover, elevated plasma glucose levels decrease serum uric acid levels by increasing urinary uric acid excretion. However, it is unknown whether serum uric acid and glucose levels in nonobese participants (with normal glucose tolerance [NGT]) are related. In this retrospective clinical study, we investigated the association of longitudinal changes in serum uric acid and glucose levels in nonobese healthy participants for 10 years.

Methods: In this single-center retrospective study, we used annual Ningen Dock (health checkup) medical records of 84 participants obtained at Kiryu Kosei General Hospital, Japan over the 10 years from 2008–2018. We analyzed the correlation between differences in annual serum uric acid (Δserum uric acid) and glycated hemoglobin (ΔHbA1c) levels over the 10-year observational period, using levels measured in 2008 as baseline.

Results: On multiple regression analysis, uric acid level was associated with gender but not with age or HbA1c level. The Δuric acid level was not associated with gender or age but was independently associated with ΔHbA1c level. Concomitant with these results, Δserum uric acid level (2008–2017) was positively and significantly associated with the ΔHbA1c (2008–2017) (r=0.272, p=0.018). Moreover, Δserum uric acid level (2008–2018) was positively and significantly associated with the ΔHbA1c (2008–2018) (r=0.371, p=0.001).

Conclusions: In nonobese participants with NGT, long-term longitudinal changes in serum uric acid and blood glucose levels are positively correlated.

Keywords 75-g oral glucose tolerance test, uric acid, glycated hemoglobin, normal glucose tolerance

Insulin resistance accompanied by hyperinsulinemia is a clinical feature of type 2 diabetes (T2D). Hyperinsulinemia induces hyperuricemia by reducing uric acid excretion in the proximal tubules of the kidney[1]. Thus, serum uric acid levels are closely related to plasma insulin levels in patients with T2D[2]. However, the relationship between uric acid level and plasma glucose level in nonobese participants with normal glucose tolerance (NGT) is unknown.

It is important to evaluate information obtained from human health checkups in the year in which the examination was conducted. In general, however, the results of physical examinations that are provided to examinees span several years. We asked whether there was any clinical implications in evaluating the results of physical examinations over a longer period of time. Accordingly, we examined the relationship between uric acid and HbA1c levels over a 10-year period. Specifically, as an index of change over 10 years, we determined the amount of change in each year's measurement results based on the 2008 test results, and examined whether changes in values had clinical significance.

Here, we investigated the association of longitudinal serum uric acid level changes with plasma glucose

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control in healthy nonobese participants over 10 years using multiple regression analysis. As a result, although there was no significant correlation between HbA1c and uric acid levels, there was a significant correlation between HbA1c and uric acid fluctuation values over a 10-year period. We also examined the correlation between values of HbA1c and uric acid levels by year.

Methods

In this single-center retrospective study, we used the medical records of all participants who underwent annual Ningen Dock (health checkup, at Kiryu Kosei General Hospital in 2008–2018), comprising a 75-g oral glucose tolerance (75g OGTT), glycated hemoglobin (HbA1c), and serum uric acid tests.

Fig. 1 shows a flowchart of the participant selection procedure (2008–2018). A total of 523 participants were screened. After exclusion of participants who were not diagnosed with NGT (n=162), those who did not undergo 75g OGTT annually for 10 years (n=252), and those who did not maintain NGT for 10 years (n=25), the final number of participants maintaining NGT for 10 years and included in the analysis was 84 (**Fig. 1**).

Differences in serum uric acid levels measured in 2008 and consecutive years 20XX (2009 through 2018) were calculated as Δserum uric acid. Further, differences in HbA1c measured in 2008 and consecutive years 20XX (2009 through 2018) were calculated as ΔHbA1c. The relationship between all calculated Δserum uric acid and ΔHbA1c was then analyzed.

On Day 1 of hospitalization, the study participants were requested to fast overnight (for 12 h). On Day 2, blood samples were drawn at 8:00 am including fasting plasma glucose (FPG) and 2 h after the oral administration of 75 g of glucose as 75g OGTT (2-h PG). NGT was defined as a fasting plasma glucose (FPG) <110 mg/dL and 2-h PG <140 mg/dL.

Our study conforms to the Declaration of Helsinki (as 2-K015) and was approved by the Ethics Committee at Kiryu Kosei General Hospital (Japan). At each Ningen Dock, participants were asked for permission to use their Ningen Dock data in future clinical studies and presentations. Thus, all eligible participants provided written informed consent before participation.

All statistical data were analyzed using the SPSS software (version 10.0, SPSS Inc., Chicago, IL, USA). All numerical values are expressed as means±SD. Pearson's correlation coefficients were calculated to estimate linear correlations between variables. All tests for significance and the resulting p values were two-sided, with a level of significance set at 5%.

Results

Participant characteristics in 2008 and 2018

Table 1 summarizes the characteristics of 84 NGT participants in 2008 and 2018 (male, 64; female, 20). Age breakdown by gender was as follows: 0 respondents were aged 30–44, 36 males and 5 females were aged 45–64, 27 males and 14 females were aged 65–80, and 1 male and 1 female were aged 80 and older. Participant characteristics remained fairly constant over the observation period (2008–2018), including body weight, body height, body-mass index, systolic blood pressure, diastolic blood pressure, waist circumference, serum creatinine, high-density lipoprotein, low-density lipoprotein, triglyceride, estimated glomerular filtration, serum uric acid, fasting plasma glucose, 2-h postchallenge plasma glucose, glycated hemoglobin, and hemo-

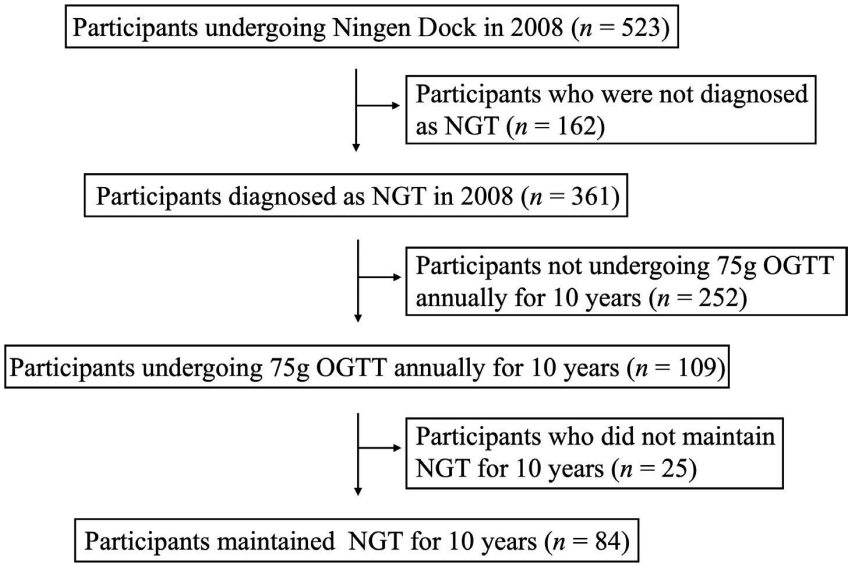

Fig. 1. Procedure for Selecting Study Subjects
Flowchart showing the selection procedure of study subjects.

Table 1. Participant Characteristics Observed in 2008 and 2018

	Normal range		Male (*n*=64)		Female (*n*=20)	
	Male	Female	2008	2018	2008	2018
Age (years old)			53.5±7.6	63.5±7.6	58.6±9.5	68.6±9.5
Body height (cm)			170.4±5.6	169.2±5.7	154.3±6.5	151.9±7.3
Body weight (kg)			68.2±10.3	68.0±11.3	52.4±5.4	52.1±7.5
Body-mass index (kg/m^2)	18.5≤ <25.0		23.4±2.8	23.7±3.3	22.1±2.5	22.6±2.9
Systolic blood pressure (mmHg)	≤129		121.4±15.4	123.9±14.4	120.2±14.4	121.4±9.9
Diastolic blood pressure (mmHg)	≤84		77.3±10.3	76.8±9.8	74.9±8.6	74.8±6.9
Waist circumference (cm)	≤84.9	≤89.9	87.1±8.3	87.2±8.4	82.9±6.5	81.4±7.8
Serum creatinine (mg/dL)	≤1.0	≤0.7	0.88±0.14	0.90±0.20	0.59±0.08	0.60±0.08
Estimated glomerular filtration rate (mL/min/1.73m^2)	60≤		73.5±12.6	69.5±14.8	82.2±13.2	75.6±11.4
Uric acid (mg/dL)	2.1–7.0		6.2±1.3	6.0±1.3	4.6±0.9	4.7±0.9
Triglyceride (mg/dL)	30–149		147.3±111.7	122.2±66.0	90.8±36.8	102.0±82.3
High density lipoprotein (mg/dL)	40≤		60.3±15.1	63.9±16.7	80.9±20.6	78.6±20.3
Low density lipoprotein (mg/dL)	60–119		131.0±30.5	120.4±30.0	132.2±26.2	113.0±38.6
Hemoglobin (g/dL)	13.1–16.3	12.1–14.5	15.0±1.1	14.9±1.4	12.8±1.0	13.1±1.0
Fasting plasma glucose (mg/dL)	≤99		94.6±7.8	99.0±10.3	93.7±7.4	96.6±5.5
2-h postchallenge glucose (mg/dL)	<140		115.7±25.8	127.5±37.3	113.0±18.3	122.9±25.4
Glycated hemoglobin (%)	≤5.5		5.5±0.3	5.6±0.4	5.6±0.2	5.7±0.3

Participant characteristics observed in 2008 and 2018. Values are presented as mean ± standard deviation.

globin (Hb) levels.

Multiple regression analysis of the relationship between uric acid level and associated variables in 2018

Table 2a shows that uric acid level was associated with gender but was not associated with age or HbA1c level.

Multiple regression analysis of the relationship between Δuric acid level and associated variables in 2018

Table 2b shows that Δuric acid level was not associated with gender or age but was independently associated with ΔHbA1c level.

Correlation between serum uric acid and HbA1c level

As shown in **Table 3a**, serum uric acid levels were not associated with HbA1c levels from 2008 through 2018.

Correlation between changes in serum uric acid and HbA1c levels

The Δserum uric acid level (2008–2017) was positively and significantly associated with ΔHbA1c (2008–2017) (r=0.272, p=0.018). The Δserum uric acid level (2008–2018) was positively and significantly associated with the ΔHbA1c (2008–2018) (r=0.371, p=0.001) (**Table 3b**).

Further, the regression coefficient of univariate linear regression between Δserum uric acid level (2008–2018) and ΔHbA1c level (2008–2018) demonstrated a positive correlation (r=0.371) (**Fig. 2**).

Table 2a. Multiple Regression Analysis of the Relationship Between Uric Acid Level and Associated Variables in 2018

	r	*p* value
Gender	−0.4231	0.0001
Age	−0.1940	0.2730
HbA1c	0.0540	0.1786

Uric acid level was associated with gender but not with age or HbA1c level.

Table 2b. Multiple Regression Analysis of the Relationship Between ΔUric Acid Level and Associated Variables in 2018

	r	*p* value
Gender	−0.0007	0.8884
Age	−0.0481	0.8430
ΔHbA1c	0.3710	0.0001

The Δuric acid level was not associated with gender or age but was independently associated with ΔHbA1c level.

Table 3a. Correlation Between Serum Uric Acid and HbA1c Levels

	Years	*r*	*p*
UA vs HbA1c	2008	0.186	0.267
	2009	0.151	0.644
	2010	0.134	0.550
	2011	0.134	0.241
	2012	0.016	0.894
	2013	0.004	0.969
	2014	0.083	0.464
	2015	0.028	0.804
	2016	0.121	0.300
	2017	0.033	0.773
	2018	0.067	0.625

Correlation between serum uric acid and HbA1c levels.

Discussion

In this clinical study, we found that longitudinal changes in serum uric acid and HbA1c levels (i.e., Δserum uric acid and ΔHbA1c) were positively associated (r=0.371, p=0.001) in the 10th year of observation, despite not being correlated at the beginning of the clinical observation (r=0.186, p=0.267). Δserum uric

acid was weakly associated with ΔHbA1c (r=0.272, p=0.018) 8 years post observation. Thus, this longitudinal retrospective observational study reveals that serum uric acid and HbA1c show similar increasing trends in nonobese subjects with NGT.

Hyperinsulinemia caused by insulin resistance increases sodium reabsorption and decreases uric acid excretion in the renal tubules, leading to increased serum uric acid levels[1]. However, hyperglycemia decreases serum uric acid levels by reducing renal tubular function and increasing the uric acid excretion from the kidney[2]. Nevertheless, the relationship between uric acid level and plasma glucose level in nonobese participants with NGT has not been extensively studied. Thus, we conducted a 10-year-long longitudinal retrospective observational study on nonobese subjects with NGT using 10 years of annual follow-up data for each participant.

Table 3b. Correlation Between Changes in Serum Uric Acid and HbA1c Levels

	Years	*r*	*p* value
ΔUA vs ΔHbA1c	2008	0.186	0.267
	2008–2009	0.061	0.107
	2008–2010	0.080	0.590
	2008–2011	0.163	0.155
	2008–2012	0.023	0.999
	2008–2013	0.042	0.674
	2008–2014	0.022	0.836
	2008–2015	0.187	0.107
	2008–2016	0.221	0.059
	2008–2017	0.272	0.018
	2008–2018	0.371	0.001

Correlation between changes in serum uric acid and HbA1c levels.

The participants underwent an annual 75g OGTT, and the results confirmed their glucose tolerance condition remained NGT over the observation period. 10-year analysis of the annual follow-up data on serum uric acid and HbA1c levels revealed a positive correlation between Δserum uric acid and ΔHbA1c after 8 years, although the a relationship between the two was lacking until then. Thus, a cross-sectional study alone would have failed to find a relationship between Δserum uric acid and ΔHbA1c levels.

Of note, however, the r value was low, at 0.37, which does not suggest a robust correlation. The r value did increase year by year in the longitudinal study, suggesting that it may have increased further if the observation period had been extended.

Regarding clinical significance, this observational study emphasizes that when evaluating serum uric acid and HbA1c levels, retrospective long-term evaluation of longitudinal and annual changes is important. Moreover, if an increasing trend in serum uric acid levels is observed over time, changes in HbA1c should be analyzed, even when the change in uric acid level is within the normal range. Thus, purine and glucose metabolism tests results must be analyzed together while providing treatment options and health advice to NGT participants. Furthermore, our study suggests the existence of common regulators, other than insulin resistance, that modulate serum uric acid and glucose levels in nonobese subjects with NGT.

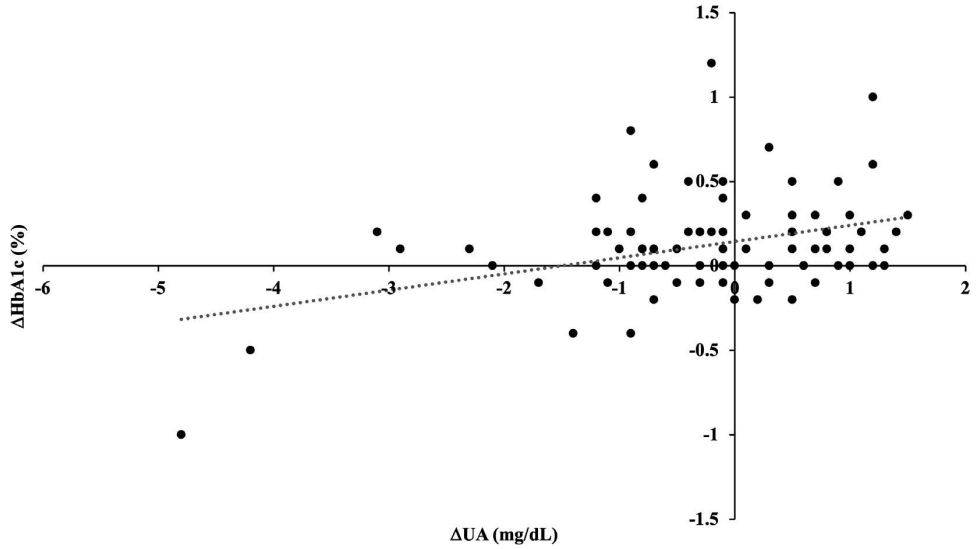

Fig. 2. Correlation Between ΔSerum Uric Acid Level (2008–2018) and ΔHbA1c Level (2008–2018)

Regression coefficients of the univariate linear regression analysis between Δserum uric acid level and ΔHbA1c level. The regression coefficients between Δserum uric acid level and ΔHbA1c level demonstrated a positive correlation (r=0.371).

Limitations

Several limitations of our study warrant mention. First, the study cohort was small and it was not possible to consider males and females separately. In addition, the age structure differed between males and females, and we cannot rule out the possibility that this difference affected the present results. Thus, these results should be verified in larger cohorts. Second, the study may have be affected by selection bias in patient characteristics such as ethnicity, age, weight, and gender. As there was a significant difference in gender in this study, it is possible that the lower uric acid levels in women compared to men may have influenced the results. Future studies should include a wider demographic range.

Conclusion

This retrospective longitudinal observation indicated that the degree of increase in serum uric acid is positively associated with the degree of HbA1c change in nonobese subjects with NGT.

Acknowledgments

None.

Conflict of Interest

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported. This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Short Report

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Prevalence of Asymptomatic Uterine Fibroids in Japanese Women

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Abstract

Objective: To examine the prevalence of asymptomatic uterine fibroids (UFs) in Japanese women visiting a medical checkup center and assess the associations between the prevalence of asymptomatic UF and parity.

Methods: Transvaginal ultrasound was performed on 3,682 healthy Japanese women 19–81 years old at our center between April 2021 and March 2022. We examined the overall and age group prevalence of asymptomatic UFs. Furthermore, we determined the relationship between the prevalence of asymptomatic UFs and parity.

Results: The overall prevalence of asymptomatic UFs was 39.2%. By age group, women in their 50s had the highest rate at 50.5%, followed by those in their 60s (39.6%) and those in their 40s (39.1%). The prevalence of asymptomatic UFs was significantly higher in postmenopausal women than in premenopausal women (44.7% vs. 36.0%, respectively, $p<0.001$), and in nulliparous women than in parous women (51.1% vs. 36.5%, respectively, $p<0.001$). The highest rate of asymptomatic UFs was seen in nulliparous women in their 50s (70.9%). The prevalence of asymptomatic UFs decreased significantly as parity increased ($p<0.001$).

Conclusions: The prevalence of asymptomatic UFs in Japanese women was 39.2%. Asymptomatic UFs are more common in women in their 50s. Prevalence was higher in postmenopausal than premenopausal women. Women with asymptomatic UFs were more likely to be nulliparous than parous.

Keywords asymptomatic uterine fibroids, prevalence, postmenopausal, parity

Uterine fibroids (UFs), also known as uterine leiomyomas or uterine myomas, are common among women. Almost all UFs are benign tumors and non-cancerous. Symptoms sometimes occur and are a substantial burden on affected women; however, many are asymptomatic[1]. In a recent epidemiological systematic review of UFs, prevalence rates varied widely, from 4.5% to 68.6%[2]. Racial and regional differences may also exist[3]. In addition, differences in prevalence may also be related to differences in survey methods and in the fibroid status of the study participants, namely symptomatic individuals requiring treatment versus asymptomatic individuals requiring no treatment[2]. Despite the fact that many women have uterine myomas, epidemiological studies on these tumors are still insufficient. In Japan, although a few epidemiological studies have reported on symptomatic UFs, few studies have been done on asymptomatic UFs. It is known that uterine myomas are more common among women with lower parity[2,4,5]. Of note, given that Japan has one of the lowest birth rates[6], it is assumed that UFs are more prevalent in modern Japanese women. Therefore, we aimed to investigate the prevalence of asymptomatic UFs and to determine their association with parity in healthy Japanese women at our center.

Methods

Participants were 3,682 healthy Japanese women (19–81 years old) who underwent transvaginal ultrasound (TVUS) at our center between April 2021 and March 2022. The participants included women who had been treated for myoma symptoms in the past but currently had no symptoms of UFs. They were residents of Kanagawa Prefecture; in particular, about 90% lived in the Shonan area within 10 km of our center. Eleven non-Japanese women who underwent TVUS were excluded from the study. Gynecologists (authors)

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confirmed the presence of UFs with a minimum diameter of 8 mm by TVUS (SonovistaGX30, 5.0-MHz, Konica Minolta). The presence or absence of myomas was determined by the TVUS diagnosis at the time of the study, not the presence or absence of self-reported past myomas. Information on childbirth and gynecological history was obtained from a self-administered gynecological questionnaire at the time of examination. Chi-square and Cochran–Armitage tests were used to analyze the data. All comparisons were two-tailed. A *p*-value of less than 0.05 was considered to be statistically significant. Data were collected using Microsoft Excel (2019). All statistical analyses were performed in R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria). The study design was in accordance with the guidelines of the Declaration of Helsinki and was approved by the ethics review board of our hospitals (Decision no: TGE02340-008).

Results

The median age of the 3,682 participants was 48 years (interquartile range, 42–56). The median age of the 1,442 participants with asymptomatic UFs was 50 years (interquartile range, 45–56), and the median age at menopause of the 1,357 menopausal participants was 51 years (interquartile range, 49–53). The overall prevalence of asymptomatic UFs was 39.2% (**Table 1**). By age group, women with asymptomatic UFs in their 50s were the most common (50.5%), followed in order by those in their 60s (39.6%) and 40s (39.1%). Prevalence reached the highest rate of 52.4% in those in their early 50s. We then counted participants with asymptomatic UFs with a minimum diameter of 20 mm. The results showed that the overall prevalence of asymptomatic UFs was 21.3% (**Supplemental Table**). By age group, asymptomatic UFs were most common in women in their 50s (30.2%). **Table 2** shows the association between the prevalence of asymptomatic UFs and parity based on menopausal status. Asymptomatic UFs were most common in postmenopausal and nulliparous women (62.0%). Although the participants included more premenopausal than postmenopausal women,

Table 1. Prevalence of Asymptomatic Uterine Fibroid with a Minimum Diameter of 8 mm

Age (years)	All participants (*n*=3682)			Nulliparous participants (*n*=677)		
	n (%)	Presence of fibroids		*n* (%)	Presence of fibroids	
		No	Yes (Prevalence %)		No	Yes (Prevalence %)
All	3682 (100)	2240	1442 (39.2)	677 (100)	331	346 (51.1)
<30	60 (1.6)	56	4 (6.7)	57 (8.4)	53	4 (7.0)
30–39	542 (14.7)	439	103 (19.0)	115 (17.0)	85	30 (26.1)
30–34	118 (3.2)	103	15 (12.7)	42 (6.2)	36	6 (14.3)
35–39	424 (11.5)	336	88 (20.8)	73 (10.8)	49	24 (32.9)
40–49	1411 (38.3)	860	551 (39.1)	247 (36.5)	107	140 (56.7)
40–44	671 (18.2)	453	218 (32.5)	117 (17.3)	59	58 (49.6)
45–49	740 (20.1)	407	333 (45.0)	130 (19.2)	48	82 (63.1)
50–59	1137 (30.9)	563	574 (50.5)	213 (31.5)	62	151 (70.9)
50–54	641 (17.4)	305	336 (52.4)	134 (19.8)	32	102 (76.1)
55–59	496 (13.5)	258	238 (48.0)	79 (11.7)	30	49 (62.0)
60–69	457 (12.4)	276	181 (39.6)	39 (5.8)	20	19 (48.7)
60–64	306 (8.3)	181	125 (40.8)	34 (5.0)	17	17 (50.0)
65–69	151 (4.1)	95	56 (37.1)	5 (0.7)	3	2 (40.0)
≥70	75 (2.0)	46	29 (38.7)	6 (0.9)	4	2 (33.3)

Table 2. Prevalence of Asymptomatic Uterine Fibroids with a Minimum Diameter of 8 mm by Parity

Participants (*n*=3682)		Nulliparous (*n*=677)	Parous (*n*=3005)				*p*-value
	Fibroids present Yes/*n* (Prevalence %)	Number of parity					
		0	≥1	1	2	≥3	
All	1442/3682	346/677	1096/3005	301/741	635/1754	160/510	
	(39.2)	(51.1)	(36.5)				<0.001[b]
		(51.1)		(40.6)	(36.2)	(31.4)	<0.001[c]
Premenopausal	836/2325	217/469	619/1856	200/505	336/1053	83/298	
	(36.0)	(46.3)	(33.4)				<0.001[b]
		(46.3)		(39.6)	(31.9)	(27.9)	<0.001[c]
Postmenopausal	606/1357	129/208	477/1149	101/236	299/701	77/212	
	(44.7)	(62.0)	(41.5)				<0.001[b]
		(62.0)		(42.8)	(42.7)	(36.3)	<0.001[c]
p-value	<0.001[a]	<0.001[a]	<0.001[a]	0.410[a]	<0.001[a]	0.042[a]	

[a] Chi-square test for categorical variables between premenopausal and postmenopausal
[b] Chi-square test for categorical variables between nulliparous and parous
[c] Cochran–Armitage trend test for parity

the prevalence of asymptomatic UFs was significantly higher in the latter group (36.0% vs. 44.7%, respectively, using the chi-square test, $p<0.001$). The prevalence of asymptomatic UFs was significantly higher in nulliparous than parous women (51.1% vs. 36.5%, respectively, using the chi-squared test, $p<0.001$), and tended to decrease significantly as parity increased in all women (overall, premenopausal, and postmenopausal; Cochran–Armitage test, $p<0.001$).

Discussion

This is an epidemiological study on asymptomatic UFs in Japanese women. There were no hospitalized patients being treated for UF symptoms during the study. None of the women had UFs associated with excessive menstruation or severe anemia. None of the women had intrauterine tumors that were suspected to be malignant. In this study, we found that the overall prevalence of asymptomatic UFs was 39.2%. By age group, women in their 50s had the highest rate, followed by those in their 60s. Asymptomatic UFs were more common in postmenopausal women than in premenopausal women. Moreover, the prevalence of asymptomatic UF was higher in nulliparous women than in parous women.

To our knowledge, only one study has examined the prevalence of asymptomatic UFs by TVUS testing in Japan. Oda and Tanaka surveyed approximately 20,000 women during the period 1998–1999 and reported an overall prevalence of asymptomatic UFs with a minimum diameter of 20 mm of 11.3%[7]. Prevalence of UFs by age group was highest in women in their 40s, and was higher in premenopausal than in postmenopausal women. In contrast, the overall prevalence of asymptomatic UFs with a minimum diameter of 8 mm in this study was 39.2%. The most common age group for asymptomatic UFs was the 50s, and prevalence was higher in postmenopausal than in premenopausal women. We consider that the prevalence of asymptomatic UFs varies according to the minimum size of the UFs at diagnosis. Thus, we assessed prevalence in the two studies by comparison using the same minimum UF size at diagnosis as in this previous study. The results showed that the prevalence in our present study was almost double that in the previous study. Furthermore, peak prevalence was shifted from the 40s to the 50s, and prevalence was still high in the 60s. We therefore hypothesize that the prevalence of asymptomatic UFs in Japanese women has been increasing in recent years, particularly in postmenopausal women.

Most epidemiological studies on UFs have been conducted on symptomatic UFs, and a large number of studies have found that women with symptomatic UFs are more likely to be premenopausal than postmenopausal[8–10]. In contrast, few epidemiological studies on UFs have been conducted on asymptomatic UFs. According to two studies performed in healthy Japanese women, one study found that women with asymptomatic UFs did not differ between premenopausal and postmenopausal cohorts[11], whereas the other study found that women with asymptomatic UFs were more likely to be premenopausal than postmenopausal[7]. Therefore, the higher prevalence of asymptomatic UFs in postmenopausal than in premenopausal women identified in this study is inconsistent with the findings of these previous epidemiological studies in both symptomatic and asymptomatic women. Although there is no clear reason for the high incidence of asymptomatic UFs after menopause in this study, we consider that there may be several causes for this result. The two studies differed in the geography (urban Tokyo in the previous study vs. suburban Kanagawa in this study). In addition, changes in the social background of Japanese women with regard to occupation and education over the more than 20 years between the studies may have influenced their outcomes. Moreover, we hypothesize that there may be other factors contributing to the different results for prevalence by age groups in the two studies. The previous study did not report mean or median age for menopausal women[7]. However, according to the Japan Nurses' Health Study (conducted between 2001 and 2007) on the health of Japanese women, the median age at menopause was 50 years old[12]. In contrast, the median age of menopause in our present study (conducted between 2021 and 2022) was 51 years old. Although there is no simple comparison, it is possible that age at menopause has slightly increased in the last 15–20 years. Consequently, the effects of sex steroid hormones that promote fibroid development may have persisted for longer. In recent years, more women have sought uterine preservation and non-surgical options[13,14], and the use of surgery, particularly invasive total hysterectomy, has declined[15]. Given this trend, the number of women who continue to have UFs may have increased. Furthermore, a recent study suggested that the growth of UFs may not stop after menopause[16]. It is possible that UFs do not shrink uniformly after menopause. These factors may result in a higher prevalence of asymptomatic UFs, even in women aged over 50 years who have experienced menopause.

Many studies[8,17,18] in women with symptomatic UFs and one study[7] of healthy women with asymptomatic UFs have reported that women with UFs were more likely to be nulliparous than parous. Therefore, the finding that the prevalence of asymptomatic UFs was higher in nulliparous than in parous women in this study is consistent with these previous epidemiological study findings in both symptomatic and asymptomatic

women. In addition, the prevalence of asymptomatic UFs decreased significantly as parity increased in this study. The results support the previously proposed protective effect of parity on UFs[19,20]. The causes of this protective effect of parity include a reduction in ovulatory menstrual cycles due to childbirth[21], changes in exposure of the uterus to sex steroid hormones during pregnancy[22], and UF shrinkage due to ischemia of the uterus during postpartum uterine remodeling[23].

This study had some limitations. It was a small, single-center study. The participating women were healthy women who visited our medical checkup center. They were not randomly selected from the general population and were not equally divided into age groups. Accordingly, the women in this study may not necessarily belong to the same group as the general public. However, they were eligible in the order in which they visited the center, and we did not intentionally select them. In Japan, healthy women generally visit medical checkup centers (rather than hospitals or clinics) for early disease detection. Although TVUS was included as a routine examination in medical checkups, only 46% of all women examined during the study period underwent TVUS. The remaining women, who accounted for more than half of the total number of women, were thought to be mostly those who were not recommended by their organizations to take the test that year, or who were menstruating and could not take it. However, we did not know the percentage of women who refused TVUS at their own discretion. Although TVUS is a useful diagnostic method in gynecology, it is difficult to obtain cooperation from all women who visit centers to determine the prevalence of UFs. Despite these limitations, the results of this study provide an approximate reference for the prevalence of asymptomatic UFs in healthy Japanese women in recent years. However, further studies are required in this field.

In conclusion, the prevalence of asymptomatic UFs in Japanese women was 39.2%. Asymptomatic UFs are more common in women in their 50s, and prevalence was higher in postmenopausal than premenopausal women. Women with asymptomatic UFs were more likely to be nulliparous than parous.

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Conflicts of Interest

The authors have no conflicts of interest to declare in regard to this study.

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Supplemental Table. Prevalence of Asymptomatic Uterine Fibroids with a Minimum Diameter of 20 mm

Age (years)	Previous study[7]		This study	
	Participants (*n*=20951)		Participants (*n*=3682)	
	n (%)	Fibroids present (Prevalence %)	*n* (%)	Fibroids present (Prevalence %)
All	20951 (100)	2371 (11.3)	3682 (100)	783 (21.3)
<30	240 (1.1)	5 (2.1)	60 (1.6)	0 (0.0)
30–39	3037 (14.5)	202 (6.7)	542 (14.7)	35 (6.5)
30–34	1383 (6.6)	64 (4.6)	118 (3.2)	5 (4.2)
35–39	1654 (7.9)	138 (8.3)	424 (11.5)	30 (7.1)
40–49	5993 (28.6)	1150 (19.2)	1411 (38.3)	298 (21.1)
40–44	2449 (11.7)	403 (16.5)	671 (18.2)	114 (17.0)
45–49	3544 (16.9)	747 (21.1)	740 (20.1)	184 (24.9)
50–59	6662 (31.8)	818 (12.3)	1137 (30.9)	343 (30.2)
50–54	3502 (16.7)	587 (16.8)	641 (17.4)	204 (31.8)
55–59	3160 (15.1)	231 (7.3)	496 (13.5)	139 (28.0)
60–69	4367 (20.8)	183 (4.2)	457 (12.4)	93 (20.4)
60–64	2850 (13.6)	129 (4.5)	306 (8.3)	62 (20.3)
65–69	1517 (7.2)	54 (3.6)	151 (4.1)	31 (20.5)
≥70	652 (3.1)	13 (2.0)	75 (2.0)	14 (18.7)

Report

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Clinical Survey on Methods in Cancer Diagnosis in the Japan Society of Ningen Dock and Preventive Medical Care

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Abstract

To understand the actual status of cancer screening during the course of physical examinations and to evaluate the effectiveness and accuracy of cancer screening methods, we conducted a survey among the Assessment Certified Facilities membership of the Japan Society of Ningen Dock and Preventive Medical Care. The survey covered cancer cases detected in subjects who underwent physical checkups in 2021 (April 2021 to March 2022).

Cancer risk factors include lifestyle habits such as smoking (including passive smoking), alcohol consumption, lack of exercise, obesity, lack of fruit, excess salt, and infections. In this study, smoking status (current, past, and nonsmoking) and facial flushing after alcohol consumption were investigated as lifestyle habits, and *Helicobacter pylori* (*H. pylori*) infection and human papillomavirus (HPV) infection status as infectious diseases. In addition, we also examined the categorization of main cancers detected by abdominal ultrasonography.

Keywords comprehensive health checkup system, cancer screening, cancer risk factor, *Helicobacter pylori* infection

Introduction

Cancer screening is conducted in physical examinations by utilizing a variety of test methods for each target organ. However, a current issue is that health screening facilities set their own test items and the accuracy control of these items is unclear. The mission of the Japan Society of Ningen Dock and Preventive Medical Care is to improve and develop the quality of preventive medicine and to contribute to the enhancement of the health of the public. One of our activities is to investigate the actual status of cancer screening and to evaluate effective screening methods and their accuracy[1–10]. This paper reports the results of a survey on the actual status of cancers detected in 2021.

Subjects and Methods

Study design

With the goal of ascertaining the actual status of cancer screening in Comprehensive Health Checkup System (Ningen Dock) and evaluating effective cancer screening methods and their accuracy, a survey was conducted of Assessment Certified Facilities in the Japan Society of Ningen Dock and Preventive Medical Care. The survey covered cancer cases detected among those who underwent physical checkups in 2021 (April 2021 to March 2022). Of the 416 facilities, 252 responded (60.6% response rate).

Methods

1) The number of examinees, breakdown of cancers detected, and examination results by examination method were reviewed.

2) The degree of progression (stomach, esophagus, and colon) and stage classification (lung and breast) by examination method were examined.

3) Smoking status of cancer patients was investigated (classified into current smokers, past smokers, never smokers, smoking-related [current smokers + past smokers], and unknown).

4) The presence or absence of facial flushing on alcohol consumption was investigated in patients with esophageal cancer (categorized as present, absent, or

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unknown).

5) *H. pylori* infection status in gastric cancer patients was investigated (classified into current infection, previous infection [including after eradication], uninfected, and untested). Diagnostic methods for *H. pylori* infection were at the discretion of each institution.

6) HPV infection status in cervical cancer patients was investigated (classified as HPV-associated, HPV-independent, or unknown).

7) Categorization of liver, gallbladder/bile duct, and pancreatic cancers detected by abdominal ultrasonography (percentage of category 3, 4, and 5) was investigated. Abdominal ultrasonography findings that triggered detection in patients with pancreatic cancer were investigated.

8) Comparison between two groups was performed by Fisher's exact test, and $p<0.05$ was considered to indicate a significant difference.

Results

1. Overview of examinees

A total of 1,726,140 (1,006,753 men and 719,387 women) subjects were examined, of whom men accounted for 58.3%. The number of examinees by gender and age group was highest in the 45–59 age group for both men and women (**Fig. 1**).

2. Breakdown of cancer cases

A total of 6,206 cancer cases (3,652 men and 2,554 women) were reported (**Table 1**). Breast cancer was the most common cancer (1,294 cases), followed by colon, stomach, prostate, and lung. In men, prostate was the most common cancer, followed by colon, stomach, lung, and esophagus, and in women, breast was the most common, followed by colon, stomach, lung, and uterus.

Table 1. Breakdown of Cancer Cases

	men	women	total
stomach	817	230	1,047
esophagus	241	28	269
colon	822	393	1,215
lung	351	181	532
breast		1,294	1,294
cervix uteri		135	135
corpus uteri		33	33
ovary		27	27
kidney	199	55	254
urinary bladder	47	16	63
prostate	915		915
thyroid gland	25	44	69
liver	72	24	96
gall bladder	27	10	37
pancreas	78	52	130
others	58	32	90
total	3,652	2,554	6,206

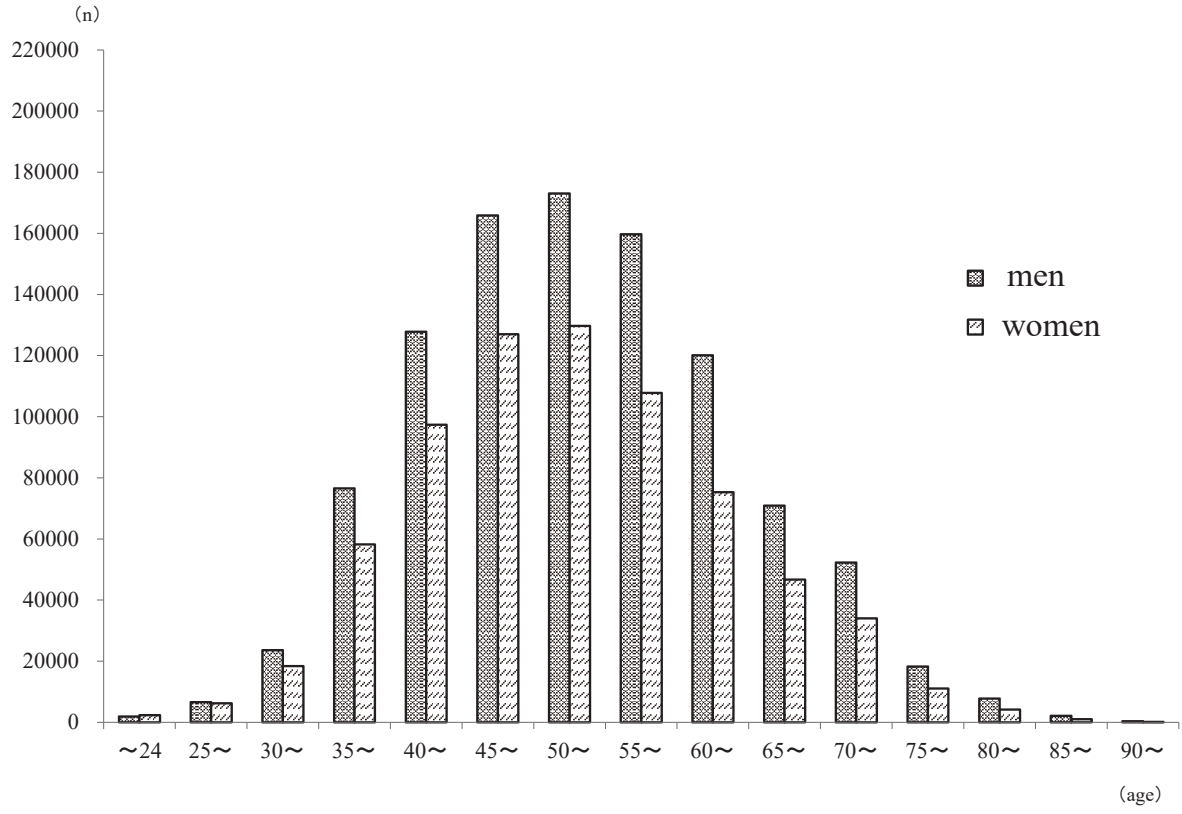

Fig. 1. Number of Examinees by Gender and Age Group
total of 1,726,140 (1,006,753 men and 719,387 women)

3. Cancer and smoking status

Among men, esophageal cancer accounted for the largest proportion of smoking-related cases (74.4%), followed by lung cancer (74.1%) and bladder cancer (65.8%). Among women, stomach cancer accounted for the largest proportion at 48.9%, followed by gall bladder and bile duct cancer at 37.5% and liver cancer at 31.8% (**Table 2**). Underlined numbers in the table indicate the rank of women.

4. Cancer screening outcomes and risk factors for gastric and esophageal cancer

The detection rate of gastric cancer was 6 times higher with endoscopy (0.112%) than with X-ray (0.016%). Esophageal cancer detection rate was 7.5 times higher with endoscopy (0.03%) than with X-ray (0.004%) (**Table 3**).

The results of gastric cancer progression showed that intramucosal cancer accounted for 55.1% of cases identified by X-ray and 72.7% of cases identified by endoscopy, with the latter accounting for a significantly higher percentage (p=0.002) (**Fig. 2**). The results of esophageal cancer progression showed that intramucosal cancer accounted for 15.8% of cases identified by X-ray and 71% of cases identified by endoscopy, the latter being significantly higher (p<0.0001) (**Fig. 3**).

The results for the *H. pylori* infection status in gastric cancer patients showed that currently infected gastric cancer accounted for 12.5% of cases (78 cases) in men and 15.7% (31 cases) in women. Previously infected gastric cancer accounted for 49.3% (308 cases) of cases and 31.0% (61 cases) of cases, respectively. On the other hand, uninfected gastric cancer accounted for 18.7% (117 cases) of cases in men and 27.9% (55 cases) of cases in women, showing a higher percentage than previously reported (**Fig. 4**)[11]. The presence or absence of facial flushing on alcohol consumption in esophageal

Table 2. Cancer and Smoking Status

n (%)

	stomach (*n*=1,196)		esophagus (*n*=246)		colon (*n*=1,021)		lung (*n*=485)		breast (*n*=1,150)	uterus (*n*=176)	prostate (*n*=750)
	men	women	men	women	men	women	men	women	women	women	men
current smoker	164 (21.4)	168 (41.9)	60 (26.9)	4 (17.4)	191 (26.5)	26 (7.5)	113 (34.9)	9 (5.6)	68 (5.9)	23 (13.1)	96 (12.8)
past smoker	317 (41.3)	28 (7.0)	106 (47.5)	3 (13.0)	265 (36.8)	35 (10.1)	127 (39.2)	27 (16.8)	115 (10.0)	19 (10.8)	317 (42.3)
never smoker	244 (31.8)	195 (48.6)	50 (22.4)	14 (60.9)	238 (33.1)	266 (76.9)	70 (21.6)	119 (73.9)	920 (80.0)	123 (69.9)	304 (40.5)
unknown	43 (5.6)	10 (2.5)	7 (3.1)	2 (8.7)	26 (3.6)	19 (5.5)	14 (4.3)	6 (3.7)	47 (4.1)	11 (6.3)	33 (4.4)
smoking-related	481 (62.7)	196 (48.9)	166 (74.4)	7 (30.4)	456 (63.3)	61 (17.6)	240 (74.1)	36 (22.4)	183 (15.9)	42 (23.9)	413 (55.1)
rank	5	1	1	4	4		2			5	

	kidney (*n*=221)		urinary bladder (*n*=55)		liver (*n*=87)		pancreas (*n*=104)		gall bladder・bile duct (*n*=34)		thyroid gland (*n*=59)	
	men	women	men	women	men	women	men	women	men	women	men	women
current smoker	37 (21.3)	3 (6.4)	8 (19.5)	1 (7.1)	20 (30.8)	2 (9.1)	17 (28.3)	5 (11.4)	2 (7.7)	0 (0)	2 (9.1)	2 (5.4)
past smoker	43 (24.7)	6 (12.8)	19 (46.3)	1 (7.1)	18 (27.7)	5 (22.7)	15 (25.0)	2 (4.5)	11 (42.3)	3 (37.5)	7 (31.8)	4 (10.8)
never smoker	85 (48.9)	38 (80.9)	10 (24.4)	12 (85.7)	23 (35.4)	15 (68.2)	23 (38.3)	35 (79.5)	10 (38.5)	3 (37.5)	13(59.1)	31 (83.8)
unknown	9 (5.2)	0 (0)	4 (9.8)	0 (0)	4 (6.2)	0 (0)	5 (8.3)	2 (4.5)	3 (11.5)	2 (25.0)	0 (0)	0 (0)
smoking-related	80 (46.0)	9 (19.2)	27 (65.8)	2 (4.2)	38 (59.5)	7 (31.8)	32 (53.3)	7 (15.9)	13 (50.0)	3 (37.5)	9 (40.9)	6 (16.2)
rank			3			3				2		

The underscores in the numbers indicate the rank of women.

Table 3. Cancer Screening Outcomes for Gastric and Esophageal Cancer

		Gastric radiography		Endoscopy	
Men	Participation number	466,962		469,496	
	Recall number	12,459	(2.7)	17,812	(3.8)
	Number of required follow-up examinations	6,264	(50.3)	11,578	(65.0)
	Number of gastric cancers	94	(0.020)	694	(0.148)
	Number of esophageal cancers	29	(0.006)	212	(0.045)
Women	Participation number	292,807		316,295	
	Recall number	5,334	(1.8)	8,023	(2.5)
	Number of required follow-up examination	3,231	(60.6)	5,373	(67.0)
	Number of gastric cancers	30	(0.010)	190	(0.060)
	Number of esophageal cancers	2	(0.001)	26	(0.008)
Total	Participation number	759,769		785,791	
	Recall number	17,793	(2.3)	25,835	(3.3)
	Number of required follow-up examinations	9,495	(53.4)	16,951	(65.6)
	Number of gastric cancers	124	(0.016)	884	(0.112)
	Number of esophageal cancers	31	(0.004)	238	(0.030)
	Estimated number of gastric cancer cases	232	(0.031)		
	Estimated number of esophageal cancer cases	58	(0.008)		

(rate: %)

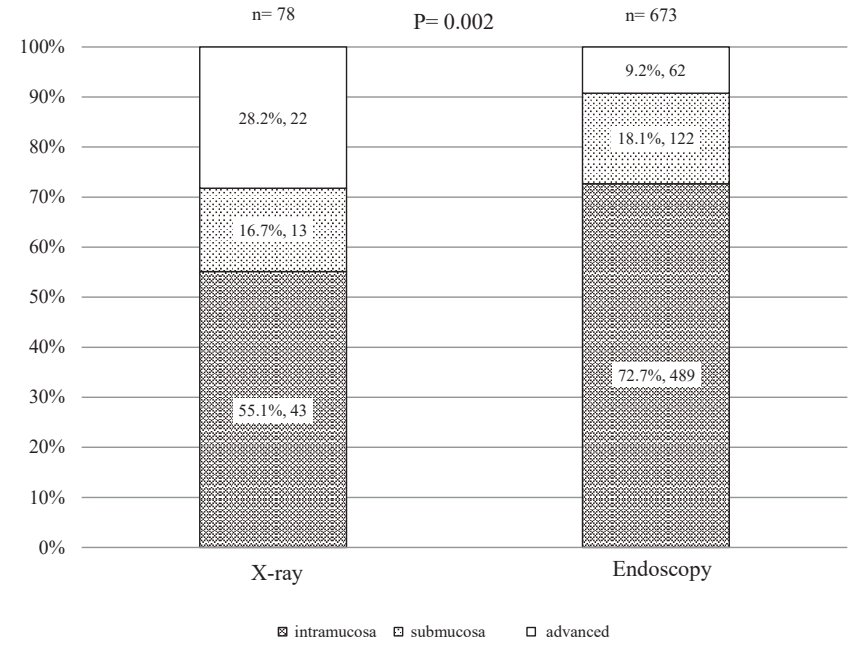

Fig. 2. Gastric Cancer Progression by Each Examination

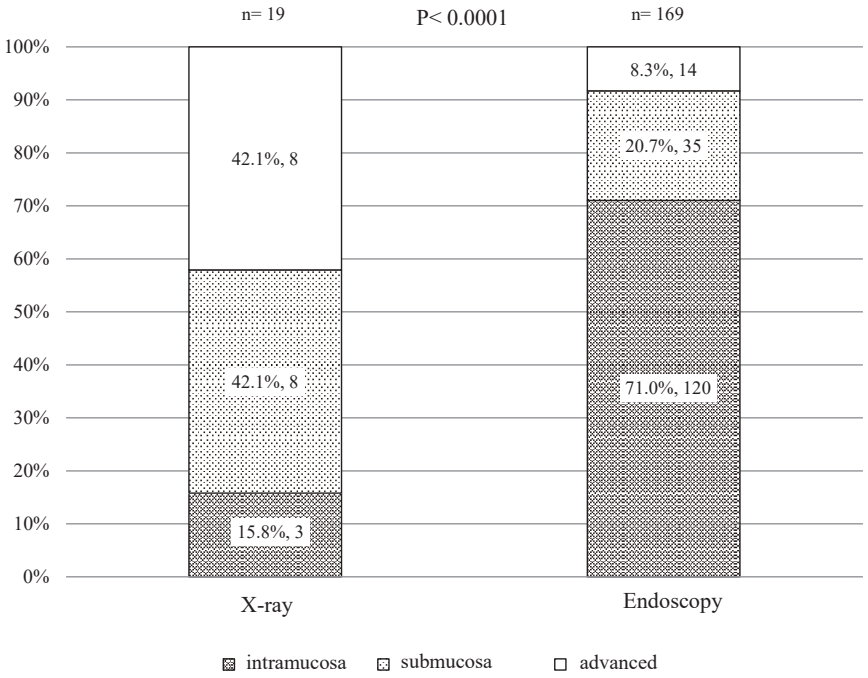

Fig. 3. Esophageal Cancer Progression by Each Examination

cancer patients was examined. Facial flushing occurred in 8.2% (17 cases) of male patients and 4.5% (1 case) of female patients. However, the presence of flushing was unknown in many cases (78.3% of males and 68.2% of females) (**Fig. 5**).

5. Cancer screening outcomes for colorectal cancer

The cancer detection rate was approximately 4.3 times higher by colonoscopy (0.289%) than by fecal occult blood testing (0.067%) (**Table 4**), and the percentage of intramucosal cancer was significantly higher in total colonoscopy (68.8%) than in fecal occult blood testing (52.4%) (p=0.036) (**Fig. 6**).

6. Cancer screening outcomes for lung cancer

The cancer detection rate by X-ray+CT scan (0.099%) was highest, followed by CT scan (0.063%). These were about 4.7 times and 3 times higher than that by X-ray (0.021%), respectively. The cancer detection rate by sputum examination was low (0.007%)

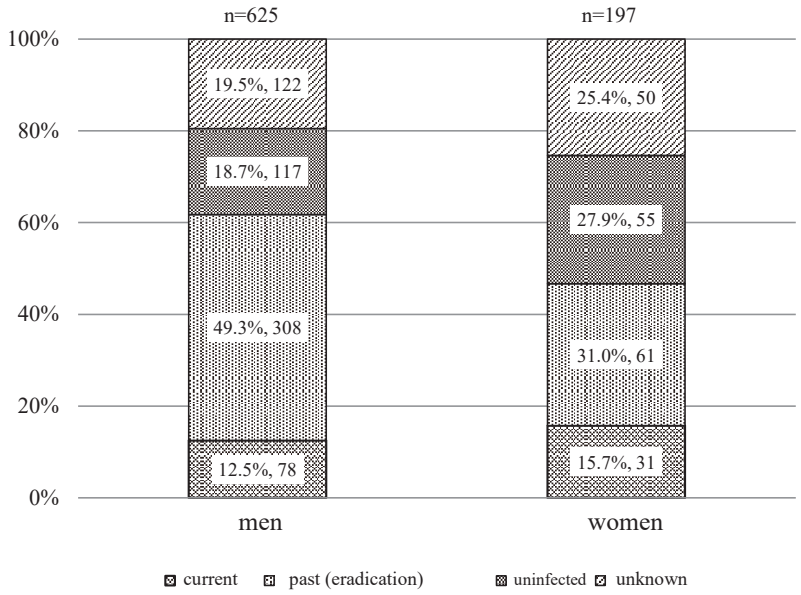

Fig. 4. *H. pylori* Infection Status in Gastric Cancer Patients

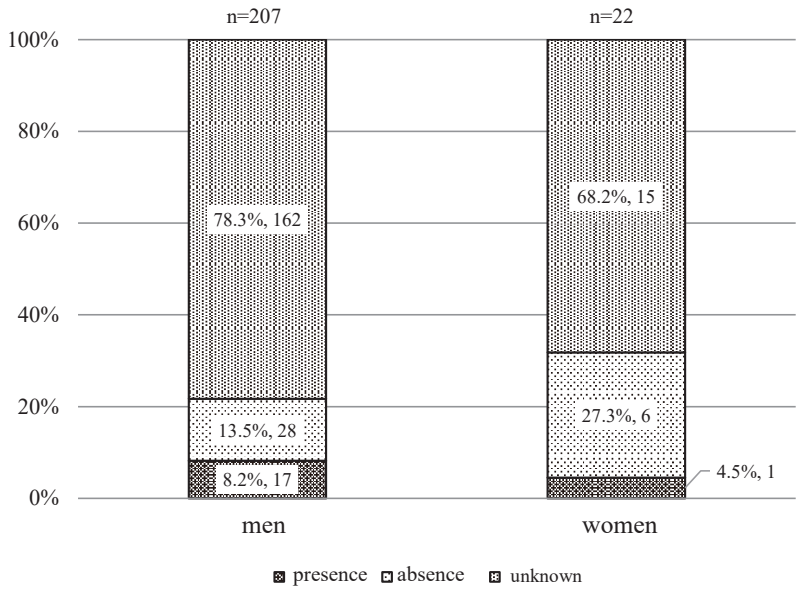

Fig. 5. The Presence or Absence of Facial Flasher in Esophageal Cancer Patients

(**Table 5**). When stage classification was examined by examination method, stage 0 accounted for 4.7% by X-ray and 16.4% by CT scan, with the latter having a significantly higher rate (p=0.0026) (**Fig. 7**).

7. Cancer screening outcomes for breast cancer

The highest cancer detection rate was by mammography+ultrasound (0.323%), followed by ultrasound 0.173%, mammography 0.167%, and palpation+mammography 0.154% (**Table 6**). When stage classification was examined by examination method, 0+I accounted for 79.9%, 79.2%, 76.9%, and 77.3% for mammography, mammography+ultrasound, ultrasound, and palpation+mammography, respectively, with no significant differences between the groups (**Fig. 8**).

8. Cancer screening outcomes and risk factors for cervical cancer

About 450,000 people were examined, and the cancer detection rate was 0.029% (**Table 7**), with a peak in the younger age group of 30–39 years and a tendency to decline with age. We examined HPV infection status in patients with cervical cancer (HPV-associated, HPV-independent, and unknown). Among cervical cancer patients, HPV-associated cases accounted for 18.4% (16 cases) and HPV-independent cases for 1.1% (1 case). However, in many cases, the presence or absence of HPV status was unknown (70 cases, 80.5%) (**Fig. 9**).

Table 4. Cancer Screening Outcomes for Colorectal Cancer

		Stool occult		Sigmoidoscopy		Total colonoscopy	
men	Participation number	975,915		5,487		15,844	
	Recall number	50,984	(5.2)	207	(3.8)	1,256	(7.9)
	Number of required follow-up examinations	27,099	(53.2)	147	(71.0)	909	(72.4)
	Number of colorectal cancers	753	(0.077)	3	(0.055)	48	(0.303)
women	Participation number	685,471		1,965		5,236	
	Recall number	31,069	(4.5)	46	(2.3)	281	(5.4)
	Number of required follow-up examinations	18,064	(58.1)	23	(50.0)	211	(75.1)
	Number of colorectal cancers	367	(0.054)	4	(0.204)	13	(0.248)
total	Participation number	1,661,386		7,452		21,080	
	Recall number	82,053	(4.9)	253	(3.4)	1,537	(7.3)
	Number of required follow-up examinations	45,163	(55.0)	170	(67.2)	1,120	(72.9)
	Number of colorectal cancers	1,120	(0.067)	7	(0.094)	61	(0.289)
	Estimated number of colorectal cancers	2,035	(0.122)	10	(0.134)	84	(0.398)

(rate: %)

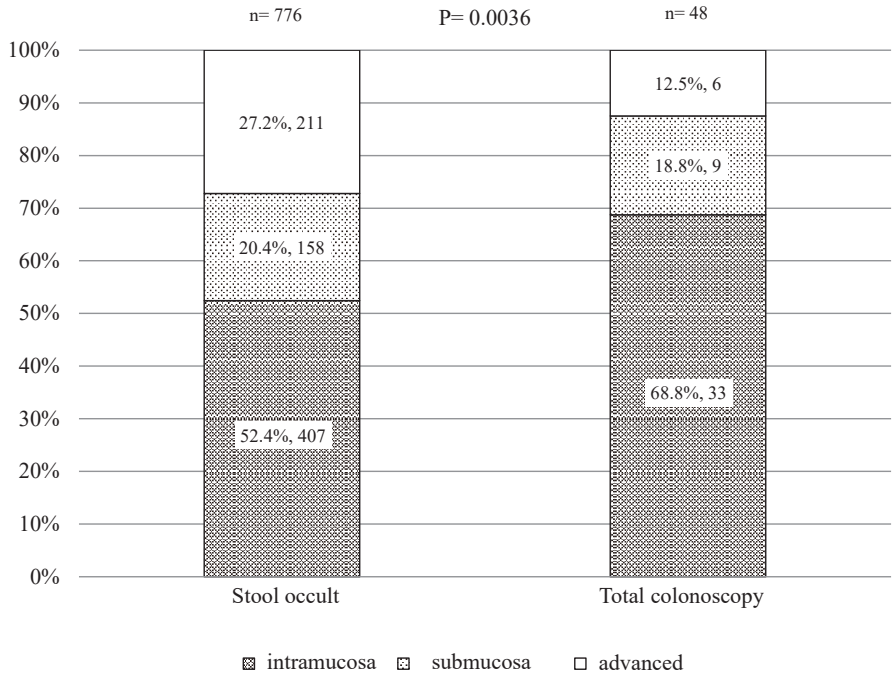

Fig. 6. Colorectal Cancer Progression by Each Examination

Table 5. Cancer Screening Outcomes for Lung Cancer

		X-ray		Sputum cytology		CT scan		X-ray+CT scan	
Men	Participation number	938,426		24,239		46,159		84,853	
	Recall number	11,921	(1.3)	37	(0.2)	1,477	(3.2)	2,555	(3.0)
	Number of required follow-up examinations	8,344	(70.0)	20	(54.1)	892	(60.4)	1,509	(59.1)
	Number of lung cancers	212	(0.023)	2	(0.008)	29	(0.063)	89	(0.105)
Women	Participation number	671,139		6,338		18,765		39,648	
	Recall number	7,942	(1.2)	7	(0.1)	669	(3.6)	1,010	(2.5)
	Number of required follow-up examinations	5,822	(73.3)	3	(42.9)	441	(65.9)	670	(66.3)
	Number of lung cancers	129	(0.019)	0	(0.000)	12	(0.064)	34	(0.086)
Total	Participation number	1,609,565		30,577		64,924		124,501	
	Recall number	19,863	(1.2)	44	(0.1)	2,146	(3.3)	3,565	(2.9)
	Number of required follow-up examinations	14,166	(71.3)	23	(52.3)	1,333	(62.1)	2,179	(61.1)
	Number of lung cancers	341	(0.021)	2	(0.007)	41	(0.063)	123	(0.099)
	Estimated number of colorectal cancer	478	(0.030)	4	(0.013)	66	(0.102)	201	(0.161)

(rate: %)

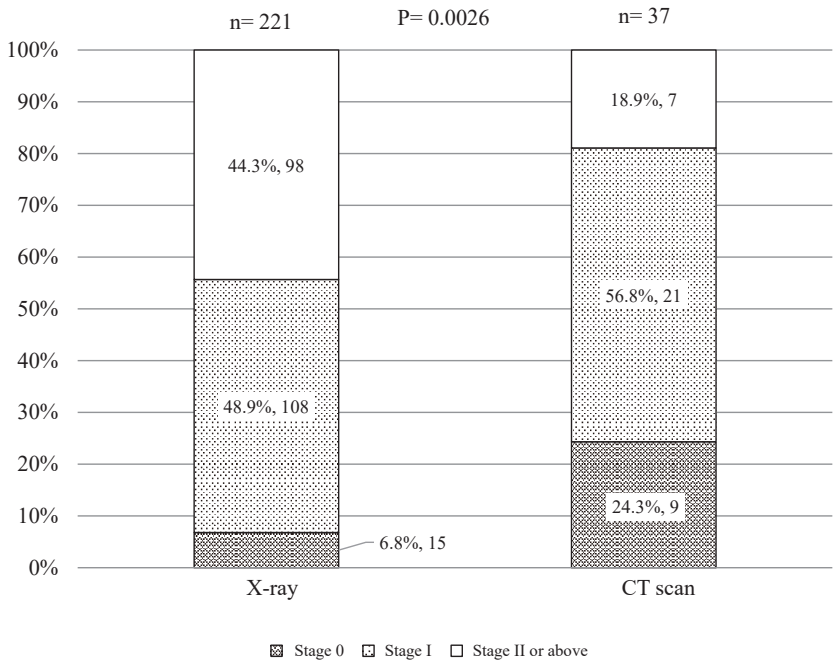

Fig. 7. Stage Classification of Lung Cancer by Each Examination

Table 6. Cancer Screening Outcomes for Breast Cancer

	Palpation+ mammography		Mammography		Ultrasound		Mammography+ ultrasound	
Participation number	83,289		251,782		198,079		124,329	
Recall number	3,423	(4.1)	11,142	(4.4)	5,187	(2.6)	5,007	(4.0)
Number of required follow-up examinations	2,377	(69.4)	8,105	(72.7)	3,515	(67.8)	3,581	(71.5)
Number of breast cancers	128	(0.154)	420	(0.167)	343	(0.173)	401	(0.323)
Estimated number of breast cancers	184	(0.221)	577	(0.229)	506	(0.255)	561	

(rate: %)

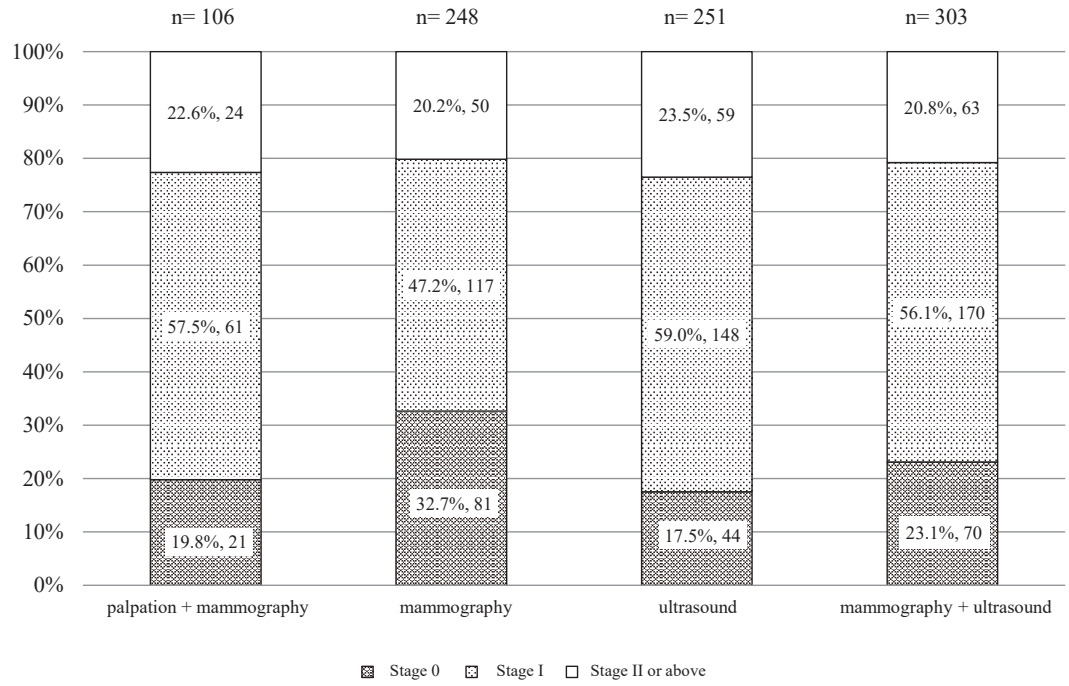

Fig. 8. Stage Classification of Breast Cancer by Each Examination

Table 7. Cancer Screening Outcomes for Cervical Cancer

	Cervical cytology	
Participation number	451,782	
Recall number	13,343	(3.0)
Number of required follow-up examinations	7,979	(59.8)
Number of cervical cancers	130	(0.029)
Estimated number of cervical cancers	217	(0.048)

(rate: %)

Table 8. Cancer Screening Outcomes for Prostate Cancer

	PSA	
Participation number	496,589	
Recall number	16,730	(3.4)
Number of required follow-up examinations	9,493	(56.7)
Number of prostate cancers	915	(0.184)
Estimated number of prostate cancers	1,613	(0.325)

(rate: %)

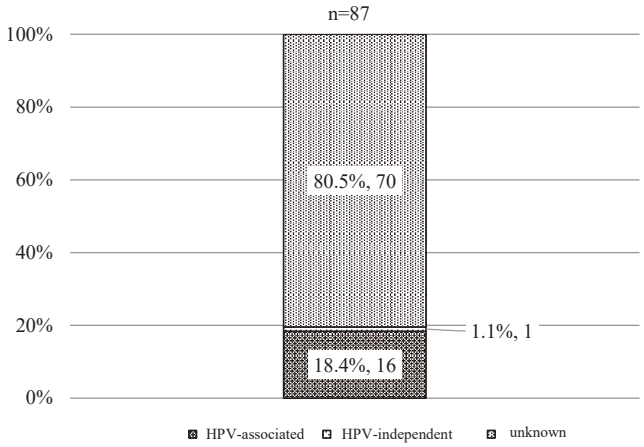

Fig. 9. HPV Infection Infection Status in Cervical Cancer Patients

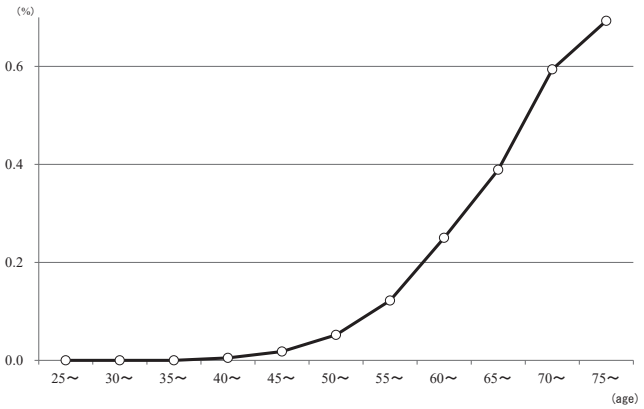

Fig. 10. Rate of Prostate Cancer Detection by Age Group

Table 9. Cancer Screening Outcomes for Thyroid Cancer by Ultrasonography

		Ultrasonography	
Men	Participation number	18,491	
	Recall number	592	(3.2)
	Number of required follow-up examinations	397	(67.1)
	Number of thyroid cancers	23	(0.124)
Women	Participation number	25,323	
	Recall number	1,157	(4.6)
	Number of required follow-up examinations	870	(75.2)
	Number of thyroid cancers	41	(0.162)
Total	Participation number	43,814	
	Recall number	1,749	(4.0)
	Number of required follow-up examinations	1,267	(72.4)
	Number of thyroid cancers	64	(0.146)
	Estimated number of thyroid cancers	88	(0.201)

(rate: %)

Table 10. Cancer Screening Outcomes and Cases of Cancer Detection by Upper Abdominal Ultrasonography

	Men		Women		Total	
Participation number	963,801		665,560		1,629,361	
Recall number	31,534	(3.3)	20,871	(3.1)	52,405	(3.2)
Number of required follow-up examinations	18,469	(58.6)	13,893	(66.6)	32,362	(61.8)
Number of cancers	417	(0.043)	165	(0.025)	582	(0.036)
Estimated number of cancers	712	(0.074)	248	(0.037)	942	(0.058)

(rate: %)

	Men	Women	Total
Liver	72	24	96
Gall bladder・bile duct	27	10	37
Pancreas	76	51	127
Kidney	198	55	253
Others	44	25	69
	417	165	582

9. Cancer screening outcomes for prostate cancer

About 500,000 people were examined for prostate cancer by PSA (prostate specific antigen), with a 3.4% recall rate. Further, follow-up examination rate was 56.7%, and cancer detection rate was 0.184% (**Table 8**). Cancer detection rates increase at an accelerating rate with age after age 50 (**Fig. 10**).

10. Cancer screening outcomes for thyroid cancer

About 43,000 people underwent thyroid cancer screening by ultrasonography, with a 4% recall rate, and 72.4% follow-up examination rate. The cancer detection rate was 0.146% (0.124% for males and 0.162% for females), with the rate for females being approximately 1.3 times higher than that for males (**Table 9**).

11. Cancer screening outcomes by upper abdominal ultrasonography

Approximately 1.62 million people underwent cancer screening by upper abdominal ultrasonography, with a 3.2% recall rate, 61.8% follow-up examination rate, and 0.036% cancer detection rate (0.043% for men and 0.025% for women). By site, kidney cancer was the most common (253 cases), followed by pancreatic cancer (127 cases) and liver cancer (96 cases) (**Table 10**).

12. Cancers detected by upper abdominal ultrasound and their categorization

Categorization (percentage of category 3, 4, and 5) in liver, gall bladder/bile duct, and pancreatic cancers was investigated. In liver cancer (51 cases), 50.0% (19 cases) of males were in category 4, followed by 36.8% (14 cases) in category 3. 46.2% (6 cases) of females were in category 4, followed by 30.8% (4 cases) in category 3 (**Fig. 11a**).

In gall bladder and bile duct cancer (15 cases), category 4 accounted for 63.6% of cases (7 cases) in males, followed by category 3 in 27.3% (3 cases), while category 4 accounted for 75.0% (3 cases) in females,

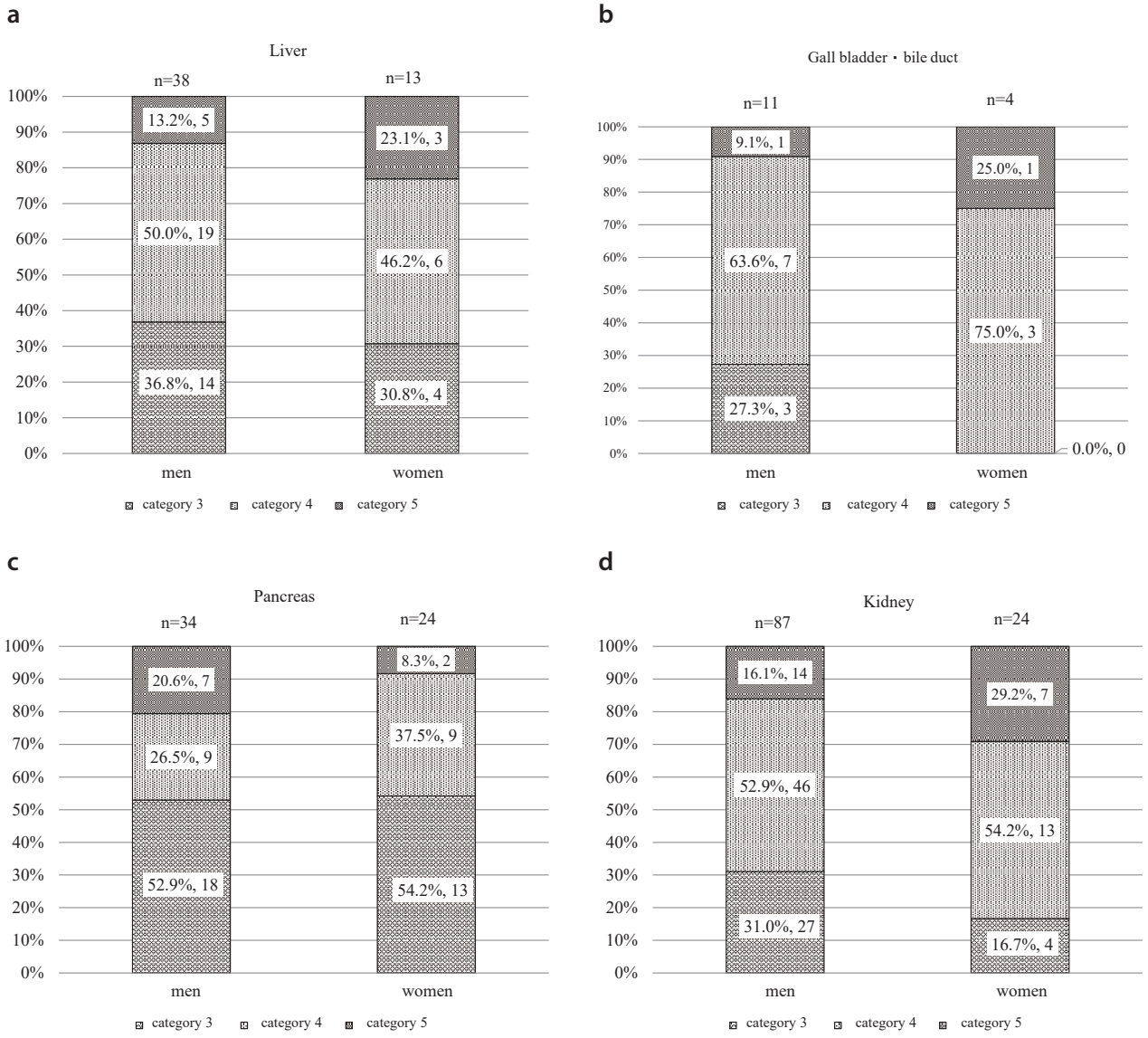

Fig. 11. Cancers Detected by Upper Abdominal Ultrasound and Their Categorization

followed by category 5 in 25.0% (1 case) (**Fig. 11b**).

In pancreatic cancer, category 3 accounted for 52.9% of cases (18 cases) in males, followed by category 4 (26.5%, 9 cases) and category 5 (20.6%, 7 cases). Similarly, among women, category 3 accounted for 54.2% of cases (13 cases), followed by category 4 (37.5%) (9

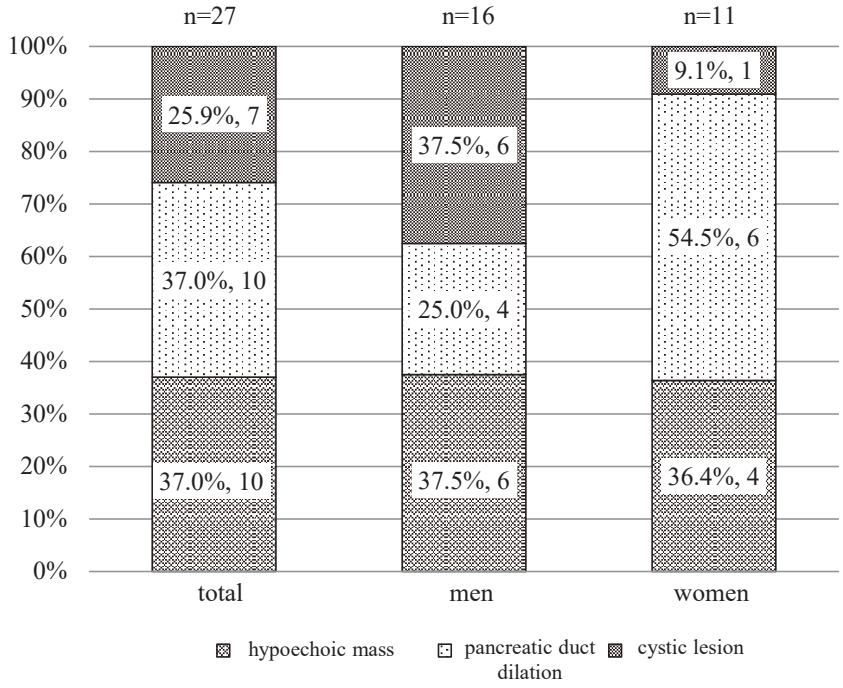

Fig. 12. Ultrasonographic Findings That Led to the Detection of Early-stage Pancreatic Cancer

Table 11. Cancer Screening Outcomes and Cases of Cancer Detection by Lower Abdominal Ultrasonography (Urinary Bladder and Prostate)

	Men		Women	
Participation number	196,905		140,495	
Recall number	4,551	(2.3)	3,948	(2.8)
Number of required follow-up examinations	2,868	(63.0)	2,768	(70.1)
Number of cancers	47	(0.024)	16	(0.011)
Estimated number of cancers	75	(0.038)	23	(0.016)

(rate: %)

	Men	Women
Bladder cancer	47	16

Table 12. Cancer Screening Outcomes and Cases of Cancer Detection by Upper Abdominal Ultrasonography (Uterus and Ovary)

	Women	
Participation number	119,500	
Recall number	2,710	(2.3)
Number of required follow-up examinations	1,815	(67.0)
Number of cancers	8	(0.007)
Estimated number of cancers	12	(0.010)

(rate: %)

Ovarian cancer	4
Uterine body cancer	3
Ovarian cancer	1

cases) and category 5 (8.3%) (2 cases) (**Fig. 11c**).

In renal cancer, category 4 accounted for 52.9% of cases (46 cases) in males, followed by category 3 (31.0%, 27 cases). Among women, category 4 accounted for the largest proportion of cases (54.2% (13 cases)), followed by category 5 (29.2% (7 cases)) (**Fig. 11d**).

Ultrasonographic findings that led to the detection of early-stage pancreatic cancer (stage I) were reviewed. Overall, hypoechoic mass and pancreatic duct dilation accounted for 37.0% of cases (10 cases), followed by cystic lesions in 25.9% (7 cases). By gender, ductal dilation tended to be more common in females (54.5%) than in males (25%) (**Fig. 12**).

13. Cancer screening outcomes by lower abdominal ultrasonography

The cancer detection rate by lower abdominal ultrasonography of the bladder and prostate was 0.011%, and all 63 cases of cancer detection were bladder cancer (**Table 11**). The cancer detection rate by lower abdominal ultrasonography of the uterus and ovaries was 0.007%, and the 12 cancer cases included 4 ovary, 3 uterine body, and 1 uterine cervix cancer (**Table 12**).

Discussion

Primary prevention of cancer is the basis of cancer control, and avoidance of risk factors can lead to a reduction in cancer incidence. Risk factors include lifestyle habits such as smoking (including passive smoking), alcohol consumption, low physical activity, obese vegetables, fruits and lack thereof, excessive intake of salted foods, and infectious diseases. The cancers judged to have a clear "Level 1" causal relationship with cancer are the oral cavity/pharynx, larynx, esophagus, lung, liver, stomach, pancreas, cervix, and bladder cancer. This result was similar with the results for smoking-related cancers, which were the top categories in the current data. These findings again demonstrate the importance of addressing smoking cessation activities among smokers.

The detection rate of gastric and esophageal cancer was significantly higher with endoscopy than with X-ray examination, and the proportion of intramucosal cancer was also significantly higher. The results for *H. pylori* infection status reflect the recent decline in the frequency of currently infected patients with gastric cancer and the increase in previously infected patients with gastric cancer (mostly post-elimination gastric cancer). However, the proportion of uninfected patients with gastric cancers was higher than previously reported[11], suggesting that previously infected patients with gastric cancers were probably contaminated. In addition, the frequency of uninfected patients with gastric cancer may increase in the future due to a decrease in the rate of *H. pylori* infection and increased recognition by endoscopists.

Compared with fecal occult blood testing, colonoscopy had a 3.6-fold higher cancer detection rate and a significantly higher percentage of intramucosal cancers, indicating once again the usefulness of voluntary screening. A study to evaluate the efficacy of colonoscopy in colorectal cancer screening in Japan (Akita pop-colon trial[12]: a randomized controlled trial to evaluate the effect of colonoscopy on mortality reduction) has been ongoing since 2009, and the results of this study are expected in the future.

Low-dose CT chest examination has a three-fold higher lung cancer detection rate than X-ray examination and a significantly higher percentage of stage 0 lung cancers, again demonstrating the usefulness of this voluntary screening method. Low-dose CT screening has been performed on only a limited basis as voluntary screening because evidence of its effectiveness in reducing mortality has been insufficient. Recently, since the reduction in lung cancer mortality was confirmed in two large randomized controlled trials[13,14] for high-risk groups, lung cancer screening guidelines recommend low-dose CT screening for heavy smokers (smoking index >600) (recommendation A).

As in the J-Start study[15], the detection rate of breast cancer in this study was highest for mammography plus ultrasonography, indicating that ultrasonography is a method that compensates for the weakness of mammography. However, there was no clear difference in the percentage of stage 0+I disease in the two groups.

There are two ways to prevent cervical cancer: regular screening (cytology) and HPV vaccination. Sakamoto *et al.*[16] performed HPV genotyping of 371 cases of invasive cervical cancer from specimens provided by multiple institutions from 1990 to 2017 to clarify HPV types in Japan. The results reported that the HPV positivity rate for invasive cervical cancer was 90.8%, and the positivity rate for high-risk HPVs, namely HPV types 16/18, was 65.4%. In our data, HPV infection was unknown in many cases and was not considered to reflect the reality of the situation.

In 2014, the Abdominal Ultrasonography Evaluation Manual[17] was published with the aim of improving and equalizing the quality of abdominal ultrasonography, to standardize the criteria for cancer (categorization), and to evaluate the accuracy and efficacy of abdominal ultrasonography (revised edition in 2021[18]). In the present study, category 3 disease accounted for a large proportion of both men and women in pancreatic cancer only. Aggressive identification of category 3 disease in the pancreatic region contributed to the detection of pancreatic cancer, suggesting the usefulness of abdominal ultrasound screening.

Conclusions

This paper presents a summary and discussion of the 2021 cancer aggregate results for the Japan Society of Ningen Dock and Preventive Medical Care. Colonoscopy and low-dose CT scan of the chest greatly contribute to early cancer diagnosis, and the usefulness of voluntary screening by both tests was confirmed. Furthermore, in order to realize early diagnosis of cancer, it is important to accurately identify cancer risk factors among health screening participants and to highlight useful examination findings in the abdominal ultrasound screening judgment manual.

Conflict of Interest

The authors have no conflicts 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

Journal of Ningen Dock and Preventive Medical Care
Official Journal of Japan Society of Ningen Dock and Preventive Medical Care

Journal of Ningen Dock and Preventive Medical Care is the official journal of Japan Society of Ningen Dock and Preventive Medical Care, in which original articles, case reports, short reports, review articles, clinical experience or practice report, and letters to the editor in English are published. Letters to the editor are to refer to papers published in the journal within approximately the preceding six months. The Editorial Board reserves the right to change submission categories at its discretion.

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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 Japan Society of Ningen Dock and Preventive Medical Care 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.

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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.

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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 4,000 words, and should be arranged as follows: Abstract, Objective, Methods, Results, Discussion, (Limitations), (Conclusions), (Acknowledgments), and References.

Case reports: A case report should not exceed 3,000 words, and be arranged as follows: Abstract (which should be a brief summary of the content without headings), Introduction, Case report, Discussion, and References.

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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 Sasamori[1]. 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. For websites, the names and initials of the first three authors, followed by "*et al.*" if there are other coauthors, title of cited page/the document, year of posting, URL, and accessed date in parentheses should be included. Examples of references are given below.

Journal: Frías JP, Davies MJ, Rosenstock J, *et al.*: Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes. N Engl J Med 2021; 385: 503–515.

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

Websites: Ministry of Health, Labour and Welfare: The National Health and Nutrition Survey in Japan. 2013, http://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h25-houkoku.pdf (in Japanese) (accessed March 1, 2022)

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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.

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Figures should be cited in the text, and numbered sequentially with Arabic numerals. A brief descriptive legend should be provided for each figure. Legends are part of the text, and should be appended to it on a

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- Specify that the use is a quotation or modification, and document the source.

Updated: November 29, 2024

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Abstract:

- ☐ Not more than 250 words.
- ☐ Arranged in the order of Objective, Methods, Results, and Conclusions.
- ☐ Up to 4 key words.

Text of paper:

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- ☐ Measurements are expressed in SI units.
- ☐ Abbreviations are spelled out at first usage.

References:

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- ☐ Format is consistent with examples in Instructions for Authors.

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If the author is a member of Japan Society of Ningen Dock and Preventive Medical Care, please write the membership number on the right of the signature line.

Abbreviations

1	**1,5-AG**	1,5-anhydroglucitol	61	**hCG**	human chorionic gonadotropin
2	**17-OHCS**	17α-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**	adrenocorticotropic 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	**CA125**	carbohydrate antigen 125	78	**MCHC**	mean corpuscular hemoglobin concentration
19	**CA19-9**	carbohydrate antigen 19-9	79	**MCV**	mean corpuscular volume
20	**cAMP**	cyclic adenosine 3', 5'-monophosphate	80	**METs**	meatbolic 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 *Staphylococcus aureus*
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	**T_3**	triiodothyronine
45	**EPO**	erythropoietin	105	**T_4**	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	**FEV_1**	forced expiratory volume in one second	110	**tPA**	tissue plasminogen activator
51	**FEV_1%**	forced expiratory volume % in one second	111	**TPHA**	*Treponema pallidum* 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	**HbA1c**	hemoglobin A1c	120	**ZTT**	zinc sulfate (turbidity) test

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