ISSN 2187-8072

NINGEN DOCK INTERNATIONAL

Official Journal of Japan Society of Ningen Dock

JAPAN SOCIETY OF NINGEN DOCK

Vol.2 No.1
2014. 9

Ningen Dock International

Official Journal of Japan Society of Ningen Dock

Editorial Board

Published by Japan Society of Ningen Dock
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The Ningen Dock International is the official English-language journal of Japan Society of Ningen Dock.
Business matters should be addressed to Japan Society of Ningen Dock.

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Polymorphism of *(Pro)renin Receptor* Gene is Associated with Renal Function in Japanese Women

Yasuhiko Shimoyama[1], Yoko Mitsuda[2], Nobuyuki Hamajima[3], Toshimitsu Niwa[1,4]

Abstract

Objective: *(Pro)renin receptor* (*PRR*), a specific receptor for renin and prorenin, has been identified as a new member of the renin-angiotensin system (RAS). We investigated an association between the rs5918007 *PRR* gene polymorphism and renal function in Japanese healthy subjects.

Methods: This study enrolled 472 Japanese subjects (288 men and 184 women) who underwent health check-ups at Nagoya University Hospital. The genotyping of the *PRR* gene single nucleotide polymorphism (SNP) rs5918007 was performed using a polymerase chain reaction with confronting two-pair primers (PCR-CTPP) assay.

Results: In female subjects, the serum creatinine level was significantly low, and estimated glomerular filtration rate (eGFR) was significantly high in (CT+TT) carriers as compared with CC carriers. In male subjects, however, there was no significant difference in serum creatinine or eGFR between the C and T genotype.

Conclusion: *PRR* SNP might affect renal function in Japanese women.

Keywords (pro)renin receptor, SNP, creatinine, eGFR

The renin-angiotensin system (RAS) is a key regulator of systemic and renal hemodynamics. RAS plays an essential role in the regulation of blood pressure and electrolyte balance[1–3]. RAS is also involved in cell growth, fibrosis, and inflammation in cardiovascular and renal tissues as locally produced and locally acting factors[1–3].

(Pro)renin receptor (PRR), a specific receptor for renin and prorenin, was identified as a member of RAS by Nguyen *et al.*[4] PRR is a 350 amino-acid protein with a single transmembrane domain, and is widely expressed in various tissues including the heart, kidney, and brain[4–6]. The binding of renin and prorenin to PRR triggers increased biosynthesis of angiotensin I, a precursor of angiotensin II, from angiotensinogen, and thereby intracellular ERK is activated by angiotensin II[7]. In addition, activation of PRR by renin and prorenin induces expression of TGF-β1, plasminogen activator inhibitor-1, fibronectin, and collagen 1[8].

The *PRR* gene is located on chromosome Xp11.4 in humans[4] and several single nucleotide polymorphisms (SNPs) of the *PRR* gene have been found. Hirose *et al.*[9] reported in the Ohasama study that the IVS5+169C>T (rs5918007) polymorphism of the *PRR* gene was associated with 24-h ambulatory blood pressure (BP) in Japanese men. Ott *et al.*[10] reported that this polymorphism was associated with BP in Caucasian men. However, there are no reports of an association between this *PRR* gene polymorphism and renal function in humans. We investigated an association between the rs5918007 *PRR* gene polymorphism and renal function in Japanese healthy subjects.

Materials and Methods

Study subjects

This study enrolled 472 Japanese subjects who underwent health check-ups at Nagoya University Hospital. The general characteristics of the subjects were as follows: they comprised 288 men and 184 women. The mean age was 50.7±12.9 (SD) years for males, and 48.2±12.6 (SD) years for females. Almost all the laboratory parameters were within the normal range for Japanese (**Table 1**). Written informed consent was obtained from all the subjects, and the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. The subject enrollment was approved by the Eth-

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Table 1. Clinical Characteristics of Subjects

		Males	Females	*p* value
n		288	184	
Age	(year)	50.7±12.9	48.2±12.6	0.042
Height	(cm)	169.5±6.1	156.6±5.9	<0.001
Body weight	(kg)	67.7±9.2	52.7±8.1	<0.001
BMI	(kg/m^2)	23.5±2.7	21.5±3.2	<0.001
Body fat ratio	(%)	18.8±5.7	23.8±6.2	<0.001
Waist circumference	(cm)	82.2±8.1	69.5±8.0	<0.001
Systolic BP	(mmHg)	124.9±15.8	119.7±19.3	0.002
Diastolic BP	(mmHg)	80.8±11.1	73.7±11.6	<0.001
Creatinine	(mg/dL)	0.87±0.13	0.65±0.11	<0.001
eGFR	($mL/min/1.73m^2$)	76.3±13.4	78.3±15.2	0.139
Albumin	(mg/dL)	4.43±0.23	4.36±0.21	0.001
Fasting glucose	(mg/dL)	94.2±14.7	87.6±11.4	<0.001
Total cholesterol	(mg/dL)	203.8±34.9	201.4±39.2	0.488
Triglyceride	(mg/dL)	120.4±88.8	88.8±47.0	<0.001
HDL cholesterol	(mg/dL)	53.1±13.8	61.1±13.3	<0.001
LDL cholesterol	(mg/dL)	126.8±33.4	122.8±34.7	0.216
Uric acid	(mg/dL)	6.23±1.22	4.65±0.99	<0.001
C-reactive protein	(mg/dL)	0.112±0.266	0.085±0.188	0.233
Current smoking	(yes / no)	78 / 209	17 / 167	<0.001
Current drinking	(yes / no)	193 / 95	46 / 138	<0.001

Data are means±SD

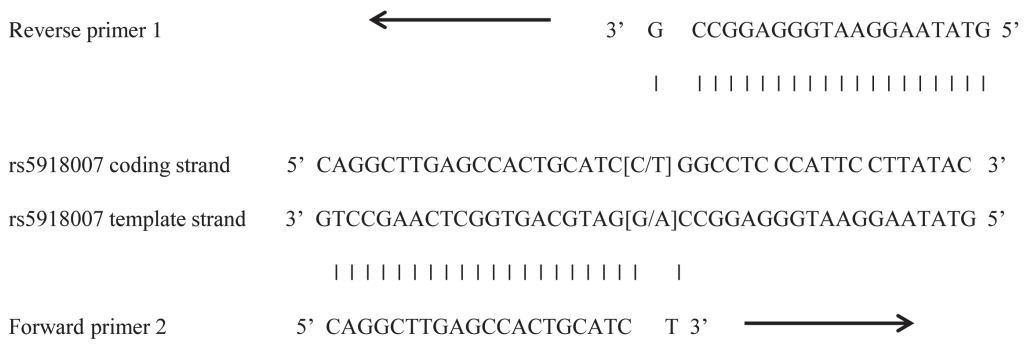

Fig. 1. Base Pairs between Primers and SNP Locus of PRR Gene

ics Committee of Nagoya University School of Medicine in 2004 for Nagoya University Hospital.

Anthropometric, laboratory measurements

Height, weight, systolic BP and diastolic BP were measured for all subjects. Body mass index (BMI) was calculated by dividing the weight (kg) by the square of height (m). The body fat percentage was measured by an electrical body-fat-percentage measuring instrument (Tanita Inc., Tokyo, Japan). Blood samples were obtained after fasting for 12 h. The following biochemical parameters were determined by standard laboratory methods based on Japan Society of Clinical Chemistry guidelines: creatinine, albumin, fasting glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, uric acid, iron and C-reactive protein. Estimated glomerular filtration rate (eGFR) was calculated by the following formula: $194 \times (\text{creatinine})^{-1.094} \times (\text{age})^{-0.287}$ (males), $194 \times (\text{creatinine})^{-1.094} \times (\text{age})^{-0.287} \times 0.739$ (females).

Genotyping of PRR SNPs

PRR rs5918007 SNPs were selected from the HapMap database. The genotyping was performed using a polymerase chain reaction with confronting two-pair primers (PCR-CTPP) assay[11]. The SNP locus of the PRR gene is shown in **Fig. 1**. Four primers (two forward and two reverse primers) were used for the PCR-CTPP assay, and PCR products were made by these primers.

Fragments with 334bp are produced between the F1 and R2 primers. If subjects have the C allele, 131bp fragments are produced between the F1 and R1 primers. If subjects have the T allele, 246bp fragments are

produced between the F2 and R2 primers. Genotyping can be determined by detecting these fragments (**Fig. 2**).
Confronting pairs of primers are as follows:
rs5918007:
5' CAGGCTTGAGCCACTGCATC(C/T)GGCCT CCCATTCCTTATAC 3'

Forward primer 1: 5' GACAGGGTCTCACTCTGTTG 3'
Forward primer 2: 5' CAGGCTTGAGCCACTGCATCT 3'
Reverse primer 1: 5' GTATAAGGAATGGGAGGCCG 3'
Reverse primer 2: 5' GATCTATGACCTCTGGCTTGTTTG 3'

The region containing this polymorphism was amplified by PCR with these primers, with the initial denature at 95° C for 5 min followed by 35 cycles at 95° C for 1 min, at 64° C for 1 min, at 72° C for 1 min and additionally at 72° C for 5 min. The products were visualized on a 2% agarose gel by electrophoresis with ethidium bromide staining. Genotyping was performed as follows: 334, 131 bp for the C genotype and 334, 246 bp for the T genotype in male subjects ; 334, 131 bp for CC genotype, 334, 246, 131 bp for CT genotype, and 334, 246 bp for TT genotype in female subjects (**Fig. 3**).

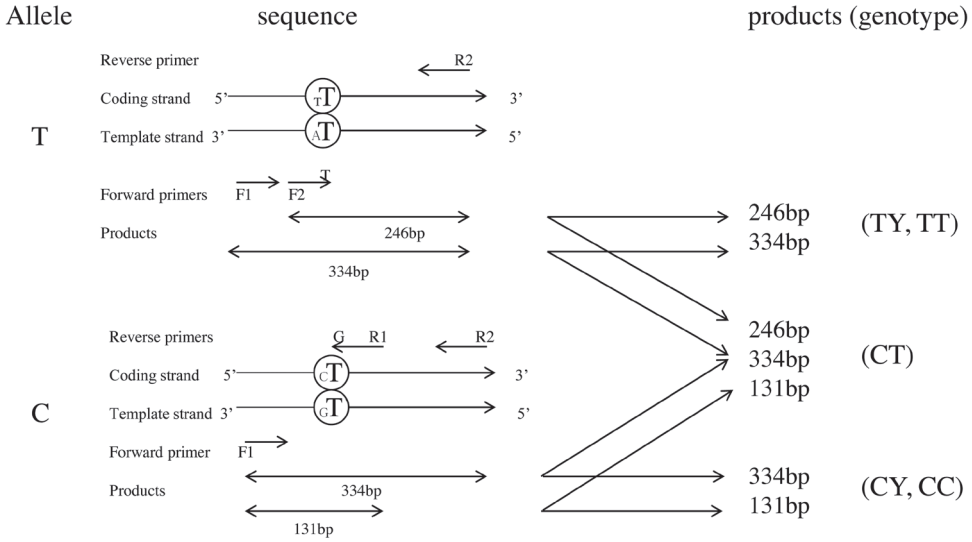

Fig. 2. PCR-CTPP Assay

Reverse primers are depicted above the coding DNA strand of PRR gene, forming base pair for T and C allele. Forward primers are depicted under the template DNA strand of PRR gene, forming base pair for T and C allele. Further, PCR products made by forward and reverse primers are depicted at the bottom of each line for T and C allele.

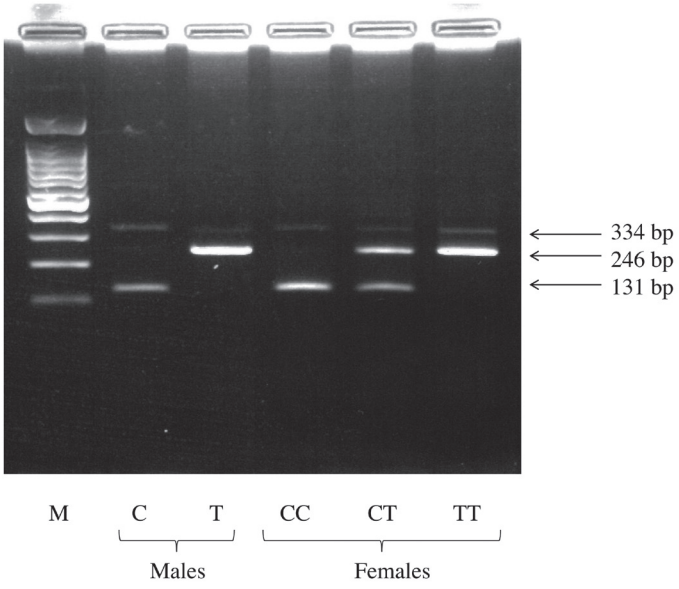

Fig. 3. Gel Showing Genotypes for *PRR* Gene rs5918007 SNPs

Lane M contains a 100 bp DNA ladder, lane C shows the C genotype in males (334, 131 bp); lane T shows the T genotype in males (334, 246 bp); lane CC shows the CC genotype in females (334, 131 bp); lane CT shows the CT genotype in females (334, 246, 131 bp) and lane TT shows the TT genotype in females (334, 246 bp).

Statistical analysis

All results are expressed as mean ± SD, and significance was defined as a p value of <0.05. The analysis was conducted using PASW statistics 20 (SPSS Japan Inc., Tokyo, Japan). Hardy-Weinberg equilibrium testing was performed using the χ^2 test. Student's t test and multiple regression analysis adjusted for age, current smoking and current drinking were performed in a comparison of the mean values between the different genotype groups. The incidence of current smoking and current drinking was compared using the Mann-Whitney U test.

Results

Incidence of PRR SNPs

Table 1 shows the clinical characteristics of the subjects. In this study, the genotype frequencies of the rs5918007 polymorphisms were 11.1% for C (n=32) and 88.9% for T (n=256) in males, and 79.3% for CC (n=146), 18.5% for CT (n=34), and 2.2% for TT (n=4) in females. The frequency of the C allele was 0.111 (males) and 0.114 (females). The allele frequency was in compliance with Hardy-Weinberg equilibrium in females (χ^2=1.37, p=0.24), which showed that there had been no bias in subject selection, and that the genotyping had been correctly performed. The incidence of hypertension or diabetes was not significantly different between the alleles in males or females.

Association of *PRR* gene SNPs with various variables

Table 2 shows associations of rs5918007 SNPs with several variables. In male subjects, BMI was significantly low in T allele carriers as compared with C carriers. In female subjects, serum creatinine was significantly low in (CT+TT) carriers as compared with CC carriers. The incidence of current smoking was significantly high in T allele carriers among male subjects. Performing multiple regression analysis adjusted for age, current smoking and current drinking (**Table 3**) revealed that in female subjects, serum creatinine was still significantly low in (CT+TT) carriers as compared with CC carriers.

In females, eGFR was significantly high in (CT+TT) carriers compared with CC carriers (**Table 2**). eGFR was already adjusted for age, and after adjusting it for current smoking and current drinking in multiple regression analysis (**Table 3**), eGFR was still significantly high in (CT+TT) carriers as compared with CC carriers. In male subjects, however, there was no significant difference in serum creatinine or eGFR between the C and T genotype. Also, there were no significant differences in BP between CC and (CT+TT) carriers for females or between C and T carriers for males.

Discussion

The present study was the first to demonstrate that in T allele carriers among Japanese female subjects, serum creatinine was significantly low and eGFR was significantly high. Thus, the *PRR* rs5918007 SNP might be associated with renal function in Japanese women. As already mentioned, *PRR* was previously identified as a member of RAS[4]. RAS activates angiotensin I and angiotensin II and the lattter plays a critical role not only in the regulation of GFR but also in the development of glomerulosclerosis by increasing glomerular capillary

Table 2. Variables According to Polymorphisms of PRR Gene

		Males			Females		
		C	T	*p* value	CC	(CT+TT)	*p* value
n		256	32		146	38	
Age	(year)	50.6±13.1	51.0±12.0	0.866	48.0±12.5	49.0±13.1	0.654
Height	(cm)	169.5±6.0	170.0±6.9	0.620	155.9±6.2	155.4±4.6	0.160
Body weight	(kg)	68.0±9.2	65.4±9.2	0.145	52.5±8.0	53.1±8.3	0.701
BMI	(kg/m^2)	23.6±2.7	22.6±2.4	0.033*	21.3±3.1	22.0±3.6	0.250
Body fat ratio	(%)	18.9±5.8	17.6±5.1	0.202	23.5±6.2	24.9±6.2	0.226
Waist circumference	(cm)	82.4±8.0	80.4±8.5	0.185	69.3±7.8	70.0±9.1	0.669
Systolic BP	(mmHg)	124.7±15.1	126.3±20.6	0.677	118.8±17.7	123.3±24.5	0.198
Diastolic BP	(mmHg)	80.7±10.7	81.9±13.8	0.557	73.1±10.8	75.7±14.3	0.214
Creatinine	(mg/dL)	0.87±0.13	0.87±0.13	0.961	0.66±0.11	0.62±0.09	0.014*
eGFR	($mL/min/1.73m^2$)	76.4±13.5	76.1±13.3	0.909	77.1±15.2	82.8±14.7	0.042*
Albumin	(mg/dL)	4.43±0.22	4.44±0.25	0.768	4.36±0.21	4.37±0.20	0.776
Fasting glucose	(mg/dL)	94.0±12.2	96.0±27.5	0.691	87.4±11.4	88.4±11.4	0.645
Total cholesterol	(mg/dL)	203.7±34.1	204.5±41.4	0.908	202.1±40.0	199.0±36.1	0.672
Triglyceride	(mg/dL)	118.8±89.4	133.6±83.5	0.374	88.9±48.8	88.4±40.2	0.950
HDL cholesterol	(mg/dL)	53.1±13.9	52.5±12.5	0.809	61.3±13.7	60.1±12.4	0.637
LDL cholesterol	(mg/dL)	127.0±33.2	125.2±35.2	0.774	122.9±35.2	122.3±33.1	0.917
Uric acid	(mg/dL)	6.25±1.25	6.08±0.95	0.358	4.64±0.98	4.70±1.03	0.770
C-reactive protein	(mg/dL)	0.109±0.251	0.141±0.363	0.521	0.092±0.204	0.059±0.101	0.351
Current smoking	(yes / no)	63 / 192	15 / 17	0.008*	12 / 134	5 / 33	0.350
Current drinking	(yes / no)	174 / 82	19 / 13	0.330	32 / 114	14 / 24	0.059

*$p<0.05$

Table 3. Multiple Regression Analysis Adjusted for Age, Current Smoking and Current Drinking

	Males (C vs T)			Females (CC vs CT+TT)		
	β	*t* value	*p* value	β	*t* value	*p* value
Height	0.032	0.583	0.560	−0.095	−1.372	0.172
Body weight	−0.078	−1.329	0.185	0.045	0.602	0.548
BMI	−0.117	−1.958	0.051	0.096	1.332	0.185
Body fat ratio	−0.079	−1.372	0.171	0.092	1.222	0.223
Waist circumference	−0.092	−1.496	0.136	0.042	0.571	0.569
Systolic BP	0.053	0.929	0.354	0.069	1.042	0.299
Diastolic BP	0.065	1.136	0.257	0.064	0.706	0.366
Creatinine	0.000	−0.006	0.995	−0.203	−2.770	0.006*
eGFR**	−0.022	−0.377	0.706	0.151	2.025	0.044*
Albumin	0.034	0.622	0.535	0.018	0.244	0.808
Fasting glucose	0.043	0.729	0.466	0.016	0.222	0.824
Total cholesterol	0.022	0.377	0.706	−0.055	−0.842	0.401
Triglyceride	0.023	0.383	0.702	−0.031	−0.425	0.672
HDL cholesterol	0.025	0.435	0.664	−0.044	−0.590	0.556
LDL cholesterol	0.000	0.000	1.000	−0.032	−0.487	0.627
Uric acid	−0.022	−0.364	0.716	−0.011	−0.150	0.881
C-reactive protein	0.034	0.575	0.566	−0.071	−0.959	0.339

β: standardized partial regression coefficient, *$p<0.05$, **adjusted for current smoking and current drinking

pressure[2]. The *PRR* rs5918007 SNP might modify activity of the *PRR* gene, and could affect renal function in Japanese women.

This SNP did not affect renal function in male subjects and the discrepancy between males and females in this regard might be due to its localization on the X chromosome. In this study, we compared findings for the C and T allele in males, and those for CC and (CT+TT) alleles in females. The (CT+TT) group consisted mostly of CT carriers, with few TT carriers. The difference in results between males and females might be due to the different genotypes in them. Regarding another possibility, sex hormones, such as testosterone and estrogen, have been reported to affect the activity of RAS[12] in different ways. Active renin might act as an antagonist to PRR-induced activation of prorenin on PRR, because both prorenin and renin bind to PRR competitively and therefore, high renin activity might attenuate the effect of PRR-activated prorenin on renal function. Therefore, the male subjects in the present study might have had higher renin activity than female subjects, which could explain the difference in the effect of active PRR on renal function between the genders.

The *PRR* rs5918007 SNP is localized in the intron of the PRR gene and might affect transcriptional activity, although there have been no reports of this. Ott *et al.*[10] reported that this polymorphism was associated with BP in Caucasian men and in the Ohasama Study, Hirose *et al.*[9] showed that it was associated with ambulatory BP in Japanese men. In contrast, the present study on the *PRR* rs5918007 SNP did not find any significant difference in BP between CC and (CT+TT) carriers among females or between C and T carriers among males. Systolic BP was lower in the present study as compared with that in the Ohasama study (males: 124.9 mmHg vs 136.6 mmHg, females: 119.7 mmHg vs 130.0 mmHg). The incidence of hypertension was also lower in the present study (males: 14.6% vs 51.0%, females: 8.7% vs 35.6%). The average age was also lower (males: 50.7 years vs 61.1 years, females: 48.2 years vs 58.7 years). The discrepancy between the present study and the Ohasama study might be due to differences in the backgrounds of the subjects such as with regard to BP and age.

The present study revealed that the *PRR* rs5918007 SNP affected renal function in women, although there was no significant difference in BP. Thus, an angiotensin II-independent pathway and/or tissue RAS might play an important role in the regulation of renal function.

In conclusion, the present study revealed for the first time that PRR might affect renal function in Japanese women. Further study is needed to clarify a mechanism for the involvement of PRR in renal function.

The authors have no conflicts of interest to declare.

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(Received February 12, 2014 ; Accepted May 1, 2014)

Original Article

Ningen Dock International 2014 ; 2 : 9-13

Reconstruction of Lung Cancer Screening CT Images for Measurement of Coronary Calcium Scores: Comparison with Coronary Calcium Scores from ECG-gated Cardiac CT

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Abstract

Objective: The coronary artery calcium (CAC) score measured by computed tomography (CT) is a strong and independent predictor of coronary events and all-cause mortality. The purpose of this study was to evaluate CAC using reconstructed images from low dose ECG-ungated CT used for lung cancer screening and to compare the results with CAC evaluation based on ECG-gated cardiac CT.

Methods: Agatston scores for CAC were determined from reconstructed low dose ECG-ungated CT and ECG-gated cardiac CT images collected for 145 subjects (101 men and 44 women) at our institution between September 2009 and July 2013.

Results: The findings showed a good correlation between low dose ECG-ungated CT and ECG-gated cardiac CT for Agatston scores in four risk categories for coronary artery disease (CAD), with a κ value of 0.963.

Conclusion: We conclude that CAC evaluation using low dose ECG-ungated CT performed for lung cancer screening has good interscan agreement with that performed using ECG-gated cardiac CT and is reliable for stratification of examinees into CAD risk categories.

Keywords Coronary artery calcium (CAC), low dose ECG-ungated CT, lung cancer screening, coronary risk factor

The coronary artery calcium (CAC) score measured by computed tomography (CT) is a strong and independent predictor of coronary events and all-cause mortality[1,2]. CAC scores determined from CT for lung cancer screening have also been shown to be clinically important, with visual assessment of CAC found to be predictive of cardiovascular death[3] and to contribute to risk stratification for coronary artery disease (CAD)[4]. In addition, populations undergoing lung cancer screening have risk factors for CAD, including old age and smoking history. Thus, it is desirable to screen for both CAD and lung cancer in one CT examination. We hypothesized that with an optimized protocol, modified low dose ECG-ungated CT (mLDCT) would be sensitive in the detection of CAC and that the CAC scores determined with it would have good concordance with those obtained with dedicated cardiac CT in health check-ups. The purpose of this study was to evaluate CAC using reconstructed mLDCT images and compare the results with those for ECG-gated cardiac CT (CACT) regarding Agatston scores for CAC[5], which are generally used to stratify patients according CAD risk.

Methods

The subjects were 101 men and 44 women without a history of major CAD (i.e. myocardial infarction, unstable angina pectoris) who underwent low dose ECG-ungated CT for lung cancer screening with optimization of reconstruction (mLDCT) and CACT for estimation of coronary artery calcium by the Agatston Method between September 2009 and July 2013 at our institution. The interval between the mLDCT and CACT scans was less than 3 months. The absence of a history of major CAD was confirmed from responses to a written questionnaire completed by each subject. All of this study

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was performed retrospectively by collecting and analyzing data from individual records. Hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg or diastolic blood pressure (DBP) of ≥90 mmHg without medication or on antihypertensive medications. Hyperlipidemia was defined as total cholesterol ≥240 mg/dL or low-density lipoprotein (LDL) cholesterol ≥ 140 mg/dL and/or high TG or low HDL, or previous use of lipid-lowering medication. The cutoff points were plasma triglyceride (TG) ≥150 mg/dL for high TG and high-density lipoprotein (HDL) cholesterol <40 mg/dL for low HDL cholesterol. Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL or HbA1c (NGSP) ≥6.5, or use of medication for diabetes. The study was approved by the Institutional Review Board of our hospital and informed consent was obtained from all subjects.

CT protocol

All subjects underwent two consecutive CT examinations with a multidetector CT system (Acquilion 64; Toshiba Medical Systems, Tochigi, Japan). CT scans were acquired during one deep inspiratory breath hold, without the use of contrast medium. The scanner was calibrated on air daily to allow reliable measurements and comparison between the imaging studies. The technical parameters for low dose ECG-ungated CT (before reconstruction) and CACT are shown in **Table 1**. In mLDCT, non-overlapping 3.0 mm datasets were reconstructed, which is the standard method used in clinical practice based on electron beam CT[6]. It is well documented[6,7] that the use of slice thickness and overlapping reconstruction have a major influence on CAC scoring, and we therefore used the same reconstruction protocol as that for CACT.

CAC Assessment

Reconstructed images were transferred to a post-processing workstation for analysis. CAC scoring was performed with commercial software (ZIO station, Tokyo, Japan). The two sets of CT scans were evaluated by observers in a blinded manner. The threshold for CAC scoring was set at a CT attenuation value of 130 H indicating potential calcification. A region of interest was circled manually for each coronary artery and a computer-driven measurement of the lesion area was automatically highlighted as the individual volume of a lesion.

The amount of coronary calcium can be quantified non-invasively using CT and calculating the Agatston score[5] or such parameters as the volume score[8] or calcium mass[9]. Among them, we chose the Agatston score because various large-scale clinical risk stratification studies have been based on it[1,10,11]. The Agatston score was obtained by multiplying the pixel area by the density score (1: 130–199 H; 2: 200–299 H; 3: 300–399 H; 4: >399 H) and summing the lesion scores[5]. Separate scores were calculated for the left main coronary artery, right coronary artery, left circumflex coronary artery, and left anterior descending coronary artery[12]. The scores were rounded and classified in a binary manner indicating the absence (Agatston score, 0) or presence (Agatston score, >0) of CAC. The scores were also placed into four categories (0, 1–100, 101–400, and >400) for CAD risk stratification[13,14].

Statistical analysis

Mean, standard deviation and median values were calculated for the Agatston scores obtained with mLDCT and CACT. A Wilcoxon signed ranks test was used to examine the significance of differences between mLDCT and CACT scores and between changes in Agatston scores. Correlations of Agatston scores from CACT with those from mLDCT (all considered to be continuous variables) were examined by Spearman correlation analysis. Agreement in CAD risk categorization based on four ranges of Agatston scores from mLDCT and CACT was assessed using the kappa value. A p-value of <0.05 was considered significant. Statistical analysis was performed using SPSS for Windows, version 13.0 (Chicago, IL).

Table 1. CT Acquisition Parameters

	Low dose ECG-ungated CT		Coronary CT
	Original	mLDCT	
Scan type	spiral	spiral	conventional half
ECG-gated	non-gated	non-gated	mid-diastole or end-systole
Rotation time (s)	0.5	0.5	0.23
Collimation	16 × 1	16 × 1	16 × 1
kVp	120	120	120
Reference mAs	30–35	30–35	46–69
Recon kernel	FC03	FC03	FC03
Slice thickness (mm)	5	3	3
Recon increments (mm)	5	3	3
Field of view (mm)	300–400	260	260

Results

A total of 145 subjects (101 men and 44 women) were enrolled in the study. **Table 2** shows their clinical characteristics. CAC was detected in 39 cases (26.9%) in mLDCT and 56 cases (38.6%) in CACT. The median (range) Agatston score was 49.4 (0–1,377) in CACT and 42.3 (0–1,394) in mLDCT. The binary findings for CAC scoring in mLDCT and CACT are shown in **Table 3**. All subjects with a positive Agatston score in mLDCT had a positive Agatston score in CACT. The sensitivity, specificity, positive predictive value and negative predictive value of mLDCT were 69.6%, 100.0%, 100.0%, and 84.0 %, respectively.

Agatston scores from CACT and mLDCT had a good correlation ($r = 0.964$, $p < 0.001$, $y = 1.471x + 2.024$; **Fig.1**). The concordance of scores from mLDCT and CACT in the four risk stratification categories for CAD is shown in **Table 4**. Of the 145 subjects, 26 were placed in a lower category based on the score from mLDCT compared to that based on CACT. A similar comparison after compensation using a linear approximation is shown in **Table 5**. After compensation, the κ value improved from 0.643 to 0.963, indicating that the linear approximation improved the concordance of Agatston scores from mLDCT and CACT. There was thus good concordance between the categorizations based on the two sets of scores.

Table 2. Clinical Characteristics of 145 Subjects Enrolled in Study

Characteristics	No (%) or mean ± SD
Age (years)	58.9 ± 10.6
Sex (male)	101 (69.7)
Body weight (kg)	65.8 ± 13.0
Body mass index	23.8 ± 3.3
Blood pressure (mmHg)	126.0 ± 16.6
Total cholesterol (mg/dL)	214.8 ± 35.7
LDL cholesterol (mg/dL)	123.7 ± 32.8
HDL cholesterol (mg/dL)	58.1 ± 15.2
Triglyceride (mg/dL)	120.8 ± 72.1
Fasting glucose (mg/dL)	104.1 ± 18.5
HbA1c (%)	5.9 ± 0.6
Risk factors	
Hypertension	61 (42.1)
Diabetes mellitus	22 (15.2)
Dyslipidemia	84 (57.9)
Current smoker	23 (15.9)
Ex-smoker	57 (39.3)
Family history of coronary artery disease	30 (20.7)

Table 3. Numbers of Subjects with Absence or Presence of CAC Using Two CT Techniques

	mLDCT	
CACT	Absence	Presence
Absence	89	0
Presence	17	39

Table 4. Numbers of Subjects in Four Agatston Score Categories for CAC by Two CT Techniques

	mLDCT				
CACT	0	1–100	101–400	>400	Total
0	89	0	0	0	89
1–100	17	18	0	0	35
101–400	0	4	11	0	15
>400	0	0	5	1	6
Total	106	22	16	1	145

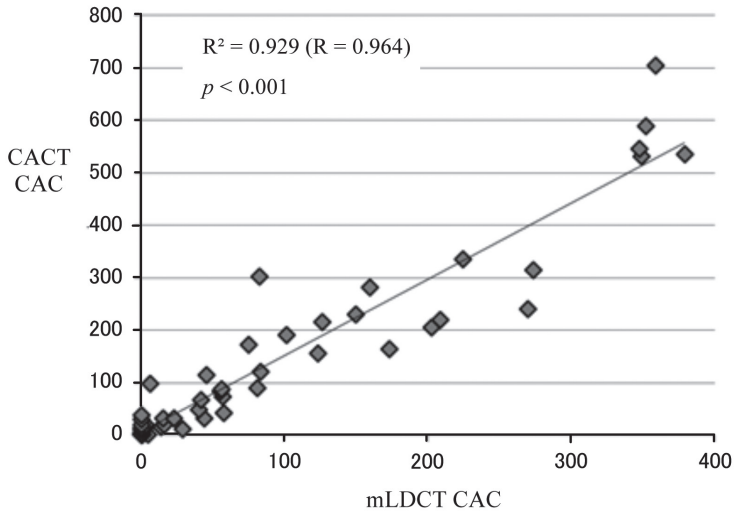

Fig.1. Interscan Correlation between Agatston Scores for CAC from mLDCT and CACT

After applying the generalized ESD test to detect the outlier of the Agatston score for this study (score of 1377 in CACT was outlier), we evaluated concordance between mLDCT and CACT scores.

Table 5. Numbers of Subjects in Four Agatston Score Categories for CAC by Two CT Techniques After Compensation Using Linear Approximation

	mLDCT				
CACT	0	1–100	101–400	>400	Total
0	89	0	0	0	89
1–100	0	34	1	0	35
101–400	0	1	13	1	15
>400	0	0	0	6	6
Total	89	35	14	7	145

Discussion

The main finding of this study was that use of mLDCT for the detection and quantification of CAC in lung cancer screening permits assignment of subjects to Agatston score categories for CAD risk stratification. These results support the idea that CAC scoring using low dose ECG-ungated CT is useful for assessment of CAD risk in persons undergoing lung cancer screening.

Several studies have evaluated CAC using ECG-ungated CT. Using the same reconstruction protocol as that in lung cancer screening (5 mm slice thickness), Sverzellati *et al.*[15] found that a cutoff value for CAC of >400 was a better predictor of cardiovascular events than pulmonary function. Wu *et al.*[16] found good agreement between CACT and low dose ECG-ungated CT for lung cancer screening, with a κ value of 0.89, similar to the result in our study. Our current results suggest that CAC scoring using low dose ECG-ungated CT is a useful and reliable tool for assessing the risk of CAD in a Japanese population, similar to the finding in a population of different ethnicity in the previous study by Wu *et al.*[16]. In addition, use of CT for lung cancer screening is increasing because randomized control trials have shown that it can reduce lung cancer mortality by up to 20%[17,18]. Thus, low dose ECG-ungated CT can be used for simultaneous screening for lung cancer and CAD in high-risk populations.

It has been well documented[6,7] that CT conditions including radiation dose, slice thickness and reconstruction method have a major influence on CAC score. Therefore, in this study, we used the same reconstruction protocol as that for CACT, non-overlapping 3.0 mm thickness and field of view of 260 mm, and evaluated CAC scores using the Agatston method. Our findings suggested that CAC scores determined by this method may be more accurate than those obtained by visual estimation. We believe that they will contribute to the standardization of CAC screening using low dose CT for lung cancer screening, and facilitate multicenter clinical trials to establish a relationship between CAC score and prognosis in Japanese populations.

The limitation of this study was that CAC could not be detected in 11.7% of the subjects using mLDCT. By CACT, while Agatston scores for these subjects ranged from 0 to 31.29, they were mainly <10, indicating that they were low risk. Compensation using a linear approximation improved agreement between mLDCT and CACT regarding assignment of scores to CAD risk categories, indicating that subjects with high CAC scores can be evaluated using the mLDCT approach. Therefore, while CACT can continue to be the reference standard for obtaining accurate CAC scores in individual patients and monitoring of CAC over time, low dose ECG-ungated CT is adequate for CAD risk stratification in screening.

In conclusion, CAC scoring based on low dose ECG-ungated CT in lung cancer screening is reliable and has good agreement with CACT for stratification of participants into CAD risk categories. Thus LDCT could be a potentially valuable tool for assessment of cardiovascular risk in large screening populations at a substantially reduced radiation dose. Lung cancer screening using low dose ECG-ungated CT is common in Japan and CAC scores obtained through its use should prove to be very useful in assessment of cardiovascular risk.

Conflict of Interest

The authors have no conflict of interest to declare.

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(Received January 28, 2014 ; Accepted May 1, 2014)

Original Article

Ningen Dock International 2014 ; 2 : 14-18

Establishing Borderline and At-risk Regions for Estimated Skeletal Muscle Mass of Legs Determined with a Body Composition Meter

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Abstract

Objective: Sarcopenia, in which decreases in muscle mass occur, may cause walking and motor function disorders among elderly individuals. We noninvasively measured lean skeletal muscle mass of the legs in healthy individuals. Results were corrected with the square of height to determine a corrected leg muscle index (CLMI). Standard deviations (SDs) were determined according to sex and age group and used to establish borderline and at-risk regions for use in preventing sarcopenia.

Subjects: The study participants comprised 5,197 healthy men (mean age, 52±11 years) and 2,237 healthy women (mean age, 50±11 years).

Methods: Leg scores were determined with a body composition meter that uses bioelectrical impedance for measurement. Leg scores were used to calculate leg skeletal muscle based on standard levels in young people. The CLMI, corrected for height, was then used to: 1) evaluate age-related changes in men and women; and 2) set a borderline region as the range 1-2 SDs below the mean in subjects 20–39 years old and an at-risk region as >2 SDs below the mean. The proportions of subjects in each age group in these regions were calculated.

Results: The CLMI of persons in their 80s was 88% of that of those in their 20s. The CMLI of women in their 80s was 95% that of subjects in their 20s. Thus, age-related decline in CLMI was more prominent in men. For men, the borderline region was 5.1–5.8 kg/m^2 and the at-risk region was <5.1 kg/m^2. For women, these regions were 4.1–4.6 kg/m^2 and <4.1 kg/m^2, respectively.

Conclusion: We set borderline and at-risk regions for use in preventing sarcopenia.

Keywords sarcopenia, leg muscle, bioelectrical impedance, ningen dock

The decrease in muscle mass with age is called sarcopenia[1], which is recognized as a medical condition[2]. Once the concept of sarcopenia had been proposed, it was widely accepted as greatly contributing to decrease in walking and motor function among elderly individuals as well as associated falls, fractures, and degenerative diseases. While many general practitioners, primarily internists, have recently become aware of sarcopenia, a comprehensive picture of the condition has yet to emerge. Our knowledge of sarcopenia has been increasing thanks to many epidemiological and clinical studies conducted outside Japan[3]. Some studies have linked it not only to physical instability, but also to immunodeficiency and insulin resistance[4]. Interventions for sarcopenia may lead to new options in the treatment of arteriosclerosis and other lifestyle-related diseases, as well as the prevention of fractures[5].

Sarcopenia has also become a topic of concern in Japan, where medical professionals are increasingly stressing the need to maintain muscle mass. Procedures for identifying sarcopenia, however, have yet to be established in this country. In Western countries, where sarcopenia research is mainstream, it has been defined as appendicular skeletal muscle mass, corrected for height, >2 standard deviations (SDs) below the mean for young, healthy reference populations[6,7], but this definition has not been firmly established. As skeletal muscle mass differs among races[8], a muscle mass for ethnic Japanese people must be determined so that it can serve as a basis for identifying sarcopenia in Japan. Bioelectrical impedance analysis (BIA) was recently developed for measuring muscle mass[8–12], and as BIA

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has been demonstrated to be a reliable and appropriate method, it is widely used not only in clinics, but also in various other settings as a convenient, noninvasive measurement procedure.

Leg scores measured by BIA represent a scored value of the skeletal muscle mass of the legs as a percentage of body weight and serve as a barometer of whether there is sufficient leg muscle to support the body. In view of the continuing aging of society, leg muscle evaluation in elderly people has become more important. To further understanding in this area, we calculated leg scores using a BIA-based body composition meter. Corrected leg muscle index (CLMI) values, corrected for height, were then used to: 1) evaluate age-related changes in men and women, and 2) set a borderline region as the range 1-2 SDs below the mean in subjects 20–39 years old and an at-risk region as >2 SDs below the mean. Finally, the proportions of subjects in each age group within in these regions were calculated.

Subjects

The study subjects were 5,197 healthy men (mean age, 52±11 years) and 2,237 healthy women (mean age, 50±11 years) who underwent a comprehensive health check-up at our medical center from April to December 2013. Informed consent was obtained from all subjects.

A profile of the subjects is shown in **Table 1**.

Methods

Leg scores were measured as follows with the subject in a standing position using a DC-250 body composition meter (Tanita, Tokyo, Japan). The 2 measurement frequencies of 6.25 kHz and 50 kHz were used. Measurement was performed using a four-electrode arrangement by which a current was passed from an electrode at the distal end of the sole of each foot and the voltage was measured with an electrode at the proximal end. Leg scores for individual subjects were expressed as values relative to the lean skeletal muscle mass of the legs of young people 20–25 years old determined with dual-energy X-ray absorptiometry (DXA), which was taken to be 100%. Nishizawa *et al.*[13] reported that muscle mass measured by the body composition meter which we used in the present study was closely correlated with that measured by DXA (n=626, r=0.979, p<0.0001).

In greater detail, leg scores, which signify %leg muscle, were determined by first estimating leg skeletal muscle (LSM) using impedance and then dividing LSM by body weight to determine the lean skeletal mass index (LSMI).

The standard LSMI in young people (standard young LSMI; average DXA leg lean weight/average body weight) was 29.23 % for men and 25.19% for women.

Leg score = calculated LSMI/standard young LSMI × 100

As explained above, we calculated the LSMI based on leg score measurement data. Height and weight are both known to affect skeletal muscle mass and research on Western subjects has shown that these factors account for approximately 60% of muscle mass of the entire body[8] so the measured skeletal muscle mass is corrected with the square of height. We therefore divided the LSMI calculated from the leg score measurement data by the square of height to determine the CLMI.

Independently in men and women, a borderline region was set as the range from 1 to 2 SDs below the mean in subjects 20–39 years old and an at-risk region as >2 SDs below the mean.

All values are expressed as mean ± SD.

Results

CLMI is shown according to sex and age group in **Table 2**. In men, CLMI began declining at 50 years old, and the CLMI for subjects in their 80s was 88% that of subjects in their 20s. In women, CLMI began declining at 60 years old, and the CLMI for subjects in their 80s was 95% that of subjects in their 20s. This data shows that age-related decline in CLMI was more prominent in men.

In men, the range of 1 to 2 SDs below the mean in subjects 20–39 years old was 5.1–5.8 kg/m^2 and defined as the borderline region. The range beginning

Table 1. Subject Profiles

	Men (n=5197)	Women (n=2237)
Age (years)	52.1 ± 10.5	49.9 ± 11.2
Body height (cm)	170.8 ± 6.0	158.3 ± 5.6
Body mass (kg)	69.6 ± 10.2	53.1 ± 8.5
BMI (kg/m^2)	23.8 ± 3.1	21.2 ± 3.2
systolic blood pressure (mmHg)	122 ± 14	113 ± 16
diastolic blood pressure (mmHg)	77 ± 10	70 ± 11
smoking rate	25%	9%
ethanol intake(g)/week	207 ± 235	136 ± 177
Leg score	92.2 ± 7.8	95.3 ± 10.4
CLMI (kg/m^2)	6.4 ± 0.7	5.0 ± 0.4

Table 2. CLMI (kg/m^2) by Sex and Age Group

	20s	30s	40s	50s	60s	70s	80s
men(*n*)	33	531	1822	1472	1043	262	34
mean ± SD	6.50±0.58	6.55±0.71	6.54±0.70	6.42±0.68	6.18±0.66	5.84±0.48	5.78±0.84
women(*n*)	26	385	769	597	339	100	21
mean ± SD	5.07±0.48	5.01±0.42	5.07±0.44	5.06±0.42	4.93±0.43	4.91±0.38	4.81±0.50

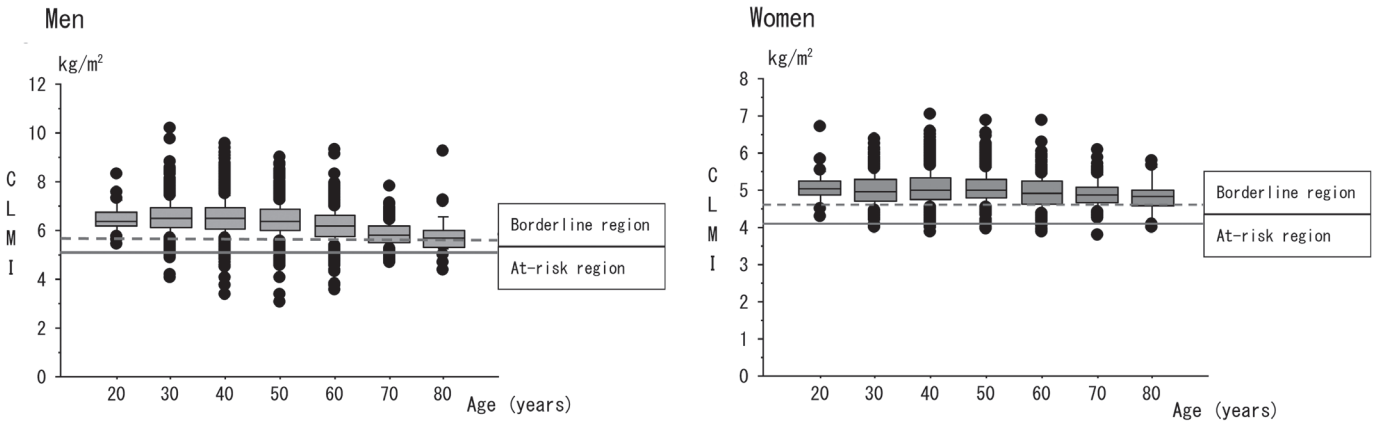

Fig.1. Corrected Leg Muscle Index (CLMI) for Different Age Groups

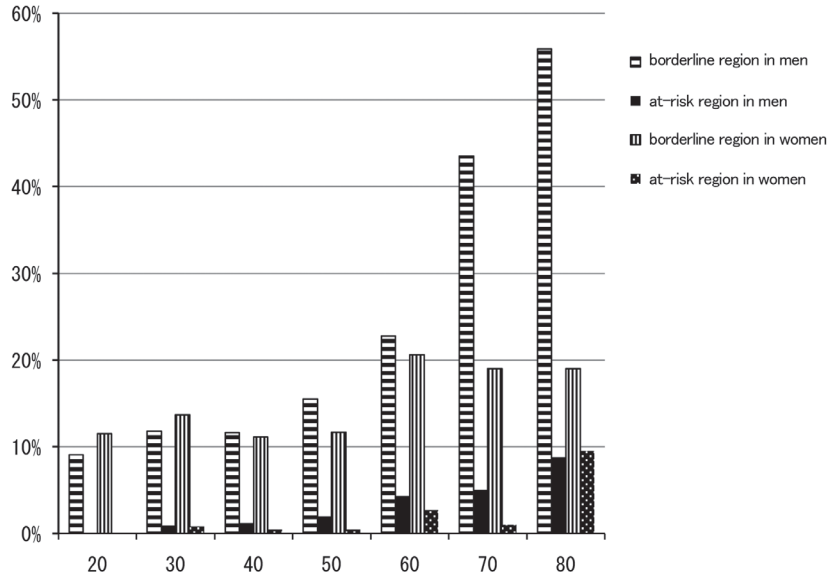

Fig.2. Percentages of Subjects in Borderline and At-risk Regions by Sex and Age Group

at >2 SDs below the mean (*i.e.*, all values <5.1 kg/m^2) was defined as the at-risk region (**Fig.1**). For women, the borderline region was defined as 4.1–4.6 kg/m^2, and the at-risk region was defined as all values <4.1 kg/m^2. The percentages of subjects in these two regions are shown by age group in **Fig.2**.

Discussion

As populations in many countries across the world age, sarcopenia has emerged in recent years as a medical term to signify the weakening of bones, joints, and muscles to the point where standing and walking become difficult, leaving sufferers bedridden or requiring long-term care, as well as an age-related imbalance

in muscle production and breakdown that results in a loss of skeletal muscle mass. To prevent the onset of sarcopenia, exercise and nutrition regimens suited to the current physical condition of the person need to be implemented well in advance.

Leg scores determined with a body composition meter, which is frequently used in health check-ups, were the primary data used for this study. They represent percentages of leg muscle relative to the mean for young, healthy individuals and are not absolute values. We calculated leg skeletal muscle mass from leg scores and then set borderline and at-risk regions for the purpose of sarcopenia prevention.

Few studies on age-related changes in skeletal muscle mass or on sarcopenia have been conducted in Japan. Sarcopenia is not defined in terms of loss of skeletal muscle mass and as skeletal muscle mass differs among races, age-related changes in the muscle mass of ethnic Japanese people must be determined.

Many methods of evaluating muscle mass are available. The procedure best suited to a particular clinical or research application is determined by the price, precision, and convenience requirements involved. Computed tomography and magnetic resonance imaging (MRI) are the gold standards for measuring skeletal muscle mass in research applications. DXA is a good alternative for research and clinical applications. BIA is used to measure body composition with and without fat mass. Inexpensive and quick, BIA is well-suited to group measurement. BIA procedures used under standard conditions have been investigated for more than a decade[14], and results correlate well with those from magnetic resonance imaging[10]. It produces appropriate measurements for adults from many ethnic backgrounds[14].

Baumgartner *et al.*[7] calculated the muscle mass of the limbs as appendicular skeletal muscle mass (ASM) using DXA, defining skeletal muscle mass index (SMI) as ASM divided by the square of height (expressed in kg/m^2). They defined the sarcopenia cutoff for men and women as an SMI >2 SDs below the mean of a young reference group of men and women. This definition of sarcopenia was closely associated with physical disability and largely independent of ethnicity, age, comorbidities, health-related behaviors, and body fat mass[15].

Tanimoto *et al.*[16] used the MC-190 multi-frequency body composition meter[17] (Tanita, Tokyo, Japan) to measure the skeletal mass of the arms, legs, trunk, and entire body of subjects in a standing position. The 4 measurement frequencies of 5, 50, 250, and 500 kHz were used. An 8-electrode arrangement was used; by which current was passed from electrodes at the distal end of the limbs and voltage was measured with electrodes at the proximal end. They studied age-related changes in skeletal muscle mass, finding that muscle mass was greater and decreased with age to a larger extent in men than in women for all sites. The decrease in muscle mass over time differed by site, and the percent decrease in muscle mass was greatest in the legs, followed by the entire body, arms, and trunk, in that order.

The effect of testosterone, which has an anabolic action and promotes muscle development, is partially responsible for the difference between men and women. In men, testosterone levels increase in puberty and result in greater muscle mass gain than in women. Men then lose a larger proportion of muscle mass as testosterone levels begin decreasing in middle age. However, some studies have found that female hormones may also be associated with muscle mass loss[8,17]. The sex-based differences in age-related muscle mass changes that have been demonstrated mean that sex must be included as a factor in evaluation of muscle mass. We therefore set borderline and at-risk regions independently for men and women and for different age groups as well.

Sanada *et al.*[18] produced reference values for sarcopenia in Japanese individuals from appendicular muscle mass data obtained from DXA. Based on the values obtained from >2 SDs below the mean for 529 Japanese men and women 18–40 years old, they defined the ASMI constituting sarcoma as ≤ 6.87 kg/m^2 for men and ≤ 5.46 kg/m^2 for women. The definitions in the present study are different because the object of measurement was only the legs.

Various limitations must be considered when interpreting the present results. First, elderly participants voluntarily underwent the health check-ups in the study and could therefore be considered relatively health-conscious. The results for our subjects might therefore be better than those obtained for the general population. Second, they lived primarily in urban areas and this may also produce values differing from the national average. Third, skeletal muscle mass was calculated by correcting leg scores for height and may therefore differ from that measured by DXA. The convenience and low cost of BIA, however, makes it applicable to obtaining data for group analysis and use in the home, so our findings are relevant to sarcopenia prevention.

Impedance measurement is particularly suited to group analysis because, unlike DXA, X-rays are not involved, the instrument is inexpensive and easy to use, and measurements are available within seconds. In addition, we believe that CLMI could be a useful measure for sarcopenia prevention and will rapidly gain wide acceptance. Our findings indicate that for men, a borderline region of 5.1–5.8 kg/m^2 and an at-risk region <5.1 kg/m^2 are appropriate. For women, the appropriate regions are 4.1–4.6 kg/m^2 and <4.1 kg/m^2, respectively.

Conflict of Interest

The authors state that they have no conflict of interest.

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(Received May 2, 2014 ; Accepted June 18, 2014)

Original Article

Ningen Dock International 2014 ; 2 : 19-26

New Low-cost Method for Detecting Abnormal Thyroid Function in Patients Making Use of a Set of Routine Tests–Testing Many More Ningen Dock Examinees and Studying Appropriate Threshold Levels

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Abstract

Objective: We developed a new, simple, low-cost screening method for detecting patients with overt thyroid dysfunction (PTDs) using a combination of six routine tests, and identified 7 new PTDs in the Ningen Dock of JR Sendai Hospital. The aim of this study was to investigate whether we can use this screening method on many more people taking Ningen Dock health check-ups and detect a lot of PTDs who should be further examined soon.

Methods: The screening was applied to 8,831 Japanese people undergoing a health check-up in the Ningen Dock of Tohoku Kousai Hospital from September 2011 to March 2013. Six types of routine test data (alkaline phosphatase, serum creatinine, total cholesterol, heart rate, lactate dehydrogenase and red blood cells), which had already been measured, were input to a personal computer.

Results: Through the measurement of serum TSH and free T4 (FT4) in 144 people who were suspected of having thyroid dysfunction in our screening (probability of >60%), we identified 14 new overt PTDs (8 Graves' disease, 2 painless thyroiditis, 4 hypothyroidism). Except for the patient with Graves' disease, none of them was suspected of having thyroid dysfunction in a medical examination. If we limit additional testing consisting of TSH and FT4 measurement to subjects with a thyroid dysfunction probability of >85%, this would greatly decrease the number of false positives and no Graves' disease patient would be overlooked.

Conclusions: The clinical usefulness of our method in screening for overt PTDs was confirmed. More than 10,000 PTDs would be discovered if this screening were performed at all Ningen Docks in Japan.

Keywords thyroid dysfunction, screening, routine tests, pattern recognition methods

There is a tendency for patients with thyroid dysfunction (PTDs) to fail to be identified[1–5]. Some thyroid specialists have emphasized that, for the early detection of PTDs, it is essential to measure serum TSH[2,5–7]. However, TSH measurement for all people visiting a clinic is expensive and including it in a full health check-up is difficult to justify in terms of cost-effectiveness[8].

We noted that PTDs usually have some abnormal routine test results. In this regard, according to the clinical practice guidelines of the Japan Thyroid Association[9], hyperthyroidism is often associated with elevated alkaline phosphatase (ALP) and lowered total cholesterol (TC), and hypothyroidism is often associated with elevated creatine kinase and TC. In view of this, assessment of the likelihood of disease from early-stage routine testing could provide important information on the possibility of thyroid dysfunction, although this would not be as good as TSH measurement. In our previous studies[10–13], we analyzed routine test data in patients with confirmed thyroid diseases and normal control subjects using three types of pattern recognition methods (PRM)[14–17] in ad-

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dition to medical statistics. We found that a set of three parameters (hypErthyroidism Combination of 3 Routine Tests (EC3RT): ALP, serum creatinine (S-Cr), TC) or a set of four parameters (hypErthyroidism Combination of 4 Routine Tests (EC4RT): ALP, S-Cr, TC, heart rate (HR)) allowed accurate screening for hyperthyroidism[10–12,18], while another set of four parameters (hypOthyroidism Combination of 4 Routine Tests (OC4RT): lactate dehydrogenase (LDH), S-Cr, TC, red blood cells (RBC)) allowed accurate screening for hypothyroidism[13,18]. In the last one, we replaced creatine kinase with LDH since the former is rarely measured as a routine test and they are closely correlated (correlation coefficient of about 0.8)[13].

Being examined under the general health check-up system, referred to as "Ningen Dock", is now mandatory for employees in Japan and more than 7 million people undergo Ningen Dock annually[7]. While people have many routine tests in it, screening thyroid tests, consisting of TSH and free thyroxine (FT4), are only performed at a limited number of institutions. Upon applying them to 2,379 Japanese people whose routine test data had already been measured in the Ningen Dock of JR Sendai Hospital, we identified 7 overt PTDs (2 Graves' disease, 2 painless thyroiditis, 3 hypothyroidism)[18–19] who then went on to treatment by thyroid specialists. None of these 7 people complained of illness.

In this paper, we report in detail on our recent screening, which was performed on many more Ningen Dock participants at Tohoku Kousai Hospital. If the clinical usefulness of this new method of screening for undetected PTDs were confirmed, its application would help to shorten the period during which patients are troubled by thyroid dysfunction and elevate their QOL. It is advantageous in terms of cost-effectiveness since it involves the secondary use of already measured routine test data and thus there is almost no additional cost. In the future, it should become part of a powerful clinical decision support system (CDSS) based on lifetime electronic medical records.

Subjects and Methods

This study was approved by Tohoku Kousai Hospital Ethical Review Board.

Subjects

The subjects were a total of 11,449 people (7,007 men, 4,442 women) who underwent a general health check-up in the Ningen Dock of Tohoku Kousai Hospital from September 2011 to March 2013. They were mostly employees, for whom an annual health check-up is mandated by law. According to the general health check-up protocol at Tohoku Kousai Hospital, they received routine tests (ALP, S-Cr, TC, LDH, RBC and HR) as well as other tests. During the period of the study, 1,543 men and 1,075 women had two health check-ups but only the data from their first one were used in the study. Consequently, the final number of subjects was 8,831 (5,464 men, 3,367 women). The mean ages were 50.5±11.3 for men and 48.6±8.8 for women.

Analyses Using Pattern Recognition Methods

The routine test data were analyzed using three types of PRM together to make the method more robust. The first method, self-organizing neural maps (SOM)[15], is a simplified model of the biological neural networks that realize feature extraction in the cortex, and also corresponds to a non-linear extension of principal component analysis in statistics. This method is superior in terms of classification capability and visualization of data distribution[10–13]. In the present study, we used the SOM_PAK package[20] after adding a facility of our own. The second method we adopted was Bayesian regularized neural networks (BRNN)[16]. This is a multi-layer neural network, but was extended to include the Bayesian probability framework for treating model parameters, to avoid defects like overfitting that are encountered in the traditional maximum likelihood approach. We used the Software for Flexible Bayesian Modeling package[21] by Neal for the BRNN. The third one we adopted was support vector machine (SVM)[17,22,23] using the LIBSVM package[22], and selected radial basis function kernels.

Implementation of Screening

Similar to our previous studies[18,19], we utilized a simple tool based on a predictive model that adopts trained model parameters and instantaneously yields classification results for examinees if their routine test (ALP, S-Cr, TC, LDH, RBC and HR) data are inputted[10–13]. We used a combination of 3 routine tests (EC3RT)[10–12,19] or 4 routine tests (EC4RT) for hyperthyroid screening, while another combination of 4 routine tests (OC4RT) was used for hypothyroid screening.

Fig. 1 shows the interface of our predictive screening tool, which displays 3 types of predictive output for an examinee for both hyperthyroidism and hypothyroidism. The calculated probabilities by BRNN and SVM are given by %. Through visual observation, we determined whether each examinee had thyroid dysfunction or not, and the degree of severity, by noting the location projected on the SOM (indicated by yellow star).

Routine tests involved measurement by the p-nitrophenyl phosphate substrate method for ALP (reference range 100–360 IU/L), an enzyme method for S-Cr (men 0.6–1.1, women 0.4–0.8 mg/dL) and TC (130–219 mg/dL), a UV method for LDH (100–230 IU/L), and an automated method for RBC (men 4.40–5.60, women 3.80–4.80 $10^6/\mu L$), performed in the same way at Tohoku University Hospital and at the Center for Health Promotion, JR Sendai Hospital, where learning data were collected to construct the predictive tool. HR

Fig. 1 CDSS Interface Used in Screening for PTDs in Health Check-up

was obtained from an electrocardiogram measured in rest and its reference range was 50–99 min^{-1}. Free T4 (FT4) and TSH were measured by the CLIA method, and their reference ranges were 0.7–1.48 ng/dL and 0.35–4.94 μIU/mL, respectively. For participants taking cholesterol-lowering drugs to treat hyperlipidemia, we used the estimated cholesterol level TC* without medication, obtained by the following formula using the measured cholesterol level TC:

$$TC^* = TC \times 1/(1 - a),$$

where the coefficient was set to 0.20 for mild statins like pravastatin sodium or 0.30 for strong statins like atorvastatin calcium hydrate[24]. If the examinee was suspected of having hyper- or hypo-thyroidism with a probability of more than 60% by SVM or BRNN, TSH and FT4 were measured with preserved blood after the health check-up. If the TSH level was more than 10 μIU/mL or less than 0.1 μIU/mL, the examinee was recommended to visit a thyroid specialist outpatient clinic[25,26]. For those who had a serum TSH of less than 0.1, the last author, who is a thyroid specialist, made a diagnosis of Graves' disease or painless thyroiditis according to the guidelines of the Japan Thyroid Association[9].

Results

Among the 8,831 examinees, it was verified from medical records that 89 (21 men, 68 women) were under treatment (or monitoring) for thyroid diseases. As shown in **Table 1**, 144 examinees were suspected of having thyroid dysfunction in the screening of 8,742 people who has no known thyroid disease. Among them, we discovered 14 new overt PTDs, and three men had mildly abnormal TSH levels (two had a serum TSH between 4.9 and 10, and one a serum TSH of 0.24 μIU/mL). **Table 2** shows FT4 and serum TSH levels as well as the results of six routine tests for these 14 patients.

Thyrotoxicosis

All of subjects 1–10 had a TSH level less than 0.01. Subjects 1 and 2 were diagnosed as having painless thyroiditis because of transient elevation of FT4 and a negative anti-TSH receptor antibody (TRAb). TRAb was positive in subjects 3–4, 6, and 8–10. Subject 5 had a negative TRAb, but there had been elevation of serum FT4 for three months so she was suspected of having Graves' disease and treated with methimazole. Subject 7 was diagnosed with Graves' disease and treated at a hospital in Fukushima Prefecture. Among 8 subjects

Table 1. Prevalence of Suspected Thyroid Diseases in Screening and Abnormal Results from Thyroid Function Tests

	Disease	Number of subjects with suspected disease (predicted probability of more than 60%)	Prevalence (%)	Number of subjects diagnosed as PTD	Prevalence (%)
Women (n=3299)	thyrotoxicosis	32	0.97	7	0.21
	hypothyroidism	14	0.42	1	0.03
Men (n=5443)	thyrotoxicosis	43	0.79	3	0.06
	hypothyroidism	55	1.00	3	0.06
Total(n=8742)		144	1.60	14	0.16

Table 2. Clinical Features of 14 Cases of Overt Thyroid Dysfunction (10 Thyrotoxicosis and 4 Hypothyroidism) Discovered in Present Screening

Subject No.	Sex	Age (years)	Diagnosis	FT4 (ng/dL) 0.7–1.48	TSH (μIU/mL) 0.35–4.94	ALP (IU/L) 100–360	S-Cre (mg/dL) m 0.6–1.1 f 0.4–0.8	TC (mg/dL) 130–219	LDH (IU/L) 100–230	RBC (10^6/μL) m 4.40–5.60 f 3.80–4.80	HR (1/min) 50–99
1	male	46	PT	1.54	<0.01	167	0.60	130	169	3.98	60
2	female	41	PT	1.76	<0.01	216	0.47	164	176	4.86	81
3	female	54	GD	3.24	<0.01	154	0.37	131	175	4.30	89
4	male	35	GD	3.30	<0.01	236	0.56	133	131	5.00	82
5	female	34	GD	3.29	<0.01	207	0.44	149	135	5.08	66
6	male	54	GD	3.56	<0.01	376	0.65	148	134	4.89	70
7	female	57	GD	3.35	<0.01	396	0.57	134	184	5.28	81
8	female	61	GD	3.65	<0.01	375	0.33	128	165	4.31	58
9	female	47	GD	2.25	<0.01	392	0.45	146	185	4.56	92
10	female	41	GD	3.78	<0.01	306	0.53	128	157	5.02	80
11	female	36	OH	0.52	384.0	230	0.81	331	238	3.60	70
12	male	53	OH	0.80	17.1	144	0.99	250	198	4.26	76
13	male	78	OH	0.97*1	18.0	181	0.87	228*2	195	3.98	64
14	male	51	OH	0.50	84.8	160	1.32	379	204	4.40	56

The symbol *1 indicates that the FT4 level was measured by another method (reference range 0.9–1.7) since measurement of FT4 in the Ningen Dock of Tohoku Kousai Hospital produced a pseudo-high level. The symbol *2 indicates that the subject is on medication for hyperlipidemia. Abbreviations: PT, painless thyroiditis; GD, Graves' disease; OH, overt hypothyroidism.

with overt Graves' disease, 4 (subjects 6–9) had a high ALP level, while in the other 4 subjects ALP was near the center of the reference range. All of these subjects except 7 and 10 had an S-Cr level below the reference range or near the lower reference limit. Two of them, subjects 8 and 10, had TC levels below the reference limit, and the 6 other subjects were near the lower reference limit. All subjects had HR levels within the reference range.

Hypothyroidism

In subject 11, all routine test levels were outside the reference ranges. Subject 14 had high TC and S-Cr levels, which were over the reference range, but the RBC level was near the lower reference limit, and the LDH level was within the reference range. Subjects 12 and 13 had high TC levels of over the upper reference limit and RBC was under the lower reference limit, but LDH and S-Cr levels were within the reference range. Subject 13 was undergoing treatment for hypercholesterolemia.

Table 3 shows the predicted results obtained by the three PRMs using EC3RT (SVM3, BRNN3) or EC4RT (SVM4, BRNN4) for subjects with hyperthyroidism, and OC4RT (SVM4, BRNN4) for subjects with hypothyroidism. Seven of the 8 subjects with Graves' disease were predicted to have hyperthyroidism with higher probability, by both SVM and BRNN, than subjects 1 and 2 with painless thyroiditis. Comparing the predicted results for females, in subjects 2–4 and 7 there was a higher probability of hyperthyroidism by EC4RT than by EC3RT. In particular, in subject 2, hyperthyroidism could be identified by EC4RT, but was overlooked if we used EC3RT. Excepting subject 3, none of the 14 subjects identified to have thyroid problems in the present screening would have been discovered by a physician using their history or a physical examination during Ningen Dock.

In subjects 11 and 14, who had FT4 levels under the lower reference limit, hypothyroidism was predicted to be highly likely by both SVM and SOM, as compared with subjects 12 and 13. Subject 13 was predicted to have hypothyroidism by SVM, but with a low probability of 43%, when drug influence was not consid-

Table 3. Predicted Probability (%) for 10 Patients with Overt Thyrotoxicosis and 4 Patients with Overt Hypothyroidism Listed in Table 2

	Subject	Sex	FT4 (ng/dL)	TSH (μIU/mL)	SVM4 (%)	BRNN4 (%)	SVM3 (%)	BRNN3 (%)
hyperthyroid	1	male	1.54	<0.01	80	70	72	48
	2	female	1.76	<0.01	72	87	71	98
	3	female	3.24	<0.01	99	100	98	100
	4	male	3.30	<0.01	99	100	93	90
	5	female	3.29	<0.01	62	50	90	100
	6	male	3.56	<0.01	96	98	96	96
	7	female	3.35	<0.01	97	99	92	100
	8	female	3.65	<0.01	89	96	100	100
	9	female	2.25	<0.01	99	100	98	100
	10	female	3.78	<0.01	97	99	95	100
hypothyroid	11	female	0.52	384	96	100		
	12	male	0.80	17.1	59	89		
	13	male	0.97*2	18.0	43	50		
	13*1	male	0.97*2	18.0	88	100		
	14	male	0.50	84.8	98	99		

The symbol *1 indicates that the subject is on medication for hyperlipidemia. The symbol *2 indicates that the FT4 level was measured by another method (reference range 0.9–1.7) since measurement of FT4 in the Ningen Dock of Tohoku Kousai Hospital produced a pseudo-high level.

Table 4. Frequency Distribution of Predicted Probabilities by SVM for 144 Subjects, who were Suspected of Being PTDs, and Discovered to be PTDs

Predictive rate interval	hyperthyroid (3 routine tests)		hyperthyroid (4 routine tests)		hypothyroid	
	number of predicted subjects	discovered patients	number of predicted subjects	discovered patients	number of predicted subjects	discovered patients
95–100%	14	6M (GD), 3F (GD) 8F (GD), 9F (GD)	10	4M (GD), 6M (GD), 3F (GD), 7F (GD), 9F (GD), 10F (GD)	4	14M (OH), 11F (OH)
90–95%	11	4M (GD), 7F (GD), 10F (GD)	5		7	
85–90%	13	5F (GD)	6	8F (GD)	8	13M (OH)*, 12M (OH)*
80–85%	12		8		7	
75–80%	7		3	1M (PT)	8	
70–75%	9	1M (PT), 2F (PT), M (slightly low TSH)	5	2F (PT), M (slightly low TSH)	8	M (slightly high TSH)
65–70%	1		7		9	
60–65%	2		4	5F (GD)	2	
0–60%*1	6		27		16	M (slightly high TSH)
Total	75		75		69	

For hyperthyroidism, predicted probabilities by both EC3RT and EC4RT are shown. The patient numbers of the discovered patients are the same as the numbers of the predicted subjects shown in Table 2. M, F, GD, PT and OH are abbreviations for male, female, Graves' disease, painless thyroiditis and overt hypothyroidism, respectively. Subject 13 (marked by an asterisk) was on medication for hyperlipidemia and the predicted probability was calculated after correction for this. Subject 12 (marked by an asterisk) has a predicted probability of 59% by SVM, but 89% by BRNN, so he was suspected of having hypothyroidism. Those who were suspected of being PTDs with predicted probabilities less than 60% by SVM (marked by the symbol*1), were evaluated by considering the prediction result together with those for SOM and BRNN.

ered, and the result was almost the same for SOM and BRNN. However, he could be identified as potentially having hypothyroidism since the probability of hypothyroidism markedly increased when drug influence is considered.

Among the144 subjects suspected of having thyroid dysfunction and measured for FT4 and TSH in preserved blood, 127 (1.45%) turned out to be false positives (39 men and 26 women for hyperthyroidism, 50 men and 12 women for hypothyroidism). Some of the hyperthyroidism false positive patients had other diseases; among them, there were 15 with high blood pressure, 13 with diabetes mellitus and 2 with hepatitis C, but about 60% of false positives had no diseases. Among the false positives for hypothyroidism, there were 29 patients with high blood pressure, 25 with hyperlipidemia, 4 with gastritis and 4 with diabetes mellitus, but 12 false positives had no diseases. Almost half of false positives were patients with an estimated glomerular filtration rate of less than 60 mL/min/1.73 m^2.

Table 4 shows a frequency distribution of predicted probability obtained by SVM for 144 subjects using EC3RT or EC4RT for hyperthyroidism and OC4RT for hypothyroidism. As shown by **Table 2** and **Table 4**, it was verified that subjects 3, 4, 6, 7, 9 and 10 with Graves' disease, who were predicted to have hyperthyroidism with a high probability of 90–100%, had high FT4 levels. In contrast, subjects 1 and 2 with painless thyroiditis, who were predicted to have hyperthyroidism with a lower probability of 70–75%, had lower FT4 levels. Although female subject 5 had a high FT4 level, she was predicted to have hyperthyroidism with a low probability of 62% by SVM and 50% by BRNN using EC4RT, because of a relatively low HR level of 66. However, with the EC3RT protocol, for all three PRMs, the high probability of hyperthyroidism was successfully predicted.

If TSH and FT4 were measured in subjects with a disease probability of >70 %, it would decrease the number of false positives to 64 (EC4RT+OC4RT) or 92 (EC3RT+OC4RT) subjects (0.73 or 1.05 %) and add no (EC3RT+OC4RT) or one (EC4RT+OC4RT) false negative. If we further limited additional testing of TSH and FT4 to subjects with a probability of >85%, it would decrease the number of false positives to 29 (EC4RT+OC4RT) or 45 (EC3RT+OC4RT) subjects (0.33 or 0.51 %), although two (EC3RT+OC4RT) or three (EC4RT+OC4RT) false negatives (two painless thyroiditis) would be added.

Discussion

In the present study, we successfully discovered 14 new overt PTDs (8 Graves' disease, 2 painless thyroiditis, 4 hypothyroidism) using a set of routine tests that had already been carried out in the Ningen Dock of Tohoku Kousai Hospital. This finding is similar to the one in our previous screening at JR Sendai Hospital[18,19], and thus the clinical usefulness of our new screening method was confirmed. Only one of the Graves' disease patients was suspected of having thyroid dysfunction by a physician in a medical examination. This indicates that a physical examination alone is inadequate for identifying PTDs, except for those with serious thyroid dysfunction, and the measurement of serum TSH is essential[2,5–7]. Accordingly, many PTDs are likely to be overlooked in Ningen Dock health check-ups in Japan since TSH is not measured in almost all of them.

Recently, the American Thyroid Association and American Association of Clinical Endocrinologists jointly proposed recommendations for TSH testing in asymptomatic individuals[25,26]. However, no consensus has been reached yet and examination issues remain, among them cost-effectiveness. In view of the present situation, we consider that our screening for PTDs is useful for finding many who would otherwise be overlooked and should be further examined soon.

It has generally been reported that, compared to male PTDs, the numbers of female PTDs with hyperthyroidism and hypothyroidism are 3–4 times greater and about 10 times greater, respectively[27–29]. It has also been reported that in screening involving the measurement of TSH during a health check-up in Ningen Dock in Japan, it was found that about 2.4 times more women than men had overt hyperthyroidism, and about an equal number of women and men had overt hypothyroidism[6]. In the general health check-up program of Ningen Dock, mammography testing for breast cancer is usually performed and during it, the surgeon palpates the thyroid gland. In this screening, the number of male examinees was 1.6 times larger than that of females, while 3.2 times more women were undergoing treatment for thyroid diseases or were being monitored for them, and thyroid dysfunction was newly discovered in 6 male and 8 female patients. These results indicate that some female PTDs may have already been discovered in a previous health check-up.

Accuracy of this screening and its dependence on thresholds

In the present study, we limited additional testing of TSH and FT4 to subjects with a probability of >60% and the prevalence of the number of false positives was 1.45 %. However, on the basis of the analysis already presented, we could recommend limiting additional testing of TSH and FT4 to subjects with a probability of >85% in our (EC3RT+OC4RT) screening model (false positives of 0.51% and two false negatives of painless thyroiditis; overlooking these would yield no clinical problems).

It is unclear whether there were false negatives in this screening or not since we measured serum TSH and FT4 not in all 8,742 participants but only in 144 who were suspected of being PTDs. Actually, the number of overt PTDs discovered in this screening seems to be somewhat less than the numbers obtained in the other studies in Ningen Dock using TSH measurement in Japan[6,7] as well as in general studies[27,28,30,31], especially for hypothyroidism. However, we still think that it was important to identify 14 PTDs who had been overlooked, undiagnosed, and remained unexamined. Further studies are needed to improve our screening method.

The present screening method could be applied in areas of Japan where TSH measurement is not routinely conducted due to its cost. The screening tool for the present method (**Fig. 1**) could be applied directly or after minor modification. None of the tests involved (particularly ALP and LDH) are routinely preformed in health screenings in other places, but for this purpose, gamma glutamyl transferase can be used instead

of ALP[11], although the accuracy of screening would be about 10% lower. In addition, creatine kinase can be used instead of LDH.

Furthermore, it would be easy to incorporate the present tool in the electronic medical records system, which displays an alert if thyroid dysfunction is suspected, similar to the case in which abnormal kidney function alert is displayed when S-Cr is elevated above the upper reference limit. As shown in **Table 4**, the overall predictive ability of our method for thyrotoxicosis is higher for both EC4RT and EC3RT. In some Ningen Docks, HR physiological examinations may be difficult to conduct together with blood testing, so it is inevitable that screening will be carried out using EC3RT only.

In these circumstances, the following question arises: Approximately how many new overt PTDs will be discovered if this screening were performed at all Ningen Docks in Japan? More than 7 million individual health check-ups are conducted in Ningen Docks in Japan[7]. Considering the results of our study on screening in two Ningen Docks, it is expected that more than 10,000 new PTDs who require further examination soon (TSH > 10 or TSH < 0.1) would be discovered.

We are sure of the clinical importance of the present new screening method in view of its cost-effectiveness - using secondary data from already performed routine tests, it is almost costless - and the fact that it succeeded in discovering many PTDs who should be further examined soon. As electronic medical records that will be used throughout the life of patients will be developed in the near future, its importance will increase further if used in a clinical decision support system, and it would also improve patients' QOL.

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research (23590811) from the Japan Society for the Promotion of Science.

Conflict of Interest

There are no conflicts of interest associated with this paper.

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(Received May 27, 2014 ; Accepted June 30, 2014)

Original Article

Ningen Dock International 2014 ; 2 : 27-34

Clinicopathological Features and Outcomes of Prostate Cancer Patients Detected using Ningen Dock (annual health check-up) System and Population-based Screening in Rural Area of Japan

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Abstract

Objective: To investigate clinicopathological features and define factors predicting survival in patients diagnosed with prostate cancer by the two major Japanese screening systems.

Methods: Between 1999 and 2010, 36 (10.4%) and 310 (89.6%) patients were diagnosed with prostate cancer in Ningen Dock and population-based screening, respectively. Clinicopathological features were recorded and factors predictive of biochemical relapse-free survival (bRFS), cause-specific survival, and overall survival were investigated.

Results: The mean ages of patients detected in Ningen Dock and population-based screening were 63 and 73 years, respectively. The proportion of localized prostate cancer was higher, with borderline statistical significance, in Ningen Dock, and significantly higher in those who had been screened at least once prior to diagnosis, compared with patients diagnosed in population-based screening, and those who had not been screened prior to diagnosis. The bRFS was significantly lower in patients who were older than the median age, in those diagnosed in population-based screening, in those with PSA above the median value, in patients at advanced clinical stages, in those with poorer Gleason scores, and in patients for whom the proportion of positive biopsy cores was > 25%. In the multivariate Cox's proportional hazards model, only clinical stage and age were significant independent prognostic factors predicting bRFS.

Conclusions: In Japan, the Ningen Dock and population-based screening systems provide complementary cover for males of different ages. Both screening systems play important roles in Japan in detecting prostate cancer at the curative stage, and improving patient outcomes.

Keywords prostate cancer, PSA screening, Ningen Dock, population-based screening

Previous randomized controlled trials[1,2] indicated a positive impact of prostate-specific antigen (PSA)-based screening in the reduction of mortality caused by prostate cancer. However, the incidence of death from prostate cancer continues to increase in Japan. In 2012, the number of such deaths was estimated to be approximately 11,600[3]. Therefore, urgent countermeasures are required to reduce under-detection rates and delay in the treatment of curable prostate cancer. In Japan, two major screening systems for early detection of prostate cancer are in operation, i.e., population-based screening and screening in "Ningen Dock" annual health check-ups. The mean ages of subjects covered by the two screening systems are different.

It may be important to expand the use of both systems to cover more men at risk of prostate cancer. Establishment of an ideal screening system requires examination of the age distributions of people subjected to the two major screening systems, and the clinicopathological features of the prostate cancer detected. In the present study, we examined differences in the clinicopathological features of and treatment strategies for prostate cancer patients detected by the two screening systems in Japan and sought factors predictive of their survival.

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Patients and Methods

Between January 1999 and December 2010, 625 patients were pathologically diagnosed with prostate cancer at the Department of Urology, Tone Central Hospital, located in a rural mountainous area of Gunma Prefecture, Japan. Of these 625 patients, 403 (64.6%) were detected by PSA screening, whereas 222 (35.5%), who had urinary symptoms, were diagnosed without such screening. Of the 403 screen-detected patients, 310 (76.9%) and 36 (8.9%) had been assessed for PSA levels during population-based screening in the Tone-Numata region and in the Ningen Dock of Tone Central Hospital, respectively. PSA levels had been measured in 31 (7.7%) of the remaining patients by private physicians, in 16 of them (4.0%) in our department during follow-up of other non-urological diseases and in 10 (2.5%) by other means.

The study population thus consisted of 310 (89.6%) patients diagnosed with prostate cancer during population-based screening, in which 80% are usually aged 60 years or older, and 36 (10.4%) patients diagnosed in Ningen Dock screening, in which approximately 70% are usually aged 40 – 59 years. Clinical stage was evaluated using the Union for International Cancer Control (UICC) TNM criteria published in 2002[4]. All Gleason scores were reviewed by a single urological pathologist (K.S.) with reference to criteria proposed by the 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma[5]. The Mann–Whitney U test or the chi-squared test was used to examine the significance of differences in the clinicopathological features of prostate cancer stratified by screening system and screening history. The significance of differences in treatment strategy, stratified by screening system and clinical stage, was also investigated using the chi-squared test.

The biochemical relapse-free survival (bRFS) rate, cause-specific survival (CSS), and overall survival (OS) were estimated using the Kaplan–Meier method. The between-group significance of observed differences was tested with the log-rank test. Age, pretreatment PSA level, screening system used, positive core biopsy rate, prior screening history, clinical stage, and Gleason score were analyzed for possible associations with bRFS, CSS, and OS. The cut-off values for continuous variables (age, PSA level, and positive core biopsy rate) used in Kaplan–Meier analyses were meticulously determined by separating patients into several subgroups. Cox's proportional hazards model was used to explore the independence of various factors shown to be significant by univariate analysis. Stepwise multiple regression analysis (with forward selection) was performed to identify independent prognostic factors predicting survival. In this analysis, all clinicopathological factors were considered to be categorical variables.

The bRFS was prospectively analyzed by serial 3-point PSA increases[6] before December 2010, and by a 25% or greater increase from the PSA nadir, with an absolute increase of 2 ng/mL or more[7], from January 2011, in patients treated by concurrent endocrine therapy. The definition of bRFS after surgery was an increase in absolute PSA level ≥ 0.2 ng/mL[7]. The definition of bRFS after radiation therapy was an increase in PSA of 2 ng/mL or more from the nadir[7]. Patients developing new lesions or exhibiting disease progression, and those with any cancer-related symptoms prior to biochemical relapse, were also included in the group with biochemical relapse.

The Institutional Ethics Committee of Tone Central Hospital approved the protocol of this study, and informed consent was obtained from each subject.

Results

Age, digital rectal examination, PSA level, and proportion of positive biopsy cores, with respect to screening system, are shown in **Table 1**. The mean patient age was significantly younger, and percentage of abnormal digital rectal examinations was significantly lower in the Ningen Dock group than in the population-based screening group. The mean patient PSA level in the Ningen Dock group was not significantly lower than that in the population-based screening group. However, the mean PSA level in the population-based screening group was twice that in the Ningen Dock group. The proportion of positive biopsy cores in the Ningen Dock group was significantly lower than that in the population-based screening group.

The distribution of clinical stages and Gleason scores with respect to screening system used and screening history are shown in **Table 2**. The proportion of patients who had been screened at least once before diagnosis by the Ningen Dock system was significantly higher (69.4%; 23/36) than that by population-based screening (44.2%) (p=0.0014). The proportion of patients with localized prostate cancer (T1 – T2/N0M0) was higher, with borderline significance, and the proportion of those with locally advanced/metastatic prostate cancer (T3 – T4/N1/M1) was lower in the Ningen Dock group than the population-based screening group (p=0.061). The proportion of patients with a Gleason score of 2 – 7 (3 + 4) was significantly higher and that of patients with a Gleason score of 7 (4 + 3) – 10 was significantly lower in the Ningen Dock group than the population-based screening group. Regarding screening history, 183 patients had been diagnosed with prostate cancer at an initial screening. The proportions of T1 – T2/N0M0 and T3 – T4/N1/M1 patients among those who had never been screened prior to diagnosis were significantly lower and

Table 1. Differences in Age, Digital Rectal Examination, PSA Level, and Percentage of Positive Biopsy Cores between Patients Diagnosed with Prostate Cancer in Ningen Dock and those in Population-based Screening

Variables	Screening system		Significance
	Ningen Dock	population-based screening	
Number of patients	36	310	
Age (years old)			
Mean±S.D.	62.75±5.99	72.57±7.14	$p<0.0001$*
Median	62	73	
Range	54-76	52-94	
Digital rectal examination [n(%)]			
abnormal	7(25.0)	130(41.9)	$p=0.0481$**
normal	27(75.0)	179(57.7)	
unknown	0(0.0)	18(0.3)	
PSA level (ng ml^{-1})			
Mean±S.D.	11.90±51.61	22.32±19.27	$p=0.232$*
Median	6.47	8.48	
Range	3.8-119.3	3.0-610.0	
% of positive biopsy cores (%)			
Mean±S.D.	22.6±19.1	34.2±25.0	$p=0.0073$*
Median	13.1	27.3	
Range	5.0-80.0	4.8-100	

PSA: prostate-specific antigen, % of positive biopsy cores (%) = biopsy cores including carcinoma/all biopsy cores taken x100, *; Mann-Whitney U test, **; χ^2 test for independence

Table 2. Impact of Screening History and Screening System on Clinicopathologic Features of Patients Diagnosed with Prostate Cancer

Clinicopathologic features	Screening system		Significance	Screening history before diagnosis		Significance
	NingenDock	population-based screening		None	Once or more	
	n (%)	n (%)		n (%)	n (%)	
Number of patients	36	310		183	162	
Clinical stage						
T1a/T1bN0M0	0 (0)	1 (0.3)	$p=0.061$; T1/T2N0M0 vs. T3-T4/N1/M1	0 (0)	1 (0.6)	$p<0.0001$; T1/T2N0M0 vs. T3-T4/N1/M1
T1c/T2N0M0	32 (88.9)	231 (74.5)		117 (63.9)	146 (90.1)	
T3N0M0	3 (8.3)	61 (19.7)		55 (30.1)	8 (4.9)	
T4/N1/M1	1 (2.8)	17 (5.5)		11 (6)	7 (4.3)	
Gleason score (primary+secondary Gleason grade)						
2-6	9 (25)	46 (14.8)	$p=0.058$; 2-7 vs. 8-10	29 (15.8)	26 (16.0)	$p=0.079$; 2-8 vs. 9-10
7a(3+4)	8 (22.2)	49 (15.8)		25 (13.7)	32 (19.8)	
7b(4+3)	7 (19.4)	60 (19.4)	$p=0.044$; 2-7a vs. 7b-10	35 (19.1)	32 (19.8)	
8	8 (22.2)	98 (31.6)		56 (30.6)	50 (30.9)	
9-10	4 (11.1)	57 (18.4)		38 (20.8)	22 (13.6)	

Clinical stage defined according to UICC TNM classification published in 2002. Significance was calculated using χ^2 test for independence.

significantly higher, respectively, compared to those who had been screened at least once. Eighteen patients (5.2%) had distant metastases. The PSA level in 17 patients with metastases detected in the population-based screening averaged 149.9 ng/mL (median, 81.9, range, 8.4 – 610 ng/mL) and that in one metastatic patient detected by the Ningen Dock system was 6.05 ng/mL.

First-line treatments stratified by screening system and clinical stage are shown in **Table 3**. Patients who were treated with radical retropubic prostatectomy or radiation therapy after < 1 year of neoadjuvant androgen deprivation therapy were classified into a radical retropubic prostatectomy and radiation therapy group, respectively. Patients who were treated with androgen deprivation therapy only for > 1 year after diagnosis were assigned to an androgen deprivation therapy group. The proportion of patients undergoing androgen deprivation therapy (LHRH agonist monotherapy) was significantly lower ($p=0.0003$) and that of patients undergoing radical retropubic prostatectomy was significantly higher ($p<0.0001$) in the Ningen Dock group than the population-based screening group.

The bRFS, CSS, and OS rates with respect to clinicopathological factors are shown in **Table 4**. The 3- and 5-year bRFS rates for all participants were 80.2% and 69.2%, respectively, at a median follow-up of 40.1

Table 3. First-line Treatments within 1 year Following Diagnosis in Ningen Dock and Population-based Screening

Screening system	First-line treatment	Clinical stage T1a/T1bN0M0 n (%)	T1c/T2N0M0 n (%)	T3N0M0 n (%)	T4/N1/M1 n (%)	Total n (%)	χ^2 test
Ningen Dock	ADT (LHRH agonist monotherapy)	0 (0%)	4 (13%)	1 (33%)	0 (0%)	5 (14%)	p=0.0003
	ADT (CAB)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1 (3%)	
	RRP	0 (0%)	21 (66%)	0 (0%)	0 (0%)	21 (58%)	
	RT	0 (0%)	7 (22%)	2 (67%)	0 (0%)	9 (25%)	
	Total	0 (0%)	32 (100%)	3 (100%)	1 (100%)	36 (100%)	
population-based screening	ADT (LHRH agonist monotherapy)	0 (0%)	109 (47%)	30 (49%)	2 (12%)	141 (45%)	p<0.0001
	ADT (CAB)	0 (0%)	6 (3%)	6 (10%)	14 (82%)	26 (8%)	
	RRP	0 (0%)	65 (28%)	0 (0%)	0 (0%)	65 (21%)	
	RT	0 (0%)	47 (20%)	22 (36%)	0 (0%)	69 (22%)	
	AS	1 (100%)	1 (0%)	0 (0%)	0 (0%)	2 (1%)	
	Refused all treatments	0 (0%)	3 (1%)	3 (5%)	1 (6%)	7 (2%)	
	Total	1 (100%)	231 (100%)	61 (100%)	17 (100%)	310 (100%)	

ADT: androgen deprivation therapy, LHRH: luteinizing hormone-releasing hormone, CAB; combined androgen blockade, RRP: retropubic radical prostatectomy, RT: radiation therapy including external beam radiation therapy, permanent implant low-dose brachytherapy, and 192-Ir high-dose rate brachytherapy, AS: active surveillance, Clinical stage defined according to UICC TNM classification published in 2002

Table 4. Impact of Various Clinical or Pathological Factors on Biochemical Relapse-free, Cause-specific, and Overall Survival in Patients Diagnosed with Prostate Cancer

Variables	All n	All Event	All Median follow-up (months)	3 years n	3 years Event	3 years Cumulative rate (%)	3 years S.E.	5 years n	5 years Event	5 years Cumulative rate (%)	5 years S.E.	Significance
Biochemical relapse-free survival												
All	346	124	39	286	60	80.2%	2.3%	160	23	69.2%	2.9%	
Age range (years old)												
52-71	169	47	41	139	30	80.4%	3.3%	87	6	74.0%	3.9%	p=0.00036
72-94	177	77	37	147	30	80.0%	3.3%	73	17	64.4%	4.3%	
Screening system												
Ningen Dock	36	4	41	32	4	88.4%	5.5%	22	0	88.4%	5.5%	p=0.00426
population-based screening	310	120	37.5	254	56	79.2%	2.5%	138	23	67.1%	3.2%	
Clinical stage												
T1/T2N0M0	264	76	40	231	33	85.3%	2.4%	129	13	76.8%	3.1%	p=0.00363; T1/T2N0M0 vs. T3N0M0
T3N0M0	64	38	45.5	46	18	70.8%	5.8%	28	10	51.3%	6.8%	p<0.00001; T1/T2N0M0 vs. T4/N1/M1
T4/N1/M1	18	10	12.5	9	9	42.9%	12.8%	3	0	42.9%	12.8%	p=0.01551; T3N0M0 vs. T4/N1/M1
PSA level (ng ml^{-1})												
3.0-8.18	173	46	40	157	19	87.0%	2.8%	83	9	77.3%	4.0%	p=0.00436
8.19-610	173	78	37	132	41	73.6%	3.6%	77	14	61.4%	4.2%	
Gleason score												
2-6	55	16	55	50	5	90.3%	4.1%	33	6	75.5%	6.5%	p=0.07667; 2-6 vs. 7, 8
7,8	230	85	38	193	37	81.3%	2.8%	106	15	70.0%	3.6%	p=0.03147; 2-6 vs. 9, 10
9,10	61	23	26	43	18	65.8%	6.8%	21	2	59.8%	7.4%	p=0.13710; 7,8 vs. 9, 10
% of positive biopsy cores												
0-24%	156	41	44	138	18	86.3%	3.0%	84	7	79.0%	3.8%	p=0.00117
25-100%	189	83	33	147	42	75.1%	3.4%	75	16	60.8%	4.2%	
Cause-specific survival												
All	346	7	55	344	2	99.3%	0.5%	225	3	97.9%	1.0%	
Clinical stage												
T1/T2aN0M0	162	0	54	162	0	100.0%		106	0	100.0%		p=0.02691; T1/T2aN0M0 vs. T3N0M0
T2bN0M0	102	0	55	102	0	100.0%		62	0	100.0%		p<0.00001; T1/T2aN0M0 vs. T4/N1/M1
T3N0M0	64	4	82.5	64	0	100.0%		53	2	96.1%	2.8%	p=0.04075; T2bN0M0 vs. T3N0M0
T4/N1/M1	18	3	35.5	16	2	85.7%	9.4%	8	1	71.4%	15.2%	p<0.00001; T2bN0M0 vs. T4/N1/M1
Screening system												
Ningen Dock	36	0	42	36	0	100.0%		23	0	100.0%		p=0.41135
population-based screening	310	7	56	308	2	99.3%	0.5%	202	3	97.6%	1.1%	
Gleason score												
2-7	179	1	64	179	0	100.0%		122	1	99.1%	0.9%	p=0.03086
8-10	167	6	51	165	2	98.6%	1.0%	103	2	96.4%	1.8%	
Overall survival												
All	346	55	55	336	10	96.7%	1.0%	217	11	91.5%	1.8%	
Age range (years old)												
52-71	169	16	56	164	5	96.6%	1.5%	112	3	93.6%	2.3%	p=0.00027
72-94	177	39	55	172	5	96.7%	1.5%	105	8	89.3%	2.8%	
Clinical stage												
T1/T2N0M0	264	29	54	258	6	97.2%	1.1%	158	6	93.2%	1.9%	p=0.01437
T3-4/N1/M1	82	26	71.5	78	4	94.9%	2.5%	59	5	86.8%	4.2%	

PSA: prostate-specific antigen, % of positive biopsy cores (%)=biopsy cores including carcinoma/all biopsy cores taken x100, Clinical stage defined according to UICC TNM classification published in 2002

months (range: 1.8 – 144.7 months). The bRFS was significantly lower in patients aged 72 – 94 years, those detected in population-based screening, those at more advanced clinical stages, those with PSA levels above the median value (8.19 ng/mL), those with poorer Gleason scores, and those in whom 25% or more biopsy cores were positive. The 3- and 5-year CSS rates in all participants were 99.3% and 97.9%, respectively, at a median follow-up of 56.6 months (range: 1.8 – 147.2 months). CSS was significantly poorer in patients at more advanced clinical stages and in those with higher Gleason scores. There was no significant difference in CSS between the Ningen Dock group and population-based screening group. The 3- and 5-year OS rates were 96.7% and 91.5%, respectively. OS in patients aged above the median (72 years) and in those with locally advanced/metastatic cancer (T3–T4/N1/M1) was significantly lower compared to patients with alternative corresponding clinical features. The decreases in OS with elapsed years after intervention in patients aged above the median and with locally advanced/metastatic cancer (T3–T4/N1/M1) were more evident, compared to those aged below the median with localized cancer (T1/T2N0M0).

Table 5 shows factors independently predicting bRFS as determined by the multivariate Cox's proportional hazards model. Candidate factors included in stepwise multiple regression were PSA level, age, screening system used (Ningen Dock or population-based screening), clinical stage, Gleason score, and positive core biopsy rate, all of which were significant factors predictive of bRFS in Kaplan–Meier analysis. Only clinical stage and age were significant independent prognostic factors predicting bRFS. Multivariate Cox's regression analysis did not identify any independent factors predictive of CSS or OS.

Discussion

The intention-to-screen analysis of the Prostate, Lung, Colorectal, and Ovarian (PLCO) study[8] did not show decreased cancer-specific mortality in the screening arm compared to that in the control arm. One of the serious flaws of this study could have been uncontrollable contamination in the control arm, which was 42% within 1 year of randomization and estimated at up to 85% within 5 years of randomization[9]. Furthermore, up to 44% of the participants were prescreened by the PSA test in the 3 years before study entry. The high contamination rate in the control cohort and the high exposure rate to PSA testing prior to randomization led to a decrease in advanced stages of prostate cancer, even in the control arm, and may have affected the scientific value of the PLCO study.

In contrast, the results of other recent randomized controlled trials, including the European Randomized Study of Screening for Prostate Cancer (ERSPC)[1] and the Göteborg[2] studies, indicate that the introduction of PSA screening over the past decade has yielded significant reductions in prostate cancer mortality. The latest intention-to-screen analysis of the ERSPC[1] indicated that the rate of mortality from prostate cancer fell by 21% in the screening arm compared to controls during a median follow-up of 11 years, which could still be too short to evaluate the life-time effects of PSA screening. The impact of PSA screening in decreasing mortality increased over time and the rate of reduction was 38% between years 10 and 11 after randomization. However, the screening interval was once every 4 years in the screening arm of the ERSPC and the findings suggested that the efficacy of a fixed screening interval of 4 years is not optimal. Therefore, in the decrease in mortality observed in the ERSPC, the impact of life-time risk reduction in cancer deaths or progression to metastatic disease may have been underestimated. The screening interval was set to 2 years in the Göteborg study[2] and the intention-to-screen analysis showed that mortality from prostate cancer decreased by 44% at a median follow-up of 14 years.

As socioeconomic features of prostate cancer patients and epidemiological aspects of the disease may vary among countries, countermeasures should be based on health policies that are specific to each country. It is therefore very important to explore the epidemiological and clinical features of prostate cancer.

Table 5. Results of using Multivariate Cox Proportional Hazards Model to Assess Predictors of Biochemical Relapse-free Survival

Variables	Estimate ± S.E.	Hazard ratio (95% CI)	*p*-value
Age range	0.57±0.19	1.77 (1.22–2.57)	0.00286
Clinical stage	0.60±0.15	1.82 (1.37–2.43)	0.00004

In the stepwise multiple regression analysis, age range from 52 to 71 years old with clinical stage of T1/T2N0M0 was coded as 1.
Similarly, age range from 72 to 94 years old with clinical stage of T3N0M0 was coded as 2.
Clinical stage of T4, N1, or M1 was coded as 3.

In Japan, only 5% – 10% of men undergo PSA-based screening. In Western countries with very high screening rates, overdiagnosis and overtreatment are significant issues, whereas delays in detection and treatment are serious problems in Japan and other Asian countries. In this regard, the proportions of prostate cancer patients with distant metastases were seen to be very high (26.1% – 76.6%) in China[10–14]. Analysis of Japanese hospital-based registries indicated that the proportion of patients with metastatic prostate cancer fell from 56% to 26% between 1975 and 2002[15], but the figure remains very high. According to the nationwide cancer registry operated by the Japanese Urological Association, the proportions of metastatic prostate cancer patients were 21.3% in 2000 and 11.6% in 2004[16,17]. Only one Japanese study has compared clinical stage distributions among patients diagnosed with prostate cancer via population-based screening or in urological outpatient clinics in the pre-PSA (1981 – 1991) and PSA (1992 – 1996) eras[18]. At 58.7%, the proportion of metastatic prostate cancer patients was highest among those diagnosed in outpatient clinics in the pre-PSA era and the next highest was 46.8% for those diagnosed in such clinics in the PSA era. Of patients detected via screening, 23.4% had metastatic cancer in the pre-PSA era and 10.6% in the PSA era.

The average age of patients diagnosed with prostate cancer via the Ningen Dock system in this study was significantly lower than that of patients detected via population-based screening. In the Göteborg study[2], the mortality rate of participants assigned to a screening group, who were screened by PSA testing, was 0.36% (27/7,578). Of the 27 patients who died, 13 had never undergone a PSA test before participating in the screening study. Of these 13, 12 (92%) were between 60 and 64 years of age at the time of registration. Therefore, if the initial PSA screening exposure is after the age of 60 years, this may increase the risk of prostate cancer-specific death. About 70% of those screened under the Ningen Dock system were aged 59 years or younger.

It may be very valuable to provide Ningen Dock screening throughout Japan. The average age of 5,995 men undergoing such screening between April 2002 and June 2009 was 55.3 years, with a median age of 55.0 years. In contrast, the average age of 19,094 men who underwent PSA testing during population-based screening in Gunma Prefecture in 2009 was 67.3 years, with a median age of 67.0 years. Nationwide surveys have yielded similar data[19,20]. Therefore, in Japan, Ningen Dock screening could play a very important role in the early detection of prostate cancer in men at risk of the disease.

Previous studies have demonstrated that the proportion of localized prostate cancer in screen-detected prostate cancer ranges from 75.0% to 93.3% and from 57.8% to 85.1% under the Ningen dock system[21–24] and in population-based screening, respectively[25–28]. In the present study, the proportion of patients with localized cancer was slightly higher in the Ningen Dock group compared to that in the population-based screening group, but no significant difference in clinical stage distribution was observed between the screening systems.

The bRFS was significantly better in younger patients, those detected under the Ningen Dock system, those at earlier clinical stages, those with lower PSA levels, those with lower Gleason scores, and those with a lower proportion of positive biopsy cores. In multivariate analysis, only clinical stage and age were significant independent factors predicting bRFS. Although, no prognostic factors predicting CSS or OS were identified in the present study, our previous study revealed a significant difference in CSS between patients (mostly asymptomatic) diagnosed with prostate cancer by means of a PSA-based screening system and patients with urological symptoms diagnosed in urological outpatient clinics[29]. Further follow-up is necessary to clarify associations of CSS or OS with the screening system used for detection and various clinicopathological factors.

The present study had some limitations. First, all data were taken from a hospital-based cancer registry and analyzed retrospectively. Therefore, it may not be representative of all Japanese populations. However, there is only one regional general hospital (Tone Central Hospital) with full-time urologists in the area. Furthermore, our database contains about 80% of all screened populations in the Tone-Numata region and therefore, the present results may be representative of other rural areas in Japan. Second, Tone-Numata may be an ideal area in which to investigate long-term outcomes in patients undergoing screening because most people have lived in the same region for many years. However, the Ningen Dock and population-based screenings were conducted in an independent manner, making it very difficult to determine the long-term screening history of men screened under both systems. It would therefore be desirable to combine the data of the two screening systems.

In conclusion, it is difficult to determine which of the 2 screening systems examined is superior overall from the present retrospective study, as each covered a different community and age group. Therefore, at present, we should provide both screening systems throughout the country according to the updated Japanese Urological Association guidelines on PSA screening[30], to ensure that appropriate screening is conducted and this should include informed consent regarding PSA screening. In future, changes in the incidence of metastatic prostate cancer and mortality from cancer within our region

will be investigated using a database of data from the 2 screening systems which is linked to our hospital-based cancer registry.

Conflicts of interest

None declared.

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(Received February 13, 2014 ; Accepted July 23, 2014)

Case Report

Ningen Dock International 2014 ; 2 : 35-38

A Case of Achalasia Detected in a Health Check-up

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Abstract

We report a case of achalasia, which was identified and diagnosed through a health check-up. The patient was 24-year-old woman who in screening during a periodic employee health check-up had an abnormal finding on the right lung in a posteroanterior (PA) view chest X-ray. As the result of a thorough investigation, the patient was diagnosed with mild aspiration pneumonia and it was accompanied by suspected esophageal dilation, which was revealed by chest computed tomography (CT). The dilation was later confirmed by barium swallow and imaging. After completing the treatment regimen for aspiration pneumonia, pneumatic dilation (PD) was performed. Post-procedural examination indicated disappearance of symptoms. Also, the 24-month post-op follow-up did not indicate re-emergence of achalasia. However, long-term follow-up is still essential. Especially in young adults, it is necessary to identify the cause of aspiration pneumonia in an investigation.

Keywords achalasia, aspiration pneumonia, health check-up

Achalasia is said to occur when there is a loss of peristalsis in the distal two thirds of the esophagus, and impaired relaxation of the lower esophageal sphincter. Patients with achalasia usually complain of dysphagia, chest pain, and regurgitation. It is extremely rare for achalasia to be diagnosed as a result of repeated mild aspiration pneumonia. The intention of this paper is to address the process of diagnosing achalasia and the pitfalls of diagnosis.

Case Report

The patient was a 24-year-old woman who underwent employee occupational health screening in a health check-up. While the patient had no past history of chronic respiratory disease, she had frequently visited an emergency department (ED) in the past 9 months due to repeated episodes of occasional nighttime coughing as well as infrequent mild fever. ED examinations had indicated acute upper respiratory inflammation and as a result, the patient had been put on pharmacotherapy. She experienced 4 similar episodes within the 9 months and consequently had continued to be treated in the ED. More recently, the symptoms had started to recur at shorter intervals. In addition to having to visit the ED, in the past 6 months the patient had also experienced postprandial burning in the chest and nocturnal emesis of a mucoid-like substance. The repeated nature of the episodes caused her to visit another clinic for examination. After consideration of her history, she was given a histamine H_2 receptor antagonist. She was advised to rest and observe the effect of the medication. On the day of the health check-up, she had general malaise and a mild fever (37.5℃), though she was not interviewed about these concerns. On the same day, she underwent a PA chest X-ray (**Fig. 1**) and blood analysis revealed a white blood cell count of 10,050/mm^3. The X-ray findings suggested that she should go through a complete physical examination. She reported to our clinic for examination 9 days after the initial health check-up.

In auscultation of the posterior–central region of the right chest a moist rale was heard. There were no other abnormal general or abdominal findings. Laboratory studies revealed a white blood cell count of 8,600/mm^3 and the C-reactive protein level was elevated at 10.0 mg/dL (normal: under 0.3 mg/dL).

Examination of the PA chest X-ray (**Fig. 1**) indicated dense air opacities at the right upper lobe of the lung. Chest computed tomography (CT) (**Fig. 2**) revealed a minimally infiltrating shadow on the right middle lobe, as well as esophageal dilation. Image analysis results, notable physical findings, and laboratory values led to a diagnosis of mild aspiration pneumonia. She was provided with antibiotics as outpatient treatment and as a

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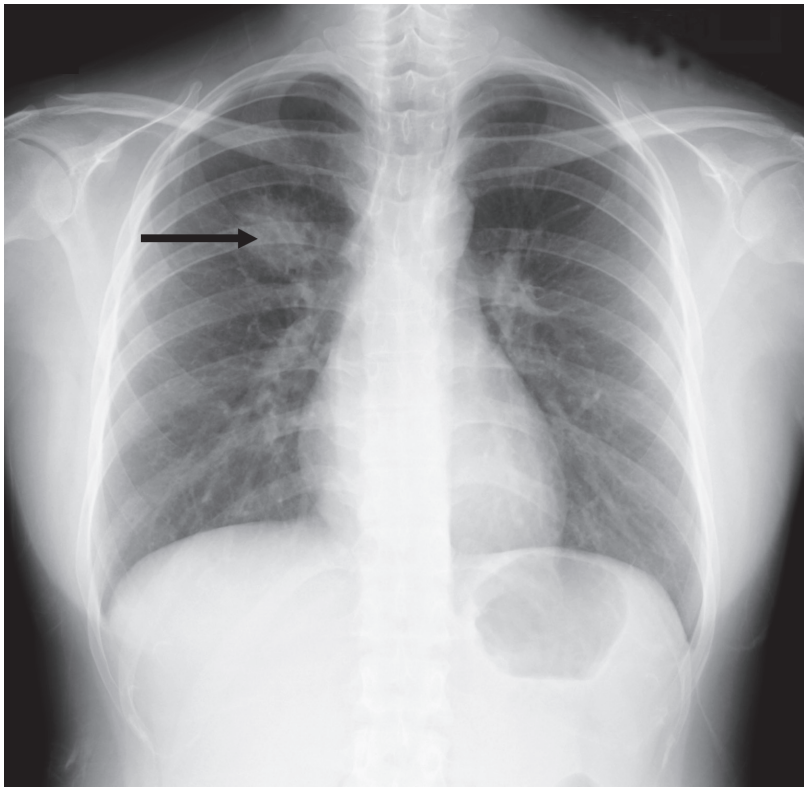

Fig. 1. Anteroposterior View of the Chest Showing Right Ground Glass Appearance (Black Arrow)

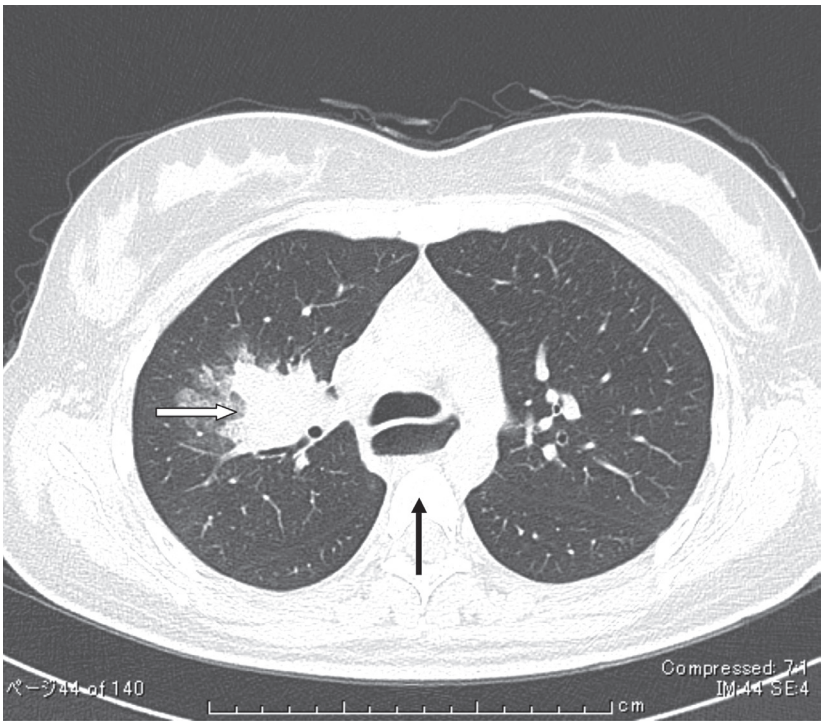

Fig. 2. Chest CT Scan Showing Right Lung Infiltrate (White Arrow) and Moderately Dilated Esophagus (Black Arrow)

result, the pneumonia was relieved after one week. Further investigation of the cause of the aspiration pneumonia based on our previous finding of esophageal dilation had led us to suspect the presence of achalasia and its symptoms as the leading cause of the aspiration pneumonia. She was advised to have a barium swallow, the result of which is shown in **Fig. 3**. According to the Japan Society of Esophageal Diseases[1] guidelines, this case of achalasia was the spindle-shaped type and grade I.

Based on these findings, she was treated using a PD (Rigiflex™ achalasia balloon dilator, Boston Scientific). There have been no signs of recurrence in the 31 months that have passed since the initial treatment.

Discussion

Long-standing achalasia presents with acute-onset aspiration pneumonia[2–4]. We performed a review of the literature based on Pub Med (1981–2012) and Japan Medical Abstracts Society (1983–2012) in order to determine the degree of aspiration pneumonia and using the key words "achalasia" and "aspiration pneumonia", found 76 articles. Only 10 cases[5–13] have been reported in the English literature and 2 cases[14,15] in the Japanese literature. These 12 cases involved severe aspiration pneumonia and highly hypomotile achalasia. The case described here is highly unusual considering that a diagnosis of achalasia has been made after such a short time interval in very few cases in the medical literature[2,3,14]. The present case is particularly rare because a diagnosis of achalasia was made following an initial diagnosis of mild aspiration pneumonia.

The patient began complaining of the initial symptoms only 9 months prior to the actual diagnosis of achalasia and furthermore, clinical findings indicating presence of aspiration pneumonia were only minimal.

In the study of Tanaka *et al.*[16], 17 out of 88 patients had received treatment for a period longer than that in our case report. The treatments were especially focused on gastric and respiratory symptoms. Respiratory symptoms including cough, sputum, and dyspnea are known to occur and are due to regurgitation of oropharyngeal secretions during sleep rather than true emesis of gastric contents[2,3]. Careful documentation of a patient's history may reveal interstitial lung disease and provide a wide differential diagnosis, and a CT finding of ground glass opacity at the lung parenchyma may be caused by something as common as aspiration[9] that af-

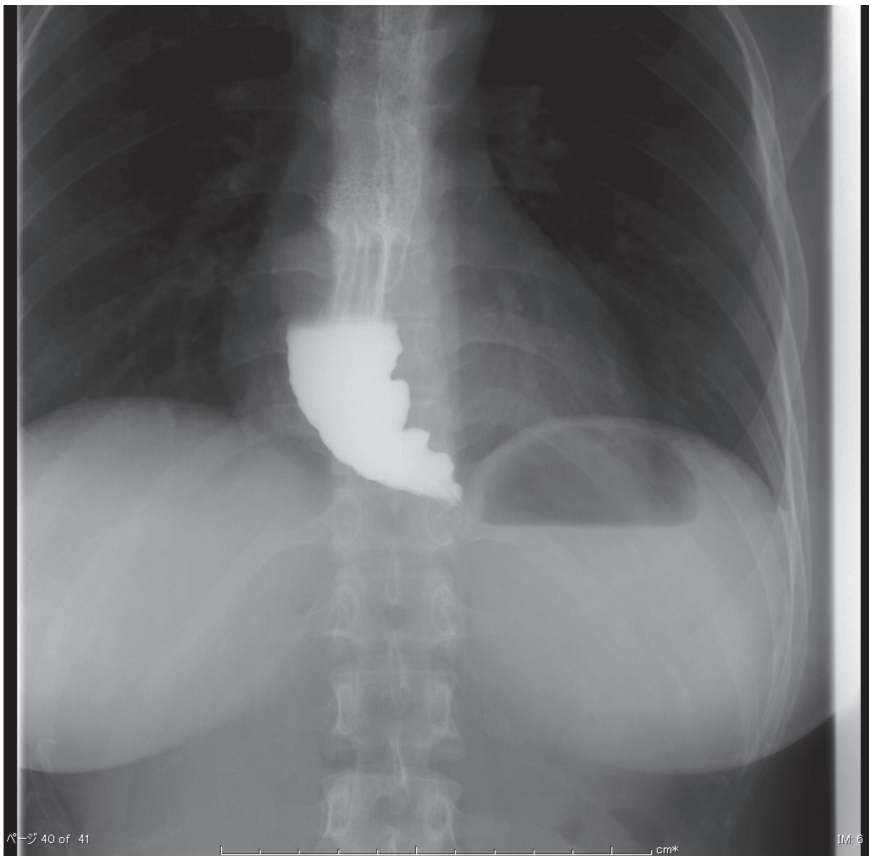

Fig. 3. Barium Esophagram Showing Dilated Esophagus with Smooth Tapering to a Narrowed Gastroesophageal Junction

fects patients of all ages and both sexes.

It is beneficial to identify the clear cause of the aspiration pneumonia and for this purpose, it should be kept in mind that it is quite possible to make a clinical diagnosis through a barium swallow[16]. Generally, the barium swallow is the most common primary diagnostic tool used.

The dilation may be described according to the two categories of type and grade[1]. In the present case, we simply analyzed CT findings to obtain the dilation size of the esophagus. An esophageal dilation size of more than 2 cm would indicate the need for a barium swallow. A diagnosis by means of endoscopy, however, would require a clear understanding of the characteristic findings for primary achalasia. Without significant training in endoscopic examination, it is a challenge for the clinician to make a proper diagnosis[1,17]. It is therefore difficult to draw up general diagnostic procedures and processes.

Recommended treatment for achalasia is primarily PD or surgical intervention and several procedures have been recommended by Kinoshita *et al.*[18]. As esophagomyotomy of any previously dilated esophagus may cause damage to the mucoid secretory function of the esophagus, they suggest an endoscopic procedure as the initial intervention, such as Heller myotomy with Dor fundoplication[19]. Tanaka, *et al.*[20], mention that in patients who are under 40 years of age, the muscular lining has often become softer and more delicate and therefore a surgical intervention may be recommended as the initial treatment. Recommendations in consideration of age suggest that PD is quite effective[18,20]. In this regard, a recent randomized trial did not show any difference in effectiveness between PD and endoscopic intervention[21]. Nonetheless, it is difficult to clearly identify the intervention that would be most suitable.

Conflicts of Interest and Source of Funding

None of the authors have any conflicts of interest to declare.

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(Received March 18, 2014 ; Accepted May 1, 2014)

Acknowledgments

We are very grateful to the following individuals who served as reviewers for the papers submitted to Ningen Dock International Vol. 2 No. 1, September 2014.
I sincerely thank their kind cooperation.

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The Regulations of the International Society of Ningen Dock

Article 1
Name
The name of the association shall be the International Society of Ningen Dock.

Article 2
Office
The Society has its principal office in Japan Society of Ningen Dock.

Article 3
Aims
The Society, an organization of Japan Society of Ningen Dock for international operations, aims to contribute to global health promotion by enhancing the development of ningen dock as a medical check-up system.

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

Article 5
Membership
1. The Society consists of the following members
   1) Regular member
      A regular member shall be a member of the International Society of Ningen Dock who agrees to the aims of the Society, and has expert knowledge, techniques, or experience in the areas associated with the Society.
   2) Supporting member
      A supporting member shall be a person, a corporation, or a group that agrees to the aims of the Society, and supports its programs.
   3) Honorary member
      An honorary member shall be recommended, from those who have significantly contributed to the areas associated with the Society, by the executive board.
2. Those who want to apply for regular or supporting membership of the Society shall submit the prescribed application form with the membership fee.
3. The board meeting will process applications mentioned in the preceding section, and promptly notify the applicants of its decision.

Article 6
Officials
1. The Society shall appoint the following honorary advisors and officials.

Honorary advisor: Number not decided
Congress president: 1
President: 1
Vice president: 3 (from Japan : 2, overseas: 1)
Board members: up to 25 (from Japan : 15 or less, overseas : 10 or less)
Auditor: 2

Article 7
Honorary advisor
1. An honorary advisor shall be appointed by the president from those who have contributed to the development of the Society for a long period, and approved by the executive board.
2. Honorary advisors shall be eligible to attend the board meeting, and to express opinions; honorary advisors will not have voting rights.

Article 8
Congress president
1. The congress president shall be recommended by the executive board and appointed by the president.
2. The congress president shall represent the Society and host the World Congress on Ningen Dock as a scientific meeting.

Article 9
President
1. The president shall be selected by and from among board members and delegated by the president of Japan Society of Ningen Dock.
2. The president shall preside the Society.

Article 10
Vice president
1. The vice president shall be appointed, from among board members, by the president.
2. The vice president shall assist the president. In the case of accident, one of the vice presidents will be appointed by the president and will temporarily take over the duties.

Article 11
Board members
1. Board members from Japan shall be selected among candidates from regular members at Japan Society of Ningen Dock.
2. Overseas board members shall be selected at the recommendation of the executive board.
3. Board members execute duties for the Society under the orders from the president.
4. Board members, together with the president and the vice president, comprise the executive board.

Article 12
Board meeting
1. The president will call a board meeting on an as-needed basis, and serves as the chairman of the meeting.
2. The board meeting will pass resolutions on important matters of the Society.
3. The board meeting shall have the right to start proceedings if the majority of all the board members (including a letter of proxy) attend the meeting.
4. The board meeting shall pass resolutions with the majority votes of attendances.

Article 13
Auditor
Auditors shall audit accounts of the Society, and report to the board meeting.

Article 14
Commissioner
For the aims of successful programs of the Society, the president will set up committees and divisions through the resolutions of the executive board, and delegate the commissioners to regular members or other members of the Society.

Article 15
Accounting
1. The fiscal year for the Society starts on April 1 every year and ends on March 31 the following year.
2. Expenses required for the Society shall be covered by the following revenues.
   1) Membership fees
   2) Grants
   3) Donations
   4) Others

Article 16
Modification of rules
The rules of the Society can be amended by the resolution of the executive board.

Article 17
Miscellaneous provisions
Detailed regulations necessary for the enforcement of the rules of the Society are defined elsewhere by the president with the approval of the executive board.

Article 18
Additional clause
The Regulations of the International Society of Ningen Dock will come into effect on September 15, 2006.

Detailed Regulations of the International Society of Ningen Dock

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

(Detailed regulations on members)

Article 1

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

Article 2

Members will be given priority in the following events :

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

Article 3

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

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

Article 4

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

Article 5

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

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

Article 6

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

(Detailed regulations on officials)

Article 7

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

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

Article 8

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

(Detailed regulations on congress and board meeting)

Article 9

Congress and board meeting will be held as follows :

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

Article 10

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

(Enforcement of the detailed regulations)

Article 11

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

INSTRUCTIONS TO AUTHORS

Ningen Dock International
Official Journal of Japan Society of Ningen Dock

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

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

Online submission system

Ningen Dock uses an online submission system called ScholoarOne Manuscripts.
Please access http://mc.manuscriptcentral.com/ningendock
This site is only in Japanese at this time.

Preparation of manuscript

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

The title, abstract, text, acknowledgments, references, tables, and figure legends should begin on separate sheets, with pages numbered, and be typed double-spaced using the 12-point font size in MS-Word.
Files for submission should be prepared in English in a Microsoft Word or other file format that may be uploaded to the online system.

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

Example file names: Manuscript.doc, Fig 1 .jpg, Table 1 .xls, etc.

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

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

Title page

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

Abstract

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

Types of articles

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

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

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

References

References should be numbered consecutively in order of appearance in the text and cited in the text using superscript numbers. For example, according to the study by 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. Examples of references are given below.

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

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

Tables

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

Figures

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 separate page. Color figures can be reproduces if necessary, but the authors will be expected to contribute towards the cost of publication.

Conflict of Interest (COI)

All authors are required to disclose any conflict of interest (COI) on the form designated by the Japan Society of Ningen Dock.

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The corresponding author will receive PDF proofs, the author should correct only typesetting errors. After correcting, page proofs must be returned promptly.

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Categories of manuscript:

- ☐ Original article (not more than 3,000 words)
- ☐ Case report (not more than 2,000 words)
- ☐ Review article (not more than 4,000 words)

Typing:

- ☐ Manuscript on A4 paper with wide margins
- ☐ Type double space using 12-point

Title page:

- ☐ Title of paper
- ☐ Full names of authors and affiliations without title of MD, PhD, etc
- ☐ Full name and address of a corresponding author including fax number, telephone number and e-mail address.
- ☐ Running title not more than 50 characters.

Abstract:

- ☐ Not more than 250 words.
- ☐ Arranged in the order of Background, Methods, Results, and Conclusion.
- ☐ Up to four key words.

Text of paper:

- ☐ Manuscript is arranged in the order of Objective, Methods, Results, Discussion, (Conclusion), (Acknowledgments), and References.
- ☐ Measurements are expressed in SI units.
- ☐ Abbreviations are spelled out at first usage.

References:

- ☐ References are numbered consecutively in order of appearance in the text and cited in the text using superscript numbers.
- ☐ Format is consistent with examples in Instructions for Authors.

Tables:

☐ Each table is given a number and a brief informative title, and appears on separate page.

☐ All abbreviations used are explained in footnotes.

Figures:

☐ Figure legends are appended to the text on a separate page.

☐ The top of the figure, the first author's name, and the figure number are indicated lightly in soft pencil on the back of the four figures.

Submission:

☐ Check list, agreement, cover letter, manuscript (title page, abstract, text, acknowledgments, and references), figure legends, tables, figures and/or photos prepared in due form.

☐ One set of the original manuscript and three sets of the copies (with original photos, if any) are submitted.

☐ All pages are numbered.

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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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Doctors (internal medicine, primary care, surgery, gynecology, ophthalmology, pediatrics, radiology, orthopedics, pharmacology, epidemiology, other:)

Nurse, Public Health Nurse, Dietician, Clinical Technologist,

Clinical Radiological Technologist, Pharmacist, Other:

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☐Supporting Member

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☐Regular Member -International

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The International Society of Ningen Dock, c/o Japan Society of Ningen Dock

Ningen Dock International Vol. 2 No. 1 September, 2014

Produced by
Scientist Press Co., Ltd.
5-8-10-605, Sendagaya, Shibuya-ku,
Tokyo 151-0051, Japan
TEL: +81-3-3354-2004
FAX: +81-3-3354-2017
E-mail: info@scientist-press.com

Printed by
SHINANO Co., Ltd.
4-32-8, Ikebukuro, Toshima-ku,
Tokyo 171-0014, Japan
TEL: +81-3-5911-3355
FAX: +81-3-5911-3356