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New Aspects of Rheumatoid Arthritis (RA) in the Comprehensive Health Checkup System, Ningen Dock

Junichi Kaburaki

Abstract

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease. Recently developed drugs and guidelines have improved the working ability and prognosis of RA patients. Survival in RA has been extended, and RA patients have increased opportunity to visit Preventive Health Care Centers to undergo the Comprehensive Health Checkup System, or Ningen Dock. The health problems experienced by patients with RA are characteristically similar to those of the general population without RA. These include frailty, sarcopenia and locomotive syndrome. Basically, cytokines which cause chronic inflammation play important roles in both arthritis and sarcopenia, including tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2). In addition, recent studies have recognized that RA is a risk factor for cardiovascular disease, as well as for bone fragility and refracture. Clinical care of these patients therefore includes explanations of exercise and diet, as recognized in the 2022 American College of Rheumatology (ACR) guidelines for exercise, rehabilitation, diet, and additional integrative interventions.

Keywords rheumatoid arthritis, chronic inflammation, frailty, sarcopenia

Rheumatoid arthritis (RA), a chronic systemic inflammatory disease, which is fundamentally characterized as an autoimmune phenomenon involving polyarthritis and joint destruction[1–3]. Given an estimated prevalence of between 0.6% and 1.0% in Japan, there at least 800 thousand patients with RA[4]. Recently, outcomes have been improved by early diagnosis and appropriate management, especially when conducted in accordance with criteria for the classification and management of RA[5–7]. The IORRA study, conducted by the Division of Rheumatology at Tokyo Women's Medical University School of Medicine, estimated a standardized mortality ratio (SMR) for RA of between 1.46 and 1.90[4]. This value is similar to the SMR in a general population without RA.

Therefore, as RA patients can now survive into older age, they have the possibility of experiencing work disability before clinical remission, and to develop other conditions such as frailty, sarcopenia and locomotive syndrome. Recently, the American College of Rheumatology (ACR) proposed in its 2022 guideline for the provision of exercise, rehabilitation, dietary support, and additional integrative interventions for RA patients to improve their physical activity, in addition to drug therapy[8]. Clinicians should also consider these management practices in Preventive Health Care Medicine for patients with RA.

The classification and clinical features of RA have been described elsewhere[1–3]. In this article, we review new aspects of RA bought to light by advances this decade.

Factors Influencing the Onset of RA

The pathophysiology of RA was reviewed in 2014[1]. More recent advances in the decade since then have clarified additional factors which influence the onset of RA[3].

The onset of RA has recently been attributed to a combination of genetic and environmental factors (**Table 1**).

Table 1. Genetic Factors and Environmental Factors in the Onset of Rheumatoid Arthritis

Genetic factors
HLA-DR gene, especially a shared epitope in HLA-DRB1
More than 100 susceptibility genes in a genome-wide association study (GWAS)
Environmental factors
Smoking
Periodontal disease
Intestinal flora

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First, the HLA-DR gene plays an important role among genetic factors[3]. In particular, the 70th to 74th amino acid sequence in the HLA-DRB1 region, called a shared epitope, is a risk factor for RA onset. The shared epitope promotes antigen presentation, leading to an autoimmune phenomenon. A genome-wide association study (GWAS) revealed at least 100 genes which confer susceptibility to RA onset. Although these genes vary among different races, FCRL3 (Fc receptor like 3), which controls the activity of PAD (peptidyl arginine deaminase) for citrullination, is found in Japanese patients with RA. This result can be associated with the clinical significance of anti-citrullinated protein antibodies (anti-CCP antibodies: ACPA) in these RA patients[1,2].

Among environmental factors, smoking is a well-known risk factor[1]. Smoking in people with the shared epitope is associated with the dose-dependent frequency of RA onset. Periodontal disease is also important in the onset of RA. *Porphyromonas gingivalis* has PAD activity, resulting in an increase in citrullinated protein in the mouth and production of ACPA, leading to arthritis[3]. Patients with unclassified arthritis or RA undergoing the Comprehensive Health Checkup System, Ningen Dock should therefore be asked whether they smoke, and if they have periodontal disease, and the importance of non-smoking and regular consultation with a dentist should be explained. In addition, recent advances have found that the intestinal flora also play a role in the onset of RA: *Prevotella corpi* can activate the immune response of Th 17 cells, leading to the autoimmune phenomenon of RA[3].

Additional Problems in the Management of RA Patients Arising in the Past Decade

In 2010, Smolen *et al.* advocated a concept for the management of RA termed the "Treatment to target (T2T)" recommendations[5]. These outlined the necessity of treating RA patients in the early stage to prevent articular destruction and subsequent deterioration in quality of life (QOL) based on the idea that previous increases in tenosynovitis and synovitis during RA progression are followed by an increase in osteitis[9].

The "T2T" recommendations state that the primary goal of RA management is to maximize long-term health-related QOL through the control of symptoms, prevention of structural damage, normalization of joint function, and participation in social and work-related activities[5]. Recent advances have achieved clinical remission, and subsequently improved prognosis and QOL in RA patients. We should keep in mind that RA patients now have increased opportunity to visit Preventive Health Care Centers, to a degree similar to that of the general population without RA.

The purpose of the T2T recommendations include economic measures to reduce absenteeism (percentage of work time missed due to RA activity) and presenteeism (percentage of impaired work time due to RA activity). Absenteeism and presenteeism are major problems for RA patients who work. Among the many patients with RA who experience work disability before clinical remission, one-third are obliged to temporarily discontinue work or retire early[10–12]. Sakai *et al.* reported that improvement in a visual analogue scale for pain, namely the global assessment visual analogue scale (pain-VAS), as well as in the Japanese version of the Healthcare Assessment Questionnaire Disability Index (J-HAQ), is strongly associated with improvements in work productivity and activity impairment (WPAI)[13].

Furthermore, new drugs such as biologic disease modifying anti-rheumatic drugs (bDMARDs) and targeted synthetic disease modifying anti-rheumatic drugs (tsDMARDs) have been developed for use in addition to methotrexate (MTX) and other conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs). Guidelines have proposed appropriate management for individual patients with RA, for example the Japan College of Rheumatology (JCR) Guideline 2020[7]. MTX is included among csDMARDs, but is considered to be an anchor drug in therapy for RA[7,14]. However, rheumatologists have commonly experienced patients whose RA was difficult to treat in spite of the use of MTX and csDMARDs, a situation which subsequently lead to the development of bDMARDs and tsDMARDs (**Table 2**)[7,15]. bDMARDs inhibit the action of inflammatory cytokines such as TNF-α and IL-6, as well as lymphocyte interaction, while tsDMARDs are known to be Janus kinase (JAK)-inhibitors which block an intracellular signal pathway.

The availability of these new drugs has allowed patients with RA to improve their ability to work and to survive longer than before, to a similar extent as the general population without RA[16]. Nevertheless, these improvements have in turn lead to the development of new problems, which will require management in the future. These problems include the management

Table 2. Approved Target Molecules of DMARDS in RA Management

bDMARDs (Biologic disease-modifying anti-rheumatic drugs)
TNF-α (tumor necrosis factor-α)
TNF-αR (tumor necrosis factor-α receptor)
IL-6R (interleukin-6 receptor)
IL-1βR (interleukin-1 β receptor)
CD80/86 (T cell)
CD 20 (B cell)
tsDMARDs (Targeted synthetic disease-modifying anti-rheumatic drugs)
JAK (Janus kinase)

DMARDs=disease-modifying anti-rheumatic drugs

of frailty, sarcopenia and locomotive syndrome, which occur in RA patients to a similar extent to their occurrence in the general population without RA. Moreover, atherosclerosis and bone fragility have now become important clinical aspects of RA.

General Concepts of Frailty, Sarcopenia and Locomotive Syndrome

Frailty is a condition of increased vulnerability due to the failure of homeostatic coping under certain stresses. It is associated with increases in various adverse events such as falls, hospitalization and mortality[17,18]. Frailty is divided into three categories: physical frailty, mental frailty and social frailty (**Fig. 1**). Various kinds of symptom are found in each category. While frailty is a broad concept which presents physical as well as mental and social manifestations, the starting point is often physical frailty, which then proceeds to sarcopenia and locomotive syndrome through chronic inflammation, and presents clinically with a range of disabling manifestations (**Fig. 2**).

The concept of sarcopenia was originally advocated by Rosenberg in 1989[19] as an age-related decrease in muscle mass. Recent reviews have characterized sarcopenia as a steady reduction in skeletal muscle mass and strength which is associated with poor physical perfor-

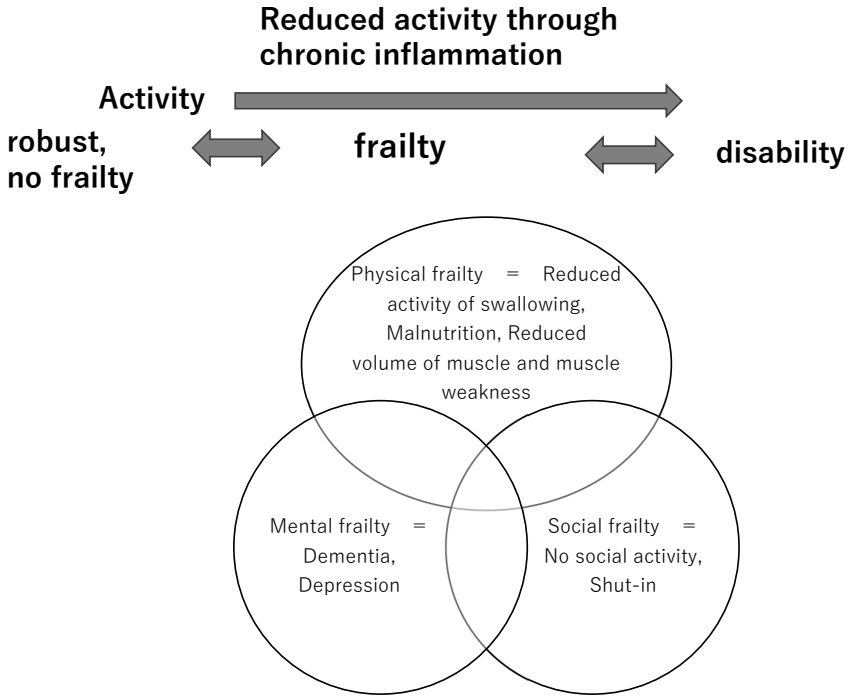

Fig. 1. Classification of Frailty and Representative Symptoms
Frailty consists of physical frailty, mental frailty and social frailty.

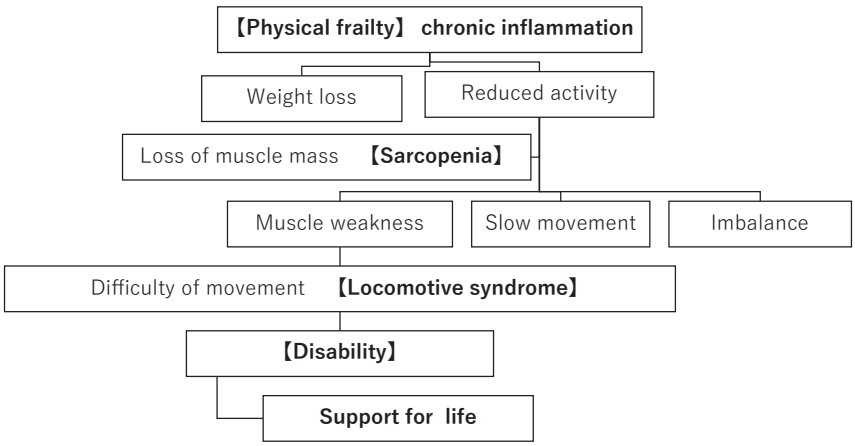

Fig. 2. Association between Physical Frailty, Sarcopenia and Locomotive Syndrome
Frailty, especially physical frailty, proceeds to sarcopenia and locomotive syndrome through chronic inflammation.

mance, functional impairment and disability, which in turn result in high mortality and significant medical costs[20].

Locomotive syndrome is a condition in which the musculoskeletal system, including bones, joints, and skeletal muscles, experiences a decrease in function[21]. This concept was proposed by the Japanese Orthopaedic Association in 2011. As a concept, locomotive syndrome is considered to overlap with physical frailty and is focused on musculoskeletal disorders, whereas sarcopenia is a concept within frailty which focuses on muscles. Locomotive syndrome is particularly applicable to insurance for the long-term care of invalids in Japan.

Given these relationships, sarcopenia should be detected early, in the Comprehensive Health Checkup System, Ningen Dock and treated with medicine, nutrition and rehabilitation to prevent the progression of frailty to locomotive syndrome. Chronic inflammation, especially inflammation involving inflammatory cytokines such as TNF-α and IL-6, plays an important role in sarcopenia as well as in arthritis (**Fig. 3**)[20,22,23].

Sarcopenia occurs by several pathways. In muscle growth, growth hormone is first released from the hypothalamus by stimulation such as exercise, and induces insulin-like growth factor-1 (IGF-1) in the liver and skeletal muscle. Then, IGF-1 activates the phosphatidylinositol 3-kinase (PI3-K)-Akt signaling pathway via insulin receptor substrate-1 (IRS-1), PI3-K and Akt through insulin-like growth factor-1 receptor (IGF-1R). Finally, IGF-1 increases protein synthesis for the prevention of sarcopenia.

In contrast, TNF-α, IL-6 and myostatin inhibit protein synthesis, and thereby promote muscle atrophy. TNF-α activates nuclear factor kappa B (NF-kB) via the TNF-α receptor (TNF-αR). IL-6 triggers the Janus kinase (JAK)-signal transducer and activator of the transcription (STAT-3) pathways via IL-6R, like gp130, while chronic exposure to IL-6 exerts inhibitory effects on IGF-1. In addition, myostatin inhibits the Akt pathway through ActRII, while myostatin promotes IL-1β (interleukin-1β) expression, especially in RA synovial fibroblasts[24]. These pathways inhibit protein synthesis in myocytes, and thereby induce sarcopenia.

Epidemiology and Clinical Characteristics

Ozeki *et al.* reported that frailty is observed in 37.6% of patients with RA but in only 15.7% of community dwellers[25]. Multivariate models have shown that body mass index (BMI) and social participation are independently associated with frailty in RA patients. Accordingly, maintaining an appropriate body weight and participating in social activities are important in preventing frailty in RA patients to the same degree that they are in the general population without RA.

It has been reported that the prevalence of sarcopenia in patients with RA is relatively high, from 25% to 44%, versus approximately only 10% in the general population without RA[19,26–30]. When RA patients develop sarcopenia, they experience imbalance, leading to falls, bone fractures and reduced QOL. Risk factors for sarcopenia in RA patients include severe functional

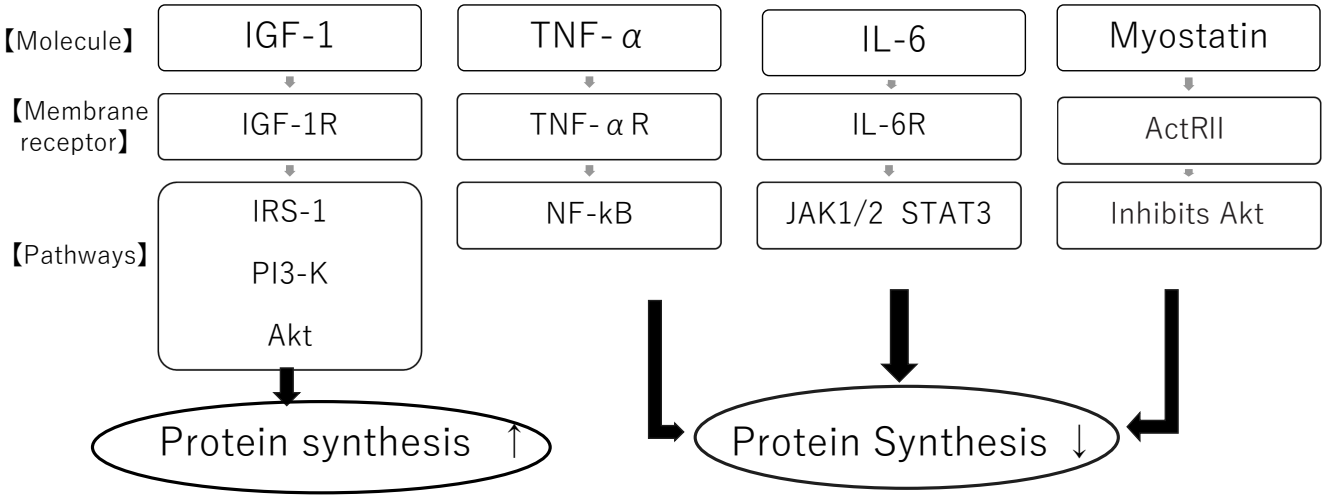

Fig. 3. Molecules and Intracellular Pathways Controlling Protein Synthesis and Breakdown in Muscle

Insulin-like growth factor 1 (IGF-1) activates the phosphatidylinositol 3-kinase (PI3-K)-Akt signaling pathway, including insulin receptor substrate-1 (IRS-1), PI3-K and Akt through insulin-like growth factor-1 receptor (IGF-1R). In this process, IGF-1 increases protein synthesis in myocytes.

To the contrary, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and myostatin have opposite effects. TNF-α activates nuclear factor kappa B (NF-kB) through the TNF-α receptor (TNF-αR). IL-6 triggers Janus kinase (JAK)-signal transducer and activator of the transcription (STAT-3) pathways through IL-6R, like gp130, while chronic exposure to IL-6 exerts inhibitory effects on IGF-1. In addition, myostatin inhibits the Akt pathway through ActRII. These pathways finally inhibit protein synthesis in myocytes.

limitations arising due to articular destruction, high C-reactive protein and positive reaction of serum rheumatoid factor, suggesting chronic inflammation as a basic mechanism[31]. In addition, several studies have reported that RA patients with sarcopenia are clinically distinguished by features such as higher Steinbrocker Stage and Health Assessment Questionnaire (HAQ) scores than those without sarcopenia[29,32,33].

When patients with RA undergo the Comprehensive Health Checkup System, Ningen Dock, it is necessary that clinicians perform not only the usual physical examination and laboratory tests, but also provide explanations of exercise and nutrition.

Medications for RA Patients with Sarcopenia

Drug therapy for sarcopenia, either with or without RA, has not been established. Given that inflammatory cytokines such as TNF-α and IL-6 and the JAK pathway have effects on muscle metabolism, bDMARDs such as TNF-α or IL-6 inhibitors, and JAK inhibitors may have the potential to treat sarcopenia in patients with RA. However, there is little evidence that medical treatment is able to influence muscle metabolism. Tournade *et al.* reported that tocilizumab, an anti-IL-6 receptor monoclonal antibody, is associated with increasing muscle mass in patients with RA[34]. However, treatment with etanercept, a TNF-αR inhibitor, did not increase muscle mass over 6 months, compared to MTX treatment[35]. Thus, although bDMARDs and JAK-inhibitors may theoretically improve muscle metabolism, we do not have sufficient evidence to show that they improve not only muscle mass but also muscle strength.

Therefore, while adequate treatment control of inflammation in RA is a prerequisite, additional adjunctive management, such as exercise and nutrition, are required to improve sarcopenia in RA patients to bring them to a similar incidence to that in the general population without RA. Moreover, steroid therapy should be avoided, as these drugs can cause steroid myopathy.

It is against this background that the ACR's 2022 guideline calls for exercise, rehabilitation, diet and additional integrative interventions for RA, in conjunction with drugs such as DMARDs[8].

Exercise Therapy

The 2022 ACR guideline reflects the ACR's strong recommendation for consistent engagement in exercise, as regular exercise results in improved physical function in RA patients[8]. The ACR also conditionally recommends consistent engagement in aerobic exercise and aquatic exercise, and consistent engagement in resistance exercise, especially very low to low intensity resistance exercise, to improve physical function. Baillet *et al.* reported that resistance training significantly improves isokinetic muscle strength, isometric muscle strength and grip strength, and also improves inflammatory state as assessed by erythrocyte sedimentation rate[36]. Moreover, it has also been reported that resistance training or a combination of aerobic exercise and resistance training effectively improves muscle strength and physical activity in patients with RA, even in patients with RA diagnosed with locomotive syndrome[37–41].

Diet Therapy

Appropriate nutritional management is required to increase muscle strength and mass, even in patients with RA. The 2022 ACR guideline states that the ACR conditionally recommends adherence to a Mediterranean-style diet over no formally defined diet, and conditionally recommends against adherence to any formally defined diet other than a Mediterranean-style diet[8]. The Mediterranean-style diet consists of vegetables, fruits, whole grains, nuts, seeds, and olive oil in conjunction with moderate amounts of low-fat dairy and fish, and limits the intake of added sugars, sodium, highly processed foods, refined carbohydrates and saturated fats.

The relationship between low protein intake and loss of muscle strength and mass has been reported in the general population[42,43]. Based on this knowledge, it has been reported that average protein intake should be at least 1–1.5 g/kg/day, and increased up to an upper limit of 2 g/kg/day during severe disease activity due to inflammation[44,45].

Among other nutrients, Vitamin D has attracted attention as a potential target for muscle as well as bone metabolism in RA patients. Vitamin D is reportedly effective in inducing improvements in muscle strength and mass[46,47].

On the other hand, obesity is a risk factor for the worsening of RA disease activity[48]. Obesity is associated with higher disease activity, impairment in physical function and poorer treatment response, in addition to poor long-term health outcomes. Obesity may also be associated with atherosclerosis, as discussed in the next section.

Other Problems Recently Found in RA Patients

Two other problems have been recently highlighted in patients with RA, apart from side effects of DMARD therapy such as infection. These are atherosclerosis and bone fragility.

It is well-known that cardiovascular risk is substantially increased in patients with RA[49–51]. In a meta-analysis, both cardiovascular morbidity and mortality were 1.5-fold higher in RA patients than in the general pop-

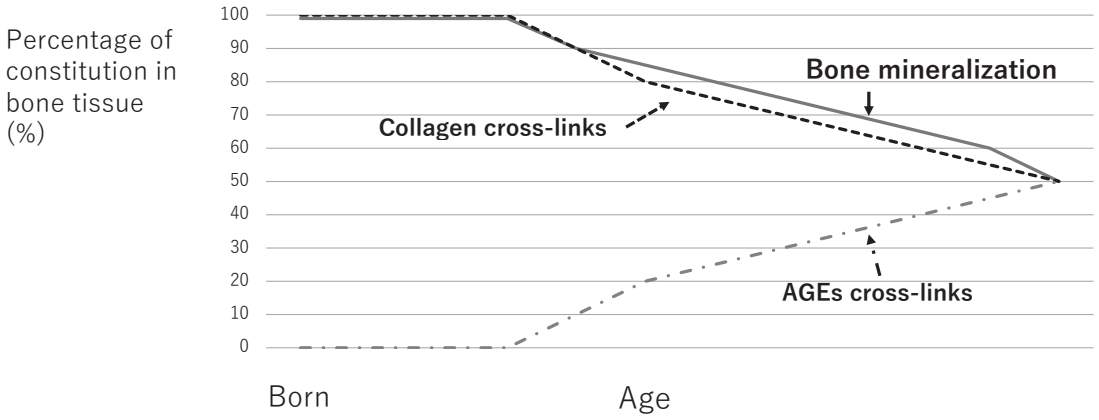

Fig. 4. Chronological Change in Bone Strength (Bone Mineralization plus Bone Quality)

Bone mineralization and normal collage cross-linking decrease, while advanced glycation end products (AGEs) and homocysteine cross-linking, indicating poor bone quality, increase with aging.

ulation. Peter *et al.* reported that the 3-year incidence of cardiovascular disease was 9.0% in patients with RA and 4.3% in the general population, corresponding to an incidence rate of 3.30 per 100 patient-years [95% confidence interval (95%CI) 2.08–4.25)] and 1.51 per 100 person-years (95%CI 1.18–1.84), respectively[52]. Moreover, compared with the nondiabetic population, nondiabetic patients with RA and those with type 2 diabetes mellitus (DM) had comparable age- and the sex-adjusted hazard ratios of 2.16 (95%CI 1.28–3.63 p=0.004) and 2.04 (95%CI 1.12–3.67 p=0.029), respectively. From that study, it is evident that the risk of cardiovascular disease in patients with RA is significantly elevated when compared with the general population, and comparable with the magnitude of risk in patients with type 2 DM. These observational studies hypothesized a common basis —namely systemic inflammation by cytokines— for both arthritis and atherosclerosis.

Moreover, a recent study by Yuan *et al.* demonstrated a genetic liability to RA in relation to both coronary artery disease and stroke[53].

Therefore, when aged patients with RA visit the Preventive Health Care Centers, close attention should be paid to the possibility of atherosclerosis, as it is in the general population without RA.

The second newly identified problem is bone fragility, which causes locomotive syndrome as well as frailty[17]. RA is a well-known cause of secondary osteoporosis[54]. Recent studies showed that bone fragility resulting in bone fracture is associated with both bone mineralization and bone quality, such as normal collagen cross-links[55–57]. Bone fragility and fracture can be caused by a reduction in normal collagen cross-links with an increase in levels of advanced glycation end products (AGEs) or homocysteine cross-links. Chronological change in bone strength is shown in **Fig. 4**. Although bone mineralization and normal collagen cross-linking, as measures of bone quality, decrease with aging, AGEs or homocysteine cross-links increase as a constituent percentage. It has been reported that patients with a history of multiple fractures and women aged 75 years are at increased risk of refracture[58,59]. Recently, Ishizu *et al.* demonstrated that a cohort of RA patients with an average age of 75.4 years who had experienced bone fragility and fracture were at increased risk (OR: 2.714 95%CI: 1.015–7.255 p=0.040) of refracture, compared to the general population without RA[60]. This result was independent of the use of steroid therapy in these RA patients.

These findings highlight the need for close attention to atherosclerosis and bone fragility, which is associated with both bone mineralization and bone quality, in the Comprehensive Health Checkup System, Ningen Dock.

Conclusion

Prognosis in RA has been improved by recently developed drugs such as bDMARDs and tsDMARDs, in addition to MTX and other csDMARDs. Accordingly, we face new problems in RA patients, such as in their ability to work. Moreover, as patients with RA survive longer than before, maintenance of a good QOL requires clinicians to take a similar degree of care for frailty, sarcopenia and locomotive syndrome, as well as for atherosclerosis and bone fragility, in these patients as they do in the general population without RA.

Conflict of Interest

The author declares no conflict of interest.

References

1. Kaburaki J, Kuwana M: How to detect early stage rheumatoid arthritis (RA) in persons undergoing Ningen Dock (Health Evaluation and Promotion). Ningen Dock International 2014; 1: 24–33.
2. Kaburaki J: Rheumatoid Arthritis and related diseases in Ningen Dock. Ningen Dock 2017; 32: 601–610. (in Japanese)

3. Nakano K: Rheumatoid arthritis-pathophysiology, clinical features and diagnosis. In: Japan College of Rheumatology (ed), Textbook of Rheumatology. 3rd ed., Nankodo, Tokyo, 2022, 90–98. (in Japanese)
4. Tanaka E: Epidemiology of rheumatoid arthritis. Nihon Naika Gakkai Zasshi 2023; 112: 1890–1899. (in Japanese)
5. Smolen JS, Aletaha D, Bijlsma JW, *et al*.: Treating rheumatoid arthritis to target: recommendations of an international task force. Ann Rheum Dis 2010; 69: 631–637.
6. Sumitomo H: Evaluation for activity of rheumatoid arthritis. In: Japan College of Rheumatology (ed), Textbook of Rheumatology. 3rd ed., Nankodo, Tokyo, 2022, 99–104. (in Japanese)
7. Kaneko Y: Rheumatoid arthritis-treatment in Internal Medicine. In: Japan College of Rheumatology (ed), Textbook of Rheumatology. 3rd ed., Nankodo, Tokyo, 2022, 105–112. (in Japanese)
8. England BR, Smith BJ, Baker NA, *et al*.: 2022 American College of Rheumatology guideline for exercise, rehabilitation, diet, and additional integrative interventions for rheumatoid arthritis. Arthritis Care Res (Hoboken) 2023; 75: 1603–1615.
9. Krijbolder DI, Matthijssen XME, van Dijk BT, *et al*.: The natural sequence in which subclinical inflamed joint tissues subside or progress to rheumatoid arthritis: a study of serial MRIs in the TREAT EARLIER trial. Arthritis Rheumatol 2023; 75: 1512–1521.
10. Young A, Dixey J, Cox N, *et al*.: How does functional disability in early rheumatoid arthritis (RA) affect patients and their lives? Results of 5 years follow-up in 732 patients from the Early RA Study (ERAS). Rheumatology (Oxford) 2000; 39: 603–611.
11. Young A, Dixey J, Kulinskaya E, *et al*.: Which patients stop working because of rheumatoid arthritis? Results of five years' follow up in 732 patients from the Early RA Study (ERAS). Ann Rheum Dis 2002; 61: 335–340.
12. Scott DL, Smith C, Kingsley S, *et al*.: What are the consequences of early rheumatoid arthritis for the individual? Best Pract Res Clin Rheumatol 2005; 19: 117–136.
13. Sakai R, Tanaka E, Inoue E, *et al*.: Association between patient-reported outcomes and impairments in work and activity in patients with rheumatoid arthritis in clinical remission: a retrospective analysis using the IORRA database. Mod Rheumatol 2023; 33: 899–905.
14. Hirata S: Clinical guidelines for rheumatoid arthritis. Nihon Naika Gakkai Zasshi 2023; 112: 1907–1913. (in Japanese)
15. Takeuchi T: Progress in the past 20 years and prospects in the future of collagen diseases. Nihon Naika Gakkai Zasshi 2023; 120 (anniversary ed): 268–278. (in Japanese)
16. Takeuchi T, Nakajima R, Komatsu S, *et al*.: Impact of adalimumab on work productivity and activity impairment in Japanese patients with rheumatoid arthritis: large-scale, prospective, single-cohort ANOUVEAU study. Adv Ther 2017; 34: 686–702.
17. Iritani A, Morimoto S: Daily custom and Mibyo in aged person. In: Association of Japanese Mibyou Medicine (ed), Textbook of Mibyou Medicine. 1st ed., NTS, Tokyo, 2018, 73–81. (in Japanese)
18. Clegg A, Young J, Iliffe S, *et al*.: Frailty in elderly people. Lancet 2013; 381: 752–762.
19. Rosenberg IH: Summary comments. Am J Clin Nutr 1989; 50: 1231–1233.
20. Torii M, Itaya T, Minamino H, *et al*.: Management of sarcopenia in patients with rheumatoid arthritis. Mod Rheumatol 2023; 33: 435–440.
21. Nakamura K: The concept and treatment of locomotive syndrome: its acceptance and spread in Japan. J Orthop Sci 2011; 16: 489–491.
22. Benatti FB, Pedersen BK: Exercise as an anti-inflammatory therapy for rheumatic disease-myokine regulation. Nat Rev Rheumatol 2015; 11: 86–97.
23. Bowen TS, Schuler G, Adams V: Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. J Cachexia Sarcopenia Muscle 2015; 6: 197–207.
24. Hu S-L, Chang A-C, Huang C-C, *et al*.: Myostatin promotes interleukin-1β expression in rheumatoid arthritis synovial fibroblasts through inhibition of miR-21-5p. Front Immunol 2017; 8: 1747.
25. Ozeki S, Takeuchi K, Yasuoka M, *et al*.: Comparison of frailty associated factors between older adult patients with rheumatoid arthritis and community dwellers. Arch Gerontol Geriatr 2021; 96: 104455.
26. Shafiee G, Keshtkar A, Soltani A, *et al*.: Prevalence of sarcopenia in the world: a systematic review and meta-analysis of general population studies. J Diabetes Metab Disord 2017; 16: 21.
27. Ngeuleu A, Allali F, Medrare L, *et al*.: Sarcopenia in rheumatoid arthritis: prevalence, influence of disease activity and associated factors. Rheumatol Int 2017; 37: 1015–1020.
28. Tada M, Yamada Y: Prevalence, incidence rate and predictors of sarcopenia in rheumatoid arthritis-analysis from the CHIKARA study-. Clinical Rheumatology and Related Research 2021; 33: 70–77. (in Japanese)
29. Torii M, Hashimoto M, Hanai A, *et al*.: Prevalence and factors associate with sarcopenia in patients with rheumatoid arthritis. Mod Rheumatol 2019; 29: 589–595.
30. Yamada M, Furuyama M, Origuchi T: The comparison of sarcopenia prevalence, nutrition and exercises between rheumatoid arthritis patients and healthy subjects. Clinical Rheumatology and Related Research 2023; 35: 95–107. (in Japanese)
31. Li TH, Chang YS, Liu CW, *et al*.: The prevalence and risk factors of sarcopenia in rheumatoid arthritis patients: a systematic review and meta-regression analysis. Semin Arthritis Rheum 2021; 51: 236–245.
32. Morley JE, Anker SD, von Haehling S: Prevalence, incidence, and clinical impact of sarcopenia: facts, numbers, and epidemiology-update 2014. J Cahexia Sarcopenia Muscle 2014; 5: 253–259.
33. Mochizuki T, Yano K, Ikari K, *et al*.: Sarcopenia-associated factors in Japanese patients with rheumatoid arthritis: a cross-sectional study. Geriatr Derontol Int 2019; 19: 907–912.
34. Tournadre A, Pereira B, Dutheil F, *et al*.: Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J Cachexia Sarcopenia Muscle 2017; 8: 639–646.
35. Marcora SM, Chester KR, Mittal G, *et al*.: Randomized phase

2 trial of anti-tumor necrosis factor therapy for cachexia in patients with early rheumatoid arthritis. Am J Clin Nutr 2006; 84: 1463–1472.
36. Baillet A, Vaillant M, Guinot M, *et al.*: Efficacy of resistance exercises in rheumatoid arthritis: meta-analysis of randomized controlled trials. Rheumatology 2012; 51: 519–527.
37. Hurkmans E, van der Giesen FJ, Vliet Vlieland TP, *et al.*: Dynamic exercise programs (aerobic capacity and/or muscle strength training) in patients with rheumatoid arthritis. Cochrane Database Syst Rev 2009; 4: CD006853.
38. Andersson SEM, lange E, Kucharski D, *et al.*: Moderate- to high intensity aerobic and resistance exercise reduces peripheral blood regulatory cell populations in older adults with rheumatoid arthritis. Immun Ageing 2020; 17: 12.
39. Lourenzi FM, Jones A, Pereira DF, *et al.*: Effectiveness of an overall progressive resistance strength program for improving the functional capacity of patients with rheumatoid arthritis: a randomized controlled trial. Clin Rehabil 2017; 31: 1482–1491.
40. Hu H, Xu A, Gao C, *et al.*: The effect of physical exercise on rheumatoid arthritis: an overview of systematic reviews and meta-analysis. J Adv Nurs 2021; 77: 506–522.
41. Matsushita I, Ishigo W, Ishida T, *et al.*: Effects and problems of locomotion training for elderly patients with rheumatoid arthritis. Clinical Rheumatology and Related Research 2023; 35: 86–94. (in Japanese)
42. Kobayashi S, Asakura K, Suga H, *et al.*; Three-generation Study of Women on Diets and Health Study Group: High protein intake is associated with low prevalence of frailty among old Japanese women: a multicenter cross-sectional study. Nutr J 2013; 12: 164.
43. McLean RR, Mangano KM, Hannan MT, *et al.*: Dietary protein intake is protective against loss of grip strength among older adults in the Framingham Offspring cohort. J Gerontol A Biol Sci Med Sci 2016; 71: 356–361.
44. Bauer J, Biolo G, Cederholm T, *et al.*: Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE study group. J Am Med Dir Assoc 2013; 14: 542–559.
45. Bauer J, Morley JE, Schols A, *et al.*: Sarcopenia: a time for action. An SCWD position paper. J Cahexia Sarcopenia Muscle 2019; 10: 956–961.
46. Minamino H, Katsushima M, Torii M, *et al.*: Serum vitamin D status inversely associates with a prevalence of severe sarcopenia among female patients with rheumatoid arthritis. Sci Rep 2021; 11: 20485.
47. Beaudart C, Buckinx F, Rabenda V, *et al.*: The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. J Clin Endocrinol Metab 2014; 99: 4336–4345.
48. Poudel D, George MD, Baker JF: The impact of obesity on disease activity and treatment response in rheumatoid arthritis. Curr Rheumatol Rep 2020; 22: 56.
49. Safiri S, Kolahi AA, Hoy D, *et al.*: Global, regional and national burden of rheumatoid arthritis 1990–2017: a systematic analysis of the Global Burden of Disease study 2017. Ann Rheum Dis 2019; 78: 1463–1471.
50. Avina-Zubieta JA, Thomas J, Sadatsafavi M, *et al.*: Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis 2012; 71: 1524–1529.
51. Avina-Zubieta JA, Choi HK, Sadatsafavi M, *et al.*: Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum 2008; 59: 1690–1697.
52. Peters MJL, van Halm VP, Voskuyl AE, *et al.*: Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis Rheum 2009; 61: 1571–1579.
53. Yuan S, Carter P, Mason AM, *et al.*: Genetic liability to rheumatoid arthritis in relation to coronary artery disease and stroke risk. Arthritis Rheumatol 2022; 74: 1638–1647.
54. Fardellone P, Salawari E, Le Monnier L, *et al.*: Bone loss, osteoporosis, and fractures in patients with rheumatoid arthritis: a review. J Clin Med 2020; 9: 3361.
55. Saito, M, Fujii K, Soshi S, *et al.*: Reductions in degree of mineralization and enzymatic collagen cross-links and increases in glycation induced pentosidine in the femoral neck cortex in cases of femoral neck fracture. Osteoporos Int 2006; 17: 986–995.
56. Saito M, Fujii K, Mori Y, *et al.*: Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in the spontaneously diabetic WBN/Kob rats. Osteoporos Int 2006; 17: 1514–1523.
57. Saito M, Fujii K, Marumo K: Degree of mineralization-related collagen crosslinking in the femoral neck cancellous bone in cases of hip fracture and controls. Calcif Tissue Int 2006; 79: 160–168.
58. Balasubramanian A, Zhang J, Chen L, *et al.*: Risk of subsequent fracture after prior fracture among older women. Osteoporos Int 2019; 30: 79–92.
59. Sujic R, Beaton DE, Mamdani M, *et al.*: Five-year refracture rates of a province-wide fracture liaison service. Osteoporos Int 2019; 30: 1671–1677.
60. Ishizu H, Shimizu H, Shimizu T, *et al.*: Rheumatoid arthritis is a risk factor for refracture in patients with fragility fractures. Mod Rheumatol 2022; 32: 1017–1022.

Original Article

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The Natural Course of Patients Four Years after Pathological Urinary Sediment Findings at Ningen Dock: The Cost and Benefit of Urine Sediment Examination

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Abstract

Objective: Urinary sediment is examined in Ningen Dock. We sought to evaluate the costs and diagnostic value associated with abnormal urinary sediment findings, in addition to estimated glomerular filtration rate (eGFR), urinary protein, and occult blood measurement, in Ningen Dock after 4 years.

Methods: In the patients who underwent Ningen Dock in 2015, urinalysis was performed using a fully-automated urinalysis system at the central laboratory of each hospital. Urine sediment was microscopically examined by laboratory technicians. Granular casts, epithelial casts, and tubular epithelium were defined as pathological findings. Seventy-six patients who showed these findings in 2015 and were re-examined in 2019. The eGFR, urinary protein, and occult blood were statistically analyzed.

Results: Pathological urinary sediment findings detected in 2015 spontaneously disappeared after 4 years in 91% of the patients. No significant change was observed in eGFR, urinary protein or occult blood in the patients who continued to have pathological findings after 4 years. At least ¥157 was spent per sample in supplies and personnel.

Conclusions: Almost the pathological urine sediment was disappeared after four years. The benefit of urine sediment examinations was small for the cost. The eGFR, urine protein and occult blood test are sufficient in the kidney function examinations in Ningen Dock.

Keywords urine sediment test, Ningen Dock, natural course, cost

A urinary sediment examination is performed routinely in Ningen Dock[1]. Assessment criteria are set for each item[2]. Detailed examination is required if at least one tubular epithelial cell, epithelial cast, granular cast, fatty cast, red blood cell cast, and/or white blood cell cast is detected per high-power field.

In this study, the value of urine sediment examination in Ningen Dock was evaluated from the natural course of pathological urine sediment examination findings that require detailed examination after 4 years. Since the Guidelines for the Diagnosis of Hematuria[3] mention that there is the possibility of renal parenchymal disease if microscopic hematuria persists for ≥3 years, we assessed the follow-up examination performed after 4 years. While most of the items examined in Ningen Dock can be measured automatically, urine sediment is examined microscopically by laboratory technicians. In the basic examination items in Ningen Dock, the urinary sediment test can be omitted if urine protein and occult test are negative. In the urinalysis laboratory, time may be saved if performing a urine sedimentation test does not need to be done. Thus, we examined the cost and benefit of urine sediment examination, through the natural course of patients after four years with pathological sediment findings.

Methods

We selected 78 cases with abnormal urine sedimentation tests in 2015 and underwent Ningen Dock again in 2019 (**Table 1**). In Ningen Dock, blood tests and urine tests are performed by the central laboratory of the affiliated hospital. Renal tests include the estimated glomerular filtration rate (eGFR: mL/min/1.73 m^2) calculated from the serum creatinine level (HMMPS method), correcting for age and sex. Proteinuria and occult hematuria are assessed for with a fully-automated

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Table 1. The Profile of Patients with Granular Casts, Epithelial Casts, And/or Tubular Epithelial Cells Which Were Severe Urinary Sediment Findings

	All subjects in 2015	Granular casts	Epithelial casts	Tubular epithelial cells
	n=9210	*n*=24	*n*=50	*n*=4
Sex (male)	67.5%	70.8%	68.0%	50.0%
Age (y)	52.1±10.7	50.2±9.3	53.7±10.4	44.0±1.8
BMI (kg/m^2)	23.0±3.4	22.7±4.4	23.8±4.8	22.9±2.1
Smoking	18.8%	33.3%	18.0%	25.0%
Ethanol intake	70.7%	66.7%	68.0%	50.0%
eGFR (mL/min/1.73m^2)	72.3±13.0	67.2±14.1	66.7±13.0	65.5±12.6
SBP (mmHg)	118.3±15.4	122.2±16.4	123.0±20.0	121.3±30.6
DBP (mmHg)	74.7±11.3	80.8±15.3	77.3±15.1	79.0±30.6
FPG (mg/dL)	100.9±17.2	102.0±19.1	104.5±22.1	91.5±5.8
HbA1c (%)	5.68±0.58	5.60±0.52	5.81±0.75	5.38±0.17
Serum uric acid (mg/dL)	5.72±0.58	5.79±1.12	6.27±1.39	6.08±1.47
Urine protein (−)	57.9%	0.0%	10.0%	25.0%
Urine protein (±)	31.6%	62.5%	52.0%	25.0%
Urine protein (+)	9.4%	37.5%	32.0%	50.0%
Urine protein (2+)	0.9%	0.0%	4.0%	0.0%
Urine protein (3+ and over)	0.1%	0.0%	2.0%	0.0%
Mean urine protein score	0.54±0.71	1.38±0.50 (*p*<0.001)	1.36±0.80 (*p*<0.001)	1.25
Urine occult (−)	76.7%	58.3%	62.0%	100.0%
Urine occult (±)	11.4%	20.8%	16.0%	0.0%
Urine occult (1+)	8.1%	12.5%	10.0%	0.0%
Urine occult (2+)	2.7%	8.3%	10.0%	0.0%
Urine occult (3+ and over)	1.1%	0.0%	2.0%	0.0%
Mean urine occult score	0.40±0.83	0.71±1.00 (*p*=0.072)	0.74±1.12 (*p*=0.004)	0

BMI: body mass index
SBP: systolic blood pressure, DBP: diastolic blood pressure
FPG: fasting plasma glucose
eGFR: estimated glomerular filtration rate

urinalysis system (AUTION MAX AX-4060, ARKRAY, Inc., Kyoto, Japan).

Urinary sediment was examined on microscopy by laboratory technicians. If there were multiple findings, the finding with the highest score was adopted according to the grading system of the Japan Society of Ningen Dock. Findings of proteinuria and occult blood of (−), (±), (1+), (2+), and (3+) were scored as 0, 1, 2, 3, and 4, respectively, and were statistically analyzed with the variance method.

The information concerning the personnel expense for laboratory technicians was obtained from the table of Wages by Occupational Categories in the Basic Survey on Wage Structure by the Ministry of Health, Labour and Welfare[4]. The mean hourly wage of part-time laboratory technicians was ¥1,842–2,266 in 2013–2015. In this study it was assumed to be ¥2,000 per hour.

The data are expressed as mean±SD. Statical analysis was used Wilcoxon signe rank test (SPSS software version 28, Japan IBM Corp, Tokyo, Japan). $p<0.05$ was considered statistically significant.

This study was conducted in compliance with the Declaration of Helsinki and was approved by the institutional review board of the Jikei University School of Medicine (approval no., 17-015). The results of this study have not been previously published.

Results

Table 1 shows the profile of Ningen Dock in 2015 and patients with granular casts, epithelial casts, and tubular epithelial cells, which were the pathological urinary sediment findings. No red blood cell casts, white blood cell casts, waxy casts, or fatty casts were observed. The 4-year courses of the pathological urinary sediment findings are shown in **Table 2**.

Granular casts were observed in 24 patients but disappeared in 19 (79%) after 4 years. Of the remaining 5, 1 (4%) continued to show granular casts, 2 (8%) showed epithelial casts, and 2 showed hyaline casts. eGFR was 67.2–67.8 and showed no significant difference. The urinary protein score improved significantly from 1.38 to 0.79 ($p=0.004$). The urinary occult blood score remained unchanged at 0.71. Epithelial casts were observed in 50 but were normalized in 37 (74%). Of the remaining 13, 9 showed hyaline casts, 1 (2%) showed granular casts, and 3 (6%) showed epithelial casts. eGFR was 66.7–67.7 and showed no significant difference. The urinary protein score improved significantly from 1.36 to 0.7 ($p<0.001$). The urinary occult

Table 2. The Natural Course of the Pathological Urinary Sediment Findings after 4 Years

	Granular casts		Epithelial casts		Tubular epithelial cells	
	2015	2019	2015	2019	2015	2019
Normal		*n*=19 (79%)		*n*=37 (74%)		*n*=4 (100%)
Granular casts	***n*=24**	*n*=1 (4%)		*n*=1 (2%)		
Epithelial casts		*n*=2 (8%)	***n*=50**	*n*=3 (6%)		
Renal tubular epithelium					***n*=4**	
Hyaline casts		*n*=2 (8%)		*n*=9 (18%)		
Mean eGFR	67.2±13.8	67.8±11.8 (ns)	66.7±12.8	67.7±14.4 (ns)	65.5±10.9	65.5±9.9
Urine protein (−): score 0	*n*=0	*n*=9	*n*=5	*n*=22	*n*=1	*n*=3
Urine protein (±): score 1	*n*=15	*n*=11	*n*=26	*n*=21	*n*=1	
Urine protein (1+): score 2	*n*=9	*n*=4	*n*=16	*n*=7	*n*=2	*n*=1
Urine protein (2+): score 3			*n*=2			
Urine protein (3+ and over): score 4			*n*=1			
Mean protein score	1.38±0.50	0.79±0.71 (*p*=0.004)	1.36±0.80	0.70±0.70 (*p*<0.001)	1.25	0.5
Urine occult (−): score 0	*n*=14	*n*=15	*n*=31	*n*=37	*n*=4	*n*=3
Urine occult (±): score 1	*n*=5	*n*=3	*n*=8	*n*=6		
Urine occult (1+): score 2	*n*=3	*n*=4	*n*=5	*n*=3		*n*=1
Urine occult (2+): score 3	*n*=2	*n*=2	*n*=5	*n*=3		
Urine occult (3+ and over): score 4			*n*=1	*n*=1		
Mean occult score	0.71±1.00	0.71±1.02 (ns)	0.74±1.12	0.50±0.98 (*p*=0.038)	0	0.5

eGFR: estimated glomerular filtration rate

Table 3. Cases Showing Granular Casts and Epithelial Casts in Both 2015 and 2019

case	age in 2015 (y)	sex	BMI in 2015 (kg/m^2)	BMI in 2019 (kg/m^2)	SBP in 2015 (mmHg)	SBP in 2019 (mmHg)	DBP in 2015 (mmHg)	DBP in 2019 (mmHg)	FPG in 2015 (mg/dL)	urine protein score 2015	urine protein score 2019	urine occult score 2015	urine occult score 2019	eGFR in 2015 (mL/min/1.73 m^2)	eGFR in 2019 (mL/min/1.73 m^2)
1	43	female	22.8	27.1	117	113	65	59	94	1	0	0	0	70	73
2	44	male	23.3	24.3	137	113	92	75	102	1	1	1	0	75	75
3	49	male	19.3	20.8	105	143	82	102	86	1	1	0	0	58	72
4	54	female	15.4	16.9	110	91	62	55	106	1	1	2	2	90	61
5	57	male	25.4	23.1	100	119	64	86	97	3	1	0	0	52	62
6	62	male	21	21.5	101	94	70	67	99	2	2	2	3	53	50
7	66	male	26.7	26.3	131	105	86	68	133	2	2	1	2	52	52
mean	53.6		22.0	22.9 (ns)	114.4	111.1 (ns)	74.4	73.1 (ns)	102.4	1.6	1.1 (ns)	0.9	1.0 (ns)	64.3	63.6 (ns)

BMI: body mass index
SBP: systolic blood pressure, DBP: diastolic blood pressure
FPG: fasting plasma glucose
eGFR: estimated glomerular filtration rate

blood score improved significantly from 0.74 to 0.5 (p=0.038). Urinary tubular epithelium was normal in all 4. Mean eGFR was 66.5 and remained unchanged. Urinary protein changed from 1.25 to 0.5, and urinary occult blood changed from 0 to 0.5, but statistical analysis was not performed, because there were only 4 cases. Seven patients (9.2%) who continued to have both granular and epithelial casts after 4 years were evaluated (**Table 3**). There was no difference of eGFR, urine protein and occult in 2015 and 2019 in those subjects. Case 4 showed a striking decrease in eGFR. Serum creatinine changed from 0.54 mg/dL to 0.75 mg/dL after 4 years; muscle mass from 31.2 kg to 32.3 kg by impedance method; systolic blood pressure decreased from 110 mmHg to 91 mmHg, and diastolic blood pressure decreased from 62 mmHg to 55 mmHg. Hemoglobin was from 14.6g/dL to 13.8g/dL; serum uric acid was from 4.7 mg/dL to 4.1 mg/dL, serum albumin was from 4.0 g/dL to 4.1 mg/dL. These data are all within the normal range. Ultrasound findings were only simple renal cysts and renal calcifications in 2015, and simple renal cysts in 2019. The cause of decreased eGFR was unknown.

Concerning the cost of examination, the supplies expense per examination is assumed to be ¥30. As for the capital cost, if a microscope worth ¥1,000,000 is used for 5 years, its cost per year is ¥200,000 by fixed installment depreciation. If it is assumed to be used for the examination of 30 samples per day on 250 days a year, the capital cost of the microscope per sample was calculated at ¥27. Since the examination takes 3 minutes

per sample, and since the hourly wage is assumed to be ¥2,000 as mentioned above, the personnel expense per sample was calculated at ¥100. The total cost of urine sediment examination per sample was calculated at ¥157 as the sum of the supplies expense of ¥30, capital cost for the microscope of ¥27, and personnel expense of ¥100.

Discussion

If pathological urinary sediment findings are observed in Ningen Dock, a detailed examination is indicated. However, there are few patients in whom eGFR is low, or the urinary protein or urinary occult blood level is extremely high. There is also no drug that can correct urinary sediment findings themselves. Therefore, we examined and analyzed the natural course of pathological urinary sediment findings after 4 years. As a result, pathological urinary sediment findings persisted in only 7 of the 78 patients, and no patient showed impairment of renal function such as a decrease in eGFR and exacerbation of the urinary protein or urinary occult blood level. Ultrasonography showed no pathological findings in the subjects with the pathological urine sediments. The pathological urinary sediment in the subjects without kidney dysfunction may appear temporary and disappear without medical intervention.

This study focused on granular casts, epithelial casts, and tubular epithelial cells. Particularly, the granular components are reported to be closely related to chronic kidney disease[5]. Ishibashi *et al.*[6] compared urinary sediment findings using three models of a urinary solid component analyzer with those by visual examination and reported that the results of examination with all 3 analyzers showed generally high concordance rates with the results of visual examination, but that the results concerning casts were unsatisfactory, and that microscopy by laboratory technician was necessary. According to Harada *et al.*[7], they adopted a fully-automatic urinary solid component analyzer, but visual re-examination was needed when the sample contained a high level of solid components or when the results of automated analysis differed from the results of qualitative examination. Hishiki *et al.*[8] reported correlations between the results obtained with an automatic urinary sediment analyzer and those by microscopy. The sensitivity and specificity were 69.0% and 95.1%, respectively, for red blood cells, 60.9% and 99.4% for white blood cells, 34.1% and 99.6% for squamous epithelial cells, and 52.6% and 77.4% for hyaline casts. Regarding the reasons for the discrepancies with the results of microscopy, they suggested the difficulty of distinguishing components with similar shapes and the lack of recognition of special shapes. As observed above, automatic urinary sediment analyzers have limitations, and microscopy by laboratory technicians is necessary for casts of special importance. On the other hand, Ichimura *et al.*[9] examined the concordance rate among technicians concerning each sediment component and reported that components with a poor concordance rate of <60% varied among technicians, which means that the diagnosis is limited concerning components that require comprehensive evaluation, including the patient history. Morita *et al.*[10] analyzed qualitative urinary protein test results and cast-positive rate by visual examination and reported that the concordance rate was 8.5% in the urinary protein (−) group, but 80.0–93.5% in the (±)–(2+) groups, and 100% in the (3+) group. Also, concerning red blood cell casts, which is a pathological finding but was excluded from analysis in this study, occult blood was positive in 89% of the 85 samples by qualitative urinalysis[11]. From these reports, tests of urinary protein and urinary occult blood are considered to be generally sufficient as Ningen Dock items of urine examination.

The cost of microscopy was calculated to be at least ¥157 per sample. This does not include the costs of electricity and the facility. It also does not include the cost for training to improve or maintain skills. Therefore, this figure is considered to be the minimum cost of examination. On the other hand, the medical treatment fee for microscopic examination of urinary sediment is 27 points. Although the balance is not in the red, the benefit of examining urinary sediment in Ningen Dock is considered small from the viewpoint of the efficient use of personnel.

Limitations of this study include that there were not many patients who had pathological urinary sediment findings because of the rarity of patients with chronic kidney disease among Ningen Dock examinees. And only those who could be followed up after 4 years were evaluated. Thus the clarification of the reality is insufficient. The references were limited to reports from Japan due to the lack of Western literature concerning this subject.

This study does not deny the significance of urinary sediment examination itself. However, the results suggest that the medical significance of urinary sediment examination is limited in the stage of screening in the healthy subjects. Dependence on an automatic urine sediment analyzer in Ningen Dock for explanation of the results on the day of examination causes a decline in the diagnostic accuracy, eventually requiring microscopy by clinical laboratory technicians. The diagnosis of the most important casts relies on the image reading ability of laboratory technicians. The items of Ningen Dock examinations related to the kidney and urinary tract are the serum creatinine level and eGFR calculated from it, urinary protein, urinary occult blood, and

abdominal ultrasonography. It is considered more important to prevent kidney and urinary tract diseases by sufficiently utilizing the results of these examinations.

Conclusions

Pathological urinary sediment findings detected in Ningen Dock (granular casts, epithelial casts, tubular epithelium) disappeared in the natural course after 4 years in 91% of the patients. Even in those who continued to have these findings after 4 years, no significant change was observed in eGFR, urinary protein, or urinary occult blood. Therefore, the significance of examining urinary sediment in Ningen Dock by spending money on the personnel and equipment is considered to be low compared to measurement of eGFR, urinary protein, and urinary occult blood.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Japan Society of Ningen Dock: Basic Test Items. https://www.ningen-dock.jp/en/other/inspection (accessed July 25, 2022)
2. Japan Society of Ningen Dock: Criteria category. https://www.ningen-dock.jp/wp/wp-content/uploads/2018/06/Criteria-category.pdf (accessed July 25, 2022)
3. Japanese Society of Nephrology, The Japanese Urological Association, Japanese Society for Pediatric Nephrology, *et al.*: Guidelines for diagnosis of hematuria. https://www.jslm.org/others/news/hugl20140523.pdf (in Japanese) (accessed July 25, 2022)
4. Ministry of Health, Labour and Welfare: Basic Survey on Wage Structure by Occupations. https://www.mhlw.go.jp/file/05-Shingikai-10701000-Daijinkanboutoukeijouhoubu-Kikakuka/shiryo2-9.pdf (in Japanese) (accessed July 25, 2022)
5. Nozawa K, Takahashi J, Watanabe Y, *et al.*: The relationship between CKD severity classification and urinary cylindrical component. The Journal of Hokkaido Medical Technologists Association 2014; 12: 12–15. (in Japanese)
6. Ishibashi N, Yoshizawa R, Fujii Y, *et al.*: Comparison of 3 models of urinary solid component analyser. Japanese Journal of Medicine and Pharmaceutical Science 2020; 77: 1189–1200. (in Japanese)
7. Harada T, Yamazaki Y, Hoshi N: Criteria for visual examination using UF-5000 at our hospital. Journal of Asahikawa Medical Center 2020; 6: 52–54. (in Japanese)
8. Hishiki K, Akiyama Y, Karube K, *et al.*: Evaluation of performance of urine sediment analyzer ARKREY AUTION EYE AI-4510. Japanese Journal of Medical Technology 2021; 70: 456–464. (in Japanese)
9. Ichimura N, Koyama S, Sato A, *et al.*: Development of internal quality control system for microscopic urinary sediment examination and evaluation of an effect of a quality improvement on intralaboratory error among staff members. Japanese Journal of Medical Technology 2018; 67: 708–715. (in Japanese)
10. Morita Y, Shukuya K, Tanaka M, *et al.*: Validation of three automated urine sediment analyzers and the efficient workflow using criteria. Japanese Journal of Clinical Laboratory Automation 2019; 44: 602–609. (in Japanese)
11. Inoue T, Nakagawa R, Watanabe T, *et al.*: Evaluation of occult blood reaction and red blood cell count in urine samples with red blood cell casts. The Ehime Journal of Medical Technology 2019; 38: 57–60. (in Japanese)

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Original Article

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Association Between Arterial Stiffness and Bone Mineral Density in the Japanese General Population

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Abstract

Objective: Cardiovascular disease and osteoporosis are major public health problems that share common pathophysiological mechanisms besides aging. This study aimed to investigate the possible association between bone mineral density (BMD) and arterial stiffness measured by cardio-ankle vascular index (CAVI) in the Japanese general population without overt cardiac disease.

Methods: The study population was 1,242 consecutive participants who underwent cardiovascular health check-ups at the University of Tokyo Hospital. After excluding participants with coronary artery disease, peripheral arterial disease, atrial fibrillation/atrial flutter, decreased left ventricular systolic function, and pacemaker implantation, 1,169 men and women were analyzed. The calcaneus BMD was assessed by quantitative ultrasound methods. Cardiovascular functions were evaluated with CAVI, left ventricular global longitudinal strain (LVGLS), peak early diastolic transmitral velocity (E)/peak early diastolic mitral annular velocity(e′) (E/e′), and carotid intima-media thickness (IMT) measurements.

Results: In univariate analysis, BMD was associated with CAVI, E/e′ and IMT along with other cardiovascular risk factors except for diabetes ($r=-0.162$, $p<0.001$, $r=-0.203$, $p<0.001$, $r=-0.113$, $p<0.001$, respectively), whereas no association was identified between BMD and LVGLS. In multivariate analysis, after adjustment for pertinent confounding variables, only CAVI remained associated with low BMD (estimated coefficient=0.0050, $p=0.004$), but the direction of association was opposite and smaller in size compared to univariate analysis.

Conclusions: This study is the first to examine the relationship between BMD and multiple cardiovascular measurements in the Japanese general population without overt cardiovascular disease and has demonstrated the partial relationship of osteoporotic state and enhanced arterial stiffness, as evaluated by CAVI.

Keywords cardiovascular disease, bone mineral density (BMD), arterial stiffness

Cardiovascular disease and osteoporosis are major public health problems that are known to share common pathophysiological mechanisms besides aging. Over the last decades, we are facing aging society. Although a global increase in life expectancy is a huge achievement driven by improvements in public health and health care, many societies across the world are not prepared for this problem. Especially in elderly people, so-called multimorbidity, the coexistence of multiple chronic diseases, is a problematic issue that needs to be solved[1]. For example, prevalence of multimorbidity ranges from 55–98% worldwide. For this reason, acting on aging biology itself rather than modifying a single disease-specific process is highly recommended.

Osteoporosis and cardiovascular disease are frequently seen comorbidities, therefore preventive strategy and early detection are important. In clinical settings, there have been large-scale clinical trials showing links between osteoporosis and cardiovascular disease. In terms of heart failure, the EPIC-Norfolk Prospective study from the United Kingdom and the Cardiovascular Health study conducted in the United States have shown that a history of osteoporosis and low bone

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mineral density (BMD) was an independent predictor for future occurrence of heart failure[2,3]. And the EPIC-Norfolk Prospective study, the same study from UK, has shown the correlation between osteoporosis and future stroke risk[4].

Similarly, several studies have investigated the correlations between osteoporosis and artery diseases, namely atherosclerosis and arteriosclerosis. First, in animal models, both osteoprotegerin-deficient mice and apoprotein E-deficient mice have a greater ability to develop early vascular calcification, osteoporosis, and increased risk of cardiovascular disease (CVD)[5]. Second, in humans, two studies have shown the links between bone mineral density and atherosclerosis measured by carotid intima-media thickness (IMT)[6,7]. On the other hand, the correlation between BMD and arterial stiffness, usually measured by pulse wave velocity (PWV) or cardio-ankle vascular index (CAVI), is under controversial debate, and only one study from China showed the correlation between CAVI and BMD in the general population[8]. In the current study, we explored the relationship between BMD and multiple cardiovascular parameters to investigate a possible association between the osteoporotic state and arterial stiffness in the Japanese population.

Methods

Study population

The study population was derived from 1,242 consecutive subjects who participated in extensive cardiovascular health check-ups from August 2014 to May 2018 at the University of Tokyo Hospital. Among a total of 1,242 participants who underwent laboratory testing, lifestyle questionnaires, ultrasonographic examination and BMD measurement, patients with a history of coronary artery disease, peripheral arterial disease, atrial fibrillation or atrial flutter, decreased LV systolic function (LV ejection function <50%), valvular disease, or pacemaker implantation were excluded. Informed consent was obtained from all study participants. The study was approved by the ethics committee of the University of Tokyo.

Risk factor assessment and definition

Cardiovascular risk factors were ascertained through direct examination and interviews conducted by healthcare professionals. Hypertension was defined as systolic BP (SBP) ≥140 mmHg or diastolic BP (DBP) ≥90 mmHg, or the use of antihypertensive medication. Diabetes mellitus was defined by a fasting blood glucose ≥126 mg/dL or the current use of insulin or oral hypoglycemic agents. Hyperlipidemia was defined as total serum cholesterol >240 mg/dL or the use of lipid-lowering medications. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Venous blood samples were collected under fasting conditions on the same day as cardiovascular examinations. The glomerular filtration rate (GFR) was estimated in the equation: $eGFR=194\times age^{-0.287}\times creatinine^{-1.094}+$ ($\times 0.739$ if female). Predictive indices for insulin resistance, Triglyceride-glucose (TyG) index and TG/HDL-C, were calculated as follows: TyG index=ln [Fasting triglyceride (mg/dL) × Fasting glucose (mg/dL)]/2, and TG/HDL-C=Fasting triglyceride/HDL cholesterol, respectively.

Quantitative ultrasound assessment of calcaneus

Although dual-energy X ray absorptiometry (DXA) is recommended for BMD evaluation by numerous guidelines and World Health Organization (WHO) criteria, quantitative ultrasound (QUS) has been developed and proven to be an alternative method to DXA for assessing osteoporotic status[9,10]. In this study, the SOS (speed of sound, m/s) of the calcaneus bone was measured using an ultrasound bone densitometer (CM-300, Furuno Electric Co. Ltd., Nishinomiya, Japan). The T-score was calculated from the manufacturer-specific SOS reference population database. %YAM (young adult mean) represents % value relative to the standard SOS of the young age. Subjects were screened for presence of low BMD and classified into osteopenia and osteoporosis based on WHO criteria.

Measurement of CAVI

CAVI was measured using an automatic vascular screening system (Vasera VS-1500, Fukuda Denshi, Tokyo, Japan) with the patients in the supine position after 5 minutes of rest. The electrocardiogram, phonocardiogram, and SBP and DBP of the brachial and ankle arteries were simultaneously recorded. The formula to determine CAVI was as follows:

$CAVI=a[(2\rho/\Delta P)\times \ln (SBP/DBP)\ PWV^2]+b$, where $\Delta P=SBP-DBP$, ρ=blood density, PWV=pulse wave velocity, and a and b are scale conversion constants to match aortic PWV. The mean of the right and left CAVI values was used for analysis.

Measurement of carotid IMT

Carotid IMT measurement was performed with patients in the supine position using high resolution B-mode ultrasonography with linear-array 7.5 MHz transducer (Aplio 300, Toshiba Medical Systems, Tokyo, Japan). The maximal IMT in both right and left common carotid artery was recorded according to the current guideline[11], and the mean was used for analysis as max IMT.

Echocardiographic assessment or echocardiographic examination

Two-dimensional echocardiographic examination was performed utilizing a commercially available system (Aplio 300, Toshiba Medical Systems, Tokyo, Japan) by registered cardiac sonographers blinded to

the participants' clinical information according to a standardized protocol. The dimensions of the cardiac chambers and left ventricular ejection function (LVEF) were measured in the standard manner[12]. LV diastolic function assessment was performed based on the current guidelines[13]. Transmitral diastolic flow was obtained from an apical four-chamber view. Pulsed-wave Doppler examination of mitral inflow was performed to measure early (E) peak velocity, and peak early diastolic mitral annular velocity (e′) was also measured from tissue Doppler imaging in the lateral and the septal mitral annulus. The mean value of E/e′ was used for analysis, and impaired diastolic function was defined as mean E/e′>14.

Speckle-tracking analysis was performed offline using vendor-independent commercially available software (2D Cardiac Performance Analysis; Tomtec Imaging System, Germany). LV global longitudinal strain (LVGLS) was calculated by averaging the negative peak of longitudinal strain from 18 ventricular segments in all 3 apical views, including the 4-chamber, 2-chamber and long-axis views, according to the current guidelines[14]. As for the definition of strain, negative strain denotes shortening for LV which indicates that a smaller value (a larger absolute value) shows better LV contraction.

Statistical analysis

Data are expressed as mean±SD for continuous variables and as number (percentage) for categorical variables. Statistical significance was set at $p<0.05$. The differences in baseline characteristics between men and women were determined by Student's *t*-tests for continuous variables and χ^2 tests for categorical variables. Linear regression analysis was performed to evaluate associations between BMD and each dependent variable, and Pearson's correlation coefficients were calculated. Multivariate regression analysis was used to determine the effects of BMD on cardiovascular measurements before and after incorporating potential confounding factors. All statistical analyses were performed with JMP 15 statistical software (SAS Institute, Cary, NC, USA).

Results

Flow chart of participant enrollment and exclusion

A population flow chart is summarized in **Fig. 1**. We enrolled 1,242 participants who underwent extensive cardiovascular health check-ups in our institute from 2014 to 2018.

After exclusion of cardiovascular diseases, such as coronary artery disease (n=29), peripheral arterial disease (n=1), atrial fibrillation or atrial flutter (n=14), decreased LV systolic function (n=6), pacemaker implantation (n=1), and missing clinical data (n=22), the final study group comprised 1,169 participants without overt cardiovascular disease. None of the participants had a history of valvular disease.

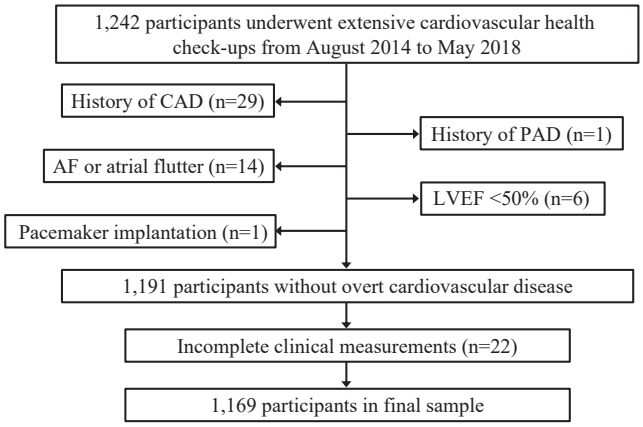

Fig. 1. Flow Chart of Participant Enrollment and Exclusion

Abbreviations: CAD indicates coronary artery disease; PAD, peripheral arterial disease; AF, arterial fibrillation; and LVEF, left ventricular ejection fraction.

Baseline characteristics of men and women

Table 1 shows the baseline characteristics of the participants stratified by sex. The prevalence of hypertension, diabetes, and smoking habit was significantly higher in men, whereas the prevalence of hyperlipidemia and osteoporosis was higher in women. Similarly, the gender differences were recognized in most of the demographics and laboratory data except for estimated glomerular filtration rate (eGFR) and thyroid stimulating hormone (TSH). Men had higher blood pressure, and women had higher levels of brain natriuretic peptide (BNP), HDL cholesterol, and lipoprotein (a). Regarding cardiovascular measurements, CAVI and max IMT were higher in men, while LVGLS and mean E/e′ were lower in men. Regarding BMD, %YAM was higher in male subjects in accordance with the lower prevalence of osteoporosis in men.

Linear regression analysis for BMD

Table 2 shows linear regression analysis between BMD and the clinical variables from baseline characteristics. BMI ($r=+0.129$, $p<0.001$), eGFR ($r=+0.069$, $p=0.018$), TyG index ($r=+0.030$, $p<0.001$), albumin ($r=+0.164$, $p<0.001$), uric acid ($r=+0.120$, $p<0.001$) were positively correlated with BMD, whereas age ($r=-0.349$, $p<0.001$), systolic blood pressure ($r=-0.060$, $p=0.004$), HDL cholesterol ($r=-0.099$, $p<0.001$), BNP ($r=-0.170$, $p<0.001$), lipoprotein (a) ($r=-0.066$, $p<0.001$) were negatively correlated with BMD. Among cardiovascular parameters, mean CAVI, mean E/e′ and max IMT showed negative correlation with BMD ($r=-0.162$, $p<0.001$, $r=-0.203$, $p<0.001$, $r=-0.113$, $p<0.001$, respectively), nonetheless LVGLS demonstrated no correlation with BMD.

Table 1. Baseline Characteristics of Men and Women

	Men (*n*=651)	Women (*n*=518)	*p* value
Age, years	61.4±11.6	63.4±11.6	0.001*
Body mass index (BMI), kg/m^2	24.5±3.3	22.3±3.5	<0.001*
Systolic blood pressure, mmHg	121.6±14.7	117.7±15.9	<0.001*
Diastolic blood pressure, mmHg	76.6±10.9	73.43±10.3	<0.001*
Current smoking, *n* (%)	90 (13.8)	25 (4.8)	<0.001*
Alcohol intake, *n* (%)	426 (65.4)	187 (36.1)	0.062
Hypertension, *n* (%)	251 (38.6)	155 (29.9)	0.002*
Hyperlipidemia, *n* (%)	208 (32.0)	221 (42.7)	<0.001*
Diabetes mellitus, *n* (%)	97 (14.9)	22 (4.3)	<0.001*
Osteoporosis, *n* (%)	5 (0.77)	61 (11.78)	<0.001*
Creatinine, g/dL	0.91±0.49	0.66±0.17	<0.001*
eGFR, mL/min/1.73 m^2	71.2±15.9	72.7±15.5	0.097
Fasting plasma glucose, %	103.8±22.1	94.6±15.0	<0.001*
Hemoglobin A1c, %	5.91±0.68	5.76±0.51	<0.001*
LDL-cholesterol, mg/dL	123.9±30.6	126.4±30.3	0.162
HDL-cholesterol, mg/dL	58.9±15.5	74.0±19.0	<0.001*
Triglyceride (TG), mg/dL	127.8±94.2	91.4±55.2	<0.001*
non HDL-cholesterol, mg/dL	141.8±33.2	139.3±32.4	0.211
TG/HDL-C	2.53±2.49	1.43±1.36	<0.001*
TyG index	4.65±0.31	4.47±0.26	<0.001*
Lipoprotein (a), mg/dL	11.3±10.9	14.3±12.8	<0.001*
Albumin, g/dL	4.3±0.3	4.2±0.2	<0.001*
Uric acid, mg/dL	6.2±1.2	4.9±1.1	<0.001*
Thyroid stimulating hormone (TSH), μIU/mL	2.3±1.9	2.3±1.6	0.879
Serum iron, μg/mL	113.5±35.5	99.6±30.4	<0.001*
Brain natriuretic peptide (BNP), pg/mL	19.9±21.3	27.5±29.1	<0.001*
%YAM	82.9±14.8	77.6±13.7	<0.001*
mean CAVI	8.16±1.18	7.90±1.02	<0.001*
LVGLS, %	−20.60±2.39	−22.09±2.90	<0.001*
LVEF, %	68.33±6.57	69.95±6.45	<0.001*
mean e′, cm/s	8.16±2.14	8.21±2.44	0.662
mean E/e′	8.56±2.36	9.67±3.14	<0.001*
max IMT, mm	1.08±0.34	0.98±0.25	<0.001*

Values represent mean±SD or number of participants and prevalence rate (%) in parentheses.
Abbreviations: eGFR indices estimated glomerular filtration rate; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; TyG, triglyceride-glucose; CAVI, cardio-ankle vascular index; LVGLS, left ventricular global longitudinal strain; LVEF, left ventricular ejection fraction; e′, early diastolic mitral annular velocity; E, early diastolic transmitral flow velocity; and max IMT, maximal intima-media thickness.

Correlation between osteoporotic state and cardiovascular parameters

Fig. 2 shows the correlations between the cardiovascular parameters and BMD in a categorized manner, namely normal (*n*=524), osteopenia (*n*=383), and osteoporosis (*n*=262). Variables other than LVGLS had significant correlations with osteoporotic state (CAVI, $p<0.001$; mean E/e′, $p<0.001$; max IMT, $p<0.001$).

Correlation between BMD and cardiovascular parameters in multivariate analysis

In **Table 3**, multivariate analysis of BMD for cardiovascular parameters was performed with the variables that showed significant correlations with BMD in the linear regression analysis shown in **Table 2**. After adjusting for age and sex (Model 1), BMI, diabetes, hyperlipidemia, hypertension, current smoking, and alcohol intake (Model 2), albumin, eGFR, HDL-cholesterol, uric acid, serum iron, and BNP (Model 3), lipoprotein (a) and TyG index (Model 4), the correlation between BMD and CAVI remained statistically significant (Model 1, estimated coefficient=+0.0037, $p=0.041$; Model 2, estimated coefficient=+0.0046, $p=0.009$; Model 3, estimated coefficient=+0.0046, $p=0.009$; Model 4, estimated coefficient=+0.0050, $p=0.004$); however the association between BMD and the remaining cardiovascular parameters (LVGLS, mean E/e′, max IMT) fell short of statistical significance.

Correlation between BMD and cardiovascular parameters in multivariate analysis stratified by sex

As gender difference was not negligible in this study population, we stratified subjects into two groups by sex. In sub-analyses stratified by sex (**Table 4, 5**), the correlation between BMD and CAVI was attenuated but still statistically significant in all adjusted models in men (Model 1, estimated coefficient=+0.0056, $p=0.015$; Model 2, estimated coefficient=+0.0055,

Table 2. Linear Regression Analysis for BMD

	r	*p* value
Age, years	−0.349	<0.001*
Body mass index (BMI), kg/m^2	+0.129	<0.001*
Systolic blood pressure, mmHg	−0.060	0.004*
Diastolic blood pressure, mmHg	+0.040	0.170
Creatinine, g/dL	+0.033	0.266
eGFR, mL/min/1.73 m^2	+0.069	0.018*
Fasting plasma glucose, %	−0.017	0.555
Hemoglobin A1c, %	−0.051	0.082
LDL-cholesterol, mg/dL	−0.009	0.747
HDL-cholesterol, mg/dL	−0.099	<0.001*
Triglyceride (TG), mg/dL	+0.048	0.103
non HDL-cholesterol, mg/dL	+0.006	0.830
TG/HDL-Cholesterol	+0.047	0.110
TyG index	+0.030	<0.001*
Albumin, g/dL	+0.164	<0.001*
Uric Acid, mg/dL	+0.120	<0.001*
Thyroid stimulating hormone (TSH), μIU/mL	−0.032	0.278
Serum iron, μg/mL	+0.066	0.024*
Brain natriuretic peptide (BNP), pg/mL	−0.170	<0.001*
Lipoprotein (a) , mg/dL	−0.066	<0.001*
mean CAVI	−0.162	<0.001*
LVGLS, %	−0.035	0.231
LVEF, %	−0.033	0.256
mean e′, cm/s	+0.207	<0.001*
mean E/e′	−0.203	<0.001*
max IMT, mm	−0.113	<0.001*

r=Pearson's correlation coefficient

Abbreviations: eGFR indices estimated glomerular filtration rate; LDL-cholesterol, low-density lipoprotein-cholesterol; HDL-cholesterol, high-density lipoprotein-cholesterol; TyG, triglyceride-glucose; CAVI, cardio-ankle vascular index; LVGLS, left ventricular global longitudinal strain; LVEF, left ventricular ejection fraction; e′, early diastolic mitral annular velocity; E, early diastolic transmitral flow velocity; and max IMT, maximal intima-media thickness.

p=0.016; Model 3, estimated coefficient=+0.0059, *p*=0.011), while as for women, the correlation between BMD and CAVI virtually all vanished. Regarding the other parameters (LVGLS, mean E/e′, max IMT), no correlation with BMD was observed in all models (Models 1–3), which was consistent with the results shown in **Table 3**.

Discussion

In this study, we explored the relationship between BMD and cardiovascular variables including CAVI, LVGLS, E/e′, and IMT in the population without overt cardiac disease. Each of the parameters are of importance in terms of arterial aging (arteriosclerosis for CAVI and atherosclerosis for IMT), or preclinically impaired cardiac function (systolic function for LVGLS and diastolic function for E/e′) respectively. In univariate analysis, BMD was associated with CAVI, E/e′, and IMT along with other cardiovascular risk factors except for diabetes, whereas no association was identified between BMD and LVGLS. In multivariate analysis, after adjustment for pertinent confounding variables, only CAVI remained associated with low BMD, but unexpectedly the direction of association was opposite and smaller in size compared to univariate analysis. This can be explained that the observed association between BMD and CAVI in univariate analysis was due to confounding through the association between age and BMD or CAVI, but still does not completely deny the clinical significance of the correlation between CAVI

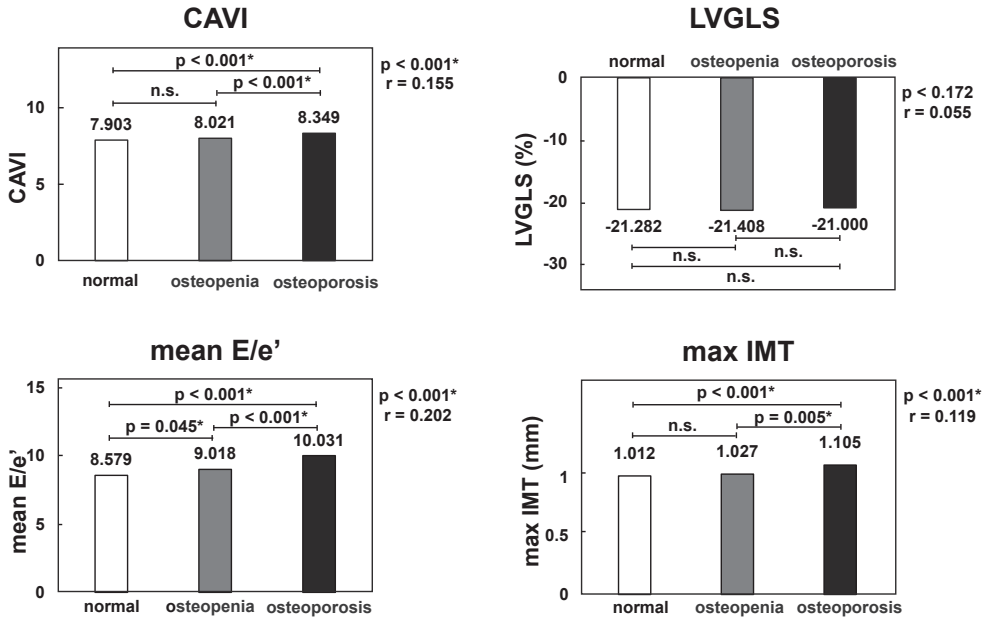

Fig. 2. Correlation Between Osteoporotic State and Cardiovascular Parameters

normal (*n*=524), osteopenia (*n*=383), osteoporosis (*n*=262)

Abbreviations: CAVI indices cardio-ankle vascular index; LVGLS, left ventricular global longitudinal strain; e′, early diastolic mitral annular velocity; E, early diastolic transmitral flow velocity; and max IMT, maximal intima-media thickness.

Table 3. Correlation Between BMD and Cardiovascular Parameters in Multivariate Analysis

parameters	Model	B	Lower 95% CI	Upper 95% CI	*p* value
CAVI	Model 1	0.0037	0.0002	0.0072	0.041*
	Model 2	0.0046	0.0012	0.0080	0.009*
	Model 3	0.0046	0.0012	0.0080	0.009*
	Model 4	0.0050	0.0016	0.0084	0.004*
LVGLS	Model 1	−0.0049	−0.0159	0.0061	0.382
	Model 2	−0.0067	−0.0175	0.0041	0.225
	Model 3	−0.0073	−0.0180	0.0035	0.185
	Model 4	−0.0067	−0.0175	0.0041	0.221
mean E/e′	Model 1	−0.0033	−0.0136	0.0070	0.529
	Model 2	−0.0045	−0.0146	0.0056	0.379
	Model 3	−0.0035	−0.0135	0.0065	0.493
	Model 4	−0.0036	−0.0136	0.0065	0.487
max IMT	Model 1	0.0004	−0.0008	0.0015	0.499
	Model 2	0.0003	−0.0008	0.0015	0.593
	Model 3	0.0004	−0.0007	0.0016	0.463
	Model 4	0.0004	−0.0007	0.0015	0.502

Model 1: adjusted for age and sex
Model 2: adjusted for Model 1 plus BMI, diabetes, hyperlipidemia, hypertension, current smoking and alcohol intake
Model 3: adjusted for Model 2 plus albumin, eGFR, HDL-cholesterol, uric acid, serum iron and BNP
Model 4: adjusted for Model 3 plus lipoprotein (a) and TyG index
Abbreviations: B indicates regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-cholesterol, high-density lipoprotein-cholesterol; TyG, triglyceride-glucose; BNP, brain natriuretic peptide; CAVI, cardio-ankle vascular index; LVGLS, left ventricular global longitudinal strain; e′, early diastolic mitral annular velocity; E, early diastolic transmitral flow velocity; and max IMT, maximal intima-media thickness.

Table 4. Correlation Between BMD and Cardiovascular Parameters in Multivariate Analysis Stratified by Sex (Men)

parameters	Model	B	Lower 95% CI	Upper 95% CI	*p* value
CAVI	Model 1	0.0056	0.0011	0.0100	0.015*
	Model 2	0.0055	0.0010	0.0101	0.016*
	Model 3	0.0059	0.0014	0.0104	0.011*
LVGLS	Model 1	−0.0052	−0.0176	0.0073	0.416
	Model 2	−0.0055	−0.0180	0.0070	0.386
	Model 3	−0.0051	−0.0175	0.0073	0.419
mean E/e′	Model 1	0.0005	−0.0101	0.0112	0.919
	Model 2	0.0010	−0.0097	0.0117	0.854
	Model 3	0.0012	−0.0095	0.0119	0.830
max IMT	Model 1	0.0004	−0.0012	0.0020	0.640
	Model 2	0.0006	−0.0010	0.0022	0.486
	Model 3	0.0005	−0.0011	0.0022	0.515

Model 1: adjusted for age, BMI, diabetes, hyperlipidemia, hypertension, current smoking and alcohol intake
Model 2: adjusted for Model 2 plus albumin, eGFR, HDL-cholesterol, uric acid, serum iron and BNP
Model 3: adjusted for Model 3 plus lipoprotein (a) and TyG index
Abbreviations: B indicates regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-cholesterol, high-density lipoprotein-cholesterol; TyG, triglyceride-glucose; BNP, brain natriuretic peptide; CAVI, cardio-ankle vascular index; LVGLS, left ventricular global longitudinal strain; e′, early diastolic mitral annular velocity; E, early diastolic transmitral flow velocity; and max IMT, maximal intima-media thickness.

and BMD. Overall, this study is the first to examine the relationship between BMD and multiple cardiovascular measurements in the Japanese general population without overt cardiovascular disease and has partially demonstrated the independent relationship of osteoporotic state and enhanced arterial stiffness, as evaluated by CAVI.

In general, cardiovascular disease and osteoporosis are two major factors that severely affect the quality of life and mortality of middle-aged and elderly people. There is growing evidence that the coincidental occurrence of both diseases may be related to common pathophysiological mechanisms regardless of age[15]. Indeed, bone formation and vascular calcification share underlying biological mechanisms, mainly through inflammation and the RANK (receptor activator of nu-

Table 5. Correlation Between BMD and Cardiovascular Parameters in Multivariate Analysis Stratified by Sex (Women)

parameters	Model	B	Lower 95% CI	Upper 95% CI	*p* value
CAVI	Model 1	0.0018	−0.0038	0.0073	0.535
	Model 2	0.0010	−0.0045	0.0066	0.714
	Model 3	0.0017	−0.0038	0.0072	0.538
LVGLS	Model 1	−0.0062	−0.0268	0.0144	0.556
	Model 2	−0.0090	−0.0296	0.0116	0.391
	Model 3	−0.0086	−0.0293	0.0121	0.417
mean E/e′	Model 1	−0.0077	−0.0283	0.0129	0.464
	Model 2	−0.0095	−0.0299	0.0110	0.363
	Model 3	−0.0096	−0.0300	0.0108	0.354
max IMT	Model 1	−0.0001	−0.0017	0.0014	0.869
	Model 2	−0.0001	−0.0016	0.0015	0.947
	Model 3	−0.0001	−0.0017	0.0015	0.925

Model 1: adjusted for age, BMI, diabetes, hyperlipidemia, hypertension, current smoking and alcohol intake
Model 2: adjusted for Model 2 plus albumin, eGFR, HDL-cholesterol, uric acid, serum iron and BNP
Model 3: adjusted for Model 3 plus lipoprotein (a) and TyG index
Abbreviations: B indicates regression coefficient; 95%CI, 95% confidence interval; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-cholesterol, high-density lipoprotein-cholesterol; TyG, triglyceride-glucose; BNP, brain natriuretic peptide; CAVI, cardio-ankle vascular index; LVGLS, left ventricular global longitudinal strain; e′, early diastolic mitral annular velocity; E, early diastolic transmitral flow velocity; and max IMT, maximal intima-media thickness.

clear factor-kappaB (NFκB))/RANKL (RANK ligand)/OPG (osteoprotegerin) system[16]. Recently, it was proven by positron emission tomography that vascular inflammation precedes vascular calcification in both the intimal and medial layers of the vasculature wall[17]. As for intimal layer calcification, it is explained by the atherosclerotic state, which is characterized by the death of lipid-laden macrophages and the sequential formation of calcium phosphate crystals. For the calcification in the medial layer, in addition to saturated fatty acids, inflammatory mediators such as interleukin (IL)-1β, IL-6, IL8, insulin-like growth factor (IGF)-1, and tumor necrosis factor (TNF)-α are responsible for inducing osteogenic differentiation of vascular smooth muscle cells (VSMCs)[18]. In this process, the RANK/RANKL/OPG system that belongs to TNF family is postulated to be important inducers and inhibitors of VSMC calcification. As inducers, the binding of RANKL to RANK receptor promotes differentiation of preosteoclasts into mature osteoclasts through the activation of the NFκB intracellular signaling pathway, a process that is also important in bone resorption in osteoporosis. As inhibitors, on the contrary, OPG, which is protective of bone tissue through inhibition of the RANK/RANKL system, not only protects bones from resorption, but also hampers the differentiation of preosteoclasts into mature osteoclasts in VSMCs. OPG as well as RANK and RANKL proteins are detected in atherosclerotic plaques, and RANKL was also shown to be elevated in heart failure patients[19,20]. It should be also noted that angiotensin II, which is a well-known promoter of atherosclerosis and arterial stiffness, has been reported to activate osteoclasts[21], leading to osteoporosis through the RANK/RANKL pathway[22]. These pathophysiological mechanisms could be a plausible explanation for the clinical trials that showed the association between low BMD and cardiovascular diseases.

In our study, we observed an association of BMD and arterial stiffness after adjusting for other pertinent cardiovascular risk factors and the variables that had significant univariate linear correlation with BMD, however the direction of association was opposite and smaller in size compared to univariate analysis. Although several studies have suggested an association between PWV or CAVI and BMD, these studies were mostly conducted in specific populations, for example, postmenopausal women, patients with chronic kidney disease, patients undergoing hemodialysis, or hypertensive patients[23–25]. In contrast, few studies have been conducted in a general population, and there is only one study from China suggesting an association between BMD and arterial stiffness as assessed by CAVI[8]. Hence the results of our study are in accordance with the controversial debate which has been conducted worldwide.

The degree of arterial stiffness is often used as an important predictor for the prognosis of cardiovascular diseases. Therefore, early detection of arterial stiffness before the structure becomes abnormal gives us crucial information to prevent cardiovascular events. Compared to PWV, CAVI is a newer noninvasive indicator of arterial stiffness, which is performed by integrating ECG, phonocardiogram, and arterial pulse waveform techniques[26]. CAVI can reflect the overall arterial elasticity from the origin of the aorta to the ankle artery without being affected by blood pressure. In 2001, Framingham's study showed that patients with low BMD had more severe abdominal aortic calcification after adjusting for age and cardiovascular risk factors[27].

It has been observed that the association between osteoporosis and vascular calcification is also detected in coronary and peripheral arteries[28]. If we focus on the results of univariate analysis in terms of clinical interpretation, these findings support the results of our study showing that low BMD is associated with increased risk of arterial stiffness, with the concept that calcification of the aorta and peripheral arteries foster arterial stiffness and central blood pressure augmentation, which can enhance CAVI and ultimately develop cardiac dysfunction, that could have been evaluated by LVGLS or E/e′ if the participants of the current study were in the advanced stage of cardiovascular diseases.

Another important finding of our study is that the association between BMD and arterial stiffness vanished in female subjects. The reason for this lack of association in women is unclear. One possible explanation is that the prevalence of osteoporosis in women is higher than in men and the clinical condition of osteoporosis gets worse in the postmenopausal state. To address this possibility, we performed a sub-analysis focusing on women >50 years, but the observed insignificant association between BMD and CAVI in women persisted after adjustment for these factors (data not shown). Several epidemiological studies have reported similar results. For example, in the Cardiovascular Health Study, there was no evidence of a significant association between BMD measured by DXA scan and incident heart failure in women, whereas significant association was found in nonblack men >65 years old[2]. Also, the EPIC-Norfolk study found that lower BMD in the calcaneus measured by quantitative ultrasound was associated with a higher incidence of heart failure, however the association was not significant in women[3]. In these studies, the reason for the lack of association of BMD and heart failure in women was uncertain; nonetheless the effect of estrogen could have substantial effect on these bone-cardiovascular interactions. Overall, it can be postulated in our study that estrogen could overshadow other potentially weaker factors linking atrial stiffness, such as BMD, in female subjects.

In fact, estrogen deficiency may have a crucial role in the association between osteoporosis and cardiovascular diseases. First, estrogen receptors are widely distributed not only in osteoclasts and osteoblasts, but also in vascular endothelial and smooth muscle cells[29]. Second, in the postmenopausal state, the decline in production of estrogen causes secretion of pro-inflammatory cytokines such as IL-6, IL-1, and TNF-α. These proinflammatory cytokines increase the level of RANKL, and the action of RANKL results in bone loss and vascular calcification, causing calcium to transfer from bone to the blood vessel walls[30]. Although these mechanisms are plausible, further research is needed.

The present study has some limitations. First, there is a selection bias, as the participants are from single center. Second, the adjustment may not have been sufficient to account for covariates and hence the results are susceptible to residual confounding. Third, due to the nature of cross-sectional studies, our study has not yet revealed the causal relationship between arterial stiffness and osteoporosis. Fourth, we lack the information for anti-osteoporotic medications and supplement intakes. Fifth, we used quantitative ultrasound of the heel as a measure of osteoporosis, although DXA is the standard method recommended in current guidelines. Finally, the selection processes in this study resulted in a bias for healthy subjects and would lead to smaller variances in BMD and arterial stiffness, making it difficult to reach significant differences.

Conclusion

Although our study is in accordance with the controversial debate and could not reach a solid conclusion showing a correlation between BMD and CAVI, the results give us an indication that increased arterial stiffness is likely to be comorbid with osteoporosis in the Japanese general population. During treatment, clinicians should pay attention to arterial stiffness in patients with low BMD and vice versa.

Acknowledgement

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Conflicts of Interest

The authors have no conflicts of interest to declare.

References

1. Marengoni A, Angleman S, Melis R, *et al.*: Aging with multimorbidity: a systematic review of the literature. Ageing Res Rev 2011; 10: 430–439.
2. Fohtung RB, Brown DL, Koh WJ, *et al.*: Bone mineral density and risk of heart failure in older adults: the cardiovascular health study. J Am Heart Assoc 2017; 6: e004344.
3. Pfister R, Michels G, Sharp SJ, *et al.*: Low bone mineral density predicts incident heart failure in men and women: the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk prospective study. JACC Heart Fail 2014; 2: 380–389.
4. Myint PK, Clark AB, Kwok CS, *et al.*: Bone mineral density and incidence of stroke: european prospective investigation into cancer-norfolk population-based study, systematic review, and meta-analysis. Stroke 2014; 45: 373–382.

5. Sprini D, Rini GB, Di Stefano L, *et al.*: Correlation between osteoporosis and cardiovascular disease. Clin Cases Miner Bone Metab 2014; 11: 117–119.
6. Kajikawa M, Oda N, Kishimoto S, *et al.*: Increasing risk of osteoporotic fracture is associated with vascular dysfunction and abnormal vascular structure in both men and women. Circ J 2017; 81: 862–869.
7. Hamada M, Kajita E, Tamaki J, *et al.*: Decreased bone mineral density and osteoporotic fractures are associated with the development of echogenic plaques in the carotid arteries over a 10-year follow-up period: the Japanese Population-based Osteoporosis (JPOS) cohort study. Maturitas 2020; 131: 40–47.
8. Zhang M, Bai L, Kang J, *et al.*: Links between arterial stiffness and bone mineral density in middle-aged and elderly Chinese individuals: a cross-sectional study. BMJ Open 2019; 9: e029946.
9. Frost ML, Blake GM, Fogelman I: Quantitative ultrasound and bone mineral density are equally strongly associated with risk factors for osteoporosis. J Bone Miner Res 2001; 16: 406–416.
10. Hans D, Dargent-Molina P, Schott AM, *et al.*: Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. Lancet 1996; 348: 511–514.
11. Stein JH, Korcarz CE, Hurst RT, *et al.*: Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008; 21: 93–111; quiz 189–190.
12. Lang RM, Badano LP, Mor-Avi V, *et al.*: Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2015; 28: 1–39.e14.
13. Nagueh SF, Smiseth OA, Appleton CP, *et al.*: Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016; 29: 277–314.
14. Voigt JU, Pedrizzetti G, Lysyansky P, *et al.*: Definitions for a common standard for 2d speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. J Am Soc Echocardiogr 2015; 28: 183–193.
15. Towler DA: Commonalities between vasculature and bone: an osseocentric view of arteriosclerosis. Circulation 2017; 135: 320–322.
16. García-Gómez MC, Vilahur G: Osteoporosis and vascular calcification: a shared scenario. Clin Investig Arterioscler 2020; 32: 33–42.
17. New SE, Aikawa E: Cardiovascular calcification: an inflammatory disease. Circ J 2011; 75: 1305–1313.
18. Kawakami R, Nakagami H, Noma T, *et al.*: RANKL system in vascular and valve calcification with aging. Inflamm Regen 2016; 36: 10.
19. Leistner DM, Seeger FH, Fischer A, *et al.*: Elevated levels of the mediator of catabolic bone remodeling RANKL in the bone marrow environment link chronic heart failure with osteoporosis. Circulation Heart failure 2012; 5: 769–777.
20. Ueland T, Yndestad A, Øie E, *et al.*: Dysregulated osteoprotegerin/RANK ligand/RANK axis in clinical and experimental heart failure. Circulation 2005; 111: 2461–2468.
21. Shapiro Y, Boaz M, Matas Z, *et al.*: The association between the renin-angiotensin-aldosterone system and arterial stiffness in young healthy subjects. Clin Endocrinol (Oxf) 2008; 68: 510–512.
22. Shimizu H, Nakagami H, Osako MK, *et al.*: Angiotensin II accelerates osteoporosis by activating osteoclasts. FASEB J 2008; 22: 2465–2475.
23. Tanna N, Patel K, Moore AE, *et al.*: The relationship between circulating adiponectin, leptin and vaspin with bone mineral density (BMD), arterial calcification and stiffness: a cross-sectional study in post-menopausal women. J Endocrinol Invest 2017; 40: 1345–1353.
24. Masugata H, Senda S, Inukai M, *et al.*: Association between bone mineral density and arterial stiffness in hypertensive patients. Tohoku J Exp Med 2011; 223: 85–90.
25. Raggi P, Bellasi A, Ferramosca E, *et al.*: Pulse wave velocity is inversely related to vertebral bone density in hemodialysis patients. Hypertension 2007; 49: 1278–1284.
26. Saiki A, Sato Y, Watanabe R, *et al.*: The role of a novel arterial stiffness parameter, cardio-ankle vascular index (CAVI), as a surrogate marker for cardiovascular diseases. J Atheroscler Thromb 2016; 23: 155–168.
27. Kiel DP, Kauppila LI, Cupples LA, *et al.*: Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. Calcif Tissue Int 2001; 68: 271–276.
28. Hyder JA, Allison MA, Wong N, *et al.*: Association of coronary artery and aortic calcium with lumbar bone density: The MESA Abdominal Aortic Calcium Study. Am J Epidemiol 2009; 169: 186–194.
29. Mendelsohn ME, Karas RH: The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999; 340: 1801–1811.
30. Khosla S: The bone and beyond: a shift in calcium. Nat Med 2011; 17: 430–431.

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Original Article

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Predictive Factors for Diabetes Development in Ningen Dock Examinees with Very Mild Glucose Intolerance

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Abstract

Objective: This study aimed to identify predictive factors for diabetes development in Ningen Dock examinees with very mild glucose intolerance.

Methods: We retrospectively analyzed medical records, interview responses, and Ningen Dock results of 11,313 examinees from April 2015 to March 2016 and followed them until March 2021.

Results: Over five years, 2.6% of examinees were newly diagnosed with diabetes. Those who developed diabetes had statistically significantly higher median age, body weight, and BMI compared to those who did not develop diabetes ($p<0.001$). Almost all laboratory values, including FPG and HbA1c, were worse in the former group. When their FPG and HbA1c levels were not high (Ningen Dock category A or B), their incidence rate of developing diabetes over the next five years was extremely low (0.09–0.7%). We conducted multivariate analysis regarding predictive factors for the development of diabetes among examinees in Ningen Dock category B, which identified BMI ≥25 kg/m^2 (OR 5.356, $p<0.001$), LDL-C ≥140 mg/dL (OR 2.415, $p=0.023$), smoking (OR 2.320, $p=0.042$), and family history of diabetes (OR 2.510, $p=0.016$) as significant predictive factors. The incidence rate of developing diabetes over the next five years increased from 0.7% to 12.5% when these predictive factors were present.

Conclusions: FPG and HbA1c levels are the most important variables in predicting the development of diabetes. However, individuals with multiple risk factors should be carefully monitored for the development of diabetes, even if their FPG and HbA1c levels are not high.

Keywords diabetes, risk factor, life style habits, Ningen Dock

Diabetes is known to be associated with reduced life expectancy and reduced healthy life expectancy[1–5]. However, epidemiologic studies of diabetes in Japanese populations have largely taken a cross-sectional approach, with few longitudinal studies available. In addition, even when longitudinal studies are conducted, they may be hampered by imprecise diagnostic criteria, limited study populations, or inadequate follow-up data[6–9]. Therefore, longitudinal studies of diabetes in Japanese populations are extremely valuable.

One of the major challenges of the Ningen Dock, an annual medical examination that originated and is now widely used in Japan, is to diagnose diabetes at an early stage and prevent its progression[10,11]. Our center is a hospital-based medical check-up center located in Mitaka City, Tokyo, Japan, which receives approximately 15,000 Ningen Dock examinees annually. All examinees receive detailed medical interviews conducted by medical professionals, and their medical history and visit records are strictly recorded. As it provides a comprehensive health examination, detailed examination data of the entire body is recorded annually. Approximately 80% of our examinees return annually for follow-up examinations, allowing for continuous and longitudinal data collection. In addition, on the day of the examination at our center, almost all examinees have a discussion with a doctor to explain the results and receive health advice from a public health nurse. Therefore, the Ningen Dock database of our center, although from a single institution, has a large number of examinees, making it one of the most suitable populations for conducting longitudinal studies. Moreover, if a population at risk for developing diabetes can be identified, this population would be the most optimal group to implement immediate preventive measures.

Several risk factors and predictive models for diabetes have been proposed[12–17], but their target patients

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and accuracy have been mixed. While fasting plasma glucose (FPG) and HbA1c levels are the greatest predictors of diabetes in these studies, little is known about non-glycemia-related predictors/indicators, especially in patients who develop diabetes from a very mildly impaired glucose tolerance state. In our study, we aimed to use our extensive database to identify predictors that can be used more practically and easily, with the goal of implementing approaches to prevent the progression of diabetes from very early stages.

Methods

Among the 14,458 Ningen Dock examinees at our center between April 2015 and March 2016, we excluded examinees who had previously been diagnosed with diabetes and those whose FPG and HbA1c levels at their first visit were greater than 126 mg/dL and 6.5%, respectively. We also excluded examinees who did not return to our center after March 2016. This retrospective study is based on the records of the remaining 11,313 examinees. We analyzed the medical records, interview responses, and Ningen Dock results of these individuals between April 2015 and March 2021 and determined whether they developed diabetes during this period. For questions regarding lifestyle habits such as diet, exercise, and sleep, we used the answers to a questionnaire for specific health checkups and specific health guidance by the Japan Ministry of Health, Labour and Welfare[18], as well as sleep time records provided by the examinees.

The definition of the development of diabetes in this study is based on the following criteria: 1) examinees who were newly diagnosed with diabetes after their first visit between April 2015 and March 2016 and up to their last visit to our center by March 2021, or 2) those whose FPG and HbA1c levels were both above 126 mg/dL and 6.5% at their last visit to our center, although they had not received an official diagnosis of diabetes until that time.

The severity classification of glycemic factors used in this study were based on the criteria categories published by the Japan Society of Ningen Dock[19]. These categories define the following four states: A (normal), with FPG≤99 mg/dL and HbA1c≤5.5%; B (slightly abnormal), with 1) 100 mg/dL≤FPG≤109 mg/dL and HbA1c≤5.9%, or 2) FPG≤99 mg/dL and 5.6%≤HbA1c≤5.9%; C (requires re-examination and lifestyle improvement), with 1) 110 mg/dL≤FPG≤125 mg/dL, 2) 6.0%≤HbA1c≤6.4%, 3) FPG≥126 mg/dL and HbA1c≤6.4%, FPG≤125 mg/dL and HbA1c≥6.5%; and D (medical consultation required), with FPG≥126 mg/dL and HbA1c≥6.5% (**Table 1**).

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY).

Table 1. Glycemic Criteria Categories Published by the Japan Society of Ningen Dock

	Reference interval
Category A	FPG ≤ 99 mg/dL and HbA1c ≤ 5.5%
Category B	1) or 2) 1) 100 mg/dL ≤ FPG 109 mg/dL and HbA1c ≤ 5.9% 2) FPG ≤ 99 mg/dL and 5.6% ≤ HbA1c ≤ 5.9%
Category C	1) or 2) or 3) or 4) 1) 110 mg/dL ≤ FPG ≤ 125 mg/dL 2) 6.0% ≤ HbA1c ≤ 6.4% 3) FPG ≥ 126 mg/dL and HbA1c ≤ 6.4% 4) FPG ≤ 125 mg/dL and HbA1c ≥ 6.5%
Category D	FPG ≥ 126 mg/dL and HbA1c ≥ 6.5%

FPG, fasting plasma glucose

Characteristics of examinees with and without the development of diabetes were compared using the Mann–Whitney U test and the chi-squared test where appropriate. The cumulative incidence of diabetes was analyzed using the Kaplan–Meier method. Multivariate logistic regression analysis was performed to examine the development of diabetes in examinees with glycemic category B by Ningen Dock using the following factors: age, male sex, BMI 25 kg/m^2 or higher, systolic blood pressure 140 mmHg or higher, LDL-C 140 mg/dL or higher, current smoking status, skipping breakfast 3 or more times per week, exercising 2 or more times per week, sleeping less than 6 hours, having sleep apnea syndrome, and having a family history of diabetes. These factors were selected on the basis of previous studies as known predictors of the development of diabetes[12–17,20]. Statistical significance was defined as $p<0.05$.

The study was approved by the Institutional Review Board of Nomura Hospital (R4-10). Informed consent was obtained from patients using an opt-out procedure.

Results

The clinical characteristics of the 11,313 examinees who visited our center between April 2015 and March 2016 are summarized in **Table 2**. Their median age was 50 years (range: 19–92), and 6,552 of them were male. The median values of body weight, BMI, and waist circumference were 62.2 kg, 22.4 kg/m^2, and 81.8 cm, respectively. As shown in **Fig. 1**, the cumulative incidence of newly diagnosed diabetes during the 5-year period between April 2015 and March 2021 was 2.6% (296 examinees).

We compared the characteristics of the 296 examinees who developed newly diagnosed diabetes with those who did not (**Table 3**). The median age of examinees with diabetes was 54 years, which was significantly higher than the median age of examinees without diabetes of 50 years ($p<0.001$). The median body weight (73.2 kg vs. 62.0 kg, $p<0.001$) and BMI (25.7 kg/m^2 vs. 22.3 kg/m^2, $p<0.001$) of examinees with diabetes

Table 2. Characteristics of 11,313 Ningen Dock Examinees Between April 2015 and March 2016

		All examinees (*n*=11313)	Male examinees (*n*=6552)	Female examinees (*n*=4761)
Age, years		50 (19–92)	50 (20–92)	49 (19–92)
Anthropometric data	Body weight, kg	62.2 (30.1–137.7)	68.2 (42.3–137.7)	52.4 (30.1–105.6)
	BMI, kg/m^2	22.4 (12.8–45.2)	23.3 (15.8–45.2)	20.9 (12.8–45.2)
	Abdominal circumference, cm	81.8 (54.0–138.6)	84.0 (61.3–138.6)	77.7 (54.0–121.7)
	SBP, mmHg	117 (75–196)	120 (78–192)	112 (75–196)
	DBP, mmHg	71 (31–128)	74 (38–128)	67 (31–124)
Laboratory data	TC, mg/dL	210 (87–412)	208 (87–412)	212 (111–378)
	LDL-C, mg/dL	123 (33–284)	126 (34–284)	120 (33–260)
	HDL-C, mg/dL	67 (26–168)	60 (26–161)	77 (33–168)
	TG, mg/dL	85 (19–1920)	101 (28–1920)	68 (19–1068)
	FPG, mg/dL	98 (61–163)	100 (61–163)	95 (61–144)
	HbA1c, %	5.4 (3.8–8.0)	5.4 (3.8–8.0)	5.4 (4.0–6.9)
	UA, mg/dL	5.5 (0.6–13.5)	6.3 (0.6–13.5)	4.5 (0.6–9.0)
	AST, IU/L	21 (10–281)	22 (10–281)	20 (10–180)
	ALT, IU/L	18 (5–886)	22 (5–886)	15 (5–190)

Data are presented as number or median (range).
Data in this table are described using data from visits between April 2015 and March 2016.
BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FPG, fasting plasma glucose; UA, uric acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase

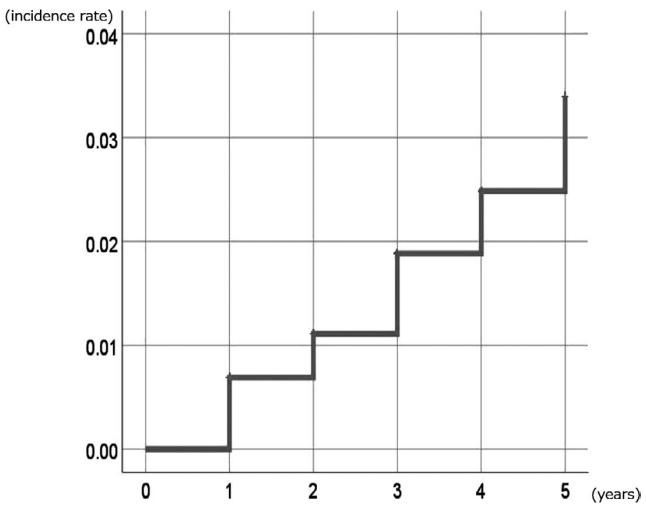

Fig. 1. Cumulative Incidence of Newly Diagnosed Diabetes During the 5-year Study Period

This figure shows the cumulative incidence of newly diagnosed diabetes over the 5-year period between April 2015 and March 2021. Out of 11,313 examinees, 296 developed diabetes, resulting in a cumulative incidence rate of 2.6%.

were also significantly higher than those of examinees without diabetes. The proportions of male examinees (78% vs. 57%, $p<0.001$), smokers (26% vs. 18%, $p<0.001$), examinees with sleep apnea syndrome (3% vs. 1%, $p<0.001$), and examinees with a family history of diabetes (42% vs. 24%) were significantly higher in examinees with diabetes than in examinees without diabetes. There were no significant differences in lifestyle factors such as dietary habits, exercise habits, and sleep habits between examinees with and without diabetes. As shown in **Table 3**, laboratory test results, including blood pressure, cholesterol, blood glucose, uric acid, and liver function tests, were worse in examinees with diabetes. Notably, the differences in median FPG (119 mg/dL vs. 98 mg/dL, $p<0.001$) and HbA1c (6.0% vs. 5.4%, $p<0.001$) were extremely higher in examinees with diabetes.

Fig. 2 shows the impact of FPG and HbA1c in the development of diabetes. The incidence rates of developing diabetes during the 5-year follow-up period were 0.09%, 0.7%, and 18% for categories A, B, and C, respectively (**Fig. 3**).

We performed multivariate analysis to identify predictive factors for the development of newly diagnosed diabetes over a 5-year period in the examinees with glycemic category B (**Table 4**). Our results showed that BMI≥25 kg/m^2 (odds ratio [OR] 5.356, 95% confidence interval [CI]: 2.359–12.158; $p<0.001$), LDL-C≥140 mg/dL (OR 2.415, 95%CI: 1.127–5.177; $p=0.023$), current smoker (OR 2.320, 95%CI: 1.031–5.221; $p=0.042$), and family history of diabetes (OR 2.510, 95%CI: 1.186–5.310; $p=0.016$) were significant predictive factors for the development of diabetes.

The incidence rate for diabetes in examinees with glycemic category B increased from 0.7% to 12.5% when they had multiple risk factors, such as BMI≥25 kg/m^2, LDL-C≥140 mg/dL, smoking, and family history of diabetes, as shown in **Fig. 3**.

Discussion

In this study, 296 (2.6%) of 11,313 examinees developed newly diagnosed diabetes during a 5-year follow-up period. The incidence rate of diabetes in Japanese is valuable data in itself, as most studies of diabetes have focused only on its prevalence[6,7,21,22] while limited studies focused on its incidence rate[8,9,23–25].

Table 3. Characteristics of Examinees With and Without Development of Diabetes

		Examinees who developed diabetes (*n*=296)	Examinees who did not develop diabetes (*n*=11017)	*p* value
Age, years		54 (20–82)	50 (19–92)	<0.001
Gender (male), *n* (%)		230 (78)	6322 (57)	<0.001
Anthropometric data	Body weight, kg	73.2 (38.1–127.4)	62.0 (30.1–137.7)	<0.001
	BMI, kg/m^2	25.7 (16.7–42.1)	22.3 (12.8–45.2)	<0.001
	SBP, mmHg	126 (90–195)	116 (75–196)	<0.001
	DBP, mmHg	77 (43–124)	71 (31–128)	<0.001
	SBP ≥ 140 mmHg, *n* (%)	52 (18)	876 (8)	<0.001
Laboratory data	TC, mg/dL	215 (126–389)	209 (87–412)	0.0014
	LDL-C, mg/dL	132 (59–284)	123 (33–260)	<0.001
	LDL-C ≥ 140 mg/dL, *n* (%)	122 (41)	3353 (30)	<0.001
	HDL-C, mg/dL	56 (30–119)	67 (26–168)	<0.001
	TG, mg/dL	134 (35–606)	84 (62–1920)	<0.001
	FPG, mg/dL	119 (77–163)	98 (61–155)	<0.001
	HbA 1c, %	6.0 (5.1–8.0)	5.4 (3.8–6.7)	<0.001
	UA, mg/dL	6.4 (2.1–9.9)	5.5 (0.6–13.5)	<0.001
	AST, IU/L	24 (13–76)	21 (10–281)	<0.001
	ALT, IU/L	27 (9–149)	18 (5–886)	<0.001
Lifestyle	Current smoker, *n* (%)	78 (26)	1985 (18)	<0.001
	Skipping breakfast 3 or more times per week, *n* (%)	50 (17)	1576 (14)	N.S.
	Physical activity 2 or more times per week, *n* (%)	70 (24)	2830 (26)	N.S.
	Sleep duration <6 hours, *n* (%)	88 (30)	2788 (25)	N.S.
Comorbidity and family history	Present history of sleep apnea syndrome, *n* (%)	10 (3)	111 (1)	<0.001
	Family history of hypertension, *n* (%)	127 (43)	4621 (42)	N.S.
	Family history of diabetes, *n* (%)	125 (42)	2660 (24)	<0.001
	Family history of dyslipidemia, *n* (%)	25 (8)	891 (8)	N.S.

Data are presented as number or median (range).
Data in this table are described using data from visits between April 2015 and March 2016.
BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FPG, fasting plasma glucose; UA, uric acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; N.S., not significant

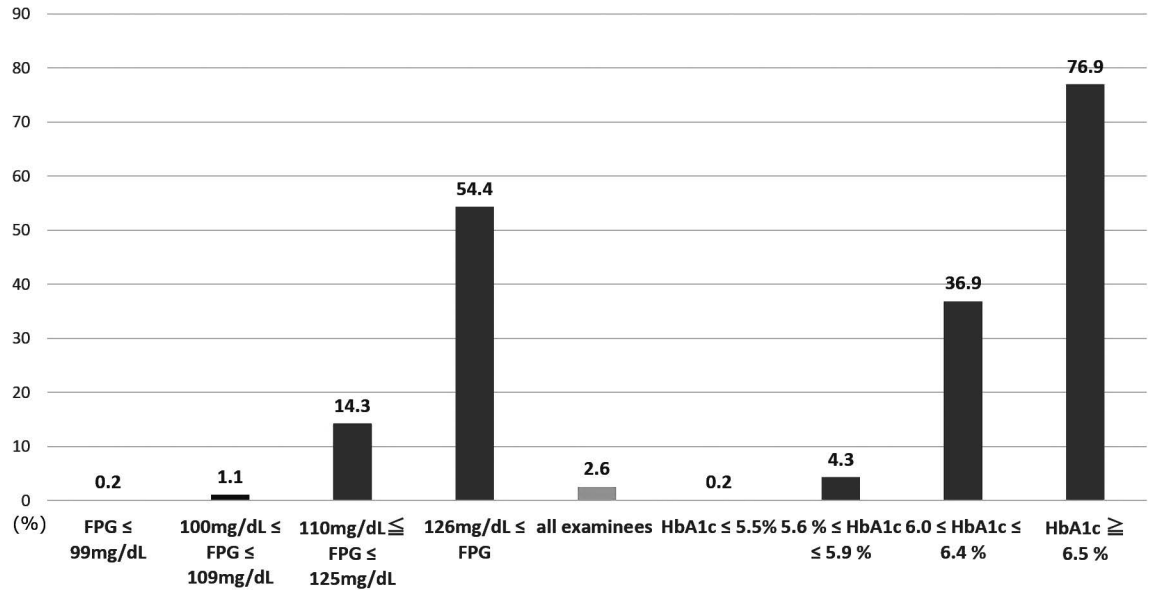

Fig. 2. Predicted Incidence Rate Over the Next Five Years Based on the Levels of FPG and HbA 1c Levels

This figure shows the predicted incidence rate of new-onset diabetes over the next five years for each individual variable. The predicted incidence rate increases as both FPG and HbA 1c levels increase. Both FPG and HbA 1c are strong predictors of new-onset diabetes.

Table 4. Multivariate Analysis for the Development of Diabetes During the Study Period in Examinees in Glycemic Category B

	Odd ratio	95% Confidence Interval	*p* value
Age, years	1.027	0.984–1.071	0.222
Male sex	1.070	0.449–2.549	0.879
BMI ≥ 25 kg/m^2	5.356	2.359–12.158	<0.001
SBP ≥ 140 mmHg	1.085	0.364–3.231	0.884
LDL-C ≥ 140 mg/dL	2.415	1.127–5.177	0.023
Current smoker	2.320	1.031–5.221	0.042
Skipping breakfast 3 or more times per week	0.582	0.190–1.783	0.343
Exercise 2 or more times per week	0.369	0.109–1.247	0.109
Sleep duration <6 hours	1.479	0.678–3.228	0.325
Present history of sleep apnea syndrome	1.646	0.361–7.499	0.520
Family history of diabetes	2.510	1.186–5.310	0.016

This analysis was conducted using data from visits between April 2015 and March 2016.
BMI, body-mass index; SBP, systolic blood pressure; LDL-C, low-density lipoprotein cholesterol

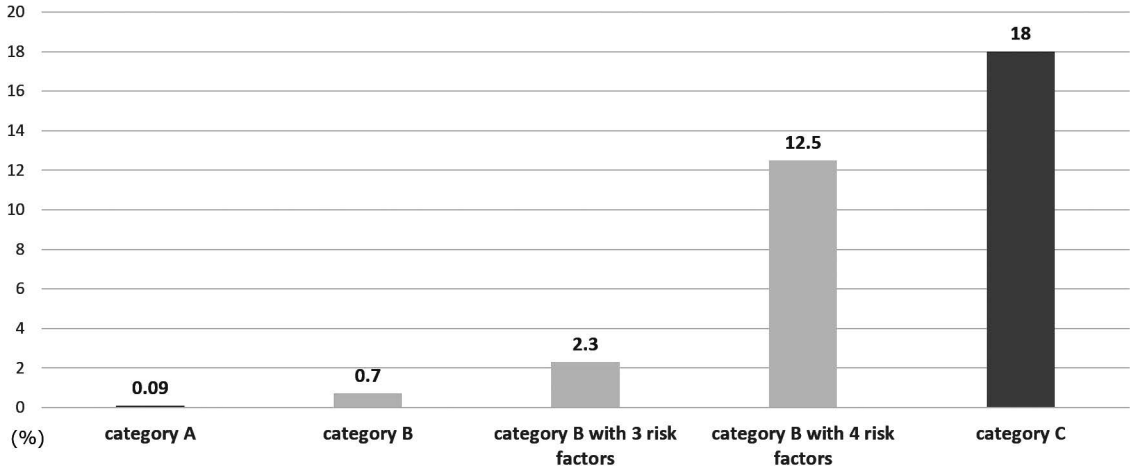

Fig. 3. Predicted Incidence Rate Over the Next Five Years Based on the Glycemic Criteria Category

This figure shows the predicted incidence rate of new-onset diabetes over the next five years for each individual category. The incidence rate of diabetes is extremely low in categories A and B, and extremely high in category C. On the other hand, it is clear that the incidence rate increases substantially when multiple risk factors accumulate even in category B.

In a comparative analysis between examinees who developed diabetes and those who did not, almost all of the variables we compared were worse in the former group (**Table 3**). As previously reported and as expected[12–17,20], there were significant differences in weight, as well as in biochemical tests for variables known to be associated with lifestyle diseases, between the group of individuals who developed diabetes and those who did not. It is believed that health and lifestyle counseling targeting these variables can be expected to prevent the onset of diabetes in the future.

We would like to refer to the diabetes criteria category published by the Japan Society of Ningen Dock[19]. This categorization is based on the results of FPG and HbA1c (**Table 1**). It is clear from **Fig. 2** and previous studies[7,12,20,25] that FPG and HbA1c are the strongest risk factors for developing diabetes. The incidence rates of developing diabetes during the 5-year follow-up period were 0.09%, 0.7%, and 18% for categories A, B, and C, respectively (**Fig. 3**). This result confirms the appropriateness of the cut-off values for this categorization. This could mean that factors other than this category, i.e., factors other than FPG and HbA1c, are not needed to determine the risk of developing diabetes in the future. However, some patients with very mild glucose intolerance, such as those in category B, may develop diabetes, albeit only a mild case, and using this category alone would put them in the low-risk group for developing diabetes and exclude them from healthcare counselling for lifestyle improvement. Therefore, we decided to investigate risk factors for developing diabetes in patients with very mild impairment of glucose tolerance.

In the multivariate analysis (**Table 4**), BMI≥25 kg/m^2, LDL-C≥140 mg/dL, smoking, and family history of diabetes were identified as risk factors for developing diabetes in examinees in glycemic category B. Although less attention may be necessary for examinees in categories A and B due to their lower incidence rate of developing diabetes, it is noteworthy that the incidence rate

increased from 0.7% to 12.5% when there were multiple risk factors, such as BMI≥25 kg/m^2, LDL-C≥140 mg/dL, smoking, and a family history of diabetes in examinees in category B, as shown in **Fig. 3**.

BMI, LDL-C, and smoking have traditionally been known as risk factors for the development of diabetes, and all of these factors, unlike family history, are relatively easy to improve with intervention. Therefore, these factors are considered to be extremely important in a setting such as Ningen Dock, where the results can be reviewed on the day of the examination and health guidance can be provided promptly on the spot.

In this study, lifestyle habits such as diet, exercise, and sleep were not identified as risk factors for the development of diabetes. However, this could have been be somewhat influenced by the number of patients and the selection of factors for analysis, and we do not mean to downplay these lifestyle habits. In fact, these factors are known to be closely related to BMI and LDL-C, which were also identified as risk factors in this study. In this study, only the variables in the questionnaire[18] were examined with regard to diet and exercise habits, and the study did not examine the details of dietary content as, for example, in the famous Hisayama cohort study[23,24]. It is possible that some of the details of dietary habits may play a significant role in the development of diabetes. Sleep, in particular, is often neglected, but several papers have already pointed out its association with the development of diabetes[26–28], and we have also published a paper to that effect[29]. We will continue to study this point.

We should mention some limitations of this study. First, this is a single-center retrospective study, and our sample was limited to the examinees of Ningen Dock, so we cannot generalize our results to all Japanese people. The diagnosis of diabetes is partly based on self-reported history. Several antihypertensive drugs such as angiotensin receptor blockers and lipid-improving drugs such as statins are known to influence the development of diabetes[8,9]. However, in this study, blood pressure and lipid values were not taken into account with or without oral medications. On the other hand, this study also has many strengths. Our sample size of 11,313 was large enough for statistical analysis, and we followed these examinees for five years. Longitudinal studies are valuable even if they are retrospective, because there have been few longitudinal studies of diabetes in Japan. We accurately recorded all results of Ningen Dock examinations and responses to medical interviews, including medical history. In addition, most of the examinees undergo Ningen Dock annually at our center, which allowed us to conduct a precise study. We believe that our study is unique and valuable because of these strengths.

Conclusions

We followed 11,313 Ningen Dock examinees, and 2.6% of them were newly diagnosed with diabetes during the 5-year study period. FPG and HbA1c levels appear to be the most important variables in predicting the development of diabetes. However, it is important to note that even if FPG and HbA1c are not high, careful attention should be paid to individuals who have multiple risk factors such as BMI≥25 kg/m^2, LDL-C≥140 mg/dL, smoking, and a family history of diabetes.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Nomura Hospital (R4-10). Patient consent was obtained using informed consent documents with an opt-out procedure.

Competing interests

None.

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References

1. Turin TC, Murakami Y, Miura K, *et al.*: Diabetes and life expectancy among Japanese—NIPPON DATA80. Diabetes Res Clin Pract 2012; 96: e18–e22.
2. Gregg EW, Cheng YJ, Saydah S, *et al.*: Trends in death rates among U.S. adults with and without diabetes between 1997 and 2006: findings from the National Health Interview Survey. Diabetes Care 2012; 35: 1252–1257.
3. Rawshani A, Rawshani A, Franzén S, *et al.*: Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med 2018; 379: 633–644.
4. Wubishet BL, Byles JE, Harris ML, *et al.*: Impact of diabetes on life and healthy life expectancy among older women. J Gerontol A Biol Sci Med Sci 2021; 76: 914–921.
5. Feng X, Astell-Burt T: Impact of a type 2 diabetes diagnosis on mental health, quality of life, and social contacts: a longitudinal study. BMJ Open Diabetes Res Care 2017; 5: e000198.
6. Ohmura T, Ueda K, Kiyohara Y, *et al.*: Prevalence of type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in the Japanese general population: the Hisayama Study. Diabetologia 1993; 36: 1198–1203.
7. Kato M, Takahashi Y, Matsushita Y, *et al.*: Diabetes mellitus defined by hemoglobin A1c value: risk characterization for incidence among Japanese examinees in the JPHC Diabetes Study. J Diabetes Investig 2011; 2: 359–365.
8. Cederberg H, Stančáková A, Yaluri N, *et al.*: Increased risk of diabetes with statin treatment is associated with impaired insulin sensitivity and insulin secretion: a 6 year follow-up study of the METSIM cohort. Diabetologia 2015; 58: 1109–1117.
9. Nazarzadeh M, Bidel Z, Canoy D, *et al.*: Blood pressure lowering and risk of new-onset type 2 diabetes: an individual participant data meta-analysis. Lancet 2021; 398: 1803–1810.

10. Lu J: Ningen Dock: Japan's unique comprehensive health checkup system for early detection of disease. Glob Health Med 2022; 4: 9–13.
11. Okada R, Tsushita K, Wakai K, *et al*.: Lower risk of progression from prediabetes to diabetes with health checkup with lifestyle education: Japan Ningen Dock study. Nutr Metab Cardiovasc Dis 2017; 27: 679–687.
12. Nanri A, Nakagawa T, Kuwahara K, *et al*.: Development of risk score for predicting 3-year incidence of type 2 diabetes: Japan Epidemiology Collaboration on Occupational Health Study. PLoS One 2015; 10: e0142779.
13. Wilson PW, Meigs JB, Sullivan L, *et al*.: Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 2007; 167: 1068–1074.
14. Lai H, Huang H, Keshavjee K, *et al*.: Predictive models for diabetes mellitus using machine learning techniques. BMC Endocr Disord 2019; 19: 101.
15. Schmidt MI, Duncan BB, Bang H, *et al*.: Identifying individuals at high risk for diabetes: the Atherosclerosis Risk in Communities study. Diabetes Care 2005; 28: 2013–2018.
16. Lindström J, Tuomilehto J: The diabetes risk score: a practical tool to predict type 2 diabetes risk. Diabetes Care 2003; 26: 725–731.
17. Dall TM, Narayan KMV, Gillespie KB, *et al*.: Detecting type 2 diabetes and prediabetes among asymptomatic adults in the United States: modeling American Diabetes Association versus US Preventive Services Task Force diabetes screening guidelines. Popul Health Metr 2014; 12: 12.
18. Japan Ministry of Health, Labour and Welfare: The questionnaire for specific health checkups and specific health guidance. https://www.mhlw.go.jp/seisakunitsuite/bunya/kenkou_iryou/kenkou/seikatsu/dl/hoken-program2_02.pdf (accessed April 14, 2023) (in Japanese)
19. Japan Society of Ningen Dock: Criteria Category (April, 2022). https://www.ningen-dock.jp/wp/wp-content/uploads/2018/06/Criteria-Category.pdf (accessed April 14, 2023)
20. Noda M, Kato M, Takahashi Y, *et al*.: Fasting plasma glucose and 5-year incidence of diabetes in the JPHC diabetes study: suggestion for the threshold for impaired fasting glucose among Japanese. Endocr J 2010; 57: 629–637.
21. Nanri A, Mizoue T, Noda M, *et al*.: Rice intake and type 2 diabetes in Japanese men and women: the Japan Public Health Center-based Prospective Study. Am J Clin Nutr 2010; 92: 1468–1477.
22. Maegawa H, Hidaka H, Okayama A, *et al*.: Risk factors for non-insulin dependent diabetes mellitus in a rural Japanese population: incidence and risk factors. Journal of the Japan Diabetes Society 1992; 35: 241–248. (in Japanese)
23. Kiyohara Y, Shinohara A, Kato I, *et al*.: Dietary factors and development of impaired glucose tolerance and diabetes in a general Japanese population: the Hisayama Study. J Epidemiol 2003; 13: 251–258.
24. Kimura Y, Yoshida D, Hirakawa Y, *et al*.: Dietary fiber intake and risk of type 2 diabetes in a general Japanese population: the Hisayama Study. J Diabetes Investig 2021; 12: 527–536.
25. Mukai N, Doi Y, Ninomiya T, *et al*.: Cut-off values of fasting and post-load plasma glucose and HbA1c for predicting Type 2 diabetes in community-dwelling Japanese subjects: the Hisayama Study. Diabet Med 2012; 29: 99–106.
26. Kuwahara K, Imai T, Miyamoto T, *et al*.: Sleep duration modifies the association of overtime work with risk of developing type 2 diabetes: Japan Epidemiology Collaboration on Occupational Health Study. J Epidemiol 2018; 28: 336–340.
27. Tasali E, Leproult R, Spiegel K: Reduced sleep duration or quality: relationships with insulin resistance and type 2 diabetes. Prog Cardiovasc Dis 2009; 51: 381–391.
28. Shan Z, Ma H, Xie M, *et al*.: Sleep duration and risk of type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care 2015; 38: 529–537.
29. Ogawa K, Seya A, Tomita Y, *et al*.: Short sleep duration is a predictor of diabetes and bedtime irregularity of cardiovascular disease. Ningen Dock 2022; 37: 655–663. (in Japanese)

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Investigation of Factors Involved in the Prevention of Gastric Cancer Using Health Checkup Data from Our Facility

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Abstract

Objective: It has been nearly 30 years since *Helicobacter pylori* (Hp) was identified as a class I carcinogen for gastric cancer (GC). However, only a small percentage of Hp-infected patients develop cancer. Although eradication therapy is considered the most effective for preventing GC, various cases of GC have been observed after eradication. This study aimed to analyze the factors preventing GC based on health examination data.

Methods: A total of 19,278 health checkup subjects (12,916 males and 6,362 females; mean age, 51.9±11.3 years) who underwent physical examinations at our health checkup center from 2012 to 2018 were included. Patients with a history of GC were excluded. Multiple logistic regression analysis was used to analyze the association between GC and background factors (age, sex, smoking history, alcohol consumption history, and family history of GC), lifestyle factors (dietary preferences, exercise, and sleep), and lifestyle-related disease factors (body-mass index, metabolic syndrome, abdominal circumference, visceral fat, body fat, lipids, glucose tolerance, liver function, and fatty liver). We also compared GC incidence between Hp-infected and post-Hp eradication patients.

Results: Age and salt intake were significantly associated with GC (p=0.001 and 0.003, respectively). GC incidence was 1.53% among Hp-infected patients and 0.52% among post-Hp eradication patients, approximately one-third of that in those who did not undergo eradication.

Conclusion: Aging and a high salt intake may be associated with GC, suggesting that a low-sodium diet can prevent GC, especially among the elderly. The results of this study also suggest that Hp eradication therapy is effective in preventing GC.

Keywords *Helicobacter pylori*, gastric cancer, age, salt

Gastric cancer (GC) is the fifth-most common cancer and the third-most common cause of cancer mortality worldwide[1]. In Japan, GC is the third-most common cancer in terms of both incidence and death[2,3]. Its prognosis is dismal, with an average 5-year survival rate of <20% mainly due to late diagnosis because the early stages are often symptomless[4]. In Japan, extensive programs have been established for the early detection of GC. Owing to the implementation of GC screening as a public cancer prevention measure in 1983, the incidence and mortality rates of GC have been declining since 1985[5]. Furthermore, eradication therapy for patients with gastritis caused by *Helicobacter pylori* (Hp) infection, classified as a class I human carcinogen for GC in 1994[6], was covered by insurance in 2013, resulting in a significant increase in the number of patients receiving Hp eradication therapy and possibly contributing to the decrease in the incidence and mortality rates of this disease.

Over the decades, risk factors for GC have been studied. Hp infection is a well-known risk factor for GC[6]. Hp is a gram-negative bacterium capable of colonizing the human gastric mucosa and eliciting an immune response in the host[6]. Multifocal atrophic gastritis, a type of gastritis associated with infection, may be linked to a precancerous process[6]. Various molecular biological factors associated with Hp have been reported to be involved in gastric carcinogenesis[6]. However, <1% of patients with Hp infection develop GC. Other factors are also associated with GC development: environmental factors, including age, high salt intake, smoking,

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and consumption of processed meat, may be involved in gastric carcinogenesis[4]. The consumption of fresh fruits and vegetables has been associated with reduced cancer risk[4]. Furthermore, host factors, including the genetic polymorphisms of genes linked to inflammatory responses, are also involved in gastric carcinogenesis[4]. Nevertheless, these elements do not fully explain the risk factors associated with the development of GC. It has been recently reported that lower high-density lipoprotein (HDL) and higher low-density lipoprotein (LDL) cholesterol levels were associated with an increased risk of GC[7]. Impaired fasting glucose and high hemoglobin A1c levels are also reportedly associated with an increased risk of GC[8,9]. These reports indicate that lifestyle-related diseases may be involved in gastric carcinogenesis.

This study aimed to explore the factors associated with GC, including environmental factors, lifestyle habits, and underlying lifestyle-related diseases, using data from seven years of physical examinations at our institution. Through this study, we hope to contribute to future GC prevention and the ideal approach for GC screening.

Methods

Study design

This retrospective study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Jikei University School of Medicine (approval date: February 1st, 2021; approval number: 31-349 [9928]), which waived the requirement for informed consent owing to the nature of this study. A total of 19,278 health subjects registered in the Ningen Dock—the Japanese health checkup system—at the Center for Preventive Medicine, Jikei University Hospital between 2012 and 2018 were included. The Ningen Dock is a periodic health checkup program for adults. It is comprehensive and examines the following factors: physical characteristics (height, body weight, and waist circumference), blood pressure, complete blood count, blood biochemistry, urinalysis, electrocardiography, chest radiography, spirometry, abdominal ultrasonography, upper gastrointestinal tract barium meal or endoscopic examination, visual acuity test, tonometry, fundic examination (retinal photography), visual field test, hearing assessment, physical examination by a medical doctor, and a nurse's medical interview. There were 12,916 males and 6,362 females, with a mean age of 51.9±11.3 (range, 22–90) years. Subjects with a history of stomach cancer and those already receiving treatment for lifestyle-related diseases such as hypertension, dyslipidemia, or diabetes were excluded from the present study. The study profile is shown in **Fig. 1**. First, we analyzed factors related to GC in the whole subject group and subsequently in Hp-positive subjects, as Hp is a potent gastric carcinogen. In our facility, the serum anti-Hp antibody test (Hp test) is provided as an optional examination, and 3,534 people underwent the Hp test as an optional examination along with the Ningen Dock. Of these, 232 who previously received Hp eradication therapy were excluded. A total of 959 participants tested positive for Hp. In the whole subject group, 31 (0.2%) patients with GC and 19,247 without were enrolled. In the Hp-positive group, 14 (1.5%) patients with GC and 945 without were enrolled (**Table 1**). The mean age of the

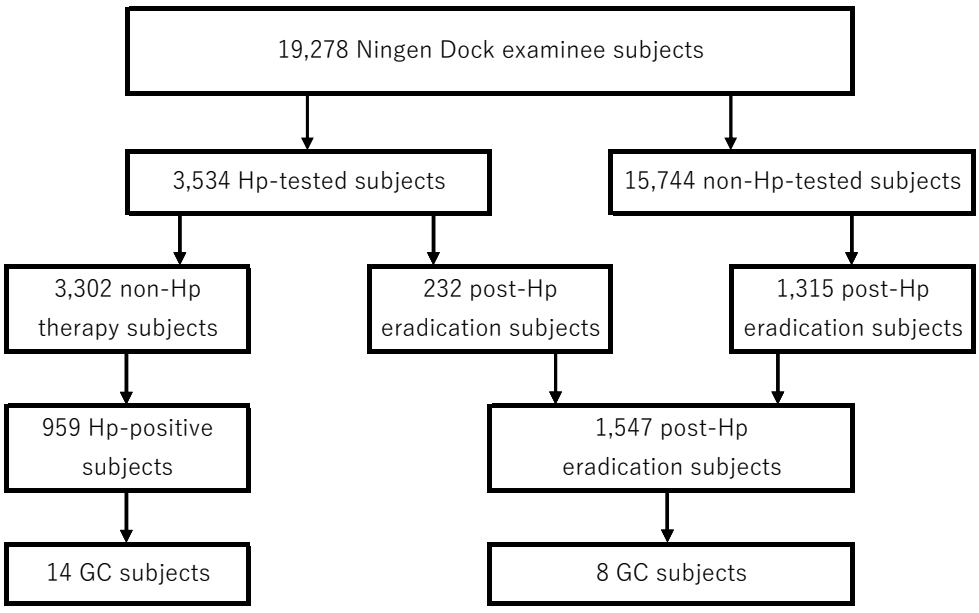

Fig. 1. Study Profile

Table 1. Subject Characteristics

	Total number of subjects	Number of subjects with gastric cancer	Number of subjects without gastric cancer
Total	19278	31 (0.16%)	19247
Male	12916	22 (0.17%)	12894
Female	6362	9 (0.14%)	6353
Hp-positive	959	14 (1.46%)	945
Smoker	3406	5 (0.14%)	3401
Excessive alcohol intake	1709	1 (0.06%)	1708
Family history of gastric cancer	1663	5 (0.30%)	1658
Obesity	4931	5 (0.10%)	4926
Hypertension	1338	4 (0.30%)	1334
Dyslipidemia	8131	16 (0.20%)	8115
Diabetes mellitus	1191	4 (0.34%)	1187

*$p<0.05$

Hp-positive group was 55.0±13.0 years.

Moreover, the incidence of GC after Hp eradication was compared with that of patients infected with Hp. A total of 959 Hp-positive and 1,547 post-Hp eradication patients, including 232 who had undertaken the Hp test described above, were enrolled.

Target factors for the analysis of related factors to GC

To detect GC-related factors, the following aspects were investigated: background factors, including age, gender, smoking history, drinking history, body-mass index (BMI), body fat percentage (BFP), and family history of GC; lifestyle factors, including dietary preference, daily activity, exercise habits, sleep, and hobbies; factors involving lifestyle-related diseases, including metabolic syndrome, blood pressure, visceral fat accumulation, lipids (LDL cholesterol, HDL cholesterol and triglycerides), glucose tolerance (fasting blood glucose, hemoglobin A1c, homeostatic model assessment for insulin resistance) renal function (estimated glomerular filtration rate (eGFR)) and liver function (alanine aminotransferase, fibrosis 4 (FIB4) index, which represents the degree of liver fibrosis, and fatty liver). The corresponding data were extracted from the medical checkups registered in Ningen Dock. Data on lifestyle factors, family history, smoking, and drinking were based on information obtained from the medical interview conducted upon physical examination. The medical questionnaire used herein is presented in **Table 2**. This questionnaire is in accordance with the questionnaire for Lifestyle Health Check-ups and Health Guidance prepared by the Ministry of Health, Labour and Welfare. A smoker was defined as an individual who had ever smoked. The Brinkmann index was used to examine the association between smoking and GC. The amount (g) of ethanol consumption per week was used to examine the relationship between ethanol consumption and GC. Excessive drinking was defined as the consumption of ≥300 g of ethanol per week. According to "Health Japan 21," a national health promotion campaign by the Japanese Ministry of Health, Labour and Welfare (https://www.mhlw.go.jp), excessive alcohol was defined as a daily ethanol intake of >60 g[10]. Given the recommendation of 2 days of no alcohol consumption per week by Health Japan 21, alcohol consumption was assumed to be occurring 5 days per week. Accordingly, excessive alcohol was defined as described above which is the alcohol consumption of ≥300 g of ethanol per week. Information on family history was obtained from an interview by a nurse registered in Ningen Dock. Hp infection status was evaluated by EIA using a serum anti-Hp antibody titer test (E plate 'eiken' H. pylori antibody kit; SRL Inc., Tokyo, Japan). Titers with values ≥10 U/mL were considered positive for Hp. Patients with a BMI ≥25 kg/m^2 were considered obese, according to the diagnostic criteria of the Japan Society for the Study of Obesity (https://www.jasso.or.jp). BFP was measured by bioelectrical impedance analysis. Metabolic syndrome was diagnosed according to the diagnostic criteria established by eight academic societies: The Japanese Society of Internal Medicine, Japan Society for the Study of Obesity, Japan Atherosclerosis Society, The Japan Diabetes Society, The Japanese Society of Hypertension, The Japanese Circulation Society, Japanese Society of Nephrology, and The Japanese Society on Thrombosis and Hemostasis—as recommended by the Ministry of Health, Labour and Welfare.

Statistical analysis

A logistic regression analysis was used to identify factors associated with GC. First, a univariate regression analysis was performed to detect factors associated with GC. Subsequently, a multivariate logistic regression analysis of the variables that were significant with p value ≥0.05 in the univariate analysis was performed. The dependent factor was GC, and the independent factors were background factors, lifestyle-related factors, and lifestyle-related disease-related factors, including those mentioned above. A chi-square test was used to compare the incidence or clinicopathological characteristics

Table 2. Questionnaire for the Medical Checkup

1.	What do you tend to take a lot of in your diet?		
	Amount of food Salt Fat Sweets		
2.	How many minutes of walking or equivalent physical activity do you engage in per day?	____ min	
3.	Have you exercised with lightly sweatting for at least 30 min a day at least twice a week over the last year or more?	Yes	No
4.	Do you get enough rest through sleep?	Yes	No
5.	Do you have any hobbies?	Yes	No
6.	Answer if you smoke currently.		
	How many cigarettes do you smoke per day?	____ cigarettes	
	How many years have you smoked?	____ years	
7.	How many days per week do you drink alcohol?	____ days	
8.	What type and how much alcohol do you drink at one time?		

of GC between Hp-infected and post-Hp eradication subjects. For the comparison of age between Hp-infected and post-Hp eradication subjects, Student's *t*-test was used. Stata Software version 16 (StataCorp LP, College Station, TX, USA) was used for statistical analysis. A *p*-value of <0.05 was considered statistically significant.

Results

Table 1 presents participant characteristics; men comprised two-thirds of the entire cohort and the GC group. No Hp-negative patients had GC. Moreover, 42.2% of subjects had dyslipidemia; 25.6% of subjects were obese; and 17.7% of subjects were smokers. The incidence of GC was 0.16%, 0.17%, and 0.14% among all subjects, males and females, respectively. The incidence of GC was 1.6% among Hp-positive subjects, approximately ten times higher than the overall GC incidence. GC incidence was relatively higher among patients with a family history of GC, hypertension, and diabetes. GC was relatively less common among women, excessive drinkers, and obese people compared to the overall average. Smokers and heavy alcohol drinkers had a GC incidence of 0.2% and 0.06%, respectively.

Univariate logistic regression analysis (**Table 3**) showed significant associations between GC and age, body fat percentage, high salt intake, activity, exercise habits, LDL cholesterol levels, fasting blood glucose levels, eGFR, and high FIB4 index. Multivariate logistic regression analysis (**Table 4**) showed a significant association between GC and age and high salt intake. **Tables 5** and **6** present the results of the univariate and multivariate analyses of Hp-positive subjects. In total, 27.1% of subjects who underwent Hp testing had Hp ($n=3,534$). Among Hp-positive patients, univariate logistic regression analysis showed significant associations between GC and age, BMI, dyslipidemia, diabetes, and high FIB4 index (**Table 5**). However, multivariate logistic regression analysis did not reveal a significant association between GC and any of these factors (**Table 6**).

Fourteen GC patients were present in the Hp-positive group ($n=959$), while eight GC patients were present in the post-Hp eradication group ($n=1,547$). Accordingly, GC was prevalent in 1.53% of Hp-positive patients, but in only 0.52% of the post-Hp eradication group, which is approximately one-third of Hp-positive patients, indicating that the incidence of GC was significantly lower ($p=0.02$) after Hp eradication (**Table 7**). This suggested that Hp eradication therapy can reduce the risk of GC, because the GC incidence post-eradication therapy was approximately 33% of that before eradication. **Table 8** shows the clinicopathological characteristics of GC subjects who were Hp-positive or post-Hp eradication. No factors, such as age, sex, tumor size, tumor macroscopic type and tumor histological type, were significantly different between the Hp-positive and post-Hp eradication subject groups. However, age was slightly higher in the post-Hp eradication group than the Hp-positive group, and there was a higher proportion of females in the Hp-positive group than in the post-Hp eradication group. Tumor macroscopic type was mostly early GC 0-IIc in both the groups, but the Hp-positive group included more types than did the post-Hp eradication group.

Discussion

In the present study, aging and a high salt intake were found to be factors associated with GC, as seen in previous reports[1,4]. Therefore, a low-sodium diet is important for the prevention of GC, especially in the elderly. However, among Hp-positive patients, we did not find any factor associated with GC, suggesting that Hp factors themselves, including the strain, as well as the genes and proteins it holds, are more likely to have a strong influence on gastric carcinogenesis than host background factors, lifestyle factors, or factors related to lifestyle-related diseases.

Shah *et al.* mentioned in their review that older age is likely a secondary risk factor to longer exposure to potential carcinogens, increased susceptibility to mucosal damage, delayed healing of the gastric mucosa, increased incidence of mucosal cancer stem cell markers, increased prevalence of chronic active gastritis,

Table 3. Univariate Logistic Regression Analysis

Factor	Odds ratio	SE	Z score	*p*-value	95%CI
Background factors					
Age	1.11	0.02	6.71	0.001*	[1.077, 1.145]
Sex	1.20	0.48	0.47	0.639	[0.554, 2.617]
BMI	0.93	0.05	−1.29	0.196	[0.832, 1.038]
Body fat	0.93	0.03	−2.10	0.036*	[0.874, 0.995]
Family history of gastric cancer	2.04	0.99	1.46	0.145	[0.782, 5.320]
Lifestyle factors					
Smoking	0.90	0.44	−0.22	0.822	[0.344, 2.335]
Alcohol intake	1.00	0.01	0.11	0.911	[0.998, 1.002]
Dietary intake	0.48	0.23	−1.50	0.133	[0.184, 1.249]
Salt intake	2.95	1.09	2.93	0.003*	[1,433, 6.087]
Fat intake	0.52	0.281	−1.21	0.227	[0.183, 1.496]
Sugar intake	0.64	0.29	−0.98	0.329	[0.263, 1.564]
Physical activity	1.00	0.01	2.12	0.034*	[1.000, 1.006]
Sleep	1.63	0.65	1.23	0.217	[0.751, 3.543]
Exercise	2.18	0.79	2.17	0.030*	[1.078, 4.418]
Hobby	0.65	0.27	−1.06	0.291	[0.290, 1.450]
Factors associated with lifestyle-related diseases					
Visceral fat accumulation	0.97	0.36	−0.09	0.930	[0.463, 2.021]
Metabolic syndrome	1.23	0.61	0.41	0.679	[0.463, 3.263]
Blood pressure	1.01	0.01	1.10	0.273	[0.990, 1.037]
LDL cholesterol	0.99	0.01	−2.11	0.035*	[0.975, 0.999]
HDL cholesterol	1.01	0.01	0.32	0.752	[0.984, 1.023]
Triglyceride	1.01	0.01	0.98	0.327	[0.999, 1.004]
Dyslipidemia	1.46	0.53	1.06	0.291	[0.722, 2.958]
FBG	1.01	0.01	2.00	0.046*	[1.001, 1.021]
HbA1c	1.40	0.25	1.93	0.054	[0.995, 1.978]
HOMA-IR	0.76	0.23	−0.90	0.370	[0.425, 1.375]
Diabetes mellitus	2.40	1.29	1.62	0.105	[0.833, 6.896]
eGFR	0.97	0.01	−2.49	0.013*	[0.946, 0.993]
ALT	0.97	0.02	−1.76	0.079	[0.929, 1.004]
FIB4 index	1.51	0.15	3.99	0.001*	[1.231, 1.839]
Fatty liver	0.88	0.40	−0.29	0.773	[0.361, 2.134]

*$p < 0.05$

SE, standard error; CI, confidence interval; BMI, body-mass index; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; and FIB4, fibrosis 4

Table 4. Multivariate Logistic Regression Analysis

Factors	Odds ratio	SE	Z score	*p*-value	95%CI
Age	1.12	0.03	4.99	0.001*	[1.073, 1.175]
Body fat	0.96	0.04	−1.2	0.231	[0.889, 1.029]
Salt intake	3.61	1.55	2.99	0.003*	[1.557, 8.388]
Physical activity	1.01	0.01	0.92	0.356	[0.998, 1.007]
Exercise	1.309	0.56	0.63	0.529	[0.566, 3.028]
LDL cholesterol	0.99	0.01	−1.06	0.291	[0.977, 1.007]
FBG	1.01	0.01	0.69	0.493	[0.988, 1.024]
eGFR	1.01	0.02	0.16	0.869	[0.972, 1.033]
FIB4 index	0.93	0.28	−0.22	0.822	[0.515, 1.694]

*$p < 0.05$

SE, standard error; CI, confidence interval; LDL, low-density lipoprotein; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; and FIB4, fibrosis 4

intestinal metaplasia, and mucosal atrophy, especially among Hp-positive patients[14]. This may explain our results which show no association between age and GC among Hp-positive patients. High salt intake has been reported to increase GC risk[15]. Shah *et al.* suggested that there are several mechanisms via which salt may contribute to carcinogenesis[14]. High salt concentration has been shown to disrupt the mucosal barrier of the stomach and lead to inflammation and atrophy. Experimental studies in mice and gerbils have shown increased rates of Hp colonization among mice undergoing a high salt diet due to alterations in their protective

Table 5. Univariate Logistic Regression Analysis in Hp-positive Subjects

Factor	Odds ratio	SE	Z score	*p*-value	95%CI
Background factors					
Age	1.09	0.03	3.25	0.001*	[1.035, 1.150]
Sex	0.58	0.30	−1.03	0.301	[0.209, 1.622]
BMI	0.8	0.08	−2.28	0.023*	[0.660, 0.969]
Body fat	0.93	0.05	−1.39	0.154	[0.846, 1.029]
Family history of gastric cancer	2.21	1.45	1.21	0.225	[0.613, 7.983]
Lifestyle factors					
Smoking	0.81	0.62	−0.28	0.780	[0.180, 0.030]
Alcohol intake	1.00	0.00	−0.55	0.584	[0.996, 1.002]
Dietary intake	0.18	0.19	−1.66	0.098	[0.023, 1.371]
Salt intake	1.64	0.86	0.95	0.343	[0.590, 4.569]
Fat intake	0.99	0.64	−0.02	0.985	[0.276, 3.536]
Sugar intake	0.40	0.31	−1.20	0.229	[0.089, 1.782]
Physical activity	1.00	0.01	0.53	0.599	[0.996, 1.007]
Sleep	1.21	0.67	0.34	0.731	[0.410, 3.568]
Exercise	1.23	0.66	0.39	0.694	[0.435, 3.495]
Hobby	0.62	0.37	−0.31	0.417	[0.195, 1.969]
Factors associated with lifestyle-related diseases					
Visceral fat accumulation	0.46	0.30	−1.20	0.232	[0.129, 1.64]
Metabolic syndrome	0.44	0.46	−0.78	0.435	[0.057, 3.430]
BP	0.99	0.02	−0.74	0.460	[0.953, 1.022]
LDL cholesterol	1.00	0.01	−0.29	0.769	[0.980, 1.015]
HDL cholesterol	1.01	0.15	1.00	0.319	[0.986, 1.044]
Triglyceride	1.00	0.00	0.43	0.666	[0.995, 1.007]
Dyslipidemia	4.12	2.52	2.31	0.021*	[1.241, 13.690]
FBS	1.01	0.02	0.87	0.382	[0.988, 1.031]
HbA1c	1.62	0.46	1.70	0.089	[0.929, 0.283]
HOMA-IR	0.71	0.31	−0.79	0.432	[0.308, 1.65]
Diabetes mellitus	3.98	2.75	2	0.045*	[1.030, 15.407]
eGFR	0.98	0.02	−0.98	0.329	[0.942, 1.020]
ALT	0.97	0.03	−1.04	0.297	[0.920, 1.026]
FIB4 index	2.98	0.99	3.25	0.001*	[1.552, 5.705]
Fatty liver	1.09	0.6	0.16	0.877	[0.369, 3.217]

*$p<0.05$

Hp, *Helicobacter pylori*; SE, standard error; CI, confidence interval; BMI, body-mass index; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; and FIB4, fibrosis 4

Table 6. Multivariate Logistic Regression Analysis in Hp-positive Subjects

Factor	Odds ratio	SE	Z score	*p*-value	95%CI
Age	1.06	0.05	1.29	0.197	[0.968, 1.168]
BMI	0.86	0.12	−1.12	0.263	[0.660, 1.120]
Dyslipidemia	2.07	1.66	0.91	0.362	[0.432, 9.958]
Diabetes mellitus	1.02	0.94	0.02	0.985	[0.168, 6.164]
FIB4 index	5.27	5.19	1.69	0.092	[0.001, 4.779]

Hp, *Helicobacter pylori*; BMI, body-mass index; and FIB4, fibrosis 4

Table 7. Incidence of Gastric Cancer in the Current Study

H. pylori status of subjects	Incidence of gastric cancer
H. pylori-positive	1.46% (14 out of 959 patients)
Post-eradication of *H. pylori*	0.52%* (8 out of 1547 patients)

H. pylori, *Helicobacter pylori*; *$p=0.02$

mucin layer[16,17]. High salt intake has also been found to increase CagA expression, a potent virulence factor in Hp and a known risk factor for GC development in Hp-infected patients[18]. Studies also suggest that salt intake may enhance the effect of other carcinogens, including *N*-nitroso compounds[19,20]. The National Health and Nutrition Examination Survey in 2018[21] reported that the average salt intake in Japan was 11 g for males and 9.3 g for females. Dietary Intake Standards for Japanese 2020 by the Ministry of Health, Labour and Welfare[22] recommends a salt intake of less than 7.5 g for males and 6.5 g for females for health maintenance. Reduced salt intake may also be effective in inhibiting gastric carcinogenesis, although further studies are needed to clarify how many grams of sodium an individual must limit themselves to in order to reduce the risk of gastric carcinogenesis.

Hp infection has been shown to increase the risk of

Table 8. Clinicopathological Characteristics of Gastric Cancer Subjects

	Current Hp infection	Post-Hp eradication	*p* value
Age (years old)	64.7±7.4	71.9±6.3	0.073
Sex			N/A
Male	8	8	
Female	6	0	
Tumor size (mm)			0.675
20>	9	4	
20≤	5	4	
Tumor macroscopic type			0.248*
0-IIc	10	7	
0-IIb	1	1	
0-IIa	1	0	
Combination	2	0	
Tumor histological type			0.370**
tub1	5	3	
tub2	1	1	
poorly differenciated	3	1	
Combination	5	3	

*0-IIc vs. others, **poorly vs. others

GC three- to six-fold[23–25]. Shah *et al.* suggested that the underlying mechanism of how Hp increases this risk is unclear, but there are two possible pathways: the direct modulation of gastric mucosa through virulence factors, including CagA and VacA, and the indirect action of Hp on gastric epithelial cells[16]. What is clear is that Hp infection induces a state of chronic active inflammation that can last decades. This chronic, active gastritis can subsequently promote gastric carcinogenesis, typically via the Correa model, suggesting that chronic gastric inflammation leads to a cascade of mucosal atrophy, metaplasia, dysplasia, and eventually carcinoma[26]. Host genetic factors likely play a role as well: specific gene polymorphisms in genes encoding tumor necrosis factor-α, interleukin (IL)-1, IL-8, and IL-10 have been associated with an increased risk of GC in Hp-infected patients[27].

Smoking and heavy alcohol intake have been shown to increase the risk of GC[14]. However, our current study did not show an association between smoking or heavy alcohol intake and GC. Recently, the number of smokers and heavy alcohol drinkers has been decreasing in Japan[28,29]. According to the National Health and Nutrition Survey in Japan 2019 conducted by the Ministry of Health, Labour and Welfare, the adult smoking rate was 16.7% in 2019, demonstrating a significant decrease in smoking, especially among men (from 55.3% in 1989 to 27.1% in 2019)[28]. As for alcohol consumption in Japan, according to the "Sake Bookmark" of the Liquor Tax Division of the Taxation Department of the National Tax Administration Agency, per capita consumption of alcoholic beverages has been decreasing since 1992[29]. This is thought to be due to increased health awareness among the public and the progress made in quitting smoking and moderating alcohol consumption through Lifestyle Health Check-ups and Health Guidance initialized in 2008. In this program, specialist staff, including public health nurses, nutritionists, and so on, provide support for those who are at high risk of developing lifestyle-related diseases and for whom lifestyle-related diseases can be prevented to a great extent by improving their lifestyle[30]. Similarly, Health Japan 21 (the second term), which was initialized in 2013 and includes the "Basic Direction for Comprehensive Implementation of National Health Promotion" established by the Ministry of Health, Labour and Welfare, is also thought to have decreased the number of smokers and drinkers[10]. Moreover, Correa mentioned in his review that no clear association has been found between alcohol consumption and GC[4], consistent with the results of this study.

Previous studies have reported a GC incidence of 7%–17% and 0.5%–1% among Hp-positive and Hp-negative individuals, respectively[11,12]. GC incidence was lower in this study than in the aforementioned reports (1.53% and 0% in Hp-positive and Hp-negative subjects, respectively). Accordingly, it was suggested that some factors other than Hp infection affected GC carcinogenesis. No factors associated with GC in Hp-positive subjects were found in the current study, although further studies to clarify these factors are warranted.

This study supports the suggestions of previous studies that Hp eradication therapy reduces the incidence of GC by approximately one-third[13], although Ford *et al.* report a significant but limited effect of eradication therapy on primary cancer prevention for simple Hp gastritis without gastric cancer or peptic ulcer in their meta-analysis[33]. Our results suggest that Hp plays a possible role in GC carcinogenesis. However, it is not the only factor that induces GC; aging and a high salt intake were also important factors inducing GC, although factors associated with GC among Hp-positive

patients were unclear in this study.

Limitations

According to the guidelines of the Japanese Society of *Helicobacter* Research[31], using multiple tests enhances the accuracy of infection diagnosis. However, in clinical practice, according to a notice from the Ministry of Health, Labour and Welfare[32], only one additional test can be covered by insurance when Hp infection is suspected, even if either of the tests yields negative results. Since no other test apart from the serum antibody method was available in our facility, only subjects who tested positive for Hp by this method were enrolled as Hp-positive patients in the present study. This is considered a limitation of the current study. Thus, at least two or more testing methods need to be performed to accurately categorize patients within the Hp-infected group.

Another limitation is that the intake of salt, sugar, fat, and overall dietary intake was not calculated, but rather assessed subjectively using a questionnaire. For a more precise study, it is necessary to conduct an analysis based on quantified dietary intake.

Conclusion

Aging and a high salt intake are possibly associated with GC, suggesting that a low-sodium diet is important for the prevention of GC, especially in the elderly. Hp eradication therapy could be effective in preventing GC, although further studies with a larger number of GC patients are needed for more conclusive evidence.

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Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Smyth EC, Nilsson M, Grabsch HI, *et al.*: Gastric cancer. Lancet 2020; 396: 635–648.
2. Cancer and Disease Control Division, Ministry of Health, Labour and Welfare: Cancer Incidence of Japan 2019. 2019, https://www.mhlw.go.jp/content/10900000/000942181.pdf (accessed October 15, 2023) (in Japanese)
3. Foundation for Promotion of Cancer Research: Cancer Statistics in Japan. 2023, https://ganjoho.jp/public/qa_links/report/statistics/pdf/cancer_statistics_2023_pre_E.pdf (accessed October 15, 2023)
4. Correa P: Gastric cancer: overview. Gastroenterol Clin North Am 2013; 42: 211–217.
5. Center for Cancer Control and Information Services, National Cancer Center, Japan: Age-adjusted morbidity and mortality rates. 2021, https://ganjoho.jp/reg_stat/statistics/stat/cancer/5_stomach.html (accessed April 9, 2023) (in Japanese)
6. International Agency for Research on Cancer: IARC monographs on the evaluation of carcinogenic risks to humans. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Press, Lyon, 1994.
7. Pih GY, Gong EJ, Choi JY, *et al.*: Associations of serum lipid level with gastric cancer risk, pathology, and prognosis. Cancer Res Treat 2021; 53: 445–456.
8. Yamagata H, Kiyohara Y, Nakamura S, *et al.*: Impact of fasting plasma glucose levels on gastric cancer incidence in a general Japanese population: the Hisayama study. Diabetes Care 2005; 28: 789–794.
9. Ikeda F, Doi Y, Yonemoto K, *et al.*: Hyperglycemia increases risk of gastric cancer posed by *Helicobacter pylori* infection: a population-based cohort study. Gastroenterology 2009; 136: 1234–1241.
10. Ministry of Health, Labour and Welfare: Health Japan 21. 2013, https://www.mhlw.go.jp/www1/topics/kenko21_11/b5.html (accessed April 9, 2023) (in Japanese)
11. Kawai S, Wang C, Lin Y, *et al.*: Lifetime incidence risk for gastric cancer in the *Helicobacter pylori*-infected and uninfected population in Japan: a Monte Carlo simulation study. Int J Cancer 2022; 150: 18–27.
12. Hamajima N, Goto Y, Nishio K, *et al.*: *Helicobacter pylori* eradication as a preventive tool against gastric cancer. Asian Pac J Cancer Prev 2004; 5: 246–252.
13. Fukase K, Kato M, Kikuchi S, *et al.*: Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet 2008; 372: 392–397.
14. Shah D, Bentrem D: Environmental and genetic risk factors for gastric cancer. J Surg Oncol 2022; 125: 1096–1103.
15. D'Elia L, Rossi G, Ippolito R, *et al.*: Habitual salt intake and risk of gastric cancer: a meta-analysis of prospective studies. Clin Nutr 2012; 31: 489–498.
16. Fox JG, Dangler CA, Taylor NS, *et al.*: High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances *Helicobacter pylori* colonization in C57BL/6 mice. Cancer Res 1999; 59: 4823–4828.
17. Kato S, Tsukamoto T, Mizoshita T, *et al.*: High salt diets dose-dependently promote gastric chemical carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils associated with a shift in mucin production from glandular to surface mucous cells. Int J Cancer 2006; 119: 1558–1566.
18. Loh JT, Torres VJ, Cover TL: Regulation of *Helicobacter pylori* cagA expression in response to salt. Cancer Res 2007; 67: 4709–4715.
19. Takahashi M, Nishikawa A, Furukawa F, *et al.*: Dose-dependent promoting effects of sodium chloride (NaCl) on rat glandular stomach carcinogenesis initiated with

N-methyl-*N*'-nitro-*N*-nitrosoguanidine. Carcinogenesis 1994; 15: 1429–1432.
20. Tatematsu M, Takahashi M, Fukushima S, *et al*.: Effects in rats of sodium chloride on experimental gastric cancers induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine or 4-nitriquinoline-1-oxide. J Natl Cancer Inst 1975; 55: 101–106.
21. Ministry of Health, Labour and Welfare: National Health and Nutrition Examination Survey in 2018. https://www.mhlw.go.jp/bunya/kenkou/kenkou_eiyou_chousa.html (accessed August 19, 2023) (in Japanese)
22. Ministry of Health, Labour and Welfare: Dietary Intake Standards for Japanese 2020. https://www.mhlw.go.jp/content/10904750/000586565.pdf, 270 (accessed August 19, 2023) (in Japanese)
23. Vohlonen I, Pukkala E, Malila N, *et al*.: Risk of gastric cancer in *Helicobacter pylori* infection in a 15-year follow-up. Scand J Gastroenterol 2006; 51: 1159–1164.
24. *Helicobacter* and Cancer Collaborative Group: Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 2001; 49: 347–353.
25. Kikuchi S, Wada O, Nakajima T, *et al*.: Serum anti-*Helicobacter pylori* antibody and gastric carcinoma among young adults. Research Group on Prevention of Gastric Carcinoma among Young Adults. Cancer 1995; 75: 2789–2793.
26. Correa P, Haenszel W, Cuello C, *et al*.: A model for gastric cancer epidemiology. Lancet 1975; 2: 58–60.
27. Shanks AM, El-Omer EM: *Helicobacter pylori* infection, host genetics and gastric cancer. J Dig Dis 2009; 10: 157–164.
28. Ministry of Health, Labour and Welfare: The National Health and Nutrition Survey in Japan. Ministry of Health, Labour and Welfare, Tokyo, 2019, 191–208.
29. National Tax Agency: Sake Bookmark 2019. The Liquor Tax Division of the Taxation Department of the National Tax Administration Agency. National Tax Agency, Tokyo, 2020, 3.
30. Ministry of Health, Labour and Welfare: About Lifestyle Health Check-ups and Health guidance. Revised 2023, https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000161103.html (accessed April 9, 2023) (in Japanese)
31. The Japanese Society for *Helicobacter* Research Guideline Development Committee (ed): Diagnosis Methods. Guidelines for the diagnosis and treatment of Hp infection. Revised 2016, Sentan-Igakusya, Tokyo, 2016, 32. (in Japanese)
32. Notification No. 1031-5 issued by the Medical Division, Health Bureau, Ministry of Health, Labour and Welfare. October 31, 2022, https://www.mhlw.go.jp/web/t_doc?dataId=00tc7124&dataType=1&pageNo=1 (accessed August 19, 2023) (in Japanese)
33. Ford AC, Yuan Y, Moayyedi P: *Helicobacter pylori* eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut 2020; 69: 2113–2121.

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The Favorable Effect of Continuous Glucose Monitoring (CGM) on Hyperglycemia and Glycemic Fluctuations in Elderly People with Untreated Glucose Intolerance

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Abstract

Objective: To evaluate the effect of a continuous glucose monitoring (CGM) device, the FreeStyle Libre™, on blood glucose (BG) fluctuation in elderly people with untreated glucose intolerance.

Methods: Sixty participants aged 60 years or older with glucose intolerance (6.5%≤HbA1c or 125 mg/dL≤BG) who were not receiving any treatments for diabetes were enrolled in the study. They were placed on the CGM device for 14 days. During these 14 days, they were asked to keep a detailed diary regarding daily events, focusing on diet and exercise. On the 7th day, an educational review session was performed to assess a weekly summary of their BG levels and daily events. On the 14th day, the BG results were summarized. Fifty-five participants completed the trial, and their response to a questionnaire was obtained.

Results: Even with glucose intolerance (HbA1c 6.7±0.48), large diurnal blood glucose fluctuations and frequent hyperglycemia were observed during the first week of the study period. The mean daily BG level significantly improved from 129.9±7.5 mg/dL in the first week to 119.1±6.18 mg/dL in the second week ($p<0.01$). The number of hyperglycemic events exceeding 180 mg/dL decreased, from 6.8±5.8 in the first week to 4.3±4.0 in the second week ($p<0.01$). The peak BG level also decreased, from 236±73.6 mg/mL in the first week to 218±67.8 mg/dL in the second week ($p<0.01$). Twenty-five of 55 participants (45%) lost more than 1 kg of weight. Forty-nine participants (89%) positively responded to CGM use as effective in the post-enrollment survey. For 47 (85%), review on the 7th day was needed. Fifty participants (91%) wanted to continue using CGM. The majority of participants stated that the CGM system helped them understand the importance of dietary carbohydrate intake, which subsequently improved their glycemic control after the review session and counseling.

Conclusion: In elderly people with untreated glucose intolerance, various patterns of elevated BG were observed. However, the CGM universally improved their hyperglycemia and glucose fluctuations, regardless of the cause. The CGM system may become a valuable tool in improving untreated glucose intolerance in elderly people, who are highly motivated to restore and maintain their health status.

Keywords glucose intolerance, untreated diabetes, elderly people, CGM (continuous glucose monitoring device)

The number of people with glucose intolerance increases with age. Of interest, the number of patients with overt diabetes with fasting blood glucose (FBG) of 140 mg/dL or higher does not increase, while the number of those who have mild glucose intolerance (FBG less than 140 mg/dL) increases with age[1]. Further, hemoglobin A1c (HbA1c) less than 7.5 in those not undergoing treatment is often identified in regular health assessments. This is probably because HbA1c 7.5 is the goal of glycemic control in elderly diabetics. Although many elderly people with untreated glucose intolerance (FBG less than 140 mg/dL or HbA1c less than 7.5) are identified on regular health assessments, there are very limited data with regard to their daily glycemic fluctuations. To date, the Clinical Practice Guideline for Diabetes for the Elderly 2023[2] also states that it is important to embody individual goals in the treatment of geriatric diabetes, but does not clearly indicate a method for embodying them[2]. Conventional dietary and exercise guidance can

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be empirically attempted, but this strategy often fails to achieve optimal glucose control and may complicate inappropriate weight loss, which may further lead to muscle wastage/weakness. In addition, blind dietary restrictions may cause symptomatic hypoglycemia and frailty in elderly people.

In this study, we investigated daily glycemic fluctuations using a continuous glucose monitoring (CGM) system —the FreeStyle Libre™— in elderly people with untreated glucose intolerance diagnosed in routine health assessments.

The purpose of this study was to identify glycemic fluctuations in elderly people with untreated glucose intolerance and to evaluate the effectiveness and usefulness of the CGM system and appropriate counseling to improve their hyperglycemia and BG fluctuations.

Subjects and Methods

Among participants who underwent routine health assessments at our health check center, those who were 60 years of age or older with glucose intolerance (6.5%≤HbA1c or 125 mg/dL ≤BG) were invited to participate in the study. Patients who were already receiving diabetic treatments were excluded. A total of 60 participants who agreed to the study were enrolled.

Estimated blood glucose (BG) levels were obtained using a CGM (FreeStyle Libre Continuous Glucose Monitoring system)[3,4]. A sensor was affixed to the posterior upper arm for 14 days. Glucose levels in the interstitial fluid were obtained by simply holding the reader over the sensor and scanning. The measurements were also recorded in a smartphone app[5].

While wearing the device for 14 days, participants were asked to keep a detailed diary regarding their daily events, focusing on diet and exercise. On the 7th day, CGM results were reviewed and interim counseling and guidance for lifestyle modification were provided. On the 14th day, final CGM results were reviewed and discussed with the participants. After completion of the study, each participant was requested to complete a questionnaire.

Mean daily BG, frequency of hyperglycemia, duration of hyperglycemia and maximal BG were compared between the first and second weeks. Statistical analyses were performed using the HAD program by the paired Student *t*-test. BG levels of 180 mg/dL or higher and 70 mg/dL or lower were defined as hyperglycemia and hypoglycemia, repectively[6–8].

This study was approved by the Ethics Committee of Ashiya Municipal Hospital (No.22.2022/5/11) and were consistent with the Japanese Ethical Guideline For Life Science And Medical Research involving Human Participants[9]. Written informed consent was obtained from each participant prior to enrollment.

Results

The characteristics of the participants are shown in **Table 1**.

A summary of the trial is shown in **Fig. 1**. Five of 60 participants did not complete the study due to equipment failure (n=2), sensor detachment (n=2), and inappropriate scanning (n=1).

Fifty-five participants with 14 days of blood glucose (BG) data were included in the study. Forty-nine of the 55 participants demonstrated some improvement in BG profile.

Weekly summaries from five representative cases of this study are shown in **Figs. 2–6**.

Case 1 (**Fig. 2**) was a 70-year-old male with BMI 26.3 kg/m^2, abdominal circumference 86 cm, HbA1c 6.7% and FBG 145 mg/dL. He walked for 30 minutes every morning and evening, ate Japanese food for all three meals, had a snack at 3 p.m. and performed all household chores by himself. He was very active and often went out for social events. He was surprised to see the high BG levels from the CGM during the first week. These showed prolonged periods of high BG levels during the first week, with an average daily BG level of 140–160 mg/dL. After lifestyle and dietary guidance on the 7th day, his BG significantly decreased to 100–120 mg/dL and no further hyperglycemic events were observed in the second week.

Case 2 (**Fig. 3**) was a 76-year-old male, with a BMI 24.5 kg/m^2, abdominal circumference 87 cm, HbA1c 7.0% and FBG 138 mg/dL. He was receiving treatment for hypertension, but not for diabetes. He did radio calisthenics every morning. On the 7th day, lifestyle guidance was provided. He was advised to walk more than 8,000 steps daily. He was also advised to limit high carbohydrate meals such as noodles and rice. Additionally, he was instructed to avoid snacks after dinner and encouraged exercise for 10 minutes daily. With these suggestions, significant BG improvement was observed in the second week.

Case 3 (**Fig. 4**) was a 60 year-old male, with BMI 26.1 kg/m^2, abdominal circumference 98 cm, HbA1c 6.1% and FBG 125 mg/dL. He was under medical treatment for hypertension and hyperlipidemia. He worked as a

Table 1. Participant Characteristics

Characteristic	n=60
Male	40
Female	20
Age (years)	68.2±7.9
BMI (kg/m^2)	23.4±3.4
Waist circumference (cm)	85.1±12.2
Fasting blood glucose (mg/dL)	124±19.3
HbA1c (%)	6.7±0.48

Values are mean±standard deviation.

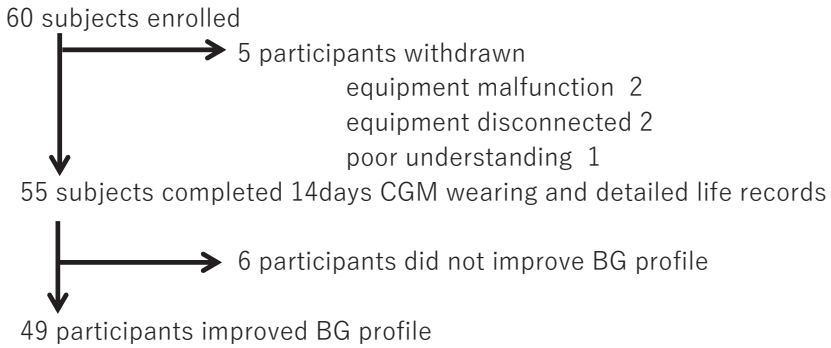


Fig. 1. Summary of the Trial

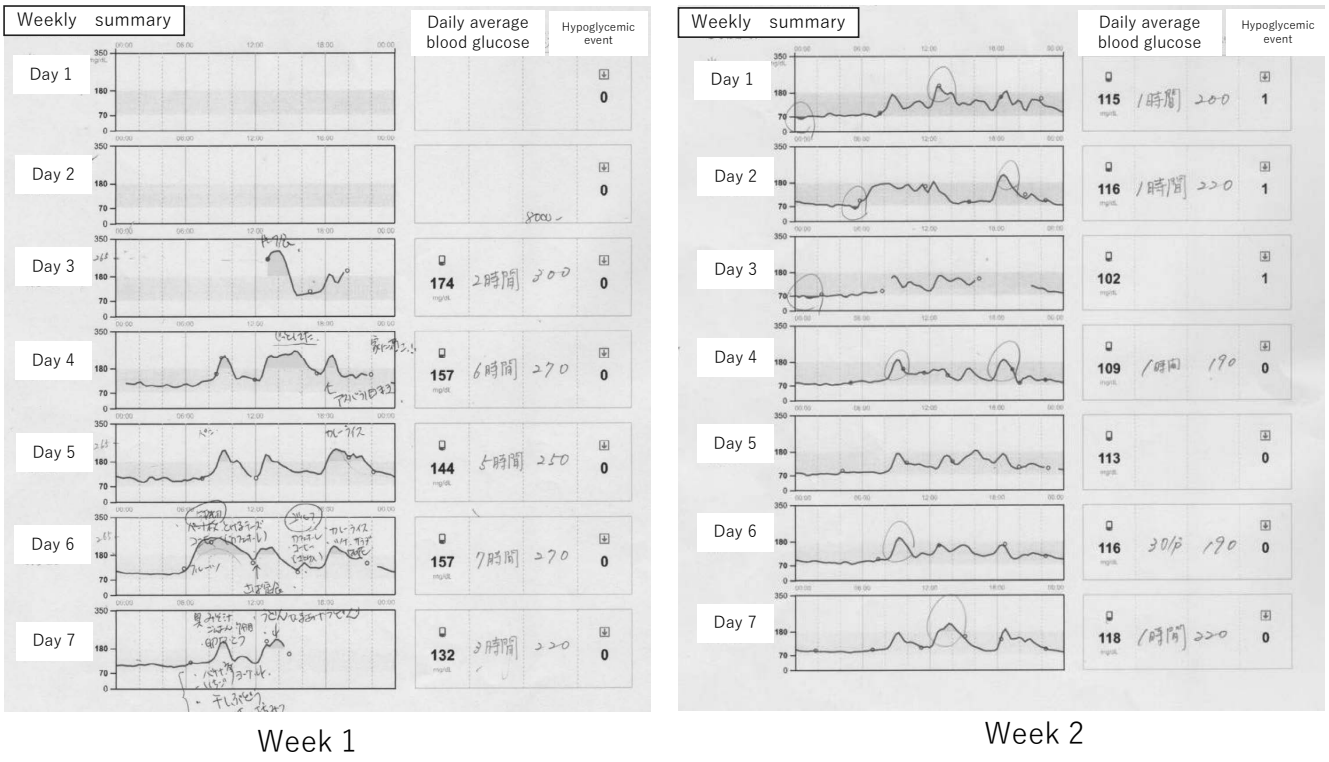
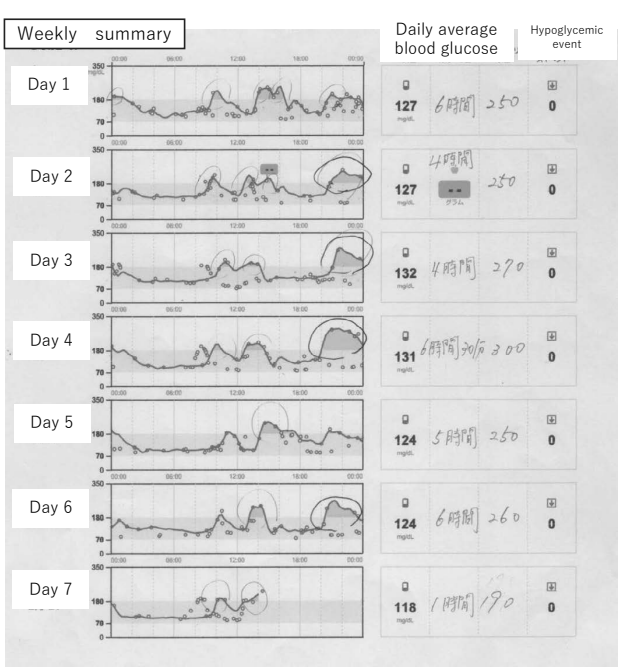


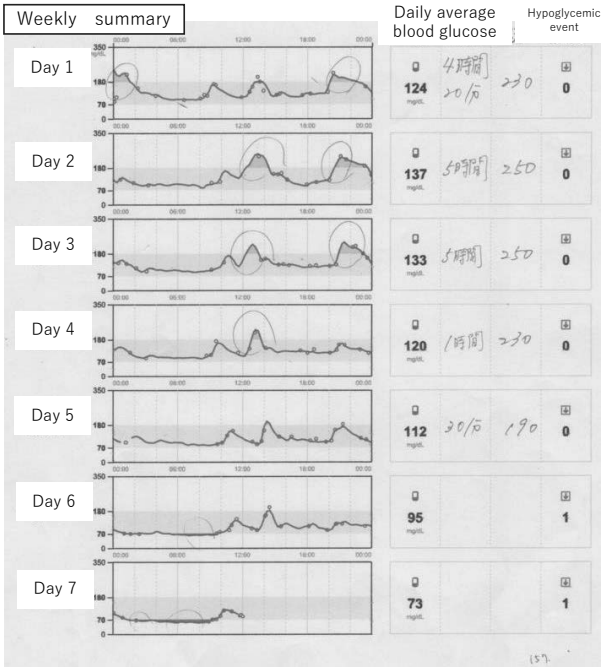
Fig. 2. Case 1. 70 y.o. Male (BMI 26.3 kg/m^2, HbA1c 6.7%, FBG 145 mg/dL)

security guard and returned home late in the evening, having dinner at 8:00–9:00 p.m. He drank sweetened canned coffee 4 to 5 times a day at work. On the 7th day, instruction was given to him to replace canned coffee with unsweetened tea and to reduce carbohydrate intake at dinner. Improvement in the glycemic profile was observed in the second week.

Case 4 (**Fig. 5**) is an 80-year-old male, with a BMI 24.5 kg/m^2, abdominal circumference 92 cm, HbA1c 7.0% and FBG 124 mg/dL. He did not have a significant medical history. He walked 4,000 steps daily. For breakfast, he ate bread with jam and butter, vegetable juice, yogurt with cereal, banana, mandarin oranges, and honey. For lunch, he ate noodles such as udon, soba and ramen. For dinner, he typically ate sushi or yakitori with beer. He used to eat ice cream after dinner. We advised him to walk 30 minutes twice daily, not eat ice cream, cut down the fruit intake in the morning, stop having cereals for breakfast, and avoid simple carbohydrate meals for lunch. He also demonstrated improvement in glycemic profile.

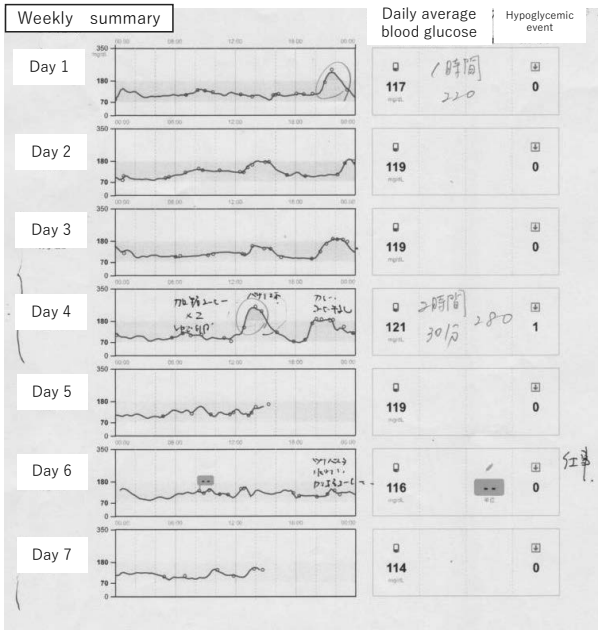
Case 5 (**Fig. 6**) is a 69-year-old female with BMI 27.4 kg/m^2, abdominal circumference 88.0 cm, HbA1c 6.5% and FBG 101 mg/dL. She was on anticoagulants due to the history of coronary artery disease. She was treated for hyperlipidemia and obstructive sleep apnea. She exercised in a gym four times a week. Although she reduced sugar intake in each meal, she remained hyperglycemic after lunch and dinner. Her carbohydrate restriction was insufficient, so we provided

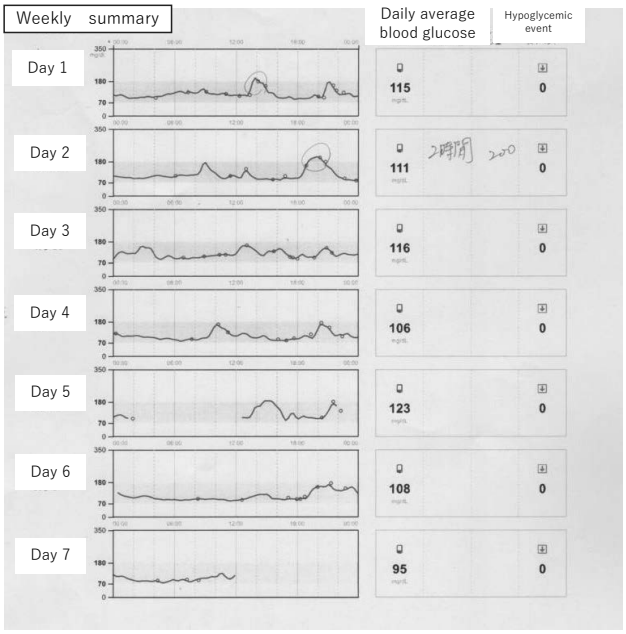




Week 1 Week 2

Fig. 3. Case 2. 76 y.o. Male (BMI 24.5 kg/m^2, HbA1c 7.0%, FBG 138 mg/dL)





Week 1 Week 2

Fig. 4. Case 3. 60 y.o. Male (BMI 26.1 kg/m^2, HbA1c 6.1%, FBG 125 mg/dL)

detailed dietary counseling, including replacing sports drinks with plain water or unsweetened tea during the exercise. She lost 1 kg of weight in two weeks and her glucose profile improved.

As in these five representative cases, 49 of the 55 participants (89%) showed significant improvement in mean daily BG level in the second week compared to that in the first week. Changes in mean daily BG in these 55 cases after initiating CGM are shown in **Fig. 7**. BG levels began to fall on the fourth day without

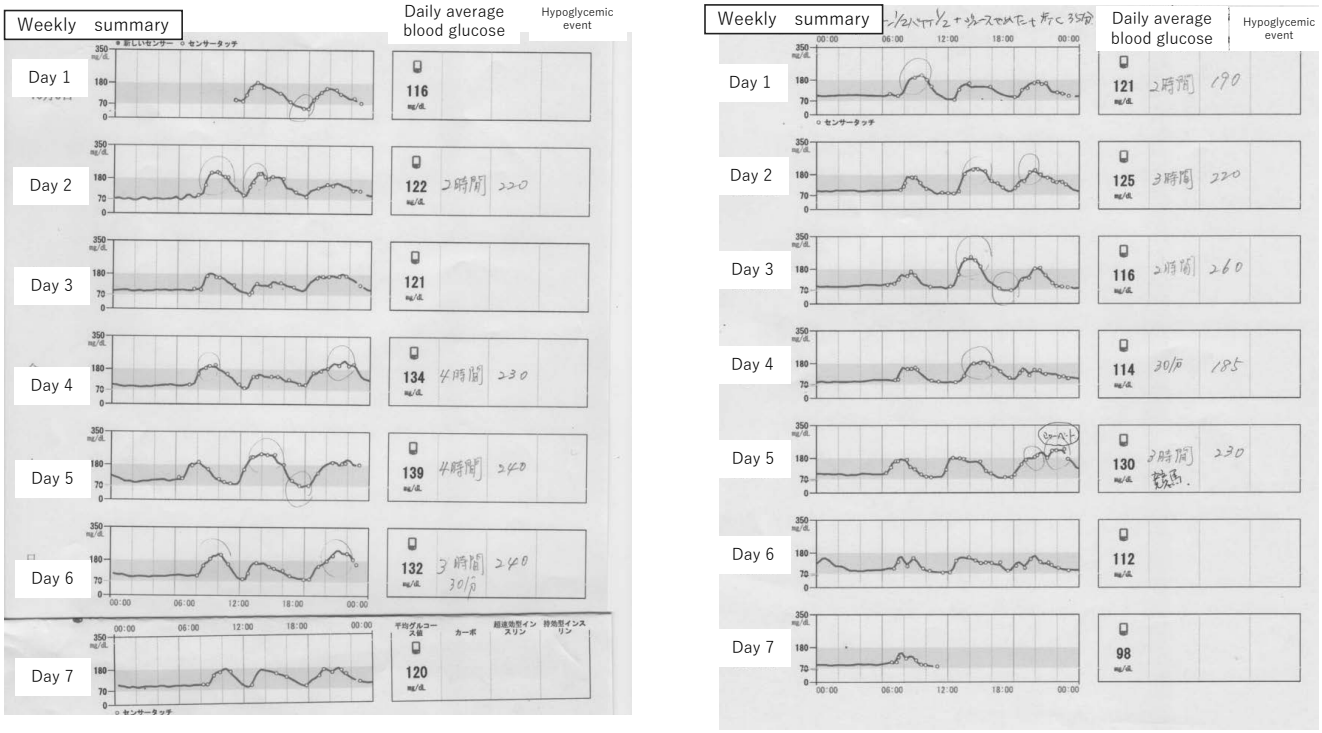


Fig. 5. Case 4. 80 y.o. Male (BMI 24.5 kg/m^2, HbA1c 7.0%, FBG 124 mg/dL)

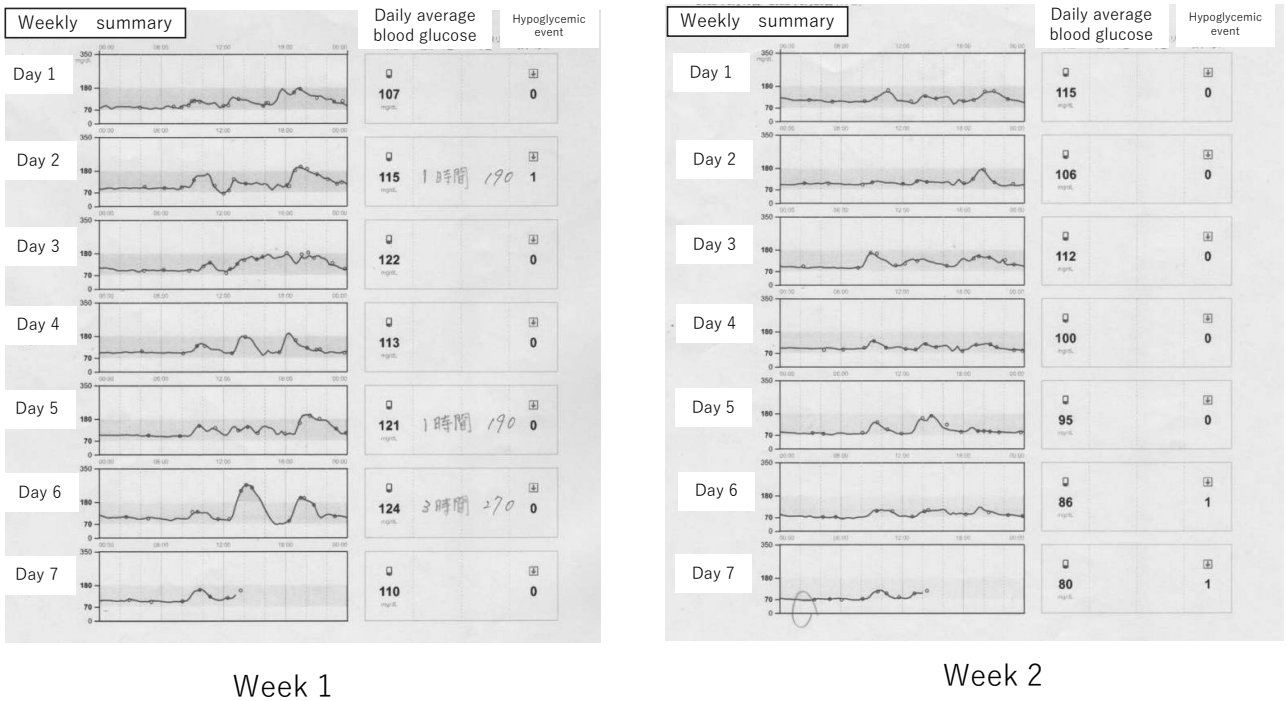


Fig. 6. Case 5. 69 y.o. Female (BMI 27.4 kg/m^2, HbA1c 6.5%, FBG 101 mg/dL)

interventions and further decreased after the interim interview on the 7th day. The CGM application seemed to change behavioral attitude in the participants, many of whom demonstrated improved glucose metabolism without further treatment, which impressed them with satisfaction.

Table 2 shows mean daily glucose, number of hyperglycemic events at or above 180 mg/dL, duration of BG

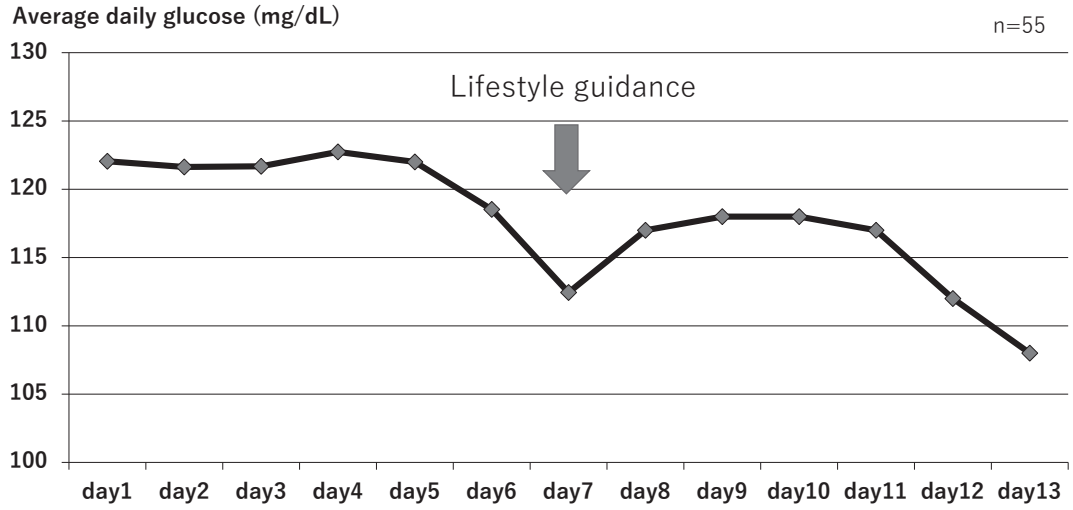


Fig. 7. Changes in Average Daily Blood Glucose while Wearing CGM

Table 2. Effect of CGMS on Hyperglycemia

	Week 1	Week 2	*p* value
Average daily glucose (mg/dL)	129.9±7.5	119.1±6.18	0.0008
Number of hyperglycemic events	6.8±5.8	4.3±4.0	0.0098
Duration of hyperglycemia (hour)	14.2±25.0	8.2±21.0	0.16
Peak blood glucose (mg/dL)	236±73.6	218±67.8	0.0003

n=55.
p values were calculated by the paired *t*-test.
Hyperglycemia: blood glucose ≥180 mg/dL

Table 3. Questionnaire Results After Participation in This Study

Weight loss ≥1 kg	25/55 (45%)	
Is CGM effective in improving your health?	Yes 49 (89%)	
Do you need a review on the 7th day?	Yes 47	No 8 (85%)
Would you like to apply CGM again?	Yes 50	No 5 (91%)

What food raises blood sugar the most?
Baked sweet potato, Udon, Curry, Amazake, Red rice, Katsudon, Rolled sushi, Inari sushi, Croquette, Yakisoba, Breakfast fruit, Quick meal, Convenience store bento, Boiled root vegetables, Rice balls, Chirashi sushi, Ice cream, Rice crackers

Table 4. Lessons Learned from CGM

- I learned what food raises my blood sugar.
- I wanted to know my blood glucose levels immediately after eating.
- I recognized I had been taking too much food.
- Self-control in diet helped to reduce my belly size.
- I learned how much exercise and dietary intake I need every day.
- I learned the relationship between my blood sugar levels and life events, such as choice of food intake, exercise, and physical condition.
- I found I had been taking too many carbs.
- I thought my lifestyle was just fine, but I realized I did not have appropriate knowledge.
- After reviewing my lifestyle, I learned I should not take so much sweet and greasy food.
- I tried to cut down on snacks, but I didn't cut down enough to meet my goal.
- My problem was a lack of regular exercise.

at or above 180 mg/dL, and peak BG level in the first and second weeks of the study. Statistically significant improvements were observed in the weekly average of daily glucose (129.9 mg/dL vs 119.1 mg/dL, $p<0.01$), number of hyperglycemic events (6.8 vs 4.3, $p<0.01$) and peak BG (236 mg/dL vs 218 mg/dL, $p<0.01$) between the first and second weeks. Duration of BG at or above 180 mg/dL did not differ due to large variance. No adverse effects were observed with wearing the CGM device.

The responses to the post-CGM study questionnaires are presented in **Table 3**. Twenty-five participants (45%) lost more than 1 kg of weight, 49 (89%) gave a positive response to the usefulness of CGM, 47 (85%) noted that the interim counseling and guidance on the 7th day were helpful, and 50 (91%) expressed a desired to use CGM again. Many participants surprised that noodles, rice, fruit, and baked potatoes significantly raised their BG levels. Typical comments after CGM use are shown in **Table 4**. One of the most common responses was that they learned the importance of proper dietary carbohydrate intake and regular physical activity to improve glycemic profile.

Discussion

BG fluctuations are affected by multiple factors such as age, sex, genetic background, weight, muscle mass,

fat mass, metabolic efficiency and physical activity. They are also influenced by social factors such as work, exercise, sleep, physical/mental stress, and dietary habits. The CGM system has a strong advantage over a traditional self-monitoring of BG (SMBG) in that it is convenient for patients and can continuously measure BG levels with no time lag.

With CGM, many participants who had glucose intolerance (6.0%≤HbA1c<7.5%) demonstrated large amplitudes of diurnal BG fluctuations and prolonged/high frequency and hyperglycemia, which may not have been detected otherwise.

Previous studies demonstrated remarkable clinical benefits of CGM use in people with type II diabetes[10–14]. However, there are few reports with regard to the effects of CGM in patients with untreated glucose intolerance. The results presented in this study suggest that introducing the CGM system to elderly people with untreated glucose intolerance is beneficial in improving glycemic profile. Cases of mild glucose intolerance followed by diabetes mellitus are frequent. They would be exposed to large-amplitude diurnal BG fluctuation and hyperglycemia during the period of mild glucose intolerance. The earlier achievement of normal glycemic control will be of benefit to them.

By recording life events, factors that were responsible for hyperglycemia and BG fluctuations were identified. Of interest, these factors were found very specific for each individual. After reviewing BG pattern and lifestyle, a tailored counseling was performed to set a clear and practical goal for each participant. Goal attainment must be collaborative and attainable. Individual lifestyle and ability should be taken into account. Lawlor and Hornyak proposed the SMART goal, which incorporates four important elements for behavioral change: 1) The goal is specific and defines exactly what is to be achieved (Specific); 2) The goal is measurable and there is tangible evidence when it has been achieved (Measurable); 3) The goal is achievable but the participant feels a bit challenged (Achievable); 4) The goal is achievable in a short period of time (Time bound)[15,16]. The CGM may be an important tool in achieving the goal setting that meets this SMART goal law.

For elderly people with mild glucose intolerance, utilizing the CGM system may be effective in preventing or delaying the onset of overt diabetes via appropriate education and counseling. In this study, not only an improvement in BG control but also suppression of carbohydrate intake was achieved in two weeks, and about half of the participants lost 1 kg of weight or more. With continuation of this behavioral change, improvements in other health aspects such as hypertension, dyslipidemia, and abnormal liver function are anticipated, which may further prevent geriatric syndrome.

It has been reported that the long-term effect of this intervention persists even six months after its cessation[17]. Since the participants in this study are planned to have follow-up visits for annual health assessments, long-term effectiveness of intervention with CGM will be evaluated. We also plan to include a CGM application as an optional test for patients who receive health assessments in our center.

We would like to study further applications of CGM in clinical practice. These further studies will be expanded to include various health conditions such as untreated glucose intolerance in middle aged people, gestational diabetes, diet management after gastrectomy, obesity, and emaciation.

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Disclosure

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

References

1. Ito C: Epidemiology of type II diabetes. In: Kadowaki T, Ogawa Y, Shimomura I (ed), Igaku No Ayumi (Suppl, Diabetes and metabolic syndrome: state of arts 2004), Ishiyaku Publishers, Tokyo, 2004, 177–180. (in Japanese)
2. Japan Geriatrics Society, Japan Diabetes Society (ed): Geriatric Diabetes Practice Guideline. VI. Control goals and treatment policies for geriatric diabetes. Nankodo, Tokyo, 2023, 85–101. (in Japanese)
3. Hirota Y: Flash glucose monitoring. Journal of the Japan Diabetes Society 2018; 61: 809–811. (in Japanese)
4. Edelman SV, Argento NB, Pettus J, *et al.*: Clinical implications of real-time and intermittently scanned continuous glucose monitoring. Diabetes Care 2018; 41: 2265–2274.
5. Abbott: Abbott Diabetes Care FreeStyle Libre Software. https://www.freestylelibre.com (accessed December 25, 2023)
6. Bergenstal RM, Ahmann AJ, Bailey T, *et al.*: Recommendations for standardizing glucose reporting and analysis to optimize clinical decision making in diabetes: the Ambulatory Glucose Profile (AGP). Diabetes Technol Ther 2013; 15: 198–211.
7. American Diabetes Association: 6. Glycemic targets. Diabetes Care 2017; 40 (Suppl 1): S48–S56.

8. Battelino T, Danne T, Bergenstal RM, *et al*.: Clinical targets for continuous glucose monitoring data interpretation: recommendations from the international consensus on time in range. Diabetes Care 2019; 42: 1593–1603.
9. Ministry of Health, Labour and Welfare: Japanese Ethical Guidelines for Life Science and Medical Research Involving Human Participants, March 2022 provisional translation. http://www.mhlw.go.jp/content/000909926.pdf (accessed December 25, 2023) (in Japanese)
10. Vigersky RA, Fonda SJ, Chellappa M, *et al*.: Short- and long-term effects of real-time continuous glucose monitoring in patients with type 2 diabetes. Diabetes Care 2012; 35: 32–38.
11. Ogawa W, Hirota Y, Osonoi T, *et al*.: Effect of the FreeStyle Libre™ flash glucose monitoring system on glycemic control in individuals with type 2 diabetes treated with basal-bolus insulin therapy: an open label, prospective, multicenter trial in Japan. J Diabetes Investig 2021; 12: 82–90.
12. Kröger J, Fasching P, Hanaire H: Three European retrospective real-world chart review studies to determine the effectiveness of flash glucose monitoring on HbA1c in adults with type 2 diabetes. Diabetes Ther 2020; 11: 279–291.
13. Vigersky R, Shrivastav M: Role of continuous glucose monitoring for type 2 in diabetes management and research. J Diabetes Complications 2017; 31: 280–287.
14. Yaron M, Roitman E, Aharon-Hananel G, *et al*.: Effect of flash glucose monitoring technology on glycemic control and treatment satisfaction in patients with type 2 diabetes. Diabetes Care 2019; 42: 1178–1184.
15. DeWalt DA, Davis TC, Wallace AS, *et al*.: Goal setting in diabetes self-management: taking the baby steps to success. Patient Educ Couns 2009; 77: 218–223.
16. Blaine Lawlor K, Hornyak MJ: SMART goals: how the application of SMART goals can contribute to achievement of student learning outcomes. Developments in Business Simulation and Experiential Learning: Proceedings of the Annual ABSEL Conference 2012; 39: 259–267.
17. Nishimura A, Harashima S, Hosoda K, *et al*.: Long-term effect of the color record method in self-monitoring of blood glucose on metabolic parameters in type 2 diabetes: a 2-year follow-up of the color IMPACT Study. Diabetes Ther 2018; 9: 1501–1510.

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Original Article

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The Correlation Between Brachial-ankle Pulse Wave Velocity (baPWV) and Atherosclerosis Risk Factors, as well as the Risk Score for Development of Atherosclerotic Diseases in Ningen Dock Health Checkup: A Prospective Collaborative Study at Six Health Checkup Facilities

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Abstract

Objectives: A prospective collaborative study was conducted on subjects who underwent consecutive comprehensive health checkup system (Ningen Dock) at six health checkup facilities. This study aimed to achieve the following objectives:

1) To examine the correlation between Brachial-ankle Pulse Wave Velocity (baPWV) values and atherosclerosis risk factors.

2) To investigate the secular changes in baPWV values in subjects who underwent Ningen Dock for three consecutive years.

3) To assess the association between baPWV values and atherosclerotic disease risk scores (Suita score, Hisayama score).

Methods: A total of 14,933 subjects were enrolled in 2019, and cross-sectional analysis was conducted. Additionally, longitudinal analysis was carried out on 4,578 subjects who had baPWV measurements for three consecutive years (2019–2021).

Results: Regarding the relationship between atherosclerosis risk factors and baPWV values, the strongest correlation was found with four factors: hypertension, glucose metabolism abnormalities, lipid abnormalities (excluding LDL-C), and obesity. These factors remained independent variables in multiple regression analysis. baPWV values increased significantly in both males and females annually. Among 4,249 subjects (40 to 80 years old during the three-year study period), baPWV values increased significantly as each risk score classification elevated, despite each risk score remained unchanged.

Conclusion: baPWV values were robustly correlated with the clustering of atherosclerosis risk factors and risk scores for atherosclerotic disease. Furthermore, baPWV values increased annually even when risk scores remained unchanged, suggesting the importance of continuous baPWV measurement for monitoring the progression of atherosclerosis.

Keywords baPWV, atherosclerosis risk factor, Suita score, Hisayama score

The primary objective of lifestyle-related disease management is to prevent the onset of atherosclerotic vascular disease, and the method used to monitor the progression of atherosclerosis is a very important issue. Brachial-ankle Pulse Wave Velocity (baPWV) measurement is a non-invasive and convenient vascular function test, widely recognized for primarily reflecting the stiffness of large arteries[1,2]. In our previous research, we have reported both cross-sectional and longitudinal results on the usefulness of

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baPWV values in health checkups[3–8]. Among these, we have also reported that baPWV values are affected by various factors, and that it is necessary to standardize the measurement conditions and interpret the results[4]. Furthermore, since baPWV values are greatly influenced by blood pressure, we analyzed the results of a comparative study with the cardiac-ankle vascular index (CAVI) adjusted by the stiffness parameter β[9], and found that the baPWV value remained more useful for health guidance in the comprehensive health checkup system (Ningen Dock)[6]. Subsequently, we also reported on the relationship between longitudinal changes in baPWV values and clustering of atherosclerosis risk factors[7], and that the effects of smoking can be detected through secular changes in baPWV values[8]. A recent report has suggested that CAVI values adjusted by the stiffness parameter β attenuate the predictive effectiveness for the onset of atherosclerotic diseases compared to baPWV values[10].

In this study, we conducted a prospective study on subjects who underwent Ningen Dock at multiple health checkup facilities nationwide. We examined the relationship between the clustering of atherosclerosis risk factors and baPWV values. Additionally, we explored the association of baPWV values with the Suita score and Hisayama score, both of which are predictive scores for the development of atherosclerotic disease[11,12].

Methods

Subjects of this study had undergone Ningen Dock and measurement of baPWV values at six health checkup facilities participating in this study in 2019, and a cross-sectional analysis was carried out. Furthermore, for those who underwent Ningen Dock for three consecutive years (2019 to 2021), and measurement of baPWV values, the secular changes of baPWV values and the relationship between baPWV values and atherosclerotic disease risk scores (Suita score and Hisayama score) were examined.

baPWV was measured using BP-203RPEIII form (Colin Fukuda, Tokyo) under conditions of maximum rest by the same measurement method as previously reported[7,8].

The relationship between clustering of atherosclerosis risk factors and baPWV values was examined using the following criteria. Hypertension: systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg; glucose intolerance: fasting blood glucose ≥110 mg/dL or HbA1c ≥6.0%; dyslipidemia: HDL-C <40 mg/dL or triglyceride ≥150 mg/dL; and obesity: BMI ≥25 kg/m^2 or waist circumference ≥90 cm.

A stepwise multiple regression analysis was performed to determine independent variables for predicting baPWV values. Systolic blood pressure, diastolic blood pressure, pulse pressure, fasting glucose, HbA1c, triglyceride, HDL-C, BMI, waist circumference, LDL-C, and smoking habits were used as analysis variables.

In addition, baPWV cut-off values were calculated from the relationship between the number of atherosclerosis risk factors and baPWV values by receiver operating characteristic (ROC) curve analysis.

Furthermore, we examined the relationship between baPWV values and the risk classification of the Suita score and Hisayama score, which are atherosclerotic disease risk scores. Since the Hisayama score targets people aged 40 to under 80, subjects who were aged 40 to under 80 during the three-year study period were analyzed using both scores. According to the risk classification of the Hisayama score, a Suita score ≤40 (predicted probability ≤1%) was low risk, ≤60 was medium risk (predicted probability ≤9%), and ≥61 was high risk. The Hisayama score according to the Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2022[13], defines diabetes, chronic kidney disease (CKD), and peripheral vascular disease (PAD) as high-risk diseases, and classification is carried out by risk score after excluding high-risk diseases (Hisayama score 1). However, since the initial report assessed risk using the score only[12], we also examined the relationship between baPWV values and risk stratification based solely on the score (Hisayama score 2).

Statistical Analysis

All analyses were performed using IBM SPSS Statistics version 21 (IBM, Armonk, NY, USA). All data are expressed as mean±SD. A p-value <0.05 was considered statistically significant.

The optimal cut-off values, determined by ROC curve analysis, were based on the Youden index. Wilcoxon signed rank test or McNemar's test were performed to compare the 1st and 3rd year data of subjects who underwent Ningen Dock for three consecutive years. Friedman's test with Bonferroni correction was employed to analyze the secular changes of baPWV values, Suita scores, Hisayama scores in subjects who underwent Ningen Dock for three consecutive years. The Kruskal–Wallis test with Bonferroni correction was used to analyze the relationship between risk classification of the Suita or Hisayama score and the first year baPWV value in subjects who underwent Ningen Dock for three consecutive years. A Mann Whitney u test was performed to compare the first year baPWV values between high-risk disease patients and score-based high-risk subjects in the Hisayama score high-risk group.

This study was conducted prospectively and analyzed with the approval of the Ethics Committee of Olive

Takamatsu Clinic (2019-01) and each participating health checkup facility, in accordance with the Declaration of Helsinki.

Results

Table 1 shows the background characteristics of a total of 14,933 subjects, 9,090 males and 5,843 females, who were enrolled in the first year (2019) at 6 health checkup facilities. **Fig. 1** shows the relationship between baPWV values and the number of atherosclerosis risk factors. It was confirmed that baPWV values increased with an increase in the number of atherosclerosis risk factors in both sexes, which was consistent with our previous results[4,6,7]. **Table 2** shows independent variables for predicting baPWV values by stepwise multiple regression analysis. Age, systolic blood pressure (mmHg), fasting glucose (mg/dL), BMI (kg/m^2), triglyceride (mg/dL), and waist circumference (cm) were selected in both sexes, but LDL-C and smoking habit were not. The correlation between baPWV value and number of atherosclerosis risk factors shown in **Fig. 1** was also better without considering LDL-C and smoking habit. **Table 3** shows the optimal cut-off values of baPWV values for the number of atherosclerosis risk factors in subjects enrolled in 2019 by ROC analysis. The AUC (area under the curve) was greatest in both sexes when the number of atherosclerosis risk factors was differentiated between 0 and 1 to 4, and the optimal cut-off values were 1,401 cm/sec and 1,308 cm/sec, respectively.

Table 4 shows the first and 3rd year characteristics of subjects who underwent Ningen Dock for three consecutive years and measurement of baPWV values. A total of 4,578 subjects, 2,974 males and 1,604 females, underwent Ningen Dock for three consecutive years and measurement of baPWV values. The number of subjects who underwent Ningen Dock and continued baPWV measurement for three consecutive years decreased to 30.7% of the 14,933 enrolled in the first year. The number of atherosclerosis risk factors was unchanged in both sexes. The correlation between the number of atherosclerosis risk factors and baPWV values was similar in subjects at first year enrollment. Three-year baPWV values showed a significant increase every year (males: 1st year 1,542±290 cm/sec, 2nd year 1,561±305 cm/sec, 3rd year 1,586±324 cm/sec; females: 1st year 1,428±274 cm/sec, 2nd year 1,450±290 cm/sec, 3rd year 1,467±301 cm/sec) (**Table 5**).

A total of 4,249 subjects, consisting of 2,769 males and 1,480 females, aged 40 to under 80 during three-year study period, were analyzed for the Suita and Hisayama scores. Suita scores were statistically slightly increased in both males and females; however, the probability of developing coronary artery disease remained unchanged at 2–3% for males and ≤1% for females. On the other hand, baPWV values showed a significant increase as the risk level of the Suita score worsened (**Table 6**). **Table 7** shows the change in Hisayama

Table 1. Background Characteristics of Subjects Enrolled at Six Health Checkup Facilities in 2019

		Male (*n*=9090)	Female (*n*=5843)
Age (years old)		56.8±10.8	55.6±10.7
Height (cm)		169.6±6.3	156.7±5.8
Body weight (kg)		70.1±11.4	55.8±10.3
BMI (kg/m^2)		24.3±3.4	22.7±4.0
Waist circumference (cm)		86.2±9.2	80.6±80.6
Systolic blood pressure (mmHg)		123±14	117±15
Diastolic blood pressure (mmHg)		77±10	71±11
Fasting glucose (mg/dL)		105±19	97±14
HbA1c (%)		5.8±0.7	5.7±0.5
LDL-C (mg/dL)		127±30	128±31
HDL-C (mg/dL)		58±15	70±17
Triglyceride (mg/dL)		128±93	90±55
baPWV (cm/sec)		1537±302	1431±292
Subject numbers of smoking habit (%)		2254 (24.8)	483 (8.3)
Number of atherosclerosis risk factors		1.6±1.1	0.9±1.0
Subjects numbers in each number of atherosclerosis risk factor (%)	0	1765 (19.4)	2650 (45.4)
	1	2580 (28.4)	1781 (30.5)
	2	2636 (29.0)	929 (15.9)
	3	1593 (17.5)	409 (7.0)
	4	516 (5.7)	74 (1.3)

mean ± SD

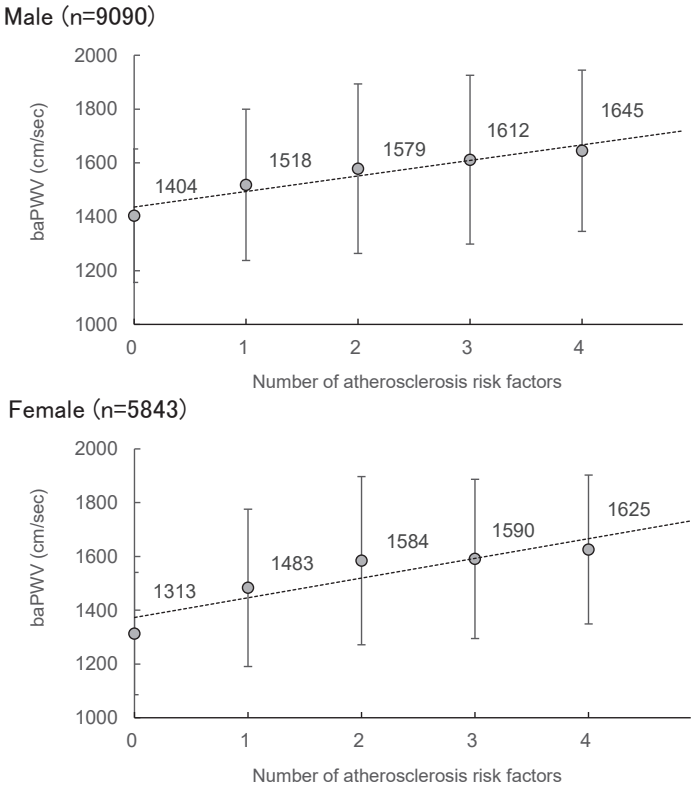

Fig. 1. Relationships Between baPWV Values and the Number of Atherosclerosis Risk Factors in Subjects Enrolled in 2019

Table 2. Independent Variables Selected for Predicting baPWV Values in Subjects Enrolled in 2019 by Stepwise Multiple Regression Analysis

Male

	Standardized coefficients (β)	Unstandardized coefficients	*p* value
Age (years old)	0.461	0.236	<0.001
Systolic blood pressure (mmHg)	0.326	0.254	<0.001
Fasting glucose (mg/dL)	0.093	0.202	<0.001
BMI (kg/m^2)	−0.195	1.566	<0.001
Triglyceride (mg/dL)	0.054	0.026	<0.001
Waist circumference (cm)	0.084	0.582	<0.001
Diastolic blood pressure (mmHg)	0.045	0.349	<0.001
HbA1c (%)	0.034	6.077	0.009

Female

	Standardized coefficients (β)	Unstandardized coefficients	*p* value
Age (years old)	0.500	0.259	<0.001
Systolic blood pressure (mmHg)	0.438	0.254	<0.001
Triglyceride (mg/dL)	0.054	0.053	<0.001
Fasting glucose (mg/dL)	−0.191	1.374	<0.001
BMI (kg/m^2)	0.071	0.196	<0.001
Waist circumference (cm)	0.114	0.530	<0.001
Pulse pressure (mmHg)	−0.063	0.372	<0.001
HDL-C (mg/dL)	−0.047	0.180	<0.001

score over three years. No significant change was seen in males, whereas statistically significant increases in the 2nd and 3rd years compared to the 1st year were seen in females. baPWV values showed a significant increase in both sexes as the risk level worsened for Hisayama score 2, and in males for Hisayama score 1. For Hisayama score 1, baPWV values were significantly higher at high risk due to high scores than at high risk

Table 3. Cut-off Values for baPWV Values in Relation to the Number of Atherosclerosis Risk Factors in Subjects Enrolled in 2019 as Determined by Receiver Operating Characteristic (ROC) Analysis

Male

Numbers of atherosclerosis risk factors (*n*)		Cut-off value (cm/sec) (AUC (95% CI))
0 (1765)	1–4 (7325)	1401 (AUC 0.68 (0.67–0.69))
0–1 (4345)	2–4 (4745)	1467 (AUC 0.63 (0.62–0.64))
0–2 (6981)	3–4 (2109)	1492 (AUC 0.61 (0.60–0.63))
0–3 (8574)	4 (516)	1483 (AUC 0.62 (0.60–0.65))

Female

Numbers of atherosclerosis risk factors (*n*)		Cut-off value (cm/sec) (AUC (95% CI))
0 (2650)	1–4 (3193)	1308 (AUC 0.73 (0.72–0.75))
0–1 (4431)	2–4 (1412)	1386 (AUC 0.71 (0.70–0.73))
0–2 (5360)	3–4 (483)	1397 (AUC 0.69 (0.67–0.72))
0–3 (5769)	4 (74)	1413 (AUC 0.72 (0.68–0.76))

Table 4. Background Characteristics in the First and Third Years of Subjects Who Underwent Ningen Dock for Three Consecutive Years (2019–2021)

		Male (*n*=2974)			Female (*n*=1604)		
		first year	3rd year	*p*	first year	3rd year	*p*
Age (years old)		56.9±10.5	58.9±10.5	*p*<0.001	55.0±10.6	57.0±10.6	*p*<0.001
Height (cm)		169.7±6.2	169.6±6.2	*p*<0.001	157.1±5.8	156.9±5.9	*p*<0.001
Body weight (kg)		69.8±11.1	69.5±11.3	*p*=0.006	56.0±10.4	56.0±10.6	*p*=0.507
BMI (kg/m^2)		24.2±3.4	24.1±3.4	*p*=0.783	22.7±4.0	22.7±4.0	*p*=0.005
Waist circumference (cm)		86.1±9.1	86.2±9.3	*p*<0.001	81.2±10.3	81.5±10.3	*p*<0.001
Systolic blood pressure (mmHg)		122±14	124±14	*p*<0.001	117±15	119±16	*p*<0.001
Diastolic blood pressure (mmHg)		78±10	78±10	*p*<0.001	71±10	73±10	*p*<0.001
Fasting glucose (mg/dL)		106±19	106±19	*p*=0.145	98±14	99±14	*p*=0.004
HbA1c (%)		5.8±0.6	5.8±0.6	*p*=0.824	5.7±0.5	5.7±0.5	*p*=0.087
LDL-C (mg/dL)		126±30	124±30	*p*=0.012	128±31	127±31	*p*=0.065
HDL-C (mg/dL)		58±15	58±15	*p*=0.158	68±16	69±16	*p*=0.085
Triglyceride (mg/dL)		128±94	124±90	*p*=0.008	90±54	90±53	*p*=0.593
baPWV (cm/sec)		1542±290	1586±324	*p*<0.001	1428±274	1467±301	*p*<0.001
Subject number of smoking habit (%)		747 (25.1)	673 (22.6)	*p*=0.053	131 (8.2)	122 (7.6)	*p*=0.615
Number of atherosclerosis risk factors		1.6±1.1	1.7±1.1	*p*=0.149	0.9±1.0	0.9±1.0	*p*=0.160
Subjects numbers in each number of atherosclerosis risk factor (%)	0	568 (19.1)	509 (17.1)	*p*=0.077	745 (46.4)	675 (42.1)	*p*=0.067
	1	848 (28.5)	881 (29.6)	*p*=0.442	477 (29.7)	516 (32.2)	*p*=0.228
	2	863 (28.5)	875 (29.6)	*p*=0.792	246 (15.3)	271 (16.9)	*p*=0.291
	3	520 (17.5)	555 (18.7)	*p*=0.300	114 (7.1)	125 (7.8)	*p*=0.518
	4	175 (5.9)	154 (5.2)	*p*=0.270	22 (1.4)	17 (1.1)	*p*=0.522

mean±SD

due to high-risk disease in males. On the other hand, only one or two females were at high risk due to high scores. High-risk disease subjects in the first year consisted of 700 males (319 diabetes, 308 CKD, 9 PAD, 57 diabetes and CKD, and 7 diabetes and PAD) and 263 females (86 diabetes, 108 CKD, 8 PAD, 7 diabetes and CKD, and 1 diabetes and PAD). Similar trends were seen in the 2nd year and 3rd year.

Discussion

One of the main purposes of Ningen Dock is to prevent the development of atherosclerotic vascular diseases, such as coronary artery disease and cerebrovascular diseases, due to the worsening of lifestyle-related disease. As atherosclerosis advances with age, it is important to conduct examinations that can monitor the progression of atherosclerosis before the onset of atherosclerotic vascular disease.

Table 5. Relationships Between baPWV Values and Number of Atherosclerosis Risk Factors, and Secular Changes in baPWV Values in Subjects Who Underwent Ningen Dock for Three Consecutive Years (2019–2021)

Number of ARFs	1st year		2nd year		3rd year	
	n	baPWV (cm/sec)	*n*	baPWV (cm/sec)	*n*	baPWV (cm/sec)
Male (*n*=2974)						
0	568	1421±244	541	1438±253	509	1442±271
1	848	1523±278	866	1550±305	881	1581±329
2	863	1581±303	856	1590±307	875	1617±319
3	520	1606±291	519	1620±311	555	1650±320
4	175	1647±280	192	1672±305	154	1689±349
mean baPWV (cm/sec)		1542±290		1561±305*		1586±324$^{**,\#}$
mean number of ARFs		1.6±1.1		1.6±1.2		1.7±1.1
Female (*n*=1604)						
0	745	1318±224	707	1324±228	675	1334±235
1	477	1491±271	482	1521±297	516	1544±311
2	246	1539±267	253	1565±285	271	1567±310
3	114	1616±286	138	1604±290	125	1617±270
4	22	1603±347	24	1678±306	17	1668±395
mean baPWV (cm/sec)		1428±274		1450±290*		1467±301**
mean number of ARFs		0.9±1.0		0.9±1.0		0.9±1.0

mean±SD, ARFs: atherosclerosis risk factors
$^{*}p<0.005$ 1st year vs 2nd year, $^{**}p<0.001$ 1st year vs 3rd year, $^{\#}p<0.001$ 2nd year vs 3rd year

Table 6. Relationship Between Risk Classification of Suita Score and baPWV Value, and Secular Changes in Suita Score in Subjects (aged 40–80 During the Three-year Study Period) Who Underwent Ningen Dock for Three Consecutive Years (2019–2021)

Suita score	1st year		2nd year		3rd year	
	n	baPWV (cm/sec)	*n*	baPWV (cm/sec)	*n*	baPWV (cm/sec)
Male (*n*=2769)						
Low risk	841	1372±180	717	1363±172	624	1360±174
Middle risk	1540	1570±255**	1543	1578±271**	1600	1593±279**
High risk	388	1811±310$^{**,\#}$	509	1798±312$^{**,\#}$	545	1837±341$^{**,\#}$
mean score		45.4±9.4		46.7±9.3**		47.4±9.1$^{**,\#}$
Female (*n*=1480)						
Low risk	1126	1363±211	1064	1368±218	1013	1373±212
Middle risk	345	1673±274**	400	1681±287**	447	1694±308**
High risk	9	1738±190**	16	1963±282**,†	20	1878±310**,†
mean score		33.6±9.3		34.9±9.2**		35.7±9.4$^{**,\#}$

mean±SD
$^{**}p<0.001$ Low risk vs Middle risk, Low risk vs High risk, 1st year vs 2nd year, 1st year vs 3rd year
$^{\#}p<0.001$ Middle risk vs High risk, 2nd year vs 3rd year, $^{\dagger}p<0.05$ Middle risk vs High risk

We have frequently and consistently reported on the usefulness of baPWV as a non-invasive, convenient, and highly reproducible measurement of arterial stiffness[3–8]. In this study, we aimed to investigate longitudinal changes in baPWV in subjects who underwent Ningen Dock, across multiple health checkup facilities. While our previous studies already revealed a robust correlation between baPWV values and clustering of atherosclerosis risk factors, this study reaffirmed the same findings. Regarding atherosclerosis risk factors, we considered various conditions such as smoking habits, LDL-C levels, and obesity measures including BMI and waist circumference. After careful evaluation, we identified four factors that demonstrated the strongest correlation with baPWV values: blood pressure, diabetes, triglycerides and HDL-C as lipid abnormalities, and obesity, which includes both BMI and waist circumference (**Fig. 1** and **Table 5**). The factors influenc-

Table 7. Relationship Between Risk Classification of Hisayama Score 1 and 2 and baPWV Value, and Secular Changes in Hisayama Score 2 in Subjects (40–80 Years Old During the Three-year Study Period) Who Underwent Ningen Dock for Three Consecutive Years (2019–2021)

Hisayama score 1

	1st year		2nd year		3rd year	
	n	baPWV (cm/sec)	*n*	baPWV (cm/sec)	*n*	baPWV (cm/sec)
Male (*n*=2769)						
Low risk	425	1340±154	391	1346±173	350	1353±176
Middle risk	1381	1516±237**	1292	1518±245**	1248	1545±276**
High risk	963	1674±314**,#	1086	1693±322**,#	1171	1705±333**,#
Female (*n*=1480)						
Low risk	947	1345±205	923	1358±216	870	1361±209
Middle risk	322	1628±280**	317	1650±294**	348	1673±311**
High risk	211	1565±257**,†	240	1597±297**,†	262	1603±301**,†

Hisayama score 2

	1st year		2nd year		3rd year	
	n	baPWV (cm/sec)	*n*	baPWV (cm/sec)	*n*	baPWV (cm/sec)
Male (*n*=2769)						
Low risk	485	1350±167	442	1355±187	407	1358±177
Middle risk	1859	1540±257**	1822	1543±259**	1790	1563±281**
High risk	425	1783±304**,#	505	1815±319**,#	572	1832±330**,#
mean score		10.7±2.0		10.7±2.0		10.6±1.9
Female (*n*=1480)						
Low risk	1049	1354±206	1034	1366±218	989	1371±214
Middle risk	430	1642±275**	444	1673±292**	489	1691±306**
High risk	1	1847	2	2137	2	1817
mean score		2.6±1.8		2.7±1.9**		2.7±1.9**

mean±SD
** $p<0.001$ Low risk vs Middle risk, Low risk vs High risk, 1st year vs 2nd year, 1st year vs 3rd year
# $p<0.001$ Middle risk vs High risk, † $p<0.05$ Middle risk vs High risk

ing baPWV values in multiple regression analysis were consistent with previous reports[4,5], demonstrating that factors such as smoking habits and LDL-C have minimal impact on baPWV values (**Table 2**). Nevertheless, our recent report shows that the impact of smoking on baPWV values can be detected through the analysis of changes in baPWV values over a 10-year period[8]. Although each atherosclerosis risk factor contributes differently to baPWV values, the robust correlation between the clustering of atherosclerosis risk factors and baPWV values suggests the significance of continuous baPWV measurements in preventive strategies during Ningen Dock.

Furthermore, we also found that baPWV values increased annually even when atherosclerotic disease development risk scores remained unchanged. This emphasizes the importance of continuous baPWV measurements for monitoring atherosclerosis progression in Ningen Dock. Additionally, the area under the curve (AUC) analysis identified cut-off values of baPWV for different numbers of atherosclerosis risk factors. The highest values were observed for subjects with zero or one to four risk factors. For male, the cutoff value was 1,401 cm/sec, and for females, it was 1,308 cm/sec. These values are consistent with the currently accepted normal range of 1,400 cm/sec (**Table 3**)[2].

A notable finding of our study was the annual increase in baPWV values among subjects who underwent baPWV measurements for three consecutive years during Ningen Dock, despite the fact that atherosclerotic disease development prediction scores, such as the Suita score and Hisayama score, remained unchanged (**Table 6, 7**). The Suita score, a risk score used to predict the probability of developing coronary artery disease in a 10-year period by combining atherosclerotic risk factors[11], has been incorporated into the JAS Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017 for urban residents in our country. The Suita score has indicated that the inclusion of CKD in the scoring system enhances the accuracy of coronary artery disease prediction in Japan. On the other hand, the Hisayama score, which assesses absolute risk, including not only coronary artery disease but also atherothrombotic cerebral infarction, was newly

incorporated into the JAS Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2022[13]. The initial report on the Hisayama score assessed risk using the score only[12]. However, in the 2022 version of the guidelines, this was modified to analyze risk classification by score after excluding high-risk diseases such as diabetes. The analysis results revealed a robust correlation between risk score classification and baPWV values, implying that elevated baPWV values are associated with the development of atherosclerotic diseases. In the future, it is expected that a risk score incorporating baPWV values will be developed, further confirming the usefulness of baPWV values.

Conclusion

baPWV values were robustly correlated with the clustering of atherosclerosis risk factors and risk scores for atherosclerosis disease. Furthermore, baPWV values increased annually even when the risk scores remained unchanged, suggesting the importance of continuous baPWV measurements for monitoring the progression of atherosclerosis.

Conflict of Interest

The authors have no conflict of interest to declare.

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References

1. Yamashina A, Tomiyama H, Takeda K, *et al*.: Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002; 25: 359–364.
2. Yamashina A, Tomiyama H, Arai T, *et al*.: Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. Hypertens Res 2003; 26: 615–622.
3. Fukui T, Momoi A, Yasuda T: Usefulness for Measurement of baPWV/ABI in the patients of early stage of type II diabetes. Official Journal of the Japanese Society of Human Dry Dock 2003; 18: 159–162. (in Japanese)
4. Fukui T, Momoi A, Yasuda T: Attention for the interpretation of measurements of brachial-ankle pulse wave velocity. Ningen Dock 2005; 19: 29–32.
5. Fukui T, Momoi A, Yasuda T: Is it possible to detect the influence of cigarette smoking on arterial stiffness by the measurements of brachial-ankle pulse wave velocity? Ningen Dock 2006; 21: 58–62. (in Japanese)
6. Fukui T, Abe Y, Yasuda T, *et al*.: Comparison of the measurements of brachial-ankle pulse wave velocity (baPWV) and cardio-ankle vascular index (CAVI) as an examination for aortic stiffness. Ningen Dock 2008; 23: 70–76. (in Japanese)
7. Fukui T, Yamauchi K, Maruyama M, *et al*.: Ten-year longitudinal study of brachial-ankle pulse wave velocity (baPWV) in middle-aged Japanese males: analysis of relationship with clustering of atherosclerosis risk factors. Ningen Dock International 2015; 2: 70–75.
8. Fukui T, Yamauchi K, Matsumura S, *et al*.: Effects of smoking for 10 years on brachial-ankle velocity (baPWV), a large arterial stiffness index. Ningen Dock 2020; 35: 578–585. (in Japanese)
9. Shirai K, Utino J, Otsuka K, *et al*.: A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). J Atheroscler Thromb 2006; 13: 101–107.
10. Tomiyama H, Ohkuma T, Ninomiya T, *et al*.: Brachial-ankle pulse wave velocity versus its stiffness index β-transformed value as risk marker for cardiovascular disease. J Am Heart Assoc 2019; 8: e013004. doi: 10.1161/JAHA.119.013004
11. Nishimura K, Okamura T, Watanabe M, *et al*.: Predicting coronary heart disease using risk factor categories for a Japanese urban population, and comparison with the Framingham risk score: the Suita study. J Atheroscler Thromb 2014; 21: 784–798.
12. Honda T, Yoshida D, Hata J, *et al*.: Development and validation of modified risk prediction models for cardiovascular disease and its subtypes: the Hisayama Study. Atherosclerosis 2018; 279: 38–44.
13. Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2022. https://www.j-athero.org/jp/wp-content/uploads/publcations/pdf/GL2022_s/jas_gl2022_3_230210.pdf (accessed October 15, 2023)

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HDL Subclass Is Associated with Metabolic Dysfunction-associated Fatty Liver Disease in Japanese

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Abstract

Objective: Recently, metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed as a newly defined disease entity. Although high-density lipoprotein cholesterol (HDL-C) is one of the diagnostic criteria for MAFLD, it is uncertain how HDL subclasses are associated with MAFLD in Japanese.

Methods: Among subjects who underwent a health examination at our hospital, 1,879 subjects (men: 1,238, women: 641) who participated in HDL2-C and HDL3-C evaluation were included in this cross-sectional study. The association between HDL2-C, HDL3-C, HDL2-C/HDL3-C ratio and MAFLD was investigated using multiple logistic regression analysis.

Results: HDL2-C, HDL3-C, and HDL2-C/HDL3-C ratio were significantly lower in MAFLD subjects in men, while HDL2-C and HDL2-C/HDL3-C ratio were significantly lower and HDL3-C was significantly higher in MAFLD subjects in women. Further, as the number of MAFLD diagnostic components increased, all indices decreased in men, while HDL2-C and HDL2-C/HDL3-C ratio decreased as the number of MAFLD diagnostic components increased in women. In multiple logistic regression analysis, HDL2-C and HDL2-C/HDL3-C ratio was negatively associated with MAFLD in both men and women, while HDL3-C was positively associated.

Conclusion: Our results indicate that HDL2-C and the HDL2-C/HDL3-C ratio are negatively associated with MAFLD, while HDL3-C is positively associated with MAFLD in both men and women. The associations between these indices and MAFLD vary between genders and with the number of MAFLD diagnostic components.

Keywords metabolic dysfunction-associated fatty liver disease, HDL2-cholesterol, HDL3-cholesterol, HDL2-cholesterol/HDL3-cholesterol ratio

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease globally[1] and is a primary cause of cirrhosis and liver cancer[2]. Its widespread occurrence can be attributed to the increasing prevalence of sedentary lifestyles, low physical activity, and excessive calorie intake from imbalanced and unhealthy diets[3]. NAFLD is characterized by the presence of fat accumulation in over 5% of hepatocytes, as observed on imaging or histological examination, with no apparent causes such as alcohol consumption, viral hepatitis, genetic liver conditions, or long-term use of steatogenic medications[4]. A definitive diagnosis typically requires a liver biopsy. NAFLD is strongly linked to metabolic syndrome (MetS), which encompasses factors like elevated blood glucose, high blood pressure, abdominal obesity, and dyslipidemia[5]. This condition elevates the risk of cirrhosis and associated complications. Given the increasing prevalence of NAFLD, there is a pressing need for a clearly defined "positive" diagnosis.

Recently, a group of international experts proposed renaming the condition from NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD). The new diagnostic criteria involve evidence of liver fat accumulation along with any of the following three conditions: being overweight or obese, having type 2 diabetes mellitus (T2DM), or showing signs of metabolic dysfunction[6].

High-density lipoprotein (HDL) particles are a heterogeneous population characterized by density, size,

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composition, and surface charge, and they are constantly changing. There are several methods for identifying different types of HDL particles, but one of the most representative methods is separation of the main subfractions of HDL2 and HDL3 using ultracentrifugation[7]. Pre-β HDL, the simplest form of discoidal-shaped HDL, is secreted by the liver and intestine. These particles rapidly take up cholesterol and phospholipids, and thereby convert into larger HDL subclasses. Pre-β HDL undergoes rapid lipidation, resulting in the formation of HDL3. In the maturation process, ingested free cholesterol is esterified by lecithin-cholesterol-acyl transferase (LCAT). HDL3 is associated with anti-oxidative and anti-inflammatory activities, as well as ATP-binding cassette transporter A1 (ABCA1)-cholesterol efflux. On the other hand, HDL2 is involved in ATP-binding cassette subfamily G member 1 (ABCG-1)-mediated cholesterol efflux[8].

We previously demonstrated the importance of analyzing the levels of HDL cholesterol (HDL-C) subclasses, as well as the overall HDL-C levels, when evaluating an individual's lifestyle habits[9]. Both HDL2-C and HDL3-C have been reported to exhibit inverse associations with the incidence of CHD. However, the relative values of these two HDL subclasses as risk predictors remain unknown. Lagos *et al.* recently reported that lower HDL-C levels in patients with MetS resulted from reductions in both the large and small HDL subclasses[10]. As the number MetS components increases, the HDL phenotype includes a greater percentage of small HDL3 and a lesser percentage of large HDL2 molecules, resulting in a lower HDL2/HDL3 ratio. Furthermore, we reported that the HDL2-C/HDL3-C ratio was associated with MetS components, insulin resistance, and high-molecular-weight adiponectin levels and was thus useful for evaluating MetS in Japanese subjects[11]. Because metabolic factors are included in the diagnostic criteria for MAFLD, a relationship between MAFLD and HDL subclasses is anticipated, but this relationship is not well understood.

Visceral obesity is a part of a condition that involves dysfunction in the expansion of subcutaneous fat and the storage of TG in abnormal locations. It is closely associated with the clustering of risk factors related to heart and metabolic health. Some of the metabolic changes closely linked to this condition include high levels of TG, increased availability of free fatty acids, the release of inflammatory substances from fat tissue, insulin resistance and inflammation, higher production and release of very low-density lipoproteins by the liver, slower removal of TG-rich lipoproteins, the presence of small and dense low-density lipoprotein (LDL) particles, and lower levels of HDL-C[12]. A recent study also demonstrated that obesity markedly affects HDL metabolism, composition, and subclass distribution linked to changes in liver and adipose tissue[13].

Given our[9,11] and other reports[10] and noting that HDL-C measurement alone is not satisfactory for risk assessment[14–19], it has become apparent that not only HDL-C, but also HDL subclasses and ratios should be investigated with respect to the coronary heart disease (CHD) risk, obesity, MetS, and lifestyle habits. The diagnosis of MAFLD is based on the presence of fatty liver, and HDL-C is one of the diagnostic criteria for MAFLD. However, the relationship between HDL subclass and MAFLD is not understood at all. Therefore, in this study, we investigated the relationship between HDL subclasses and MAFLD in subjects undergoing annual health check-ups.

Subjects and Methods

Subjects

Of 1,991 subjects (men: 1,323, women: 668) who underwent an annual health examination at the Health Evaluation and Promotion Center of Tokai University Hachioji Hospital between April 2018 and October 2020, 84 men and 27 women were excluded due to incomplete data, leaving 1,879 subjects (men: 1,238, women: 641) for inclusion in this cross-sectional study. Medical history and information on smoking, physical activity, and alcohol intake were obtained using self-administered questionnaires and interviews conducted by nurses.

Verbal consent for the use of anonymized health records was obtained from all study subjects. The study protocol was approved by the Ethics Committee of Tokai University School of Medicine (protocol number 20R-279).

Measurements

Waist circumference (WC) was measured at the level of the umbilicus during slight expiration while in the standing position. Blood pressure (BP) was measured in the upper right arm using an automatic BP monitor device (TM-2655P; A&D, Tokyo, Japan) while the participant was seated. Blood samples were collected in heparin-coated tubes early in the morning after overnight fasting. Fasting plasma glucose (FPG) levels were measured with an L-type Glu 2 kit using the hexokinase/glucose-6-phosphate dehydrogenase method (Wako Pure Chemicals). Fasting immunoreactive insulin (FIRI) levels were measured using a fluorescence enzyme immunoassay (ST AIA-PACK IRI; Toso, Tokyo, Japan). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: FPG (mg/dL) × FIRI (μIU/mL)/405[20]. LDL-C, HDL-C, and TG levels were measured using visible spectrophotometry (Determiner L LDL-C, Determiner L HDL-C, and Determiner L TG II, respectively; Kyowa Medex, Tokyo,

Japan). Uric acid (UA) levels were measured with an L-Type UA.M kit using the uricase-*N*-(3-sulfopropyl)-3-methoxy-5-methylaniline method (Wako Pure Chemicals, Osaka, Japan). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and g-glutamyltranspeptidase (GGT) were measured using the JSCC transferable method with L-Type AST.J2 and L-Type ALT.J2 (Wako Pure Chemicals, Osaka, Japan) and Labofit g-GT (Kanto Chemical, Tokyo, Japan), respectively. Serum high-sensitivity C-reactive protein (hsCRP) levels were measured using latex agglutination turbidimetry.

HDL2-C and HDL3-C levels were determined via ultracentrifugation. Briefly, after plasma was centrifuged using an L-60 centrifuge (Beckman Coulter, Brea, USA) at 22,300×g for 4 h at a plasma density of 1.063 kg/L and solvent density of 1.125 kg/L, adjusted by adding solid KBr, 40% volume from the top was aspirated, yielding HDL (a) and HDL3 (b) fractions. The cholesterol concentration of each fraction was measured, and HDL2-C was calculated as follows: [(a)−(b)×1.54]×0.6[21].

Diagnostic criteria for fatty liver

Fatty liver was diagnosed when any two of the following three ultrasonic criteria were met: liver and kidney echo discrepancy and presence of increased liver echogenicity (bright), unclear intrahepatic duct structure, and liver far-field echo decay[22].

Definition of metabolic dysfunction associated with fatty liver disease

Diagnosis of MAFLD was based on evidence of hepatic steatosis, in addition to one of the following three criteria: overweight/obesity, presence of T2DM, or evidence of metabolic dysregulation[6].

Statistical analyses

Data are expressed as mean±standard deviation or median (interquartile range). Normality was examined using the Kolmogorov–Smirnov test. Student's *t*-test was used to compare mean values between the two groups. The chi square test was used for categorical variables. Bonferroni's multiple comparison test was used to compare mean values across three or more groups. Subjects were classified as nonsmokers or current smokers. High physical activity was defined as participation in any bodily movement equivalent to 3.0 metabolic equivalents (METs) (such as walking on flats at 67 m/min or walking with a dog) for ≥60 min/day. Alcohol consumption was determined per units of sake consumed per day, with one unit (180 mL) considered equivalent to 25 g of alcohol.

A multiple logistic regression analysis to calculate odds ratios (ORs) for quantifying the association of HDL2-C, HDL3-C, HDL2-C/HDL3-C ratio and MAFLD was performed. BMI, WC, systolic BP, diastolic BP, FPG, FIRI, LDL-C, HDL2-C, HDL3-C, TG, and hsCRP were used as independent variables. Another set of the same analysis was performed using HDL2-C/HDL3-C ratio instead of HDL2-C and HDL3-C as independent variables.

All statistical analyses were performed using SAS Studio version 3.4 (SAS Institute, Cary, NC, USA). All *p*-values were two-tailed, and a *p*-value <0.05 was considered statistically significant.

Results

All variables evaluated in this study are shown in **Table 1**, with the data stratified according to sex and the presence of MAFLD. The mean age and BMI of subjects who were diagnosed with MAFLD was 57.1 years and 26.4 kg/m^2 in men, and 61.6 years and 27.3 kg/m^2 in women, respectively. The prevalence of MAFLD was 41.1% in men and 17.2% in women. Most of the variables, except for HDL3-C and hsCRP in men and age in women, were accentuated in MAFLD subjects.

Table 2 (a) shows the prevalence of underlying diseases with medication when stratified by sex and the presence of MAFLD. The prevalence of DM, HT, and dyslipidemia was significantly higher in the MAFLD subjects, while the prevalence of stroke, heart disease, and chronic kidney disease was not statistically different in both men and women.

Table 2 (b) and (c) show alcohol consumption, smoking status and physical activity stratified by sex and the presence of MAFLD. In terms of alcohol consumption, there was no significant difference in the presence of MAFLD among men. Among women, there were more MAFLD cases among those who consumed alcohol at up to 100 g per week, but there was no consistent trend observed for alcohol intake beyond that threshold. The prevalence of current smokers was not significantly higher in the presence of MAFLD in both men and women. The proportion of subjects with one hour or more of daily physical activity was significantly lower among both men and women who met the criteria for MAFLD.

Fig. 1 shows a comparison of HDL2-C, HDL3-C, HDL2-C/HDL3-C ratio and the presence of MAFLD in men. HDL2-C and HDL2-C/HDL3-C ratio were significantly lower in MAFLD subjects. The relationship between the number of MAFLD diagnostic components and HDL2-C, HDL3-C, HDL2-C/HDL3-C ratio was also investigated after subjects were stratified according to the number of MAFLD diagnostic components. All indices decreased as the number of MAFLD diagnostic components increased.

Fig. 2 shows a comparison of HDL2-C, HDL3-C, HDL2-C/HDL3-C ratio and the presence of MAFLD in women. HDL2-C and HDL2-C/HDL3-C ratio

Table 1. Characterization of Study Subjects

	Men		Women	
	MAFLD no (*n*=729)	MAFLD yes (*n*=509)	MAFLD no (*n*=529)	MAFLD yes (*n*=112)
Age	53.9±13.1	57.1±11.9**	59.2±13.5	61.6±9.8
BMI (kg/m^2)	22.8±2.4	26.4±2.8**	21.6±2.7	27.3±3.8**
Waist circumference (cm)	82.3±7.1	91.9±7.8**	78.8±8.8	93.0±9.0**
Systolic BP (mmHg)	125.7±17.0	131.8±17.3**	122.6±19.7	137.0±17.9**
Diastolic BP (mmHg)	78.5±11.9	83.1±12.5**	72.9±12.9	80.0±12.0**
FPG (mg/dL)	100.1±15.5	108.1±21.9**	95.8±13.7	109.5±24.6**
FIRI (μIU/mL)	4.3 [3.00,6.00]	7.70 [5.50,11.40]**	4.50 [3.40,6.30]	10.30 [7.35,14.0]**
HOMA-IR	1.10 [0.70,1.50]	2.00 [1.40,3.00]**	1.10 [0.80,1.50]	2.65 [1.90,3.95]**
HbA1c (NGSP)	5.6±0.5	5.9±0.7**	5.6±0.4	6.0±0.7**
TG (mg/dL)	87.0 [64.0,118.0]	123.0 [91.0,181.0]**	74.0 [55.0,100.0]	115.0 [87.0,161.0]**
HDL-C (mg/dL)	64.3±15.6	53.7±12.3**	77.8±17.1	62.7±14.2**
HDL2-C (mg/dL)	38.4±13.3	29.5±9.7**	51.0±15.8	36.8±12.5**
HDL3-C (mg/dL)	22.5±4.0	22.1±3.7	21.7±3.9	23.1±3.8**
HDL2-C/HDL3-C	1.74±0.66	1.34±0.42**	2.42±0.85	1.61±0.55**
LDL-C (mg/dL)	120.9±29.5	125.2±29.3*	124.1±29.3	137.2±35.7*
Non-HDL-C (mg/dL)	137.5±32.2	148.3±33.1**	137.7±32.3	158.8±38.5**
AST (U/L)	22.5±7.1	27.8±14.2**	21.3±6.4	25.2±10.2**
ALT (U/L)	21.3±9.7	35.6±25.3**	17.3±11.8	29.9±19.5**
GGT (U/L)	26.0 [19.0,40.0]	38.0 [27.0,62.0]**	18.0 [13.0,25.0]	27.0 [21.0,40.5]**
UA (mg/dL)	5.9±1.1	6.3±1.3**	4.7±1.0	5.3±1.1**
PLT	23.4±5.8	24.2±5.3*	24.4±5.9	26.0±6.0**
hsCRP (mg/dL)	0.04 [0.02,0.07]	0.07 [0.04,0.13]	0.03 [0.02,0.07]	0.11 [0.06,0.18]**
No. of MAFLD	1.4±1.2	4.4±1.4**	1.1±1.3	4.9±1.4**

Variables are expressed as mean±standard deviation or median [interquartile range]. MAFLD, metabolic dysfunction-associated fatty liver disease; BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; FIRI, fasting immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; HDL2-C/HDL3-C, high-density lipoprotein 2 cholesterol/ high-density lipoprotein 3 cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non high-density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; UA, uric acid; PLT, platelet; hsCRP, high-sensitivity C-reactive protein

*$p<0.05$, **$p<0.01$ by paired *t*-test.

were significantly lower and HDL3-C was significantly higher in MAFLD subjects. HDL2-C and HDL2-C/HDL3-C ratio decreased as the number of MAFLD diagnostic components increased.

Determinants of MAFLD were analyzed by multiple logistic regression analysis (**Table 3**). BMI, WC, systolic BP, diastolic BP, FPG, FIRI, LDL-C, HDL2-C, HDL3-C, TG, and hsCRP were used as independent variables in **Table 3** (a). Five variables (BMI, FPG, TG, HDL2-C, and HDL3-C) were selected by a stepwise procedure in men. Six variables (BMI, SBP, FPG, TG, HDL2-C, and HDL3-C) were selected by a stepwise procedure in women. BMI had a positive and significant association with MAFLD in both men and women. HDL2-C had a negative and HDL3-C had a positive association with MAFLD in both men and women.

BMI, WC, systolic BP, diastolic BP, FPG, FIRI, LDL-C, HDL2-C/HDL3-C ratio, TG, and hsCRP were used as independent variables in **Table 3** (b). Four variables (BMI, FPG, TG, HDL2-C/HDL3-C ratio) were selected by a stepwise procedure in men. Five variables (BMI, SBP, FPG, LDL-C, HDL2-C/HDL3-C ratio) were selected by a stepwise procedure in women. BMI had a positive and strong association with MAFLD in both men and women. HDL2-C/HDL3-C ratio had a strong negative association with MAFLD in both men and women.

Discussion

We showed that in MAFLD subjects, HDL2-C and HDL2-C/HDL3-C ratio were lower in both men and women, while HDL3-C was higher in women. As the number of MAFLD diagnostic components increased, all indices decreased in men, and in women, HDL2-C and HDL2-C/HDL3-C ratio decreased. Multiple logistic regression analysis showed a negative association of HDL2-C and HDL2-C/HDL3-C ratio, and a positive association of HDL3-C with MAFLD in both genders.

In this study, differences in HDL3-C were observed between men and women. In both genders, HDL2-C and the HDL2-C/HDL3-C ratio showed a decreasing trend as the number of cases meeting the diagnostic criteria for MAFLD increased. In men, there was no difference in HDL3-C regardless of whether they met the criteria for MAFLD or not; however, as the number of cases meeting the diagnostic criteria for MAFLD increased, there was a tendency for HDL3-C to decrease. Our results in women may contradict a previous report which suggested a negative association between HDL3 and the status of carotid artery

Table 2. Background of Study Subjects

(a) Diseases under treatment

MAFLD	Men			Women		
	No	Yes	*p*-value	No	Yes	*p*-value
DM	46 (6.3%)	57 (11.2%)	0.00324	13 (2.5%)	10 (8.8%)	0.00217
HT	190 (26.1%)	186 (36.5%)	<0.0001	89 (16.8%)	38 (33.9%)	<0.0001
Dyslipidemia	119 (16.3%)	140 (27.5%)	<0.0001	98 (18.5%)	37 (33.0%)	<0.0001
Stroke	26 (3.6%)	20 (3.9%)	0.8614	15 (2.8%)	3 (2.7%)	1.0000
Heart disease	60 (8.2%)	36 (7.1%)	0.7617	21 (4.0%)	8 (7.1%)	0.2234
Chronic kidney disease	7 (1.0%)	4 (0.8%)	0.5168	3 (0.6%)	0 (0.0%)	0.9706

MAFLD, metabolic dysfunction associated fatty liver disease; DM, diabetes mellitus; HT, hypertension
p-value by Chi-squared test.

(b) Alcohol

Ethanol (g/w)|MAFLD	Men			Women		
	No	Yes	*p*-value	No	Yes	*p*-value
0	315 (43.2%)	216 (42.4%)	0.83670	394 (74.5%)	92 (82.1%)	0.02271
0–100	103 (14.1%)	73 (14.3%)		44 (8.3%)	10 (8.9%)	
100–200	115 (15.8%)	78 (15.3%)		53 (10.0%)	2 (1.8%)	
200–300	127 (17.4%)	95 (18.7%)		30 (5.7%)	5 (4.5%)	
300–400	19 (2.6%)	8 (1.6%)		0 (0.0%)	1 (0.9%)	
400–	50 (6.9%)	39 (7.7%)		8 (1.5%)	2 (1.8%)	

MAFLD, metabolic dysfunction associated fatty liver disease
p-value by Chi-squared test.

(c) Smoking status and physical activity

MAFLD	Men			Women		
	No	Yes	*p*-value	No	Yes	*p*-value
Current smoker	140 (19.2%)	125 (24.6%)	0.0245	40 (7.6%)	11 (9.8%)	0.442
Physical activity of one hour or more a day	334 (45.8%)	187 (36.7%)	0.0015	251 (47.4%)	36 (32.1%)	0.0031

MAFLD, metabolic dysfunction associated fatty liver disease
p-value by Chi-squared test.

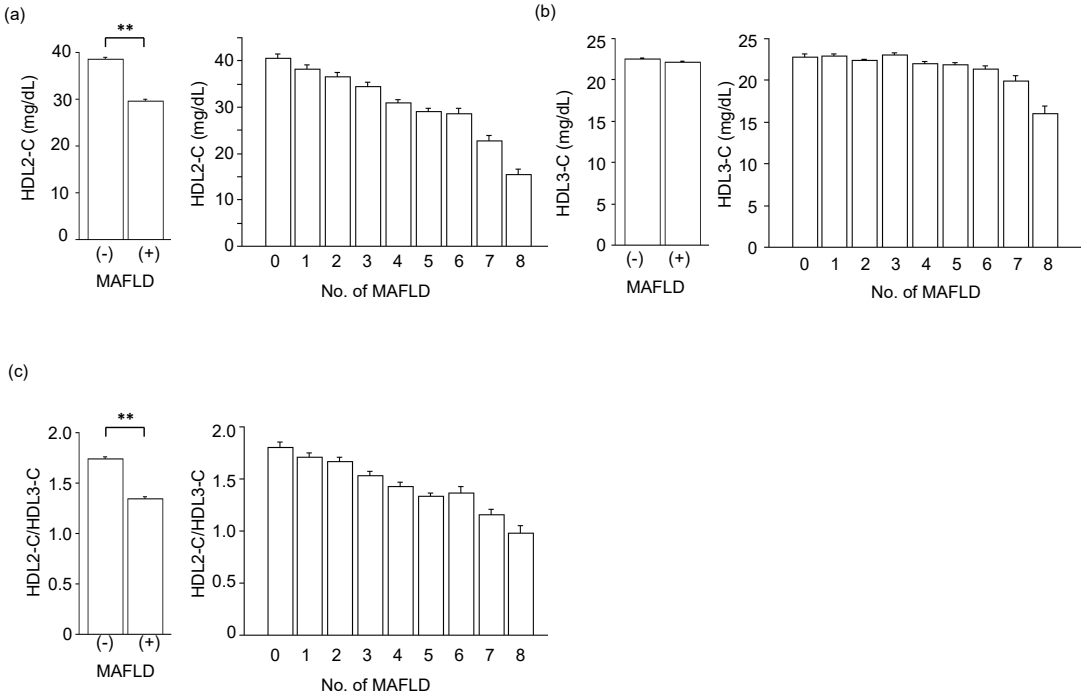

Fig. 1. Bar Graph of Mean HDL2-C (a), HDL3-C (b), and HDL2-C/HDL3-C Ratio in Men after Stratifying Subjects According to the Presence of MAFLD or Number of MAFLD Diagnostic Components

HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; MAFLD, metabolic dysfunction-associated fatty liver disease

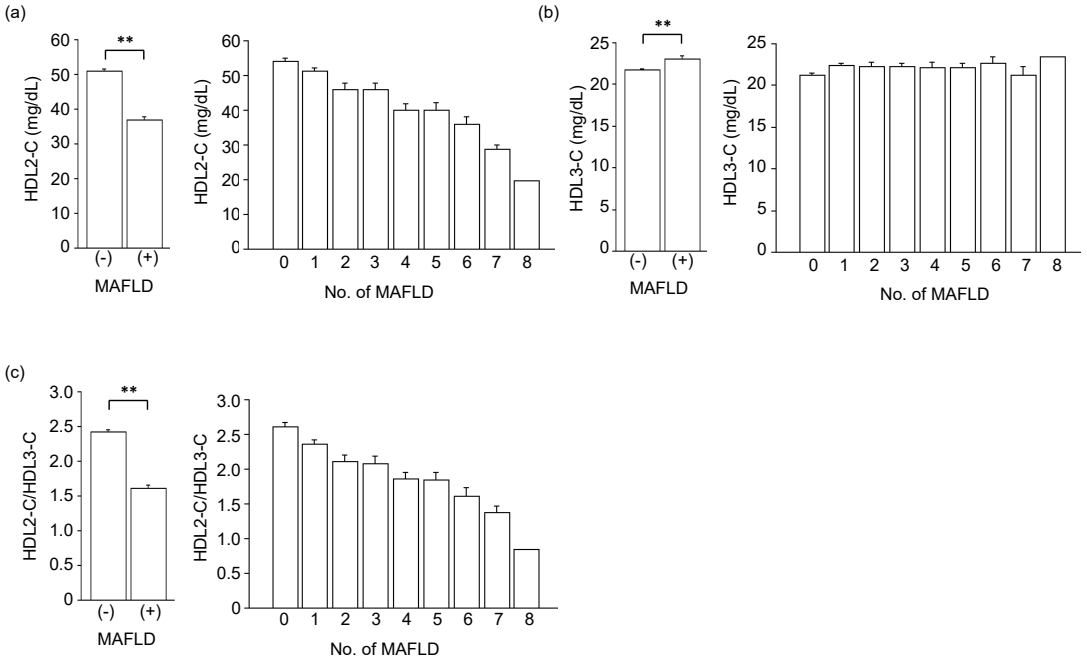

Fig. 2. Bar Graph of Mean HDL2-C (a), HDL3-C (b), and HDL2-C/HDL3-C Ratio in Women after Stratifying Subjects According to the Presence of MAFLD or Number of MAFLD Diagnostic Components

HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; MAFLD, metabolic dysfunction-associated fatty liver disease

Table 3. Multiple Logistic Regression Analysis for MAFLD

(a)

	Men					Women				
	RC	SE	OR	95%CI	*p*	RC	SE	OR	95%CI	*p*
BMI	0.5634	1.2544	1.757	1.629–1.894	<0.0001	0.5653	0.0651	1.760	1.549–1.999	<0.0001
SBP	Not selected					0.0216	0.00859	1.022	1.005–1.039	<0.0001
FPG	0.0283	0.0385	1.029	1.019–1.038	<0.0001	0.0859	0.00869	1.024	1.007–1.042	0.0121
TG	0.00249	0.00464	1.002	1.000–1.005	0.0201	0.0079	0.0284	1.008	1.002–1.014	0.0057
HDL2-C	−0.0551	0.00107	0.946	0.931–0.962	<0.0001	−0.043	0.0136	0.957	0.932–0.983	0.0055
HDL3-C	0.0779	0.00842	1.081	1.037–1.127	0.0002	0.2054	0.0433	1.228	1.128–1.337	<0.0001

Variable selection was made by a stepwise procedure. BMI, body mass index; SBP, systolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; HDL2-C/HDL3-C, high-density lipoprotein 2 cholesterol/high-density lipoprotein 3 cholesterol; RC, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval

Independent variables were BMI, waist circumference, systolic and diastolic blood pressure, FPG, fasting immunoreactive insulin, low-density lipoprotein cholesterol (LDL-C), TG, HDL2-C, HDL3-C, and high-sensitivity C-reactive protein.

(b)

	Men					Women				
	RC	SE	OR	95%CI	*p*	RC	SE	OR	95%CI	*p*
BMI	0.5667	0.0386	1.762	1.634–1.901	<0.0001	0.5482	0.0630	1.730	1.529–1.958	<0.0001
SBP	Not selected					0.0205	0.00854	1.021	1.004–1.038	0.0163
FPG	0.0287	0.00467	1.029	1.020–1.039	<0.0001	0.0258	0.00871	1.026	1.009–1.044	0.0030
TG	0.00239	0.00104	1.002	1.000–1.004	0.0210	Not selected				
LDL-C	Not selected					0.0115	0.00468	1.012	1.002–1.021	0.0137
HDL2-C /HDL3-C	−1.2039	0.1758	0.300	0.213–0.423	<0.0001	−1.3351	0.281	0.263	0.152–0.456	<0.0001

Variable selection was made by a stepwise procedure. BMI, body mass index; SBP, systolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL2-C, high-density lipoprotein 2 cholesterol; HDL3-C, high-density lipoprotein 3 cholesterol; HDL2-C/HDL3-C, high-density lipoprotein 2 cholesterol/high-density lipoprotein 3 cholesterol; RC, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval

Independent variables were BMI, waist circumference, systolic and diastolic blood pressure, FPG, fasting immunoreactive insulin, LDL-C, TG, HDL2-C/HDL3-C ratio, and high-sensitivity C-reactive protein.

disease, but no association for HDL2, HDL-C, or Apo A-I levels[19]. In women, the average value of HDL3-C was significantly higher in MAFLD cases compared to non-MAFLD cases, but there was no observed decrease in HDL3-C with an increase in the number of cases meeting the diagnostic criteria for MAFLD. The reason for this gender difference is not clear. Previously, in our analysis of the relationship between HDL subclasses and lifestyle habits, we reported that HDL2-C increases with exercise and alcohol consumption but decreases with smoking[9]. On the other hand, we found that HDL3-C increases only with alcohol consumption. Alcohol intake levels did not show a significant difference between subjects with or without MAFLD in men, and a large proportion of women did not consume alcohol, potentially indicating that alcohol consumption alone may not explain the observed differences. On comparison with the previous report, we suggest that HDL3-C level is lower in Japanese than Caucasans[19], suggesting significant racial variations. Further research specifically targeting Japanese subjects is necessary.

Obesity often comes with decreased levels of HDL-C and an increase in TG-rich lipoproteins[23], a condition commonly referred to as atherogenic dyslipidemia. A key feature of this dyslipidemia is reduced clearance of TG-rich lipoproteins due to a relative deficiency of insulin-sensitive lipoprotein lipase[24–26]. The rise in TG-rich lipoproteins is a contributing factor to the low HDL-C levels observed in obesity. In individuals who are obese and insulin-resistant, HDL becomes enriched with TG, and there is an increase in hepatic lipase activity[27–29]. This results in the breakdown of triglyceride-rich HDL into smaller HDL3 particles, which are more susceptible to rapid catabolism[30].

While the extent of obesity and the distribution of visceral fat may vary between Japanese and Westerners, there have been reports indicating an inverse correlation between HDL3 and various events[19,31–33]. Interestingly, in patients undergoing blood dialysis, the distribution of HDL2b and HDL3 subclasses is associated with macrovascular events, and a proinflammatory state has been shown to influence this subclass distribution[32]. The relationship between proinflammatory states and insulin resistance is well known[34]. In this study, hsCRP, an indicator of inflammation, and HOMA-IR, reflecting insulin resistance, were elevated in both male and female participants with MAFLD. However, the average values of HDL3-C and the relationship between MAFLD status and HDL3-C level did not exhibit a clear trend in males as compared to females. Therefore, this phenomenon may not be solely explained by a pro-inflammatory state.

The major limitation of this study was its cross-sectional design, as it prevented the assessment of causality between HDL2-C, HDL3-C, HDL2-C/HDL3-C ratio and MAFLD. Fatty liver was diagnosed using ultrasonography alone. Additional imaging, such as CT and MRI, might be needed for a more accurate diagnosis of fatty liver. All subjects in this study were middle-aged Japanese individuals; thus, the effects of ethnicity on the relationship between HDL2-C, HDL3-C, HDL2-C/HDL3-C and MAFLD were not assessed. Finally, our dataset was not large; therefore, our findings may not be generalizable to all Japanese individuals.

Conclusion

Our results indicated that HDL2-C and the HDL2-C/HDL3-C ratio are negatively associated with MAFLD, while HDL3-C is positively associated with MAFLD in both men and women. The associations between these indices and MAFLD vary between genders and with the number of MAFLD diagnostic components. The concentration of HDL-C is included in the diagnostic criteria for MAFLD, but the addition of HDL2-C and HDL3-C analysis may allow a more detailed evaluation of MAFLD.

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Conflicts of Interest

There are no conflicts of interest.

References

1. Younossi Z, Anstee QM, Marietti M, *et al.*: Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018; 15: 11–20.
2. Yki-Järvinen H: Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014; 2: 901–910.
3. Inoue Y, Qin B, Poti J, *et al.*: Epidemiology of obesity in adults: latest trends. Curr Obes Rep 2018; 7: 276–288.
4. Chalasani N, Younossi Z, Lavine JE, *et al.*: The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018; 67: 328–357.
5. Huang TD, Behary J, Zekry A: Non-alcoholic fatty liver disease: A review of epidemiology, risk factors, diagnosis and management. Intern Med J 2020; 50: 1038–1047.
6. Eslam M, Newsome PN, Sarin SK, *et al.*: A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol 2020; 73: 202–209.
7. Glomset JA: The plasma lecithins:cholesterol acyltransferase reaction. J Lipid Res 1968; 9: 155-167.
8. Stadler JT, Marsche G: Obesity-related changes in high-density lipoprotein metabolism and function. Int J Mol Sci 2020; 21: 8985.

9. Moriyama K, Takahashi E: Relationships of high-density lipoprotein 2 and 3 cholesterols with lifestyle habit factors in Japanese adults. Ningen Dock International 2014; 1: 54–62.
10. Lagos KG, Filippatos TD, Tsimihodimos V, *et al*.: Alterations in the high density lipoprotein phenotype and HDL-associated enzymes in subjects with metabolic syndrome. Lipids 2009; 44: 9–16.
11. Moriyama K, Negami M, Takahashi E: HDL2-cholesterol/HDL3-cholesterol ratio was associated with insulin resistance, high-molecular-weight adiponectin, and components for metabolic syndrome in Japanese. Diabetes Res Clin Pract 2014; 106: 360–365.
12. Tchernof A, Després JP: Pathophysiology of human visceral obesity: an update. Physiol Rev 2013; 93: 359–404.
13. Stadler JT, Lackner S, Mörkl S, *et al*.: Obesity affects HDL metabolism, composition and subclass distribution. Biomedicines 2021; 9: 242.
14. Castelli WP: Cardiovascular disease and multifactorial risk: challenge of the 1980s. Am Heart J 1983; 106: 1191–1200.
15. Barter PJ, Caulfield M, Eriksson M, *et al*.: Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007; 357: 2109–2122.
16. Boden WE, Probstfield JL, Anderson T, *et al*.; AIM-HIGH Investigators: Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med 2011; 365: 2255–2267.
17. Voight BF, Peloso GM, Orho-Melander M, *et al*.: Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet 2012; 380: 572–580.
18. Rohatgi A, Khera A, Berry JD, *et al*.: HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 2014; 371: 2383–2393.
19. Kim DS, Burt AA, Rosenthal EA, *et al*.: HDL-3 is a superior predictor of carotid artery disease in a case-control cohort of 1725 participants. J Am Heart Assoc 2014; 3: e000902.
20. Matthews DR, Hosker JP, Rudenski AS, *et al*.: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419.
21. Bronzert TJ, Brewer HB Jr: New micromethod for measuring cholesterol in plasma lipoprotein fractions. Clin Chem 1977; 23: 2089–2098.
22. Farrell GC, Chitturi S, Lau GK, *et al*.: Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol 2007; 22: 775–777.
23. Ashen MD, Blumenthal RS: Clinical practice. Low HDL cholesterol levels. N Engl J Med 2005; 353: 1252–1260.
24. Clemente-Postigo M, Queipo-Ortuño MI, Fernandez-Garcia D, *et al*.: Adipose tissue gene expression of factors related to lipid processing in obesity. PLoS ONE 2011; 6: e24783.
25. Walton RG, Zhu B, Unal R, *et al*.: Increasing adipocyte lipoprotein lipase improves glucose metabolism in high fat diet-induced obesity. J Biol Chem 2015; 290: 11547–11556.
26. Klop B, Elte JWF, Cabezas MC: Dyslipidemia in obesity: mechanisms and potential targets. Nutrients 2013; 5: 1218–1240.
27. Carr MC, Hokanson JE, Zambon A, *et al*.: The contribution of intraabdominal fat to gender differences in hepatic lipase activity and low/high density lipoprotein heterogeneity. J Clin Endocrinol Metab 2001; 86: 2831–2837.
28. Chatterjee C, Sparks DL: Hepatic lipase, high density lipoproteins, and hypertriglyceridemia. Am J Pathol 2011; 178: 1429–1433.
29. Blades B, Vega GL, Grundy SM: Activities of lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma of patients with low concentrations of HDL cholesterol. Arterioscler Thromb 1993; 13: 1227–1235.
30. Rashid S, Barrett PHR, Uffelman KD, *et al*.: Lipolytically modified triglyceride-enriched HDLs are rapidly cleared from the circulation. Arterioscler Thromb Vasc Biol 2002; 22: 483–487.
31. Degoricija V, Potočnjak I, Gastrager M, *et al*.: HDL subclasses and mortality in acute heart failure patients. Clin Chim Acta 2019; 490: 81–87.
32. Lee WC, Chen JB, Moi SH, *et al*.: Association of proportion of the HDL-cholesterol subclasses HDL-2b and HDL-3 and macrovascular events among patients undergoing hemodialysis. Sci Rep 2021; 11: 1871.
33. Martin SS, Khokhar AA, May HT, *et al*.: HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative. Eur Heart J 2015; 36: 22–30.
34. Grimble RF: Inflammatory status and insulin resistance. Curr Opin Clin Nutr Metab Care 2002; 5: 551–559.

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Case Report

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Incidental Detection of a Papillary Fibroelastoma During a Health Check-up

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Abstract

An asymptomatic 69-year-old woman exhibited slight ST-segment depression in the inferior leads on an electrocardiogram during a health check-up. Echocardiography revealed a small mobile tumor originating from the anterolateral papillary muscle of the left ventricle. As brain computed tomography revealed a cerebral infarction, urgent surgery was performed. The resected tumor was histologically diagnosed as a papillary fibroelastoma (PFE). The postoperative course was uneventful with no tumor recurrence two years post-surgery. It is extremely rare to find PFE during a health check-up. We emphasize that even minor abnormal findings during health check-ups should be monitored vigilantly.

Keywords papillary fibroelastoma, health check-up, transesophageal echocardiography

Primary cardiac tumors are rare, with an incidence of 0.02%[1], and approximately three-quarters are benign[2]. Papillary fibroelastoma (PFE) is the third-most common primary cardiac tumor, accounting for 8% of all cases of primary cardiac tumor following myxoma and lipoma[3]. PFE is known to cause embolic complications such as stroke or myocardial infarction[4], and commonly originates from the heart valves[5]. Herein, we report a surgical case of PFE arising from the anterolateral papillary muscle of the left ventricle that was incidentally detected during a health check-up.

Case Report

A 69-year-old woman presented to our hospital for a health check-up. The patient was in her usual state of good health. Physical examination revealed a blood pressure of 144/76 mmHg and a heart rate of 75 beats per minute with sinus rhythm. The electrocardiogram (ECG) showed slight ST-segment depressions in leads II, III, and aVF (**Fig. 1**). Transthoracic echocardiography (TTE), which was performed based on minor abnormal ECG findings, revealed a highly echoic mobile mass arising from the anterolateral papillary muscle (ALPM) of the left ventricle (**Fig. 2**). Left ventricular ejection fraction was 81% and no asynergy of the left ventricle was detected. The differential diagnosis of the mass included cardiac tumors, thrombi, or vegetation. Since the mass was highly mobile, thrombus formation was unlikely to have occurred. Because she was afebrile and there was no evidence of infection, vegetation was unlikely. Transesophageal echocardiography

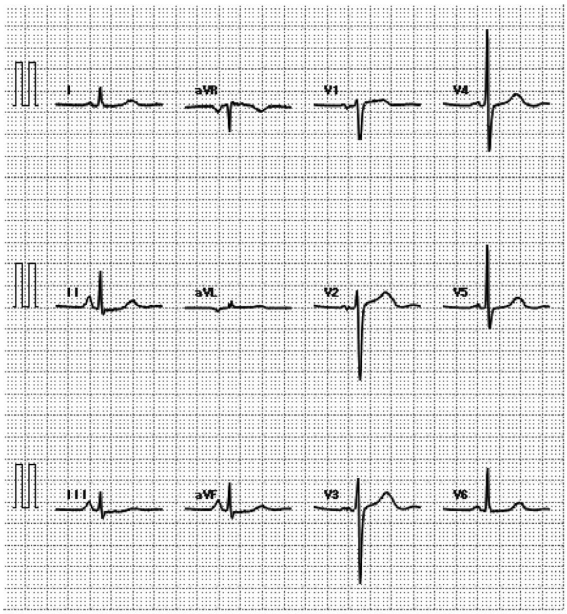

Fig. 1. Electrocardiogram Showing Slight ST-segment Depression in Leads II, III, aVF

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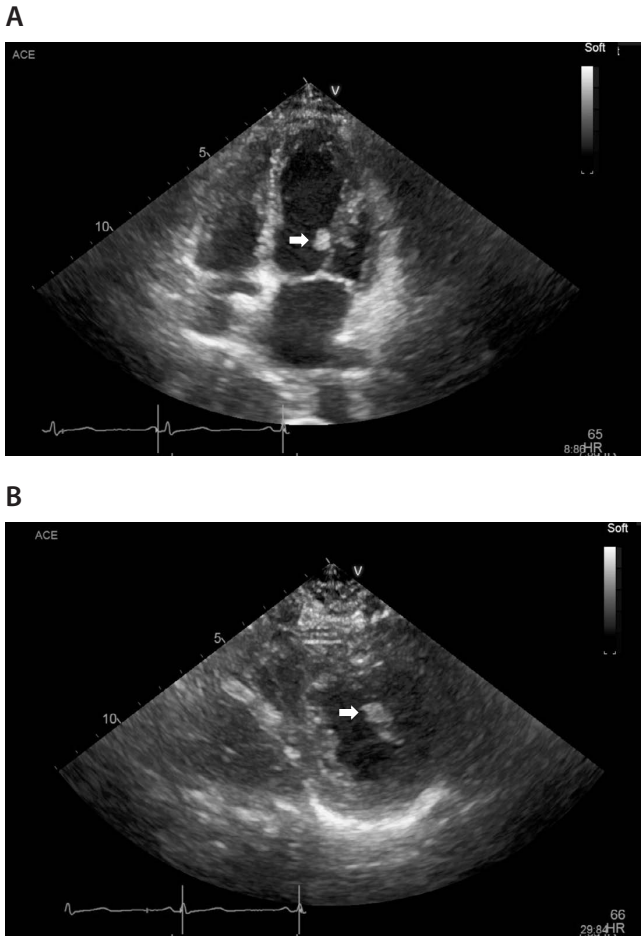

Fig. 2. Transthoracic Echocardiogram Showing a Hyperechoic Mass (Arrow) Arising from the Head of the Anterolateral Papillary Muscle

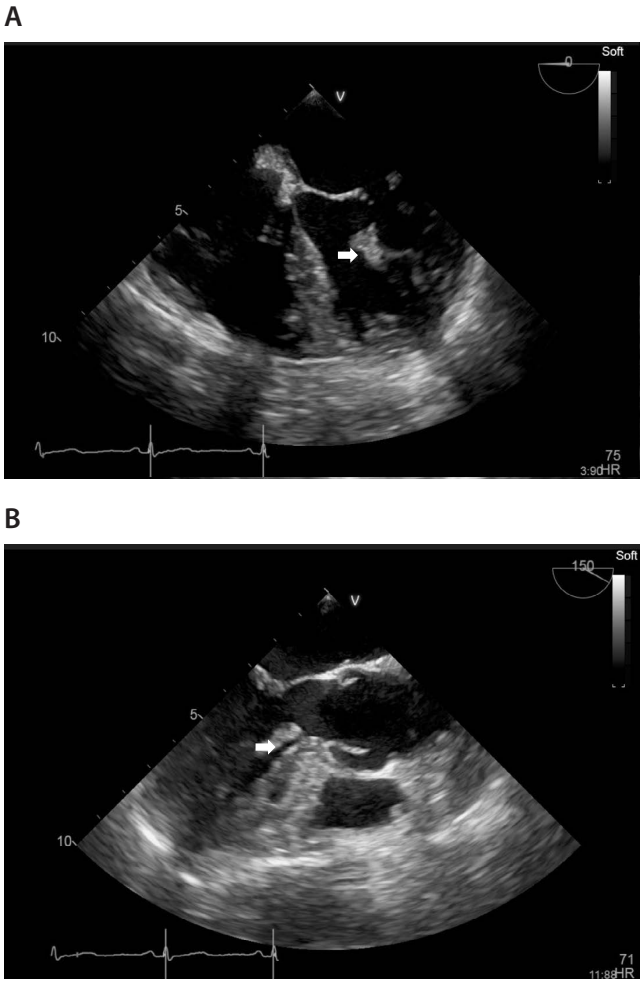

Fig. 3. Transesophageal Echocardiogram Showing a Stretched Mass (Arrow) Located in the Left Ventricular Outflow Tract During Systole

(TEE) was performed for further examination. TEE results also revealed a mobile and stretched mass, 12×6 mm in size, adhering to the head of the ALPM. The mass had moved from under the mitral valve to the left ventricular outflow tract (LVOT) (**Fig. 3**). Based on the appearance of the mass on TTE and TEE, PFE was the most likely diagnosis. Cardiac computed tomography (CT) revealed no significant coronary artery stenosis. Head CT demonstrated a low-density area suggestive of an old cerebral infarction at the border of the left parietal and occipital lobes (**Fig. 4**). Despite the absence of symptoms, the mass was urgently removed to avoid further embolization.

The operation was performed through a median sternotomy, and cardiopulmonary bypass was established via aorto-bicaval cannulation. After cardioplegic cardiac arrest was achieved, the left atrium was opened using a transseptal approach. Inspection of the left ventricle through the mitral valve revealed a jelly-like tumor with multiple fronds originating from the head of the ALPM (**Fig. 5**). The tumor was completely excised with surgical margins. When placed in saline, the excised tumor had a sea anemone appearance with multiple fronds, consistent with PFE (**Fig. 6**). PFE was confirmed via histopathological analysis (**Fig. 7**). The postoperative course was uneventful. No recurrence of PFE was observed two years after surgery.

Discussion

PFE is the third-most common benign cardiac tumor and usually originates from the heart valve tissue. When a left-heart PFE is found, early surgery is recommended because left-heart PFE frequently presents with embolic neurological symptoms. Ngaage *et al.* advocated that since PFE is frequently located in the high-flow and high-pressure LVOT of the heart, the risk of embolism is comparatively high[5]. In the present case, the tumor moved back and forth from beneath the mitral valve to the LVOT, and preoperative head CT revealed cerebral infarction, probably due to embolization of the PFE. PFE has a high embolic risk, given the friability of its tissue matrix[6].

TTE is a simple, convenient, and non-invasive tool

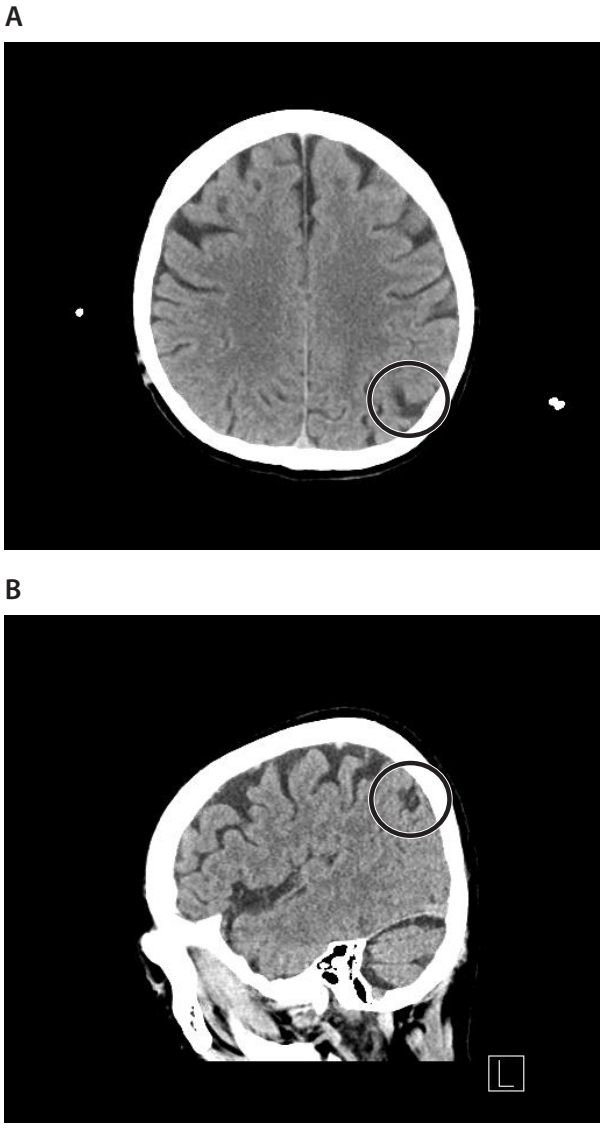

Fig. 4. Head Computed Tomography Showing a Low Density Area (Circle) Suggestive of Old Infarction at the Border of the Left Parietal Lobe and Occipital Lobe

for investigating abnormal findings. Although TEE is difficult to perform, it is useful for obtaining detailed information. A case-control study reported that the sensitivity and specificity of TTE were 88.9% and 87.8%, with an overall accuracy of 88.4% for the detection of PFEs >0.2 cm. When PFEs <0.2 cm were included, the overall sensitivity of TTE was 61.9% and that of TEE was 76.6%[7]. Therefore, TEE is recommended for differentiating cardiac tumors, especially small tumors.

Approximately 85% of PFEs arise from heart valves in the following order of frequency: aortic, mitral, tricuspid, and pulmonary valves. The remaining 15% of tumors originate from nonvalvular sites, commonly left-sided endocardial surfaces[8]. PFE arising from the papillary muscles of the left ventricle are rare. Tamaru *et al.* reviewed nine such cases and insisted that PFEs located on the papillary muscle tend to cause thrombosis compared with those located at other sites[9].

There are only a few reports on the diagnosis of cardiac tumors during health check-ups[10,11]. In our case, minor ST-segment depression of the ECG during a health check-up triggered the discovery of PFE and led to successful tumor resection. Since cardiac CT demonstrated no significant stenosis of the coronary arteries, the mechanism of ST segment depression is unknown in the present case. ST-segment elevation has been reported to occur due to myocardial invasion in patients with metastatic cardiac tumors[12,13]. An important aspect of health check-ups is to avoid overlooking hidden diseases. Therefore, secondary inspections should be performed proactively, even if abnormal findings appear less significant in health check-ups.

Conflict of Interest

No financial support was received for this study from any specific company.

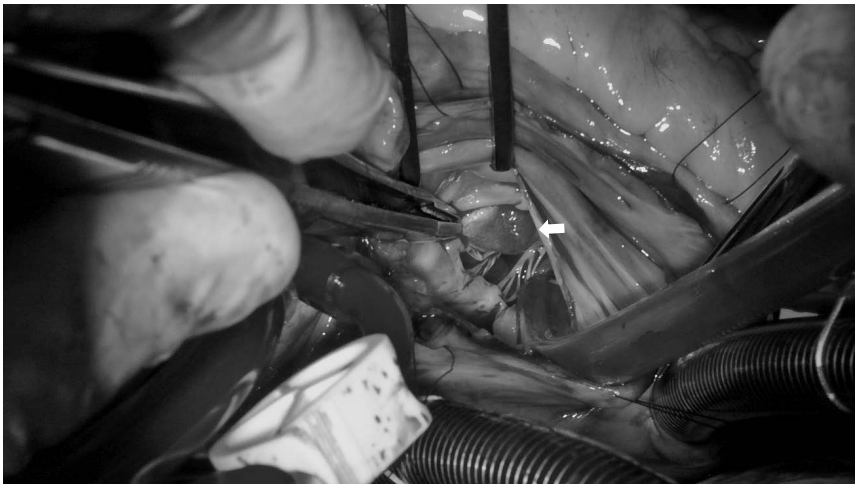

Fig. 5. Surgeon's View: A Jelly-like Tumor with Multiple Fronds (Arrow) Originating from the Head of the Anterolateral Papillary Muscle

Fig. 6. Macroscopic Image of the Formalin-fixed Tumor Immersed in Water Demonstrates a Sea Anemone-like Appearance with Complex Branching of the Multiple Fronds

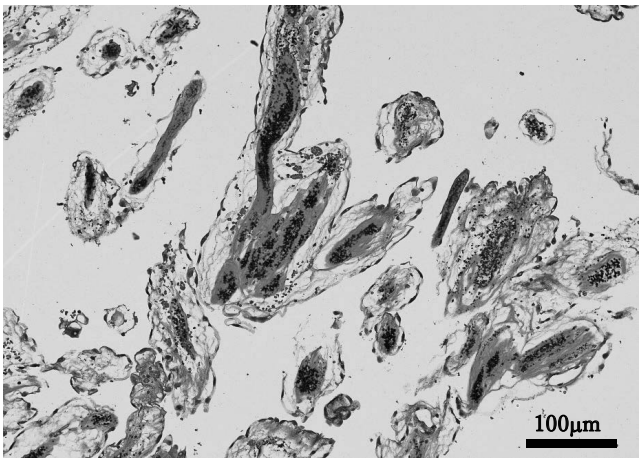

Fig. 7. Histopathological Analysis of the Tumor with Elastica van Gieson Stain Shows Avascular Fronds Lined by Endothelial Cells, of Which Central Stalks Are Composed of Fibroelastic Tissue

Disclosure

The authors have no potential conflict of interest to disclose.

Ethical Consensus and Patient Consent Statement

Written informed consent was obtained from the patient and her family for the publication of this case report and accompanying images.

References

1. Reynen K: Frequency of primary tumors of the heart. Am J Cardiol 1996; 77: 107.
2. Majano-Lainez RA: Cardiac tumors: a current clinical and pathological perspective. Crit Rev Oncog 1997; 8: 293–303.
3. Ragland MM, Tak T: The role of echocardiography in diagnosing space-occupying lesions of the heart. Clin Med Res 2006; 4: 22–32.
4. Oda T, Aoyagi S, Yoshida S, *et al.*: A surgical case of papillary fibroelastoma originating from the left atrial ridge. J Cardiol Cases 2015; 11: 18–20.
5. Ngaage DL, Mullany CJ, Daly RC, *et al.*: Surgical treatment of cardiac papillary fibroelastoma: a single center experience with eighty-eight patients. Ann Thorac Surg 2005; 80: 1712–1718.
6. Kamdar F, Win S, Manivel JC, *et al.*: A rare nonvalvular left ventricular papillary fibroelastoma: a case report. J Cardiol Cases 2014; 9: 8–10.
7. Sun JP, Asher CR, Yang XS, *et al.*: Clinical and echocardiographic characteristics of papillary fibroelastomas: a retrospective and prospective study in 162 patients. Circulation 2001; 103: 2687–2693.
8. Gowda RM, Khan IA, Nair CK, *et al.*: Cardiac papillary fibroelastoma: a comprehensive analysis of 725 cases. Am Heart J 2003; 146: 404–410.
9. Tamaru N, Abe K, Anami M, *et al.*: A papillary fibroelastoma on a papillary muscle of the left ventricle. Pathology 2006; 38: 174–177.
10. Bryde R, Landolfo K, Ray JC, *et al.*: Subaortic membrane papillary fibroelastoma. Case Rep Cardiol 2020; 2586730. doi: 10.1155/2020/2586730.
11. Strecker T, Agaimy A, Zelzer P, *et al.*: Incidental finding of a giant asymptomatic right atrial tumor. Int J Clin Exp Pathol 2014; 7: 4528–4530.
12. Akgun T, Gulsen K, Cinier G, *et al.*: Electrocardiographic characteristics of metastatic cardiac tumors presenting with ST-segment elevation. J Electrocardiol 2020; 59: 93–99.
13. Cheon DY, Park KH, Hong SE, *et al.*: Metastatic cardiac tumor manifested by persistent ST-segment elevation with coexisting reciprocal changes on electrocardiography. Int Heart J 2014; 55: 466–468.

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Breast Screening Assessment Manual

Japan Society of Ningen Dock
Health Screening Assessment and Guidance Manual Drafting Committee
Breast Working Group (WG)

Chapter 1 Mammography Assessment Manual

Introduction

The incidence of breast cancer has increased in recent years in Japan, and breast cancer ranks first among malignant tumors in women. Over 90,000 women are diagnosed with breast cancer each year. The lifetime risk of developing breast cancer is estimated to be 1 in 9[1].

Breast cancer screening using mammography is the only screening method proven to lower the rate of breast cancer mortality[2]. While the benefits of mammographic screening have been studied and the practice has become standard for breast cancer testing in Western countries with high rates of breast cancer, several issues, such as the age at which the test should be performed and how dense breast tissue should be evaluated, are still being discussed[3].

In Japan, breast cancer screening using mammography started in 2000, and was conducted in combination with the clinical breast examination in women aged ≥50 years every 2 years. Since 2004, the test has been available every other year for women aged ≥40 years. However, as breast cancer screening with a clinical breast examination alone has been found to be ineffective for lowering breast cancer mortality, today, an increasing number of municipalities have omitted the clinical breast examination and opted for mammography only.

Voluntary breast cancer screening provided as part of a medical checkup package for Ningen Dock and other services has become widely available today, and supplements the government's population-based screening rate (approximately 20% for those aged 40–74 years). The current overall breast cancer screening rate is 47.4% (2019), which has yet to reach the target of 50%[4]. Due to the increase in breast cancer incidence, the nation is taking efforts to increase population-based breast cancer screening rates, and Ningen Dock plays an important role in this process. However, as described in more detail in the ultrasound screening section, validating the accuracy of breast cancer screening within Ningen Dock is fraught with challenges, because of the lack of standardized terms and criteria for screening across testing facilities.

Here, we standardized terms related to breast cancer screening and created assessment classes for interpreting screening results to improve the accuracy of breast cancer screening. Mammography Guidelines Ver. 4 (Japan Radiological Society/Japanese Society of Radiological Technology)[5] was used to standardize and maintain consistency of breast cancer screening terms.

Ⅰ. Assessment procedure

1. Identify the lesion location from the parts list
2. Assess the results
3. Select the category
4. Select the assessment class

Assessment should be conducted according to the above steps. The reason (category) for selecting the assessment class and name should be noted.

1. Lesion location

The lesion location should be noted based on the view during screening.

(See Mammography Guideline Ver. 4)

1) Location on mediolateral oblique (MLO) view (Fig. 1)

L: Region caudal to the perpendicular line stretching posterior to the center of the nipple

M: Region delineated by the line drawn cranially from the perpendicular line of the same length as that to the lower margin of the breast and the line drawn parallel to the perpendicular line

U: Region cranial to M

S: Subareolar region (corresponding to the main duct); site 2 cm from the central nipple

X: Axillary region

Locations between two regions should be marked under the main site.

Locations that cover the entire breast should be marked as W.

2) Location on craniocaudal (CC) view (Fig. 2)

I: Region medial to the perpendicular line from the center of the nipple

O: Region lateral to the perpendicular line from the center of the nipple

S: Subareolar region

Locations between two regions should be marked under the main site.

Locations that cover the entire breast should be marked as W.

3) Bidirectional view (Fig. 3, 4)

For the bidirectional view, MLO and CC images should both be taken.

Findings should be noted in the following order: "Bilateral differences," "Location on MLO view," and "Location on CC view."

Locations between two regions should be marked under the main site.

If findings can only be identified in one view, "N" to indicate "no findings" should be noted in the view in which the findings are not observable to indicate that they were only visible in one view.

2. Assessment methods

Mammography interpretations should be double-checked by two physicians (ideally mammography

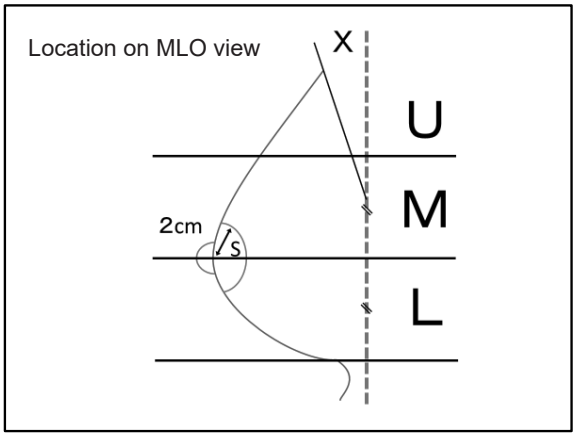


Fig. 1. Location on MLO View

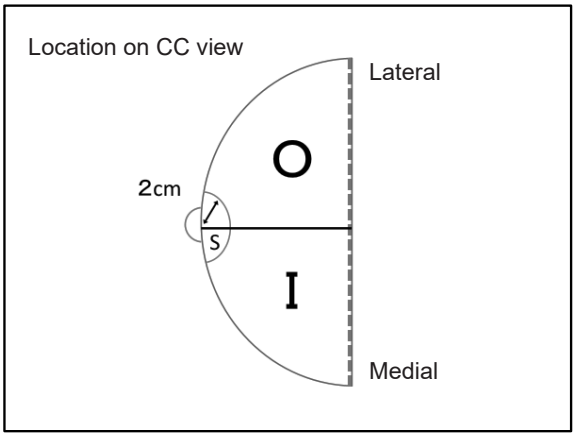


Fig. 2. Location on CC View

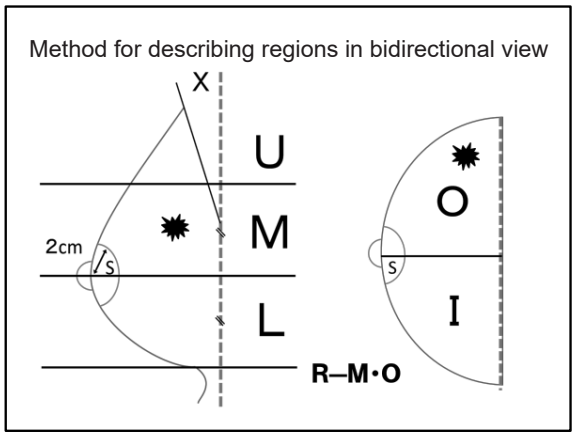


Fig. 3. Location on Bidirectional View

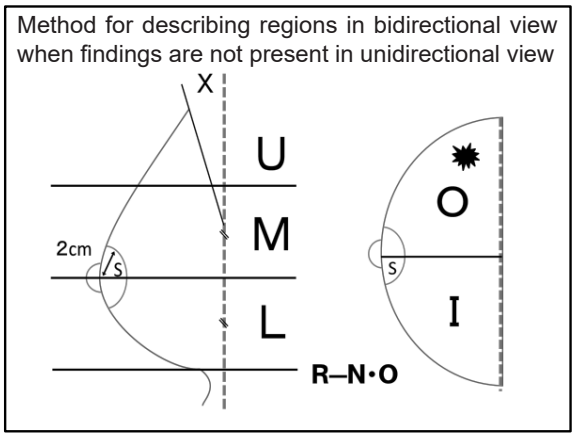


Fig. 4. Location When There Are No Findings on One View of the Bidirectional View

interpreting physicians certified by the Japan Central Organization on Quality Assurance of Breast Cancer Screening).

The assessment should be made separately on the left and right.

In the bidirectional view, the assessment should not be made individually for each view. Instead, a category should be selected as the overall assessment result from the two views for each breast. Similar findings identifiable in both directions increase the chances of finding a lesion.

Assessment should be conducted with past images for comparison, if available.

The possibility of malignancy should be considered when selecting the category. Categories ≥3 require further examination.

An image that cannot be appropriately assessed should be categorized as "unassessable."

A reading physician should be consulted as soon as possible to determine whether an unassessable case should be reinterpreted or assessed using another testing method.

3. Categories

1) Uninterpretable

Uninterpretable cases (Category N) can be further divided into two categories:

N-1: Cases requiring re-examination and re-imaging owing to body movement, poor imaging conditions, or positioning

N-2: Cases that are expected to be categorized as N after a re-examination owing to assessment of breast or areolar shape via clinical breast examination (palpation).

2) Interpretable

Category 1	Negative
Category 2	Benign, findings that do not require further examination
Category 3	Benign, but malignancy cannot be ruled out
Category 4	Suspicious abnormality
Category 5	Highly suggestive of malignancy

3) Uninterpretable

Uninterpretable cases should be categorized as N.

4. Assessment class

※The assessment class conforms to the Assessment Classes of the Japanese Society of Ningen Dock.

II. Breast composition[6]

Fatty, scattered fibroglandular density, heterogeneously dense, and extremely dense

No method exists to assess the degree of the mixture of fibroglandular and fatty tissue on mammography. Heterogeneously dense and extremely dense breast tissue are common in Japan; these breast composition types account for over 60% of Japanese women. In general, dense breast tissue (heterogeneously dense and extremely dense) decreases with age, especially after menopause. Scattered areas of fibroglandular density and fatty breasts are common in overweight people.

Mammography has low sensitivity in those with dense breast tissue compared to non-dense breast tissue; thus, dense breast tissue itself is a risk factor for breast cancer, according to some reports.

However, there is no consensus on whether ultrasound should be added in women with dense breast tissue[7,8].

Assessment Class		Category
Assessment Class A	Negative	(Includes physiological changes.) Corresponds to Category 1. Does not require further examination or re-examination.
Assessment Class B	Mild abnormality	Of Category 2; corresponds to clearly benign. Does not require further examination or re-examination.
Assessment Class C	Requires re-examination (in 6/12 months) *	If malignancy is suspected, always make a judgment of Class D. Of Categories 2 and 3; corresponds to a condition that is determined not to affect vital prognosis as assessed in a 1-year re-examination, and thus requires a re-examination in 1 year as a general rule. The time to re-examination can be at the discretion of the physician; however, if re-examination is required within 3 months, it should be classed as "required detailed examination." Unassessable cases should be categorized as N, and the interpreting physician should make a recommendation on re-examination or examination using another modality.
Assessment Class D	Requires detailed examination/treatment	Corresponds to Categories 3, 4, 5.
Assessment Class E	In treatment	Receiving chemotherapy or radiotherapy in a medical institution, with the aim of improving the result of the corresponding examination.

* Class D if re-examination cannot be performed in the same facility.

※Categories were developed with the intention to assess benign or malignant tumors, and assessment class is an indicator of breast assessment findings and patient's actions.

III. Terms for mammography findings

Terms for mass, calcification, and other findings (categorized into glandular parenchyma, skin, and lymph node findings) were standardized based on the "Mammography Guidelines[5]."

References

1. Cancer Information Service, National Cancer Center: Latest Cancer Statistics: Cancer-related deaths, 2017. https://ganjoho.jp/reg_stat/statistics/stat/summary.html#a25 (in Japanese) (accessed June 18, 2021)
2. Cancer Information Service, National Cancer Center: Breast Cancer Screening, 2019. https://ganjoho.jp/med_pro/pre_scr/screening/screening_breast.html (in Japanese) (accessed June 18, 2021)
3. Checka CM, Chun JE, Schnabel FR, *et al.*: The relationship of mammographic density and age: implications for breast cancer screening. AJR Am J Roentgenol 2012; 198: 292–295.
4. Ministry of Health, Labour and Welfare: Intensive campaign to raise cancer screening rate to 50%. https://www.gankenshin50.mhlw.go.jp/campaign_30/ (in Japanese) (accessed June 18, 2021)
5. Japanese Society of Radiological Technology (ed): Mammography Guideline Ver. 4, Igakushoin, Tokyo, 2021. (in Japanese)
6. The American College of Radiology: https://www.acr.org/Quality-Safety/Resources/BI-RADS/Mammography (accessed January 12, 2017)
7. Boyd NF, Guo H, Martin LJ, *et al.*: Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007; 356: 227–236.
8. Japan Association of Breast Cancer Screening, Japan Breast Society, The Japan Central Organization on Quality Assurance of Breast Cancer Screening: Countermeasures. Proposals on tackling "dense breast tissue" issues in breast cancer screening, 2017. https://www.qabcs.or.jp/news/entry-900.html (in Japanese) (accessed June 18, 2021)

Chapter 2 Ultrasound Assessment Manual

Introduction

The incidence of breast cancer has increased in recent years in Japan, and breast cancer ranks first among malignant tumors in women. In 2019, 91,605 women were diagnosed with breast cancer. The lifetime risk of developing breast cancer is estimated to be 10.6% for Japanese women or 1 in 9[1].

The J-START trial, which tested the effectiveness of ultrasound combined with mammography in screening for breast cancer in Japanese women aged ≥40 years[2] showed that adding ultrasound to mammography increases the detection rate of breast cancer; however, conclusions are yet to be reached on whether it decreases mortality, given it requires long-term follow-up observation. Furthermore, as the study did not directly compare mammography with ultrasound, ultrasound alone is not used for population-based screening. According to the Japanese Breast Cancer Society 2017 Report on clinical statistical studies on registered patients with mammary cancer in Japan, a sizable portion of patients with breast cancer (5.3% of patients with breast cancer overall, n=4,947) are aged ≤39 years and are not eligible for population-based screening.

Given that the aim of breast cancer screening is to lower the breast cancer mortality rate, the lack of evidence for ultrasound screening should be thoroughly explained to people undergoing ultrasound. However, ultrasound screening is generally important in women who are poor candidates for mammography (young women with dense breast tissue, for whom the detection sensitivity of breast cancer is low[3]) and are more sensitive to the effects of radiation exposure, women who have undergone breast augmentation surgery, are pregnant or breastfeeding, high-risk young women, and women who cannot be screened or screened sufficiently using mammography because of dense breast tissue or who want to avoid it by preference.

Furthermore, the terms and criteria used in voluntary breast cancer screening in Ningen Dock are not standardized across screening facilities and cannot be easily validated for screening accuracy. It is important to standardize techniques, systems, and process indicators (data submitted to national data by the Japan Association of Breast Cancer Screening) to ensure accuracy. To promote accuracy in Ningen Dock, The Health Screening Assessment and Guidance Manual Drafting Committee Breast WG (Sakurai Team) standardized "location" and "finding and diagnosis names" for presenting screening assessment criteria. Location, finding, and diagnosis names are in the screening standard format of the Japan Medical Health Management Assessment Association, which comprises 10 health screening-related organizations.

In the Breast Ultrasound Ningen Dock Screening Assessment Manual, diagnoses were described with reference to the Japan Association of Breast and Thyroid Sonology Breast Ultrasound Guideline (ver. 4)[4]. We plan to promptly update the Breast Ultrasound Ningen Dock Screening Assessment Manual when the breast ultrasound guideline is revised. We hope that the present assessment manual will be widely used in health screening facilities to improve accuracy.

I. Screening methods

See the Guideline for Breast Ultrasound Diagnosis and Management, Ver. 4, for ultrasound devices and examination methods.

II. Assessment procedure

1. Identify the lesion location from the parts list

2. Select the corresponding assessment result from the list of findings or diagnoses
3. Select the category
4. Select the assessment class

Assessment should be conducted according to the above steps. The reason (category) for selecting the assessment class, finding, and diagnosis should be noted.

1. Lesion location

To describe the lesion location in terms of breast region, methods that use a clock axis centered on the nipple and methods conforming to the Japanese Breast Cancer Society "General Rules for Clinical and Pathological Recording of Breast Cancer"[5] should be used in screening.

Given that the recording method in "General Rules for Clinical and Pathological Recording of Breast Cancer" is used in clinical practice, the present guidelines also recommend this method.

Specifically, this method entails first noting whether the lesion is on the right or left (or both) breast(s).

The region where the lesion is located should be classified under one of the following:

Upper medial····· region A
Lower medial····· region B
Upper lateral····· region C
Lower lateral····· region D
Areolar····· region E
Axillary····· region C'
Whole breast····· region W

The breast is divided mediolaterally and superoinferiorly into quadrants, and the areolar and axillary regions are marked as separate regions, as shown above.

An alphabetical letter is assigned to each quadrant to obtain regions A–D (**Fig. 1**). The areolar region, including the nipple, is designated region E, and the axillary tail of the breast is designated C'.

If multiple lesions are present across multiple regions, they should be indicated in descending order from the region (main seat) with the greatest number of lesions.

Example: If the main seat is in region A but lesions are also found in region C, this should be noted as →AC

2. Findings and diagnosis

Findings should be classified as masses or non-mass lesions. See Guideline for Breast Ultrasound Diagnosis and Management, Ver. 4, for details of the classifications and diagnostic methods.

History of the finding (e.g., first examination, new onset, detection, and growth since the last examination) should be noted to the best possible extent.

See the table below and notes on diagnosis.

Apart from the most typical cases, ultrasound alone has limitations for making a diagnosis; thus, while it is mandatory to note the finding and category, noting the diagnosis is optional.

3. Categories

※ The screening categories below are based on the Guidelines for Breast Ultrasound Diagnosis and Management.

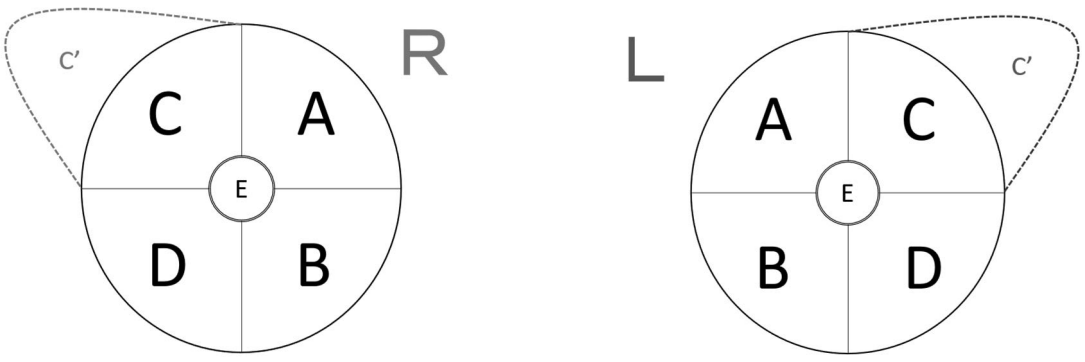
Category 1:	Negative
Category 2:	Benign findings that do not require further testing*1
Category 3:	Benign, but malignancy cannot be ruled out
Category 4:	Suspicious abnormality
Category 5:	Highly suggestive of malignancy
Category 0:	Unassessable case*2

*1 "Category 2 Benign," used widely in diagnostic categories, includes "findings that do not require further testing."

*2 "Unassessable" refers to cases that cannot be assessed owing to device malfunction or factors related to the examinee or examiner that require re-examination or other examination and are classified as Assessment Class C with the necessary details.

4. Assessment class (Breast screening assessment and management)

※ Assessment classes comply with the Japan Medical Health Management Assessment Association health screening standard format and the Japanese Breast Cancer Society "Breast Cancer Detailed Screening Report Manual based on Categories and Diagnosis Categories[6]."



Right Left

Fig. 1. Location

Breast ultrasound findings (Major items)	Standard terms (Mid-level items)	Recommendation category	Assessment Class	Page in Guideline for Breast Ultrasound Diagnosis and Management, Ver 4.
Mammary tumor＊1	Noninvasive ductal carcinoma	4, 5	D	p99
	Invasive ductal breast carcinoma		D	p100
	Mucinous carcinoma		D	p104
	Invasive lobular carcinoma		D	p105
	Breast cancer		D	p99
Indistinguishable (Benign/malignant) mammary tumor＊2	Breast tumor Intraductal lesion	3	D	p110
Mammary tumor＊3	Fibroadenoma	2	B	p105
Other benign diseases of the breast gland＊4	Mastopathy	2	B	p108
	Fibrous disease		B	p110
	Hamartoma		B	p111
	Hematoma		B, D	
	Mastitis		B, D	
	Subareolar abscess		B, D	
Axillary lymph node enlargement＊5	Axillary lymph node enlargement	2, 3, 4, 5	B, D	p116
Intramammary lymph node＊6	Intramammary lymph node	1, 2, 3	A, B, D	p116
Lactation change＊7	Lactation change	1	A	p97
Gestational change	Gestational change	1	A	p97
Foreign-body granuloma	Foreign-body granuloma	2	B	p112
Postoperative breast	Postoperative breast	2	B	
Other breast findings＊8	Lipoma	2	B	
	Mondor's disease		B	
	Fat necrosis		B	p111
Other breast findings＊9	Subcutaneous tumor	2, 3, 4, 5	B, D	
	Skin mass			
Other breast findings＊10	Other than the above (includes supernumerary nipples, etc.)	2, 3, 4, 5	B, D	

＊1: Malignant tumors are assessed as Category 4 or 5, Assessment Class D if the histology type can be predicted.
＊2: Masses that are not distinguishable (between benign or malignant), such as masses that cannot be identified as a tumor and phyllode tumor, are assessed as Category 3, Assessment Class D.
＊3: Typical fibroadenomas and other tumors that can be easily determined to be benign tumors are assessed as Category 2.
＊4: Other benign diseases (mastopathy, fibrous disease, hamartoma, hematoma, mastitis, and subareolar abscess) that are clearly diagnosable are assessed as Assessment Class B.
＊5: Axillary lymph node enlargement that clearly indicates reactive lymph nodes are assessed as Category 2, Assessment Class B.
In other cases, assume the possibility of dormant breast cancer, malignant lymphoma, or breast cancer metastasis, and assess as Categories 3, 4, or 5, Assessment Class D.
＊6: Intramammary lymph nodes that appear normal are assessed as Category 1, Assessment Class A; clear reactive enlargement as Category 2, Assessment Class B; and others as Category 3, Assessment Class D.
＊7: Clear lactation changes or gestational changes are assessed as Category 1, Assessment Class A.
Foreign-body granuloma after breast augmentation surgery or post-mastectomy findings that clearly indicate a change from foreign bodies or surgery is assessed as Category 2, Assessment Class B.
＊8: Other subcutaneous findings should not be mentioned to examinees at the breast cancer screening; however, lipoma, fat necrosis to traumatic injury, and Mondor's disease are assessed as Category 2, Assessment Class B.
＊9: Subcutaneous tumors and skin tumor alone should not be mentioned to examinees at the screening; however, clearly benign cases are assessed as Category 2, and suspected metastasis after breast cancer surgery as Categories 3, 4, or 5, Assessment Class D.
＊10: Other breast findings (including supernumerary nipples) should be noted and assessed.

Assessment Class		Category	Management
Assessment Class A	Negative	Corresponds to screening Category 1	Does not require further examination or follow-up
Assessment Class B	Mild abnormality Does not require detailed examination	Corresponds to screening Category 2; clearly benign or findings do not require further testing	Does not require further examination or follow-up
Assessment Class C	Requires re-examination (in 6/12 months)*		Small masses and non-mass lesions with possibility of ductal carcinoma *in situ* are Category 2, and screening again in 6 or 12 months is allowed Unassessable cases are Category 0, and require the interpreting physician's recommendation for re-examination or examination by another modality
Assessment Class D	Requires detailed examination/treatment	Corresponds to screening Categories 3, 4, 5	Requires detailed examination
Assessment Class E	In treatment Generally not used in Ningen Dock Breast Screening assessment		Not in scope of breast cancer screening

* Cases that cannot be re-examined in the same facility are categorized as Class D.
※ See the Ultrasound Category Assessment Checklist for details

Table of English terms

Standard terms
Mass
Cystic pattern
Mixed pattern
Solid pattern
Non-mass lesions
Abnormal lactiferous duct (bilateral, multiple)
Abnormal lactiferous duct (segmental, local)
Hypoechoic area (bilateral, multiple)
Hypoechoic area (segmental, local)
Architectural distortion
Multiple small cysts (bilateral, scattered)
Multiple small cysts (segmental, local)
Echogenic foci
Coarse calcifications
Diagnosis
Ductal carcinoma *in situ*: DCIS
Invasive ductal carcinoma
Mucinous carcinoma
Invasive lobular carcinoma
Breast cancer
Breast tumor
Fibroadenoma
Mastopathy
Fibrous disease
Hamartoma
Hematoma
Mastitis
Subareolar abscess
Axillary lymph node enlargement
Intramammary lymph node
Lactation change
Gestational change
Foreign-body granuloma
Postoperative breast
Lipoma
Mondor's disease
Fat necrosis
Subcutaneous tumor
Skin tumor

References

1. Cancer Information Service, National Cancer Center: Latest Cancer Statistics: Cancer-related deaths, 2017. https://ganjoho.jp/reg_stat/statistics/stat/summary.html#a25 (in Japanese) (accessed June 18, 2021)
2. Ohuchi N, Suzuki A, Sobue T, *et al.*: Sensitivity and specificity of mammography and adjunctive ultrasonography to screen for breast cancer in the Japan Strategic Anticancer Randomized Trial (J-START): a randomised controlled trial. Lancet 2016; 387: 341–348.
3. Checka CM, Chun JE, Schnabel FR, *et al.*: The relationship of mammographic density and age: implications for breast cancer screening. AJR Am J Roentgenol 2012; 198: 292–295.
4. Japanese Society of Breast and Thyroid Sonology: Breast Ultrasound Diagnosis (Diagnostic ultrasound) Guideline. Revised 4th ed, Nankodo, Tokyo, 2020. (in Japanese)
5. Japanese Association for Breast Cancer: Clinical and Pathological Regulations for Handling Breast Cancer. 18th ed, Kanehara Shuppan, Tokyo, 2018. (in Japanese)
6. Japan Association for Breast Cancer Screening: Ultrasound Screening Accuracy Management Committee: "Guide to Breast Cancer Screening Using Ultrasound: Accuracy Control Manual". 2nd ed, Nankodo, Tokyo, 2020. (in Japanese)

Chapter 3 Clinical Breast Examination (Visual Inspection and Palpation)

Breast cancer screening with clinical breast examination (visual inspection and palpation only) is not recommended owing to its high false-positive and false-negative rate and ineffectiveness in increasing the survival rate of patients with breast cancer. Clinical breast examination (visual inspection and palpation) should only be performed in combination with mammography (ultrasound).

A non-negligible percentage of examinees attend breast cancer screening with subjective symptoms such as mass, abnormal nipple discharge, and mastalgia; thus, information on such symptoms should be collected before the clinical breast examination.

In performing a clinical breast examination, acquiring the following information in a medical interview is helpful:

All examinees
1. Age at menarche
2. Number of pregnancies and births
3. Age at first birth
4. Family history of breast cancer (age at onset, side), family history of ovarian cancer
5. Previous biopsies

Premenopausal examinees
1. Date of the most recent menstruation, cycle
2. Use of hormone therapies

Postmenopausal examinees
1. Age at menopause
2. Use of hormone replacement therapy

Professionals in the examination room should consider the examinee's privacy. A female assistant must accompany a male examining physician. If possible, a special gown should be provided as examinees will be required to remove the clothes from their upper body for the examination.

A. Visual inspection

In a sitting position, have the examinee place both hands on their waist to examine the presence or absence of laterality of the breasts and papilla, skin tone, breast deformity, and skin indentation. Next, have the examinee raise both hands and check for the presence or absence of laterality of the breasts and constriction. In the case of an inverted nipple, inquire about when it started. Unilateral papillary erosion may be a sign of Paget's disease, whereas bilateral papillary erosion may be atopic dermatitis.

B. Palpation

Palpation with the flats of the hand is the common method. Palpate gently in case of a premenstrual examinee as some may experience significant pain even with a light touch.

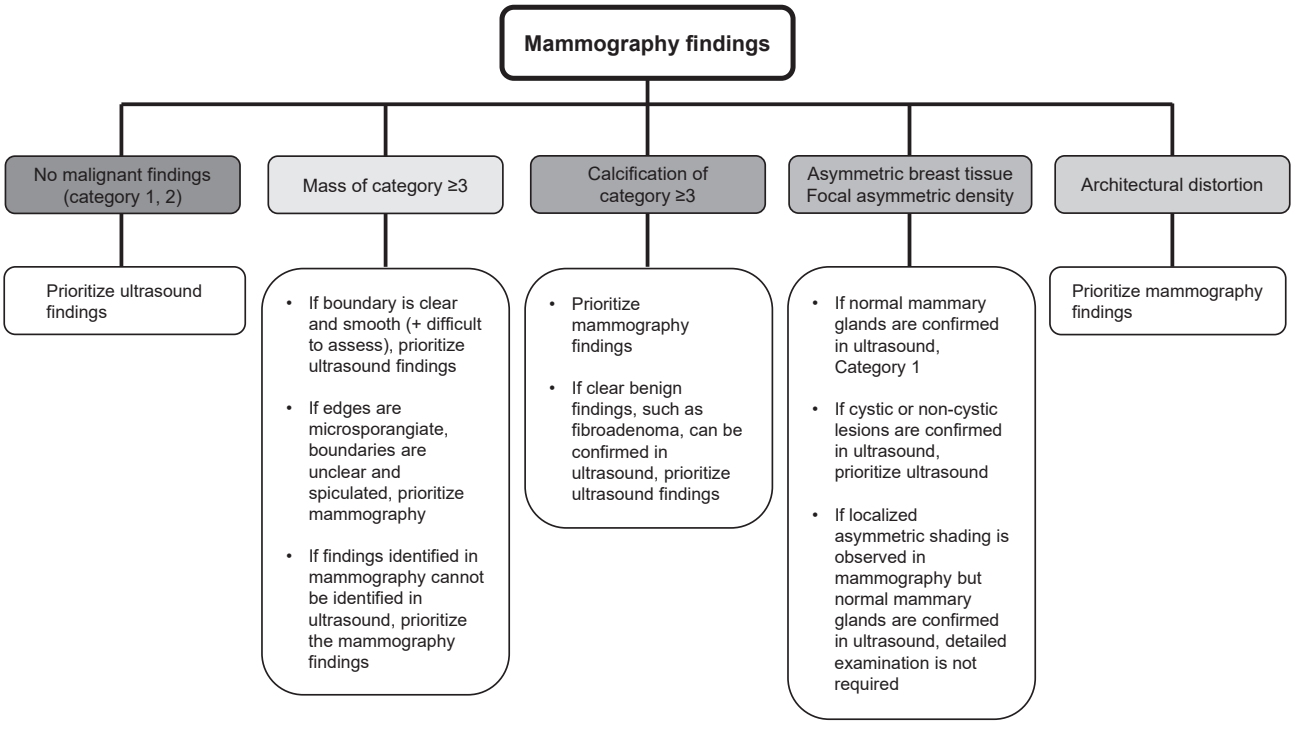
Palpation is performed in the sitting and supine positions. In the sitting position, have the examinee raise both hands for palpation. Large or drooping breasts are better examined in the supine position. If a mass or induration is observed, note the location, size, margins, surface properties, mobility, and the presence or absence of a dimpling sign. Bilateral breast comparison is important. In the premenopausal breast, an induration observed on the upper lateral sides of both breasts may indicate normal mammary gland tissue. However, when the examinee reports subjective symptoms such as a mass, due caution must be paid to avoid overlooking the sign and assessing the examinee as negative.

Palpation of the axillary lymph nodes is performed in a sitting position, with the arms lowered and the body relaxed. The axillary lymph nodes are not usually palpable, but in rare cases, lymph node metastases may precede the mammary mass. Additionally, soft lymph nodes may be palpated in the presence of inflammation in the upper arm, such as in patients with atopic dermatitis.

Overall Assessment Flowchart: Combined Mammography and Ultrasound Screening

In population-based breast cancer screening, a mammography examination is performed once every 2 years for women aged ≥40 years. However, since a study reported that breast cancer can be difficult to detect in dense breast tissue in Ningen Dock screening[1], an increasing number of facilities have begun performing both mammography and ultrasound. The rise in this approach is in part because patients have recently started to request combined screening with mammography and ultrasound.

In addition, a single-center, randomized controlled trial of Japanese patients aged 40–49 years comparing mammography+ultrasound vs. mammography alone showed that 1.5 times more breast cancers were detected in examinees who underwent mammography+ultrasound[2]. In combination screening, there are cases where both examinations are performed at one facility and others where the two are performed at separate facilities. For patient convenience and accuracy of the assessment, it would be best if the two can be performed in one facility. In addition, a "simultaneous combination method" is possible, in which an ultrasound is performed while viewing the mammography result, as is a "separate combination method," in which an ultrasound is performed without information from



※ If two or more tests or visual inspection and palpation are combined with mammography, findings should be noted under the mammography section of the Test Findings Sheet.

Fig. Flowchart of the Mammography+Ultrasound Overall Assessment

mammography. The simultaneous combination method would certainly increase the accuracy of screening and the reliability of the overall assessment.

To obtain the final judgment of the comprehensive assessment, the mammography examination (investigation) and ultrasound should be judged separately, and the judgment of the modality with findings should be given priority. Alternatively, after obtaining each separate judgment, the findings of both should be comprehensively evaluated to obtain the final judgment. The latter comprehensive judgment is preferable from the viewpoint of screening accuracy, especially specificity.

Below is a flowchart of the comprehensive judgment process for mammography and ultrasound. For the final overall assessment, the mammography and ultrasound results are assessed separately and assessment of the modality with findings may be given priority or the findings of both may be reassessed together for the overall assessment. The latter overall assessment method is preferable from the viewpoint of screening accuracy, especially specificity.

A flowchart of the mammography+ultrasound overall assessment is shown below.

References

1. Yomiuri Shimbun: "Unreported difficulties in distinguishing breast cancers," June 12, 2016, morning edition. (in Japanese)
2. Ohuchi N, Suzuki A, Sobue T, *et al.*: Sensitivity and specificity of mammography and adjunctive ultrasonography to screen for breast cancer in the Japan Strategic Anticancer Randomized Trial (J-START): a randomized controlled trial. Lancet 2016; 387: 341–348.
3. Japan Association of Breast Cancer Screening Overall Judging Committee (ed): Mammography and Ultrasound Comprehensive Judgment Manual. Shinohara Publishing, Tokyo, 2015. (in Japanese)

Test Findings Sheet for the Various Examinations (Reference)

Ultrasound Findings Sheet

[Examinee information]

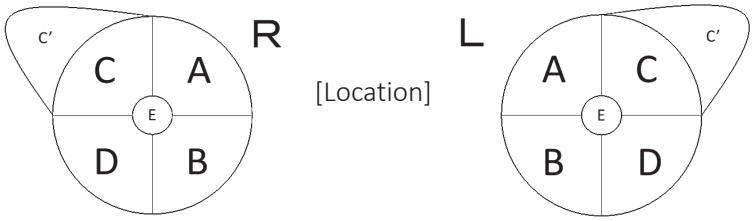
Name:_____ ID: _____

Birthdate: ____/____/____

Age: ____ years

[Date of examination] ____/____/____

[Test facility]



R ☐No abnormalities

[Tumor lesion
New Growth Unchanged Shrinkage]
☐Cystic ☐Mixed ☐Solid
☐Location R ( )
Large diameter
× Short diameter × Height mm

[Tumor lesion
New Growth Unchanged Shrinkage]
☐Cystic ☐Mixed ☐Solid
☐Location R ( )
Large diameter
× Short diameter × Height mm

[Tumor lesion
New Growth Unchanged Shrinkage]
☐Cystic ☐Mixed ☐Solid
☐Location R ( )
Large diameter
× Short diameter × Height mm

[Nontumor lesion]
☐Location R ( )
☐Abnormal lactiferous duct
☐Hypoechoic region
☐Architectural distortion
☐Multiple small cysts

L ☐No abnormalities

[Tumor lesion
New Growth Unchanged Shrinkage]
☐Cystic ☐Mixed ☐Solid
☐Location L ( )
Large diameter
× Short diameter × Height mm

[Tumor lesion
New Growth Unchanged Shrinkage]
☐Cystic ☐Mixed ☐Solid
☐Location L ( )
Large diameter
× Short diameter × Height mm

[Tumor lesion
New Growth Unchanged Shrinkage]
☐Cystic ☐Mixed ☐Solid
☐Location L ( )
Large diameter
× Short diameter × Height mm

[Nontumor lesion]
☐Location L ( )
☐Abnormal lactiferous duct
☐Hypoechoic region
☐Architectural distortion
☐Multiple small cysts

Last examination (/ /)
☐MG ☐Ultrasound ☐MG + Ultrasound

Last examination
☐Negative ☐Benign
☐Requires detailed examination

[Notes at examination]
Pregnant / Breastfeeding /
Post-breast augmentation surgery /
Post-mastectomy
Other ( )

[Diagnosis]

R: Right		L: Left
☐	Malignant mammary tumor	☐
☐	Indistinguishable (benign/malignant) mass	☐
☐	Benign mass (fibroadenoma)	☐
☐	Cyst	☐
☐	Axillary lymph node enlargement	☐
☐	Other ( )	☐

Right	[Ultrasound categories]	Left
☐1	**Negative**	☐1
☐2	**Benign, does not require detailed examination**	☐2
☐3	**Benign, but malignancy cannot be ruled out**	☐3
☐4	**Suspicious abnormality**	☐4
☐5	**Highly suggestive of malignancy**	☐5
☐0	**Unassessable**	☐0

Testing technician's comments

Assessing physician:

Assessing physician:

Testing technician's name:

Mammography Findings Sheet

[Examinee information]

Name: ID:

Birthdate: : / / Age: ____ years

[Date of examination: / /]

☐MMG Direction: /

☐Ultrasound: /

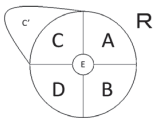
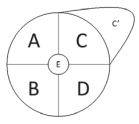
☐Visual inspection and palpation: /

Visual inspection and palpation alone is not recommended

Mammography

	Right	Left
Location		
	Breast composition: Fatty, scattered fibroglandular density, heterogeneously dense, extremely dense	**Breast composition:** Fatty, scattered fibroglandular density, heterogeneously dense, extremely dense
	U M L S X W A B C D E C′	U M L S X W A B C D E C′
	Diffuse Regional Segmental Aggregated Fibrous Other ( )	Diffuse Regional Segmental Aggregated Fibrous Other ( )
Findings	Tumor lesion Calcified lesion Local Asymmetrical Architectural distortion Mastopathy Other ( )	Tumor lesion Calcified lesion Local Asymmetrical Architectural distortion Mastopathy Other ( )
Category	1) No findings 2) Benign 3) Probably benign 4) Suspicious of malignancy 5) Strongly suspicious of malignancy 6) Unassessable → Imaging conditions Positioning Not eligible for mammography (Breast cancer surgery, breast augmentation surgery, pacemaker implantation)	1) No findings 2) Benign 3) Probably benign 4) Suspicious of malignancy 5) Strongly suspicious of malignancy 6) Unassessable → Imaging conditions Positioning Not eligible for mammography (Breast cancer surgery, breast augmentation surgery, pacemaker implantation)
Assessment	1) Negative 2) Benign 3) Probably benign 4) Suspicious malignancy 5) Strongly suspicious malignancy 6) Unassessable	
Physician's name	Primary interpreting physician	Secondary interpreting physician / Imaging technician

Visual inspection/Palpation

●Mass ○Induration Size × cm Hardness Hard / Tender Margins Unclear / Clear Surface Irregular / Unsmooth Flat hand + / − Pain on pressure + / − 	+ −	Mass	+ −	●Mass ○Induration Size × cm Hardness Hard / Tender Margins Unclear / Clear Surface Irregular / Unsmooth Flat hand + / − Pain on pressure + / − L 
	+ −	Induration	+ −	
	+ −	Diffuse nipple	+ −	
	+ −	Inverted	+ −	
	+ −	Secretion	+ −	
	+ −	Skin changes	+ −	
	+ −	Redness	+ −	
	+ −	Axillary lymph nodes	+ −	
	+ −	Supraclavicular lymph nodes	+ −	

Right	Diagnosis at the time of examination	Left
1) Negative 2) Benign 3) Probably benign 4) Suspicious of malignancy 5) Strongly suspicious of malignancy		1) Negative 2) Benign 3) Probably benign 4) Suspicious of malignancy 5) Strongly suspicious of malignancy

Assessment on visual inspection and palpation:

Physician's comments [Diagnosing physician's name]

Overall assessment

Abnormal findings	☐Mammography ☐Ultrasound ☐Visual inspection and palpation (Assessment by visual inspection and palpation alone is not recommended)
Assessment class	☐A Negative ☐B Mild abnormalities ☐C Requires re-examination (in 6/12 months) ☐D Requires detailed examination/treatment ☐E In treatment
Physician's comment	[Diagnosing physician's name]

Ultrasound　Category Assessment Checklist (Reference)

Right　(R)

Mass	Category ≥ 3	Category ≤ 2
☐ Cystic pattern		☐ Category 2
☐ Mixed pattern	☐ >15 mm	☐ ≤15 mm
☐ Solid pattern	☐ Halo, with rupture of gland margin	☐ ≤20 mm small D/W ratio (approximately ≤0.5) and clear and smooth margins
	☐ Multiple echogenic foci	☐ With coarse calcifications
		☐ Anterior arch-shaped echogenic focus and posterior shadowing
	☐ D/W ≥0.7, >5 mm	☐ D/W ≥0.7, ≤5 mm
	☐ D/W <0.7, >10 mm	☐ D/W <0.7, ≤10 mm

Non-mass lesions	Category ≥ 3	Category ≤ 2
Abnormal lactiferous duct	☐ Segmental, local duct dilation	☐ Bilateral, multiple duct dilation
		☐ Segmental, local dilation without hypoechoic regions or floating echoes
Intraductal hypoechoic region	☐ Segmental, local	☐ Bilateral, multiple
	☐ Echogenic foci	
Architectural distortion	☐ Architectural distortion	
Multiple small cysts	☐ Segmental, aggregated with intraductal hypoechoic regions or abnormal lactiferous duct	☐ Bilateral, scattered
		☐ Segmental, aggregated without intraductal hypoechoic regions or abnormal lactiferous duct

Left　(L)

Mass	Category ≥ 3	Category ≤ 2
☐ Cystic pattern		☐ Category 2
☐ Mixed pattern	☐ >15 mm	☐ ≤15 mm
☐ Solid pattern	☐ Halo, with rupture of gland margin	☐ ≤20 mm small D/W ratio (approximately ≤0.5) and clear and smooth margins
	☐ Multiple echogenic foci	☐ With coarse calcifications
		☐ Anterior arch-shaped echogenic focus and posterior shadowing
	☐ D/W ≥0.7, >5 mm	☐ D/W ≥0.7, ≤5 mm
	☐ D/W <0.7, >10 mm	☐ D/W <0.7, ≤10 mm

Non-mass lesions	Category ≥ 3	Category ≤ 2
Abnormal lactiferous duct	☐ Segmental, local duct dilation	☐ Bilateral, multiple duct dilation
		☐ Segmental, local dilation without hypoechoic regions or floating echoes
Intraductal hypoechoic region	☐ Segmental, local	☐ Bilateral, multiple
	☐ Echogenic foci	
Architectural distortion	☐ Architectural distortion	
Multiple small cysts	☐ Segmental, aggregated with intraductal hypoechoic regions or abnormal lactiferous duct	☐ Bilateral, scattered
		☐ Segmental, aggregated without intraductal hypoechoic regions or abnormal lactiferous ducts

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(Observers)

Chairperson

Takashi Wada: Professor, Jikei University　School of Medicine

Deputy chairperson　(Imaging)

Masaki Adachi: Preventive Medicine Research Center, Saitama Medical University Hospital Visiting Professor/Advisor

There are no conflicts of interest related to companies that must be declared in the preparation of this breast screening manual.

April 2022

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Yoshiaki Maruyama	(3)
Yoshiko Mizuno	(1)

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 International is the official journal of Japan Society of Ningen Dock, in which original articles, case reports, short reports, review articles, and clinical experience or practice report in 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 International uses an online submission system called ScholoarOne Manuscripts.
Please access https://mc.manuscriptcentral.com/ndi

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.

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

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- ☐ Up to 4 key words.

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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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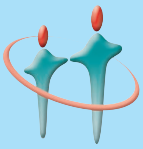
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