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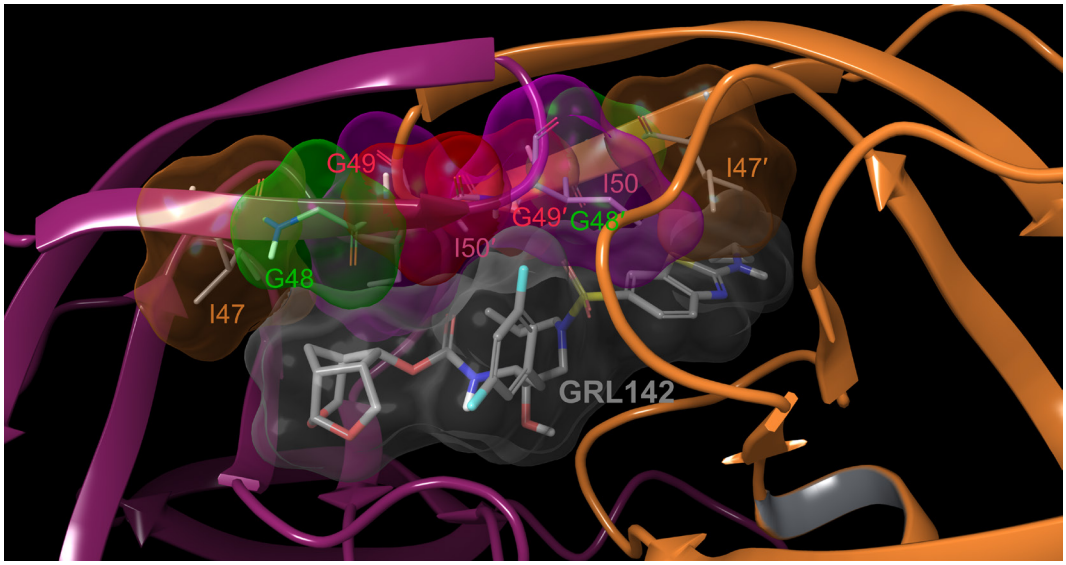

The van der Waals surface interactions of GRL-142, an experimental anti-HIV-1 protease inhibitor, with the HIV-1 protease flap residues as determined in a crystal structure (protein data bank identifier 5TYS) are shown (Pages iv and 360)

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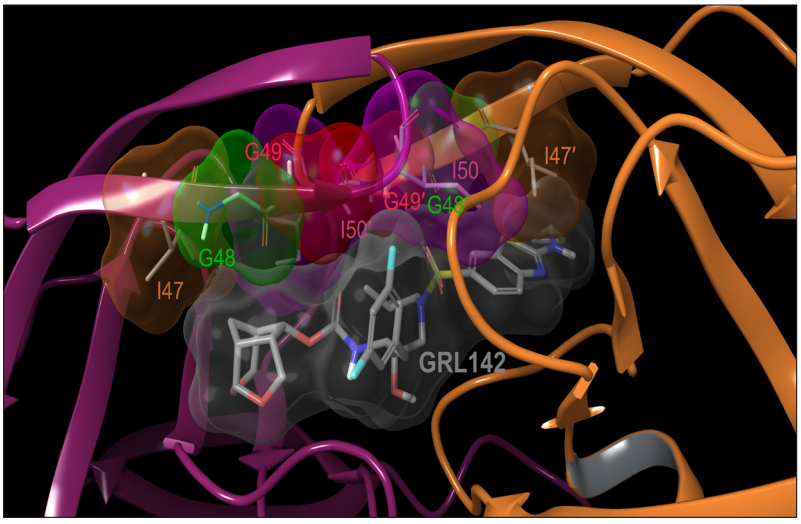

The van der Waals surface interactions of GRL-142, an experimental anti-HIV-1 protease inhibitor, with the HIV-1 protease flap residues as determined in a crystal structure (protein data bank identifier 5TYS) are shown. GRL-142 has optimal van der Waals surface contacts with the shown residues in the HIV-1 protease flap. These interactions, besides increasing the binding affinity between GRL-142 and HIV-1 protease, are partly responsible for keeping a closed conformation of the protease flap. The surface of GRL-142 is shown in gray; the surfaces of I47, G48, G49 and I50 are shown in aquamarine, green, red and magenta, respectively. Part of the protease secondary structure, in ribbon representation, are shown in orange and maroon colors. (Page 360)

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MINI-REVIEW
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HIV-1 protease inhibitors and mechanisms of HIV-1's resistance

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Abstract: Current anti-HIV drugs have significantly improved the prognosis of HIV infected patients so much so that it is now considered a chronic disease, and adherence to medications keeps non-detectable amounts of the virus in the body. However, HIV is still able to generate drug resistance substitutions. Protease inhibitors (PIs) in combination with other classes of anti-HIV drugs constitute an important part of the anti-HIV drug regimen. This article discusses some of the common resistance substitutions against PIs, mechanistic insight on resistance, and potential new inhibitors that can show efficacy against current resistant variants.

***Keywords*:** HIV-1, anti-HIV therapy, protease inhibitors, drug resistance, darunavir

Introduction

Since the identification of human immunodeficiency virus (HIV) as the causative agent for acquired immunodeficiency syndrome (AIDS) in the 1980s, significant progress has taken place not only in the diagnosis and treatment, but also in the education of vulnerable populations and in public health initiatives to combat the spread of the virus. The first set of anti-HIV drugs, AZT, ddI, and ddC targeted the reverse transcriptase of the virus (*1-3*). These drugs were crucial in the initial management of the disease and gave hope to millions of patients worldwide.

Subsequently, drugs targeting the viral protease and integrase were approved (*4-7*). More extensive understanding of the mechanism of HIV replication, cellular pathways involved, and involvement of viral reservoirs have increased the number of potential anti-HIV targets. More than twenty-five drugs have now been approved over the years, and many of them are combined for optimal effective doses (*4,8*). In spite of remarkable progress across many different areas to tackle the disease, several significant challenges in diagnosis and treatment remain. One of the continuing challenges is in HIV being able to generate drug resistance-associated amino acid substitutions that decrease the efficacy of treatment over a period of time (*9*). Research continues for the discovery and development of more potent, safe and long-acting inhibitors.

Protease inhibitors in clinical use

In December 1995, saquinavir (SQV) became the first protease inhibitor (PI) approved for the treatment of HIV-1-infected individuals by the USA Food and Drug Administration (FDA) (*10*). This was a watershed moment in the clinical care and disease management of HIV-1-infected patients. Till that time, AZT, ddI and ddC that targeted the HIV-1 reverse transcriptase, were in clinical use (*4*). However, treatment failed in many instances because of the resistance developed by HIV-1. Following SQV, other protease inhibitors such as ritonavir, lopinavir, atazanavir, fosamprenavir, and tipranavir secured FDA approvals between 1996 and 2005 (*4*). In 2006, darunavir (DRV) became the last PI approved for clinical use. DRV exerted its activity not only because it binds to the active site formed by the protease dimer, but also because it binds to protease monomers and prevents the formation of dimers (*11,12*). The high genetic barrier towards development of resistance of currently used anti-HIV medications, their much-improved safety profile and simplified dosing regimen have led to more rigorous standards for the initiation of clinical trials and the eventual approval of newer inhibitors.

Amino acid substitutions in protease that give rise to resistance

Drug-resistance-associated amino acid substitutions in HIV-1 protease result in a wide range of resistance and are somewhat arbitrarily categorized into major and minor substitutions (*9*). The major substitutions appear earlier during drug treatment and may result in a significant decrease in the efficacy of the drug. Minor substitutions often appear later, and may not

necessarily make the virus more resistant, but is thought to contribute to improving the replication fitness of the virus. Though the difference between major and minor substitutions is not clearly defined, mostly because of a lack of mechanistic understanding of how these substitutions contribute to resistance, such classification has helped in a simplistic understanding – hence we will use the same terminology with the caveat that some of the so called minor substitutions might be significantly contributing to HIV's resistance and the mechanism of their impact and contribution has not been fully deciphered so far. The following are some of the well-known resistance-generating major substitutions: D30N for nelfinavir; V32I for atazanavir and lopinavir; M46I/L for indinavir; I47V/A for darunavir, lopinavir, and tipranavir; G48V for saquinavir; I50L/V for atazanavir, darunavir, lopinavir and fosamprenavir; I54L/V/M for atazanavir, darunavir, and lopinavir; V82A for atazanavir, lopinavir, and indinavir; I84V for atazanavir, darunavir, lopinavir, fosamprenavir, and indinavir; and L90M for nelfinavir and saquinavir (Figure 1). Some residues commonly classified as minor resistant substitutions are L10F, K20M, L24I, L33F, M36I, and A71V (Figure 1). Some substitutions may be major for some PIs while such substitutions may be classified as minor for others. Clinically, substitutions may arise as a drug-resistance-conferring substitution(s) for one inhibitor, but can cause resistance against other inhibitors – a phenomenon known as cross-resistance.

New protease inhibitors to combat drug resistance

The quest to discover anti-HIV inhibitors that are more potent and long-acting with improved safety and pharmacological properties is an area of vigorous basic, pre-clinical, and clinical research. The design, synthesis, and virological assays of new chemical entities are required to improve the potency, resistance, and safety profile of PIs. In that regard, inhibitors that form polar interactions with backbone atoms of the protease have been examined (*13-15*). The rationale is that interactions of inhibitors with the protease backbone atoms are likely to be mostly maintained even if HIV-1's protease-encoding gene undergoes amino acid substitutions. Besides having moieties that have interactions with the backbone atoms, inhibitors with macrocyclic moieties that can more effectively occupy the active site cavity have also been explored (*15*). The crystal structure analysis of GRL-216, a protease inhibitor with a *bis*-tetrahydrofuran (*bis*-THF) and macrocyclic moiety showed that it not only formed several polar interactions with key protease residues, but also more effectively packed the S1′-S2′ binding pocket and formed better van der Waals (vdW) contacts with Val82 and Ile84 than did darunavir (*15,16*). Design and synthesis of inhibitors with more interactions with the protease flaps have also been explored. GRL-078 and GRL-079 have an alkylamine at the C-5 position of the P2-tetrahydropyrano-tetrahydrofuran (Tp-THF) and have a P2′ alkyl-aminobenzothiazole (Abt) moiety (*17*). These P2 and P2′ substituents form additional vdW interactions with the protease flaps, and are probably responsible for their impressive potencies against HIV_{NL4-3} and multi-drug-resistant HIV-1 variants (*17*). We reported GRL-044, a PI with a P2-Tp-THF, P1-methoxybenzene, and P2'-isopropyl-aminobenzothiazole (Ip-Abt) (*18*). The antiviral EC_{50} values of GRL-044 against wild-type $HIV\text{-}1_{NL4-3}$ and HIV-2 were 0.0028 and 0.0004 nM,

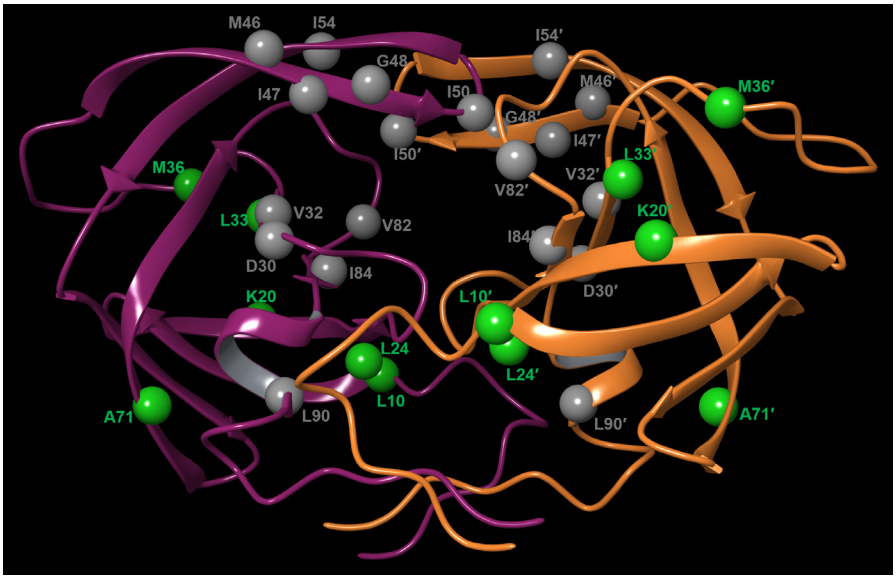

Figure 1. Drug resistance-associated mutations in HIV-1 protease. The drug-resistance-associated mutations are shown by small spheres corresponding to the locations of the alpha carbons. Mutations that are in general classified as major are shown in gray spheres and those generally classified as minor are shown in green spheres. The protease ribbons are shown in orange and maroon colors.

respectively. GRL-044 had antiviral EC_{50} values ranging from 0.065 to 19 nM against various PI-resistant HIV-1 variants (*18*). In selection assays, the emergence of HIV-1 variants resistant to GRL-044 was significantly delayed compared to that against DRV (18). Structural analyses showed that the larger size of GRL-044 over DRV, enabled GRL-044 to occupy a larger volume of the active-site hydrophobic cavity in the protease and contributed to the greater potency of GRL-044 against HIV-1 (*18*) (Figure 2 A-D). GRL-121 and GRL-142 were discovered by further optimizing the interactions of these inhibitors with HIV-protease (*19,20*). Both GRL-121 and GRL-142 have a P2 moiety consisting of 6-5-5-ring-fused crown-like tetrahydropyranofuran (Tp-THF) and a P2′ moiety consisting of a cyclopropyl aminobenzothiazole (Cp-Abt) moiety. The only difference between these inhibitors is in the P1 moiety: GRL-121 has a phenyl group whereas GRL-142 has a *bis*-fluorobenzyl group. While GRL-121 had impressive anti-HIV activity (*20*), GRL-142 had much better potency against wild-type and many drug-resistant variants with IC_{50} values in attomolar to picomolar ranges (*19*). X-ray crystal structure showed that GRL-142 had polar and non-polar interactions with many protease residues, and these interactions must be responsible for its strong binding to the active site in HIV-1 protease (Figure 3 A and B). GRL-142 was initially discovered as a protease inhibitor, but subsequent research demonstrated that it also had activity against HIV-1 integrase (*21*). The activity of GRL-142 against two viral proteins (protease and integrase) essential in the HIV-replication life-cycle is likely responsible for its attomolar to nanomolar activity against many HIV-1 variants. We further explored the effects of the number and location of fluorine substituents on the P1-phenyl moiety and replacement of Cp-Abt of GRL-142 with isopropyl-aminobenzothiazole (Ip-Abt) or isopropyl-aminobenzoxazole (Ip-Abo) as a P2′ ligand (*22*). While these molecules had very good activity against wild-type and many drug-resistant variants, they were not better than those of GRL-142 (*22*).

Darunavir and Val32Ile substitution in HIV-1 protease

DRV is the most widely used PI and has a high genetic barrier against the development of resistance by HIV-1. The presence of multiple mutations is often needed for HIV to develop resistance against DRV. It was reported that the combination of V32I, L33F, I54M and I84V substitutions (Figure 1) in the protease developed high resistance levels against DRV (*23*). HIV-1 having only V32I substitution is sensitive to DRV, in line with structural studies that show that Ile32 has a better vdW contact with DRV than does Val32 (*24*). Moreover, HIV-1 with V32I substitution had reduced viral fitness. We found that V32I substitution rarely occurs in HIV-1 protease but when it does occur, it triggers HIV-1 to accumulate additional substitutions such as L33F, I54M and I84V that allow HIV-1 to develop high-level resistance against DRV (*24*). Since DRV is the only PI once recommended as a first-line therapy, the study suggested that for patients already having V32I substitution, initiating or continuing DRV therapy might be potentially problematic.

Mechanisms of HIV-1's resistance to protease inhibitors

Crystal structures of HIV-1 protease and the PIs have been instrumental in deciphering the structural basis of drug resistance (*25-27*). These structures have illuminated the changes in interactions between the PIs

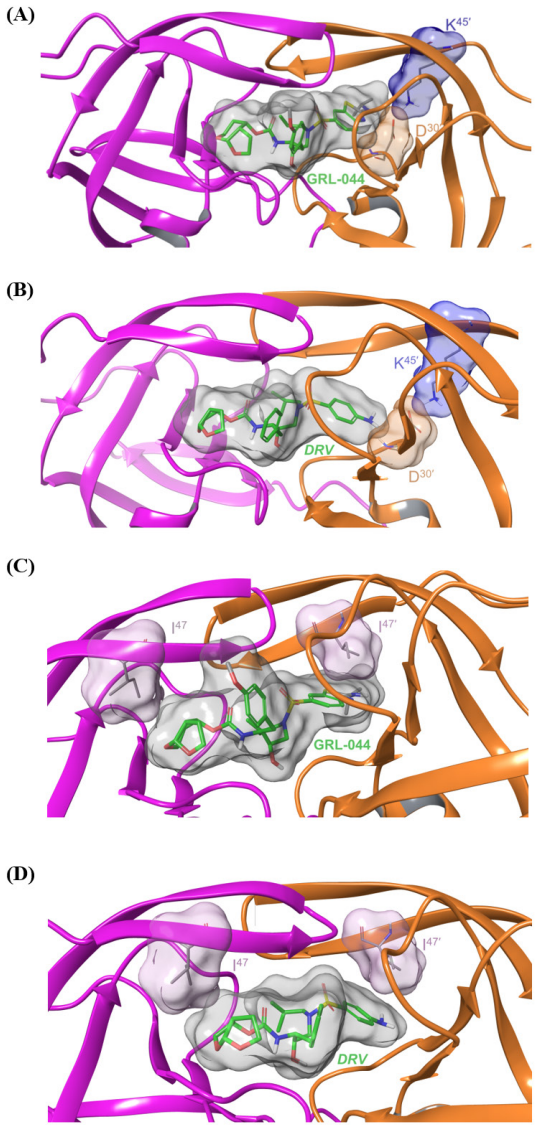

Figure 2. van der Waals surface interactions of GRL-044 and DRV. Comparison of the vdW surface interactions of GRL-044 and DRV with selected protease residues is shown in panels **A-D**. GRL-044 and DRV surfaces are in gray. D30', K45', and I47 surfaces are in orange, blue and plum, respectively. (Figure reproduced from Aoki *et al*. *Glob Health Med*. 2019; 1:36-48.)

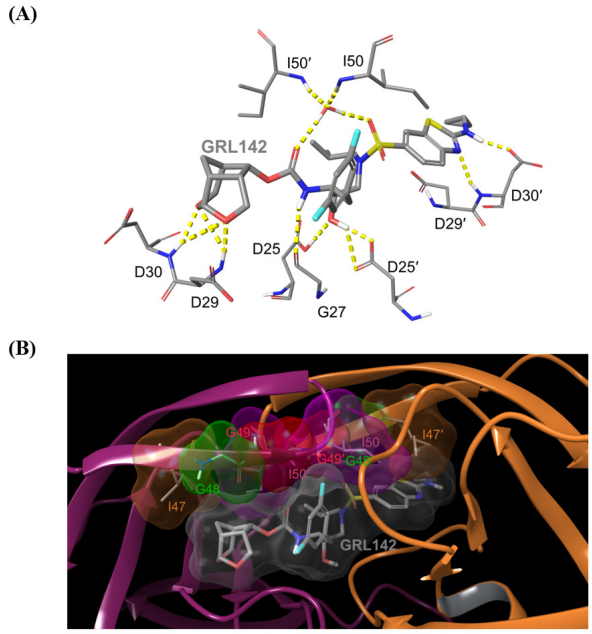

Figure 3. Polar and nonpolar interactions of GRL-142. Panel **A**: The hydrogen bond interactions (yellow dashed lines) of GRL-142, a highly potent PI, with active site residues are shown. Panel **B**: The van der Waals surface interactions of GRL-142 with HIV-1 protease flap residues are shown. The surface of GRL-142 is shown in gray; the surfaces of I47, G48, G49 and I50 are shown in aquamarine, green, red and magenta, respectively.

and HIV-1 protease with various substitutions. Pawar *et al*. determined the x-ray structure of GRL-1111 with HIV-1 protease with V32I, I47V and V82I substitutions (*26*). Though there is a significant loss of activity for the triple mutant (K_i: 31.4 ± 3.9 nm) compared to inhibition of PR_{wt} (K_i: 2 to10 pm), the x-ray-derived structure of GRL-1111 with the triple mutant protease showed only minor changes in interactions compared to interactions with PR_{wt} (*26*). Ghosh *et al*. overlayed the crystal structure of GRL-06579 with PR_{wt} against mutant proteases having two to eleven substitutions (*25*). These mutant proteases were crystalized with different PIs. Despite of the differences in inhibitors and protease amino acid sequences, the overlay of the alpha-carbon positions of the protease backbone produced root mean square deviation (RMSD) in the range of 0.5 -1.1 Å (*25*). This and other studies have shown that there is minimal structural fluctuation in the secondary structures of inhibitor-bound wild-type and drug-resistance-associated proteases. However, the side-chain configurations and contacts of these residues with the inhibitor are different between the wild-type and mutant proteases.

Recently, for an experimental PI and a keto-DRV-bound HIV-1 protease, a combination of x-ray and neutron diffraction was used to unambiguously assign the position of hydrogen atoms and the protonation/deprotonation states of the catalytic aspartates enabling differentiation of the tetrahedral intermediate as an oxyanion or a gem-diol (*28*). The study provided important insight on peptide hydrolysis catalyzed by HIV-1-protease and on the design of more potent inhibitors to overcome HIV-1 resistance (*28*).

Molecular dynamics (MD) simulations have shed light on the dynamical features of the protease-inhibitor complexes. They have helped understand the local variations and perturbations in the structural interactions. Foulkes-Murzycki *et al.* carried out MD simulations and proposed that the distal residues, many of which are in the hydrophobic core of the protease with limited solvent accessibility can easily exchange one hydrophobic vdW contact between PR_{wt} residues for another vdW contact between mutated residues with negligible energetic penalty (*29*). They postulated that changes in these hydrophobic contacts might be responsible for conformational changes in the protease (*29*). Rana *et al.* carried out MD and binding energy calculations to study the effect of M46I mutation on generating resistance against SQV (*30*). This resistance-associated mutation has clinically been observed in certain populations. They observed that M46I mutation induced wider opening of the protease flaps and significantly decreased the vdW interactions and free energy of binding of SQV to PR_{M46I} compared to PR_{wt} (*30*).

Wong-Sam *et al*. studied a protease with 10 mutations (L10F, V32I, M46I, I54L, L63P, A71V, L76V, I84V, L89V and L90M) that show resistance to LPV and DRV (*27*). They generated crystals of an apo form of this protease, and one bound to GRL-519, a potent experimental PI. The apo crystal structure of the mutant protease shows a highly curled flap conformation, and MD simulations showed extremely large fluctuations of the protease flaps (*27*). The total number of hydrogen bonds and non-polar interactions were identical for GRL-519 complexes with wild-type and the 10-mutant protease. However, the nature of interactions were different at the active site, flap-core interface, hydrophobic core, hinge region and the 80s loop (*27*). The study highlighted that mutations in highly resistant protease variants work cooperatively to reduce binding and generate resistance against PIs.

Discussion

As per the UN AIDS fact sheet (*https://www.unaids.org/en/resources/fact-sheet*), nearly forty million people globally were living with HIV-1 in 2023 out of which nearly thirty-one million had access to antiretroviral therapy. Protease inhibitors along with other classes such as nucleoside and non-nucleoside reverse transcriptase, fusion, attachment, and integrase strand transfer inhibitors have made significant impact in the quality of life and longevity of HIV-1-infected individuals. Still there is a long way to go. Darunavir, the last approved PI, has been in clinical use for more than fifteen years. Recently, it is used in combination with other nucleoside and/or integrase inhibitors (*4*). Though DRV has a high genetic

barrier against the emergence of resistance, mutations can still accumulate and decrease its potency (*9*). A lot of progress has been made in simplifying the dosage intervals and requirements. The recent FDA approval of lenacapavir, a long-acting HIV-1 capsid inhibitor which can be administered subcutaneously, has increased the available options (*31*). One of the continuing challenges with protease inhibitors is that most of them are readily metabolized by cytochrome P450-3A4. Ritonavir is a potent inhibitor of cytochrome P450-3A4, and is used as a pharmacokinetic booster when another P450-3A4-sensitive PI is administered. A side effect of ritonavir is that it causes a bad taste in the mouth – a factor that might also have negatively impacted wider adoption of nirmatrelvir, an inhibitor targeting the main protease of SARS-CoV-2 (*32*). A new anti-HIV-1 protease inhibitor not only needs to have significantly better potency against drug-resistant variants, but needs to have no toxicity and favorable pharmacokinetics and dosing requirements to be considered for clinical utility.

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Adverse reactions to mRNA COVID-19 vaccine in people with allergies in Japan

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Abstract: mRNA vaccines emerged as a new therapeutic modality during the COVID-19 pandemic. Individuals with allergies often experience anxiety about potential adverse reactions to these vaccines. This study aims to elucidate the relationship between adverse reactions and various allergies, asthma, or atopic disorders. Data from approximately 20,000 Japanese healthcare workers participating in a prospective cohort study were analyzed. The number of vaccinated individuals was 19,792 (first dose), with systemic reactions occurring in 35.8% after the first dose and 75.3% after the second dose. Participants with allergies were categorized into groups: food and/or drug allergies (n = 806), asthma and/or atopic disorders (n = 2,370), asthma (both past medical history [PMH] and present illness [PI]) (n = 1,983), and atopic disorders (PI) (n = 567). Most systemic reactions in those with food and/or drug allergies occurred within the first three days of vaccination. Logistic regression analysis showed that food and/or drug allergies, asthma (PMH and PI), and asthma and/or atopic disorders were significantly associated with systemic reactions (odds ratios [95% confidence interval]: 1.65 [1.43-1.91], 1.36 [1.23-1.49], and 1.32 [1.21-1.45], respectively, for the first dose). These findings suggest the risk of systemic reactions after COVID-19 vaccination in individuals with the specified allergies, potentially contributing to vaccine hesitancy. Medical professionals should clearly communicate the risks and benefits of vaccination to those with allergies to alleviate their concerns. Additionally, our study's data may be useful for making decisions whether or not to get vaccinated in those with allergies and inform the development of future mRNA vaccines.

***Keywords*:** vaccine safety, vaccine hesitancy, asthma, atopy, food allergy, drug allergy

Introduction

The COVID-19 pandemic accelerated the development of vaccines, leading to the rise of mRNA vaccines as a promising new modality distinct from traditional vaccines such as recombinant protein vaccines, inactivated vaccines, live attenuated vaccines, and toxoid vaccines. mRNA COVID-19 vaccines have been effective in reducing transmission globally and have shown promise against other viruses, such as respiratory syncytial virus (*1*), and are being evaluated for influenza (*2*). Moreover, multiple mRNA cancer vaccines are under clinical trials for various cancers (*3*).

Initially, concerns about the safety and efficacy of the newly developed COVID-19 vaccines were prevalent, especially among individuals with a history of allergies or comorbidities. As vaccination campaigns progressed and more safety data were gathered (*4*), large-scale international studies confirmed the good safety profile of COVID-19 vaccines (*5,6*). However, there is a lack of comprehensive data on the safety of mRNA vaccines for individuals with allergic diseases in Japan, despite several small-scale studies. According to the Rheumatology and Allergy Control Committee Report, about one in two Japanese individuals has some form of allergy (*7*). Anaphylaxis, a severe allergic reaction, is one of the most clinically significant adverse reactions following COVID-19 vaccination. Studies (*8,9*) showed that most individuals who experienced anaphylaxis after vaccination had a history of allergies to foods, medical products, or insect stings. Additionally, higher incidences of certain adverse reactions (*e.g.*, vomiting, local swelling, redness, and pain) (*10*) or prolonged reactions (*e.g.*, fatigue, malaise, headache, and chills) (*11*) have been observed in allergic individuals compared to those without allergies. These reports could contribute

to vaccine hesitancy among those with a history of allergies.

Given these concerns, we investigated the incidence of local and systemic reactions to COVID-19 mRNA vaccination in individuals with various allergies (food and/or drug allergies, asthma [past medical history (PMH) and present illness (PI)], asthma and/or atopic disorders, and atopic disorders) using data from a large-scale cohort study of hospital-based healthcare workers in Japan from February 17, 2021 to January 30, 2022.

This report focuses on adverse reactions following BNT162b2 vaccination in individuals with food and/or drug allergies, asthma (PMH and PI), atopic disorders (PI), and asthma and/or atopic disorders.

Materials and Methods

Original prospective cohort study and population

An original prospective cohort study, titled "Cohort Survey at the Beginning of SARS-CoV-2 Vaccination in Japan" (For details, see UMIN000073345 (*12*)), aimed to evaluate the safety of the BNT162b2 mRNA COVID-19 Vaccine (Pfizer-BioNTech) in healthcare workers. Participants included doctors, nurses, pharmacists, medical technologists, radiological technologists, physical therapists, care workers, clerks, and other staff from the National Hospital Organization (52 sites), Japan Community Health Care Organization (27 sites), and Japan Organization of Occupational Health and Safety (21 sites) member hospitals. The study was part of the "Emerging/Re-emerging Infectious Diseases and Vaccination Policy Promotion Research Project" of the Ministry of Health, Labor and Welfare (MHLW) of Japan, conducted between February 17, 2021 and January 30, 2022 (preparation ongoing as of June 10, 2024). Approximately 20,000 participants completed their primary vaccination series, consisting of two doses at least three weeks apart.

Data collection from study participants

In the prospective cohort study (*12*), participants who received either the first dose only or both doses completed daily symptom diaries for eight days (Day 1 to Day 8) after each dose and continued for up to 28 days after the last dose. Diaries were collected from 19,792 participants after the first dose and 19,592 participants after the second dose. Post-vaccination adverse reactions recorded included injection-site reactions (redness, swelling, induration, pain, warmth, and pruritus), systemic reactions (headache, malaise, and rhinorrhea), and body temperature. If symptoms persisted beyond Day 8, participants continued recording until resolution. Each participant's history of food allergy, drug allergy, asthma, and atopic disorders was obtained before vaccination.

Subgroup analysis

Participant characteristics were analyzed, and the incidence rate of each reaction after each dose was calculated by sex and age group. Adverse reactions occurring between Day 1 and Day 8 post-vaccination were used for subgroup analysis to examine differences in the time-to-onset and duration of each reaction based on the presence or absence of food and/or drug allergies or allergic diseases (asthma and/or atopic disorders). Bar charts were created for each reaction to illustrate any significant differences between individuals with food and/or drug allergies or asthma and/or atopic disorders and those without.

Odds ratios from logistic regression model

To assess the associations of food and/or drug allergies, asthma, atopic disorders, or asthma and/or atopic disorders with local and systemic reactions after BNT162b2 vaccination, the odds ratio (OR) with a 95% confidence interval (CI) for each reaction was calculated using a logistic regression model.

Statistical analysis

Categorical variables are presented as numbers and percentages. Reaction duration is defined as the time between symptom onset and resolution. Pearson's chi-squared test or Fisher's exact test was used to determine statistically significant differences in reactions between individuals with and without food and/or drug allergies or asthma and/or atopic disorders. A p-value less than 0.05 was considered statistically significant. Logistic regression analysis, adjusted for age and sex, was used to calculate the OR with a corresponding 95% CI to describe the association of food and/or drug allergies, asthma, atopic disorders, or asthma and/or atopic disorders with each reaction. All statistical analyses were performed using R statistical software (version 4.0.3).

Ethical statement

The study was approved by the Tokushukai Group Ethics Committee (TGE01643-701) prior to initiation and conducted in accordance with the principles of the Declaration of Helsinki (trial registration number: UMIN000043377). Informed consent was obtained from all participants in the original prospective cohort study.

Results

Participant characteristics

The study included 19,792 participants who received the first dose and 19,592 who received the second dose of the vaccine. Among those receiving the first dose, 13,108

(66.2%) were female. Over 90% of participants who received both doses fell into the age groups 20-29, 30-39, 40-49, and 50-59 years. Participants with allergies were categorized into four groups: food and/or drug allergies (806 for the first dose and 791 for the second dose), asthma and/or atopic disorders (2,370 and 2,335), asthma (PMH and PI) (1,983 and 1,955), and atopic disorders (567 and 556). Among the 13,108 vaccinated females, 4.7% had a history of food and/or drug allergies (compared to 2.8% of the 6,684 vaccinated males). A slightly higher percentage (5.3%) of individuals with food and/or drug allergies was observed in the 50-59 years group (Table 1).

Local and systemic reactions to COVID-19 vaccine primary series

Incidence of adverse reactions following vaccination

Local reactions, including redness, swelling, induration, pain (92.0%), warmth, and pruritus, occurred in 92.5% of participants after the first dose. Systemic reactions, including headache, malaise, and rhinorrhea, were observed in 35.8% of participants after the first dose. For the second dose, local reactions, including pain (89.5%), occurred in 90.7% of participants, while systemic reactions occurred in 75.3% of participants. The incidence of specific systemic reactions increased significantly after the second dose compared to the first: headache (21.3% to 53.1%), malaise (23.2% to 68.8%), fever ≥ 37.5°C (3.3% to 38.1%), and fever ≥ 38.0°C (0.9% to 21.3%) (Table 2).

Subgroup analysis of adverse reactions by age and sex

Adverse reactions were more common in females than males after each dose. Participants aged 70 years and older experienced fewer adverse reactions compared to younger participants. In the 20-29 years age group, fever ≥ 37.5°C was reported more frequently after the second dose than the first dose (49.7% *vs.* 5.8%), with incidence decreasing with age. Systemic reactions (headache, malaise, and rhinorrhea) after the second dose were more prevalent in the 20-29 and 30-39 years age groups (approximately 80%) compared to other age groups (Table 3).

Subgroup analysis of food and/or drug allergies

Among vaccinated participants, 806 and 791 individuals had a history of food and/or drug allergies for the first and second doses, respectively. Local reactions were similar between participants with and without food and/or drug allergies. However, systemic reactions (headache, malaise, and rhinorrhea) were more frequent in individuals with these allergies, particularly after the second dose. The incidence of fever ≥ 37.5°C after the second dose was relatively high at 42.7% (Supplemental Table S1a, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=95*).

Table 1. History of food and/or drug allergies, asthma (PMH and PI), asthma and/or atopic disorders, and atopic disorders by sex and age group

	Sex		Age group				
First dose (n = 19,792)	Male n = 6,684 n (%)	Female n = 13,108 n (%)	20-29 n = 4,152 n (%)	30-39 n = 4,753 n (%)	40-49 n = 4,934 n (%)	50-59 n = 4,236 n (%)	60-69 n = 1,559 n (%)	≥ 70 n = 158 n (%)
History of food and/or drug allergies* (n = 806)	189 (2.8)	617 (4.7)	135 (3.3)	152 (3.2)	223 (4.5)	226 (5.3)	65 (4.2)	5 (3.2)
History of asthma and/or atopic disorders** (n = 2,370)	765 (11.4)	1605 (12.2)	542 (13.1)	636 (13.4)	616 (12.5)	430 (10.2)	131 (8.4)	15 (9.5)
Asthma (PMH and PI) (n = 1,983)	633 (9.5)	1350 (10.3)	418 (10.1)	510 (10.7)	521 (10.6)	393 (9.3)	126 (8.1)	15 (9.5)
Atopic disorders (PI) (n = 567)	195 (2.9)	372 (2.8)	175 (4.2)	185 (3.9)	134 (2.7)	64 (1.5)	9 (0.6)	0
Second dose (n = 19,592)	Male n = 6,630 n (%)	Female n = 12,962 n (%)	20–29 n = 4,075 n (%)	30–39 n = 4,702 n (%)	40–49 n = 4,887 n (%)	50–59 n = 4,218 n (%)	60–69 n = 1,553 n (%)	≥70 n = 157 n (%)
History of food and/or drug allergies* (n = 791)	188 (2.8)	603 (4.7)	130 (3.2)	149 (3.2)	219 (4.5)	223 (5.3)	65 (4.2)	5 (3.2)
History of Asthma and/or atopic disorders** (n = 2,335)	757 (11.4)	1578 (12.2)	527 (12.9)	629 (13.4)	606 (12.4)	428 (10.1)	131 (8.4)	14 (8.9)
Asthma (PMH and PI) (n = 1,955)	626 (9.4)	1329 (10.3)	406 (10.0)	504 (10.7)	513 (10.5)	392 (9.3)	126 (8.1)	14 (8.9)
Atopic disorders (PI) (n = 556)	193 (2.9)	363 (2.8)	170 (4.2)	183 (3.9)	131 (2.7)	63 (1.5)	9 (0.6)	0

*: 1) food allergy to wheat, egg, shrimp, and crab, among others, and 2) drug allergy to penicillin, contrast media, fluoroquinolones, *etc.* **: Multiple choices allowed. PMH: past medical history, PI: present illness.

Table 2. Incidence of local and systemic reactions after each dose

Local and systemic reactions	First dose *n* = 19,792 *n* (%)	Second dose *n* = 19,592 *n* (%)
Local reactions	18,316 (92.5)	17,765 (90.7)
Redness	2,742 (13.9)	3,123 (15.9)
Swelling	2,470 (12.5)	2,758 (14.1)
Induration	2,106 (10.6)	1,972 (10.1)
Pain	18,203 (92.0)	17,534 (89.5)
Warmth	2,544 (12.9)	3,722 (19.0)
Pruritus	1,585 (8.0)	2,335 (11.9)
Systemic reactions	7,078 (35.8)	14,759 (75.3)
Headache	4,225 (21.3)	10,411 (53.1)
Malaise	4,584 (23.2)	13,478 (68.8)
Rhinorrhea	2,020 (10.2)	2,829 (14.4)
Fever ≥ 37.5 ℃	654 (3.3)	7,470 (38.1)
Fever ≥ 38.0 ℃	174 (0.9)	4,179 (21.3)

Local reactions include redness, swelling, induration, pain, warmth, and pruritus. Systemic reactions include headache, malaise, and rhinorrhea. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental Tables S2a and S2b.

We investigated the relationship between food and/or drug allergies and the onset and duration of adverse reactions. Most systemic reactions occurred within the first three days after vaccination, with some lasting more than four days. Significant differences in the onset and duration of malaise and headache, as well as the duration of rhinorrhea, were observed between those with and without food and/or drug allergies. Fevers (both ≥ 37.5°C and ≥ 38.0°C) generally occurred within 1-3 days post-vaccination, with a small percentage lasting beyond four days (Figures 1A, 1B, 1C and Figure 2A, 2B, and 2C).

Subgroup analysis of asthma and/or atopic disorders

Among vaccinated participants, 2,370 and 2,335 individuals had a history of asthma and/or atopic disorders for the first and second doses, respectively. Systemic reactions were more frequent in individuals with these conditions compared to those without (Supplemental Table S1b, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=95*). Most local and systemic reactions occurred within the first three days post-vaccination, with some reactions persisting beyond four days. Significant differences in the onset and duration of malaise and headache were observed after the second dose between individuals with and without asthma and/or atopic disorders. Most fevers (≥ 37.5°C) occurred within 1-3 days post-vaccination, with a small percentage lasting beyond four days for both doses (Figures 3A, 3B, 3C and Figures 4A, 4B, and 4C).

Subgroup analysis using logistic regression models for food and/or drug allergies, asthma, atopic disorders, and asthma and/or atopic disorders

Food and/or drug allergies were associated with systemic reactions (ORs [95% CI]: 1.65 [1.43-1.91] for the first dose and 1.53 [1.26-1.85] for the second dose). Asthma and/or atopic disorders were associated with systemic reactions (ORs: 1.32 [1.21-1.45] for the first dose and 1.25 [1.12-1.40] for the second dose). Asthma (PMH and PI) was associated with systemic reactions (ORs: 1.36 [1.23-1.49] for the first dose and 1.30 [1.15-1.46] for the second dose). Atopic disorders (PI) were not associated with nearly all local and systemic reactions. Food and/or drug allergies, asthma and/or atopic disorders, and asthma (PMH and PI) were associated with fever ≥ 37.5°C (Table 4).

Discussion

The original prospective cohort study, "Cohort Survey at the Beginning of SARS-CoV-2 Vaccination in Japan", was based on Japan's criteria for reporting adverse reactions following COVID-19 vaccination. These criteria were developed by doctors involved in a 2006 clinical trial for a new influenza vaccine and have since been used to evaluate vaccine reactions in Japan. In contrast, the FDA has its own independent criteria, leading to differences in grading the severity of pain, redness, swelling, induration, and fever between Japan and the FDA (Supplemental Tables S2a and S2b for further details, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=95*).

As a preventive measure against COVID-19, vaccination efforts accelerated globally until Spring 2023. Despite the demonstrated safety and effectiveness of COVID-19 vaccines, high incidences of adverse reactions, such as injection site pain, headache, and malaise, are well-documented (*13,14*). Severe allergic reactions to COVID-19 vaccines are rare but can occur (*15*). Safety concerns about vaccination are a significant factor in vaccine hesitancy.

This study examined the safety of the BNT162b2 vaccine in the Japanese population. Most reactions to the BNT162b2 vaccine reported in our study were mild and transient, consistent with the known safety profile of the vaccine (*14,16*). Fever was more common after the second dose and among younger age groups compared to older participants (60-69 and ≥ 70 years). A notable finding from our data, compared to a US online cohort study with 19,586 participants (*17*), was the incidence of fatigue. The US study reported higher fatigue rates after the second dose in both males and females (female: ~35% for the first dose and 60% for the second dose; male: ~22% and 45%). Our study showed a similar trend, with fatigue after the second dose occurring almost three times more often than after the first dose in Japan (female: 26.2% and 73.5%; male: 17.1% and 59.6%). Additionally, this study explored the relationships between food and/or drug allergies, asthma, and atopic disorders with local and systemic reactions. Consistent with other studies (*18,19*), food and/or drug allergies and asthma and/or atopic disorders were more common in

Table 3. Local and systemic reactions after each dose by sex and age group

	Sex		Age group					
First dose (n = 19,792)	Male n = 6,684 n (%)	Female n = 13,108 n (%)	20-29 n = 4,152 n (%)	30-39 n = 4,753 n (%)	40-49 n = 4,934 n (%)	50-59 n = 4,236 n (%)	60-69 n = 1,559 n (%)	≥ 70 n = 158 n (%)
Local reactions (n = 18,316)	5,955 (89.1)	12,361 (94.3)	3,863 (93.0)	4,429 (93.2)	4,649 (94.2)	3,883 (91.7)	1,368 (87.7)	124 (78.5)
Redness (n = 2,742)	523 (7.8)	2,219 (16.9)	572 (13.8)	655 (13.8)	731 (14.8)	565 (13.3)	200 (12.8)	19 (12.0)
Swelling (n = 2,470)	532 (8.0)	1,938 (14.8)	574 (13.8)	595 (12.5)	627 (12.7)	499 (11.8)	160 (10.3)	15 (9.5)
Induration (n = 2,106)	460 (6.9)	1,646 (12.6)	401 (9.7)	538 (11.3)	549 (11.1)	456 (10.8)	142 (9.1)	20 (12.7)
Pain (n = 18,203)	5,933 (88.8)	12,270 (93.6)	3,843 (92.6)	4,429 (93.2)	4,610 (93.4)	3,839 (90.6)	1,361 (87.3)	121 (76.1)
Warmth (n = 2,544)	425 (6.4)	2,119 (16.2)	748 (18.0)	629 (13.2)	585 (11.9)	454 (10.7)	119 (7.6)	9 (5.7)
Pruritus (n = 1,585)	174 (2.6)	1,411 (10.8)	383 (9.2)	376 (7.9)	387 (7.8)	326 (7.7)	106 (6.8)	7 (4.4)
Systemic reactions (n = 7,078)	1,689 (25.3)	5,389 (41.1)	1,523 (36.7)	1,853 (39.0)	1,886 (38.2)	1,394 (32.9)	394 (25.3)	28 (17.6)
Headache (n = 4,225)	768 (11.5)	3,457 (26.4)	968 (23.3)	1,106 (23.3)	1,135 (23.0)	806 (19.0)	196 (12.6)	14 (8.9)
Malaise (n = 4,584)	1,146 (17.1)	3,438 (26.2)	1,042 (25.1)	1,177 (24.8)	1,205 (24.4)	902 (21.3)	247 (15.8)	11 (7.0)
Rhinorrhea (n = 2,020)	492 (7.4)	1,528 (11.7)	372 (9.0)	529 (11.1)	543 (11.0)	427 (10.1)	141 (9.0)	8 (5.1)
Fever > 37.5°C (n = 654)	127 (1.9)	527 (4.0)	239 (5.8)	199 (4.2)	144 (2.9)	56 (1.3)	16 (1.0)	0
Fever > 38.0°C (n = 174)	40 (0.6)	134 (1.0)	71 (1.7)	43 (0.9)	41 (0.8)	14 (0.3)	5 (0.3)	0
Second dose (n = 19,592)	Male n = 6,630 n (%)	Female n = 12,962 n (%)	20–29 n = 4,075 n (%)	30–39 n = 4,702 n (%)	40–49 n = 4,887 n (%)	50–59 n = 4,218 n (%)	60–69 n = 1,553 n (%)	≥70 n = 157 n (%)
Local reactions (n =17,765)	5,703 (86.0)	12,062 (93.1)	3,691 (90.6)	4,280 (91.0)	4,509 (92.3)	3,830 (90.8)	1,342 (86.4)	113 (72.0)
Redness (n = 3,123)	566 (8.5)	2,557 (19.7)	567 (13.9)	758 (16.1)	840 (17.2)	706 (16.7)	236 (15.2)	16 (10.2)
Swelling (n = 2,758)	607 (9.2)	2,151 (16.6)	575 (14.1)	658 (14.0)	720 (14.7)	581 (13.8)	204 (13.1)	20 (12.7)
Induration (n = 1,972)	441 (6.7)	1,531 (11.8)	323 (7.9)	478 (10.2)	529 (10.8)	475 (11.3)	152 (9.8)	15 (9.6)
Pain (n = 17,534)	5,644 (85.1)	11,890 (91.7)	3,645 (89.4)	4,227 (89.9)	4,446 (91.0)	3,789 (89.8)	1,317 (84.8)	111 (70.7)
Warmth (n = 3,722)	722 (10.9)	3,000 (23.1)	967 (23.7)	893 (19.0)	861 (17.6)	762 (18.1)	216 (13.9)	23 (14.6)
Pruritus (n = 2,335)	247 (3.7)	2,088 (16.1)	424 (10.4)	533 (11.3)	585 (12.0)	596 (14.1)	183 (11.8)	14 (8.9)
Systemic reactions (n = 14,759)	4,256 (64.2)	10,503 (81.0)	3,287 (80.7)	3,826 (81.4)	3,795 (77.7)	2,969 (70.4)	831 (53.5)	51 (32.5)
Headache (n = 10,411)	2,444 (36.9)	7,967 (61.5)	2,499 (61.3)	2,769 (58.9)	2,667 (54.6)	1,985 (47.1)	467 (30.1)	24 (15.3)
Malaise (n = 13,478)	3,950 (59.6)	9,528 (73.5)	3,063 (75.2)	3,505 (74.5)	3,485 (71.3)	2,658 (63.0)	724 (46.6)	43 (27.4)
Rhinorrhea (n = 2,829)	671 (10.1)	2,158 (16.6)	644 (15.8)	723 (15.4)	709 (14.5)	583 (13.8)	162 (10.4)	8 (5.1)
Fever ≥37.5°C (n = 7,470)	1,976 (29.8)	5,494 (42.4)	2,026 (49.7)	2,116 (45.0)	1,833 (37.5)	1,227 (29.1)	256 (16.5)	11 (7.0)
Fever ≥38.0°C (n = 4,179)	1,074 (16.2)	3,105 (24.0)	1,216 (29.8)	1,209 (25.7)	1,000 (20.5)	643 (15.2)	106 (6.8)	5 (3.2)

Local reactions include redness, swelling, induration, pain, warmth, and pruritus. Systemic reactions include headache, malaise, and rhinorrhea. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental S2a and S2b.

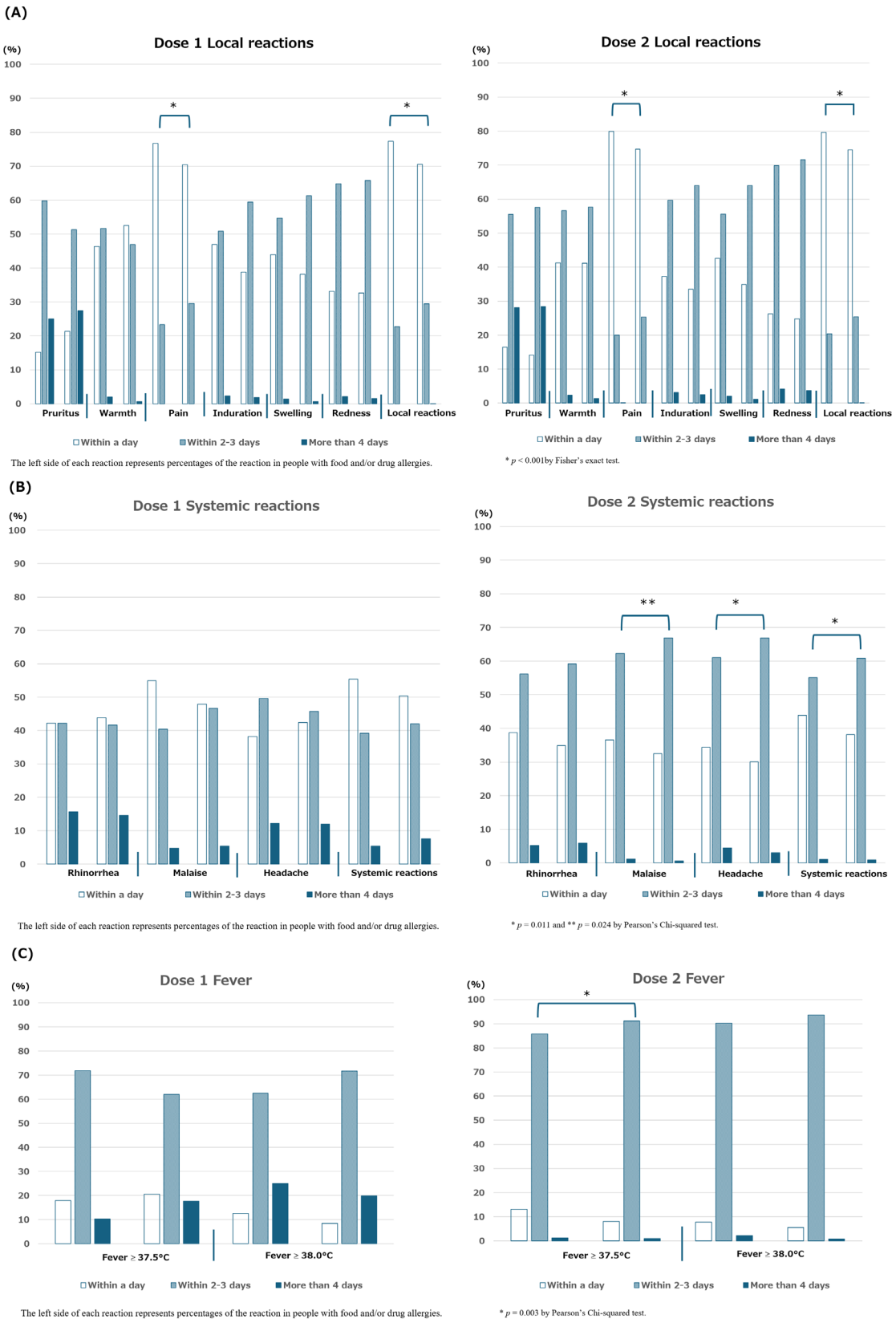

Figure 1. Time-to-onset of adverse reactions in participants with a history of food and/or drug allergies. (A) Local reactions include redness, swelling, induration, pain, warmth, and pruritus. **(B)** Systemic reactions include headache, malaise, and rhinorrhea. **(C)** Fever. Allergies include 1) food allergy to wheat, egg, shrimp, and crab, *etc.*, and 2) drug allergy to penicillin, contrast media, and fluoroquinolones, *etc*. Pearson's chi-squared test or Fisher's exact test is used, as appropriate. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental Tables S2a and S2b.

Figure 2. Duration of adverse reactions in participants with a history of food and/or drug allergies. (A) Local reactions include redness, swelling, induration, pain, warmth, and pruritus. **(B)** Systemic reactions include headache, malaise, and rhinorrhea. **(C)** Fever. Allergies include 1) food allergy to wheat, egg, shrimp, and crab, among others, and 2) drug allergy to penicillin, contrast media, and fluoroquinolones, among others. Pearson's chi-squared test or Fisher's exact test is used, as appropriate. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental Tables S2a and S2b.

Figure 3. Time-to-onset of adverse reactions in participants with a history of asthma and/or atopic disorders. (A) Local reactions include redness, swelling, induration, pain, warmth, and pruritus. **(B)** Systemic reactions include headache, malaise, and rhinorrhea. **(C)** Fever. Pearson's chi-squared test or Fisher's exact test is used, as appropriate. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental Tables S2a and S2b.

females than males. As previously reported (*13,17,20,21*), injection site pain was the most common local reaction (92.0% for the first dose and 89.5% for the second dose). Logistic regression model results indicated that food and/or drug allergies (ORs: 1.65 for the first dose and 1.53 for the second dose), asthma and/or atopic disorders (ORs: 1.32 for the first dose and 1.25 for the second dose), and asthma (PMH and PI) (ORs: 1.36 for the first

Figure 4. Duration of adverse reactions in participants with a history of asthma and/or atopic disorders. (A) Local reactions include redness, swelling, induration, pain, warmth, and pruritus. **(B)** Systemic reactions include headache, malaise, and rhinorrhea. **(C)** Fever. Pearson's chi-squared test or Fisher's exact test is used, as appropriate. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental Tables S2a and S2b.

dose and 1.30 for the second dose) were associated with systemic reactions (headache, malaise, and rhinorrhea). These systemic reactions can significantly impact daily activities, leading people to instinctively avoid vaccines due to fear of unpleasant side effects. An internet survey on COVID-19 vaccine hesitancy in Japan (*22*)

Table 4. Associations between some types of allergies and adverse reactions

History of food and/or drug allergies	First dose OR (95% CI)	Second dose OR (95% CI)
Local reactions	1.20 (0.89-1.62)	1.05 (0.81-1.37)
Redness	1.26 (1.04-1.51)	1.34 (1.13-1.60)
Swelling	1.51 (1.25-1.82)	1.39 (1.16-1.67)
Induration	1.52 (1.25-1.85)	1.64 (1.35-2.00)
Pain	1.03 (0.79-1.36)	0.95 (0.75-1.20)
Warmth	1.54 (1.28-1.85)	1.62 (1.38-1.90)
Pruritus	1.37 (1.09-1.71)	1.50 (1.24-1.81)
Systemic reactions	1.65 (1.43-1.91)	1.53 (1.26-1.85)
Headache	1.56 (1.34-1.83)	1.37 (1.18-1.59)
Malaise	1.71 (1.47-1.99)	1.52 (1.28-1.81)
Rhinorrhea	1.62 (1.33-1.97)	1.42 (1.18-1.70)
Fever ≥ 37.5 ℃	1.60 (1.14-2.23)	1.26 (1.09-1.46)
Fever ≥ 38.0 ℃	1.20 (0.59-2.46)	1.24 (1.05-1.47)
History of asthma and/or atopic disorders	First dose OR (95% CI)	Second dose OR (95% CI)
Local reactions	1.37 (1.14-1.65)	1.19 (1.01-1.39)
Redness	1.16 (1.03-1.32)	1.21 (1.08-1.36)
Swelling	1.26 (1.11-1.42)	1.26 (1.12-1.41)
Induration	1.34 (1.18-1.52)	1.37 (1.20-1.57)
Pain	1.33 (1.11-1.59)	1.14 (0.98-1.33)
Warmth	1.16 (1.02-1.31)	1.23 (1.10-1.36)
Pruritus	1.20 (1.03-1.39)	1.13 (0.99-1.29)
Systemic reactions	1.32 (1.21-1.45)	1.25 (1.12-1.40)
Headache	1.22 (1.10-1.35)	1.24 (1.13-1.35)
Malaise	1.31 (1.18-1.44)	1.15 (1.04-1.26)
Rhinorrhea	1.37 (1.20-1.56)	1.38 (1.24-1.55)
Fever ≥ 37.5 ℃	1.28 (1.03-1.59)	1.13 (1.03-1.23)
Fever ≥ 38.0 ℃	0.75 (0.45-1.23)	1.09 (0.98-1.21)
Asthma (PMH and PI)	First dose OR (95% CI)	Second dose OR (95% CI)
Local reactions	1.41 (1.15-1.73)	1.17 (0.99-1.40)
Redness	1.20 (1.06-1.36)	1.30 (1.15-1.47)
Swelling	1.30 (1.14-1.48)	1.30 (1.14-1.47)
Induration	1.39 (1.22-1.60)	1.47 (1.28-1.69)
Pain	1.34 (1.11-1.62)	1.14 (0.97-1.34)
Warmth	1.21 (1.06-1.38)	1.26 (1.12-1.41)
Pruritus	1.28 (1.09-1.50)	1.20 (1.04-1.38)
Systemic reactions	1.36 (1.23-1.49)	1.30 (1.15-1.46)
Headache	1.28 (1.14-1.42)	1.25 (1.13-1.38)
Malaise	1.34 (1.20-1.48)	1.18 (1.06-1.32)
Rhinorrhea	1.44 (1.25-1.65)	1.41 (1.25-1.59)
Fever ≥ 37.5 ℃	1.32 (1.05-1.67)	1.16 (1.05-1.27)
Fever ≥ 38.0 ℃	0.83 (0.48-1.41)	1.13 (1.01-1.27)
Atopic disorders (PI)	First dose OR (95% CI)	Second dose OR (95% CI)
Local reactions	1.14 (0.81-1.63)	1.51 (1.07-2.14)
Redness	1.04 (0.81-1.32)	0.93 (0.73-1.19)
Swelling	1.19 (0.94-1.51)	1.19 (0.94-1.50)
Induration	1.23 (0.95-1.59)	1.04 (0.78-1.38)
Pain	1.19 (0.84-1.68)	1.41 (1.03-1.95)
Warmth	0.86 (0.66-1.12)	1.13 (0.91-1.39)
Pruritus	1.06 (0.78-1.44)	0.90 (0.68-1.20)
Systemic reactions	1.22 (1.03-1.45)	1.11 (0.90-1.38)
Headache	1.15 (0.94-1.40)	1.07 (0.89-1.27)
Malaise	1.29 (1.07-1.56)	1.15 (0.94-1.40)
Rhinorrhea	1.11 (0.85-1.45)	1.16 (0.92-1.46)
Fever ≥ 37.5 ℃	1.08 (0.71-1.64)	0.99 (0.83-1.18)
Fever ≥ 38.0 ℃	1.00 (0.44-2.28)	0.98 (0.80-1.20)

PMH: past medical history, PI: present illness, OR: odds ratio, 95% CI: 95% confidence interval. Local reactions include redness, swelling, induration, pain, warmth, and pruritus. Systemic reactions include headache, malaise, and rhinorrhea. Allergies include 1) food allergy to wheat, egg, shrimp, and crab, *etc.* and 2) drug allergy to penicillin, contrast media, and fluoroquinolones, *etc*. Japan's criteria for evaluating some adverse reactions are different from those prescribed by the Food and Drug Administration. See Supplemental Tables S2a and S2b.

analyzed 23,142 responses and found that over 70% of respondents cited concerns about adverse reactions as the primary reason for not getting vaccinated. However, our results showed that most participants, including those with allergies, completed their primary vaccination series (two doses).

While there are several large-scale studies on the BNT162b2 vaccine involving populations outside Japan, this study is significant as it focuses on a regional, large-scale cohort of approximately 20,000 Japanese healthcare workers. It examines the incidence of local and systemic reactions after the BNT162b2 vaccination in subgroups with allergies (food and/or drug allergies, asthma and/or atopic disorders, asthma [PMH and PI], and atopic disorders (PI)), highlighting the patterns of adverse reactions in Japan. However, the sample size for those aged ≥ 70 years was relatively small (158 for the first dose and 157 for the second dose), which may not provide sufficient data to fully assess adverse reaction incidence in this age group.

In our study, individuals with food and/or drug allergies, asthma, and asthma and/or atopic disorders were more likely to develop systemic adverse reactions following vaccination, though these reactions were generally not severe, such as anaphylaxis. Previous reports indicate that most anaphylaxis cases after receiving the BNT162b2 vaccine occurred in individuals with a history of food allergy, drug allergy, or insect stings (*8*). Among 25,929 individuals who received the first dose of the BNT162b2 vaccine, seven cases of anaphylaxis were reported, all of whom had a history of allergies or anaphylaxis and recovered after treatment (*9*). Although a study on a naturally hypersensitive porcine model suggested that complement activation might contribute to rare (pseudo) allergic reactions induced by COVID-19 mRNA vaccines (*23*), the mechanisms underlying these allergic reactions remain unclear.

Some highly allergic patients have successfully received COVID-19 mRNA vaccines under medical supervision (*24*). Several reports (*25,26*) indicate that individuals who experienced anaphylaxis after the first dose of the BNT162b2 vaccine were able to safely receive the second dose in a supervised setting.

The WHO Global Advisory Committee on Vaccine Safety defines immunization stress-related response (ISRR) as vaccination-induced stress and anxiety that may present as adverse events (*27*). Healthcare workers should be aware of ISRR, which can cause anxiety-induced reactions in some individuals following vaccination. To mitigate stress and anxiety related to COVID-19 vaccination, healthcare workers should clearly communicate the risks and benefits of the vaccines to those experiencing vaccine-related anxiety. In our study, all participants received the BNT162b2 vaccine shortly after its approval in Japan. As previously reported (*28*), some participants may have experienced ISRR, leading to more frequent reporting of adverse reactions during the study compared to subsequent doses.

Our study has several limitations. Firstly, a large cohort of approximately 20,000 healthcare workers is not a representative sample of the general population. Compared to the general population, healthcare workers would have the ability to find, understand, and use health-related information, and inform health-related decisions and take actions for themselves and others. This leads to a positive attitude toward vaccines in healthcare workers. On the other hand, in the general population, inadequate health literacy would lead to less interest in vaccination, having difficulty expressing what they have the symptoms or concerns, or having difficulty in symptom perception. This population bias may limit the external validity of our findings. The dataset shows more females and younger individuals, and few older adults and no children included. This selection bias may impact the study results. However, we believe this regional large-scale cohort study provides valuable insights into adverse reactions following vaccination in the Japanese population. Secondly, the self-reported nature of adverse reactions may introduce response biases. Participants might underreport or overreport symptoms, but we assume healthcare workers are more likely to report symptoms accurately and consistently. Despite the limitations of self-reporting, we believe this study achieves high reporting reliability.

mRNA technology has been used in developing vaccines for various infectious diseases (*e.g.*, CMV infection, influenza A & B, RSV infection, herpes zoster, HIV infection) and various cancers (*29*). Vaccine hesitancy, often driven by concerns about adverse reactions to new mRNA vaccines, could significantly impact the acceptance of such vaccines. The findings of this study could serve as a reference for evaluating new mRNA vaccines and aid in their acceptance by the public.

Systemic reactions were more common in individuals with food and/or drug allergies, asthma (PMH and PI), and asthma and/or atopic disorders. These reactions could significantly impact daily activities, potentially leading to vaccine hesitancy. As a way to manage and reduce stress and anxiety during vaccination consultations, healthcare workers and allergy specialists provide allergic patients with information that systemic reactions may be more common than in nonallergic patients, but most symptoms are generally mild and transient. The WHO (*30*) recommends that healthcare workers use a structured approach in motivational interviewing during vaccination consultations. To promote vaccine confidence, healthcare workers provide patients with consistent and accurate information about vaccine safety and benefits in a respectful and positive manner.

This study provides valuable data for medical and allergy specialists to explain vaccine-related adverse reactions to individuals with allergies, thereby reducing stress and anxiety. Additionally, as mRNA technology continues to be used in developing vaccines for various infectious diseases and various cancers, the results of this study could help enhance the acceptance of new mRNA vaccines. Real-world vaccination data for children and older adults need to be collected and analyzed to further the development of new mRNA vaccines in the future.

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Imaging of unicentric hyaline-vascular variant of Castleman disease: Emphasis on perilesional fat stranding and fatty proliferation

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Abstract: The hyaline-vascular variant of Castleman disease (HVCD) is relatively uncommon and demonstrates no specific clinical or laboratory findings; therefore, its preoperative diagnosis warrants a radiological evaluation. This study aimed to review imaging findings of HVCD, focusing on perilesional fat stranding and fatty proliferation. Patients with a pathologically confirmed HVCD diagnosis who had undergone CT were recruited from five hospitals from January 2000 to March 2023. Three experienced radiologists assessed CT findings, including lesion location, lesion size, calcification, enhanced pattern, feeding vessel visualization, and arterial enhancement. Perilesional fat stranding, fatty proliferation, neighboring fascial thickening, and surrounding lymphadenopathy were the primary targets of analysis. Moreover, the intensities and apparent diffusion coefficient (ADC) values on MRI and the maximum standardized uptake value (SUVmax) on ^{18}F-fluorodeoxyglucose positron emission tomography (PET) were evaluated. This study enrolled 43 patients (mean age 41.3 years ± 14.6 [standard deviation], 23 women). All lesions were well-defined round masses. Calcification and feeding vessels were detected in 21% (9/43) and 86% (36/43) of the patients, respectively. Perilesional fat stranding and fatty proliferation were observed in 44% (19/43) and 19% (8/43), respectively, with fatty proliferation detected only in retroperitoneal HVCD. Neighboring fascial thickening and surrounding lymphadenopathy were identified in 21% and 60%, respectively. The mean ADC value and SUVmax were 0.884×10^{-3} mm^2/s and 5.0, respectively. Retroperitoneal HVCD cases with perilesional fatty proliferation demonstrated a higher visceral fat ratio than those without ($p = 0.046$). Perilesional fat stranding and fatty proliferation were new characteristics of HVCD, especially in retroperitoneal cases.

***Keywords*:** Castleman disease, liposarcoma, fat, CT, MRI

Introduction

Castleman disease is a rare lymphoproliferative pathologic condition affecting lymph nodes and immune cells (*1*). It exists in two clinical forms: unicentric and multicentric. The former is usually asymptomatic, and its localization is restricted to a single node or nodal region. Meanwhile, the multicentric form typically manifests with general symptoms, such as fever, unintended body weight loss, fatigue, and appetite loss, as well as non-specific manifestations of anemia, multiple lymphadenopathies in several regions, and an enlarged spleen or liver. The two major histopathological types of Castleman disease are the hyaline-vascular and plasma cell variants. Previously, unicentric and multicentric forms corresponded to the former and latter types, respectively. However, some researchers currently believe that both histological types exist in each clinical form (*1*).

Several imaging features, particularly CT results, have been previously reported for the hyaline-vascular variant of Castleman disease (HVCD). These CT results included a solitary hypervascular round mass, well-defined margins indicating a noninvasive nature, calcification, especially arborizing shapes, and surrounding lymphadenopathies around the lesion (*2-11*). In addition, a clinical case of

HVCD masquerading as a dedifferentiated focus in a well-differentiated liposarcoma that demonstrated prominent fatty proliferation around a round mass was encountered. We have experienced similar cases in other hospitals, and PubMed research using the terms "Castleman[Mesh] AND fat[TIAB]" as of 9 June 2023 (time of presentation at a scientific meeting) and 30 April 2024 (time of manuscript preparation) has not yet reported these results well except for one case report (*12*).

The present study aimed to retrospectively analyze the imaging findings of unicentric HVCD in a large case series, mainly focusing on perilesional fat stranding and fatty proliferation.

Materials and Methods

Ethical approval

The institutional ethics review boards in five hospitals approved this multi-institutional study, which complied with the tenets of the Declaration of Helsinki and the Standards for Reporting Diagnostic Accuracy (*13*). Due to the retrospective design, they waived individual written informed consent.

Patient population

Patients were initially selected with a keyword search ("Castleman" and "CT or MR") in the radiological reporting systems from January 2000 to March 2023 in five hospitals. Data of patients with a pathologically confirmed Castleman disease diagnosis were retrieved. Inclusion criteria were surgically treated or biopsied unicentric lesions on CT. Exclusion criteria were nonpathologically confirmed cases, multicentric lesions on imaging, or absence of preoperative CT scans. Individuals with confirmed unicentric plasma cell variants were also excluded (Figure 1). Finally, preoperative CT findings of 43 cases with unicentric HVCD, including 42 surgically treated cases and one biopsied case, were analyzed. Preoperative MRI and positron emission tomography (PET) data, which were not indispensable, were evaluated. Patient demographics and clinical findings were extracted from respective hospital electronic records. Preoperative laboratory data, such as serum white blood cell (WBC) count, C-reactive protein (CRP), lactate dehydrogenase (LDH), and soluble interleukin-2 receptor (sIL2R), were analyzed.

Image acquisition and interpretation

CT imaging was conducted with 16–320-row multidetector systems (Mx8000 IDT 16, Brilliance 64, Philips Medical Systems, Best, Netherland; Aquilion, Aquilion Lightning, Aquilion ONE, Aquilion PRIME, Canon Medical System, Tochigi, Japan; LightSpeed QX/i, LightSpeed Ultra, Optima CT660, GE, Milwaukee, WI, USA; Emotion 6, Emotion 16, Siemens, Erlangen, Germany), including non-contrast examinations alone (n = 4), non-contrast and two-phase dynamic contrast (n = 12), non-contrast and three-phase dynamic (n = 12), non-contrast and portal venous phase contrast (n = 8), and portal venous phase contrast examinations alone (n = 7).

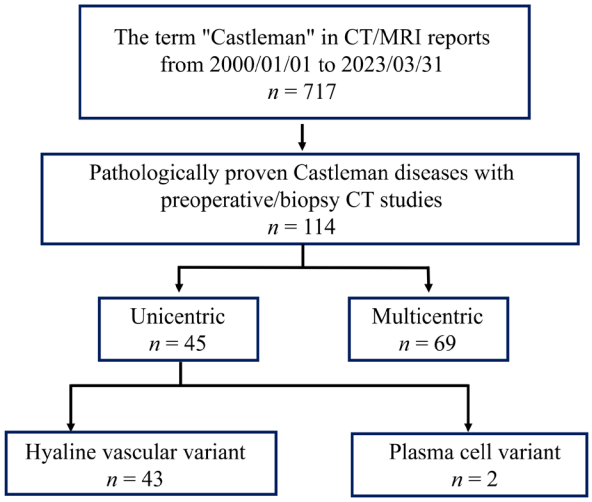

Figure 1. Flowchart of patient selection.

MRI was conducted in 22 patients with 1.5-T (n = 19) or 3-T machines (n = 3) (Achieva, Ingenia1, Ingenia 2, Philips Medical System; MAGNETOM Vision, MAGNETOM Spectra, Emotion 6, Skyra, Siemens, Signa Genesis, Signa HDxt, GENESIS Signa, GE; MRT200PP5, MRT100L2, Canon Medical System). Ten cases received contrast enhancement, including six with dynamic evaluations. One case underwent a dynamic contrast study with MRI alone. Diffusion-weighted images (DWI) and the apparent diffusion coefficient (ADC) values were obtained for 18 and 12 patients, respectively. Supplemental Table S1 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=94*) summarizes detailed scan parameters and contrast methods of CT and MRI studies.

Sixteen patients underwent PET/CT (Emotion Duo, Biograph64_mCT, Biograph16_TruePoint, Siemens; Aquiduo, Canon Medical System) after fasting for at least 10 h and receiving 3 MBq/kg of ^{18}F- fluorodeoxyglucose (FDG). Whole-body supine images were taken 90–120 min after ^{18}F-FDG injection, and the standardized uptake value (SUV) was determined in 15 cases.

Imaging analyses

Three experienced radiologists (S.H., T.W., and M.M., with 15, 21, and 40 years of experience in body imaging, respectively) independently evaluated CT and MRI images. Evaluated CT results reflected lesion location, maximum size, attenuation on non-contrast studies, calcification (arborizing, punctate, another shape, or none), enhanced pattern on portal venous phase

images of contrast-enhanced studies (homogeneous, inhomogeneous, or rim-like), and feeding vessels. Lesions' signal intensities were analyzed on T1WI, T2WI, and DWI in MRI studies. Flow void signals (intra-lesion, peri-lesion, both, or none) were estimated on T2WI. Vivid arterial or rim-like enhancements were observed during dynamic CT or MRI. Moreover, perilesional fat stranding, perilesional fatty proliferation (concentric pattern, eccentric pattern, or absent), neighboring fascial thickening, including abdominal peritoneal thickening, and surrounding lymphadenopathy, were analyzed on CT and MRI images. The outcomes in which agreement was achieved by two or more of the three radiologists were evaluated. The relevance of the findings was decided by a consensus in a case of disagreement among the three radiologists. Additionally, time-course changes in the lesion size were evaluated when multiple studies of CT and MRI were available preoperatively.

The ADC values of DWI on MRI and the maximum SUV (SUVmax) on ^{18}F-FDG-PET were determined by placing region-of-interest circles of the largest size possible to cover the lesion areas.

Visceral and subcutaneous fat volumes were calculated, to determine the relationship between perilesional fatty proliferation and visceral fat ratio (=visceral fat volume / (subcutaneous fat volume + visceral fat volume)), using medical image analysis software (AZE Virtual Place, Canon Medical System) in patients with retroperitoneal HVCD. Axial CT images obtained at the umbilical level were used for data analysis.

Statistical analysis

Mann–Whitney's *U*-test was used to evaluate the relationship of WBC, CRP, LDH, sIL2R, ADC values, and SUVmax with perilesional fat stranding and fatty proliferation. The difference between perilesional fatty proliferation and the visceral fat ratio was also evaluated in patients with retroperitoneal HVCD using the same test. Statistical Package for the Social Sciences (SPSS) for Windows version 26 (IBM SPSS Inc., Chicago, IL) was used for statistical analyses. *P*-values of < 0.05 indicated statistical significance.

Results

Patient characteristics and clinical findings

The study sample included 43 patients (mean age ± standard deviation: 41.3 ± 14.6 years, range: 16–77 years; 23 women).

All patients demonstrated preoperative serum WBC counts within normal limits. Serum CRP was elevated in three of them (5.7–16.4 mg/dL). Serum LDH levels were normal in all 41 patients with available data. Among 28 individuals in whom the measurements were performed, four patients showed increased serum sIL2R levels (571–1,190 U/mL).

Radiological findings

Among 43 lesions of HVCD, 16 (10 on the left side and 6 on the right), 14, 6, 4, 2, and 1 were located in the retroperitoneum, mediastinum, mesentery or mesocolon, neck, axilla, and chest wall, respectively. The mean diameter of lesions in HVCD was 4.4 ± 1.9 cm (mean ± standard deviation, range: 1.3–9.7 cm).

Table 1 summarizes the radiological findings of the enrolled patients. All lesions were homogeneously iso-attenuated to the skeletal muscle in 36 CT examinations, including non-contrast studies. All lesions analyzed in our study were a well-defined round soft-tissue mass. Calcification was detected in 9 (21%) of 43 patients, including arborizing configuration in 4 patients. Among 39 patients, enhanced patterns on portal venous phase contrast images were homogeneous in 31, inhomogeneous in 5, and rim-like enhanced in 3 patients. Cystic degeneration and necrotic changes were observed in five and two patients, respectively. Feeding vessels were visualized around lesions in 36 of 43 patients. Among all 22 patients that underwent MRI, none demonstrated hyperintense areas indicating hemorrhage on T1WI, and the obtained signal intensities on both T1WI and T2WI were comparable to those of lymph nodes. Flow void signals were detected inside the lesion in 5 of 22 patients, at the periphery encircling them in 9, and at both locations in 3 patients. The signal intensities on DWI were higher and similar to those of normal lymph nodes in 11 and 7 of 18 patients, respectively. All 25 lesions evaluated using dynamic CT or MRI were hypervascular in the arterial phase, and 6 of them demonstrated a rim-like enhancement.

Notably, 44% of the enrolled patients (19/43, Figures 2–6) and 75% of patients with retroperitoneal HVCD (12 of 16 cases) demonstrated perilesional fat stranding. Perilesional fatty proliferation was observed in 8 of 43 patients (19%), where all followed a concentric pattern. The eccentric pattern of perilesional fatty proliferation was not observed. All 8 lesions were located in the retroperitoneum (8 of 16 retroperitoneal cases, 50%; Figures 2–4), which was not characteristic of the other areas. Perilesional fat stranding was observed in all eight patients with perilesional fatty proliferation. Neighboring fascial thickening and surrounding lymphadenopathy were identified in 9 and 26 of the enrolled patients, respectively. The ADC value of 13 lesions was 0.884 ± 0.134 × 10^{-3} (mean ± standard deviation, range: 0.689–1.143 × 10^{-3}) mm^2/s.

The SUVmax of 15 lesions was 5.0 ± 3.5 (mean ± standard deviation, range: 2.1–16.3). The mean SUVmax value was 5.7 (n = 7) in patients with perilesional fat stranding and 4.4 (n = 8) in the opposite group; however, the Mann–Whitney *U*-test revealed no statistically

Table 1. Imaging findings in the hyaline-vascular variant of Castleman disease

	Overall (n = 43)	Retroperitoneum (n = 16)	Mediastinum (n = 14)	Mesentery/Mesocolon (n = 6)	Neck (n = 4)	Axilla/Chest wall (n = 3)
Calcification						
arborizing configuration	9% (4/43)	20% (3/16)	7% (1/14)	0	0	0
punctate	7% (3/43)	20% (3/16)	0	0	0	0
other shapes	5% (2/43)	7% (1/16)	7% (1/14)	0	0	0
none	79% (34/43)	53% (9/16)	86% (12/14)	100% (6/6)	100% (4/4)	100% (3/3)
Enhancement pattern on portal venous phase images						
homogeneous	79% (31/39)	86% (13/15)	64% (7/11)	100% (6/6)	75% (3/4)	67% (2/3)
inhomogeneous	13% (5/39)	7% (1/15)	36% (4/11)	0	0	0
rim-like enhancement	8% (3/39)	7% (1/15)	0	0	25% (1/4)	33% (1/3)
Visualization of feeding vessels						
present	84% (36/43)	97% (15/16)	86% (12/14)	83% (5/6)	50% (2/4)	67% (2/3)
absent	14% (6/43)	6% (1/16)	7% (1/14)	17% (1/6)	50% (2/4)	33% (1/3)
not assessable	2% (1/43)	-	7% (1/14)	-	-	-
Vivid arterial enhancement						
present	100% (25/25)	100% (12/12)	100% (8/8)	100% (5/5)	-	-
absent	0	0	0	0	-	-
Rim-like enhancement on arterial phase images						
present	24% (6/25)	33% (4/12)	0	40% (2/5)	-	-
absent	76% (19/25)	67% (8/12)	100% (8/8)	60% (3/5)	-	-
Flow void signals						
Present, intra-lesion	23% (5/22)	30% (3/10)	50% (2/4)	0	0	0
Present, peri-lesion	40% (9/22)	20% (2/10)	25% (1/4)	60% (3/5)	100% (2/2)	100% (1/1)
both	14% (3/22)	30% (3/10)	0	0	0	0
absent	23% (5/22)	20% (2/10)	25% (1/4)	40% (2/5)	0	0
Perilesional fat stranding						
present	44% (19/43)	75% (12/16)	29% (4/14)	50% (3/6)	0	0
absent	56% (24/43)	25% (4/16)	71% (10/14)	50% (3/6)	100% (4/4)	100% (3/3)
Perilesional fatty proliferation						
concentric pattern	19% (8/43)	50% (8/16)	0	0	0	0
eccentric pattern	0	0	0	0	0	0
absent	81% (35/43)	50% (8/16)	100% (14/14)	100% (6/6)	100% (4/4)	100% (3/3)
Neighboring fascial thickening						
present	21% (9/43)	31% (5/16)	14% (2/14)	33% (2/6)	0	0
absent	79% (34/43)	69% (11/16)	86% (12/14)	67% (4/6)	100% (4/4)	100% (3/3)
Surrounding lymphadenopathy						
present	60% (26/43)	62% (10/16)	64% (9/14)	50% (3/6)	50% (2/4)	67% (2/3)
absent	40% (17/43)	38% (6/16)	36% (5/14)	50% (3/6)	50% (2/4)	33% (1/3)

significant differences between the two groups (p = 0.613). Moreover, we found no evidence supporting the association of blood test findings and the ADC value with perilesional fat stranding. The mean SUVmax was 8.6 (n = 3) in the perilesional fatty proliferation group and 4.1 (n = 12) in the counterpart, indicating no remarkable differences in the outcomes of the performed Mann–Whitney U-test (p = 0.10). Blood test results and the ADC value did not significantly differ between the mentioned groups. The mean values of the visceral fat ratio in retroperitoneal HVCD cases with perilesional fatty proliferation and those without it were 0.46 (n = 8) and 0.33 (n = 8), respectively, demonstrating higher visceral fat ratios in the group showing perilesional fatty proliferation (p = 0.046).

Time-course changes in lesion size were evaluated for 23 patients. The average follow-up period was 28 months (range: 1–132 months). Seventy-four percent of lesions (17/23), exhibited no interval size changes, but five demonstrated an increase in size. Among them, one manifested a 2.9-fold increase, *i.e.*, from 1.8 cm to 5.3 cm over 3.5 years. The remaining one patient exhibited a decrease in lesion size from 3.7 cm to 3.0 cm over 7 years.

Discussion

Resection surgery is expected to treat unicentric Castleman disease completely, but accurate preoperative diagnosis of HVCD is essential to avoid an excessive intervention similar to that for malignant tumors (*1*). HVCD diagnosis is considered challenging because of its rarity, with no typical clinical symptoms, signs, or laboratory findings. The present study, including 43 cases, revealed no specific symptoms or laboratory findings associated with HVCD. Therefore, radiological evaluation is crucial for precise HVCD diagnosis.

The imaging findings of HVCD have been widely reported. Typical HVCD is incidentally detected in the neck, mediastinum, hilar region, mesentery, and

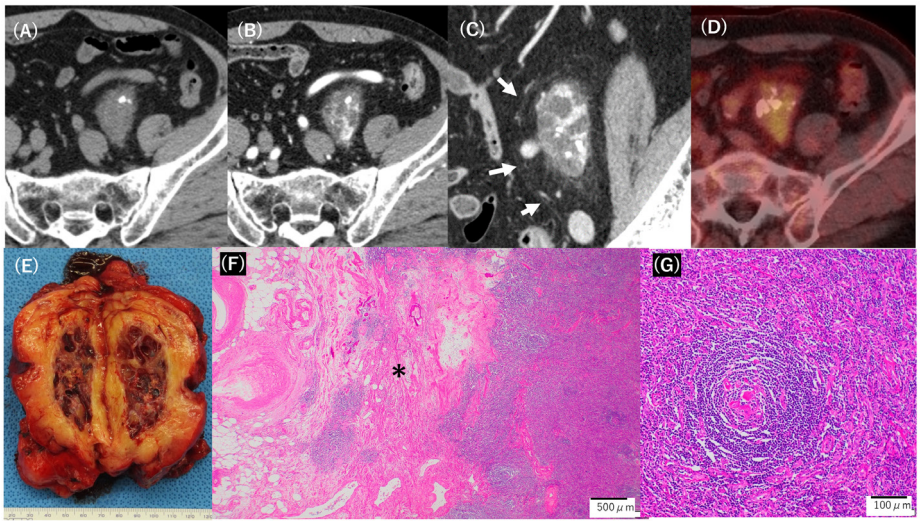

Figure 2. A 65-year-old man was admitted to our hospital due to the suspicion of retroperitoneal liposarcoma on CT before mycosis fungoides treatment. (A) In non-contrast CT, a 4.5-cm mass was observed in the left pelvic wall, which compressed the left external iliac artery anteriorly. **(B)** The mass showed a relatively strong heterogeneous contrast enhancement in contrast-enhanced CT with cystic degeneration inside. Coarse-to-nodular calcifications were observed within the mass. **(C)** The contrast-enhanced coronal MPR CT image revealed perilesional fat stranding and fatty proliferation, which were interpreted as a well-differentiated component of liposarcoma at the previous hospital. The parietal peritoneum of the pelvis was slightly thickened (arrows). **(D)** The mass was hypermetabolic, and the SUVmax was 4.49 in ^{18}F-FDG-PET /CT. **(E)** The mass and surrounding fatty tissue were removed *en bloc*. Grossly, the fat around the mass was white and hard, whereas the interior mass was reddish-brown. **(F)** Histological examination revealed fatty proliferation and dense fibrosis (*) around the mass. Lymph follicles with atrophied germinal centers and enlarged mantle zones were observed inside the mass. **(G)** Sclerotic blood vessels traversed into the germinal centers. Small vessel hypervascularization was observed between the follicles. Based on these findings, a diagnosis of hyaline-vascular Castleman's disease was made.

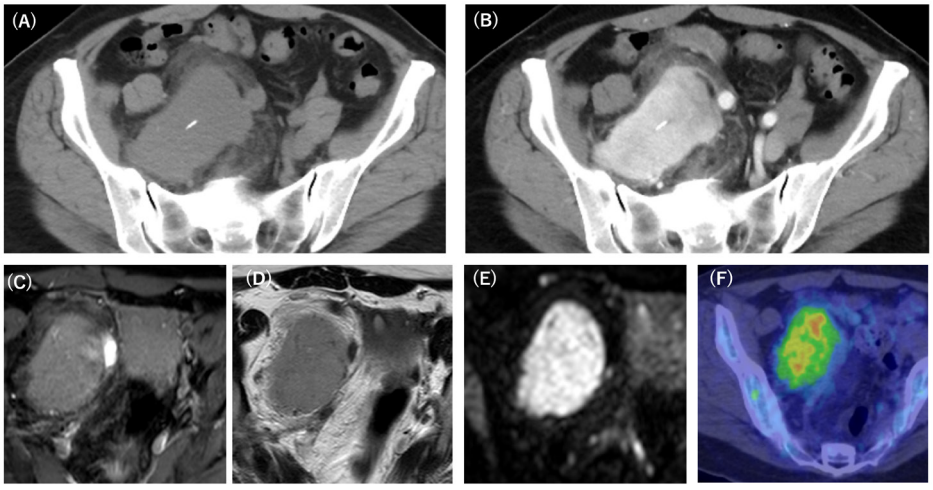

Figure 3. A 36-year-old woman was referred to our hospital because of a pelvic mass detected on an ultrasound examination for infertility. (A) A 9.0-cm mass was observed in the extraperitoneal space on the right side of the pelvis. A small linear calcification was observed in the center of the lesion. **(B)** Substantial contrast enhancement was identified in the mass on contrast-enhanced CT, accompanied by perilesional fat stranding and fatty proliferation. **(C)** Fat-saturated T1WI at the lower level of images **(A)** and **(B)** showed that the lesion had a signal equivalent to that of muscle. **(D, E)** The lesion was relatively hyperintense compared to muscle on T2WI and significantly hyperintense on DWI. The ADC value of the lesions was 0.985×10^{-3} mm^2/sec. **(F)** ^{18}F-FDG-PET/CT showed avid uptake of ^{18}F-FDG in the lesion. The mass was proven to be the hyaline-vascular variant of Castleman disease in pathological examinations.

retroperitoneum (*2*). It rarely occurs in the intestinal tract and abdominal parenchymal organs (*6,10*). It is usually diagnosed as a well-defined, round, solitary soft-tissue mass, with surrounding lymph node enlargement in 10.0%–54.5% of cases (*3,14*). Lesions are approximately 5.5 cm in diameter (*7*) and demonstrate relatively homogeneous attenuation on non-contrast CT, which is frequently comparable to skeletal muscle (*4*). The frequency of calcification is approximately 10%, which is not high, but discontinuous, coarse, and especially arborizing (dendritic)-shaped calcifications have been considered one of the characteristic imaging findings of HVCD (*2*). Contrast-enhanced CT shows abundant feeding vessels around lesions, with a strong and uniform

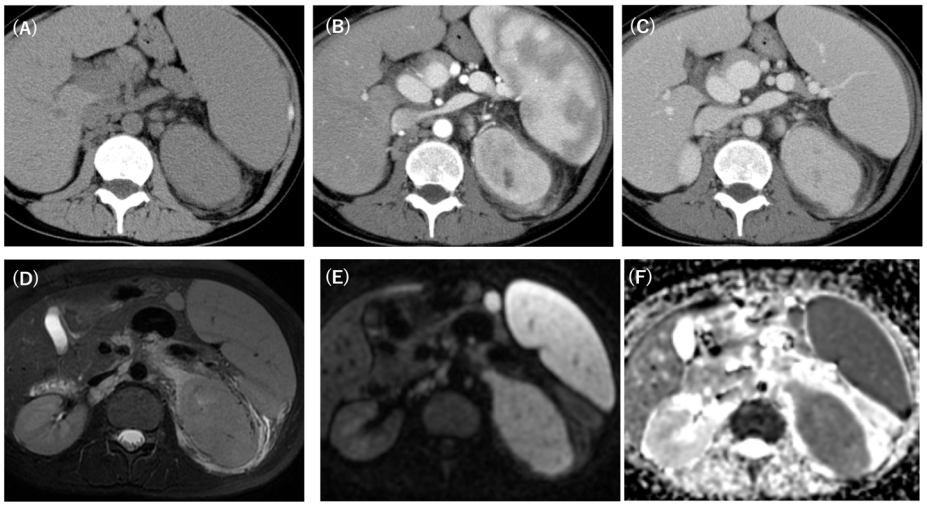

Figure 4. A 36-year-old woman with pathologically proven hyaline-vascular Castleman disease. (A) A well-defined 4.0-cm mass was identified in the retroperitoneum in front of the left psoas major muscle on non-contrast CT image. **(B)** The arterial phase image of dynamic contrast-enhanced CT showed hypervascularity with rim-like peripheral enhancement. **(C)** Portal venous phase image of contrast-enhanced CT displayed perilesional fat stranding and fatty proliferation. Thickening of the surrounding fascia was also observed. **(D)** The well-defined mass was almost isointense to the spleen, and high signal intensities, probably representing edematous changes, were observed around the lesion on fat-saturated T2WI. Encircling flow voids were noted around the mass. **(E)** DWI showed the mass of high signal intensities comparable to those of the spleen. **(F)** The ADC value of the lesion was 1.081×10^{-3} mm^2/sec.

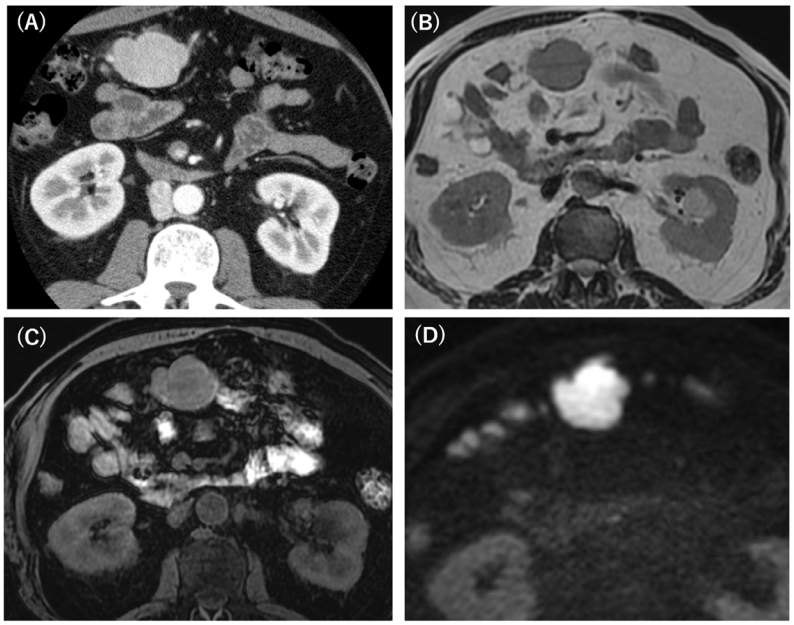

Figure 5. A follow-up CT scan of pancreatic intraductal papillary mucinous neoplasm revealed a 4.3-cm well-defined mass in the transverse mesocolon that was incidentally detected in a 49-year-old man. (A) The mass was homogeneously enhanced with perilesional fat stranding on contrast-enhanced CT. **(B)** T2WI revealed a slightly hyperintense mass compared to muscle and flow voids around it, probably due to feeding vessels. **(C, D)** The mass exhibited a similar signal to that of muscle on T1WI and markedly higher signal intensities on DWI.

contrast enhancement, but it can be heterogeneous in larger cases. Significantly, lesions of > 5 cm are heterogeneous, displaying internal fibrosis, necrosis, and degeneration (*3,7*). The signal intensity of the lesions is similar to or lower than that of a skeletal muscle on T1WI of MRI and is typically equal to or higher than that of a skeletal muscle on T2WI (*2,3,7*). The pattern of contrast effect on MRI is compatible with that seen on CT. In previous studies, feeding vessels were visualized within or around the lesion in 35.7%–51.2% of patients, which strongly indicated HVCD (*7,8*). Zheng *et al.* revealed a peripheral rim-like contrast effect in the arterial phase in three case series, where histological examinations in the peripheral portions of the tumors demonstrated increased small vessels (*15*). They reported fascial thickening around the retroperitoneal HVCD lesion in one of three

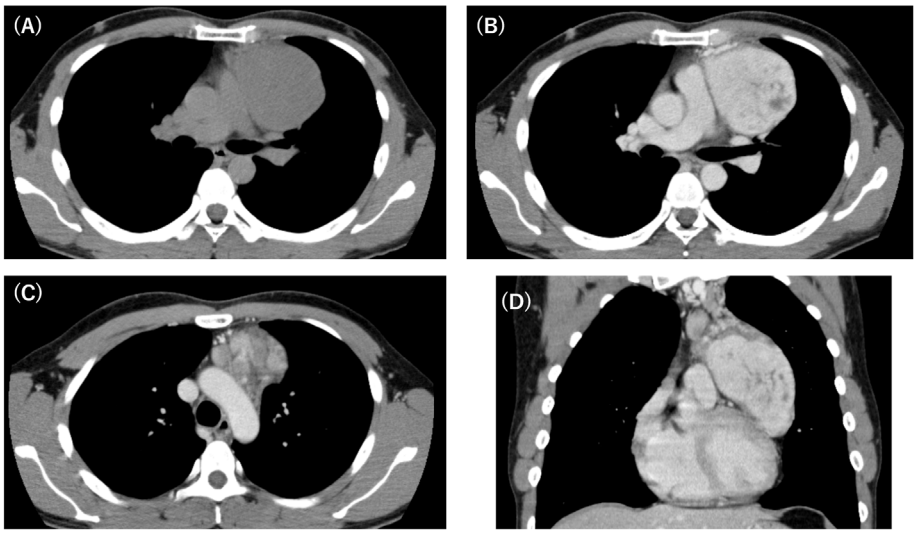

Figure 6. A 35-year-old man manifested a mediastinal mass on a chest radiograph for chickenpox. (A) A 9.7-cm mass was observed in the anterior mediastinum on non-contrast CT image. **(B)** The contrast enhancement of the mass was strong and heterogeneous. **(C, D)** Perilesional fat stranding with surrounding small lymphadenopathies was detected around the mass despite no evident fatty proliferation. The pathological diagnosis of the mass was a hyaline-vascular variant of Castleman disease.

cases (*15*). DWI revealed high signal intensities in all five cases reported by Zhao *et al.* (*7*). He *et al.* reveal avid ^{18}F-FDG uptake on PET/CT in all 18 patients in their study, with a median SUVmax value of 3.7 (2.4–5.2) (*16*).

These previously reported imaging findings were mostly consistent with the outcomes in this study. Well-defined round soft-tissue masses were observed in all evaluated cases. The frequency of calcification of the lesions was 21%, which was higher than that reported in previous studies. However, this may be associated with recent advances in CT, which enable higher spatial and contrast resolution and can be performed with thinner scanning techniques. The internal attenuation of the lesions was homogeneous in post-contrast evaluations in 79% of the assessed cases, and three cases demonstrated a rim-like enhancement. The signal intensities of lesions on T1WI, T2WI, and DWI of MRI were similar to those reported in previous studies. Feeding vessels were present around the lesions in 84% of the cases. Those who underwent dynamic studies demonstrated vivid arterial enhancement in all. Frequencies of rim-like enhancement in the arterial phase, flow void signals, and perilesional fascial thickening were not reported in previous studies, but the present study clarified these features and revealed that 60% of the cases were associated with surrounding lymphadenopathies. Meanwhile, ^{18}F-FDG accumulation rates were significantly not different from those reported in previous studies.

Perilesional fat stranding and fatty proliferation were not reported in previous studies. In the present study, perilesional fat stranding was observed in 75%, 29%, and 50% of the retroperitoneal, mediastinal, and mesentery lesions, respectively. Perilesional fatty proliferation was detected in 19% of the total cases, and 50% of the retroperitoneal lesions demonstrated perilesional fatty proliferation, but not in other body parts. All perilesional fatty proliferations had a concentric pattern. Perilesional fat stranding and fatty proliferation demonstrated no association with laboratory data, the ADC value on DWI, or the SUVmax on ^{18}F-FDG PET/CT. However, patients with a higher visceral fat ratio demonstrated perilesional fatty proliferation more frequently. The exact etiology of the indicated fat tissue changes is unknown. We speculate that mild chronic inflammation caused by HVCD may induce their development in obese patients. It is known that adipose tissue proliferates as a result of persistent weak chronic inflammation (*17*). The effect is more significant around lymph nodes because of the paracrine action between lymphoid tissue and surrounding adipose tissue (*18*). Chronic inflammation of the lungs and pleurae can cause thickening of subpleural fat, and chronic inflammatory diseases such as human immunodeficiency virus can cause hypertrophy of the omentum and mesentery (*18,19*). It is possible that HVCD, which involves lymphoid tissue, may have led to an increase in perilesional adipose tissue due to paracrine effects.

Previous studies revealed that the radiological differential diagnosis of HVCD included lymphoma, plasmacytoma, follicular dendritic cell sarcoma, soft-tissue sarcoma, thymic epithelial tumors and germ cell tumors in the mediastinum, gastrointestinal stromal tumors in the abdomen, and metastases (*2,4,9*). In dynamic studies, hypervascular lesions, such as paraganglioma, solitary fibrous tumor, and neuroendocrine tumor, were essential differential entities. Interestingly, the preoperative radiological differential diagnosis included liposarcoma in three of our eight

retroperitoneal cases with fatty proliferation. Perilesional fatty proliferation in HVCD forming concentrically around the lesions can be a differential point, whereas a dedifferentiated liposarcoma component is not always present in the center of the lesion, indicating an eccentric fatty proliferation in appearance (Supplemental Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=94*).

This retrospective study had several limitations. First, the imaging conditions were not uniform because it was conducted at five facilities having different technical settings. Second, most patients were racially homogeneous; therefore, the results of the present study may not be applicable to specific races due to different reported disease rates. Third, no comparative analysis was performed on differential diagnosis with other entities, especially dedifferentiated liposarcomas and hypervascular tumors, such as paragangliomas and solitary fibrous tumors. Further studies are warranted to address these aspects.

In conclusion, we reviewed the imaging findings of 43 patients with unicentric HVCD and identified perilesional fat stranding and fatty proliferation as new imaging characteristics. Radiologists should be aware that retroperitoneal HVCD may mimic dedifferentiated liposarcoma originating in a well-differentiated liposarcoma because of surrounding fatty proliferation.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Suicide attempt and self-harm among hospitalized children in Japan: A nationwide inpatient database study

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Abstract: This study aims to delineate the characteristics and clinical trajectories of suicide attempts and self-harm, and its gender and age differences among children. This nationwide retrospective cross-sectional study utilized data extracted from the Japanese Diagnosis Procedure Combination inpatient database spanning 2016 to 2017. Children aged 7-17 years admitted to acute care hospitals for suicide attempts and self-harm, were identified. Patient characteristics included age, gender, suicide method, and comorbid psychiatric disorders. Trajectory information included the duration of hospital stay, admission ward, psychiatric/psychological interventions, in-hospital mortality, and healthcare expenditure. Data analysis encompassed 1,704 children hospitalized for suicide attempts and self-harm. Among these, 49.4% were junior high school age, 69.0% for female, and 28.4% for underweight. Overdose emerged as the most prevalent method for suicide attempts and self-harm (49.9%). Notably, 66.0% did not receive a diagnosis of any mental illness, and 56.3% did not undergo psychiatric/psychological care during their hospitalization. Boys were more likely to use high-lethality suicide methods, such as hanging ($p < 0.001$), and die during hospitalization ($p < 0.001$). Conversely, girls were more likely to use low-lethality suicide methods, such as drug overdose ($p < 0.001$), and receive psychiatric/psychological intervention during hospitalization ($p = 0.015$). Children aged 7-12 years were more likely to use high-lethality suicide methods, such as hanging ($p < 0.001$), and be diagnosed with attention-deficit/hyperactivity disorder ($p < 0.001$) and less likely to receive psychiatric/psychological intervention ($p = 0.005$) compared with other age groups. These findings suggest the importance of developing gender and age sensitive health policies, systems, and interventions to prevent child suicide.

Keywords: acute care hospital, children, Japan, self-harm, suicide, suicide attempt, nationwide inpatient data

Introduction

Child suicide poses a significant global health concern. Among adolescents aged 15-17 years, the average global suicide rate stands at 7.4 per 100,000 (*1*). Notably, Japan reports a crude suicide rate of 16.1 per 100,000 across all age groups, surpassing rates in other developed countries such as the United States, France, the United Kingdom, and Germany (*2*). In Japan, the number of suicides among children aged 7-18 years reached 514 in 2022, marking a historic high, with a majority being adolescents (*3*). Suicide ranks as the third leading cause of death among 10-14 year-olds and the primary cause among 15-19 year-olds in Japan, while globally, it ranks fourth among 10-29 year-olds (*2,4*). Additionally, previous studies focusing on a larger population of Japanese adolescents and young adults (n = 1,540 to 2,974) found a lifetime prevalence of self-harm at approximately10%, surpassing the global rate of 6.2% (*5,6*).

Studies underscore the association of suicide attempts and self-harm among children with various factors including internalizing psychopathology, depression, anxiety, externalizing psychopathology, attention-deficit

hyperactivity disorder (ADHD), psychosis, family conflict and dysfunction, family psychopathology, lower self-esteem, parental support, peer support, bullying, and child maltreatment (*7,8*). While a Japanese study in 2020 (*9*) explored suicide attempts and self-harm among adults, shedding light on demographic trends, method of suicide attempt or self-harm, suicide completion rates, and lack of psychiatric/psychological interventions, its focus excluded children. Consequently, there remains a significant gap in understanding the characteristics and clinical trajectories of suicide attempts and self-harm among this demographic.

Additionally, previous studies indicated that the rate of suicides, type of suicidal behaviors, and its associated factors may differ between genders. Studies reported that females were more likely to commit self-harm, such as cutting a wrist, and self-poisoning; furthermore, it was associated with their internalizing behaviors, such as depression and suicidal ideation (*10-14*). Conversely, males were more likely to attempt suicide possibly owing to their externalizing behaviors, such as physical and verbal aggression and hostility (*13-15*). However, findings regarding gender and age differences have been inconsistent (*16,17*). Furthermore, whether gender differences exist in the rate, methods, demographics, and clinical trajectories – such as treatments of self-harm and self-attempt – especially in childhood remains unclear.

In addition, the characteristics and clinical trajectory among children who committed self-harm and suicide attempt may differ depending on age groups. Systematic reviews indicated that children's suicidality, especially self-harm, were common from the age of 12 years onwards, especially among girls (*13*). Reasons for rapid increase of self-harm during puberty may include depressive symptoms, alcohol misuse, substance abuse, and onset of sexual activity. Furthermore, risk-taking behaviors and emotional symptoms may be associated with their neurodevelopmental vulnerability, such as underdevelopment of the cortical brain region (*7,13,18-20*). However, limited studies have examined self-harm and suicide attempts among children younger than 12 years. Furthermore, differences in the characteristics and clinical trajectory have not been elucidated among the age groups of 7-12 (elementary), 13-15 (junior high), and 16-17 years old (senior high).

This study aims to address this gap by identifying the characteristics and clinical trajectories associated with suicide attempts and self-harm among hospitalized children aged 7-17 years, and its gender and age differences leveraging data from a nationwide inpatient database in Japan.

Participants and Methods

Study design

This nationwide retrospective cross-sectional study was conducted from 2016 to 2017, utilizing the Japanese Diagnosis Procedure Combination inpatient database. The 2016-2017 database was used as the provision of data on suicide attempts and data on self-harm were compulsorily provided only during this period.

Data source

The nationwide inpatient database encompasses data gathered from approximately 1,600 acute-care hospitals across Japan, encompassing around seven million inpatients annually, which accounts for approximately 50% of inpatients admitted to Japanese academic and community hospitals (*21*). This comprehensive database comprises discharge abstracts and administrative claims data, providing insights into patient demographics such as age and gender, alongside diagnoses categorized utilizing the International Classification of Diseases Tenth Revision codes, medications, procedures, and various clinical data, including levels of consciousness and activities of daily living. Studies have indicated a moderately high accuracy in primary diagnoses and a very high accuracy in recorded procedures within this database (*22,23*).

Information on suicide attempts and self-harm

Between 2016 and 2017, the database included information on whether patients had engaged in suicide attempts or self-harm, as well as the specific method employed, for all hospitalized patients admitted to acute-care hospitals. Suicide methods encompassed *i*) hanging, *ii*) jumping from a height, *iii*) poisoning, *iv*) drug overdose, *v*) cutting or piercing excluding wrist cutting, *vi*) wrist cutting, *vii*) other methods, and *viii*) unspecified methods. In cases where healthcare professionals did not identify any suicide attempts upon admission, they assigned the code *ix*) indicating none.

Participants

We identified children aged 7-17 years, admitted to acute-care hospitals in Japan for the first time for suicide attempts or self-harm between 2016 and 2017. Children coded as 7 (others) or 8 (unspecified) were excluded owing to the low reliability of the data concerning child suicide.

Variables

Patient characteristics encompassed age, gender, body height, body weight, current pregnancy status, smoking index, prior admission within one year to the same hospital, ambulance utilization, emergency admission, forced admission by a licensed psychiatrist, level of consciousness upon admission utilizing the Japan Coma Scale, comorbid psychiatric disorders, and suicide

methods. Comorbid psychiatric disorders included mood disorders (such as depressive disorder and bipolar affective disorder), anxiety disorders (including generalized anxiety disorder, post-traumatic stress disorder, obsessive-compulsive disorder, panic disorder, and dissociative disorder), somatoform disorder, eating disorder, ADHD, schizophrenia, and personality disorder.

Clinical trajectory information comprised length of hospital stay, admission ward (categorized into psychiatry, emergency medicine, and intensive care unit), physical interventions (such as mechanical ventilation, blood transfusion, closed heart massage, counter shock, gastric lavage, and vasopressor utilization), psychiatric/psychological interventions (including inpatient psychotherapy, cognitive therapy/cognitive behavioral therapy, inpatient group psychotherapy, psychiatric occupational therapy, psychiatric discharge guidance, and antidepressants utilization), non-psychiatric/psychological intervention, in-hospital mortality, mortality within 24 hours post-admission, discharge destination (home, another hospital/clinic, or social welfare facility), Japan Coma Scale assessment at discharge, and total healthcare costs (with the conversion rate of 1 dollar = 105 yen for the years 2016 and 2017).

Statistical analyses

Descriptive statistics for all the variables are presented. The median and interquartile range (IQR) are presented for numerical variables, while numbers and percentages are presented for categorical variables. Subsequently, Wilcoxon rank-sum tests and chi-squared tests were performed for numerical and categorical variables, respectively, to compare all the variables between the genders and age groups: 7-12 years old (Elementary), 13-15 years old (Junior high), and 16-17 years old (Senior high). If patients were hospitalized again between 2016 and 2017, information from the first admission, concerning suicide attempts or self-harm, was collected. All analyses were performed utilizing Stata version 17 software (StataCorp, College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

Ethical considerations

This study was approved by the Institutional Review Board of the University of Tokyo (No. 3501-(5)). All information collected from the database was kept anonymous.

Results

Characteristics of suicide attempt and self-harm among hospitalized children

A total of 1,704 children aged 7-17 years, were identified concerning being hospitalized with regards to suicide attempts and self-harm for the first time from 2016 to 2017 (n = 862 in 2016; n = 842 in 2017) (Table 1 and Table 2). The median age of the children was 15 years (IQR, 14-16), while 90.2% of them were adolescents (49.4% comprising junior high school age and 40.8% senior high school age, respectively). Overall, 69.0% were female, while 28.4% were classified as being underweight (exhibiting a body mass index < 18.5 kg/m^2). In relation to the route and type of admission selected, half the children utilized ambulances, 65% experienced emergency admissions, and 10% experienced forced hospitalization approved by a licensed psychiatrist. Overall, 17.5% were in a coma at the time of admission; 17.3% were going through depression; 0.8% were diagnosed with post-traumatic stress disorder, 3.1% with dissociative disorder, 2.8% with a bipolar affective disorder, 2.5% with somatoform disorder, 2.2% with an eating disorder, and 1.3% with a personality disorder. Regarding suicide attempts or self-harm, drug overdose was the most common (49.9%), followed by jumping from a height (22.3%), hanging (13.3%), wrist cutting (8.0%), cutting or piercing without wrist cutting (3.2%), and poisoning (3.2%).

Furthermore, girls (n = 1,176) were more likely to be older; underweight (BMI < 18.5); use ambulances; be less in a coma; diagnosed with psychiatric disorders such as depression and bipolar affective, panic, dissociative, somatoform, eating, and personality disorders; and forcedly admitted by a licensed psychiatrist than boys (n = 528). Moreover, Boys were more likely to use high-lethality suicide methods, such as hanging, jumping from a height, poisoning, and cutting or piercing without wrist cutting, than girls who were more likely to use low-lethality suicide methods, such as drug overdose and wrist cutting.

Clinical trajectory of suicide attempt and self-harm among hospitalized children

The median length of hospitalization was four days (IQR, 2-14 days) (Table 3 and Table 4). Overall, 19.0% of children were hospitalized in a psychiatric ward, 17.1% in an intensive care unit, and 31.0% in an intermediate intensive care unit. Regarding procedures and medications, 12.2% of children received mechanical ventilation, 6.0% received blood transfusion, 9.6% received gastric lavage, 11.4% utilized vasopressors, 41.8% received psychotherapy, 6.5% utilized antidepressants, 5.1% received psychiatric discharge guidance, and 3.9% received psychiatric occupational therapy during hospitalization. A total of 959 (56.3%) children did not receive psychiatric/psychological treatment. The in-hospital mortality rate was 9%, of which 7.3% passed on within 24 hours post-admission. Most children (87.4%) were classified as having a clear consciousness at the time of discharge, and 79.1% returned home after discharge. Their median total

Table 1. Characteristics of hospitalized children due to suicide attempt and self-harm: Gender and age comparisons (*n* = 1,704)

Variables	Total *n* = 1,704 *n* (%)	Girls *n* = 1,176 *n* (%)	Boys *n* = 528 *n* (%)	*p* value
Age (years old)				
7-12: Elementary	166 (9.7)	88 (7.5)	78 (14.8)	< 0.001
13-15: Junior high	842 (49.4)	587 (49.9)	255 (48.3)	
16-17: Senior high	696 (40.8)	501 (42.6)	195 (36.9)	
Female	1176 (69.0)	NA	NA	NA
Body mass index ((kg/m^2)				
< 18.5	398 (23.4)	252 (21.4)	146 (27.7)	< 0.001
18.5-24.9	871 (51.1)	658 (55.1)	223 (42.2)	
25.0-29.9	92 (5.4)	64 (5.4)	28 (5.3)	
≥ 30.0	39 (2.3)	30 (2.6)	9 (1.7)	
Current pregnancy	4 (0.2)	4 (0.2)	0 (0.0)	0.007
Smoking status				
Current/ ex-smokers	38 (2.2)	26 (2.2)	12 (2.3)	0.86
Ambulance use	1,116 (65.5)	728 (61.9)	388 (73.5)	< 0.001
Emergency admission	1,542 (90.4)	1,063 (90.4)	479 (90.7)	0.83
Forced admission by a licensed psychiatrist	186 (10.9)	149 (12.7)	37 (7.0)	< 0.001
Japan Coma Scale at admission				
Clear	892 (52.3)	629 (53.5)	263 (49.8)	< 0.001
Dizziness	352 (20.7)	274 (23.3)	78 (14.8)	
Somnolence	162 (9.5)	109 (9.3)	53 (10.0)	
Coma	298 (17.5)	164 (13.9)	134 (25.4)	
Comorbid psychiatric disorders				
Mood disorder				
Depressive disorder	295 (17.3)	226 (19.2)	69 (13.1)	0.002
Bipolar affective disorder	48 (2.8)	41 (3.5)	7 (1.3)	0.013
Anxiety disorder				
Generalized anxiety disorder	40 (2.3)	32 (2.7)	8 (1.5)	0.13
Post-traumatic stress disorder	14 (0.8)	12 (1.0)	2 (0.4)	0.17
Obsessive-compulsive disorder	9 (0.5)	7 (0.6)	2 (0.4)	0.57
Panic disorder	14 (0.8)	14 (1.2)	0 (0.0)	0.012
Dissociative disorder	53 (3.1)	48 (4.1)	5 (0.9)	< 0.001
Somatoform disorder	43 (2.5)	36 (3.1)	7 (1.3)	0.035
Eating disorder	37 (2.2)	36 (3.1)	1 (0.2)	< 0.001
Attention deficit hyper activity disorder	32 (1.9)	19 (1.6)	13 (2.5)	0.23
Schizophrenia	1 (0.1)	1 (0.1)	0 (0.0)	0.50
Personality disorder	23 (1.3)	22 (1.9)	1 (0.2)	0.005
Non-diagnosis of any psychiatric disorders	1,125 (66.0)	721 (61.3)	404 (76.5)	< 0.001
Methods of suicide attempt and self-harm				
Hanging	226 (13.3)	86 (7.3)	140 (26.6)	< 0.001
Jumping from a height	380 (22.3)	211 (17.9)	169 (32.1)	
Poisoning	55 (3.2)	32 (2.7)	23 (4.4)	
Drug overdose	850 (49.9)	696 (59.2)	154 (29.2)	
Cutting or piercing without wrist cutting	55 (3.2)	32 (2.7)	23 (4.4)	
Wrist cutting	137 (8.0)	119 (10.1)	18 (3.4)	

healthcare cost during hospitalization was 2,792 dollars (IQR, 1,551 to 6,816).

Gender comparisons

As shown in Table 3, boys were more likely to be hospitalized in an intensive care unit ($p < 0.001$); receive physical interventions ($p < 0.001$), such as mechanical ventilation ($p < 0.001$), blood transfusion ($p < 0.001$), closed heart massage ($p < 0.001$), counter shock ($p = 0.006$), and vasopressor use ($p < 0.001$); die during hospitalization ($p < 0.001$) or within 24 hours after admission ($p < 0.001$); be in a coma ($p < 0.001$); and have more healthcare cost than girls ($p < 0.001$). However, girls were more likely to be hospitalized in a psychiatric ward ($p < 0.001$); receive psychiatric/ psychological interventions ($p = 0.015$), which included inpatient psychotherapy ($p = 0.015$), psychiatric discharge guidance ($p = 0.009$), and antidepressant use ($p = 0.009$), and be discharged to home than boys ($p < 0.001$).

Age-group comparisons

As shown in Table 2 and Table 4, children < 12 years were more likely to be underweight ($p < 0.001$), use high-lethality suicide methods, such as hanging and jumping from a height ($p < 0.001$), have a clearer conscious level ($p < 0.001$), and use vasopressor ($p = 0.036$). They were less likely to use an ambulance service ($p < 0.001$), have

Table 2. Characteristics of hospitalized children due to suicide attempt and self-harm: Age comparisons (*n* = 1,704)

Variables	7-12 years old *n* = 166 *n* (%)	13-15 years old *n* = 842 *n* (%)	16-17 years old *n* = 696 *n* (%)	*p* value
Age (years old)				
7-12: Elementary	NA	NA	NA	NA
13-15: Junior high	NA	NA	NA	NA
16-17: Senior high	NA	NA	NA	NA
Female	88 (53.0)	587 (69.7)	501 (72.0)	< 0.001
Body mass index ((kg/m^2)				
< 18.5	74 (44.6)	193 (22.9)	131 (18.8)	< 0.001
18.5-24.9	55 (33.1)	426 (50.6)	390 (56.0)	
25.0-29.9	7 (4.2)	43 (5.1)	42 (6.0)	
≥ 30.0	2 (1.2)	18 (19.2)	19 (2.7)	
Current pregnancy	0 (0.0)	0 (0.0)	4 (0.6)	0.011
Smoking status				
Current/ ex-smokers	0 (0.0)	9 (1.1)	29 (4.2)	< 0.001
Ambulance use	87 (52.1)	556 (66.0)	473 (70.0)	< 0.001
Emergency admission	130 (78.3)	776 (92.2)	636 (91.4)	< 0.001
Forced admission by a licensed psychiatrist	15 (9.0)	92 (10.9)	79 (11.4)	0.335
Japan Coma Scale at admission				
Clear	111 (66.9)	460 (54.6)	321 (46.1)	< 0.001
Dizziness	19 (11.5)	167 (19.8)	166 (23.9)	
Somnolence	12 (7.2)	73 (8.7)	77 (11.1)	
Coma	24 (14.5)	142 (16.9)	132 (19.0)	
Comorbid psychiatric disorders				
Mood disorder	19 (11.5)	167 (19.8)	194 (22.9)	< 0.001
Depressive disorder	15 (9.0)	133 (15.8)	147 (21.2)	< 0.001
Bipolar affective disorder	2 (1.2)	18 (2.1)	22 (3.2)	< 0.001
Anxiety disorder				
Generalized anxiety disorder	1 (0.6)	19 (2.6)	20 (2.9)	0.215
Post-traumatic stress disorder	1 (0.6)	6 (0.7)	7 (1.0)	0.775
Obsessive-compulsive disorder	3 (1.8)	3 (0.4)	3 (0.4)	0.056
Panic disorder	0 (0.0)	3 (0.4)	11 (1.6)	0.014
Dissociative disorder	2 (1.2)	28 (3.3)	23 (3.3)	0.033
Somatoform disorder	7 (4.2)	24 (3.0)	12 (1.7)	0.013
Eating disorder	3 (1.8)	17 (2.0)	17 (2.2)	0.804
Attention deficit hyper activity disorder	9 (5.4)	16 (1.9)	7 (1.0)	< 0.001
Schizophrenia	0 (0.0)	0 (0.0)	1 (0.1)	0.485
Personality disorder	0 (0.0)	11 (1.3)	12 (1.7)	0.221
Non-diagnosis of any psychiatric disorders	126 (75.9)	575 (68.3)	424 (60.9)	< 0.001
Methods of suicide attempt and self-harm				
Hanging	54 (32.5)	105 (12.5)	67 (9.6)	< 0.001
Jumping from a height	43 (25.9)	198 (23.5)	139 (20.0)	
Poisoning	3 (1.7)	33 (3.9)	19 (2,7)	
Drug overdose	40 (24.0)	407 (48.3)	403 (57.9)	
Cutting or piercing without wrist cutting	3 (1.8)	34 (4.0)	18 (2.6)	
Wrist cutting	23 (13.8)	64 (7.6)	50 (7.2)	

emergency admission ($p < 0.001$), be diagnosed with a mental illness ($p < 0.001$), except ADHD ($p < 0.001$), and receive any psychiatric/psychological interventions ($p < 0.001$). In contrast, children > 13 years were more likely to be girls ($p < 0.001$), use lower-lethality suicide methods, such as drug overdose ($p < 0.001$), use an ambulance service ($p < 0.001$), be diagnosed with a mental illness ($p < 0.001$), particularly, depressive ($p < 0.001$), bipolar affective ($p < 0.001$), panic ($p = 0.014$), dissociative ($p = 0.033$), and somatoform disorders ($p = 0.013$), be hospitalized in a psychiatric ward ($p < 0.001$), receive psychiatric/psychological interventions ($p < 0.001$), particularly inpatient psychotherapy ($p < 0.001$)), and be transferred to another hospital after discharge ($p = 0.046$). Children aged 16-17 years exhibited the highest proportions of current pregnancy and smoking ($p < 0.001$), ambulance use ($p < 0.001$), diagnosed with a mental illness ($p < 0.001$), and receiving psychiatric/psychological interventions ($p < 0.001$), which included inpatient psychotherapy ($p < 0.001$) and antidepressant use ($p < 0.001$).

Discussion

This study, utilizing the Japanese nationwide inpatient database spanning 2016 to 2017, represents the first endeavor to unveil the characteristics of children hospitalized aged 7-17 years owing to suicide attempts and self-harm, along with tracing their clinical trajectory from admission to discharge. Although previous studies

Table 3. Clinical trajectory of suicide attempts among hospitalized children: Gender comparisons (*n* = 1,704)

Variables	Total *n* = 1,704 *n* (%)	Girls *n* = 1,176 *n* (%)	Boys *n* = 528 *n* (%)	*p* value
Length of hospitalization, days, median (IQR)	4 (2-14)	4 (2-13)	4 (2-15)	0.43
Admission ward				
Psychiatry	324 (19.0)	255 (21.7)	69 (13.1)	< 0.001
Intensive care unit	292 (17.1)	156 (13.3)	136 (25.8)	< 0.001
Intermediate intensive care unit	529 (31.0)	372 (31.6)	157 (29.7)	0.43
Physical interventions		35 (3.0)	67 (12.7)	< 0.001
Mechanical ventilation	208 (12.2)	96 (8.2)	112 (21.2)	< 0.001
Blood transfusion	102 (6.0)	54 (4.6)	48 (9.1)	< 0.001
Closed heart massage	102 (6.0)	35 (3.0)	67 (12.7)	< 0.001
Counter shock	16 (0.9)	6 (0.5)	10 (1.9)	0.006
Gastric lavage	163 (9.6)	133 (11.3)	30 (5.7)	< 0.001
Vasopressor use	195 (11.4)	92 (7.8)	103 (19.5)	< 0.001
Psychiatric/psychological interventions				
Inpatient psychotherapy	713 (41.8)	515 (43.8)	198 (37.5)	0.015
Cognitive therapy/cognitive behavioral therapy	0 (0.0)	0 (0.0)	0 (0.0)	NA
Inpatient group psychotherapy	24 (1.4)	20 (1.7)	4 (0.8)	0.13
Psychiatric occupational therapy	67 (3.9)	48 (4.1)	19 (3.6)	0.64
Psychiatric discharge guidance	87 (5.1)	71 (6.0)	16 (3.0)	0.009
Antidepressants use	111 (6.5)	89 (7.6)	22 (4.2)	0.009
Non-psychiatric/psychological intervention	959 (56.3)	515 (43.8)	198 (37.5)	0.015
Death during hospitalization	151 (8.9)	54 (4.6)	97 (18.4)	< 0.001
Death within 24 hours after admission	123 (7.3)	40 (3.4)	83 (15.7)	< 0.001
Discharge destination				
Home	1,347 (79.1)	978 (83.2)	369 (69.9)	< 0.001
Another hospital/clinic	183 (10.7)	128 (10.9)	55 (10.4)	
Social welfare facility	18 (1.1)	12 (1.0)	6 (1.1)	
Japan Coma Scale at discharge				
Clear	1,490 (87.4)	1,078 (91.7)	412 (78.0)	< 0.001
Dizziness	52 (3.1)	36 (3.1)	16 (3.0)	
Somnolence	5 (0.3)	5 (0.4)	0 (0.0)	
Coma	6 (0.4)	3 (0.3)	3 (0.6)	
Total healthcare cost, dollars, median (IQR)	293,240 (162,860-715,660)	278,270 (154,900-649,320)	332,685 (179,195-862,250)	< 0.001

IQR, inter quartile range. The conversion rate of 1 dollar = 105 yen for the years 2016 and 2017.

in Japan reported suicide attempts and self-harm among older persons hospitalized aged 29-60 years and those in the community aged 16-49 years (*5,9*) and suicide ideation and attempts among children aged 13-18 years, no studies have included hospitalized children with a younger age group (*24*). The findings bear significant implications for health policies, healthcare systems, and clinical practices aimed at preventing child suicide considering genders and age groups.

Overall tendency

First, this study sheds light on the demographic profile of children hospitalized for suicide attempts and self-harm. A systematic review has highlighted a rapid surge in the prevalence of self-harm during early teenage years (*13*). This escalation may be as a result of specific neurodevelopmental vulnerabilities, such as serotonin imbalance, alongside personality traits such as perfectionism and impulsivity and cognitive vulnerabilities such as impaired social problem-solving abilities. The confluence of these vulnerabilities among early adolescents precipitates an upsurge in self-destructive behaviors, especially in the prevalence of adverse social events and circumstances in both early and recent life (*e.g.*, bullying, interpersonal difficulties, parental illness or loss, strained family relationships, physical punishment, and instances of child abuse and neglect) (*7,8,13*). Additionally, the percentage of female individuals in this study (70%) exceeded that observed among adults hospitalized for similar reasons in Japan (61.0%) (*9*). Consequently, female individuals in early adolescence may face heightened risk of engaging in suicide attempts and self-harm.

Notably, this study exhibited a higher prevalence of being underweight among the children examined. In Japan, the prevalence of being underweight among young female individuals aged 20-29 years ranks highest among developed countries (*25*). Studies (*26,27*) have linked being underweight with mental health issues such as depression and suicidal ideation, which can directly elevate the risk of suicide. Being underweight is a significant clinical indicator of eating disorders, such as anorexia nervosa, among adolescents (*28,29*), indicating

Table 4. Clinical trajectory of suicide attempts among hospitalized children: Age comparisons (*n* = 1,704)

Variables	7-12 years old *n* = 166 *n* (%)	13-15 years old *n* = 842 *n* (%)	16-17 years old *n* = 696 *n* (%)	*p* value
Length of hospitalization, days, median (IQR)	5 (2-12)	4 (2-12)	3 (2-12)	0.17
Admission ward				
Psychiatry	19 (10.8)	158 (18.8)	148 (21.3)	< 0.001
Intensive care unit	36 (21.7)	151 (17.9)	105 (15.1)	0.088
Intermediate intensive care unit	27 (16.3)	256 (30.4)	246 (35.3)	< 0.001
Physical interventions				
Mechanical ventilation	23 (13.9)	111 (13.2)	74 (10.5)	0.249
Blood transfusion	10 (6.0)	49 (5.8)	43 (6.2)	0.957
Closed heart massage	11 (6.8)	62 (7.4)	29 (4.2)	0.029
Counter shock	1 (0.6)	10 (1.2)	6 (0.7)	0.569
Gastric lavage	4 (2.4)	85 (10.1)	74 (10.6)	0.004
Vasopressor use	26 (15.6)	104 (12.4)	65 (9.3)	0.036
Psychiatric/psychological interventions				
Inpatient psychotherapy	46 (27.7)	350 (41.6)	317 (45.6)	< 0.001
Cognitive therapy/cognitive behavioral therapy	0 (0.0)	0 (0.0)	0 (0.0)	NA
Inpatient group psychotherapy	2 (1.2)	13 (1.5)	9 (1.3)	0.892
Psychiatric occupational therapy	5 (3.0)	33 (3.9)	29 (4.2)	0.789
Psychiatric discharge guidance	6 (3.6)	49 (5.8)	32 (4.6)	0.365
Antidepressants use	5 (3.0)	45 (5.3)	71 (8.8)	< 0.001
Non-psychiatric/psychological intervention	118 (71.1)	481 (57.1)	360 (51.7)	< 0.001
Death during hospitalization	18 (10.8)	90 (10.7)	43 (6.2)	0.005
Death within 24 hours after admission	10 (6.0)	75 (8.9)	38 (5.5)	0.097
Discharge destination				
Home	136 (81.9)	658 (77.0)	563 (80.9)	0.046
Another hospital/clinic	11 (6.6)	91 (10.8)	81 (11.6)	
Social welfare facility	1 (0.6)	11 (1.3)	6 (0.9)	
Japan Coma Scale at discharge				
Clear	145 (87.4)	728 (86.5)	617 (88.7)	
Dizziness	2 (1.2)	19 (2.3)	31 (4.5)	
Somnolence	0 (0.0)	1 (0.1)	4 (0.6)	
Coma	1 (0.6)	4 (0.5)	1 (0.1)	0.004
Total healthcare cost, dollars, median (IQR)	351,750 (184,670- 861,270)	302,500 (160,470- 785,620)	268110 (163,470- 622,760)	0.165

IQR, inter quartile range; The conversion rate of 1 dollar = 105 yen for the years 2016 and 2017.

that being underweight may be a crucial and useful screening indicator for detecting a high-risk population for child suicide attempts as well as self-harm earlier in clinical, school, and community settings.

In this study, the proportions of being in a coma at admissions, intensive physical interventions, and mortality within 24 hours were lower than those reported in a prior study on adults (*9*). The methods of suicide attempts and self-harm among children in this study were similar to those utilized for adults in a prior study (*9*). A systematic review (*13*) exhibited that methods of self-harm, such as self-cutting, jumping from a height, and self-batteries, were heterogeneous among children. However, this study could not identify diverse and unique methods for reducing fatal self-destruction.

Additionally, this study exhibited that 66% of children were not diagnosed with any mental illness, and 56% did not receive any psychiatric/psychological interventions during hospitalization. In contrast, a prior study exhibited lower proportions: 38% of adults were not diagnosed with any mental illness, and 51% did not receive any psychiatric/psychological interventions (*9*). This may be as a result of the severe shortage of child psychiatric hospitals and healthcare professionals with sufficient skills and knowledge in child psychiatry in Japan. (*2,30,31*).

Finally, the length of hospitalization and total healthcare costs owing to child suicide attempts and self-harm were somewhat higher than those among adult patients (*9*). One conceivable reason for this is that the cost may include additional medical costs for pediatric patients as they utilize special equipment and a higher number of healthcare professionals (*32*). Additionally, the shorter length of hospitalization among children may be as a result of their lower rate of death within 24 hours post-admission as opposed to adults.

Gender differences

This study showed that methods of suicidal behaviors differed between the genders. Girls were more likely to use low-lethality methods, such as self-poisoning and wrist cutting, which indicated self-harm; conversely, boys were more likely to use high-lethality methods, such

as hanging and jumping from a height, which indicated suicide attempts. These results were consistent with those of previous studies that girls tended to have suicidal ideation and conduct self-harm using a low-lethality method (*7,13,33,34*). A longitudinal study reported the trajectories of internalizing and externalizing behaviors by genders (*14*) and demonstrated that girls had increased internalizing behaviors, such as depressive symptoms, toward age 10 and continued to maintain these behaviors until age 17. However, externalizing behaviors, such as aggression, were higher in boys than in girls at age 5, which continued till age 17. Thus, differences in behavioral tendencies between girls and boys, especially during adolescence, could directly influence differences in their suicidal behaviors. Moreover, differences in their suicidal behavior may directly result in significant differences in their status during hospital admission and subsequent medical interventions and healthcare costs. Boys were more likely to receive more physical interventions and have higher healthcare costs.

Additionally, the rates of psychiatric diagnosis and treatment differed significantly between boys and girls. Previous studies demonstrated that depression was higher in adolescent girls and mediated the relationship between individual negative life events (*e.g.*, victimization of childhood abuse, bullying, suicidal ideation) and behaviors (*10,12,35*). This suggested that girls may be more likely to manifest their distress owing to negative life events as psychiatric symptoms than men, which may lead to higher rates of psychiatric diagnosis and psychiatric/psychological treatments.

Age differences

This study revealed that 9.7% of children hospitalized due to suicide attempt or self-harm were preadolescent children < 12 years old. They were more likely to use higher-lethality suicide methods, such as hanging and jumping from a height. Results of the higher proportion of low-lethality suicide methods were consistent with those in previous studies (*7,13*). A meta-analysis revealed that suicide death among pre-adolescents was rare, being approximately 1 in 1 million children compared with 3.8 in 100,000 adolescents (*36*). However, a recent systematic and meta-analytic review revealed that approximately 17.0% of pre-adolescents with suicidal ideation attempted suicide (*13*). The results of this study provide evidence to question the view that pre-adolescents do not experience suicidal thoughts and engaged in suicidal behaviors owing to the underdevelopment of their cognitive capacity (*37,38*). Furthermore, this study suggests the necessity of paying attentions to suicidality among pre-adolescents.

In addition, pre-adolescent children and adolescents who committed self-harm and suicide attempt were more likely to be diagnosed with ADHD and mood disorders, such as depression, respectively. This result was consistent with that of a previous meta-analysis, which revealed that ADHD was a strong and unique clinical factor of suicidal ideation among pre-adolescents (*7*). This high level of impulsivity and hyperactivity may be associated with their high-lethality suicidal behaviors. In addition, particular neurodevelopmental vulnerability among pre-adolescents may be related to their more direct suicidal behaviors. Owing to limited research on pre-adolescent suicide, further research should accumulate evidence on their suicidality and its related factors.

The disparities in access to psychiatric/psychological healthcare services between the age groups should also be highlighted. This study revealed that 71.1% of pre-adolescents did not receive any psychiatric/psychological interventions during hospitalization. Furthermore, this proportion was significantly higher than that in adolescents (57.1%). This may be due to the difficulty of diagnosing mental illness among younger children. In general, children's psychiatric symptoms manifest differently from those of adults, and many symptoms are not visible and are recognized as normal during development (*39*). Thus, only 10-20% of children with mental health problems meet the criteria for a specific psychological disorder, and the majority do not receive healthcare services (*39*). In particular, mental symptoms among children aged < 12 years may be more invisible owing to their immaturity in linguistic, cognitive, and intellectual development, than those in adolescents. This may be associated with the disparities in psychiatric/psychological healthcare service's depending on age groups.

Future implications

This study provides valuable evidence for developing effective policies and systems for suicide prevention in children from genders and age groups. At present, the Japanese government considers the prevention of child suicide a national priority as the number of child suicides is at a historical high as of 2022 (*3*). In 2023, the Japanese government released a plan to strengthen measures against child suicide (*3*), and the results of this study will be useful evidence regarding promoting the measures proposed by government. This study highlights the importance of attentiveness toward being underweight as a potential predicting factor for child suicidality. Psychological screening has been recommended to identify children at high risk of suicide in educational and clinical settings. However, a recent systematic review (*40*) concluded that the benefit and harm of such screening remained uncertain, especially for children and adolescents. Use of being underweight as an indicator for screening the risk of child suicide attempts and self-harm may be feasible and acceptable for frontline providers owing to its less invasive and stigma-free approach. In addition, this study suggests

the necessity of developing age- and gender-sensitive policies and interventions to effectively prevent child suicidality. This study revealed that boys and pre-adolescents tended to commit higher-lethality suicide attempts, while girls and adolescents tended to commit lower-lethality suicide attempts. Previous studies have indicated that psychopathology that lead to suicidal behaviors may differ based on gender and developmental stages (*7,13*). Thus, careful attention, interventions, and policies for the prevention of suicidality among children based age groups and gender are crucial.

Additionally, this study reported that the majority of children hospitalized due to suicide attempt and self-harm did not receive psychological treatment or interventions during hospitalization. These results indicate the importance of enhancing psychiatric/psychological treatments and psychosocial interventions. A meta-analysis examined the efficacy of psychosocial interventions for children with suicidal behaviors and demonstrated that psychosocial interventions, such as dialectical behavior, cognitive-behavioral, and mentalization-based therapies, reduced their suicidality (*41*). To provide sufficient psychiatric/psychological healthcare services for children hospitalized due to suicide attempt and self-harm, a policy to increase child psychiatric hospitals and healthcare professionals with sufficient skills and knowledge in child psychopathology is urgently required in Japan. This could directly contribute to preventing their relapse of and death due to suicidal behaviors. Furthermore, this study indicated gender and age inequality in psychiatric/psychological health service utilization for children hospitalized due to self-harm and suicide attempt. Hence, this should be an important warning to healthcare professionals. Furthermore, a policy to increase psychiatric/psychological interventions, especially for boys and pre-adolescents, is necessary.

Strengths and limitations

To the best of our knowledge, this study was the first to identify the number, characteristics, and clinical trajectory of hospitalized children of various ages from 7-17 years due to suicide attempts and self-harm *via* a nationwide inpatient database. Children with high suicidality are one of the most difficult populations to approach owing to ethical issues, such as higher physical and psychological burdens and consent age for study. This study reported the actual situations of such children *via* a nationwide inpatient database, without any additional burden on the children. These findings should be used to develop an effective healthcare policy and system to prevent child suicide in Japan and also worldwide.

This study had some limitations. First, this study included only patients who had attempted suicide or self-harm and were hospitalized in acute care hospitals. Consequently, it did not include non-hospitalized patients or those who passed on prior to admission. Second, owing to the lack of rigorous terminology and definitions in this database, the recorded information on suicide attempts and self-harm did not necessarily follow the definition of "deliberate self-harm" (*42*). Third, owing to the limitations encountered concerning collecting longitudinal data utilizing the database, we could not identify the long-term trajectory of suicide attempts and self-harm, including repeated suicide attempts and mortality. Further studies collecting long-term longitudinal data are necessary. Fourth, the database did not include information on victimization through violence, social support from parents, or self-esteem, which are reportedly correlated to suicide attempts (*7*). Finally, this study used only the 2016-2017 database as only this period was available for accessing information on suicide attempt and self-harm for all hospitalized patients. Thus, we could not identify longitudinal changes in the number and related factors of children hospitalized due to suicide attempt and self-harm. However, previous research reported that the number of child suicides increased during the COVID-19 pandemic (*43*).

Conclusion

This study utilized data from the Japanese nationwide inpatient database spanning 2016 to 2017, examining the characteristics of 1,704 children hospitalized owing to suicide attempts and self-harm, alongside tracing their clinical trajectory from admission. This study's findings serve as valuable evidence to inform the development of effective changes in health policies, healthcare systems, and interventions aimed at preventing child suicide considering genders and age.

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Prevalence of physical frailty and its associated factors among elderly patients undergoing hepatobiliary pancreatic surgery in China

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Abstract: Frailty is a geriatric syndrome characterized by a multisystem physiological decline, increased vulnerability to stressors, and adverse clinical outcomes. However, there is a knowledge gap regarding the association between frailty and its influencing factors. This study aimed to understand the current status of preoperative frailty in elderly patients with hepatobiliary pancreatic disease (HBP) and analyze debilitation-related factors. We enrolled 220 participants aged ≥ 65 years who underwent HBP surgery at two hospitals in China between December 2023 and February 2024. The physical frailty of elderly participants in communities with different characteristics was compared using Kruskal-Wallis and chi-square tests. Ordinal logistic regression analysis was used to analyze the factors influencing preoperative frailty. A total of 212 patients were included in the analysis based on the inclusion and exclusion criteria, with an overall prevalence of frailty at 53 (25%). Ordinal logistic regression analysis results showed that current smoking (odds ratio [OR] = 2.584, $p = 0.006$) was an independent risk factor for preoperative frailty in elderly participants with HBP. In contrast, exercise habits (OR = 0.323, $p < 0.001$), two or more multimorbidity statuses (OR = 0.495, $p = 0.033$), and independent status (OR = 0.216, $p < 0.001$) were protective factors. Our results suggest that having good exercise habits, not smoking, and independent status can prevent frailty progression in older adults who require HBP surgery. Interventions for frail elderly patients should be supported preoperatively by strengthening exercises to improve tolerance to surgery.

***Keywords*:** frailty, hepatobiliary pancreatic surgery, aging, frailty assessment, influencing factor

Introduction

The global population is aging rapidly, with China having the world's largest aging population and Japan having the world's highest aging rate. The figures released by the Japanese Ministry of Internal Affairs and Communications in 2022 show that the population over 65 years of age has reached 36.23 million, accounting for 29.1% of the total population (*i.e.*, aging rate) (*1*). The number of Chinese adults aged 65 years and older reached 216.76 million by the end of 2023, accounting for 15.4% of the total population (*2*). Interestingly, this rapid rate of population aging has been outpaced by an increase in number of older patients needing surgical intervention as a main modality of treatment. Laparoscopic hepatobiliary pancreatic (HBP) surgery is a minimally invasive surgical method with many advantages in the treatment of HBP diseases (*3*). However, this type of surgery is difficult, especially with the long duration of pancreaticoduodenectomy, wide resection area, and high incidence of postoperative complications (*4*). To reduce hospital stay, hospital costs, and postoperative complications, it is particularly important to understand the status of patients during the perioperative period.

Fried *et al.* provided the first standardized definition of frailty as a geriatric syndrome characterized by multisystem physiological decline, increased vulnerability to stressors, and adverse clinical outcome (*5*). Frailty increases the risk of adverse outcomes, including mortality, major morbidity, and decreased functional status and quality of life (*5,6*). Although the risk of frailty increases with age, not all older adults are frail, and frailty is not exclusive to the aged (*7,8*). Most importantly, frailty is not a static state and can progressively improve depending on intervention (*7,9,10*).

A joint statement from the American College of

Surgeons and American Geriatrics Society recommends frailty assessment as part of the preoperative assessment of older surgical patients (*11*), and the recognition that frail patients have unique vulnerabilities and challenges is increasing in surgery. The relationship between frailty and post-operative outcomes in various surgical specialties has been a popular topic in recent years (*12*). Various approaches exist for measuring frailty; however, there is little agreement regarding the optimal frailty instrument. Agreement does exist that frailty measurement should be operationalized using a multidimensional approach, which is most often performed using either Fried's Frailty Phenotype or the Edmonton Frailty Scale (EFS) (*13*). Elderly patients undergoing surgery generally have been found to have a higher prevalence of frailty (25%-56%). Notably, most studies were performed on Caucasian patients in Canada and the United States (*14*). The outcomes of HPB surgery have improved tremendously over the past decade, with reduced postoperative mortality from 20% to less than 3% and 5%-6% for major liver and pancreatic surgeries, respectively. Therefore, frailty tools need to be incorporated into clinical practice to improve these outcomes (*15*). However, to date, no studies have specifically focused on the relationship between frailty and HBP among elderly patients in China. To address these important knowledge gaps, we conducted a population-based study of older adults who underwent common major HBP surgical procedures. The primary objective of this study was to understand the current status of frailty in elderly surgical patients with HBP and analyze the factors related to their debilitation.

Patients and Methods

Study design

This cross-sectional study was performed at two hospitals (First Affiliated Hospital of Chongqing Medical University and Peking University International Hospital) in China. We enrolled patients who were 65 years of age and above and had undergone HBP surgery from December 2023 to February 2024. As shown in Figure 1, 220 patients who underwent elective HBP surgery were prospectively enrolled. Written informed consent was obtained from all patients prior to enrolment in the study.

The inclusion criteria for this study were as follows: *i*) patients 65 years of age and older, *ii*) patients who were conscious and provided written informed consent, and *iii*) patients who were expected to survive for more than 3 months without serious cardiopulmonary, renal, or psychiatric disorders. The exclusion criteria were as follows: *i*) patients who did not consent to surgical treatment, *ii*) patients who died, and *iii*) patients who were transferred to another hospital before discharge. Sociodemographic data were obtained pre-operatively, and measures for evaluating self-care, nutritional risk screening, assessment of the patient's risk of depression, and frailty status were completed before surgery. Eight participants were excluded from the study because they could not be evaluated for frailty or their questionnaires were incomplete.

Prior to conducting the study, the approval from Ethics Committee of Hamamatsu University School of Medicine was obtained (NO. 23-250, 6 November 2023),

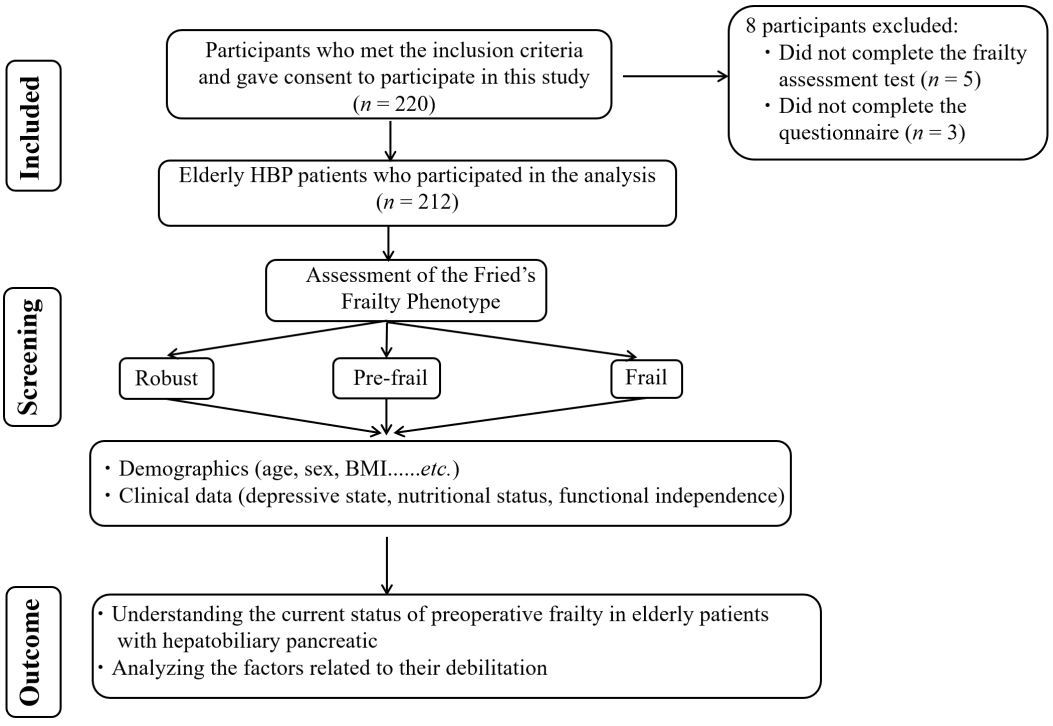

Figure 1. Study flowchart.

the First Affiliated Hospital of Chongqing Medical University (NO. 2023-337, 26 December 2023) and the Peking University International Hospital (NO. 2023-KY-0085-01, 12 December 2023). The participants' data were processed and electronically stored in accordance with the ethical principles of the Declaration of Helsinki for medical research involving human subjects. Data were stored and analyzed anonymously.

Sample size

This study is a cross-sectional design, and the sample size was calculated using the formula $n = [z^2 \alpha/2\ p(1-p)]/\delta^2$. Based on previous studies, where p = 25% (*14*), $\alpha = 0.05$, t = 1.96, and the allowable error = 0.06, the required sample size was determined to be 200 cases. Considering shedding and other factors, and additional 10% was added, bringing the total number of required cases to 220.

Research measures

Outcome parameters

Data collected preoperatively included the demographics of the participants, such as age, sex, body mass index (BMI), marital status, place of residence (rural/urban), level of education, annual tax-included income, alcohol and smoking status, sleep status, exercise habits, alone situation, and clinical data such as multimorbidity and polypharmacy, depressive state (assessed using the Patient Health Questionnaire-9 [PHQ-9]), nutritional status (by the Nutritional Risk Screening 2002), functional independence (by the Barthel Index), and frailty measures (by the Fried's Frailty Phenotype).

The Fried's Frailty Phenotype (The Fried's FP)

The Fried's FP test was used to determine the presence and degree of frailty. Preoperatively, Fried's FP was completed by the participants under the supervision of specially trained nurses. Fried's FP is a multi-dimensional screening tool comprising five domains: slow walking speed (slowness), grip strength (weakness), weight loss (shrinking), fatigue (poor endurance/energy), and low physical activity. Slowness was assessed using the timed get-up-and-go test. The area for the timed get-up-and-go test was measured 3 m from the front legs of the straight-backed armchair. The subject was instructed as follows: "sit with your back against the chair and your arms on the arm rests. On the word "go", stand upright, then walk at your normal pace to the line on the floor, turn around, return to the chair, and sit down". The time required to complete the test was time from the word "go" to time when the subject returned to the starting position. Subjects who took > 10 seconds to complete the test were classified as frail. Grip strength was measured using a Camry hand dynamometer and compared with normative data adjusted for age and sex. Participants met the "weak grip strength" criterion if their grip strength was below the 20th percentile. The subject was seated with the forearm resting on the arm of a chair and instructed to hold the dynamometer upright and squeeze it as hard as possible. Three trials in the right hand, followed by three trials in the left hand, were recorded, and the highest reading of the six was taken as the final reading. The criterion for weight loss was met if the participants suffered an unintentional loss of 2 or 3 kg in half a year. Regarding fatigue, participants were asked if they felt exhausted without any reason in the previous month and if they exercised regularly once a week for low physical activity. The scores were summed, with a score of 0 classified as non-frail, a score of 1-2 classified as pre-frail, and a score of 3-5 classified as frail.

Nutritional risk screening 2002 (NRS 2002)

This scale is a nutritional risk-screening tool developed by Kondrup *et al.* (*16*) in 2002 based on the sequential medical method, which includes three items: disease severity score, nutritional impairment score, and age score. With a total score of 0 to 7, "3" is considered nutritional risk and "4" no nutritional risk. This scale is the most widely used and clinically validated nutritional risk screening tool, and has been recommended by several nutritional associations.

Barthel Index (BI)

The BI is an ordinal scale used to measure functional disability while performing ten daily activities (*17*). It is a validated 10-item instrument that measures a patient's independence in performing the main activities of daily living, including bathing, dressing, toileting, transferring, continence, and feeding. Functional status is defined as "independent" if the participant does not require any assistance from another person for any activities of daily living. The participant is considered "partially dependent" if they require some assistance from another person for activities of daily living and "totally dependent" if they require assistance for all activities of daily living. Scores range from 0-100, with a total score of 100 indicating the highest level of independence.

Patient Health Questionnaire-9 (PHQ-9)

The PHQ-9 (*18*) was used as a self-administered screening tool to assess the severity of depressive symptoms. Unlike other depression scales, the PHQ-9 includes nine items that assess symptoms of Major Depressive Disorder (MDD), as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). The questionnaire assessed how often the subjects had been disturbed by any of the nine items during the immediately preceding two weeks. Each item of the PHQ-9 is scored on a scale of 0 to 3 (0 = not at all, 1 = several days, 2 = more than a week, 3 = nearly every day). The PHQ-9 total score ranges from

0 to 27 (scores of 0-4 indicate normal or no depressive symptoms; 5-9 indicate mild depression; 10-14 indicate moderate depression; 15-19 indicate moderately severe depression; and ≥ 20 indicate severe depression).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 29.0. Frailty was analyzed as a categorical variable. Patients were defined as robust, pre-frail, or frail based on their frailty scores, as noted above. Descriptive statistics of the baseline demographic and clinical variables were calculated using mean (standard deviation) or percentages (%). The Shapiro-Wilk test was used to assess normal distribution. Non-parametric Kruskal-Wallis and chi-square tests were used to compare continuous and categorical variables, respectively. Ordinal logistic regression was used for categorical variables to predict variables affecting frailty. Statistical comparisons were 2-sided and a p value <0.05 was considered statistically significant.

Results

Baseline characteristics

The participant characteristics and demographic data are presented in Table 1. In total, 212 elderly patients with HBP were enrolled between December 2023 and February 2024. The mean (± standard deviation) patient age was 72.46 ± 5.94 years, and 116 (54.7%) were male. The BMI of the study subjects was 22.39 ± 3.47 kg/m^2; married individuals accounted for 163 (76.9%), while unmarried, divorced, or widowed account for 49 (23.1%). One hundred eighty-three patients (86.3%) had no postsecondary education. Sixty-two (29.2%) patients lived in rural areas, and 121 (57.1%) belonged to the middle- and low-income groups (annual tax-included income < 50,000 RMB). Forty-four (20.8%) patients continued to smoke and 168 (79.2%) were non-smokers or had quit smoking. Only 36 (17.0%) patients drank alcohol, with the vast majority abstaining from alcohol consumption. One hundred and thirty-two (62.3%) had exercise habits, 102 (48.1%) had 0-1 chronic disease, and 110 (51.9%) had two or more chronic diseases. One hundred and forty-one (66.5%) patients had normal sleep, 71 (33.5%) had sleep difficulties, 182 (85.8%) were taking three or fewer drugs, and 30 (14.2%) were taking four or more drugs. One hundred eighty-one patients (85.4%) had no symptoms of depression. Sixty-one (28.8%) patients were dependent on other people for help, 171 (80.7%) lived with their family, 41 (19.3%) lived alone or in nursing homes, and 180 (84.9%) had good nutritional status.

Preoperative frailty in elderly hepatobiliary and pancreatic patients and single factor analysis

As shown in Table 1, among the elderly participants with HBP disease, there were 53 cases in the frailty group (25%), 83 in the pre-frailty group (39.1%), and 76 in the non-frailty group (35.8%). Details of the frailty scale indicators are presented in Table 2. Fatigue (42.0%) was the most common, followed by low grip strength (31.1%), slow walking speed (30.7%), weight loss (26.9%), and low activity levels, which were reported by at least 50 (23.6%) participants. Univariate analysis showed statistically significant differences in age, education level, smoking status, alcohol status, exercise habits, activity of daily living (ADL), multimorbidity and polypharmacy, sleep status, and nutritional status in the incidence of preoperative frailty among the elderly participants ($p < 0.05$), as shown in Table 1.

Factors influencing preoperative frailty in elderly hepatobiliary and pancreatic patients

To study the factors influencing preoperative frailty, ordinal logistic regression analysis was performed. The results are summarized in Table 3. The preoperative frailty of elderly participants with HBP diseases (frailty group = 1, pre-frailty group = 2, robust group = 3) was used as the dependent variable. The result of the parallel line test was $p = 0.978$, > 0.05 and which is ensuring the accuracy and reliability of the ordinal logistic regression analysis results. The results showed that current smoking status (OR = 2.584, $p = 0.006$) was an independent risk factor for preoperative frailty in elderly participants with HBP. In contrast, exercise habits (OR = 0.323, $p < 0.001$), two or more multimorbidity statuses (OR = 0.495, $p = 0.033$), and independent status (OR = 0.216, $p < 0.001$) were protective factors. Age had no significant effect on preoperative frailty.

Discussion

This study showed that 25% of the 212 elderly HBP patients were frail. Komici *et al.* (*19*) showed that a total of 34,276 HBP cancer patients were identified, and the weighted prevalence of frailty was 39%. Therefore, the number of patients included in our study was lower than that of patients with HBP cancer in a previous study. This may be related to different research objectives and evaluation standards. Komici *et al.* included patients with HBP cancer who had been treated with surgery, chemotherapy, and radiotherapy, whereas the elderly patients in this study had not yet undergone invasive treatments such as surgery. At the same time, as many different instruments are used to measure frailty, it is difficult to reliably compare those results. To date, there is no consensus regarding the assessment of frailty. There are more than 60 validated tools for screening and measuring frailty with important similarities; however, there is no defined standard assessment tool for frailty assessment in HBP surgery. Nevertheless, this wide

Table 1. Characteristics of participants in the study cohort, stratified by whether they were determined to be frail or non-frail using the Fried's Frailty Phenotype instrument before surgery

Variable	All participants *n* = 212	Robust (0) *n* = 76 (35.8%)	Pre-Frail (1-2) *n* = 83 (39.1%)	Frail (3-5) *n* = 53 (25%)	*p*-value
Age (years, x ± s)	72.46 ± 5.94	72.46 ± 5.95	71.90 ± 5.38	71.94 ± 7.68	0.018*[a]
65 ≤ age < 70 years old	83 (39.2%)	21 (25.4%)	31 (37.3%)	31 (37.3%)	< 0.001*[b]
70 ≤ age < 80 years old	101 (47.6%)	46 (45.5%)	43 (42.6%)	12 (11.9%)	
Age ≥ 80 years	28 (13.2%)	9 (32.1%)	9 (32.1%)	10 (35.8%)	
Sex (*n*, %)					0.441[b]
Male	116 (54.7%)	40 (34.5%)	43 (37.1%)	33 (28.4%)	
Female	96 (45.3%)	36 (37.5%)	40 (41.7%)	20 (20.8%)	
BMI (kg/m^2, x ± s)	22.39 ± 3.47	21.88 ± 2.72	23.11 ± 3.76	22.02 ± 3.83	0.081[a]
Marital status (*n*, %)					0.433[b]
Married	163 (76.9%)	58 (35.6%)	61 (37.4%)	44 (27.0%)	
Unmarried, divorced or widowed	49 (23.1%)	18 (36.7%)	22 (44.9%)	9 (18.4%)	
Level of education (*n*, %)					0.019*[b]
No post-secondary	183 (86.3%)	60 (32.8%)	72 (39.3%)	51 (27.9%)	
Bachelor's degree or above	29 (13.7%)	16 (55.2%)	11 (37.9%)	2 (6.9%)	
Place of residence (*n*, %)					0.925[b]
Rural	62 (29.2%)	23 (37.1%)	23 (37.1%)	16 (25.8%)	
Urban	150 (70.8%)	53 (35.3%)	60 (40.0%)	37 (24.7%)	
Annual tax-included income (RMB) (*n*, %)					0.191[b]
< 50,000	121 (57.1%)	38 (31.4%)	48 (39.7%)	35 (28.9%)	
≥ 50,000	91 (42.9%)	38 (41.7%)	35 (38.5%)	18 (19.8%)	
Smoking status (*n*, %)					0.004*[b]
Quit/non-smoker	168 (79.2%)	67 (88.2%)	67 (80.7%)	34 (64.2%)	
Current smoker	44 (20.8%)	9 (20.5%)	16 (36.4%)	19 (43.1%)	
Alcohol status (*n*, %)					0.024*[b]
Quit/non-drinker	176 (83.0%)	69 (39.2%)	62 (35.2%)	45 (25.6%)	
Current drinker	36 (17.0%)	7 (19.5%)	21 (58.3%)	8 (22.2%)	
Exercise habits (*n*, %)					< 0.001*[b]
Yes	132 (62.3%)	57 (43.2%)	54 (40.9%)	21 (15.9%)	
No	80 (37.7%)	19 (23.8%)	29 (36.2%)	32 (40.0%)	
Multimorbidity (*n*, %)					0.023*[b]
0~1	102 (48.1%)	39 (38.2%)	46 (45.1%)	17 (16.7%)	
≥ 2	110 (51.9%)	37 (33.6%)	37 (33.6%)	36 (32.8%)	
Sleep status (*n*, %)					0.003*[b]
Good	141 (66.5%)	59 (41.8%)	56 (39.7%)	26 (18.5%)	
Bad	71 (33.5%)	17 (23.9%)	27 (38.0%)	27 (38.0%)	
Polypharmacy (*n*, %)					0.038*[b]
0~3	182 (85.8%)	69 (37.9%)	73 (40.1%)	40 (22.0%)	
≥ 4	30 (14.2%)	7 (23.4%)	10 (33.3%)	13 (43.3%)	
Depressive state (*n*, %)					0.062[b]
Yes	31 (14.6%)	6 (19.4%)	13 (41.9%)	12 (38.7%)	
No	181 (85.4%)	70 (38.7%)	70 (38.7%)	41 (22.6%)	
ADL (*n*, %)					< 0.001*[b]
Independent	151 (71.2%)	69 (45.7%)	57 (37.7%)	25 (16.6%)	
Dependent	61 (28.8%)	7 (11.5%)	26 (42.6%)	28 (45.9%)	
Alone situation (n, %)					0.183[b]
Yes	41 (19.3%)	15 (36.6%)	20 (48.8%)	6 (14.6%)	
No	171 (80.7%)	61 (35.7%)	63 (36.8%)	47 (27.5%)	
Nutritional status (*n*, %)					< 0.001*[b]
Bad	32 (15.1%)	6 (18.8%)	9 (28.1%)	17 (53.1%)	
Good	180 (84.9%)	70 (38.9%)	74 (41.1%)	36 (20.0%)	

*Data are presented as mean ± standard deviation or median (interquartile range) unless indicated otherwise; represents statistical significance ($p < 0.05$). a: The value obtained by Kruskal-Wallis test; b: The value obtained by chi-square test. BMI, body mass index; ADL, activity of daily living.

range of scores and scales allows physicians to find the scale that fits their needs according to the type of surgery, local population, and resources.

As illustrated in Figure 2, fatigue was the most prevalent physical frailty factor. The prevalence values in the cases of fatigue, one of the factors of frailty, were 50 (56.2%) and 39 (43.8%) in HBP older adults with prefrailty and frailty, respectively. Of the participants in the study by Uslu *et al.*, 63.8% were frail with physical and cognitive fatigue. The higher the frailty, the higher is the fatigue (*20*). Fatigue is an often-neglected symptom that is frequently reported by older people, leading to an inability to continue functioning at a normal level. Fatigue reflects the exhaustion of physiological reserves in older individuals. Despite its clinical relevance, fatigue is typically underestimated by healthcare professionals,

Table 2. Percentage of each factor of frailty in pre-frailty and frailty participants

Item	All participants *n* (%)[a]	Pre-frailty group (*n* = 83) *n* (%)[b]	Frailty group (*n* = 53) *n* (%)[b]
Weight loss	57 (26.9%)	22 (38.6%)	35 (61.4%)
Grip strength	66 (31.1%)	21 (31.8%)	45 (68.2%)
Fatigue	89 (42.0%)	50 (56.2%)	39 (43.8%)
slow walking speed	65 (30.7%)	21 (32.3%)	44 (67.7%)
Low activity level	50 (23.6%)	24 (48.0%)	26 (52.0%)

*a: Percentage of participants in all participants; b: Percentage of participants in the pre-frail or frail groups.

Table 3. Ordinal logistic regression analysis to identify influencing factors of frailty before hepatobiliary pancreatic surgery

Variables	Groups	*B*	*SE*	*Wald* X^2	*P-Value*	*OR*	95% CI	
							lower limit	upper limit
Age	65 ≤ age < 70 years old				Reference			
	70 ≤ age < 80 years old	-1.094	0.296	13.62	< 0.001	0.335	0.188	0.596
	Age ≥ 80 years old	-0.511	0.428	1.425	0.233	0.6	0.251	1.434
Smoking status	Quit/non-smoker				Reference			
	Current smoker	0.949	0.344	7.603	0.006	2.584	1.313	5.085
Have exercise habits	No				Reference			
	Yes	-1.13	0.32	12.448	< 0.001	0.323	0.172	0.606
Multimorbidity	0~1				Reference			
	≥ 2	-0.703	0.329	4.563	0.033	0.495	0.259	0.947
ADL	Dependent				Reference			
	Independent	-1.533	0.351	19.136	< 0.001	0.216	0.109	0.427

*Represents statistical significance ($p < 0.05$), SE, standard error; OR, odds ratio; CI, confidence interval; ADL, activity of daily living.

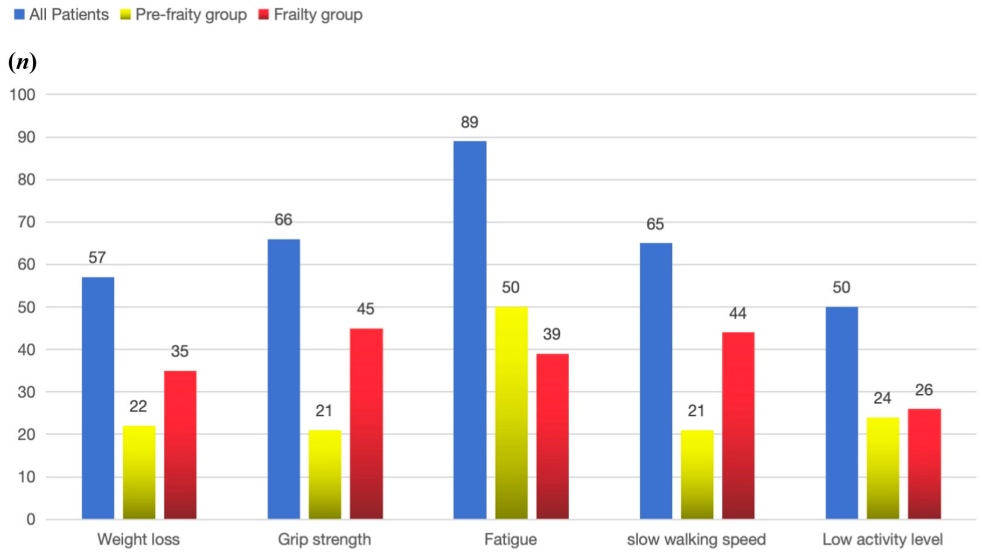

Figure 2. Overview of population of each factor of frailty in pre-frailty and frailty in elderly hepatobiliary and pancreatic patients (by the Fried's Frailty Phenotype).

mainly because reduced stamina is considered an unavoidable corollary of aging.

Although the link between marital status and frailty was not demonstrated in our study, it remains worthy of attention. In a study by Trevisan *et al.* (*21*), unmarried/divorced/widowed elderly people had no partner and needed to take care of themselves; therefore, they may pay more attention to their health status and take timely intervention measures when discovering health problems, resulting in better health conditions than married elderly people. At the same time, unmarried/divorced/widowed older adults are more likely to participate in community activities to relieve negative emotions, such as loneliness, which is beneficial for their physical health and reduces

the incidence of frailty.

Our study did not show that nutritional status is a risk factor for frailty in older adults. Both quantitative (energy intake) and qualitative (nutrient quality) assessments are important because the lack of micronutrients (Vitamin D or leucine) and macronutrients (proteins) are considered risk factors for frailty, while certain diets (*e.g.*, Mediterranean diet) can prevent or reverse frailty (*22,23*). In addition, nutrition-related biomarkers may be used to assess the nutritional status and frailty in elderly patients. Patients with better nutritional status and higher serum transferrin, total protein, and albumin levels are less likely to develop frailty (*24*). Rather than focusing on the link between nutrition and frailty, we should consider what the best options are for realistic and lasting dietary changes or what the barriers and potential solutions are to improve nutritional status in older people. Undernutrition is not the only nutritional state related to frailty; high BMI and body fat percentage can also increase the risk of aging.

BMI is known to affect the severity of frailty. A cross-sectional study of Dutch subjects showed that BMI has a U-shaped relationship with frailty prevalence (*25*). A Japanese study found that the BMI range for which the prevalence of frailty was the lowest was 21.4-25.7 kg/m^2 (*26*). These findings highlight the need to evaluate the risk of frailty in both underweight and overweight individuals. Although the association between BMI and frailty was not analyzed in our study, future studies should focus on BMI in older adults. Age is considered to be one of the independent risk factors for frailty, and the prevalence of frailty increases exponentially with age. Kojima *et al.* (*27*) investigated the age-stratified meta-analyses of four studies and showed the pooled prevalence of frailty was 1.9%, 3.8%, 10.0%, 20.4%, and 35.1% for those aged 65-69, 70-74, 75-79, 80-84, and ≥ 85 years, respectively. However, our results differ from those of previous studies. Our research results showed that the pooled prevalence of frailty was 37.3%, 11.9%, and 35.8% for those aged 65-69, 70-79, and ≥ 80 years, respectively. Therefore, the prevalence of frailty did not increase with age in the present study. Notably, old age itself does not define frailty because some patients are active despite advanced age, whereas others experience functional decline in the absence of apparent stress factors or failure to rebound following hospitalization or illness (*28*).

This study supports smoking as a causal risk factor of frailty. Liu *et al.* (*29*) also confirmed this finding. Their study indicated that a genetic predisposition to smoking is associated with the risk of frailty in aging, which supports the potential causal role of smoking in the risk of frailty. In addition, the mechanisms underlying the potential association between smoking and frailty remain unclear. The most commonly suggested explanation is chronic inflammation induced by various toxic chemicals produced by tobacco smoking, which is supported by findings of positive associations between increased levels of inflammatory markers, such as CRP and IL-6, and higher prevalence and incidence of frailty (*30*). Further studies are required to elucidate the underlying biological mechanism. However, our study did not determine whether alcohol consumption played a causal role in frailty. As this study only collected information on whether the study participants had a habit of alcohol drinking at the time of the survey, the effect of alcohol consumption against frailty may also be due to the fact that the study participants in poorer health conditions did not drink alcohol themselves or chose to abstain from alcohol when they were in poor health due to alcohol consumption, and the relationship between alcohol consumption and frailty in older adults needs to be further studied.

Regarding exercise habits, older people who did not exercise were associated with a higher frailty severity. On September 30, 2019, the International Conference of Frailty and Sarcopenia Research (ICFSR) released the International Clinical Practice Guidelines: The Recognition and Management of Physical Frailty, stating that the management of frailty should include a multi-component physical activity program with a resistance-based training component (*7*). Our study focused only on whether elderly patients had exercise habits, and did not investigate the duration, frequency, and content of exercise, which are involved in preventing frailty severity. However, there are also studies showing that a high frequency of exercise, including resistance training, are associated with exacerbation of frailty severity (*31*). This shows that it is important to choose the right type of exercise according to the physical condition of the elderly person.

This study established a link between the number of chronic diseases and the risk of frailty. Previous studies have established a link between a single disease and frailty. For example, frailty prevalence in patients with inflammatory bowel disease (IBD) was 18% (*32*) and that for patients with chronic obstructive pulmonary disease (COPD) was 36% (*33*). Interactions among diseases in patients with multimorbidity may increase the risk of frailty. The British Biological Database (*34*) showed that patients with four or more chronic diseases have a significantly increased risk of frailty. However, our study showed that multimorbidity may protect against frailty in older populations. This may be because our criteria included two or more diseases. However, most of our patients had mild hypertension or diabetes mellitus. When you have a mild disease, more attention should be paid to your physical condition, but it should become a protective factor against frailty. Future studies should focus on the number and types of diseases that cause frailty.

Our study found that self-reliance of ADL was a protective factor against frailty. If self-care is limited, it can lead to a decrease in health-promoting behavior and

motivation to exercise in the elderly and a decrease in physical activity, resulting in a decline in overall health status. At the same time, the eating ability of people with self-care abilities is affected, which can easily cause adverse events such as insufficient daily energy intake and long-term bed rest, eventually leading to frailty. A previous study (*35*) indicated that disability in ADL is an adverse outcome of frailty that places a burden on frail elderly individuals. The functional status of hospitalized older adults can be improved through multi-domain interventions. Wang *et al.* (*36*) confirmed that participation in a multidomain intervention program during hospitalization improved the functional status and decreased the length of hospitalization, medical costs, and readmission rates of frail older people. Therefore, more attention should be paid to rehabilitation training for daily activities of the elderly to achieve life independence.

The strengths and limitations of this study must be considered. As this is a population-based study, our findings may be generalizable to similarly structured healthcare systems. We also based our data on validated, accurate, and complete measures of exposure and outcomes. To our knowledge, this is the first assessment of HBP patient-centered frailty rates and influencing factors. The present study has several limitations. First, the Fried FP was used as an assessment tool to determine preoperative frailty. The questionnaire only requires five minutes to complete, yet it covers physical functioning tests. Due to the time required for completion, it is unsuitable for use in busy outpatient settings. Despite testing physical function, it is a self-reported questionnaire that contains patient-reported outcomes. Due to individual interpretations, patients may overestimate or underestimate the actual problems that exist. Second, the sample size was relatively small. It would be difficult to extrapolate our study findings to cognitively impaired patients who do not have caregivers and to potentially high-risk patients because we could not recruit them for the study. We believe that the results of this study may have been influenced by systematic selection bias. Third, our study did not identify specific diseases in the patients who underwent surgery. Depending on the disease, the frailty factors affecting the patient may be different. Fourth, our study subjects are Chinese people, and whether the same conclusions will be drawn for older people with HBP disease in different countries. Finally, we only studied the Fried's FP and its association with HBP surgery patients; future research will be required to determine if similar effect sizes are found with related frailty tools (*e.g.* the Clinical Frailty Scale), as different frailty assessment tools typically only have moderate agreement in terms of who is identified as having frailty.

The surgical population is aging, and frailty is increasingly being observed. With an increasing number of frail patients undergoing surgery, healthcare professionals should be aware of the effects of frailty and develop improved and focused preoperative management strategies for stratified frail patients. The focus of future research and the implementation of science should be threefold. First, achieving a consensus on which frailty tool should be used for screening and diagnosis in HBP surgical settings, rather than developing frailty tools, is paramount. Unfortunately, there is a lack of standardized frailty assessment criteria, and the predictive efficacy, which is also a challenge in neurosurgical procedures (*37*). Future development of objective tools to identify/measure frailty based on the newest biological and computerized technologies is indispensable (*38*). Second, the development of interventions comprising treatment goals and plans that consider preoperative frailty as a risk factor for poor functional recovery may be an important cornerstone of preoperative management. In addition, effectively managing frailty can help alleviate the economic burden of an aging population (*39*). Future research should focus on the development and implementation of interventions that can potentially improve functional and adverse outcomes in frail patients. Third, these research findings should be translated into routine clinical care through the development of collaborative pathways and evaluated using scientific implementation methodologies.

In conclusion, our study shows that good exercise habits, lack of smoking, and good nutrition can prevent the exacerbation of frailty in older adults with HBP.

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ORIGINAL ARTICLE

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Higher FIB-4 index at baseline predicts development of liver cancer in a community-based cohort with viral hepatitis

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Abstract: Hepatitis B and C (HBV and HCV) testing has been performed in Japan since 2002 and is subsidized by central and prefectural governments. A follow-up program for HBV- or HCV-infected persons was started at that time in Ishikawa Prefecture. This study analyzed the long-term follow-up data from this program. In total, 1029 participants in the Ishikawa Hepatitis Follow-up Program (HBV-infected, *n* = 535; HCV-infected, *n* = 494) were enrolled. Clinical data between the first visit and the most recent visit by March 2019 were collected. In the HBV-infected group, 384 persons (71.8%) were asymptomatic carriers, 133 (24.9%) developed chronic hepatitis, 15 (2.8%) developed compensated liver cirrhosis, and 3 (0.6%) developed decompensated liver cirrhosis. Ninety (16.8%) were treated with nucleotide/nucleoside analogs. Sixteen (3.0%) developed liver cancer. In the HCV-infected group, 427 persons (86.4%) developed chronic hepatitis, 46 (9.3%) developed compensated liver cirrhosis, and 21 (4.3%) developed decompensated liver cirrhosis. Forty-eight (9.7%) developed liver cancer. Three hundred and seventy-eight (76.5%) received antiviral therapy (a direct-acting antiviral in 166, interferon-based treatment followed by a direct-acting antiviral in 73, and interferon-based treatment in 139). The subsidy system was used by 270 persons (71.4%). Sustained virological response was confirmed in 340 persons (68.8%). A higher FIB-4 index at the first visit was a significant risk factor for liver cancer in HBV-infected and HCV-infected persons. The Ishikawa Hepatitis Follow-up Program has revealed the clinical course of HBV and HCV infection in community-dwelling individuals. The results will be used for micro-elimination at a prefectural level.

***Keywords*:** direct-acting antivirals, hepatitis B virus, hepatitis C virus, liver cancer, liver cirrhosis

Introduction

Viral hepatitis is a major public health challenge that caused an estimated 1.57 million deaths worldwide in 2019 and accounted for 2.1% of all deaths (*1*). Most mortality associated with viral hepatitis is attributable to cirrhosis and liver cancer. There are four important hepatitis viruses, namely, HAV, HBV, HCV, and HEV. While all these viruses can cause acute infection, only HBV and HCV cause chronic infection, which may progress over time to cirrhosis or liver cancer. An estimated 295.9 million people were known to have HBV infection and 57.8 million to have HCV infection worldwide in 2019 (*2*). Therefore, to reduce the mortality associated with these infections, it is important to be able to identify individuals infected with HBV or HCV, to introduce antiviral therapy, and to perform periodic surveillance for hepatic function and liver cancer.

Antiviral therapy dramatically reduces deaths from HBV- and HCV-related liver diseases (*3-5*). Highly effective direct-acting antiviral (DAA) therapy has enabled elimination of HCV in over 95% of patients treated (*6*). However, liver cancer can occur even after HCV elimination, defined as a sustained virological response (SVR). Therefore, surveillance for liver cancer is necessary after elimination of HCV as well as during persistent infection (*7*). In patients with HBV, treatment with a nucleoside/nucleotide analog reduces the viral load and ameliorates liver cirrhosis (*8*) and impedes progression to liver failure and liver cancer (*5,9,10*). Nevertheless, liver cancer can still occur after the HBV viral load is suppressed by antiviral treatment. Current HBV treatment guidelines do not recommend antiviral treatment for asymptomatic HBV carriers, which

includes those with immune-tolerant or inactive chronic HBV infection as defined by the American Association for the Study of Liver Diseases (*11*). Liver cancer is also known to occur in asymptomatic carriers, albeit with low incidence. Thus, there are multiple lines of evidence suggesting that all individuals infected with HBV or HCV should receive lifelong care.

Effective linkage of individuals with HBC or HCV infection to appropriate care is essential to achieve the World Health Organization (WHO) goal of eliminating HBV/HCV by 2030 (*12*). The first step is to provide HBV and HCV tests for as many people as possible, particularly those at high risk, and the second is to encourage infected individuals to see a liver specialist to receive the necessary care, including antiviral therapy, periodic liver function and virological monitoring, and surveillance for liver cancer.

Testing for HBV and HCV has been subsidized by central and prefectural governments in Japan since 2002 (*13,14*). In the same year, the Ishikawa Prefecture started a surveillance program for people found to be positive for HBV or HCV by government-subsidized testing. This program includes follow-up by municipal healthcare workers, who visit or telephone individuals who have tested positive for HBV or HCV to ascertain whether they are under the care of a liver specialist and encourage them to attend if not. The program was modified in 2010 and is now known as the Ishikawa Hepatitis Follow-up Program, which provides annual follow-up for people who have tested positive for HBV or HCV *via* the publicly funded testing system. This program is based at Kanazawa University Hospital, which is the sole regional core institution in Ishikawa Prefecture (*15*), and encourages people who have tested positive for HBV or HCV to attend for specialized care. Thus, the program offers linkage to care and collects long-term clinical data on people who have tested positive for hepatitis. In this study, we analyzed the long-term follow-up data for participants in the Ishikawa Hepatitis Follow-up Program to determine the clinical course of their diseases (including survival status), occurrence of liver cancer, introduction of antiviral therapy, and the use of subsidy for antiviral therapy. Our findings indicate that government-subsidized hepatitis testing has been beneficial to this local community. We hope that our findings can be used to achieve micro-elimination of HBV/HCV and reduce mortality from liver cancer at a prefectural level in Japan.

Methods

Data collection

Participants in the Ishikawa Hepatitis Follow-up Program agree to periodic collection of their data by a specialized institution or a regional core center. Accordingly, information on the following was collected by a regional core center: age, sex, aspartate transaminase (AST), alanine transaminase (ALT), and platelet count at the first visit to a specialized institution or regional core center and at the most recent visit before March 2019. The collected data were then used to calculate the AST to platelet ratio index (APRI) and FIB-4 index. Information on antiviral therapy, viral status (particularly HCV), liver status, including occurrence of liver cancer, deaths, and the cause of death between the two time points was obtained from examination letters or direct requests to specialized institutions or the regional core center. The diagnosis of compensated cirrhosis and decompensated cirrhosis was comprehensively made based on clinical data, physical examinations, or liver imaging tests by the hepatologists in specialized institutions or a regional core center. Data on subsidized antiviral therapy for participants in the program were obtained from the Ishikawa prefectural government.

Statistical analysis

The data are shown as the mean ± standard deviation and were analyzed using the Student's *t*-test, chi-squared test, the Kaplan–Meier method, receiver operating characteristic curve analysis, and Cox proportional-hazards regression analysis. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, Inc., La Jolla, CA). A p-value < 0.05 was considered statistically significant.

Ethics statement

The protocol for this study was approved by the Medical Ethics Committee of Kanazawa University (Approval No. 2018-105 (2871)) and conforms to the provisions of the Declaration of Helsinki.

Results

In Japan, municipal screening for HBV and HCV was performed as part of a national health promotion program for seniors between 2002 and 2008 and as part of a general health promotion program from 2008 onwards. In Ishikawa Prefecture, 222,029 individuals were tested for HBV and 221,967 were tested for HCV under these programs between 2002 and 2019, and 1,956 and 1,655 were positive for HBV and HCV, respectively. In Ishikawa Prefecture, starting in 2002, municipal public health staff followed up persons who were positive for viral hepatitis tests in local screening programs. The same personnel made annual home visits or telephone calls to ascertain whether individuals with a positive test had visited a specialized institution and recommended a visit to those who had not.

In 2010, the regional core center for hepatitis care coordination (Kanazawa University Hospital, the only regional core center in the prefecture) took over the

follow-up process. The new program was named as the Ishikawa Hepatitis Follow-up Program and is run in conjunction with the Ishikawa Prefecture. Persons who have tested positive for viral hepatitis and consent to participate in the program receive a leaflet in July each year from the regional core center recommending that they visit a specialized institution as well as an examination letter for their physician to complete with details of their visit. The patient brings this letter to the specialized institution, where the consulting hepatologist records in the letter the date of examination, diagnosis, liver imaging tests performed, recommendations for further testing and treatment, and the date of the next appointment. The regional core center then uses the completed examination letter to confirm whether a participant has visited a specialized institution and enters data concerning treatment and disease status into a database.

By the end of 2022, 1,726 (49.2%) of the 3,511 persons who tested positive for hepatitis from 2002 onwards had consented to participate in the follow-up program, 541 (15.4%) had declined to participate, and 1,244 (35.4%) had not responded to an invitation to participate.

Kanazawa University Hospital collects clinical data from the examination letters returned by specialized institutions, learns of any changes in the health of the study participants, and follows the long-term clinical course of persons with viral hepatitis. In this study, we aimed to clarify the clinical course in this community-based cohort with viral hepatitis over time using the data collected for participants in the Ishikawa Hepatitis Follow-up Program. However, the data provided in examination letters were insufficient. Therefore, we collected the necessary data directly from the specialized institutions, the regional core center, and municipal government.

Data for 1,029 of the 1,557 persons identified to have viral hepatitis during the study period were available for analysis. Five hundred and thirty-five were HBsAg-positive and 494 were HCV antibody-positive. The HBV-positive group included 199 men and 336 women with a mean age of 59.5 years (range, 15–82) at the first visit and an average length of observation of 6.4 years (range, 1–25). By March 2019 (the end of the 2018 financial year), 384 persons (71.8%) were diagnosed to be asymptomatic carriers, 133 (24.9%) to have chronic hepatitis, 15 (2.8%) to have compensated liver cirrhosis, and 3 (0.6%) to have decompensated liver cirrhosis. Ninety (16.8%) had been treated with nucleoside/nucleotide analogs; 6 were asymptomatic carriers, 70 had chronic hepatitis, 11 compensated liver cirrhosis, and 3 decompensated liver cirrhosis. The indication of nucleoside/nucleotide analogs treatments was decided by the hepatologists in the specialized institutions. During the observation period, 16 (3.0%) had developed liver cancer and 4 had died (2 from liver cancer and 2 of non-liver-related causes). The HBV-positive individuals who developed liver cancer were found to have APRI and FIB-4 index values at the first and most recent examination that were significantly higher than those in their counterparts who did not develop liver cancer. Moreover, the platelet count at the first examination was significantly lower in those who developed liver cancer and neither alcohol drinking nor complications of diabetes mellitus did not significantly affect development of liver cancer (Table 1). We performed a COX proportional-hazards regression

Table 1. Comparison of HBV-infected individuals according to whether they developed liver cancer

Items	Liver cancer (+)	Liver cancer (−)	*p*-value
Cases, *n*	16	519	
First examination			
Age (years)	61.5 ± 9.4	59.5 ± 10.1	NS
AST (IU/L)	31.9 ± 11.9	25.8 ± 16.2	NS
ALT (IU/L)	26.2 ± 11.4	24.8 ± 24.4	NS
Platelets ($\times 10^4/\mu L$)	13.27 ± 5.28	21.02 ± 5.61	< 0.001
Albumin (g/dL)	4.08 ± 0.78	4.35 ± 0.35	NS
APRI	1.02 ± 0.75	0.45 ± 0.34	< 0.05
FIB-4 index	3.40 ± 2.00	1.66 ± 0.83	< 0.05
Most recent examination			
Observation period (years)	8.92 ± 6.25	6.34 ± 4.29	NS
Age (years)	70.9 ± 8.1	66.4 ± 10.1	< 0.05
Alcohol intake (> 20g/day) +/-/unknown	1/11/4	47/394/68	NS
DM +/-/unknown	0/16/0	49/455/15	NS
AST (IU/L)	38.8 ± 38.5	24.0 ± 11.7	NS
ALT (IU/L)	25.0 ± 26.0	20.0 ± 11.7	NS
Platelets ($\times 10^4/\mu L$)	16.2 ± 10.9	20.7 ± 10.9	NS
Albumin (g/dL)	3.65 ± 0.85	4.26 ± 0.41	< 0.05
APRI	0.93 ± 0.67	0.43 ± 0.39	< 0.01
FIB-4 index	4.41 ± 3.19	1.95 ± 1.09	< 0.01

Liver cancer (+), developed liver cancer during the observation period. Liver cancer (−), did not develop liver cancer during the observation period. ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; HBV, hepatitis B virus; NS, not significant; DM, diabetes mellitus.

analysis to identify factors contributing to development of liver cancer in HBV-infected individuals. Univariate analysis showed that, a lower platelet count, a lower albumin level, and higher APRI and FIB-4 index values significantly increased the risk of liver cancer. Only a higher FIB-4 index remained a significant risk factor for liver cancer in multivariate analysis (Table 2). We also performed a Kaplan–Meier analysis to determine the cumulative incidence of liver cancer. Overall, the cumulative incidence was 0.56% at day 2000 after the first visit, was 4.8% at day 4000, and plateaued thereafter. However, in several cases, liver cancer was detected later than day 6000 after the first visit (Figure 1A). There was no significant sex-related difference in the cumulative incidence of liver cancer (Figure 1B). To determine the suitable cutoff of FIB-4 index at the first visit for the prediction of liver cancer, we performed the receiver operating characteristic curve (ROC) analysis, which showed that the area under curve (AUC) was 0.82 and that FIB-4 index 2.41 was a suitable cutoff with sensitivity 0.88 and specificity 0.75. Based on this analysis, we compared the cumulative incidence of liver

Table 2. Risk factors for liver cancer development in the HBV-infected group

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p*-value	Hazard ratio	95% CI	*p*-value
Age (years)	1.05	0.98–1.12	NS			
Sex (male)	1.27	0.42–3.82	NS			
NA (+)	13.36	3.67–48.64	< 0.001			
AST (IU/L)	1.01	0.99–1.03	NS			
ALT (IU/L)	1	0.98–1.02	NS			
Platelets ($\times 10^4/\mu L$)	0.72	0.63–0.82	< 0.001			
Albumin (g/dL)	0.23	0.07–0.77	< 0.05	0.55	0.17–1.81	0.33
APRI	2.87	1.69–4.86	< 0.001			
FIB-4 index	2.06	1.62–2.62	< 0.001	1.97	1.47–2.63	< 0.001
T-Bil (mg/dL)	2.62	0.7–9.9	NS			

Data at the first visit were analyzed. NA (+), nucleotide/nucleoside analogs used during the observation period. ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; CI, confidence interval; HBV, hepatitis B virus; NS, not significant; T-Bil, total bilirubin.

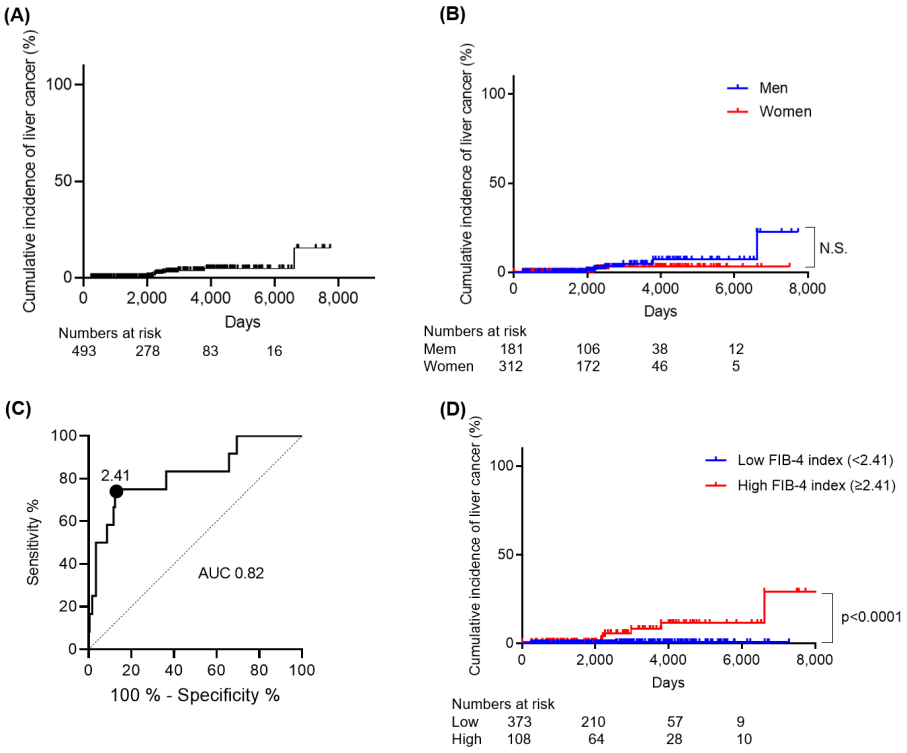

Figure 1. Cumulative incidence of liver cancer in the hepatitis B virus-infected group from the first visit. **(A)** Kaplan–Meier curve for all cases. **(B)** Comparison of Kaplan–Meier curves for the cumulative incidence of liver cancer according to sex by Cox proportional-hazards regression analysis. **(C)** Receiver operating characteristic (ROC) curve analysis for FIB-4 index at the first visit on liver cancer prediction. Area under the ROC curve was calculated. FIB-4 index at the point closest to the upper left was 2.41 (sensitivity 0.88, specificity 0.75). **(D)** Comparison of Kaplan–Meier curves for the cumulative incidence of liver cancer according to whether the FIB-4 index at the first visit was low (< 2.41) or high (≥ 2.41) by Cox proportional-hazards regression analysis. In Figure 1A, 1B and 1D, the numbers of analyzed patents were described. NS, not significant.

Table 3. Comparison of HCV-infected individuals according to whether they received antiviral treatments

Items	Antiviral treatment (−)	Antiviral treatment (+)	*p*-value
Cases, *n*	116	378	
First examination			
Age (years)	71.0 ± 7.8	60.5 ± 10.2	< 0.001
AST (IU/L)	39.7 ± 23.4	43.7 ± 33.5	NS
ALT (IU/L)	34.5 ± 22.7	47.1 ± 47.9	< 0.001
Platelets ($\times 10^4/\mu L$)	16.3 ± 6.4	18.6 ± 12.0	< 0.05
Albumin (g/dL)	4.01 ± 0.51	4.26 ± 0.37	< 0.001
APRI	1.09 ± 1.10	0.99 ± 1.06	NS
FIB-4 index	3.81 ± 2.99	2.60 ± 1.78	< 0.001
Most recent examination			
Observation period (years)	7.14 ± 4.82	8.52 ± 5.19	< 0.05
Age (years)	78.4 ± 6.9	69.1 ± 10.0	< 0.001
AST (IU/L)	64.3 ± 138.1	24.2 ± 16.1	< 0.005
ALT (IU/L)	37.7 ± 80.1	18.2 ± 14.8	< 0.05
Platelets ($\times 10^4/\mu L$)	15.2 ± 6.7	18.2 ± 6.9	< 0.001
Albumin (g/dL)	3.45 ± 0.89	4.32 ± 2.34	< 0.001
APRI	2.14 ± 5.33	0.55 ± 0.60	< 0.005
FIB-4 index	7.23 ± 15.0	2.64 ± 1.70	< 0.005
During observation period			
Liver cancer (+)/(−)	15/101	33/342[†]	*NS*

[†]Unknown: 3. Antiviral treatment (+), persons who received antiviral treatment during the observation period; Antiviral treatment (−), persons who did not receive antiviral treatment during the observation period. ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; HCV, hepatitis C virus; NS, not significant.

cancer between patients with FIB-4 index at the first visit < 2.41 and ≥ 2.41, and it revealed that the incidence was significantly higher in patients whose FIB-4 index was ≥ 2.41 than in those in whom it was < 2.41 (Figure 1D).

Next, we analyzed the clinical course in 494 HCV antibody-positive individuals who were also HCV-RNA-positive. The HCV-infected group included 129 men and 365 women who had a mean age of 63.0 years (range, 29–88) at the first visit and had been observed for a mean of 8.2 years (range, 1–27). Three hundred and seventy-eight (76.5%) of these patients had received antiviral treatment (DAA therapy only, n=166; interferon-based treatment followed by DAA therapy, *n* = 73; interferon-based treatment only, *n* = 139). SVR was confirmed in 340 cases (68.8%). Persistent infection, including no SVR after antiviral treatment, was confirmed in 154 (31.2%). The clinical data were compared between the group that had received antiviral therapy and the group that had not (Table 3). Compared with the individuals who had received antiviral treatment, those who had not were significantly older, had significantly lower ALT, albumin, and platelet values, and a higher FIB-4 index at the first examination. At the most recent examination, the AST, ALT, APRI and FIB-4 index values were significantly higher and the platelet count and albumin level were lower in the group that had not received antiviral treatment. Furthermore, we compared the clinical data obtained at the first and most recent examinations between the group that achieved SVR and the group that did not (Table 4). Individuals who achieved SVR were younger, had higher ALT and albumin levels, and had a lower FIB-4 index than those who had persistent infection at the first examination. At the most recent examination, individuals who achieved SVR were significantly younger, had significantly lower AST, ALT, APRI, and FIB-4 index values, and had a higher albumin level and platelet count than those who did not achieve SVR. The group that did not achieve SVR in Table 4 contained the two groups: one group for the patients who failed to achieve SVR after antiviral treatments and the other for those who had not received antiviral treatments, therefore, we also compared these two groups. The group who underwent antiviral treatments had some beneficial effects that the FIB-4 index and APRI values were significantly lower and the albumin level was significantly higher as shown in Table 5. By the end of March 2019, 427 individuals (86.4%) were diagnosed with chronic hepatitis, 46 (9.3%) with compensated liver cirrhosis, and 21 (4.3%) with decompensated liver cirrhosis. During the study period, 48 (9.7%) developed liver cancer and 24 (4.9%) died (of liver cancer, *n* = 6; from liver failure, *n* = 3; of a non-liver-related cause, *n* = 15). We compared the clinical features of HCV-infected persons who developed liver cancer with those of their counterparts who did not (Table 6). The APRI and FIB-4 index values at the first and most recent examinations were significantly higher in the group that developed liver cancer. The patients that developed liver cancer was significantly older, had higher AST and ALT levels, and had a lower albumin level and platelet count at the first examination than those who did not develop liver cancer. These trends were almost identical to those at the most recent examination. Although SVR was not significantly frequently observed in the group of no liver cancer occurrence compared with the group with liver cancer occurrence, there was a

Table 4. Comparison of HCV-infected individuals according to whether they achieved SVR

Items	SVR (+)	SVR (–)	*p*-value
Cases, *n*	340	154: 116 antiviral treatments (-) 38 antiviral treatments (+)	
First examination			
Age (years)	60.3 ± 10.1	69.0 ± 9.3	< 0.001
AST (IU/L)	44.1 ± 33.9	40.0 ± 25.1	NS
ALT (IU/L)	47.6 ± 48.8	36.5 ± 28.0	< 0.005
Platelets ($\times 10^4/\mu L$)	18.7 ± 12.5	16.6 ± 6.3	< 0.05
Albumin (g/dL)	4.27 ± 0.36	4.05 ± 0.48	< 0.001
APRI	0.99 ± 1.06	1.06 ± 1.07	NS
FIB-4 index	2.58 ± 1.78	3.53 ± 2.77	< 0.001
Most recent examination			
Observation period (years)	8.71 ± 5.21	7.08 ± 4.79	< 0.005
Age (years)	69.0 ± 9.9	76.2 ± 9.0	< 0.001
AST (IU/L)	23.4 ± 15.4	56.1 ± 121.0	< 0.005
ALT (IU/L)	17.0 ± 10.5	35.7 ± 71.5	< 0.005
Platelets ($\times 10^4/\mu L$)	18.5 ± 6.9	15.4 ± 6.5	< 0.001
Albumin (g/dL)	4.35 ± 2.45	3.59 ± 0.89	< 0.001
APRI	0.51 ± 0.54	1.83 ± 4.70	< 0.001
FIB-4 index	2.58 ± 1.64	6.26 ± 13.23	< 0.001

SVR (+), persons who achieved SVR; SVR (–), persons who did not achieve SVR. ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; HCV, hepatitis C virus; NS, not significant; SVR, sustained virological response.

Table 5. Comparison of HCV-infected individuals who did not achieve SVR whether they underwent antiviral treatments

Items	Antiviral treatments (+)	Antiviral treatments (–)	*p*-value
Cases, *n*	38	116	
Liver cancer (+)/(–)	4/34	14/102	NS
First examination			
Age (years)	62.7 ± 10.6	71.0 ± 7.8	< 0.001
AST (IU/L)	40.7 ± 30.0	39.7 ± 23.4	NS
ALT (IU/L)	42.7 ± 39.6	34.5 ± 22.7	NS
Platelets ($\times 10^4/\mu L$)	17.5 ± 6.1	16.3 ± 6.4	NS
Albumin (g/dL)	4.15 ± 0.36	4.01 ± 0.51	NS
APRI	0.97 ± 0.99	1.09 ± 1.10	NS
FIB-4 index	2.72 ± 1.82	3.81 ± 2.99	< 0.05
Most recent examination			
Observation period (years)	6.89 ± 4.77	7.14 ± 4.82	NS
Age (years)	69.4 ± 11.2	78.4 ± 6.9	< 0.001
AST (IU/L)	31.1 ± 20.0	64.3 ± 138.1	< 0.05
ALT (IU/L)	29.3 ± 33.2	37.7 ± 80.1	NS
Platelets ($\times 10^4/\mu L$)	16.2 ± 5.8	15.2 ± 6.7	NS
Albumin (g/dL)	4.05 ± 0.73	3.45 ± 0.89	< 0.001
APRI	0.85 ± 0.92	2.14 ± 5.33	< 0.05
FIB-4 index	3.18 ± 2.16	7.23 ± 15.00	< 0.01

tendency that SVR was frequently observed in the group with no liver cancer occurrence ($p = 0.054$). Neither alcohol drinking nor complications of diabetes mellitus did not significantly affect development of liver cancer. A Cox proportional-hazards regression analysis was performed to identify factors contributing to development of liver cancer in the HCV-infected group (Table 7). Univariate analysis identified older age, no SVR, male sex, a lower platelet count, a lower albumin level, and higher AST, ALT, APRI and FIB-4 index values at the first examination to be significant risk factors for liver cancer. In multivariate analysis, only a higher FIB-4 index and male sex remained as significant risk factors (Table 7). Finally, we performed a Kaplan–Meier analysis to clarify the cumulative incidence of liver cancer. Overall, the cumulative incidence was 3.2% on day 2500 after the first visit, 10.4% by day 5000, and plateaued thereafter (Figure 2A). The incidence of liver cancer was significantly higher in men than in women (Figure 2B). To determine the suitable cutoff of FIB-4 index at the first visit for the prediction of liver cancer, we performed ROC analysis, which showed that AUC of 0.77 and FIB-4 index 2.54 was a suitable cutoff with sensitivity 0.62 and specificity 0.88 (Figure 2C). Based on this analysis, we compared the cumulative incidence of liver cancer incidence between patients with FIB-4 index at the

Table 6. Comparison of HCV-infected individuals according to whether they developed liver cancer

Items	Liver cancer (+)	Liver cancer (−)	*p*-value
Cases, *n*	48	446	
First examination			
Age (years)	67.0 ± 8.0	62.5 ± 10.8	< 0.01
AST (IU/L)	57.4 ± 34.1	41.2 ± 30.8	< 0.005
ALT (IU/L)	62.2 ± 60.7	42.2 ± 41.0	< 0.05
Platelets ($\times 10^4/\mu L$)	13.3 ± 5.9	18.6 ± 11.3	< 0.001
Albumin (g/dL)	4.04 ± 0.35	4.22 ± 0.42	< 0.005
APRI	1.73 ± 1.37	0.93 ± 1.00	< 0.001
FIB-4 index	4.45 ± 2.44	2.71 ± 2.08	< 0.001
Most recent examination			
Observation period (years)	8.02 ± 4.74	8.22 ± 5.18	NS
Age (years)	74.7 ± 8.1	70.9 ± 10.3	< 0.005
SVR/no SVR	29/19	312/134	NS
Alcohol intake (>20g/day) +/-/unknown	5/34/9	27/363/86	NS
DM +/-/unknown	9/38/1	70/370/6	NS
AST (IU/L)	88.7 ± 201.4	27.7 ± 28.7	< 0.05
ALT (IU/L)	50.3 ± 121.6	19.9 ± 16.4	NS
Platelets ($\times 10^4/\mu L$)	13.7 ± 6.3	18.0 ± 6.9	< 0.001
Albumin (g/dL)	3.61 ± 0.84	4.18 ± 2.23	< 0.005
APRI	2.38 ± 4.24	0.77 ± 2.46	< 0.05
FIB-4 index	7.01 ± 7.67	3.37 ± 7.61	< 0.005

Liver cancer (+), persons who developed liver cancer during the observation period; Liver cancer (−), persons who did not develop liver cancer during the observation period. ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; HCV, hepatitis C virus; NS, not significant; SVR, sustained virological response; DM, diabetes mellitus.

Table 7. Risk factors for liver cancer development in the HCV-infected group

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p*-value	Hazard ratio	95% CI	*p*-value
Age at first examination	1.09	1.05–1.13	< 0.001			
Male sex	2.87	1.6–5.15	< 0.001	3.18	1.73–5.85	< 0.001
SVR achieved	0.53	0.29–0.96	< 0.05			
AST (IU/L)	1.01	1.00–1.01	< 0.01			
ALT (IU/L)	1.01	1.00–1.01	< 0.05			
Platelets ($\times 10^4/\mu L$)	0.87	0.82–0.92	< 0.001			
Albumin (g/dL)	0.47	0.30–0.74	< 0.01			
APRI	1.36	1.17–1.59	< 0.001			
FIB-4 index	1.22	1.13–1.31	< 0.001	1.12	1.15–1.34	< 0.001
T-Bil (mg/dL)	1.43	0.73–2.78	NS			

Data from the first examination were analyzed. ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; CI, confidence interval; HCV, hepatitis C virus; NS, not significant; SVR, sustained virological response; T-Bil, total bilirubin.

first visit index < 2.54 and ≥ 2.54, it revealed that the incidence was significantly higher in patients whose FIB-4 index was ≥ 2.54 than in those in whom it was < 2.54 (Figure 2D). Of note, the incidence of liver cancer was significantly lower in patients who achieved SVR during the observation period (Figure 2E). Furthermore, we also compared the cumulative liver cancer incidence between the patients who did not achieve SVR even after antiviral treatment and those who did not achieve SVR because of no antiviral treatments, and the incidences were not significantly different (Figure 2F).

We compared the clinical features of individuals who developed liver cancer between the HBV and HCV groups (Table 8). The incidence of liver cancer was found to be significantly higher in the HCV group. AST, ALT, and APRI values at the first examination were also significantly higher in the HCV group, as was the APRI value at the most recent examination.

Japan has subsidized treatment for HBV and HCV at the national and prefectural levels since 2008 (*14,16*). We investigated how many participants in the Ishikawa Hepatitis Follow-up Program used this subsidy system for antiviral therapy. Of the 378 individuals who had undergone antiviral treatment in the HCV-infected group, 270 (71.4%) used the subsidy system a total of 365 times, with an average of 1.35 times per person (Supplemental Table S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=82*). One hundred and ninety-one (70.7%) of these 270 persons used the subsidy system to undergo interferon-free DAA therapy. Seventy-eight (86.7%) of 90 persons in the HBV group used the subsidy system for treatment with nucleotide analogs.

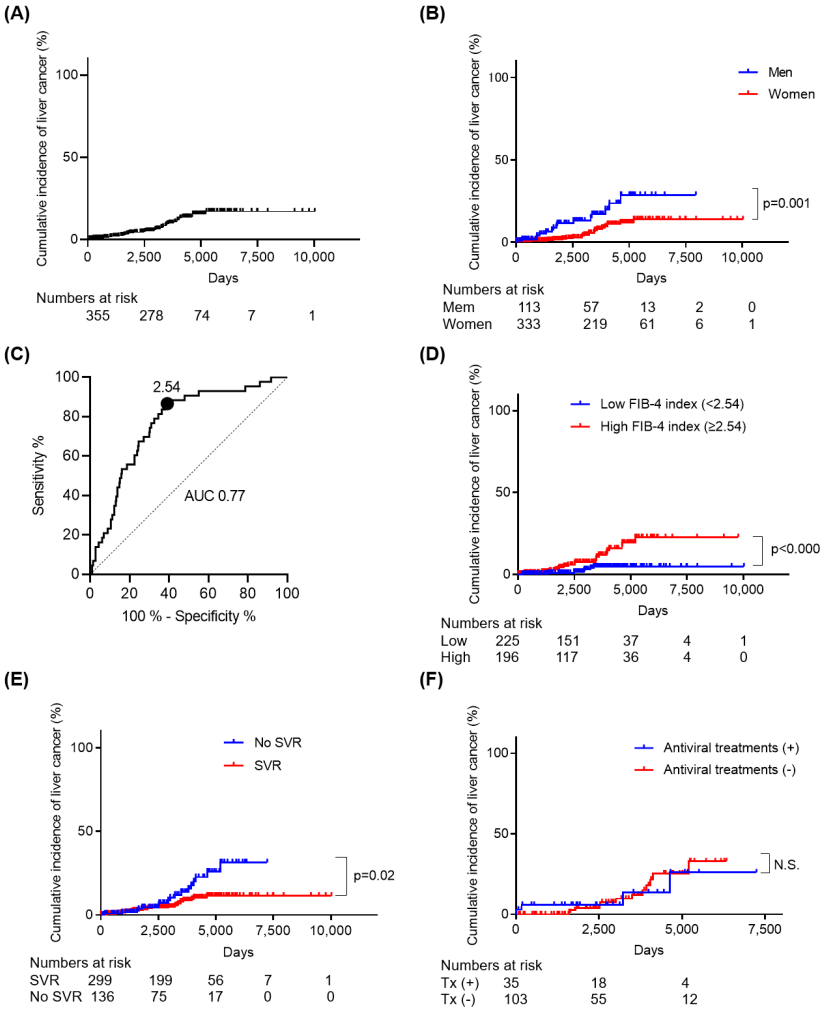

Figure 2. Cumulative incidence of liver cancer in the hepatitis C virus-infected group from the first visit. (A) Kaplan–Meier curve for all cases. **(B)** Comparison of Kaplan–Meier curves for the cumulative incidence of liver cancer according to sex by Cox proportional-hazards regression analysis. **(C)** Receiver operating characteristic (ROC) curve analysis for FIB-4 index at the first visit on liver cancer prediction. Area under the ROC curve was calculated. FIB-4 index at the point closest to the upper left was 2.54 (sensitivity 0.62, specificity 0.88). **(D)** Comparison of Kaplan–Meier curves for the cumulative incidence of liver cancer according to whether the FIB-4 index was low (< 2.54) or high (≥ 2.54) by Cox proportional-hazards regression analysis. **(E)** Comparison of Kaplan-Meier curves for the cumulative incidence of liver cancer according to whether sustained virological response (SVR) was achieved by Cox proportional-hazards regression analysis. **(F)** Comparison of Kaplan-Meier curves for the cumulative incidence of liver cancer according to whether antiviral treatments were undergone in the patients without SVR by Cox proportional-hazards regression analysis. In Figure 2A, 2B, 2D, 2E, and 2F, the numbers of analyzed patents were described. NS, not significant; Tx(+), antiviral treatments (+); Tx(-), antiviral treatments (-).

Table 8. Comparison of patients who developed liver cancer between the HBV-infected and HCV-infected groups

Items	HBV group	HCV group	*p*-value
Cases, *n*	16	48	
First examination			
Male/Female	8/8	19/29	NS
During the observation period			
Liver cancer incidence	16/535	48/494	< 0.001
First examination			
Age (years)	61.5 ± 9.4	67.0 ± 8.0	NS
AST (IU/L)	31.9 ± 11.9	57.4 ± 34.1	< 0.001
ALT (IU/L)	26.2 ± 11.4	62.2 ± 60.7	< 0.001
Platelets ($\times 10^4/\mu L$)	13.27 ± 5.28	13.3 ± 5.9	NS
Albumin (g/dL)	4.08 ± 0.78	4.04 ± 0.35	NS
APRI	1.02 ± 0.75	1.73 ± 1.37	< 0.05
FIB-4 index	3.40 ± 2.00	4.45 ± 2.44	NS
Most recent examination			
Observation period (years)	8.92 ± 6.25	8.02 ± 4.74	NS
Age (years)	70.9 ± 8.1	74.7 ± 8.1	NS
AST (IU/L)	38.8 ± 38.5	88.7 ± 201.4	NS
ALT (IU/L)	25.0 ± 26.0	50.3 ± 121.6	NS
Platelets ($\times 10^4/\mu L$)	16.2 ± 10.9	13.7 ± 6.3	NS
Albumin (g/dL)	3.65 ± 0.85	3.61 ± 0.84	NS
APRI	0.93 ± 0.67	2.38 ± 4.24	< 0.05
FIB-4 index	4.41 ± 3.19	7.01 ± 7.67	NS

ALT, alanine transaminase; APRI, AST to platelet ratio; AST, aspartate transaminase; HBV, hepatitis B virus; HCV, hepatitis C virus; NS, not significant; SVR, sustained virological response.

Discussion

In Japan, HBV and HCV are thought to be transmitted mainly *via* reuse of syringes and needles or by transfusion of contaminated blood. At the national government level, the Ministry of Health, Labour, and Welfare strongly suggests that all Japanese citizens should undergo hepatitis testing at least once in their lifetime. Nationwide hepatitis screening was started as a part of the health examination provided by municipal governments in 2002, and the cost of this screening is subsidized at the national and local government levels (*13,14*). At that time, in Ishikawa Prefecture, a program was established whereby municipal healthcare workers undertook annual and periodic follow-up for people whose government-subsidized screening test was positive for HBV or HCV. Since 2010, Kanazawa University Hospital, which is the only core institution in Ishikawa Prefecture, has provided publicly funded annual follow-up for persons with a positive HBV or HCV test through the Ishikawa Hepatitis Follow-up Program. As part of this program, a core institution can make a direct recommendation for a patient who has tested positive for hepatitis to visit a liver specialist by letter annually. The liver specialist is required to complete an examination letter and return it to the core institution, thereby confirming whether the patient has received the necessary care (*15*). A similar community-based intervention system has been implemented in Okazaki, Japan and found to be useful when encouraging HBV/HCV-infected people to attend for care by a hepatologist (*17*).

The main challenge in our follow-up system is that although we have continuously encouraged individuals with a positive hepatitis test to participate in the program, some have refused and others have not responded to our invitation to join the program. Moreover, even if persons with a positive hepatitis test agree to participate in the program, 40%–50% of examination letters are returned to the regional core center, indicating that only 40%–50% of patients visit a specialized institution each year. Furthermore, in some cases, the specialized institution receives the examination letter but does not return a completed copy to the regional core center. The regional core center enters disease and treatment data from completed examination letters into a database but can only collect a limited amount of data in this way. With advancing age, patients with viral hepatitis may develop comorbidities, such as dementia and cancers other than liver cancer, resulting in further limitations in activities of daily living or a move into a care facility for the elderly. Follow-up in a way that suits the circumstances of each patient requires an accurate knowledge of their health status. A noteworthy finding in our review of the examination letters was that some patients tried to visit a specialized institution but had seen a physician other than a hepatologist or had not undergone annual liver imaging tests. This suggests a need for more accurate data collection methods and better communication on the part of specialized institutions. We have recently started using information and communication technology to share clinical information regarding participants in this program between the core institution and specialized institutions *via* a web system that seems to be able to overcome several problems in the program. The details of this technology have been discussed elsewhere (*15*).

Noninvasive methods for diagnosis of liver fibrosis have recently been investigated. The WHO recommends transient elastography with ultrasound, APRI, and the FIB-4 index as noninvasive methods for diagnosis of liver fibrosis in patients with chronic viral hepatitis (*18*). The clinical guidelines published by the European Association for the Study of the Liver and the Asian Pacific Association for the Study of the Liver recommend noninvasive assessment of hepatic fibrosis (*19,20*). Furthermore, in the clinical guidelines for HBV and HCV issued by the American Association for the Study of Liver Diseases, APRI and the FIB-4 index are recommended for diagnosis of liver fibrosis (*11,21*). Many studies have found that a higher FIB-4 index can be useful for prediction of liver failure and/or development of liver cancer in various clinical conditions, including nonalcoholic steatohepatitis/nonalcoholic fatty liver disease (*22*) and HBV (*23*) and HCV (*24*) infection. The FIB-4 index has been found to be particularly helpful for predicting liver failure and/or development of liver cancer in various clinical situations, including symptomatic hepatitis carriers, patients with HBV treated by nucleotide/nucleoside analogs (*25,26*), and patients with HCV who have achieved SVR (*27*). This study identified a higher FIB-4 index at baseline to be a risk factor for development of liver cancer during follow-up. Therefore, individuals with a higher FIB-4 index at baseline should be followed up more intensively. Moreover, monitoring of changes in the FIB-4 index over time is reportedly useful for noninvasive real-time estimation of progression of liver fibrosis (*28,29*). FIB-4 index can be easily calculated by simple and familiar parameters, such as AST, ALT, platelet count, and age, however, transient elastography or MR electrospray seems to be a more accurate and straightforward way to assess liver fibrosis than FIB-4 index among the noninvasive methods. Therefore, FIB-4 index should be used for the first screening for liver fibrosis, then, ideally, the assessment should be followed or combined with transient elastography or MR electrospray if these are available. A regional core center can calculate the FIB-4 index for participants in the Ishikawa Hepatitis Follow-up Program using data in the returned examination letters or by information and communication technology. A regional core center can also recommend intensive follow-up for individuals with changes in their FIB-4 index over time that suggest progression of liver fibrosis. An elevated FIB-4 index has recently been reported to be related to the following: an increased risk of

cardiovascular events, cardiovascular mortality, and all-cause mortality in patients with cardiovascular disease (*30*); severity of illness and mortality in patients with COVID-19 (*31*); and an increased incidence of renal failure (*32*) and depression (*33*). The FIB-4 index can be calculated easily for individuals who attend regular follow-ups, so could be used not only for screening patients for liver disease but also for non-liver diseases as part of regular wellness checks.

This study has several important findings. Although highly effective DAAs are now available and the subsidy system can greatly reduce the copayment amount, approximately one-third of HCV-infected participants in the Ishikawa Hepatitis Follow-up Program had not yet received antiviral therapy. In a global modeling study of the HCV care cascade between 2015 and 2020, 23% of all HCV viremic patients were estimated to be diagnosed with HCV infection, and 45% of diagnosed patients were estimated to receive antiviral treatment (*34*). However, the rates of antiviral therapy for people with HCV viremia vary from region to region, being 15% in the USA (*35*), 42.2% in Canada (*36*), and 56.8%–58.1% in South Korea (*37,38*). In our cohort, 76.5% of HCV-infected persons received antiviral therapy. This rate is not satisfactory in view of the World Health Organization target of 80% of eligible people with chronic HCV infection being treated by 2030 (*39*) but is higher than those in the above mentioned reports. Although barriers to introduction of antiviral therapy vary from region to region, lower income, limited access to health services, long wait times, provider shortages, and discrimination against persons with HCV infection are thought to be common reasons (*36,37*). In addition to these social factors, we have identified clinical features of patients who have not been referred to receive antiviral therapy after diagnosis. Patients who have not received antiviral therapy are significantly older than patients who have been treated. Generally, older patients are likely to have comorbid conditions, including dementia and paralysis as a result of cerebrovascular events, resulting in limited ability to perform activities of daily living or residence in a care home, either of which makes it difficult for them to attend appointments at specialized institutions, receive antiviral therapy if necessary, and undergo periodic screening for liver function and liver cancer. We found that baseline transaminase levels were significantly higher in patients who have received antiviral therapy than in those who have not. This suggests that specialist clinicians might hesitate to treat an HCV-infected patient with normal liver function or if the patient has reduced ability to perform activities of daily living or comorbid conditions.

The risk of liver cancer is greatly reduced in HCV-infected patients who achieve SVR but can still occur after SVR. Therefore, it is recommended that surveillance for liver cancer be continued lifelong, even after achievement of SVR (*7*). Some primary care physicians may not strongly recommend surveillance for liver cancer after SVR. However, our follow-up mailing system consistently reminds patients that they should continue with periodic specialist care despite SVR. Regarding HBV-infected patients, nucleoside/nucleotide analogs treatments seemed to significantly increase risk of liver cancer occurrence as shown in Table 2. Some patients started nucleoside/nucleotide analogs treatments just after diagnosis of liver cancer, which might cause this unexpected result. Furthermore, the present study is not a prospective study to examine the effect of antiviral treatments on liver cancer occurrence, thus, our result does not show the promotional effect of antiviral treatment on liver cancer in HBV-infected patients.

In conclusion, this study has provided valuable information on the clinical course in a hepatitis-infected community-based cohort in Ishikawa Prefecture using data from the innovative Ishikawa Hepatitis Follow-up Program. An important finding was that higher FIB-4 index at baseline was significantly associated with development of liver cancer during follow-up. We hope that this analysis will be repeated in other prefectures and that the data generated will be used to achieve micro-elimination and reduce mortality from liver cancer at the prefectural level in Japan.

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Analysis of recent changes in treatment options for patients with hepatocellular carcinoma using data from a highly comprehensive Japanese national database: Impact of advances in systemic therapy and minimally invasive surgery

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Abstract: In 2011, the Ministry of Health, Labour and Welfare started providing data from the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB) for research purposes. The NDB is an exhaustive and valuable database for health policymaking and research. It provides an accurate and most recent visualization of the burden of hepatocellular carcinoma (HCC) in Japan. In this study, we analyzed the trend in HCC treatments using data from the NDB. The NDB data were retrospectively analyzed to calculate the number of patients who were diagnosed with HCC (International Classification of Diseases, version 10 code of C22.0) and underwent treatment from fiscal year (FY) 2016 to 2020. We observed the following trends in HCC treatments during the past 5 years: a slight increase in the number of liver resection (LR) cases (+5.4%), a decrease in the number of radiofrequency ablation cases (−15.2%), and a considerable decrease in the number of transarterial chemoembolization/transarterial embolization cases (−38.2%). However, the number of patients who received systemic therapy dramatically increased from 471 in FY 2016 to 1,584 in FY 2020 (+236%). Among LR cases, there was a remarkable increase in the number of laparoscopic procedures from 1,227 in FY 2016 to 2,057 in FY 2020 (+67.6%). This analysis of a national highly comprehensive database revealed a very recent visualization of HCC management in Japan, wherein the impact of recent advances in systemic therapy and minimally invasive surgery was prominent.

***Keywords*:** hepatocellular carcinoma, systemic therapy, minimally invasive surgery

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer mortality worldwide (*1*). National cancer mortality data in Japan have been reported previously (*2*), showing that the recent decrease in mortality was primarily attributable to stomach, liver, and lung cancers. Furthermore, several Japanese nationwide registries, including the Japan Liver Cancer Association formerly Liver Cancer Study Group of Japan (LCSGJ), have reported a 5-year survival rate of 53.6% (*3,4*). Nevertheless, the nationwide follow-up survey conducted by the LCSGJ was based only on data from approximately 500 specialized member institutions in Japan.

In 2011, the Ministry of Health, Labour and Welfare (MHLW) started providing data from the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB) for research purposes (*5*). The NDB is an exhaustive and valuable database for health policymaking and research and provides an accurate visualization of HCC burden in Japan.

We conducted this study to analyze data from the NDB to reveal trends in HCC treatments in Japan.

Materials and Methods

Data source

This repeated cross-sectional study used data from the NDB, which compiles and stores nationwide electronic insurance claims data for all medical goods and

services provided under the national health insurance system (*6*). Researchers can apply to the MHLW for access to NDB data and will be provided with all the data deemed necessary for their study. The MHLW provides accumulated data that contain no personally identifiable information. Details regarding insurance claims and health checkups from all hospitals and clinics in Japan are recorded in the NDB, which includes the demographics, health, diagnosis, medical and dental practice, and drug prescription information of all patients.

For the present study, we requested and obtained data with International Classification of Diseases, version 10 (ICD-10) codes related to liver cancer recorded between April 2015 and September 2021. The MHLW provided 323 million medical records, 130 million diagnostic procedure combination records, and 384,000 prescription records to our research group. The study protocol was reviewed and approved by the ethical committee of the National Center for Global Health and Medicine (approval number 004253).

Patient selection

Because the NDB is one of the most comprehensive national databases in the world and used for health policymaking and research, we did not establish exclusion criteria in this study. However, we excluded certain patients without data regarding the type of treatments from the analysis.

Measurements

Diagnostic data in the NDB are classified using ICD-10 codes. Patients who had C22.0 (liver cell carcinoma) were considered as having HCC. Patients who first had the diagnosis code in the database with a lookback period of ≥ 12 months were considered as having a new diagnosis of HCC.

Treatments

Treatments performed within 180 days after diagnosis were considered treatments for HCC. Liver resection (LR) cases were divided into open liver resection (OLR, procedure code K695) and laparoscopic liver resection (LLR, K695-2). Microwave ablation and radiofrequency ablation (RFA) were considered RFA (K697-2, K697-3). Transarterial embolization (TAE, K615) and transarterial chemoembolization (TACE, G003-3) were considered together. Patients who received sorafenib (medication code 620006778), regorafenib (622225801), lenvatinib (622416001, 622416101), ramucirumab (622417901, 622418001), atezolizumab (622594601, 629900601), bevacizumab (620004872, 620004873), and cabozantinib (622796901, 622797001) were considered as receiving systemic therapy.

Statistical analysis

The number of patients who underwent HCC treatment was analyzed in terms of number and percentage. The number of patients was calculated for each FY, which begins every April, from FY 2016 to 2020. Statistical analyses were conducted using JMP software, version 17.0 (SAS Institute Inc., Cary, NC).

Results and Discussion

We observed a gradual decrease in the number of patients who were diagnosed with HCC and underwent treatment over time (FY 2016, 14,267; FY 2017, 13,845; FY 2018, 13,246; FY 2019, 12,921; and FY 2020, 12,760). The trend of treatments is shown in Table 1 and Figure 1. The number of patients who underwent OLR, RFA, and TACE/TAE decreased over time (FY 2016, 3,449/2,650/6,470; FY 2017, 3,284/2,651/5,809; FY 2018, 3,115/2,437/5,115; FY 2019, 2,993/2,419/4,375; and FY 2020, 2,871/2,248/4,000). Nevertheless, the number of patients who underwent LLR increased (FY 2016, 1,227; FY 2017, 1,548; FY 2018, 1,644; FY 2019, 1,960; and FY 2020, 2,057), and the number of patients who received systemic therapy increased considerably (FY 2016, 471; FY 2017, 553; FY 2018, 935; FY 2019, 1,174; and FY 2020, 1,584).

Given that there was a significant increase in the number of patients who underwent LLR, we further analyzed the combination of treatments in that group. The number of patients who underwent TACE/TAE

Table 1. Trend in treatments for hepatocellular carcinoma

Treatment	2016 (*n* = 14,267)	2017 (*n* = 13,845)	2018 (*n* = 13,246)	2019 (*n* = 12,921)	2020 (*n* = 12,760)
Open liver resection	3,449 (24.2)	3,284 (23.7)	3,115 (23.5)	2,993 (23.2)	2,871 (22.5)
Laparoscopic liver resection	1,227 (8.6)	1,548 (11.2)	1,644 (12.4)	1,960 (15.2)	2,057 (16.1)
Radiofrequency ablation	2,650 (18.6)	2,651 (19.1)	2,437 (18.4)	2,419 (18.7)	2,248 (17.6)
Transarterial chemoembolization/ transarterial embolization	6,470 (45.3)	5,809 (42.0)	5,115 (38.6)	4,375 (33.9)	4,000 (31.3)
Systemic therapy	471 (3.3)	553 (4.0)	935 (7.1)	1,174 (9.1)	1,584 (12.4)

Data are reported as numbers (%).

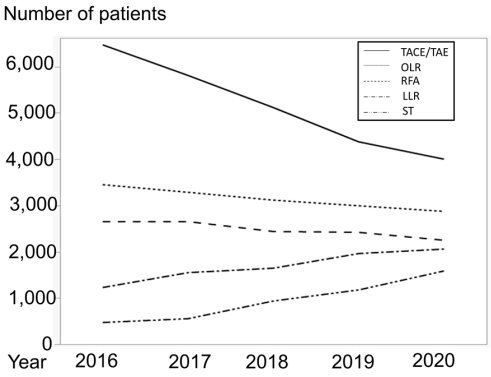

Figure 1. Trend in treatments for hepatocellular carcinoma in the national database. TACE/TAE: transarterial chemoembolization/transarterial embolization; OLR: open liver resection; RFA: radiofrequency ablation; LLR: laparoscopic liver resection; ST: systemic therapy.

and LLR was not significantly different during the study periods (FY 2016, 167; FY 2017, 135; FY 2018, 165; FY 2019, 142; and FY 2020, 144). Moreover, the number of patients who underwent RFA and LLR was not significantly different during the study periods (FY 2016, 50; FY 2017, 57; FY 2018, 76; FY 2019, 68; and FY 2020, 64).

This study demonstrated a gradual decrease in the number of patients who were diagnosed with HCC and underwent treatment over time, which in turn led to a decrease in the number of patients who underwent OLR, RFA, and TACE/TAE. Nevertheless, there was an increase over time in the number of patients who received systemic therapy and those who underwent LLR.

The global incidence of viral hepatitis-related malignancies has decreased since the 2000s because of the implementation of neonatal hepatitis B virus (HBV) vaccination programs and the availability of highly effective antiviral treatments, including nucleoside analogs and direct-acting antiviral agents for HBV and hepatitis C virus (HCV), which have been approved and publicly covered in Japan since 2008. Although several other underlying liver diseases cause HCC, such as alcohol abuse or alcoholic steatohepatitis and nonalcoholic fatty liver disease or nonalcoholic steatohepatitis, which constitutes 17.2% of patients with HCC (*7*), viral hepatitis remains one of the most important causes of HCC. This information is consistent with our finding of the gradual decrease in the total number of patients who were diagnosed with HCC and underwent treatment from FY 2016 to 2020.

Although the LCSGJ reports the number of treated patients with HCC, there is a slight delay because of inherent technical issues for data collection. The most recent report included patients from 2014 to 2015 (*4*). Moreover, the nationwide survey included only some 500 specialized member institutions treating HCC. Therefore, it would be valuable to analyze this sub-real-time exhaustive database to understand the current treatments for HCC.

The number of patients diagnosed with liver cancer recorded in cancer statistics in Japan (*8*), was 42,762 in FY 2016, 39,401 in FY 2017, 38,312 in FY 2018, and 37,296 in FY 2019. These values are comparable with those obtained from the NDB.

Sorafenib was the first approved molecular targeted drug for treating HCC (*9*). In Japan, almost all drugs were generally covered by public health insurance soon after their approval. After the approval of sorafenib in 2009, multiple regimens, including lenvatinib, regorafenib, cabozantinib, and ramucirumab, formed the mainstay of systemic therapies for advanced HCC in Japan (*10*). This may be the reason for the significant increase in the number of patients who received systemic therapy. The most recent nationwide survey conducted by the LCSGJ included patients between 2014 and 2015 (*4*), and 217 patients received systemic chemotherapy as an initial treatment, which is considered equal to systemic therapy in this study. This is probably the first report demonstrating a considerable increase in the number of patients who received systemic therapy.

Another remarkable recent trend was an increase in the number of patients who underwent minimally invasive surgery. Technical developments in LLR have enabled its wide application due to the advantages of a shorter hospital stay and reduced intraoperative blood loss and postoperative pain, especially for minor LR (*11*). This trend is consistent with our finding of an increase in the number of patients who underwent LLR irrespective of the decrease in the number of patients who underwent OLR.

The number of patients receiving TACE decreased during the study period. TACE is the first-line treatment for intermediate-stage HCC (*12*); therefore, this is probably due to the decrease in number of patients with HCC.

Regarding the limitations of our study, the data were retrospectively analyzed, and there were patients with disease overlapping with less common types of primary liver cancer. Although detailed patient characteristics and precise prognosis were unavailable, we could demonstrate the recent dynamic change of daily practice in Japan for HCC treatments based on this comprehensive database. As more promising systemic therapy regimens for HCC are being developed after the introduction of immune-checkpoint inhibitors, a follow-up survey of the NDB after 2020 will be necessary.

In conclusion, according to the national exhaustive database, there was a gradual decrease in the number of patients diagnosed with HCC over time, probably due to the decrease in the incidence of chronic hepatitis B and C infections. On the other hand, the development of treatments has resulted in an increase in the number of patients who underwent systemic therapy and LLR over time.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Patient-specific brain fluorodeoxyglucose positron emission tomography can detect the first effects of combination antiretroviral therapy in patient with HIV infection

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Abstract: Patient-specific brain fluorodeoxyglucose-positron emission tomography (FDG PET) can detect areas with abnormal FDG uptake in patients with human immunodeficiency virus (HIV) before and after combination antiretroviral therapy (cART). There were few reports about the same patients before and shortly after cART in FDG PET. It is well known that HIV-RNA levels decrease and cognitive impairments in patients with HIV tend to improve on neurocognitive performance tests 6 months after starting cART. We conducted a quantitative imaging analysis (FDG PET and voxel-based morphometry (VBM)) of eight patients at pre- and 6 months post- cART with neurocognitive performance tests. In terms of participant-specific changes between pre- and post-cART imaging, some area showed that the size of area with abnormal FDG uptake shrunk and became a nearly physiological level at 6 months post-cART. No apparent changes in VBM were observed in this short period. FDG PET might detect the first effect of cART.

***Keywords*:** FDG, PET, VBM, HIV associated neurocognitive disorder (HAND), cART

Introduction

Mild human immunodeficiency virus (HIV) associated neurocognitive disorder (HAND) are sometimes difficult to diagnose (*1-6*). Generally, these neurocognitive symptoms are highly variable and can include the cognitive, motor, or mood domains. Clinically effective combination antiretroviral therapy (cART) shows the first effectiveness 6 months after its introduction; the HIV RNA levels decline, and neurological performance tests (NPTs) tend to improve. However, persistent inflammation despite cART can cause brain damage over a period of time (*7,8*). Our prior epidemiological study indicated that after approximately 5 years of cART, the prevalence of neurocognitive impairment in people living with HIV (PLWH) increases (*9*). This suggests that the cART effect is time-limited, and cART may act as a neurotoxin (*10*).

Imaging is expected to supplement the diagnosis and follow-up of patients with HIV-induced neurocognitive disorders. Despite several studies attempting this, a definitive imaging tool has not been confirmed. Most previous imaging studies used a single imaging modality and did not include detailed tests for assessing neurocognitive performance (*11-13*). Moreover, most studies were based on group comparisons between PLWH and HIV- participants, and the group differences were rather small with milder forms of HAND.

^{18}F-2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) has been used in non-HIV-infected individuals to detect dementia, cancer, and active inflammation (*14-19*). Previous studies revealed complex FDG PET findings in PLWH (*20-26*); however, most were group studies, and the backgrounds of patients with neurocognitive disorders varied widely.

There were few studies in which the same patient was

analyzed by both FDG PET scan and NPTs with pre and shortly post cART. In this study, we analyzed whether FDG PET could detect the first cART effect in the same patient. Thus, we hypothesized that areas with abnormal FDG findings would shrink shortly after starting cART.

Patients and Methods

Patients

This study was approved by the Institutional Review Board of the National Center for Global Health and Medicine (approval number: 872, 896) and was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Eight pre-cART patients underwent the study examinations before and after receiving cART for 6 months. NPTs, neurological tests, FDG PET and magnetic resonance imaging (MRI) data acquisition, image processing, and statistical analyses were performed.

Thirty-five age-matched healthy control participants without HIV infection were also enrolled.

NPTs

Four psychologists with at least 3 years of training administered the neuropsychological tests. The Mini-Mental State Examination (MMSE), International HIV Dementia Scale (IHDS), Frontal Assessment Battery (FAB), and Self-Rating Depression Scale (SDS) were used for screening. The Category Fluency (animal), Word Fluency (starting from "ka"), Rivermead Behavioral Memory Test (RBMT) Story-Immediate Recall, RBMT Story-Delayed Recall, RBMT Picture Recognition-Delayed Recall, WAIS-III Digit Span, Trail Making Test Part A, WAIS-III Digit Symbol, and WAIS-III Block Design tests were used to assess the following six cognitive domains: "sensory perceptual/motor", "abstraction-executive", "language", "memory (learning, recall)", "attention information processing", and "complex perceptual motor skills". We regarded deficits in more than two domains with −1 standard deviation (SD) as mild HAND and those with −2 SD as HAND that interfered with daily life. Some domains had 2/3 sub-categories, and we counted "1 domain deficit" even if not all sub-categories were affected. Moreover, we performed "visuospatial" assessments as the seventh cognitive domain for reference.

Neurological tests

One neurologist performed the neurological tests for all patients. Neurological examination was performed *via* assessment, which evaluated the mental status, cranial nerves, motor strength, sensation, and reflexes, as well as activities of daily living as per Antinori's criteria. One of the six domains, sensory perceptual/motor skills, was also analyzed using these results, in addition to the NPTs.

^{18}F-FDG PET data acquisition

While resting in the supine position with their eyes covered and the noise level kept to a minimum, participants received an intravenous bolus injection of ^{18}F-FDG (5 MBq/kg). Forty-five minutes after the injection, PET-CT (Biograph Siemens 16; Siemens) imaging of the head was performed in three-dimensional-acquisition mode. Attenuation-corrected PET images were reconstructed using CT data, and a full width at half maximum Gaussian post-filter of 3.0-mm, and 53 image slices, with an interslice distance of 3 mm, were obtained. The total axial field of view was 16.2 cm with an approximate in-plane resolution of 5.8 mm.

Following the head acquisition, a whole-body PET-CT scan was performed from the vertex to the mid-thigh to check for the presence of other diseases that might affect brain metabolism.

MRI data acquisition

MRI was performed using a 3.0-Tesla Tim-Trio scanner (Magnetom Verio, Siemens, Erlangen, Germany) equipped with the standard four-channel head coil. A high-resolution, three-dimensional sagittal T1-weighted magnetization-prepared rapid gradient echo scan was acquired using the following parameters: echo time (TE) = 4.24 ms, repetition time (TR) = 1,600 ms, inversion time = 800 ms, flip angle = 15°, 256 × 256 acquisition matrix, and 1 × 1 voxels.

Image processing and statistical analyses

The PET and T1-weighted MRI images were processed and analyzed for spatially normalized to the standard brain template, using the statistical parametric mapping (SPM8) application (Wellcome Trust Centre for Neuroimaging, University College London, UK, *https://www.fil.ion.ucl.ac.uk/spm/*) implemented in MATLAB® R2010b (MathWorks, Inc., Natick, MA, USA) to detect subtle changes compared with those in the normal group. Two-sample *t* tests were used to detect differences between cART- and cART+ individuals.

The values were proportionally scaled by the whole brain means. A threshold of $p < 0.001$ (uncorrected) was used with the extent threshold for contiguous voxels set at k = 100. Two radiologists and two nuclear medicine radiologists assessed the images and separately reported abnormally decreased or increased areas. In case of any disagreement, they discussed and arrived at a consensus.

The FDG images were spatially normalized to the standard brain template, and the values were proportionally scaled by the whole brain means.

Results and Discussion

We analyzed eight individual changes pre- and 6 months post- cART (Table 1). After 6 months of cART, all patients showed that their HIV-RNA levels declined drastically. Two patients were diagnosed as mild HAND at pre-cART; one was also diagnosed as mild HAND at post-cART, however one changed to within normal limit (WNL) at post-cART. One patient's diagnosis changed from WNL at pre-cART to mild HAND at post-cART. In the analysis of the images, no apparent changes in voxel-based morphometry (VBM) were found after 6 months of cART, whereas the FDG PET findings differed dramatically. Some areas with abnormally decreased or increased FDG uptake at pre-cART shrunk and improved towards near-normal physiologically post-cART. After giving cART, brain glucose metabolism in some areas might revert to a near-normal physiological level, reflecting a brain function improvement.

In patient number (Pt. No.) 1, the diagnosis of mild HAND did not change pre- and post-cART. The decreased FDG uptake around the Sylvian fissure improved after cART, whereas that in the cingulate did not improve (Figure 1 and Figure 2). Abnormally increased uptake in the basal ganglia and thalamus improved dramatically after cART. The NPT score after cART indicated a mild neurocognitive disorder, similar to that before cART. However, the scores after cART differed slightly from those before cART. The patient exhibited improvement in the "visuospatial" domain, whereas the performance in the "attention information processing" and "complex perceptual (motor)" domains worsened.

NPT diagnosis of Pt. No.2 changed from mild HAND to WNL. The FDG PET images did not show any abnormal areas, even before starting cART.

Pts. No. 1 and 2 were diagnosed with mild HAND. After receiving cART, the diagnosis of Pt. No. 2 changed to WNL, and abnormal FDG PET findings could not be further detected at the pre-cART examination, suggesting that the functional disorder might not be strong. By contrast, the diagnosis of Pt. No. 1 remained as a mild HAND, and the abnormal findings in the anterior/posterior cingulate gyrus and precuneus did not improve after cART. Functional damage in these areas might be severe and not improve following cART.

The cingulate region has two main parts: the anterior cingulate and the posterior cingulate. The posterior cingulate cortex is closely related to the precuneus and hippocampus, which are associated with cognitive and visuospatial functions. The posterior cingulate and precuneus are well-known areas that have a reduced FDG uptake in patients with Alzheimer's disease (*27*). The anterior cingulate is associated with sympathy/empathy and focus maintenance during task performance. Some networks, such as the working memory, theory of mind, and saliency networks, are related to the anterior cingulate (*28,29*). The anterior cingulate adjusts networks to assigned tasks. Towgood *et al.* reported differences in FDG uptake in the anterior cingulate between patients in their 30s and those in their 50s, and these authors concluded that this change might relate to morphological shrinking and aging (*22*). Yuferov *et al.* reported neuroinflammatory changes in the anterior cingulate of postmortem brains from PLWH (*30*). In the current study, Pt. No. 1 showed improved abnormal findings without decreased FDG uptake in the anterior/posterior cingulate and precuneus after cART. Decreased function in these areas may irreversibly affect neuronal networks, which possibly relate aging and atrophy.

Abnormally decreased uptake around the Sylvian fissure (insula) improved after cART in Pt. No. 1. The insula is surrounded by the cerebral hemisphere and has several connections with the anterior brain areas, limbic system, middle and posterior cingulates, and the thalamus. Georgiou *et al.* showed that the decreased FDG uptake in areas around the Sylvian fissure area (insula) was related to drug use in HIV+ subjects (*21*). In our study, all participants were men, and most acquired HIV by having sex with other men; none of them had any apparent history of alcohol or drug abuse. The findings of Pt. No. 1 revealed decreased FDG uptake around the Sylvian fissure (insula) that diminished after cART initiation. Therefore, this abnormal uptake may be associated with factors other than drug use and might be reversible.

Pt. No. 3 showed abnormal FDG uptake on pre-cART images, and this finding improved after cART (Figure 1 and Figure 2). The patient had not been diagnosed with a neurocognitive disorder. FDG might detect the abnormal area before the symptoms become apparent in pre-cART. The diagnosis for Pt. No. 4 changed from WNL to mild HAND after cART. The FDG PET showed no abnormalities except for the area regarded as atrophic based on the VBM results. The patient exhibited brain atrophy before the initiation of cART.

In this study, the areas with abnormal FDG uptake shrank and reached nearly normal levels shortly after cART initiation in some areas. However, the initial cART effect might only be present for a limited time because the prevalence of neurological impairment tends to increase again after 5 years of cART. Lamers *et al.* showed that HIV DNA was detected in brain autopsy tissue following cART even if the viral load was undetectable (*7*). Blood-brain barrier impairment observed in PLWH could be attributable to HIV or secondary post-cART immune activation. In the post-cART period, these areas might reflect the direct or indirect effects of HIV on brain inflammation. FDG PET might detect the initial cART effects (*e.g.*, shrunken size and normalized levels in areas of abnormal FDG uptake) after only 6 months. Follow-up of FDG findings might be used to determine cART-related treatment effects and damage in these brain regions.

This study had several limitations. First, only 8

Table 1. Eight participants' NPTs and FDG PET findings, pre and post cART

Pt. No	Age (y)	cART	CD4 Recent (/μL)	Recent HIV/RNA (copies/mL)	NPT											FDG	
					NPT diagnosis	ADL	FAB	Language		Memory			AIP	CPM		Visuospatial		
								c	w	I	D	P		TMTA	Digit		Decreased	Increased
1	41	pre	2	1,200,000	Mild HAND	WNL	↓						↓		↓	↓↓	+	+
		post	277	ND	Mild HAND	WNL	↓						↓↓	↓	↓	↓	partially improve	partially improve
2	40	pre	571	12,000	Mild HAND	WNL	↓		↓						↓↓	–	–	–
		post	807	ND	WNL	WNL	improve		improve						↓(improve)			
3	47	pre	570	22,000	WNL	WNL											+	+
		post	655	32	WNL	WNL											improve	improve
4	47	pre	380	54,000	WNL	WNL									↓		atrophy	
		post	641	28	Mild HAND	WNL	↓			↓	↓				↓		no change	
5	41	pre	550	3,400	WNL	WNL		↓										
		post	471	< 20	WNL	WNL		improve										
6	50	pre	679	20,000	WNL	WNL								↓				
		post	729	ND	WNL	WNL								improve				
7	47	pre	358	27,000	WNL	WNL												
		post	504	ND	WNL	WNL												
8	40	pre	353	35,000	WNL	WNL												
		post	467	< 20	WNL	WNL												

NPT, neurological performance test; FDG PET, fluorodeoxyglucose positron emission tomography; cART, combination antiretroviral therapy; FAB, Frontal Assessment Battery; HAND, HIV associated neurocognitive disorder; AIP, Attention Information Processing; CPM, Complex Perceptual Motor Skills; TMTA,Trail Making Test, Part A; ND: not detected; Language c: Category Fluency, w: Word fluency; Memory I: RBMT Story-Immediate Recall; D: RBMT Story-Delayed Recall; P: RBMT Picture Recognition-Delayed Recall.

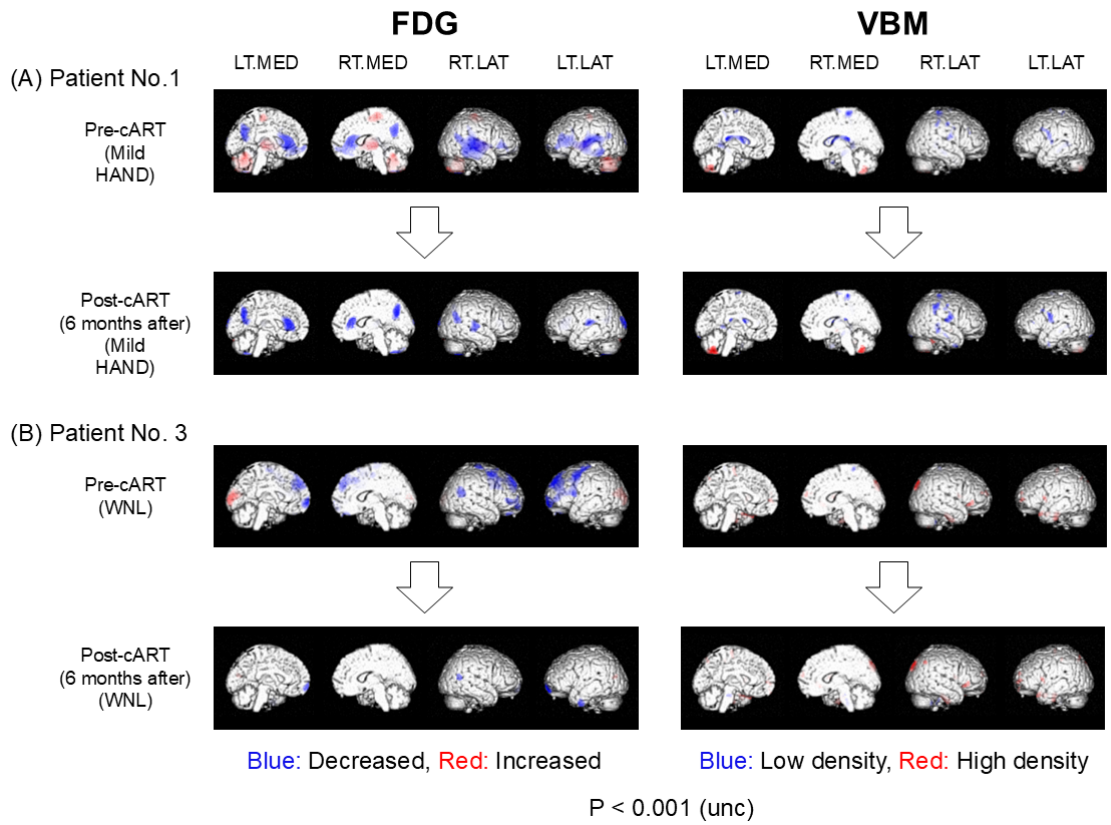

Figure 1. Comparison of FDG PET findings between pre- and post-cART images in two PLWHs. (A) Patient No. 1 shows several areas with abnormal FDG uptake in pre cART, with decreased area as blue and with increased area as red. In the post-cART images, the decreased blue area, anterior cingulate, did not improve, however, the decreased area in the left around sylvian fissure area and the increased red cerebellum and thalamus improved to normal. **(B)** Patient 3 has neurocognitive WNL and areas of abnormal FDG uptake pre-cART. The area with abnormal FDG uptake reduced in size on post-cART imaging and appeared nearly normal. cART, combination antiretroviral therapy; FDG, fluorodeoxyglucose; PET, positron emission tomography; PLWH, human immunodeficiency living with HIV; VBM, voxel-based morphometry; WNL, within normal limits.

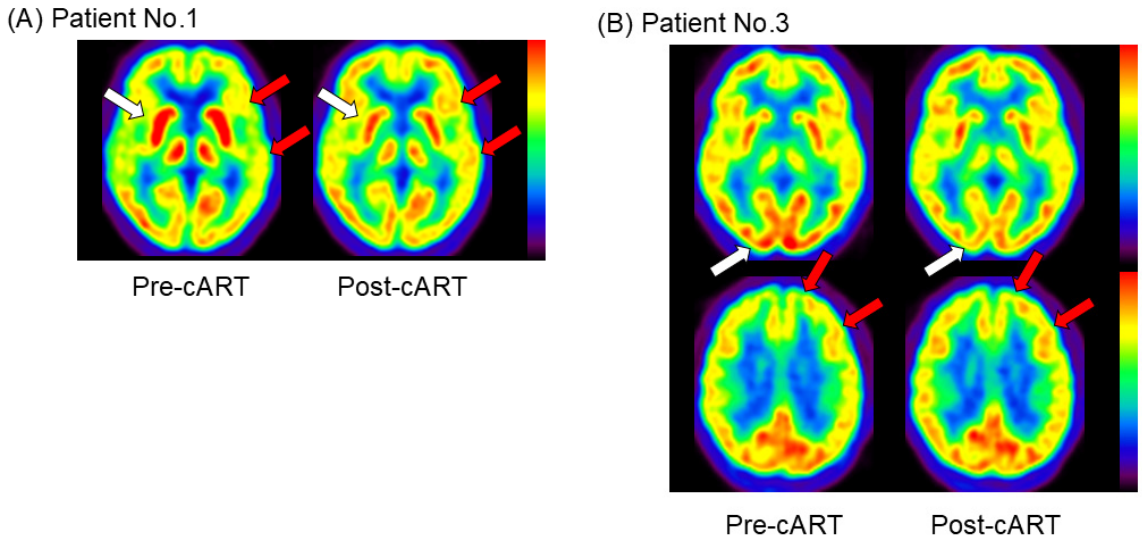

Figure 2. Decreased areas in pre-cART / improved in post-cART are shown in red arrow and increased areas in pre-cART / improved in post-cART are in white arrow. (A) Patient No. 1 showed the FDG uptake in the left around sylvian fissure areas were decreased in pre cART, however, that improved to normal after cART. The apparent increased uptake was seen in basal ganglia and thalamus in pre cART, and improved to normal in post cART. **(B)** Patient No. 3 showed decreased uptake in left frontal grey matter in pre cART and improved to normal in post cART. Uptake in the occipital areas were increased in pre cART and improved after cART. Images were spatially normalized and values were proportionally scaled by the whole brain means.

patients were analyzed with pre and 6 months post cART. Studies with larger sample sizes and longitudinal research are desirable. Second, we used Antinori's 3-HAND categories (*1*), sometimes affected by the effects of race/ethnicity, age, education, or sex. However, in this study, all PLWH and controls were age-matched Japanese men and the impact of these factors is considered to be minimal. Despite these limitations, our FDG PET data are illustrative between before and shortly after cART. More longitudinal data might be expected in the future.

In conclusion, in this study, we examined metabolic changes following cART administration in PLWH. The administration of cART tends to normalize brain metabolism in the short term; thus, FDG-PET might detect the effects of the first cART treatment.

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BRIEF REPORT

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Use of oral allylestrenol in women with recurrent spontaneous abortion: A retrospective clinical trial

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Abstract: Recurrent spontaneous abortion (RSA), defined as two or more clinically confirmed pregnancies that end before 20-24 weeks of gestation, encompasses both embryonic and fetal losses and is a significant clinical challenge. The aim of this study was to compare the efficacy of allylestrenol (AT) and progesterone in improving pregnancy outcomes in RSA. From June 2021 to June 2024, 480 participants were randomly assigned to an AT, Progesterone, or Control group. Key outcomes included early pregnancy rates, ongoing pregnancies with fetal heart activity, live birth rates after 24 weeks, and pregnancy loss before 24 weeks. Results indicated significantly higher pregnancy rates at 6-8 weeks in both the Allylestrenol (71.8%) and Progesterone groups (76.2%) compared to the Control group (57.5%). At 12 weeks, ongoing pregnancies with fetal heart activity were higher in the Allylestrenol (65%) and Progesterone groups (64%) versus the Control group (52.5%). Both treatment groups had higher live birth rates (60% and 60.6%) compared to the Control group (45%). Pregnancy loss before 24 weeks was lower in both treatment groups (31.8% and 33.1%) compared to the Control group (38.7%). No significant adverse reactions were observed, indicating good safety profiles for both treatments. These findings suggest that both treatments effectively improve pregnancy outcomes in cases of RSA with satisfactory safety, supporting their potential clinical use. However, further research is needed to explore their long-term effects and broader applicability in clinical settings.

***Keywords*:** recurrent spontaneous abortion, allylestrenol, progesterone, live birth rate

Introduction

Recurrent spontaneous abortion (RSA) is characterized by the occurrence of two or more clinically confirmed pregnancies that end before 20-24 weeks of gestation (*1*), encompassing both embryonic and fetal losses; RSA occurs in approximately 1% to 2% of couples attempting to conceive (*2*). The causes of RSA are diverse and may involve genetic abnormalities (*3*), uterine anatomical issues (*4*), hormonal imbalances (*5*), immune system disorders (*6*), and coagulation dysfunctions (*7*). To effectively manage RSA, a thorough evaluation to identify underlying causes and provide tailored treatment is essential. This may include genetic testing, hormonal therapy, corrective surgery for uterine abnormalities, immunomodulatory therapy, and lifestyle adjustments.

Progesterone has shown potential benefits in cases of recurrent spontaneous and threatened abortions (*8*). Progesterone prepares the uterine environment for embryo implantation and sustains pregnancy (*9*). Nevertheless, the use of progesterone to prevent miscarriages remains a topic of debate, given that the optimal dosing and timing have yet to be definitively established (*10*). Continued research is crucial to comprehensively understanding progesterone's role in pregnancy outcomes and to formulate effective strategies to prevent miscarriages.

Allylestrenol (AT) is a promising therapeutic option used to address conditions such as miscarriage (*11*) and preterm labor (*12*). The aim of the current study was to evaluate the effectiveness and safety of AT in the treatment of threatened miscarriages. Through a retrospective clinical trial conducted during the first trimester in women with RSA, this study sought to compare pregnancy rates, miscarriage rates, and live birth rates between a group receiving oral AT, a group

receiving progesterone treatment, and a control group. The hypothesis posited that oral administration of AT would lead to a reduction in miscarriage rates and an increase in live birth rates among women with RSA. The results of this study are expected to provide valuable insights into the potential benefits of AT in preventing miscarriages and enhancing pregnancy outcomes for women with RSA. However, further research and additional clinical trials are necessary to validate and expand upon these initial findings.

Patients and Methods

Types of study

A retrospective cohort study was conducted at the Obstetrics and Gynecology Hospital of Fudan University, with approval from the hospital's ethics committee (No. 2019-57). This clinical trial evaluated the use of AT and progesterone in women with RSA and it adhered to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines (*13*). All participants provided informed consent, and stringent measures were taken to protect their confidentiality and privacy.

Participants

Inclusion criteria: *i*) Age 20 to 40; *ii*) Body mass index (BMI) of 18-30 kg/m²; *iii*) Diagnosed with RSA; *iv*) No contraindications to continuing the pregnancy; and *v*) Normal results on chromosomal and genetic tests.

Exclusion criteria: *i*) Patients with abnormal heart, liver, lung, or kidney function, with psychiatric disorders, or tumors; *ii*) A history of chromosomal abnormalities in one's parents; *iii*) Structural abnormalities of the uterus and fallopian tubes; and *iv*) Male factor.

Intervention

Patients were administered oral AT or progesterone at a dosage of 10 mg twice daily starting from day 18-20 of the menstrual cycle. Participants were instructed to engage in clinic-directed intercourse as per the physician's guidance. Upon a positive pregnancy test, the dosage of oral AT or progesterone was increased to 10 mg three times daily, continuing until 12 weeks of gestation. During this period, ultrasound scans and blood tests were performed as necessary to monitor treatment progress and evaluate treatment efficacy up to the 12-week gestation mark.

Outcome measures

The purpose of this study was to evaluate pregnancy outcomes, including the pregnancy rate at 6-8 weeks, ongoing pregnancy with fetal heart activity at 12 weeks, the live birth rate after 24 weeks of gestation, pregnancy loss before 24 weeks of gestation, and adverse drug reactions.

Statistical analysis

The live birth rate in the progesterone treatment group was 65.8%, as observed in a randomized double-blind clinical trial. Previous research has suggested that the live birth rate could potentially exceed 70% with AT treatment (*14*). To determine the study's sample size, various parameters were considered, including a significance level of $\alpha = 0.05$, a power of $\beta = 0.1$, a test validity of 0.9, and a 5% loss to follow-up rate. Moreover, this study noted a miscarriage rate of 32.2% in the progesterone treatment group, with 50% of those cases demonstrating chromosomal abnormalities in the embryos, corresponding to 16.1% of the total sample size of 160 patients per group. For clinical trials, continuous variables with a normal distribution were verified using the Kolmogorov-Smirnov test. Data were expressed as the mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA). If the data did not follow a normal distribution, they were expressed as the median (interquartile range) and analyzed using the Kruskal-Wallis test. Categorical variables were expressed as percentages and analyzed using the Pearson χ^2 test.

Results and Discussion

This study provides compelling evidence that both AT and progesterone are effective in enhancing pregnancy outcomes among women with RSA. The improved pregnancy and live birth rates observed across these treatments underscore the clinical potential of hormone-based therapies in managing RSA. To fully understand the implications of these findings, however, one must delve into the underlying physiological and pharmacological mechanisms. For instance, exploring how these hormones support embryo implantation and early development can elucidate their roles in improving outcomes. Additionally, a comparison with similar studies in the literature would provide valuable context, revealing both consistencies and divergences that could inform the direction of future research.

Participants' demographic information and baseline characteristics

Between June 2021 and June 2024, 508 women were screened, and 480 participants ultimately met the inclusion criteria and completed the study (Figure 1). Baseline characteristics, such as age and BMI, were statistically similar across the three groups, with no significant differences in age ($p = 0.46$) or BMI ($p = 0.96$) (Table 1). This demographic comparability is essential, as it reduces the likelihood that observed outcomes are due to confounding variables rather than treatment

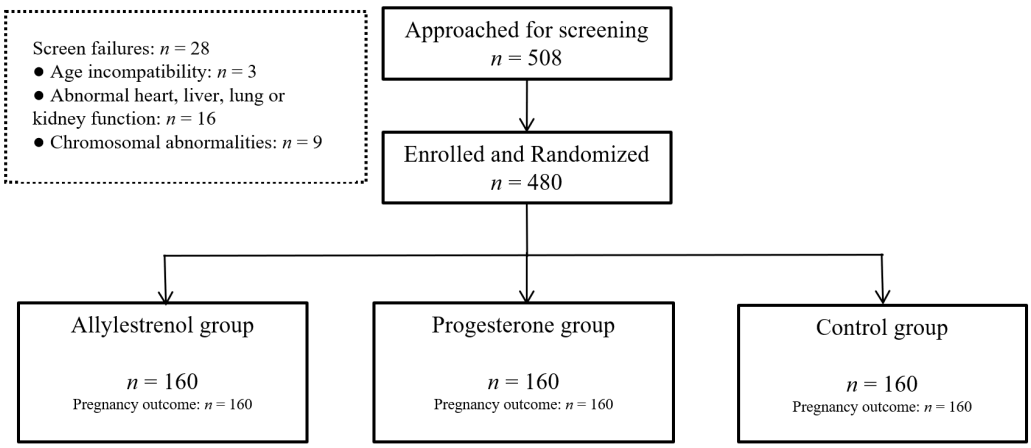

Figure 1. Overview of the study and randomization.

Table 1. The baseline characteristics of women with RSA

	Allylestrenol group (n = 160)	Progesterone group (n = 160)	Control group (n = 160)	p value
Age of women (years)	32.18 ± 1.2	32.09 ± 3.4	32.47 ± 3.1	0.46
BMI (kg/m^2)	21.40 ± 2.8	20.47 ± 1.9	21.51 ± 2.6	0.96
Number of previous miscarriages				
0	22	25	24	0.06
1	72	76	73	0.05
2	55	51	50	0.55
≥ 3	11	8	13	0.11

Abbreviations: BMI: body mass index.

effects. Such baseline uniformity ensures that differences in outcomes can be more confidently attributed to the interventions themselves. This rigor in patient selection aligns with best practices in clinical research, providing a solid foundation for the study's conclusions by enhancing internal validity and reducing potential biases.

Pregnancy rate at 6-8 weeks

The pregnancy rate at 6-8 weeks was notably higher in both the AT group (71.8%) and the Progesterone group (76.2%) compared to the Control group (57.5%) ($p < 0.0001$). These findings suggest both treatments provide critical hormonal support during early pregnancy. Further investigation into the mechanisms by which these hormones influence implantation could deepen our understanding. The slightly higher rate observed with progesterone, while not statistically significant, may indicate a potential advantage that warrants further exploration. Progesterone, known for its role in maintaining the luteal phase and promoting endometrial receptivity, has been widely studied in various conditions related to pregnancy maintenance. For instance, in patients undergoing *in vitro* fertilization (IVF), progesterone supplementation has been shown to significantly improve implantation rates and pregnancy outcomes (*15*). Similarly, AT has been used to treat threatened miscarriage and preterm labor, demonstrating efficacy in sustaining early pregnancies (*16*). Further research into the underlying mechanisms of these treatments, and particularly their effects on implantation, could deepen our understanding of their role in early pregnancy support.

Ongoing pregnancy with fetal heart activity at 12 weeks

At 12 weeks, ongoing pregnancies with detectable fetal heart activity were reported in 65% of the AT group and 64% of the Progesterone group, both of which were significantly higher than in the Control group (52.5%) ($p < 0.0001$). These results suggest comparable efficacy in sustaining pregnancies during this crucial early stage. Analyzing the physiological pathways involved could provide insights into how these treatments contribute to ongoing viability. The comparable outcomes observed in both the AT and Progesterone groups suggest that AT may offer similar benefits in promoting pregnancy viability, and particularly in women with a history of recurrent miscarriage. Although these results revealed no significant differences between AT and progesterone, the slightly higher ongoing pregnancy rate in the Progesterone group warrants further exploration, especially considering the possibility of nuanced differences in patient response or long-term outcomes.

Live birth rate after 24 weeks of gestation

The live birth rate after 24 weeks of gestation was 60% (96/160) for the AT group and 60.6% (97/160) for the Progesterone group, both of which were significantly higher than the 45% (72/160) observed in the Control group ($p < 0.0001$). This highlights the effectiveness of both treatments in facilitating live births beyond this critical threshold. Progesterone is considered a key physiological component for embryo implantation and maintaining pregnancy. It plays a crucial role in preparing the endometrium for implantation, suppressing uterine contractions, and promoting placental development. The importance of progesterone in early pregnancy is well-documented, as it stabilizes the uterine lining and supports the growing embryo. The removal of the corpus luteum, which is the primary source of progesterone in early pregnancy, or the use of progesterone antagonists such as mifepristone, can lead to pregnancy termination (*17*). Women with a history of miscarriage who experience bleeding in early pregnancy may benefit from the use of vaginal progesterone. Vaginal micronized progesterone, typically administered at a dosage of 400 mg twice daily, is associated with increased live birth rates (*18*). Comparative analyses with other studies involving similar treatments could enhance our understanding of the broader implications of these findings.

Pregnancy loss before 24 weeks of gestation

Pregnancy loss before 24 weeks was significantly lower in both treatment groups compared to that in the Control group. The AT group had a loss rate of 31.8% (51/160), while the Progesterone group had a rate of 33.1% (53/160), both of which were lower than 38.7% (62/160) in the Control group ($p = 0.0011$). These findings underscore the effectiveness of both treatments in reducing early pregnancy losses, with no significant differences between the two treatments.

In summary, both AT and progesterone significantly improve key pregnancy outcomes, including higher early pregnancy rates, increased ongoing pregnancies with fetal heart activity, and live births, while reducing losses before 24 weeks. These results indicate that both treatments are viable, effective options for enhancing pregnancy success among women with RSA (Table 2).

Adverse reactions

The incidence of adverse reactions, including nausea, vomiting, headaches, and dizziness, was similar across the AT, Progesterone, and Control groups, with no statistically significant differences. This suggests that both treatments are well-tolerated, providing reassurance regarding the safety of prolonged hormonal support in RSA management. The similar adverse reaction profiles imply that AT offers a comparable safety margin to progesterone, thus supporting its clinical utility as a potential alternative in cases where progesterone is less well-tolerated or contraindicated (Table 3).

The safety profile of both agents further supports their use as long-term interventions in this high-risk population, as tolerability is a crucial consideration in the sustained management required for patients with RSA. This comparable tolerability aligns with prior research on the use of progestational agents in pregnancy, providing further evidence that these treatments can be safely used without having significant adverse effects.

Conclusion

Our findings indicate that both AT and progesterone significantly enhance pregnancy outcomes compared to those in the Control group. The pregnancy rate at 6-8 weeks was markedly higher in the treatment groups, with AT resulting in a rate of 71.8% and progesterone resulting in one of 76.2%, both of which significantly exceeded 57.5% in the Control group ($p < 0.0001$). This suggests that both treatments are effective in increasing

Table 2. Pregnancy outcomes

Outcomes	Allylestrenol group (%)	Progesterone group (%)	Control group (%)	*p* value
Pregnancy rate at 6-8 weeks	115/160 (71.8)	122/160 (76.2)	92/160 (57.5)	< 0.0001
Ongoing pregnancy with fetal heart activity at 12 weeks	104/160 (65.0)	102/160 (64.0)	84/160 (52.5)	< 0.0001
Live birth rate after 24 weeks of gestation	96/160 (60.0)	97/160 (60.6)	72/160 (45.0)	< 0.0001
Pregnancy loss before 24 weeks of gestation	51/160 (31.8)	53/160 (33.1)	62/160 (38.7)	0.0011

Table 3. Adverse reactions during pregnancy

	Allylestrenol group ($n = 160$)	Progesterone group ($n = 160$)	Control group ($n = 160$)	*p* value
Nausea and vomiting	11	10	13	0.25
Headache	8	6	9	0.36
Dizziness	13	12	11	0.76

early pregnancy success.

These findings demonstrate that AT significantly enhances the clinical pregnancy rate and live birth rate while reducing the miscarriage rate compared to rates in the Control group. These results suggest that AT could potentially become a new standard medication for treating patients with RSA. Previous research has also corroborated the use of progesterone in preventing multiple pregnancies and associated complications, such as maternal, fetal, and neonatal morbidity and mortality (*19*). However, the use of progestogens in threatened miscarriage treatment remains controversial, with conflicting study results regarding its impact on reducing miscarriage rates (*20*). Consequently, there is an ongoing necessity for quality, large-scale studies to ensure the safety and appropriateness of medications administered to pregnant women (*21*).

The current findings indicate that both AT and progesterone are effective at improving key pregnancy outcomes, including early pregnancy rates, ongoing pregnancies with fetal heart activity, live birth rates, and reducing pregnancy loss before 24 weeks. Moreover, both treatments are well-tolerated with no significant differences in adverse reactions. These results suggest that either AT or progesterone can be considered a viable option for enhancing pregnancy success in clinical settings. Further research could delve into the long-term effects and potential benefits of these treatments in diverse populations.

In essence, oral AT has demonstrated the potential to reduce the risk of miscarriage in women experiencing preterm abortion during early pregnancy. However, further research, and particularly with larger sample sizes, is warranted to evaluate its impact on live birth rates, obstetric complications, and potential adverse drug reactions. Conducting comprehensive studies in these domains will be critical to establishing a more comprehensive understanding of the efficacy and safety of oral AT as a treatment for preterm abortion.

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LETTER

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Increase in the number of female doctors and the challenges that Japan's medical system must face

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Abstract: Japan has seen an increase in female physicians recently, yet it still lags behind other Organization for Economic Cooperation and Development (OECD) countries. A major barrier has been the historical discrimination against women in medical school admissions. In recent years, female enrolment in medical schools has risen, surpassing 40% in 2024, reflecting a broader societal shift. However, structural problems persist in the Japanese healthcare system. Although the number of doctors per capita is lower in Japan than in other countries, the number of patients is higher than in other countries, leading to overwork for doctors. As a result, only about one-third of female doctors in Japan are able to return to work after interrupting their careers to give birth or raise children. The maldistribution of physicians, both regionally and by specialty, exacerbates this issue. To sustain the rising number of female physicians, Japan must reform its medical system.

***Keywords*:** healthcare system, health policy, maldistribution, physicians in Japan, female physician

Although the female physician percentage in Japan has recently reportedly increased significantly (*1*), Japan still has one of the lowest percentages among the Organization for Economic Cooperation and Development (OECD) member countries. One reason is the long-standing discrimination against women in medical school entrance examinations in Japan (*2,3*). The 2018 admissions scandal caused a stir when it was revealed that several universities had discriminated against women in their medical school admissions practices (*2,3*). Although only four universities were mentioned, the male-to-female admission ratio to date suggests that some discrimination may have occurred at many Japanese universities over the years. According to the Basic School Survey by the Ministry of Education, Culture, Sports, Science and Technology, the female student enrollment percentage in medical schools gradually rose from the low 10% range in the 1970s to > 30% in 1994 but remained in the low 30% range for about 25 years thereafter (*4*). Since the scandal made headlines in 2018, the female student percentage enrolled in Japanese medical schools has increased nationwide, exceeding 40% in 2024 (*4*). It is gratifying to observe that the glass ceiling in Japanese society is being lifted and that freedom of choice and career path possibilities for female students are being expanded. However, it is important to address why discrimination against women persisted in Japanese medicine for so long.

Japan has fewer physicians per capita than the average for developed countries, even though it has one of the highest numbers of patients per physician and hospital beds per capita (Table 1) (*5*). This distorted structure has led to the normalization of overworking among Japanese clinicians. Doctors at universities spend much of their time on clinical work, leading to less time dedicated to research yearly (*6*). In addition to these structural issues, Japan – with the world's most aged population – will become even busier as the demand for inpatient care is projected to peak by 2040 (*7*).

In certain life stages, women are expected to balance work with childbirth and childcare. However, achieving this balance within the Japanese medical system's demanding environment is difficult, and approximately 1 in three female doctors can return to work after interrupting their careers for childbirth or childcare (*2*). This practice lacks role models owing to the lack of female doctors who continue working after becoming mothers. As of June 1, 2022, the deans of medical schools and university hospitals at all 82 university medical schools in Japan were reported to be male (*8*). Instead of remedying this, the Japanese medical community – with its doctor shortage – has justified accepting men to medical schools over women. Surprisingly, even after the scandal of discrimination against women in entrance examinations, many female doctors in Japan felt that men would inevitably receive preferential treatment in medical school entrance examinations (*3*). However, the enrolment of women in medical schools has increased

Table 1. Comparison between the OECD average and Japan

Indicators	OECD Average	Japan
Estimated number of in-person consultations per doctor, 2021 (or nearest year)	1,788 (32 countries)	4,288
Hospital beds per 1, 000 population, 2021 (or nearest year)	4.3 (38 countries)	12.6
Practicing doctors per 1, 000 population, 2021 (or nearest year)	3.7 (37 countries)	2.6

Data source: OECD Health Statistics 2023 (*5*). OECD, Organization for Economic Cooperation and Development.

since the scandal, and Japan's medical system will not be able to sustain itself unless it ensures that these women can properly develop their careers and continue working.

One issue that must be addressed as the female physician numbers increase is the regional maldistribution. The growing number of female Japanese physicians is concentrated in urban areas, where working part-time while raising children is easier (*1*). This follows the recent trend of male and female physicians concentrating in urban areas. In Japan, the medical profession's popularity, owing to its stability, has led to high competition during medical school entrance examinations. This has led students to become more career-oriented and to prefer urban areas over the countryside for their career paths (*9*). Physicians from urban areas are also less inclined to work in rural areas than those from rural regions (*9*). Regarding children's education, Japanese doctors gather in areas with better-rated high schools and university medical schools (*10*). Thus, if the current admissions and medical systems remain in place, this tendency for regional maldistribution may worsen. The Japanese government has attempted to address these problems by establishing a small number of regional quotas in medical school entrance examinations that offer preferential entrance examinations and reduced or exempted tuition fees if students agree to work in certain regions after graduation (*11*). However, these regional quotas burden students, as the mandatory working period after graduation is quite long. As a result, in certain unpopular regions, the student numbers have fallen below capacity (*11*). In the future, it may be necessary to consider more natural ways to reduce competition in the general entrance examination and to re-examine the entrance examination system to make it easier for students from diverse regions to be accepted. This could be achieved, for example, by using a quota system with fewer obligations than regional quotas or by enhancing the entrance examinations themselves. Improving the rural working environments is difficult unless the structural geographic physician maldistribution is eliminated.

Another issue that must be addressed as the female physician numbers increase is the medical specialty maldistribution. Owing to the Japanese medical working environment (as described above), female doctors often choose specialties where balancing work and family life is easier (*2*). In Japan, there are few restrictions on medical school graduates' choices when specializing. Despite the overall medical school system's increased capacity over the past decade, the number of physicians in busy and resource-limited specialties such as surgery has not increased (*12*). The maldistribution of physicians by department for reasons such as work-life balance will not improve unless strong measures are implemented to secure physicians in each department and drastic reforms are made to improve the working environment for all medical personnel.

To increase the number of female doctors welcomed in Japan, the country's medical system must be reformed and modernized. For example, there is still much that can be done for Japanese healthcare — such as reducing unnecessary medical care demands by reviewing the out-of-pocket expenses for patients and consolidating excessive medical facilities. Digitalization, which has lagged behind in overall healthcare in Japan (*7*), should also be promoted to reduce labor and increase efficiency

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