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# JUNTENDO MEDICAL JOURNAL

# 順天堂醫事雜誌

June 2023

Abstract

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# JUNTENDO MEDICAL JOURNAL

## 順天堂醫事雜誌



### The History of *Juntendo Medical Journal*

This *Juntendo Medical Journal* has been published under the Japanese name *Juntendo Igaku* (順天堂医学) from 1964 to 2012. However, the origin of *Juntendo Medical Journal* dates back to the oldest medical journal in Japan, *Juntendo Iji Zasshi* (順天堂醫事雜誌), which had been published between 1875 and 1877 (total of 8 issues). Between 1885 and 1886, Juntendo issued a limited release of a research journal titled *Houkoku* [*Juntendo Iji Kenkyukai*] (報告) for a total of 39 issues.

In 1887, *Juntendo Iji Kenkyukai Houkoku* (順天堂醫事研究會報告) was published with the government's approval and we used to regard this as the first issue of *Juntendo Medical Journal*. Since then, *Juntendo Medical Journal* has undergone a series of name changes: *Juntendo Iji Kenkyukai Zasshi* (順天堂醫事研究会雜誌), *Juntendo Igaku Zasshi* (順天堂医学雜誌), and *Juntendo Igaku* (順天堂医学).

Now in commemoration of the 175th anniversary of Juntendo University, starting with the first volume issued in 2013 (Volume 59 Number 1), we return to *Juntendo Medical Journal*'s original Japanese title in 1875-*Juntendo Iji Zasshi* (順天堂醫事雜誌). We also reconsidered the numbering of the journal and set the first issue in 1875 as the initial publication of *Juntendo Medical Journal*. The Volume-Number counting system and the English name *Juntendo Medical Journal* started in 1955 from the January 10 issue. Although this is not our intension, we will retain the Volume-Number counting system to avoid confusion. However, Volume 59 Number 1 will be the 882nd issue, reflecting the sum of all issues to date: 8 issues of *Juntendo Iji Zasshi* (順天堂醫事雜誌), 39 issues of *Houkoku* [*Juntendo Iji Kenkyukai*] (報告) (47 issues combined), and 834 issues from *Juntendo Iji Kenkyukai Houkoku* (順天堂醫事研究會報告) in 1887 to the present.

出典：小川秀興（OGAWA Hideoki, M.D., Ph.D.）：順天堂醫事雜誌（Juntendo Medical Journal）2013；59：6-10.

本誌は昭和39年（1964年）から平成24年（2012年）末まで『順天堂医学』として刊行されてきた．しかし，その起源は明治8年（1875年）から10年（1877年）にかけて発刊された日本最古の医学誌『順天堂醫事雜誌』（計8巻）にある．さらに明治18年（1885年）から19年（1886年）まで，会員限定配本として順天堂醫事研究會の雑誌『報告』（計39集）が発行されている．

その後『順天堂醫事研究會報告』が明治20年（1887年）に官許を受けて公刊されたので，順天堂ではこれを通刊1号としてきた．以来，『順天堂醫事研究会雜誌』，『順天堂医学雜誌』，『順天堂医学』と名称を変更して刊行されてきた．

今般，順天堂が創立175周年を迎える平成25年（2013年）の59巻1号を期して，本来の名称である『順天堂醫事雜誌』と復刻し，その起源である明治8年（1875年）第1巻をもって創刊号（通刊第1号）とすることとした．従来の巻号と欧文誌名は，昭和30年（1955年）1月10日発行のものを1巻1号としており，欧文誌名もこれより付け始めたもので不本意であるが，混乱を避けるためにこれらを継承する．ただし，通刊数は明治8年（1875年）から19年（1886年）にかけて刊行された『順天堂醫事雜誌』8巻分と順天堂醫事研究會の雑誌『報告』39集，計47巻分を通巻834号に加え，59巻1号を通刊882号とした．

出典：小川鼎三，酒井シヅ：順天堂医学　1980；26：414-418.
小川秀興：順天堂醫事雜誌　2013；59：6-10.

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***From the illustrator***: I came across a little unusual colored roses at a flower shop. Probably this kind of rose would have been created by artificially repeated crossbreeding, however the roses were pretty modern and beautiful. So, I painted a picture of roses like a botanical art without putting them in a vase.

Abstract

Juntendo Medical Journal
2023. 69(3), 180–187

# Development of Ryanodine Receptor (RyR) Inhibitors for Skeletal Muscle and Heart Diseases

HIROYUKI MATSUKAWA, TAKASHI MURAYAMA

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Ryanodine receptors (RyR) are intracellular calcium ($Ca^{2+}$) release channels on the sarcoplasmic reticulum of skeletal and cardiac muscles that play a central role in excitation–contraction coupling. Genetic mutations or posttranslational modifications of RyR causes hyperactivation of the channel, leading to various skeletal muscle and heart diseases. Currently, no specific treatments exist for most RyR-associated diseases. Recently, high-throughput screening (HTS) assays have been developed to identify potential candidates for treating RyR-related muscle diseases. These assays have successfully identified several compounds as novel RyR inhibitors, which are effective in animal models. In this review, we will focus on recent progress in HTS assays and discuss future perspectives of these promising approaches.

***Key words***: ryanodine receptor, $Ca^{2+}$ release channel, drug development, high-throughput screening

## Introduction

The ryanodine receptor (RyR) is a calcium ($Ca^{2+}$) release channel present in the endo/sarcoplasmic reticulum of various cells including skeletal muscle, heart, and brain. It forms a huge (>2 MDa) homotetrameric protein complex that comprised a large cytoplasmic structure with six transmembrane segments at the C-terminus forming a cation-channel domain[1, 2]. Three major isoforms (RyR1–3) of RyR have been identified in mammals: RyR1 is mainly present in skeletal muscle, RyR2 in the heart, and RyR3 in various tissues at small amounts[3–5]. RyR is activated by $Ca^{2+}$ to release $Ca^{2+}$ from the ER, referred to as $Ca^{2+}$-induced $Ca^{2+}$ release[6, 7]. RyR1 also mediates depolarization-induced $Ca^{2+}$ release, which is gated via physical interaction with a L-type voltage-dependent $Ca^{2+}$ channel, specifically, the dihydropyridine receptor[8, 9].

Genetic mutations in RyR cause various skeletal muscle and heart diseases, including malignant hyperthermia (MH) and central core disease for RyR1,[10, 11] and catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy for RyR2[12, 13]. The predominant underlying mechanism for these diseases is hyperactivation of the channel. Hyperactivation of RyR by posttranslational modifications may also be implicated in several diseases such as muscular dystrophy and heart failure[14, 15]. Therefore, RyR inhibitors are therapeutic candidates for these diseases.

In this review, we will briefly summarize several existing RyR inhibitors (Figure 1). Next, we will describe recent advances in high-throughput screening (HTS) for development of RyR inhibitors and discuss future perspectives.

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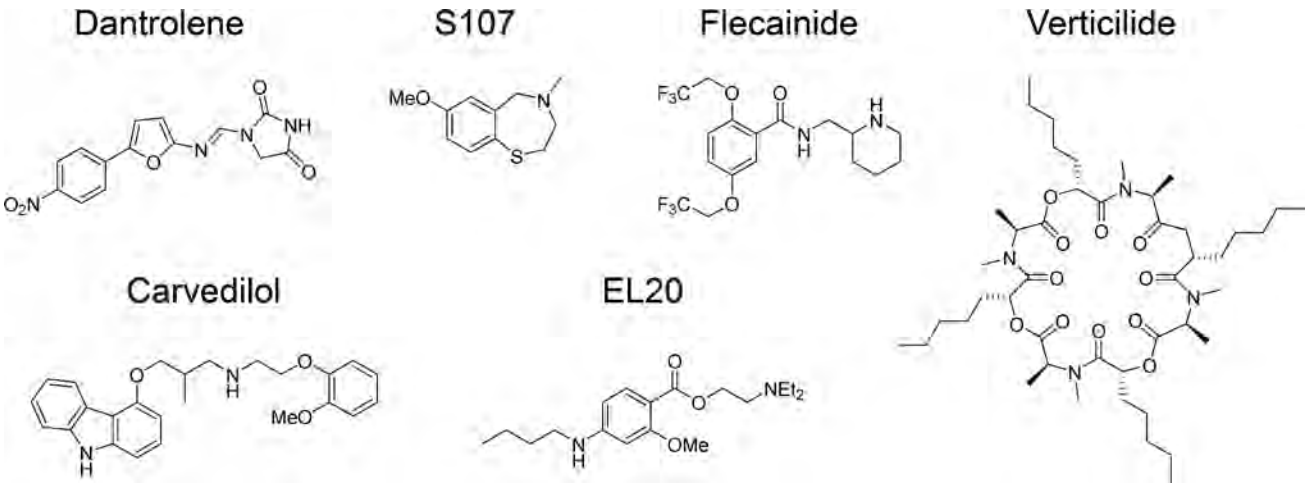


**Figure 1** Structure of existing RyR inhibitors

## Existing RyR inhibitors

### Dantrolene

Dantrolene is the only approved drug for MH[16, 17]. It was first synthesized in 1967 as a muscle relaxant[18]. Later, it was found to prevent $Ca^{2+}$ release by directly interacting with RyR1[19, 20]. Dantrolene greatly reduced the mortality of patients with MH from 70–80% to < 10%[21]. However, dantrolene has several disadvantages for clinical use including poor solubility in saline[22] and a long plasma half-life (~12 h), which prolongs side effects such as muscle weakness[23].

### Rycals

FK506 binding protein 12 (FKBP12) and FKBP12.6 bind to RyR1 and RyR2, respectively, to stabilize the channel, while posttranslational modifications (e.g., oxidation, nitrosylation, and phosphorylation) dissociate FKBP12/12.6 to cause leaky channels[15, 24]. Rycals (S107 and S48168/ARM210) are benzothiazepine derivatives that prevent dissociation of FKBP12/12.6 from RyR channels[25, 26]. Rycals exhibited therapeutic effects in various models of skeletal muscle diseases including RyR1-related myopathy, Duchenne muscular dystrophy, and sarcoglycanopathy, as well as in aging[27–29]. A recent cryo-EM structure revealed the putative binding site of ARM210 in the RY1&2/P1 domain, to which it binds cooperatively with ATP to stabilize the closed state of RyR1[30].

### Flecainide

Flecainide is a class Ic antiarrhythmic drug that prolongs the duration of the cardiac action potential by blocking the sodium channel Nav1.5 in the heart[31]. Watanabe and colleagues[32] reported that flecainide prevents mouse and human CPVT. Among class I antiarrhythmic agents, they found that only flecainide and propafenone showed antiarrhythmic activity against CPVT[33]. They also showed that flecainide inhibited RyR2 channel activity[32, 33]. Consistently, a N-methylated flecainide analog, which had less RyR2 inhibitory activity, did not prevent arrhythmias in CPVT mice[34]. However, the effect of flecainide on RyR2 is still under debate[35]. Bannister et al.[36] demonstrated that flecainide did not inhibit the physiologically relevant, luminal-to-cytosolic flux of cations through the RyR2 channel, although it partially blocked the cytosolic-to-luminal cation flux. Furthermore, Salvage et al.[37] recently demonstrated that flecainide activated RyR2 at lower concentrations, but was inhibitory at higher concentrations.

### Carvedilol

Carvedilol is a $\beta$-blocker used for chronic heart failure and CPVT. Compared with metoprolol, carvedilol extended the survival of patients with heart failure in clinical studies[38]. Mochizuki et al.[39] reported that carvedilol improved intracellular $Ca^{2+}$ concentration and systolic dysfunction in heart failure by correcting the interdomain interaction of RyR2. Zhou et al.[40] reported that carvedilol and its novel analogs (with minimal $\beta$-blocking activity)

suppressed proarrhythmic store overload-induced $Ca^{2+}$ release. This suggests that RyR2 inhibitory activity is responsible for the enhanced efficacy of carvedilol compared with other $\beta$-blockers in the treatment of arrhythmogenic heart diseases.

### EL20

Tetracaine is an ester-type local anesthetic and a nonselective RyR inhibitor within the millimolar range[41]. Because tetracaine is a potent inhibitor for voltage-gated sodium ($Na^+$) channels, it is not clinically used as a RyR inhibitor. Klipp et al.[42] synthesized derivatives of tetracaine and identified EL20, which suppressed RyR2 in the nanomolar range in the absence of calmodulin (CaM) in sheep. In R176Q CPVT model mice, EL20 suppressed the induction of ventricular tachycardia. Word et al.[43] further demonstrated that EL20 suppressed abnormal $Ca^{2+}$ homeostasis in induced pluripotent stem cell-derived cardiomyocytes from a patient with CPVT. Since EL20 did not affect electrocardiogram parameters in wild-type mice, it may prevent CPVT without affecting conduction properties of the heart[42].

### Verticilide

Verticilide is a compound isolated from Verticillium sp. FKI-1033[44]. Shiomi et al.[45] reported that verticilide inhibited RyR within the micromolar range, and more selectively inhibited insect RyR (half maximal inhibitory concentration [$IC_{50}$] = 4.2 μM) than mammalian RyR2 ($IC_{50}$ = 53.9 μM). Batiste et al.[46] synthesized derivatives of verticilide and found that an enantiomer (*ent*-1) inhibited RyR2 at the submicromolar range ($IC_{50}$ = 0.1 μM). Furthermore, *ent*-1 significantly reduced spontaneous $Ca^{2+}$ release in cardiomyocytes isolated from CPVT model mice and prevented ventricular arrhythmias, suggesting *ent*-1 may be a novel therapeutic candidate for CPVT. Structure-function relationships of *ent*-1 have shown that a high degree of N-methyl amide content is critical for its activity[47].

## High-throughput screening assays

As discussed, several RyR inhibitors have been identified and tested for the treatment of muscle and/or heart diseases. Most of these compounds also act on other targets (channels and receptors), which might cause side effects in clinical use. Therefore, the development of novel compounds that selectively inhibit RyR are an urgent need. High-throughput screening is a powerful method for the rapid evaluation of thousands to millions of chemical compounds. However, development of HTS assays targeted to RyR have been slowed by the lack of appropriate screening platforms. Recently, two groups developed HTS assays for RyR modulators using different approaches.

### Fluorescence energy transfer (FRET)-based HTS assay

RyR are tightly regulated by endogenous associated proteins, such as CaM and FKBP12/12.6[1,2]. Dissociation of these molecules from RyR may change its structural state, leading to $Ca^{2+}$ leakage. Rebbeck et al.[48] developed a HTS assay for RyR1 modulators using time-resolved fluorescence resonance energy transfer (TR-FRET). They measured FRET between donor fluorospheres (bound to FKBP 12.6) and acceptor fluorospheres (bound to CaM). Substantial FRET signals were detected between the two proteins, reflecting their close proximity determined from structural data[49,50]. They screened a compound library consisting of 727 small molecule compounds and identified five compounds that significantly altered FRET. Of these, two compounds (tacrolimus and ebselen) were known RyR1 modulators, and three (cefatrizine PG, disulfiram, and chloroquine) were new RyR1 modulators[48]. These hit compounds also showed RyR1 modulating activity in a [$^3$H]ryanodine binding assay, suggesting that structure-based HTS assays are effective at detecting functional modulators of RyR1. Rebbeck et al.[51] further improved their strategy by miniaturizing the screening format to the industry standard of 1536-well plates. Using a larger library of 1,280 compounds, chloroxine and myricetin were identified as novel RyR1 inhibitors. They demonstrated that the two drugs significantly inhibited $Ca^{2+}$ leakage from the sarcoplasmic reticulum via RyR1, with only slight effects on $Ca^{2+}$ release in E-C coupling. Similar strategies between CaM and a biosensor peptide (DPc10) have been used to identify novel RyR2 inhibitors[52].

### Endoplasmic reticulum $Ca^{2+}$-based HTS assay

Murayama and colleagues[53,54] developed a HTS

assay to search for novel RyR1 inhibitors using an endoplasmic reticulum (ER) $Ca^{2+}$ concentration measurement ($[Ca^{2+}]_{ER}$) (Figure 2A). Generally, $[Ca^{2+}]_{ER}$ is determined by the balance between $Ca^{2+}$ release by $Ca^{2+}$ release channels (RyR or 1,4,5-trisphosphate [$IP_3$] receptors) and $Ca^{2+}$ uptake by sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (or SERCA) $Ca^{2+}$ pumps. They found that under resting conditions, expression of hyperactive MH-mutant RyR1 reduces $[Ca^{2+}]_{ER}$ in HEK293 cells by $Ca^{2+}$ leakage[55,56]. Addition of RyR1 inhibitors (dantrolene and tetracaine) to cells increased $[Ca^{2+}]_{ER}$ by preventing $Ca^{2+}$ leakage[53]. They measured $[Ca^{2+}]_{ER}$ using R-CEPIA1er, a fluorescent ER $Ca^{2+}$ indicator[57], in a 96-well microplate reader. Using this assay platform, they screened a chemical compound library of well-characterized drugs (1,535 compounds) and identified several compounds as potential RyR1 inhibitors[53] (Figure 2B). Of these, oxolinic acid selectively inhibited RyR1 among the three RyR isoforms.

Oxolinic acid is a first-generation quinolone antibacterial drug that has been used to treat urinary tract infections with no major side effects[58]. Mori et al.[59] synthesized a series of modifications to oxolinic acid at the 1-N position and benzene ring to successfully develop Cpd1, which exhibited > 70-fold greater potency (half maximal effective concentration [$EC_{50}$] = 12 nM) than oxolinic acid ($EC_{50}$ = 810 nM) (Figure 2C). Cpd1 preserved RyR1

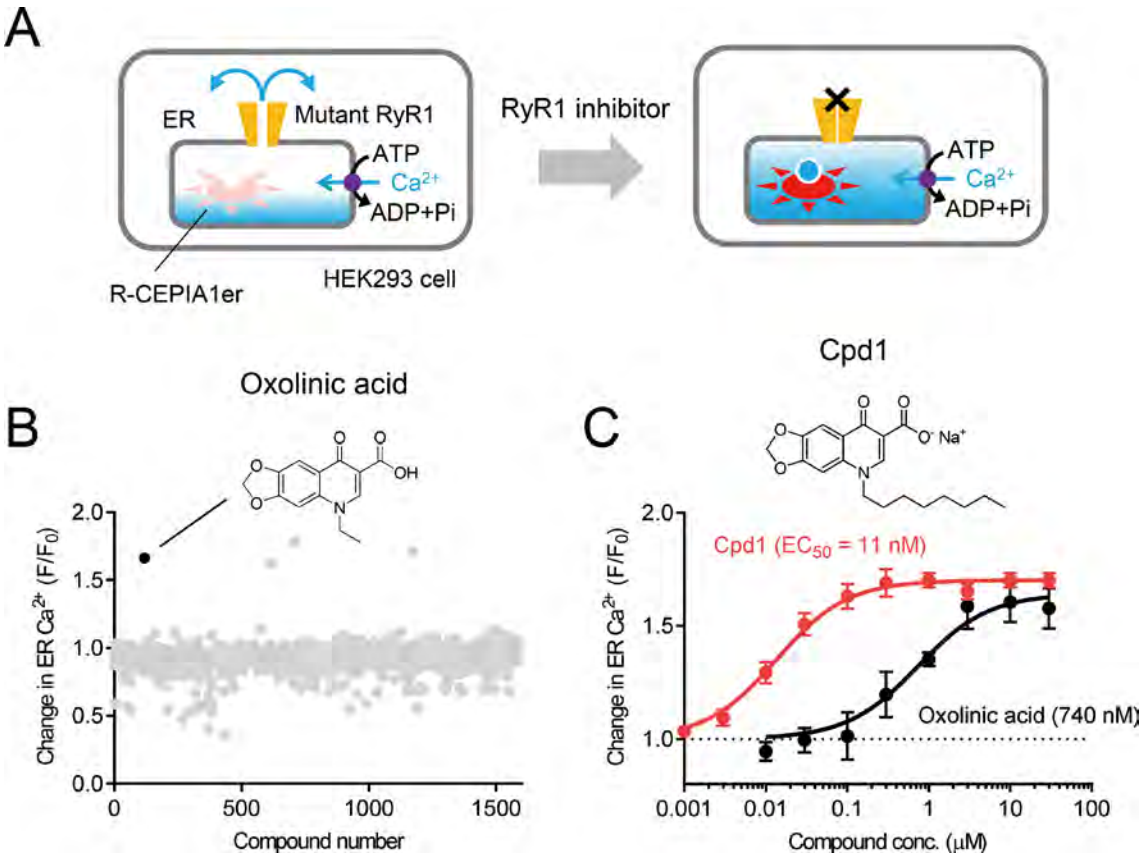


**Figure 2** High-throughput assays for identifying RyR1 inhibitors by endoplasmic reticulum $Ca^{2+}$ measurement (A) Schematic drawing of an endoplasmic reticulum (ER) $Ca^{2+}$-based assay. Stable HEK293 cells were generated that express the gain-of-function mutant, RyR1, and R-CEPIA1er, a genetically encoded ER $Ca^{2+}$ indicator. $Ca^{2+}$ leakage via mutant RyR1 channels causes a reduction in ER $Ca^{2+}$ content, therefore R-CEPIA1er fluorescence is low. RyR1 inhibitors prevent $Ca^{2+}$ leakage, which causes an increase in ER $Ca^{2+}$ content (via active transport through a $Ca^{2+}$ pump) and increases R-CEPIA1er fluorescence. (B) Typical results for a high-throughput assay for RyR1 inhibitors. Oxolinic acid was identified and shown to increase R-CEPIA1er fluorescence. (C) Development of Cpd1. Among many oxolinic acid derivatives, Cpd1 exhibited 70-fold greater potency than oxolinic acid. (This figure and its legend were modified from Murayama et al., Mol Pharmacol, 2018; 94: 722-730[53]; Murayama and Kurebayashi, Curr Protoc Pharmacol, 2019; 87: e71[54]; Mori et al., Eur J Med Chem, 2019; 179: 837-848[59]. The authors have permission to reproduce images from the copyright owner.)

selectivity among the three RyR isoforms. Ishida et al.[60] recently developed derivatives of oxolinic acid with greater water solubility.

Yamazawa et al.[61] tested the therapeutic effects of Cpd1 using multiple MH mouse models carrying different RyR1 mutations (R163C, G2434R, and R2509C). Cpd1 effectively prevented and treated fulminant MH crisis and death triggered by isoflurane anesthesia (Figure 3A, B). It has been shown that environmental heat stress causes a rise in body temperature and death in MH model mice[62–65]. Cpd1 effectively treated heat stroke and prevented death in MH mice[61]. Low water solubility[22] and a long plasma half-life[23] are disadvantage of dantrolene in clinical use. Cpd1 showed > 30-fold greater solubility in saline (845 μg/mL) than dantrolene (26 μg/mL) and much faster clearance *in vivo* ($t_{1/2}$ of ~10 min) compared with dantrolene (~10 h)[61] (Figure 3C, 3D). These findings suggest that Cpd1 has therapeutic effects *in vivo*, and certain advantages over dantrolene.

## Conclusions and future perspectives

Hyperactive RyR channels generated by genetic mutations or posttranslational modifications may cause various muscle and heart diseases. In addition to existing RyR inhibitors, novel RyR inhibitors are increasingly being identified by HTS approaches. Currently, two different methods are available, namely a FRET-based assay and ER $Ca^{2+}$-based assay. Several compounds identified by these approaches are effective not only *in vitro* but also *in vivo* in animal models, indicating they are promising approaches.

HTS typically aims to screen hundreds of thousands to millions of compounds. Miniaturizing the

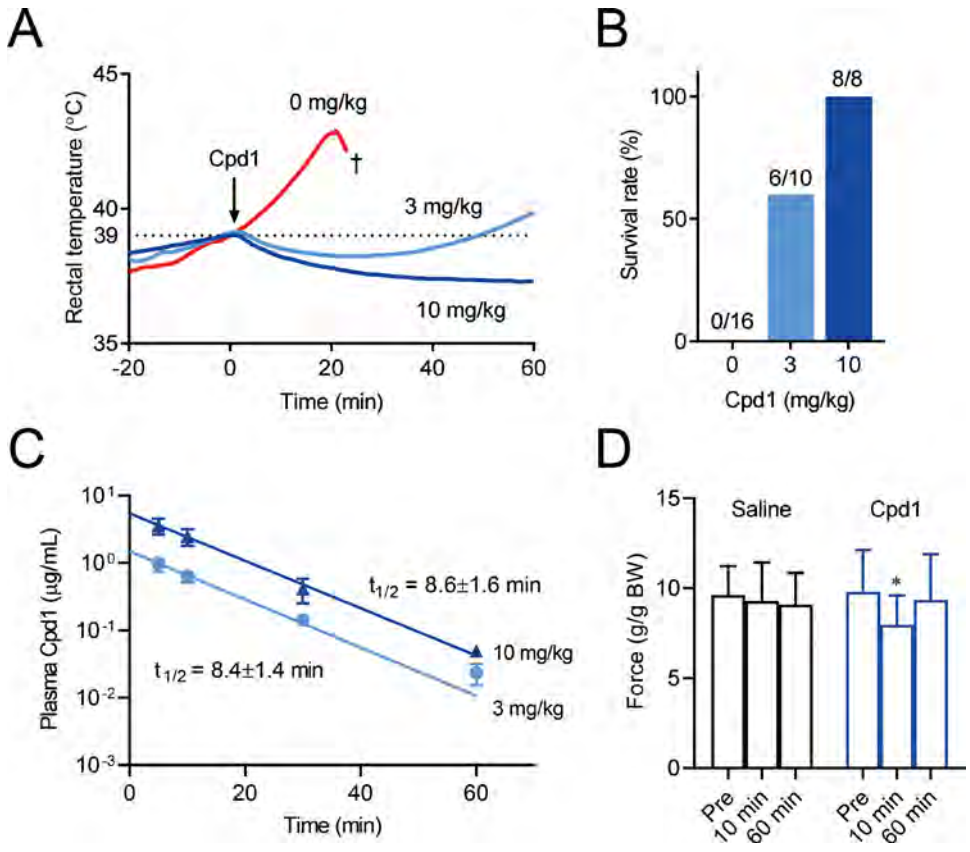


**Figure 3** Therapeutic effects of Cpd1 on malignant hyperthermia model mice
(A) Cpd1 was administered to malignant hyperthermia (MH) model mice during isoflurane-induced episodes of MH. Cpd1 reduced the body temperature of the mice. (B) Cpd1 improved survival rate in isoflurane-induced MH episodes. (C) Change in plasma Cpd1. Cpd1 rapidly declined from plasma with a half-life of ~10 min. (D) Cpd1 and muscle weakness in mice. Muscle weakness was observed at 10 min after administration of Cpd1, but disappeared by 60 min. (This figure and its legend were modified from Yamazawa T et al., Nat Commun, 2021; 12: 4293[61] under a CC BY license [Creative Commons Attribution 4.0 International license]).

screening format to 1536-well plates has been successful in a FRET-based assay[51] and is currently underway in an ER $Ca^{2+}$-based assay. These improvements will accelerate identification of good hit compounds.

Recent advances in structural biology using cryo-EM have revealed the detailed structure of RyR channels including binding sites for ligands or drugs at near-atomic resolution[30, 66]. Structure-based drug development could further improve drug affinity and selectivity, leading to the development of clinically useful, novel drugs in the near future.

RyR are also involved in the function of various tissues including brain, smooth muscles, and lymphocytes[3-5]. Since RyR inhibitors might act on RyR in these tissues to cause side effects, caution must be taken in clinical use.

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## Author contributions

HM and TM wrote the manuscript. Both authors approved the final manuscript.

## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Abstract

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# Highly Colistin-resistant *Aeromonas jandaei* from a Human Blood Sample

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*Aeromonas* species are Gram-negative rods known to cause infections such as gastroenteritis, bacteremia and wound infections. Colistin is one of few treatments for multidrug-resistant Gram-negative bacteria. However, colistin-resistant bacteria carrying the mobilized colistin resistance (*mcr*) gene are a threat in healthcare settings worldwide. In recent years, colistin-resistant *Aeromonas* species have been detected in environmental and clinical samples. We analyzed the genomic characteristics of one highly colistin-resistant *A. jandaei* isolated from a blood sample in Nepal, which harbored four novel *mcr-like* genes on its chromosome. Our study strongly suggests that *A. jandaei* is a reservoir of colistin-resistant genes. Inappropriate use of drugs in medicine and food production should be reduced and continued global surveillance for colistin-resistant bacteria is necessary.

***Key words***: *Aeromonas jandaei*, colistin resistance, phosphoethanolamine transferases, *mcr*, Gram-negative bacteria

## Taxonomy of the *Aeromonas* genus

The *Aeromonas* genus are Gram-negative, straight, rigid, nonsporeforming rods which belong to the family *Aeromonadaceae*. They are facultatively anaerobic and widely distributed in aquatic environments and food samples. They are frequently isolated from drinking water, wastewater, seawater, livestock, vegetables, seafood and fish[1)]. Although not generally considered marine organisms, they grow naturally in marine systems in contact with freshwater and are found at all salinities except extreme[2)]. The etymology of the word "*Aeromonas*" is that "*Aero*" means "gas(-producing)" and "*monas*" means "*monad*." The word "monad" is derived from the Greek word "monos," meaning "one." Type species of the genus is *A. hydrophila*, and "*hydrophila*" means "water-loving." Historically, species in this genus were classified in the family *Pseudomonadaceae* or the family Vibrionaceae[3-4)]. After the analysis of 16S ribosomal RNA of the *Ganmamaproteobacteria*, the *Aeromonas* genus became independent from the family *Vibrionaceae* and the family *Aeromonadaceae* was founded in 1992[5)].

A Phylogenetic dendrogram of the major type strains of the family *Aeromonadaceae* and clinically important Gram-negative rods is shown (Figure 1).

## *Aeromonas* species as human pathogens

*Aeromonas* species were initially known as fish pathogens. For example, *Aeromonas salmonicida*, discovered in the 19th century, means "salmon-killer." In the family *Vibrionaceae*, of which *Aeromonas* species were once classified, *Vibrio cholae*

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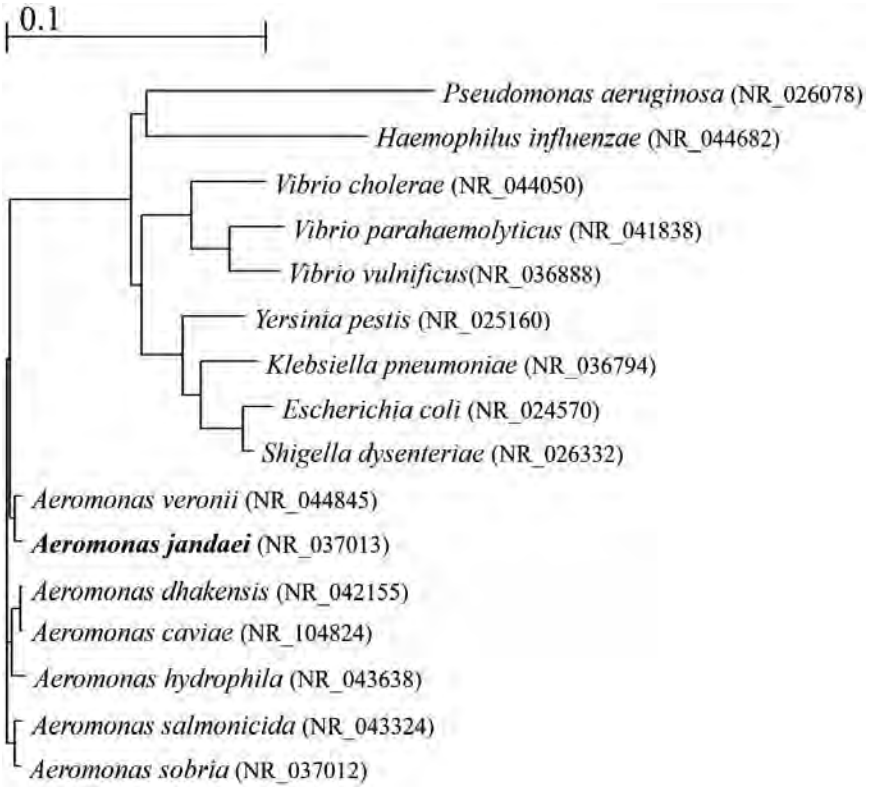


**Figure 1** Phylogenetic dendrogram of 16S rRNA of the Gram-negative rods including the family *Aeromonadaceae*. This Figure is created using the CLUSTAL OMEGA program (https://www.ebi.ac.uk/ Tools/msa/clustalo/). GenBank accession numbers were indicated in parenthesis.

and *Vibrio parahaemmolyticus* cause gastrointestinal symptoms, and *Vibrio vulnificus* causes wound infections, especially necrotizing soft tissue infection. Similarly, 19 of 36 *Aeromonas* species are pathogenic to humans, causing a broad spectrum of infections including gastroenteritis, bacteremia, and wound infections[6]. *A. jandaei*, named after *J. Michael Janda*, was originally isolated from clinical samples including blood, wounds, and stools in the USA in 1991[7].

## Emergence of mobilized colistin resistance (MCR)

The emergence of bacterial antimicrobial resistance (AMR) is a widespread problem. According to a report by the UK government, 10 million people could be killed by AMR every year[8]. In this context, colistin is once again gaining attention as a "last resort" antimicrobial agent against infections with multidrug-resistant Gram-negative bacteria including *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, by interacting with lipid A to disrupt the outer membrane of Gram-negative bacteria[9].

Colistin was discovered from *Bacillus polymyxa* var. *colistinus*, which was from soil of Fukushima, Japan in 1947 and approved by the U.S. Food and Drug Administration (FDA) in 1959[10]. However, due to its kidney toxicity, colistin has long been restricted for use in humans. On the other hand, it is used in livestock. As a result, colistin resistant *Escherichia coli* and *Klebsiella pneumoniae* encoding the mobilized colistin resistance gene *mcr-1* on a plasmid were discovered in livestock and humans in China in 2015[11]. To date, the *mcr* genes have spread across five continents and variants *mcr-1* to *mcr-10* have been found in *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Salmonella* spp., *Citrobacter* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Kluyvera* spp., *Aeromonas* spp., *Providencia* spp., and *Raulotella* spp.[12-13]. Transmission of *mcr*-positive bacteria may occur by contact with reservoirs of *mcr*, ingestion of products associated with contaminated animals or plants, and international food trade and travel[13]. A meta-analysis of colistin-resistant *E. coli* estimated *mcr* prevalence rates of

15.8%, 14.9%, 7.4%, and 4.2% among chickens, pigs, healthy humans, and clinical isolates, respectively[14]. In Southeast Asia, colistin use as an antibiotic in livestock was so routine that in one rural village survey in Vietnam in 2017, 29 of 36 households tested had colistin-resistant *E. coli* harboring *mcr-1* or *mcr-3*[15].

## Emergence of colistin-resistant *Aeromonas jandaei*

Previous studies have reported that *mcr* related genes were detected in environments, animals, and clinical samples of *Aeromonas* spp. including *A. allosaccharophila*, *A. bivalvium*, *A. caviae*, *A. hydrophila*, *A. jandaei*, *Aeromonas media*, *A. salmonicida* and *A. veronii*[16-20]. According to the U.S. Department of Agriculture (USDA) study of *mcr* prevalence in 2018, 15 of 5,169 samples were positive for *mcr*: one for *E. coli*, nine for *A. hydrophila*, and five for *A. jandaei*. Of these, all the *Aeromonas* species harbored *mcr-3-like* genes, but only *A. jandaei* harbored *mcr-7-like* gene[16]. An *A. jandaei* strain isolated from retail fish in China harbored two genes encoding phosphoethanolamine transferase *eptAv3* and *eptAv7* similar to *mcr-3* and *mcr-7*, respectively[21]. These findings suggest that *Aeromonas* spp. was a reservoir of colistin-resistant Gram-negative bacteria.

## Colistin-resistant clinical isolates of *Aeromonas jandaei* in Nepal

We obtained *A. jandaei* strain JUNP479 (Genbank accession number: AP024466) isolated from a blood sample obtained from an inpatient at Tribhuvan University Teaching Hospital, Nepal, in October 2019[22].This strain was resistant to ceftriaxone (MIC 8 mg/L) and intermediate resistant to imipenem (MIC 2 mg/L), but was susceptible to ceftazidime (MIC 0.5 mg/L), meropenem (MIC ≤ 0.25 mg/L), aztreonam (MIC ≤ 0.25 mg/L), amikacin (MIC 4 mg/L), gentamicin (MIC ≤ 0.25 mg/L), ciprofloxacin (MIC ≤ 0.25 mg/L), and tetracycline (MIC 1 mg/L). The MICs of colistin and tigecycline were 128 mg/L and 64 mg/L, respectively[22].

## Four novel *mcr-like* genes of *A. jandaei* JUNP479

Whole genome sequencing revealed that *A. jandaei* JUNP479 has a chromosome size of 4,534,922 bp, with 49.93% GC content and a plasmid size of 6,224 bp. This highly colistin-resistant clinical isolate encoded a class C β-lactamase $bla_{FOX}$, and four novel variants of genes encoding phosphoethanolamine lipid A transferases, designated *eptAv3.2*, *eptAv3.3*, *eptAv3.4*, and *eptAv7.2*. The amino acid sequences of EptAv3.2, EptAv3.3, and EptAv3.4 were 80.7%, 95.7%, and 84.7% identical to that of MCR-3.1, respectively, and 98.1%, 80.4%, and 84.0% identical to that of EptAv3, respectively. The amino acid sequence of EptAv7.2 was 79.9%, and 97.6% identical to the sequences of MCR-7.1 and EptAv7, respectively. All four genes encoding phosphoethanolamine transferases were located on the chromosome, with *eptAv3*.3 and *eptAv3.4* forming a tandem structure. The genomic environments surrounding *eptAv3.2*, *eptAv3.3*, and *eptAv3.4* in *A. jandaei* JUNP479 were similar to those in *A. veronii* WP2-S18-CRE-03 (AP021940), whereas that surrounding *eptAv7.2* in *A. jandaei* JUNP479 was similar to that in *A. jandaei* 3348 (CP043321)[22] (Figure 2). The mobile elements including transposases and insertion sequences were not detected in the 40,000 bp around the *mcr-like* genes on the chromosome of *A. jandaei* JUNP479. The *mcr-like* genes were introduced into plasmid pHSG398 (Takara Bio, Shiga, Japan) and cloned into *E. coli* DH5α (Takara Bio, Shiga, Japan) and *A. hydrophila* IOMTU903. *E. coli* transformants with *eptAv3.2*, *3.3*, or *3.4*, and in *A. hydrophila* transformants with *eptAv-7.2* increased colistin resistance (Table 1). Real-time PCR showed that the expressions of *eptAv3.2*, *3.3*, and *3.4* in *E. coli* transformants, and the expression of *eptAv7.2* in *A. hydrophila* transformants were increased[22]. Phylogenetic analysis revealed that MCR-3.1 in *E. coli* (NG_055505) showed close phylogenetic distance to MCR-3 family and EptAv3.3 in *Aeromonas* spp., whereas MCR-7.1 in *Klebsiella pneumoniae* (NG_056413) showed considerable phylogenetic distance from MCR-7 family and EptAv7 variants in *Aeromonas spp*[22] (Figure 3).

## Discussion and Conclusion

This is the first report of a highly colistin-resistant *A. jandaei* JUNP479 with four novel genes encoding MCR-like phosphoethanolamine transferases isolated in a medical setting in Nepal. Infections caused by *A. jandaei* are less common than those caused *A. caviae*, *A. veronii*, *A. hydrophila*

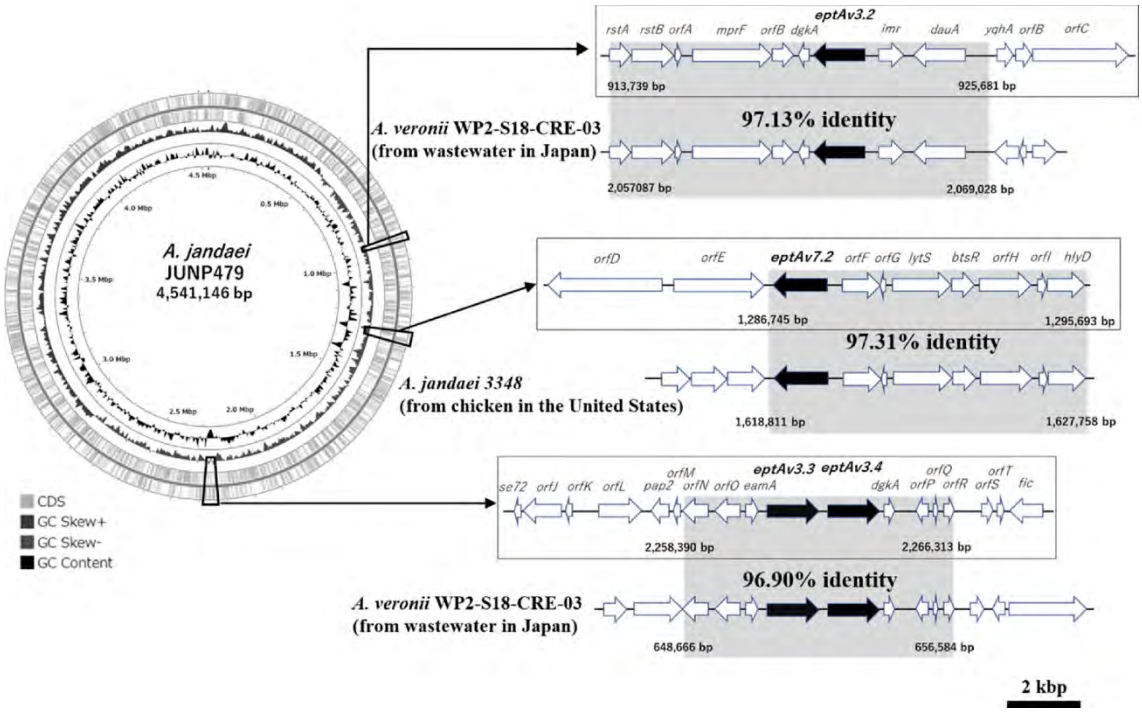


**Figure 2** Genomic environments surrounding *eptAv3.2*, *eptAv3.3*, *eptAv3.4* and *eptAv7.2* in *Aeromonas jandaei* JUNP479.All four genes encoding phosphoethanolamine transferases were contained in the chromosome. The genomic environments surrounding *eptAv3.2*, *eptAv3.3* and *eptAv3.4* were similar to those in *Aeromonas veronii* (*A. veronii*) WP2-S18-CRE-03 (accession no. AP021940), whereas that surrounding *eptAv7.2* was similar to that in *Aeromonas jandaei* (*A. jandaei*) 3348 (CP043321)[22].

**Table 1** Colistin susceptibility profiles of *E. coli* and *A. hydrophila* transformants[22]

| | MIC(mg/L) | |
|---|---|---|
| plasmid(s) | *E. coli* DH5α | *A. hydrophila* |
| pHSG398 | 0.125 | 0.063 |
| pHSG398/*eptAv3.2* | 2 | 0.063 |
| pHSG398/*eptAv3.3* | 1 | 0.063 |
| pHSG398/*eptAv3.4* | 0.5 | 0.063 |
| pHSG398/*eptAv7.2* | 0.125 | 4 |

and *A. dhakensis* in clinical situations[8–9]. However, when the genomic environments were compared in this study, it turned out that other *Aeromonas* spp. also harbored colistin-resistant genes. Moreover, *mcr-3-like* genes are also commonly found in *Aeromonas* spp. and *E. coli*, suggesting the *mcr* genes may transmit between *Aeromonas* spp. and *Enterobacteriaceae*. On the other hand, *mcr-7-like* genes will mainly spread among *Aeromonas* spp. Our study strongly suggests that *Aeromonas* spp. is a reservoir of colistin-resistant genes, including *mcr-3-like* genes and *mcr-7-like* genes. *Aeromonas* spp. have the possibility to become a threat in healthcare settings, therefore, it is necessary to continue global surveillance of colistin-resistant *Aeromonas* spp. If we humans continue to routinely use colistin in livestock, agriculture, aquaculture, and medicine, we could lose our "last defense" against multidrug-resistant Gram-negative bacteria.

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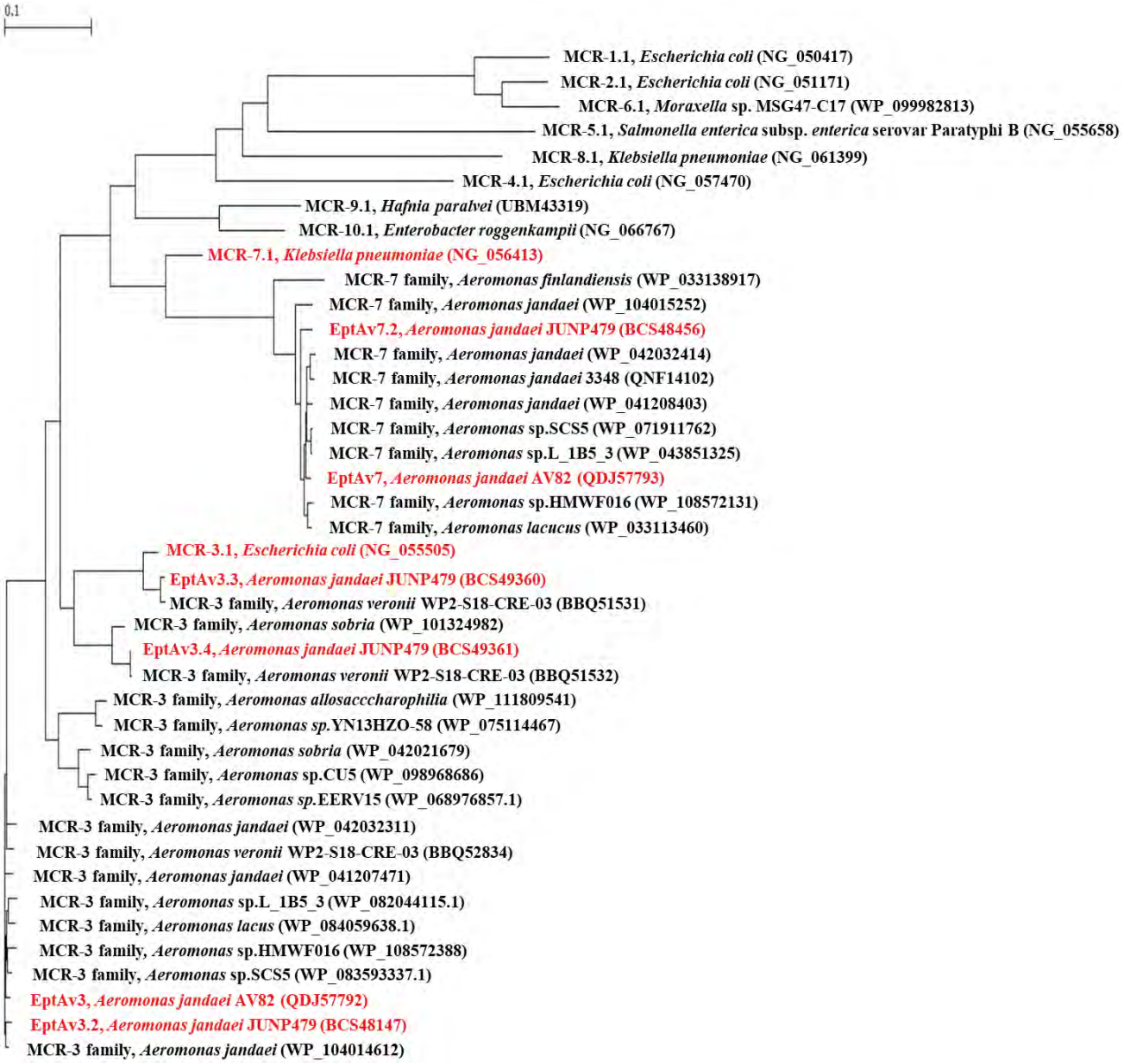


**Figure 3** Phylogenetic dendrogram of MCR and EptAv variants. MCR-3.1, MCR-7.1 and EptAv variants were indicated in red. GenBank accession numbers were indicated in parenthesis[22].

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## Author contributions

TKo summarized the data and drafted the manuscript. SS and JaBS collected samples and analyzed the data. MT and TH performed sequencing and analyzed the data. JeBS, TT and TKi designed the study and supervised the manuscript. All authors read and approved the final manuscript.

## Conflicts of interest statement

No conflict of interest.

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Abstract

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# Salivary Alpha-amylase Activity and Mild Cognitive Impairment Among Japanese Older Adults: The Toon Health Study

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***Key words***: salivary alpha-amylase, mild cognitive impairment, psychological stress, cross-sectional study

## Commentary

Dementia is an important global issue, and early detection and intervention are critical. There is growing interest in identifying mild cognitive impairment (MCI) and intervening in patients' lifestyle-related behaviors in clinical and community settings to prevent the progression of dementia[1–3]. Previous studies have shown associations between psychological stress and cognitive decline[4], increased risk of developing MCI[5], and dementia[6]. One of the human stress-response systems is the sympathetic-adrenal-medullary (SAM) axis[7, 8]. Salivary alpha-amylase (sAA) is a biomarker of psychological stress, as it indicates activation of the SAM axis[9]. Psychological stress is thought to increase $\beta$-adrenergic activity via activation of the SAM axis, which leads to an increase in sAA levels[9].

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Previous studies have further shown that elevated β-adrenergic activity leads to amyloid-β (Aβ) peptide production[10, 11], and Aβ peptide production and deposition play an important role in the pathogenesis of Alzheimer's disease (AD)[12]. We therefore hypothesized that sAA, an objective marker of the SAM axis, is associated with MCI via β-adrenergic activity, which would suggest that psychological stress contributes to cognitive decline. We conducted a large cross-sectional study to investigate this association in the elderly.

This cross-sectional study was a part of the Toon Health Study and our analysis involved 865 participants aged ≥ 65 years. Saliva samples were collected in the morning and the levels of salivary alpha-amylase were assayed. We evaluated MCI using the Japanese version of the Montreal Cognitive Assessment: a score of < 26 indicated MCI. In the statistical analysis, a multivariable-adjusted logistic regression analysis using sex-specific quartiles of sAA was performed to calculate the odds ratio (OR) and 95% confidence interval (CI) of MCI after adjusting for age, sex, drinking status, smoking status, body mass index, hypertension, diabetes mellitus, physical activity, education, social support, social network, and heart rate variability.

We found that sAA was significantly associated with MCI. The age- and sex-adjusted OR (95% CI) of MCI for the highest quartile group compared to the lowest was 1.56 (1.05-2.32). As shown in Figure 1, this significant association remained after adjusting for confounding factors. Moreover, the multivariable-adjusted OR (95% CI) for the 1-standard deviation increment of log-transformed sAA was 1.24 (1.07-1.44).

In summary, we found a significant association between sAA levels and MCI in elderly Japanese community dwellers. Our results are strongly consistent with previous studies that found associations between high levels of self-reported psychological distress and cognitive impairment[4] and dementia[6]. Further, our results suggest that, since sAA is an objective marker of psychological stress, this stress contributes to cognitive decline. See the full article for further details: Yamane N. et al. Salivary Alpha-Amylase Activity and Mild Cognitive Impairment among Japanese Older Adults: The Toon Health Study. J Prev Alzheimers Dis 9, 752-757 (2022). https://doi.org/10.14283/jpad.2022.51.

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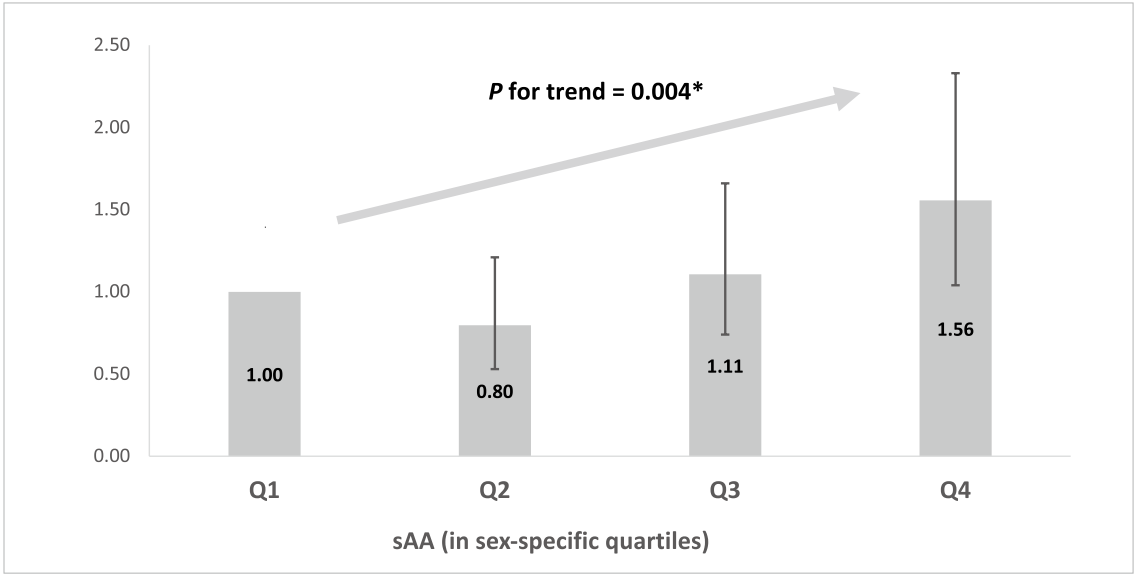


**Figure 1** Association between MCI and sAA
*P for trend associated 1SD increment of log-transformed sAA. Abbreviations: MCI, mild cognitive impairment; sAA, salivary alpha-amylase.

JP19K10670, and 25293142, and the 8020 Promotion Foundation, Japan in 2016 and 2017. The funders had no role in designing or conducting the study; in collecting, analyzing, or interpreting data; in preparing the manuscript; or in reviewing or approving the manuscript.

## Author contributions

TT and AI contributed to the study design. NY and AI analyzed and interpreted the data, and NY was the major contributor to writing the manuscript. All authors read and approved the final manuscript.

## Conflicts of interest statement

Dr. Ikeda reports grants from JSPS KAKENHI received during the study period. Dr. Saito reports grants received from the 8020 Promotion Foundation during the study period. Dr. Tanigawa reports grants received from JSPS KAKENHI during the study period. The remaining authors have nothing to disclose.

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Reviews

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# The Practice of Online Medical Care at Juntendo Hospital in Response to the Coronavirus Pandemic

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The numbers of coronavirus (COVID-19) infections have exploded in Japan since mid-March 2020, making it difficult for outpatients to visit our hospital (Juntendo Hospital in Tokyo). For this reason, the hospital expanded the use of online medical care in May 2020 to ensure uninterrupted medication treatment for outpatients who could not attend in person. Although the number of outpatient visits in person was reduced, patients were still able to consult our clinic and receive their medication through online medical care via audio–video systems. This paper discusses the background to this situation, as well as the guidelines, the medical fee system, and the advantages and disadvantages of online medical care in Japan.

***Key words***: coronavirus pandemic, remote medical care, online medical consultation recommendation, preclinical consultation

The use of online medical care has widely expanded nationwide in response to the coronavirus pandemic. Juntendo Hospital is a general hospital affiliated with a private university and located near Tokyo Station, which is convenient not only for patients in the neighborhood but also for those in rural areas. The hospital has 1,051 beds and approximately 3,700 outpatients per day. Our hospital had been providing online medical care prior to the pandemic, but the number of patients was limited, and the recent pandemic prompted the hospital to begin a full-scale operation of online medical care. This paper reviews the implementation of online medical care at our hospital, with reference to the various laws, guidelines, and medical fee systems surrounding online medical care in Japan.

## Guidelines for the appropriate implementation of online medical treatment

### Environment surrounding online medical care

Information and telecommunication devices have made great advances in recent years, and their use has spread rapidly in Japan. The relationship between medical care through the use of these devices and Article 20 of the *Medical Practitioners Act* (Act No. 201 of 1948), which prohibits medical treatment without examination, was clarified in a 1997 notice, "Medical examinations applying ICT [Information and Communications Technology]," issued by the Ministry of Health, Labour and Welfare (MHLW). This has made it possible to provide medical care by using modern telecommunications devices (e.g., online medical care).

In addition, from the perspective of information

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security and other issues when medical information is handled electronically, *Security Guidelines for Medical Information Systems* were published by the MHLW in 2005 and have been revised several times. The use of telecommunications devices in medical practice can reform the way in which physicians work and help to overcome the uneven distribution of physicians in Japan. *Guidelines for the Appropriate Implementation of Online Medical Treatment* were established by the MHLW in March 2018. They were partly revised in January 2022 to clarify minimum requirements, recommendations, and concepts, and to promote the implementation of online medical care with which physicians, patients, and related parties can feel comfortable in terms of safety, necessity, and effectiveness.

In a further interpretation of the Medical Practitioners Act, the "Medical examinations applying ICT" notice was revised in 2003 and 2011. Article 1-2 of the *Medical Care Act* (Act No. 205 of 1948) stipulates that medical care must be provided by hospitals, clinics, long-term healthcare facilities, dispensing pharmacies, and other facilities that provide medical care (hereinafter referred to as "medical institutions") and in the homes of medical care recipients (meaning a home or "other place" as specified by an Order of the MHLW). The Enforcement Regulations on the *Medical Care Act* (Order of the Ministry of Health and Welfare No. 50 of 1948) stipulate that the "other places" prescribed by Order of the MHLW, as provided in Article 1-2, paragraph 2, of the Act, are nursing homes for the elderly, intensive care homes for the elderly, low-cost homes for the elderly, fee-based homes for the elderly, and places where medical care recipients can live with medical treatment.

## Definitions of terms used in the guidelines for the appropriate implementation of online medical treatment, and scope of the guidelines

This section defines the terms related to online medical care.

**Remote medical care**

Remote medical care is an umbrella term that refers to health-promoting medical care via telecommunications devices. This includes telehealth medical consultations that can be performed by non-doctor, such as giving general information on common diseases and symptoms.

**Online medical care**

As a part of remote medical care, online medical care refers to the act of examining and diagnosing patients and communicating the results of diagnoses, and prescriptions, in real time from physician to patient via telecommunications devices.

**Online medical consultation recommendation**

An online medical consultation recommendation is a form of remote medical care in which a physician examines a patient via telecommunications devices and recommends in real time that the patient visit a medical institution. It involves the minimum medical judgment appropriate for the individual patient's physical and mental condition. This includes determining and recording the name of the suspected disease on the basis of the patient's signs and symptoms; collecting information on the patient's physical and mental condition, such as by interview; and selecting the appropriate department to be consulted. Follow-up and non-clinical recommendations, including home treatment with over-the-counter drugs, can also be implemented. With such a recommendation, the patient is not informed of their diagnosis or prescribed drugs.

**Preclinical consultation**

A preclinical consultation is an act of confirming a patient's symptoms and medical information via real-time exchange between the physician and patient via an audio-video system. This may occur when a physician other than the physician who already has a direct relationship with the patient (e.g., a family doctor through regular face-to-face visits) intends to practice online medical care from the first online consultation onward (except in cases where the physician already has enough medical information on the patient).

Online medical care will be followed when appropriate information can be obtained and both the physician and the patient mutually agree that such care is feasible.

**Coverage of online medical care**

Figure 1 illustrates the relationships among remote medical care, online medical care, online medical consultation recommendation, and telehealth medical

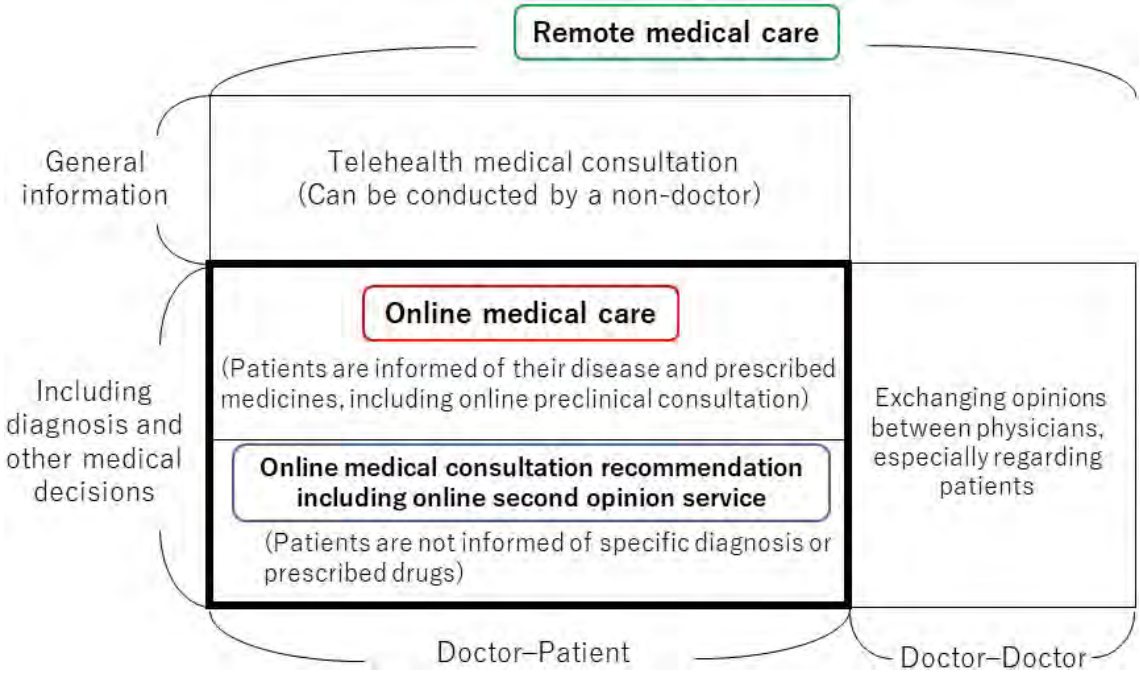


**Figure 1** Relationship between online medical care, online medical consultation recommendation, and remote medical care

consultation. The *Guidelines for the Appropriate Implementation of Online Medical Treatment* cover the online medical care and online medical consultation recommendation shown in this figure. They do not include remote medical care in the form of telehealth medical consultation. The doctor–doctor relationship in the figure refers to the exchange of opinions between physicians, especially regarding patients, which is not included in online medical care.

## Online medical care at our hospital

This section describes the practice of online medical care at our hospital.

### Steps to take before starting online medical care

Ambulatory care physicians first take e-learning courses on online medical care. Thereafter, they practice online medical care while referring to the *Guide to Online Clinical Practice in Primary Care*[1] (v. 1.0, released on 20 May 2020). Online medical care is a medical practice that uses telecommunications devices with video communication functions and is quite different from telephone medical care.

Physicians next refer to *Guidance for Primary Care Initial Clinical Practice in Clinics and Hospitals for Coronavirus (COVID-19) Infections*[2] (v. 3.0 released on 7 November 2020; v. 2.0 released on 30 April 2020). There are two types of online medical care: "online medical care in normal times" and "online medical care as a provisional measure". The provisional measure, declared by MHLW as an emergency response to the rapid spread of the coronavirus, started in April 2020 and lasted until March 31, 2022. In accordance with the MHLW policy, our hospital also started implementing the provisional measure in April 2020 and expanded its use from May 2020. During this period, online medical care was provided, and medical fees were calculated on the basis of the provisional measure.

Five conditions must be considered to determine whether online medical care is appropriate in outpatient settings:[1] (1) whether or not a doctor–patient relationship has been established; (2) whether or not an online medical care provider is available; (3) whether or not the patient is registered with a medical institution; (4) whether it is a first visit or a follow-up visit in terms of medical fees; and (5) whether the patient's symptoms are acute or chronic, and mild or severe. The primary care physician should refer to the *Guide to Online Clinical Practice in Primary Care* to determine which cases are easy to treat appropriately online and which cases should be carefully considered and therefore should be treated in person.

**Implementing online medical care**

The ambulatory care physician first determines whether the patient is eligible for online medical care. The physician then contacts the hospital's Online Medical Care Support Department to coordinate the examination. The physician and the Support Department set the day and time of the online medical examination. Specifically, the Support Department contacts the patient before the day of the examination to set up the examination. We have implemented online medical care via Zoom (Zoom Video Communications Japan, https://explore.zoom.us/ja/about/). The advantages of Zoom are: (1) it is free of charge within 40 minutes whereas using an existing online medical care system would cost a lot of money; (2) Easy to set up; (3) Easy to operate; (4) Security updates are frequent. On the contrary, the drawback of Zoom is that if the meeting ID and password are leaked to outsiders, others can access the meeting. Considering the above, the following security measures are taken when using Zoom: (1) Always use the latest version of the software by updating; (2) Zoom connection is checked in advance; (3) Meeting IDs and passwords are sent directly to patients by e-mail. On the day of the examination, the patient visits the Support Department remotely to see the physician. Patient reception, Zoom setup and management, and payment are done by the Support Department. Figure 2 shows the numbers of online medical care patients at our hospital from April 2020 to April 2022. Although the number of patients was small at the beginning, as of April 2022, approximately 200 patients were receiving online medical care per month. In addition to online medical care, an online second opinion service was started in August 2020. About three or four online second opinions are provided per month.

**Fees for online medical care**

Consultation fees for online medical care were in line with those for online medical care in normal times until April 2020, but thereafter provisional special measures were put in place and continued for 2 years (until 31 March 2022) (Table 1). No fee was set for the first visit in normal times, but in April 2020, under the provisional measure, a fee of 214 points was approved. Note that one point is worth 10 yen. The fee was increased to 251 points in April 2022. The fee for follow-up online consultations has remained more or less the same. In addition, initially, in the provisional measure period, the fee for online treatment of specific diseases was approximately 1.5 times that in normal times, but in April 2022 it was decreased to 87% of the face-to-face consultation fee for specific diseases, thus

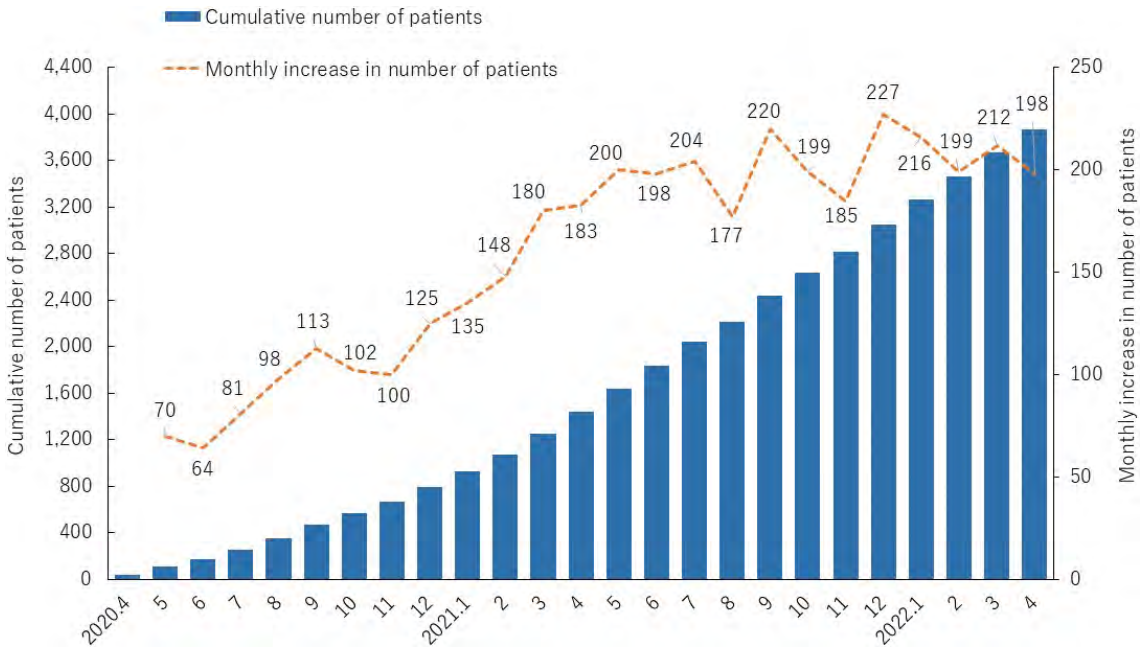


**Figure 2** Numbers of online medical care patients at our hospital from April 2020 to April 2022.The bar and line graphs show the cumulative number of patients and the number of patients for each month, respectively.

**Table 1** Fees for online medical care

| | | Normal times (until April 2020) | Provisional special measures (from April 2020 to end March 2022) | From April 2022 |
|---|---|---|---|---|
| Medical fees | First visit | – | 214 points | 251 points |
| | Follow-up visit | 71 points | 74 points | 73 points |
| | Medical treatment of specific diseases | 100 points | 147 points | 87% of the face-to-face consultation fee |
| Patient requirements | | Deemed eligible by physician on the basis of the *Guidelines for the Appropriate Implementation of Online Medical Treatment*, including patients with intractable diseases or chronic headache. | Deemed eligible on the basis of the revised Guidelines for the *Appropriate Implementation of Online Medical Treatment* (prescribing restrictions are imposed on a patient's first visit). | |
| E-learning requirements for physicians | | Mandatory | Deferred | Mandatory |

If the health care service concerned is covered by health insurance, patients must undergo face-to-face medical examination once every 3 months both in normal times and in provisional measures. One point is worth 10 yen.

further increasing the number of patients eligible for online medical care. There are many types of fee for specific diseases. For example, the fee for outpatient guidance and management of intractable diseases is 270 points for face-to-face consultation, but decreases to 235 points for online medical care. Initially, in normal times, patients eligible for online medical care were limited to those whose physicians considered them eligible on the basis of the *Guidelines for the Appropriate Implementation of Online Medical Treatment*, including patients with intractable diseases or chronic headaches. However, in April 2020, patient requirements have been eased merely to those whose physicians considered them eligible on the basis of the Guidelines, and this expanded definition has continued since April 2022. However, prescribing restrictions are imposed on a patient's first visit. For physicians, e-learning was mandatory for online medical care in normal times, but during the provisional measure it was deferred. E-learning again became mandatory in April 2022.

Online medical care fees for the first visit are shown in Table 1. In addition to the fee for the first visit, a system utilization fee, determined at the discretion of each hospital, can be charged, and prescriptions can be made at the time of the first visit. However, narcotics, psychotropics, and high-risk drugs cannot be prescribed to patients whose underlying medical conditions are not known, and prescriptions for more than 8 days' supply cannot be made. Our hospital collects fees for both first and follow-up visits through a postpaid credit card service and a medication delivery service, thus reducing the burden on patients by not requiring them to come in.

## Advantages and disadvantages of online medical care

### Advantages of online medical care

The primary advantage of online medical care is that patients can receive medical care without coming to the hospital. This saves time and effort. For example, the time required to travel back and forth between the medical institution and the patient's home or office can be saved, especially if the patient is visiting from a remote location, and the cost of transportation to and from the hospital can be greatly reduced.

Second, it saves time spent waiting for payments. Our hospital has been able to save even more time by using a postpaid credit card service, a medication delivery service, and a system called "walk-through examinations". Walk-through examinations can further reduce waiting time by allowing patients to be examined on a day different from the day of their consultation resulting in less waiting time than usual. Online medical care also makes it possible for patients to visit the hospital in between working hours and allows them to see the doctor in

a place where their privacy can be protected.

Another advantage is that the patient can relax during the examination. In a hospital, patients may get nervous or excited in an unfamiliar place, but at home, they can relax when receiving treatment. In addition, there is no risk of infection at home.

Furthermore, the fees for online medical care are inexpensive, and patients have the advantage of paying less than they would in an actual face-to-face visit.

**Disadvantages of online medical care**

Compared with face-to-face consultations, the first disadvantage of online medical care is that it is difficult to exchange information during the consultation. Visual examination and questioning are the primary focus of the consultation, and palpation and auscultation cannot be performed directly by the physician. However, nowadays, it may be possible for patients to communicate their physical information to physicians via various devices. Another disadvantage is that the patient must first visit a medical institution where online medical care is to be performed or a nearby medical institution for, for example, clinical examination, imaging, or blood collection. This means that if online medical care is to be performed while the physician is viewing the data, the examination results must be available in advance. Regardless, the patient must visit the relevant medical institution in person.

## Concluding remarks

As mentioned above, our hospital's online medical care has developed rapidly in response to the coronavirus outbreak, and at the time of writing (December 2022) it is being implemented stably. Patient satisfaction is high, and physicians have become accustomed to online medical consultations and are thus able to provide effective care. However, online medical care alone is not always sufficient, and it is important to provide face-to-face consultations on a regular basis whenever possible. Although online medical care will become increasingly useful with the development of information technology, combining it with regular face-to-face consultations is desirable so that emergency lesions and serious diseases not be overlooked.

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## Author contributions

RK read and approved the final manuscript.

## Conflicts of interest statement

The author declares that there are no conflicts of interest.

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Original Articles

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# Concomitant Septal Myectomy with Aortic Valve Replacement for Severe Aortic Stenosis with Left Ventricular Outflow Tract Obstruction

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***Objectives***: Septal myectomy confers survival benefits on patients with hypertrophic cardiomyopathy. However, its role in the treatment of severe aortic stenosis (sAS) with left ventricular outflow tract obstruction (LVOTO) remains under investigation. Another challenging question in the era of transcatheter aortic valve replacement is who would benefit more from traditional surgical aortic valve replacement (SAVR) with myectomy. Therefore, this study aimed to investigate myectomy cases at our hospital in Japan.

***Methods***: A total of 740 patients who underwent SAVR for sAS between 2012 and 2019 were identified. The demographics and baseline echocardiographic findings were retrospectively compared between patients who underwent concomitant myectomy and those who did not. The myectomy group was further assessed for factors predisposing to LVOTO, operative details, echocardiographic changes, and prognosis. The resected septa were histopathologically analyzed.

***Results***: The myectomy group mostly comprised elderly females with a small hypercontractile heart. Myectomy with SAVR led to statistically significant improvements in concentric left ventricular hypertrophy and LVOTO parameters. Survival was comparable with that reported in previous reports, even in the elderly subset (≥ 75 years). The septa showed mild fibrosis.

***Conclusions***: Myectomy can be safely performed with SAVR for sAS with LVOTO, even in the elderly, and it effectively improves LVOTO. Special attention should be paid to elderly females with relatively more severe AS and a small yet extra-hypertrophic and extra-hypercontractile heart. Such patients warrant comprehensive assessment of LVOTO, and despite its invasiveness, SAVR may be potentially more beneficial by allowing direct observation of LVOTO and ancillary myectomy.

***Key words***: left ventricular outflow tract obstruction, aortic stenosis, myectomy, aortic valve replacement, hypertrophic cardiomyopathy

## Introduction

Septal myectomy is an open-heart procedure wherein part of the interventricular septum (IVS) is removed when it is associated with left ventricular outflow tract (LVOT) obstruction (LVOTO)[1]. Classically, LVOTO accompanies the obstructive subtype of hypertrophic cardiomyopathy (HCM)[2]. Therefore, myectomy has been performed on patients with HCM and recommended as the gold standard treatment for them, as it improves long-term survival[3, 4]. More recently, LVOTO has been recognized as a manifestation of a wider array of disease entities such as aortic stenosis (AS), left ventricular hypertrophy (LVH), asymmetric septal hypertrophy (ASH), and systolic anterior motion of the mitral valve (SAM)[5, 6]. It has also been reportedly associated with various conditions of acutely reduced pre- or afterload under severely hyper- or hypokinetic left ventricle (LV)[7, 8] and may appear as treatment-resistant shock that paradoxically worsens upon ionotropic support[9, 10].

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These findings may indicate that more conditions may reap the benefits of septal reduction therapy.

Just as myectomy is recommended for patients with LVOTO, aortic valve replacement (AVR) has been recommended as the gold standard treatment for patients with severe AS (sAS)[11] because it improves long-term survival[12]. Historically, myectomy has been concomitantly performed with surgical AVR (SAVR) on patients with sAS who are at risk of LVOTO[13,14], as latent LVOTO that has been masked by sAS in a state of dual obstruction of LV can be unmasked after AVR, or LVOTO can develop *de novo*[9,10]. For example, with the increasing use of transcatheter AVR (TAVR) for sAS, more studies have reported sudden incidence and worsening of LVOTO immediately after TAVR in an event called "suicide LV[15,16]," which may have been prevented if risks were known beforehand.

Some authors have suggested that intraoperative decisions for myectomy are critical in patients at risk of LVOTO. Kayalar *et al*. analyzed cases of concomitant myectomy with SAVR for sAS and reported that myectomy is safe and effective and that 72% of decisions for myectomy were intraoperative. Therefore, they proposed that myectomy be considered in the setting of ASH even if the obstruction has not been previously demonstrated[17]. Similarly, Lim *et al*. reported that an intraoperative finding of ASH was common during SAVR for sAS and that myectomy was performed in these patients without any additional risks[18]. Although the prevalence of LVOTO is currently unknown, consequences of overt LVOTO are concerning, from perplexing manifestations in the acute phase to need for repeat surgery in the chronic phase[19]. Therefore, overt LVOTO must be treated, and latent LVOTO should be detected.

This study aimed to characterize factors predisposing to LVOTO and investigate the safety and effectiveness of myectomy in patients with sAS and LVOTO, to better serve this unique patient group that may particularly benefit from myectomy with SAVR.

## Patients and Methods

### Patients

The study was approved by the Institutional Review Board of Juntendo University Hospital (approval # E22-0301). The requirement of informed consent was waived due to general consent obtained at the time of admission and the retrospective observational nature of the study. A total of 740 patients who underwent SAVR for sAS between 2012 and 2019 were identified from our consecutive patient list. SAS was defined as an aortic valve (AV) area (AVA) $\leq$ 1.0 cm$^2$, AV systolic mean pressure gradient (mPG) $\geq$ 40 mmHg, or AV peak velocity $\geq$ 4.0 m/s on preoperative transthoracic echocardiography (TTE). Cases of combined and repeat surgeries were included.

### Clinical data collection and analysis

Patient details were collected from a review of medical records. Overall, 68 patients were excluded due to partial TTE reports. One patient was excluded because of a preoperative complication of infective endocarditis. The remaining 671 patients were divided into the following two groups: myectomy group that underwent SAVR with myectomy and AVR group that underwent SAVR without myectomy. The demographics, basic physical characteristics, and baseline TTE findings were compared between the two groups, followed by a single-arm cohort study of the myectomy group for comorbidities, operative details, TTE changes, complications, and prognosis.

Before an intergroup comparison, each TTE parameter was compared with its reference value for each sex, as proposed by the American Society of Echocardiography and the European Association of Cardiovascular Imaging[20]. Patients from each group were first classified into female and male subgroups, and the respective reference value was subtracted from each measurement. The results were combined and compared against zero, and *P*-value was calculated using paired Student's *t*-test as described in the Statistical Analysis section below.

Relative wall thickness (RWT), LV mass (LVM), and LV mass index (LVMI) were calculated following society recommendations[20]. Since LVOTO may arise from undiagnosed HCM or ASH, frequencies of positive echocardiographic criteria, i.e., IVS or posterior wall (PW) $\geq$ 15 mm for HCM, and IVS-to-PW ratio (IVS/PW) > 1.3 for ASH, were additionally analyzed with frequencies of sigmoid septum and SAM[21,22]. In this study, a high LVOT systolic peak pressure gradient (pPG) was defined

as ≥ 30 mmHg and a high LVOT peak velocity as > 1.0 m/s. Given that patient age may affect the results, we conducted subset analyses of patients aged ≥ 75 years at the time of surgery.

### Surgical procedures

Surgery was performed using median sternotomy and standard cardiopulmonary bypass techniques. Decisions for myectomy were made by the operating surgeons based on preoperative diagnosis of HCM or TTE findings, or intraoperative observations of SAM or septal protrusion into the LVOT.

### Histopathological analysis

The resected septa were sent to the pathology core facility and preserved in paraffin-embedded tissue blocks. Several of these blocks were randomly chosen and cut into 4 $\mu$m-thick slices. Masson's trichrome staining was used to identify fibrotic tissues. To quantify the degree of fibrosis, digital images of the stained slices were acquired using a multifunctional color laser machine, Bizhub c368 (Konica Minolta, Tokyo, Japan). Subsequently, the blue stain, indicative of collagenous connective tissue, was recognized and overlaid green using an image analysis software, KS400 (Carl Zeiss AG, Oberkochen, Germany). Finally, the software was programmed to calculate the ratio of green area to purple tissue background to quantify the degree of fibrosis.

### Statistical analysis

Statistical analyses were performed using R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria), EZR[23], and Microsoft Excel 2016 (Microsoft Corporation, Redmond, Washington, USA). Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively. Discrete numerical, non-normally distributed variables are presented as median (minimum, maximum). Continuous numerical and categorical variables were compared using Student's *t*-test and Fisher's exact test, respectively, between unpaired groups, and using paired Student's *t*-test and McNemar's test with continuity correction, respectively, between paired groups. Survival rate was calculated using Kaplan–Meier analysis. A *P*-value < 0.05 was considered statistically significant.

## Results

### Patient demographics and baseline clinical characteristics

Patient demographics and baseline clinical characteristics are summarized in Table 1. Forty-three patients (6.4%) underwent myectomy with SAVR. The average age at surgery and the proportion of female patients were significantly higher in the myectomy group than in the AVR group ($P < 0.05$ and $P < 0.001$, respectively). Given the female predominance, the average body surface area was significantly smaller in the myectomy group than

**Table 1** Baseline clinical characteristics of patients with severe aortic stenosis undergoing aortic valve replacement

| Characteristics | All patients (n = 671) | AVR (n = 628) | Myectomy (n = 43) | *P*-value |
|---|---|---|---|---|
| Age [years] | 72.8 ± 9.3 | 72.5 ± 9.4 | 76.2 ± 6.6 | < 0.05 |
| Sex, n (%) | | | | |
| Female | 334 (49.8) | 301 (47.9) | 33 (76.7) | < 0.001 |
| Male | 337 (50.2) | 327 (52.1) | 10 (23.3) | |
| Body mass index [kg/m$^2$] | 22.9 ± 3.6 | 22.8 ± 3.5 | 23.6 ± 3.9 | 0.163 |
| Body surface area [m$^2$] | 1.57 ± 0.19 | 1.58 ± 0.19 | 1.51 ± 0.17 | < 0.05 |
| Etiology, n (%) | | | | |
| Degenerative | 481 (71.7) | 442 (70.4) | 39 (90.7) | < 0.05 |
| Bicuspid valve | 133 (19.8) | 130 (20.7) | 3 (7.0) | |
| Rheumatic | 28 (4.2) | 28 (4.5) | 0 (0.0) | |
| Artificial valve dysfunction | 29 (4.3) | 28 (4.5) | 1 (2.3) | |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively, and were compared using Student's *t*-test and Fisher's exact test, respectively, between the groups.
AVR, aortic valve replacement.

in the AVR group ($P < 0.05$). Degenerative etiology was most frequent in both the groups. The elderly subset analysis also revealed strong female predominance in the myectomy group (Table 2).

**Baseline echocardiography**

Baseline TTE parameters are presented in Table 3. Compared with the normal reference values, the group of all patients had a significantly thicker IVS, PW, and RWT; and a significantly larger LVM and LVMI, as marked with asterisk signs (all $P < 0.001$). In contrast, only the myectomy group had a smaller left ventricular dimension during diastole (LVDd) and systole (LVDs), as marked with hash signs (both $P < 0.001$), and a higher left ventricular ejection fraction (LVEF) ($P < 0.001$). In short, the patients undergoing SAVR for sAS had concentric LVH, and the myectomy group additionally exhibited a smaller LV size and stronger systolic function than the normal references.

Compared with the AVR group, the myectomy group had a smaller LVDd and LVDs (both $P < 0.001$); a thicker IVS ($P < 0.001$), PW ($P < 0.01$), and RWT ($P < 0.001$); a higher LVEF ($P < 0.001$); and a larger AVA, AVA index (AVAI) (both $P < 0.001$), AV mPG ($P < 0.05$), and AV peak velocity ($P < 0.01$). Therefore, compared with the AVR counterpart, the myectomy group had an even smaller but more hypertrophic LV, an even higher LVEF, and more severe AS in terms of AV mPG and AV peak velocity. For the number of AV cusps, tricuspid was most frequent in both the groups.

The elderly subset analysis revealed comparable results (Table 4). However, statistical significance of larger AV mPG and AV peak velocity in the myectomy group diminished, possibly due to the small sample size (n = 24).

Compared with the normal reference values, baseline TTE parameters from each gender subgroup within the myectomy group exhibited the same significant trends as the whole myectomy group (smaller LVDd and LVDs, thicker IVS, PW, and RWT, and larger LVM and LVMI, all $P < 0.05$ with some $< 0.01$ or $< 0.001$), but only the female group had a higher LVEF (paired Student's *t*-test, $P < 0.001$). Baseline TTE parameters from female and male subgroups within the myectomy group were similar to each other (LVDd, LVDs, IVS, PW, RWT, LVM, LVMI, AVA, AVAI, AV mPG, AV peak velocity, all $P \geq 0.05$), except LVEF (unpaired Student's *t*-test, female 73% vs. male 66%, $P < 0.05$).

**Comorbidities**

Comorbidities of patients in the myectomy group are listed in Table 5. Hypertension was most frequently observed. The Charlson Comorbidity Index score had a median of 4 (range, 2 to 9). Functional status was mostly New York Heart Association functional classification II (67%). The data for the elderly subset were comparable.

**Table 2** Baseline clinical characteristics of elderly subset with severe aortic stenosis undergoing aortic valve replacement (aged ≥ 75 years)

| Characteristics | All patients (n = 333) | AVR (n = 309) | Myectomy (n = 24) | *P*-value |
|---|---|---|---|---|
| Age [years] | 79.4 ± 3.3 | 79.3 ± 3.2 | 80.8 ± 4.0 | < 0.05 |
| Sex, n (%) | | | | |
| Female | 171 (51.4) | 149 (48.2) | 22 (91.7) | < 0.001 |
| Male | 162 (48.6) | 160 (51.8) | 2 (83) | |
| Body mass index [kg/m$^2$] | 22.7 ± 3.1 | 22.8 ± 3.2 | 22.5 ± 2.8 | 0.733 |
| Body surface area [m$^2$] | 1.54 ± 0.17 | 1.55 ± 0.17 | 1.43 ± 0.14 | < 0.01 |
| Etiology, n (%) | | | | |
| Degenerative | 287 (86.2) | 263 (85.1) | 24 (100.0) | 0.391 |
| Bicuspid valve | 27 (8.1) | 27 (8.7) | 0 (0.0) | |
| Rheumatic | 12 (3.6) | 12 (3.9) | 0 (0.0) | |
| Artificial valve dysfunction | 7 (2.1) | 7 (2.3) | 0 (0.0) | |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively, and were compared using Student's *t*-test and Fisher's exact test, respectively, between the groups.
AVR, aortic valve replacement.

**Table 3** Baseline echocardiographic findings of patients with severe aortic stenosis undergoing aortic valve replacement

| Characteristics | All patients (n = 671) | AVR (n = 628) | Myectomy (n = 43) | *P*-value |
|---|---|---|---|---|
| LVDd [mm] | 47.3 ± 6.6 | 47.6 ± 6.6 | 43.0 ± 5.2$^{\#\#\#}$ | < 0.001 |
| LVDs [mm] | 30.8 ± 7.4 | 31.2 ± 7.4$^{**}$ | 25.3 ± 3.9$^{\#\#\#}$ | < 0.001 |
| IVS [mm] | 11.4 ± 1.8$^{***}$ | 11.3 ± 1.8$^{***}$ | 12.5 ± 1.7$^{***}$ | < 0.001 |
| IVS ≥ 15 [mm], n (%) | 29 (4.3) | 25 (4.0) | 4 (9.3) | 0.107 |
| PW [mm] | 11.3 ± 1.7$^{***}$ | 11.2 ± 1.7$^{***}$ | 12.1 ± 1.4$^{***}$ | < 0.01 |
| PW ≥ 15 [mm], n (%) | 21 (3.1) | 19 (3.0) | 2 (4.7) | 0.638 |
| IVS/PW > 1.3, n (%) | 7 (1.0) | 6 (1.0) | 1 (2.3) | 0.372 |
| RWT | 0.49 ± 0.10$^{***}$ | 0.48 ± 0.10$^{***}$ | 0.57 ± 0.10$^{***}$ | < 0.001 |
| LVM [g] | 201.3 ± 62.1$^{***}$ | 201.8 ± 62.8$^{***}$ | 194.2 ± 51.0$^{***}$ | 0.441 |
| LVMI [g/m$^2$] | 127.8 ± 35.3$^{***}$ | 127.7 ± 35.4$^{***}$ | 129.7 ± 33.6$^{***}$ | 0.719 |
| LVEF [%] | 63.6 ± 11.9 | 63.1 ± 12.0 | 71.1 ± 7.4$^{***}$ | < 0.001 |
| AVA [cm$^2$] | 0.74 ± 0.19 | 0.73 ± 0.19 | 0.84 ± 0.25 | < 0.001 |
| AVAI [cm$^2$/m$^2$] | 0.47 ± 0.13 | 0.47 ± 0.12 | 0.56 ± 0.16 | < 0.001 |
| AV mPG [mmHg] | 45.2 ± 17.8 | 44.8 ± 17.8 | 51.2 ± 16.9 | < 0.05 |
| AV peak velocity [m/s] | 4.4 ± 0.8 | 4.3 ± 0.8 | 4.7 ± 0.8 | < 0.01 |
| AV cusp, n (%) | | | | |
| Artificial | 29 (4.3) | 28 (4.5) | 1 (2.3) | |
| Bicuspid | 133 (19.8) | 130 (20.7) | 3 (7.0) | |
| Tricuspid | 468 (69.7) | 435 (69.3) | 33 (76.7) | < 0.05 |
| Quadricuspid | 1 (0.1) | 1 (0.2) | 0 (0.0) | |
| Unknown | 40 (6.0) | 34 (5.4) | 6 (14.0) | |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively, and were compared using Student's *t*-test and Fisher's exact test, respectively, between the groups. Some variables were compared with their normal reference values using paired Student's *t*-test, as described in 'Patients and Methods'. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ larger than the reference value. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ smaller than the reference value.
AVR, aortic valve replacement; NA, not applicable or unavailable; LVDd, left ventricular dimension during diastole; LVDs, left ventricular dimension during systole; IVS, interventricular septum; PW, posterior wall; RWT, relative wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; AVA, aortic valve area; AVAI, aortic valve area index; AV, aortic valve; mPG, mean pressure gradient.

### Operative details

Operative details of patients in the myectomy group are presented in Table 6. The procedural time of the myectomy group was similar to that of the AVR group (242 minutes vs. 269 minutes, $P = 0.09$). Most of the implanted valves were of bioprosthetic type and 19 or 21 mm in diameter. Left atrial appendage resection was the most frequent concomitant procedure performed with SAVR in addition to myectomy. The data for the elderly subset were also comparable. Of note, 2 of the 5 concomitant mitral valve plasty procedures were added intraoperatively based on the new onset of SAM (4.7%).

### Changes in echocardiography

Table 7 presents the TTE parameters of patients in the myectomy group in preoperative and two postoperative periods. Immediately postoperative and 1-year postoperative TTE were obtained on the median postoperative day (POD) 7 (range, 1 to 88) and POD 366 (range, 193 to 534), respectively. Surgery significantly reduced the IVS, PW, and RWT after 1 year, although they remained thicker than the reference values ($P < 0.001$). Similarly, surgery significantly reduced the LVM and LVMI after 1 year, although these values remained larger than the reference values ($P < 0.001$). In short, concentric LVH improved over time, but not completely to normal. The frequencies of IVS ≥ 15

**Table 4** Baseline echocardiographic findings of elderly subset with severe aortic stenosis undergoing aortic valve replacement (aged ≥ 75 years)

| Characteristics | All patients (n = 333) | AVR (n = 309) | Myectomy (n = 24) | *P*-value |
|---|---|---|---|---|
| LVDd [mm] | 46.6 ± 6.1$^{\#\#}$ | 47.0 ± 6.0$^{\#}$ | 41.3 ± 4.9$^{\#\#}$ | < 0.001 |
| LVDs [mm] | 30.1 ± 7.0 | 30.6 ± 7.0 | 24.4 ± 4.1$^{\#\#\#}$ | < 0.001 |
| IVS [mm] | 11.3 ± 1.8$^{***}$ | 11.2 ± 1.7$^{***}$ | 12.4 ± 1.8$^{***}$ | < 0.01 |
| IVS ≥ 15 [mm], n (%) | 13 (3.9) | 10 (3.2) | 3 (12.5) | 0.058 |
| PW [mm] | 11.3 ± 1.6$^{***}$ | 11.2 ± 1.6$^{***}$ | 12.2 ± 1.6$^{***}$ | < 0.01 |
| PW ≥ 15 [mm], n (%) | 7 (2.1) | 6 (1.9) | 1 (4.2) | 0.411 |
| IVS/PW > 1.3, n (%) | 4 (1.2) | 3 (1.0) | 1 (4.2) | 0.260 |
| RWT | 0.49 ± 0.10$^{***}$ | 0.48 ± 0.09$^{***}$ | 0.60 ± 0.12$^{***}$ | < 0.001 |
| LVM [g] | 195.5 ± 55.9$^{***}$ | 196.6 ± 57.1$^{***}$ | 180.8 ± 35.5$^{***}$ | 0.181 |
| LVMI [g/m$^2$] | 126.9 ± 33.1$^{***}$ | 126.9 ± 33.4$^{***}$ | 127.8 ± 30.2$^{***}$ | 0.895 |
| LVEF [%] | 64.3 ± 11.9 | 63.7 ± 12.0 | 72.1 ± 6.6$^{***}$ | < 0.01 |
| AVA [cm$^2$] | 0.72 ± 0.17 | 0.71 ± 0.17 | 0.81 ± 0.21 | < 0.01 |
| AVAI [cm$^2$/m$^2$] | 0.47 ± 0.12 | 0.46 ± 0.11 | 0.57 ± 0.14 | < 0.001 |
| AV mPG [mmHg] | 44.2 ± 16.9 | 43.8 ± 16.9 | 49.1 ± 16.2 | 0.140 |
| AV peak velocity [m/s] | 4.3 ± 0.8 | 4.3 ± 0.8 | 4.6 ± 0.8 | 0.073 |
| AV cusp, n (%) | | | | |
| Artificial | 7 (2.1) | 7 (2.3) | 0 (0.0) | |
| Bicuspid | 27 (8.1) | 27 (8.7) | 0 (0.0) | |
| Tricuspid | 284 (85.3) | 261 (84.5) | 23 (95.8) | 0.482 |
| Quadricuspid | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Unknown | 15 (4.5) | 14 (4.5) | 1 (4.2) | |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively, and were compared using Student's *t*-test and Fisher's exact test, respectively, between the groups. Some variables were compared with their normal reference values using paired Student's *t*-test, as described in 'Patients and Methods'. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ larger than the reference value. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ smaller than the reference value.
AVR, aortic valve replacement; NA, not applicable or unavailable; LVDd, left ventricular dimension during diastole; LVDs, left ventricular dimension during systole; IVS, interventricular septum; PW, posterior wall; RWT, relative wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; AVA, aortic valve area; AVAI, aortic valve area index; AV, aortic valve; mPG, mean pressure gradient.

mm, PW ≥ 15 mm, and IVS/PW > 1.3 had decreased to zero at 1 year. The presence of sigmoid septum or SAM was recorded only when echo technicians were able to identify them, and the frequencies of these were not significantly altered at 1 year. LVOT hemodynamic parameters were not recorded for all patients for the same reason, and yet, comparison using available data (n = 16) revealed a moderate decrease in LVOT pPG ($P = 0.172$) and a significant decrease in LVOT peak velocity ($P < 0.05$) at 1 year. Therefore, parameters indicative of LVOTO improved after surgery. The LVEF decreased immediately after surgery but not at 1 year and remained higher than the reference value at 1 year ($P < 0.05$). As a result of SAVR, the smaller AVA and AVAI and larger AV mPG and AV peak velocity in sAS improved immediately after surgery and remained stable after 1 year.

The elderly subset analysis revealed comparable results (Table 8). However, statistical significance of changes in the RWT and LVOT peak velocity at 1 year diminished, possibly due to the small sample sizes again (n = 12 and n = 7, respectively).

**Complications and prognosis**

Complications and prognosis of patients in the myectomy group are summarized in Table 9. Intraoperative or 30-day all-cause mortality was not observed. The median stay in the intensive care unit or in the hospital after surgery were 1 day

**Table 5** Comorbidities of patients with severe aortic stenosis undergoing aortic valve replacement with concomitant myectomy

| Characteristics | Myectomy (n = 43) | Subset ≥ 75 yo (n = 24) |
|---|---|---|
| Comorbidities, n (%) | | |
| Hypertension | 31 (72.1) | 19 (79.2) |
| Dyslipidemia | 28 (65.1) | 16 (66.7) |
| Diabetes mellitus | 10 (23.3) | 4 (16.7) |
| Cerebrovascular disease | 5 (11.6) | 4 (16.7) |
| Peripheral vascular disease | 4 (9.3) | 4 (16.7) |
| Dialysis | 2 (4.7) | 0 (0.0) |
| Connective tissue disease | 1 (2.3) | 1 (4.2) |
| COPD | 1 (2.3) | 0 (0.0) |
| Hypertrophic cardiomyopathy | 1 (2.3) | 0 (0.0) |
| Liver dysfunction | 1 (2.3) | 0 (0.0) |
| Prior myocardial infarction | 1 (2.3) | 0 (0.0) |
| Charlson Comorbidity Index, median (range) | 4 (2, 9) | 5 (3, 9) |
| Symptoms, n (%) | | |
| NYHA I | 9 (20.9) | 4 (16.7) |
| NYHA II | 29 (67.4) | 16 (66.7) |
| NYHA III | 4 (9.3) | 3 (12.5) |
| NYHA IV | 1 (2.3) | 1 (4.2) |

Discrete numerical, non-normally distributed variables and categorical variables are presented as median (minimum, maximum) and number (%), respectively.
yo, years old; COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association.

(range, 1 to 14) and 16 days (range, 7 to 77), respectively. Most of the discharge disposition locations were home (86%). There was one in-hospital mortality (2.3%). A 74-year-old woman, with the Charlson Comorbidity Index of 5, died on POD 73 from worsening mitral regurgitation, pneumonia, and sepsis. As non-lethal complications, a complete atrioventricular block requiring a new permanent pacemaker (PPM) implantation was found in three patients, and a ventricular septal defect at the myectomy site, thromboembolic stroke on POD 10, and DeBakey type IIIb aortic dissection on POD 5 in one patient each. The median follow-up interval was 1,038 days (range, 12 to 2,577).

Kaplan–Meier plot of the myectomy group is displayed in Figure 1. The survival rates at postoperative year 1, 3, 5, and 7 were 97%, 94%, 86%, and 86%, respectively. The data for the elderly subset were comparable.

### Fibrosis in resected tissues

Figure 2A shows histological preparation of resected IVS. Figure 2B shows image processing using the image analysis software. Spotty fibrotic tissues were clearly visible on gross examination of the stained slides (blue in Figure 2A). However, the degree of fibrosis calculated using the software varied greatly across tissue blocks (data not shown), and upon microscopic observation at the pathology core facility, most were observed to have slight to moderate fibrosis.

## Discussion

The patients' demographics, such as female predominance (77%) and older age at surgery (76 years old), as well as the etiology (mainly degenerative) and comorbidity (hypertension most common) were in line with the previous report on patients with sAS in need of myectomy[17]. These observations were replicated across the Pacific despite large sociomedical and genetic differences, although the female predominance may in part stem from longer lifespan of women worldwide[24]. Echocardiographically, patients in the myectomy group were

**Table 6** Operative details of patients with severe aortic stenosis undergoing aortic valve replacement with concomitant myectomy

| Characteristics | Myectomy (n = 43) | Subset ≥ 75 yo (n = 24) |
|---|---|---|
| Procedural time [minutes] | 241.8 ± 94.2 | 216.4 ± 66.4 |
| Cardiopulmonary bypass duration [minutes] | 115.0 ± 44.3 | 99.7 ± 31.9 |
| Aortic cross-clamp duration [minutes] | 91.8 ± 38.3 | 79.8 ± 27.0 |
| Valve prosthesis type, n (%) | | |
| Bioprosthesis | 42 (97.7) | 24 (100.0) |
| Mechanical | 1 (2.3) | 0 (0.0) |
| Valve prosthesis size, n (%) | | |
| 19 [mm] | 15 (34.9) | 10 (41.7) |
| 21 [mm] | 15 (34.9) | 8 (33.3) |
| 23 [mm] | 10 (23.3) | 6 (25.0) |
| 25 [mm] | 3 (7.0) | 0 (0.0) |
| Concomitant procedures, n (%) | | |
| LAA resection | 41 (95.3) | 23 (95.8) |
| Chordal cutting | 8 (18.6) | 3 (12.5) |
| CABG | 6 (14.0) | 3 (12.5) |
| MVP | 5[a] (11.6) | 1 (4.2) |
| TAP | 2 (4.7) | 2 (8.3) |
| MVR | 1 (2.3) | 1 (4.2) |
| Repeat cardiac procedure, n (%) | 1 (2.3) | 0 (0.0) |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively. [a] Two of the 5 MVP procedures were added intraoperatively based on the new onset of SAM (4.7%); one observed before and the other after weaning from the cardiopulmonary bypass.
yo, years old; LAA, left atrial appendage; CABG, coronary artery bypass grafting; MVP, mitral valve plasty; TAP, tricuspid annuloplasty; MVR, mitral valve replacement.

further characterized by a small-in-size, yet extra-hypertrophic and extra-hypercontractile heart and more severe AS compared with the AVR counterpart. This may be explained by bidirectional causality between sAS and LVH; sAS causes chronically raised afterload, which stimulates compensatory LVH. Inversely, LVH in a compensatory phase causes a higher flow across the AV, which leads to more severe form of AS. Myectomy with SAVR relieves them both. Figure 3 shows retrospectively analyzed factors predisposing to LVOTO. Most of the patients in the myectomy group (93%) had at least one of these factors. We presume predictive values of these factors, and await further studies for confirmation.

With regard to the effectiveness of myectomy, this study showed that myectomy with SAVR effectively improved concentric LVH and LVOTO. AVR alone reduces the PW and LVMI in sAS[25]; therefore, how the addition of myectomy affected the improvement of concentric LVH is unclear. In contrast, the improvement of LVOTO may be reasonably attributable to myectomy rather than AVR, because myectomy has been known to relieve LVOTO[4], and conversely, AVR has been known to unmask it[15, 16].

Regarding procedural safety, some authors recommend alcohol septal ablation for elderly patients with HCM as an alternative to myectomy. However, survival rates and complication profiles were not particularly worse for our patients in the myectomy group, including the elderly subset. Thirty-day all-cause mortality was not observed, and although one in-hospital mortality was observed, the risk (2.3%) could have been overestimated due to the small sample size (n = 43). Moreover, the long-term survival was comparable to that reported previously[17]. The frequency of new PPM implanta-

**Table 7** Echocardiographic changes in patients with severe aortic stenosis undergoing aortic valve replacement with concomitant myectomy

| Characteristics | Preoperative (n = 43) | Immediately postoperative (n = 43) | *P*-value | 1 year postoperative (n = 25) | *P*-value |
|---|---|---|---|---|---|
| LVDd [mm] | 43.0 ± 5.2$^{\#\#\#}$ | 41.1 ± 4.2$^{\#\#\#}$ | < 0.05 | 43.2 ± 3.7$^{\#\#\#}$ | 0.964 |
| LVDs [mm] | 25.3 ± 3.9$^{\#\#\#}$ | 25.8 ± 2.9$^{\#\#\#}$ | 0.410 | 26.3 ± 3.0$^{\#\#\#}$ | 0.172 |
| IVS [mm] | 12.5 ± 1.7$^{***}$ | 12.3 ± 2.1$^{***}$ | 0.409 | 11.0 ± 1.2$^{***}$ | < 0.001 |
| ≥ 15 [mm], n (%) | 4 (9.3) | 4 (9.3) | 1.00 | 0 (0.0) | NA |
| PW [mm] | 12.1 ± 1.4$^{***}$ | 11.7 ± 1.6$^{***}$ | < 0.05 | 10.9 ± 0.9$^{***}$ | < 0.001 |
| ≥ 15 [mm], n (%) | 2 (4.7) | 1 (2.3) | 1.00 | 0 (0.0) | NA |
| IVS/PW > 1.3, n (%) | 1 (2.3) | 1 (2.3) | NA | 0 (0.0) | NA |
| RWT | 0.57 ± 0.10$^{***}$ | 0.57 ± 0.11$^{***}$ | 0.931 | 0.51 ± 0.07$^{***}$ | < 0.05 |
| Sigmoid septum, n (%) | 11 (25.6) | 1 (2.3) | < 0.01 | 4 (16) | 0.450 |
| SAM, n (%) | 8 (18.6) | 5 (11.6) | 0.450 | 1 (4) | 0.248 |
| LVOT pPG [mmHg] | 18.7 ± 30.1 | 12.1 ± 13.6$^{a}$ | 0.163 | 7.5 ± 11.8$^{b}$ | 0.172 |
| ≥ 30 [mmHg], n (%) | 5 (11.6) | 3 (13.0)$^{a}$ | 1.00 | 1 (6.3)$^{b}$ | NA |
| LVOT peak velocity [m/s] | 1.92 ± 1.34 | 1.65 ± 0.87$^{a}$ | 0.251 | 1.2 ± 0.67$^{b}$ | < 0.05 |
| > 1.0 [m/s], n (%) | 23 (53.5) | 17 (73.9)$^{a}$ | 1.00 | 10 (39)$^{b}$ | 1.00 |
| LVM [g] | 194.2 ± 51.0$^{***}$ | 173.8 ± 41.5$^{***}$ | < 0.001 | 164.9 ± 28.6$^{***}$ | < 0.01 |
| LVMI [g/m$^2$] | 129.7 ± 33.6$^{***}$ | 116.9 ± 27.4$^{***}$ | < 0.001 | 111.2 ± 24.5$^{***}$ | < 0.01 |
| LVEF [%] | 71.1 ± 7.4$^{***}$ | 66.8 ± 7.4$^{**}$ | < 0.01 | 67.6 ± 8.1$^{*}$ | 0.081 |
| AVA [cm$^2$] | 0.84 ± 0.25 | 1.82 ± 0.63 | < 0.001 | 1.84 ± 0.56 | < 0.001 |
| AVAI [cm$^2$/m$^2$] | 0.56 ± 0.16 | 1.22 ± 0.39 | < 0.001 | 1.22 ± 0.31 | < 0.001 |
| AV mPG [mmHg] | 51.2 ± 16.9 | 11.7 ± 4.8 | < 0.001 | 9.8 ± 3.8 | < 0.001 |
| AV peak velocity [m/s] | 4.7 ± 0.8 | 2.4 ± 0.5 | < 0.001 | 2.2 ± 0.4 | < 0.001 |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively, and were compared using paired Student's *t*-test and McNemar's test with continuity correction, respectively, between the groups (preoperative vs. immediately postoperative, and preoperative vs. 1 year postoperative). Some variables were compared with their normal reference values using paired Student's *t*-test, as described in 'Patients and Methods'. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ larger than the reference value. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ smaller than the reference value. [a] n = 23. [b] n = 16.

LVDd, left ventricular dimension during diastole; LVDs, left ventricular dimension during systole; IVS, interventricular septum; PW, posterior wall; RWT, relative wall thickness; SAM, systolic anterior motion of the mitral valve; LVOT, left ventricle outflow tract; pPG, peak pressure gradient; LVM, left ventricular mass; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; AVA, aortic valve area; AVAI, aortic valve area index; AV, aortic valve; mPG, mean pressure gradient.

tion (7.0%) was similar to that in SAVR alone (8.5%)[26], despite the possibility of either SAVR or myectomy affecting the conduction system. The frequency of iatrogenic ventricular septal defect, a rare but important complication (2.3%), was slightly greater than in patients with HCM after myectomy[27], but its significance remains unclear due to the small sample size. Overall, this study showed that myectomy with SAVR was relatively safe.

Histologically, myocardial fibrosis (MF) has been found in various diseases including hypertension and HCM[28]. Therefore, we had expected significant MF in resected IVS. Instead, pathology reports indicated slight to moderate MF. In HCM, MF is common[29] and strongly associated with the occurrence of systolic dysfunction[30], ventricular tachyarrhythmia[31], and major adverse events[32]. In AS, MF has a significant negative correlation with symptomatic improvements[33], systolic function[34], and long-term survival after SAVR[35]. Therefore, the hypercontractile heart of patients in the myectomy group here actually corresponds with mild MF as demonstrated. The decisions for surgery were perhaps made sufficiently early, because they were made before MF became significant enough to cause hemodynamic decompensation.

**Table 8** Echocardiographic changes in elderly subset with severe aortic stenosis undergoing aortic valve replacement with concomitant myectomy (aged ≥ 75 years)

| Characteristics | Preoperative (n = 24) | Immediately postoperative (n = 24) | *P*-value | 1 year postoperative (n = 12) | *P*-value |
|---|---|---|---|---|---|
| LVDd [mm] | 41.3 ± 4.9$^{\#\#}$ | 39.8 ± 4.0$^{\#\#\#}$ | 0.115 | 42.3 ± 4.1$^{\#}$ | 0.377 |
| LVDs [mm] | 24.4 ± 4.1$^{\#\#\#}$ | 25.2 ± 2.9$^{\#\#\#}$ | 0.360 | 25.9 ± 2.6$^{\#}$ | 0.148 |
| IVS [mm] | 12.4 ± 1.8$^{***}$ | 12.4 ± 2.1$^{***}$ | 0.935 | 11.1 ± 1.0$^{***}$ | < 0.05 |
| ≥ 15 [mm], n (%) | 3 (12.5) | 2 (8.3) | 1.00 | 0 (0.0) | NA |
| PW [mm] | 12.2 ± 1.6$^{***}$ | 11.4 ± 1.2$^{***}$ | < 0.05 | 11.1 ± 0.8$^{***}$ | < 0.01 |
| ≥ 15 [mm], n (%) | 1 (4.2) | 0 (0.0) | NA | 0 (0.0) | NA |
| IVS/PW > 1.3, n (%) | 1 (4.2) | 1 (4.2) | NA | 0 (0.0) | NA |
| RWT | 0.60 ± 0.12$^{***}$ | 0.58 ± 0.09$^{***}$ | 0.378 | 0.53 ± 0.07$^{***}$ | 0.060 |
| Sigmoid septum, n (%) | 7 (29.2) | 0 (0.0) | NA | 2 (16.7) | NA |
| SAM, n (%) | 4 (16.7) | 3 (12.5) | 1.00 | 1 (8.3) | 1.00 |
| LVOT pPG [mmHg] | 18.0 ± 30.8 | 10.7 ± 14.5$^{a}$ | 0.177 | 12.1 ± 17.3$^{b}$ | 0.360 |
| ≥ 30 [mmHg], n (%) | 2 (8.3) | 1 (4.2)$^{a}$ | 1.00 | 1 (8.3)$^{b}$ | NA |
| LVOT peak velocity [m/s] | 1.58 ± 1.24 | 1.41 ± 0.87$^{a}$ | 0.358 | 1.54 ± 0.94$^{b}$ | 0.511 |
| > 1.0 [m/s], n (%) | 7 (29.2) | 8 (33.3)$^{a}$ | 1.00 | 6 (50)$^{b}$ | 0.480 |
| LVM [g] | 180.8 ± 35.5$^{***}$ | 162.9 ± 30.1$^{***}$ | < 0.05 | 161.2 ± 27.9$^{***}$ | < 0.01 |
| LVMI [g/m$^2$] | 127.8 ± 30.2$^{***}$ | 115.3 ± 22.7$^{***}$ | < 0.05 | 114.6 ±26.7$^{***}$ | < 0.05 |
| LVEF [%] | 72.1 ± 6.6$^{***}$ | 68.1 ± 7.1$^{**}$ | 0.053 | 69.0 ± 6.7$^{*}$ | 0.111 |
| AVA [cm$^2$] | 0.81 ± 0.21 | 1.75 ± 0.63 | < 0.001 | 1.80 ± 0.56 | < 0.001 |
| AVAI [cm$^2$/m$^2$] | 0.57 ± 0.14 | 1.23 ± 0.44 | < 0.001 | 1.25 ± 0.32 | < 0.001 |
| AV mPG [mmHg] | 49.1 ± 16.2 | 12.3 ± 5.5 | < 0.001 | 10.9 ± 4.1 | < 0.001 |
| AV peak velocity [m/s] | 4.6 ± 0.8 | 2.4 ± 0.5 | < 0.001 | 2.3 ± 0.4 | < 0.001 |

Continuous numerical and categorical variables are presented as mean ± standard deviation and number (%), respectively, and were compared using paired Student's *t*-test and McNemar's test with continuity correction, respectively, between the groups (preoperative vs. immediately postoperative, and preoperative vs. 1 year postoperative). Some variables were compared with their normal reference values using paired Student's *t*-test, as described in 'Patients and Methods'. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ larger than the reference value. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ smaller than the reference value. [a] n = 11. [b] n = 7.

LVDd, left ventricular dimension during diastole; LVDs, left ventricular dimension during systole; IVS, interventricular septum; PW, posterior wall; RWT, relative wall thickness; SAM, systolic anterior motion of the mitral valve; LVOT, left ventricle outflow tract; pPG, peak pressure gradient; LVM, left ventricular mass; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; AVA, aortic valve area; AVAI, aortic valve area index; AV, aortic valve; mPG, mean pressure gradient.

Lastly, to further investigate effectiveness of myectomy, a randomized controlled trial should be performed where LVOTO risk factors and severity of LVOTO are matched between myectomy and non-myectomy groups. However, difficulties in such studies are multifold. As already known, LVOTO is an extremely heterogeneous disease state, and various associated findings have been reported but universally accepted risk factors are yet to be defined. Intraoperative decisions for myectomy are also yet to be standardized. Thus, it would be technically challenging to prepare appropriate groups for comparison while controlling all possible confounding factors. In more practical terms, a patient's assignment to non-myectomy group would create an ethical dilemma for the operating surgeons as they leave hypertrophied IVS untouched, knowing the high risk of postoperative LVOTO. On the whole, LVOTO risk factors require additional investigations. For more comprehensive risk assessment, additional use of operator-independent imaging modalities may help.

## Limitations

The biggest limitation of this study was that it was a retrospective single-arm study because of

**Table 9** Complications and prognosis of patients with severe aortic stenosis undergoing aortic valve replacement with concomitant myectomy

| Characteristics | Myectomy (n = 43) | Subset ≥ 75 yo (n = 24) |
|---|---|---|
| 30-day all-cause mortality, n (%) | 0 (0.0) | 0 (0.0) |
| Days in ICU, median (range) | 1 (1, 14) | 1 (1, 9) |
| Days until discharge, median (range) | 16 (7, 77) | 16 (7, 36) |
| Disposition, n (%) | | |
| Home | 37 (86.0) | 20 (83.3) |
| Transfer | 5 (11.6) | 4 (16.7) |
| NA[a] | 1 (2.3) | 0 (0.0) |
| Complications, n (%) | | |
| Complete atrioventricular block | 3 (7.0) | 2 (8.3) |
| Ventricular septal defect | 1 (2.3) | 1 (4.2) |
| Stroke | 1 (2.3) | 1 (4.2) |
| Aortic dissection | 1 (2.3) | 0 (0.0) |

Discrete numerical, non-normally distributed variables and categorical variables are presented as median (minimum, maximum) and number (%), respectively. [a] One in-hospital mortality.
yo, years old; ICU, intensive care unit; NA, not applicable.

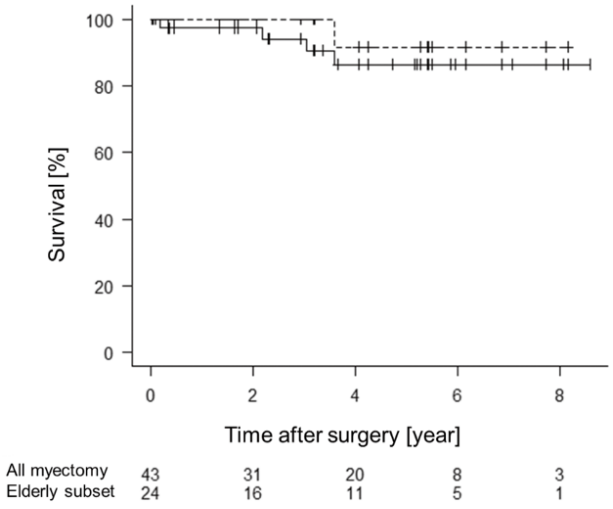


**Figure 1** Kaplan–Meier plot of the myectomy group and its elderly subset (aged ≥ 75 years). Solid line, myectomy group; dotted line, elderly subset. The survival rates at postoperative year 1, 3, 5, and 7 were 97%, 94%, 86%, and 86%, respectively, in the myectomy group, whereas they were 100%, 100%, 92%, and 92% in the elderly subset.

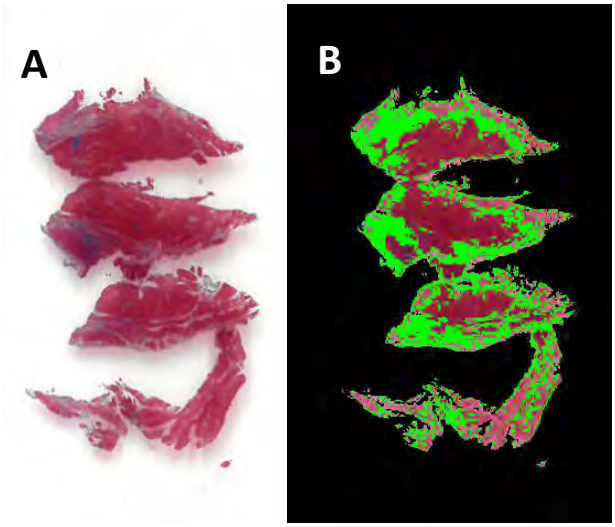


**Figure 2** Fibrosis in resected tissue. (A) Masson's trichrome staining of the resected interventricular septum in patients with severe aortic stenosis undergoing aortic valve replacement. (B) Same histopathological slide analyzed for the degree of fibrosis.

the difficulty in controlling confounding factors as described above. It was conducted at a single center; therefore, extrapolation of the results obtained here to wider patient populations may require further confirmation.

## Conclusions

Myectomy can be safely performed with SAVR for sAS with LVOTO, even in elderly patients (≥ 75 years), and is effective in improving concentric LVH and LVOTO. To detect surgically amenable entities contributing to LVOTO and avoid "suicide LV," we first propose that clinicians should be aware of such entities in various clinical situations with hemodynamic instability. Second, elderly female patients with relatively more severe AS and

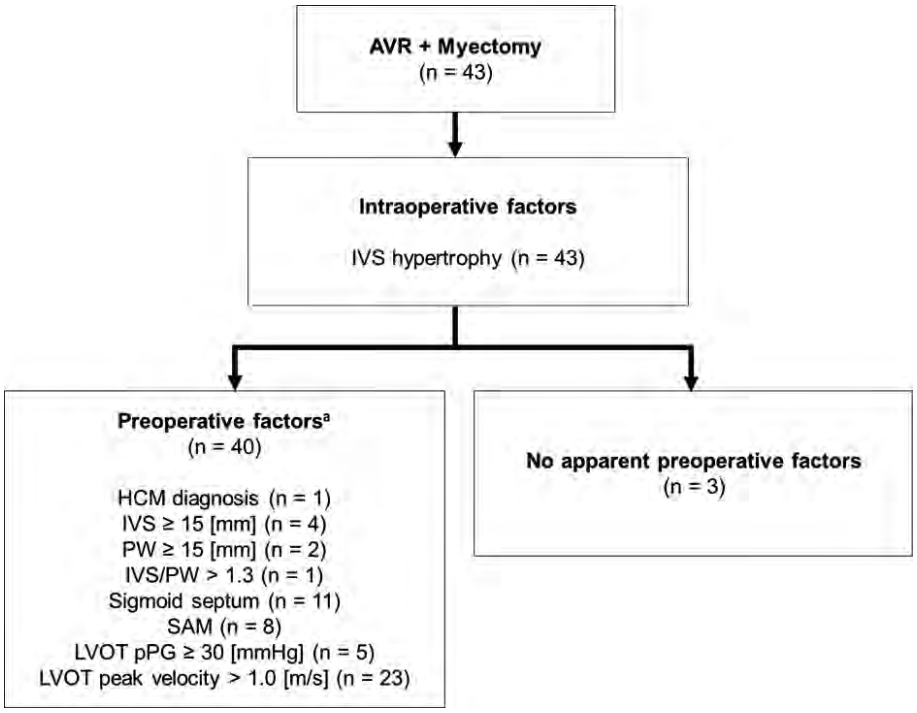


**Figure 3** Risk factors for LVOTO in patients with severe aortic stenosis undergoing aortic valve replacement with concomitant myectomy. [a] Some patients had multiple factors. AVR, aortic valve replacement; IVS, interventricular septum; HCM, hypertrophic cardiomyopathy; PW, posterior wall; SAM, systolic anterior motion of the mitral valve; LVOT, left ventricular outflow tract; pPG, peak pressure gradient.

a small yet extra-hypertrophic and extra-hypercontractile heart are of particular interest. For these patients, a comprehensive assessment of LVOTO risk factors would be necessary. It is critical to acknowledge and identify such a patient group because, despite its invasiveness, SAVR may be potentially more beneficial for them by allowing direct observation of LVOT and ancillary myectomy.

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## Author contributions

AU collected, analyzed, and interpreted the data. AU also processed the histological slices and is the main author of this manuscript. SM, TK, and MT supervised and reviewed the process. All authors read and approved the final manuscript.

## Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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Original Articles

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# A Diving Accident Checklist in Izu Peninsula can be Associated with Some Pitfalls

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***Objective***: We retrospectively investigated the degree of completion of the checklist during or immediately after diving accident, who were transported by a physician-staffed helicopter emergency medical service (HEMS).

***Method***: From May 2016 to December 2020, we conducted a retrospective the diving accident checklist review of all patients with diving accident, who were transported by HEMS. If all questions of the diving accident checklist were answered, full marks were 40 points. Subjects were divided into two groups: the Arrest group, which included subjects who became cardiac arrest in prehospital setting, and the Control group.

***Results***: A total of 86 patients with diving accident were transported by the HEMS. Among these patients, there were 16 subjects in the Arrest group and 70 in the Control group. Average total score in the Arrest group were significantly smaller than those in the Control group.

***Conclusion***: Degree of completion of the diving accident checklist in cases with cardiac arrest was low in comparison with cases without cardiac arrest. To improve this, further approach based on several remedies will be required in the future.

***Key words***: aviation, decompression illness, meetings, cardiac arrest

## Introduction

The Izu peninsula, which is a popular location for recreational scuba diving, is located near Tokyo. Accordingly, significant number of diving accidents has been occurring there[1]. Search and rescue for patients with diving accidents consisting of drowning, decompression illness (DCI), barotrauma and/or occasional endogenous disease, is mainly conducted by professional divers who belong to local dive shops and/or the coast guard[2]. After reaching shore, transportation to the hospital is carried out by the fire department for recompression treatment with hyperbaric oxygen (HBO) therapy. A physician-staffed helicopter emergency medical service (HEMS), of which base hospital is Juntendo Shizuoka Hospital, is necessary for such cases to diagnosis at scene and appropriate transport because there are no suitable hospitals for recompression in the Izu peninsula[3,4]. The HEMS can transport patients from the scene to a suitable hospital within 15 to 20 min. In contrast, a ground ambulance would take at least 1.5 h to reach the receiving hospitals[1]. In January 2011, our hospital, which is a leader of the Izu peninsula medical control council (MCC) system, began to hold meetings concerning the management of patients with DCI to establish a cooperative medical system for such patients in the Izu peninsula[5]. Representatives from the fire department, coast guard, HEMS, and professional divers belonging to local dive shops in the Izu peninsula joined the meeting. At this meeting, we share information on the diving profile using a diving accident checklist (Figure 1) newly developed by our own hospital; and review the proper, prompt management of patients with DCI, including

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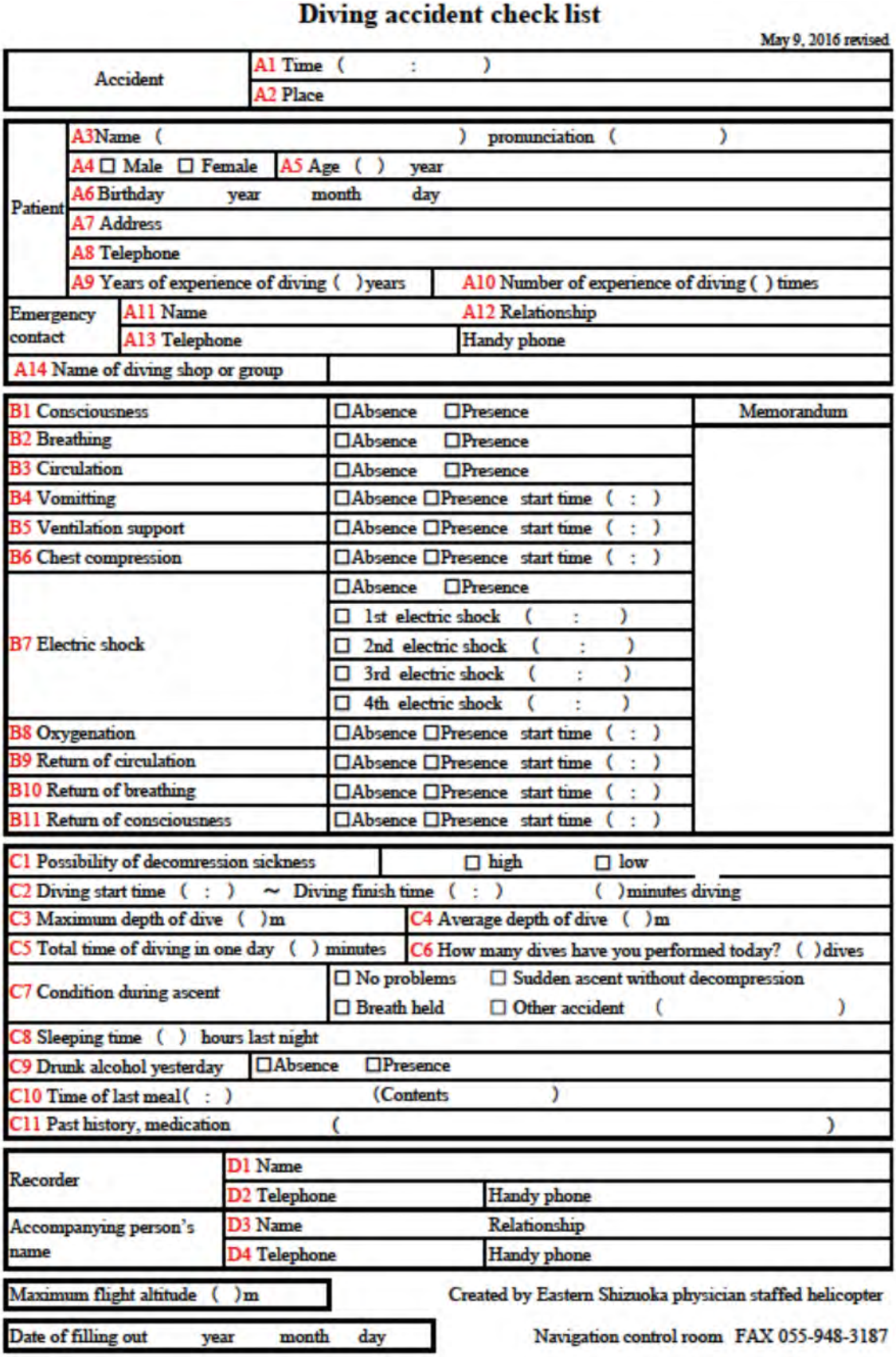

**Diving accident check list**

May 9, 2016 revised

| | | |
|---|---|---|
| Accident | A1 Time ( : ) | |
| | A2 Place | |
| Patient | A3 Name ( ) pronunciation ( ) | |
| | A4 ☐ Male ☐ Female | A5 Age ( ) year |
| | A6 Birthday year month day | |
| | A7 Address | |
| | A8 Telephone | |
| | A9 Years of experience of diving ( ) years | A10 Number of experience of diving ( ) times |
| Emergency contact | A11 Name | A12 Relationship |
| | A13 Telephone | Handy phone |
| A14 Name of diving shop or group | | |

| | | |
|---|---|---|
| B1 Consciousness | ☐Absence ☐Presence | Memorandum |
| B2 Breathing | ☐Absence ☐Presence | |
| B3 Circulation | ☐Absence ☐Presence | |
| B4 Vomitting | ☐Absence ☐Presence start time ( : ) | |
| B5 Ventilation support | ☐Absence ☐Presence start time ( : ) | |
| B6 Chest compression | ☐Absence ☐Presence start time ( : ) | |
| B7 Electric shock | ☐Absence ☐Presence | |
| | ☐ 1st electric shock ( : ) | |
| | ☐ 2nd electric shock ( : ) | |
| | ☐ 3rd electric shock ( : ) | |
| | ☐ 4th electric shock ( : ) | |
| B8 Oxygenation | ☐Absence ☐Presence start time ( : ) | |
| B9 Return of circulation | ☐Absence ☐Presence start time ( : ) | |
| B10 Return of breathing | ☐Absence ☐Presence start time ( : ) | |
| B11 Return of consciousness | ☐Absence ☐Presence start time ( : ) | |

| | |
|---|---|
| C1 Possibility of decomression sickness | ☐ high ☐ low |
| C2 Diving start time ( : ) ~ Diving finish time ( : ) | ( ) minutes diving |
| C3 Maximum depth of dive ( ) m | C4 Average depth of dive ( ) m |
| C5 Total time of diving in one day ( ) minutes | C6 How many dives have you performed today? ( ) dives |
| C7 Condition during ascent | ☐ No problems ☐ Sudden ascent without decompression |
| | ☐ Breath held ☐ Other accident ( ) |
| C8 Sleeping time ( ) hours last night | |
| C9 Drunk alcohol yesterday | ☐Absence ☐Presence |
| C10 Time of last meal ( : ) | (Contents ) |
| C11 Past history, medication | ( ) |

| | | |
|---|---|---|
| Recorder | D1 Name | |
| | D2 Telephone | Handy phone |
| Accompanying person's name | D3 Name | Relationship |
| | D4 Telephone | Handy phone |

Maximum flight altitude ( ) m

Created by Eastern Shizuoka physician staffed helicopter

Date of filling out year month day

Navigation control room FAX 055-948-3187

**Figure 1** A diving accident checklist

Personal information, vital signs, and prehospital treatments, diving profile, information of recorder and key person, in the diving accident checklist was classified into item A, B, C and D respectively. There were 14 questions in item A, 11 in B, 11 in C, and 4 in D, and each question was assigned 1 point. If all questions are answered, full marks are 40 points.

early transportation[3,5]. After commencement of using the diving accident checklist, the list was deemed useful for helping the receiving hospital diagnose decompression sickness and determine the recompression table[6]. While, we noticed that some diving accident checklists had many missing data, especially in severely ill case. Accordingly, our purpose was to clarify some of the weak points associated with the diving check list by investigating the degree of completion of the checklist during or immediately after diving accident, who were transported by the HEMS.

## Methods

The protocol of this retrospective study using opt-out system was approved by our institutional review board, and the examinations were conducted according to the standards of good clinical practice and the Declaration of Helsinki. The approval number was 298.

The implementation of the diving accident checklist, including patients' personal identifying information, diving profile, years of experience with diving, vital signs, and prehospital treatments, was started in January 2013 after an agreement to use the checklist was made at a meeting[6]. The checklist was filled out by medical staff of the HEMS with the cooperation of the fire department, coast guard, HEMS, and/or professional divers. The information obtained via the checklist was deemed useful at the receiving hospital for HBO therapy, especially the diving profile[6], as inert gas bubbles are known to cause decompression sickness, and inert gas accumulation is regulated by the diving time, diving depth, ascent speed, and individual factors, such as dehydration, stress and age[7-9]. Estimating the inert gas accumulation based on values included in the diving accident checklist can help confirm a diagnosis of decompression sickness as well as determine recompression tables[10]. This checklist was revised in May 2016 based on attendant opinions at a meeting (https://www.jshm.net/file/genatsu/shizuokacheck.pdf).

From May 2016 to December 2020, we conducted a retrospective diving accident checklist review of all patients with diving accident, who were transported by the HEMS. Excluded criteria was the patients with diving accident, who were transported by the ground ambulance. Personal information, vital signs, and prehospital treatments, diving profile, information of recorder and key person, in the diving accident checklist was classified into items A, B, C and D respectively. Item A mainly consisted of personal information, including the patient's diving history and name of the associated diving shop. Item B mainly consisted of the patient's vital signs, prehospital treatments and prehospital clinical course. Item C mainly consisted of the patient's diving profile and risk factors of decompression sickness, and Item D consisted of information obtained from recorders and individuals accompanying the patient. There were 14 questions in item A, 11 in B, 11 in C, and 4 in D, and each question was assigned 1 point (Figure 1). Accordingly, if all questions were answered, full marks were 40 points. In the case of the patients, who did not have the diving accident checklist even the patients had had diving accident and transported by the HEMS, their total scores were 0 point. We also collected the following data for each subject: sex, age, chief complaint, existence of cardiac arrest or not, and final outcome (survival or death). Subjects were divided into two groups: the Arrest group, which included subjects who became cardiac arrest in prehospital setting, and the Control group, which included subjects who did not become cardiac arrest in prehospital setting. Because patients with cardiac arrest required multiple managements, such as chest compression, tracheal intubation, bag valve mask ventilation and securing a venous route at the rendezvous point, we hypothesized that it would be difficult to fill out a diving accident checklist in such a situation. The variables were compared between the two groups.

The JMP 15.0 software program (SAS Japan Incorporation, Tokyo, Japan) was used to perform the statistical analyses. A statistical analysis was performed using Student's unpaired t-test, the chi-squared test or a contingency table analysis. $P$ values of $<0.05$ were considered to be statistically significant. Data are shown as the mean ± standard deviation.

## Results

During the investigation period, a total of 86 patients with diving accident were transported by the HEMS. Among these patients, 16 had cardiac arrest in the prehospital setting and these were

assigned as the Arrest group, and remaining 70 were assigned as the Control group. Nineteen patients did not have the diving checklist (5 patients in the Arrest group and 14 in the Control group). The all subjects in the Arrest group finally died and the all subjects in the Control group survived. Results of analysis between the two groups were shown in Table 1. Sex was not statistical difference between the two groups. The average age in the Arrest group was significantly greater than that in the Control group. Average points in the item B, item C and total score in the Arrest group were significantly smaller than those in the Control group. Average points in the item A and item D and in the Arrest group were smaller than those in the Control group, however, these differences were not significant. After excluding subjects who did not have the diving accident checklist, the same tendencies remained (Table 2).

## Discussion

The present study showed that degree of completion of the diving accident checklist in the most severely ill cases (cardiac arrest in the prehospital setting) was low in comparison with cases without cardiac arrest. The diving accident checklist was useful for diagnosing decompression sickness and determining the therapeutic recompression table[6, 10]. In addition to diving accidents, such a checklist has also been used in other emergency situations as well. In acute life-threatening situations in France, a checklist is now commonly used by firefighters on the spot to request the dispatch of physicians to the scene of the accident. The physician on site must ascertain the patient's needs in order to preserve the life and vital functions and also ensure that the patient is sent to the appropriate emergency healthcare facility[11]. In Italy, the use of a checklist for quality assurance in the treatment of acute myocardial infarction in the coronary care unit has helped provide information essential for the evaluation of therapeutic protocols; it might also help improve the cooperation between the emergency department, attending cardiologists, and family physicians[12]. This framework in Italy is similar to that used in the present study. However,

**Table 1** Results of analysis

| | | Cardiac arrest (n=16) | Control (n=70) | p value |
|---|---|---|---|---|
| Sex (male/female) | | 11/5 | 45/25 | 0.7 |
| Age | | 51.5 ±11.4 | 43.2 ± 13.5 | <0.05 |
| Checklist | A item (full score 14 points) | 6.3 ± 4.9 | 8.5 ± 5.1 | 0.05 |
| | B item (full score 11 points) | 4.3 ± 3.9 | 6.6 ± 3.8 | < 0.05 |
| | C item (full score 11 points) | 4.0 ± 3.3 | 6.7 ± 3.8 | < 0.01 |
| | D item (full score 4 points) | 1.0 ± 1.2 | 1.7 ± 1.5 | 0.1 |
| | In total (full score 40 points) | 15.7± 12.2 | 23.6± 12.8 | <0.01 |

Data are shown as the mean ± standard deviation.

**Table 2** Results of analysis after excluding subjects without diving accident checklist

| | | Cardiac arrest (n=11) | Control (n=56) | p value |
|---|---|---|---|---|
| Sex (male/female) | | 7/4 | 36/20 | 0.6 |
| Age | | 53.5 ±9.4 | 42.3 ± 13.8 | 0.01 |
| Checklist | A item (full score 14 points) | 9.2 ± 2.5 | 10.6 ± 3.1 | 0.05 |
| | B item (full score 11 points) | 6.2 ± 3.1 | 8.2 ± 2.1 | < 0.05 |
| | C item (full score 11 points) | 5.9 ± 2.1 | 8.4 ± 2.1 | < 0.01 |
| | D item (full score 4 points) | 1.4 ± 1.2 | 2.1 ± 1.5 | 0.1 |
| | In total (full score 40 points) | 22.9 ± 6.7 | 29.5 ± 5.4 | <0.01 |

Data are shown as the mean ± standard deviation.

the present study highlighted several flaws associated with filling out the diving accident checklist.

There were several considerable reasons concerning low degree of completion of the diving accident checklist in case of cardiac arrest. First, in cardiac arrest case during or immediately after diving, this is impossible to obtain information of diving profile from the victim directly. Second, we experienced that an instructor, who had become buddy with the victim, became panic so that it was impossible to make hearing of diving profile. We also experienced that an instructor, who had become buddy with the victim, was restrained by policemen due to cardiac arrest through suspected negligence in the pursuit of social activities. In such cases, it was impossible to make hearing of diving profile immediately. Third, a patient with cardiac arrest in the prehospital setting required many medical interventions, such as monitoring, bag valve mask ventilation, chest compression, electrical shock, securing airway, securing venous route and infusion of adrenaline every four minutes. In addition, reporting the patient's condition to medical staff at the receiving hospital via phone and filling out the paper-based ambulance report form were also required. In this situation, emergency medical technicians and/or medical staffs of the HEMS had few time to obtain information from the instructor who had become buddy with the victim. The fourth, recording the diving accident checklist was cooperation matter , and this was not essential document unlike the paper-based ambulance report form which was essential to record[13]. The fifth, due to COVID-19 pandemic, the regular meeting was postponed to avoid three Cs, namely: 'closed spaces with poor ventilation', 'crowded spaces with many people', and 'close contact' for 2 years[14]. As a result, medical staffs of the HEMS or emergency medical technicians might forget existence of the diving accident checklist as diving accidents are relatively rare, with only around 10 cases occurring each year[1].

The present study highlighted weak points associated with filling out the diving accident checklist. As one of solution, staffs of the control room provide instruction of the checklist when the HEMS dispatches to a diving accident to recall the checklist. Another solution may be focus on fulfilling diving profile in the checklist (Item C in the present study), which staffs of the receiving hospital for HBO thought as most useful, to become shortening recording the checklist during transportation. Information of patients private profile or information of prehospital medical interventions, were also recorded in the paper-based ambulance report form, which could be faxed or transcribed later. If the victim or instructor had a dive computer records, obtaining information from the dive computer might be useful to fulfill the diving checklist[15]. However, the dive computer records only provide the diving profile, so not all items on the diving checklist at present would be able to be filled out in this manner. Finally, if the COVID-19 pandemic continues, web conference may be useful to update information of DCI or diving accident including results of the present study[16].

The present study is associated with several limitations, including the small population size, single-institute setting and retrospective nature. We did not evaluate patients with decompression illness who were transported via ground ambulance when the HEMS was unable to fly (e.g. at night or in times of bad weather or overlapping requests). When patients with decompression sickness are transported via ground ambulance, there is sufficient time to fill out the diving accident checklist, even if the patient is in cardiac arrest. Finally, we did not attempt any of the remedies mentioned above, so further efforts will need to be made to improve the diving accident checklist.

## Conclusion

The present study clarified issues with the degree of completion of the diving accident checklist in cases with cardiac arrest was low in comparison with cases without cardiac arrest. To improve this, further approaches, such as shortening the checklist and focusing on the diving profile and/or obtaining data from a diving computer, will be required in the future.

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## Author contributions

Study concept and design (YY); acquisition of the data (MO, ST, YN); analysis of the data (MO, YY); drafting of the manuscript (MO, YY); critical revision of the manuscript (ST, YN); and approval of the final manuscript (MO, ST, YN, YY).

## Conflicts of interest statement

The authors declare no conflicts of interest in association with this study.

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Original Articles

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# A Machine Learning Prediction Model for Non-cardiogenic Out-of-hospital Cardiac Arrest with Initial Non-shockable Rhythm

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***Objectives***: The purpose of this study was to develop and validate a machine learning prediction model for the prognosis of non-cardiogenic out-of-hospital cardiac arrest (OHCA) with an initial non-shockable rhythm.

***Design***: Data were obtained from a nationwide OHCA registry in Japan. Overall, 222,056 patients with OHCA and an initial non-shockable rhythm were identified from the registry in 2016 and 2017. Patients aged <18 years and OHCA caused by cardiogenic origin, cancer, and external factors were excluded. Finally, 58,854 participants were included.

***Methods***: Patients were classified into the training dataset (n=29,304, data from 2016) and the test dataset (n=29,550, data from 2017). The training dataset was used to train and develop the machine learning model, and the test dataset was used for internal validation. We selected XGBoost as the machine learning classifier. The primary outcome was the poor prognosis defined as cerebral performance category of 3–5 at 1 month. Eleven prehospital variables were selected as outcome predictors.

***Results***: In validation, the machine learning model predicted the primary outcome with an accuracy of 90.8% [95% confidence interval (CI): 90.5–91.2], a sensitivity of 91.4% [CI: 90.7–91.4], a specificity of 74.1% [CI: 69.2–78.6], and an area under the receiver operating characteristic value of 0.89 [0.87–0.92]. The important features for model development were the prehospital return of spontaneous circulation, prehospital adrenaline administration, and initial electrical rhythm.

***Conclusions***: We developed a favorable machine learning model to predict the prognosis of non-cardiogenic OHCA with an initial non-shockable rhythm in the early stage of resuscitation.

***Key words***: cardiac arrest, non-shockable rhythm, machine learning, prediction model, termination of resuscitation

## Introduction

A large number of out-of-hospital cardiac arrests (OHCAs) occur worldwide. The number of patients with OHCA in Europe and the United States is 275,000 and 42,000 per year[1], respectively. Emergency physicians and researchers have contributed a great deal of efforts and research to improve resuscitation. Unfortunately, the prognosis for patients with OHCA is still poor, especially for those with an initial non-shockable rhythm. In many cases, medical professionals must decide to stop resuscitation. However, it is difficult to accurately predict the prognosis for patients with OHCA at an early stage based on the scene of the emergency and the complex information it involves. The legitimacy of the decision to stop resuscitation is unclear, but the fact is that it is ultimately left to the individual judgment of medical professionals.

In recent years, the usefulness of prognostic models using machine learning has been reported on for patients with OHCA[2–4]. Hirano et al. demonstrated the favorable performance of a machine learning model for predicting outcomes in patients with OHCA and an initial shockable rhythm[5]. Reliable prognostication of this specific population with

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a high probability of cardiogenic cause might support clinicians' treatment choices, such as extracorporeal cardiopulmonary resuscitation, percutaneous coronary intervention, and temperature management. However, there has been no report of a machine learning model that can support clinician decision-making for discontinuing resuscitation specifically for patients with non-shockable rhythms, a population that has poor prognostic outcomes among patients with OHCA.

In this study, we aimed to develop and validate a machine learning model for patients with non-cardiogenic OHCA and non-shockable rhythms.

## Materials and Methods

### Study design, data sources, and ethical approval

This retrospective cohort study used data from the All-Japan Utstein Registry, a nationwide prospective OHCA registry established in 2005 by the Fire and Disaster Management Agency in Japan. The registry is based on a set of international Utstein-style guidelines and includes data on all patients with OHCA transported by emergency medical services in Japan. Survival and cerebral performance category (CPC) results 1 month after onset were included in this registry from 2016 to 2017.

The study protocol was approved by the Ethics Committee of Juntendo Urayasu Hospital (protocol number: U20-0011), and the requirement for informed consent was waived owing to the retrospective design.

### Study population and outcomes

A flow diagram of the study population selection is shown in Figure 1. We extracted 250,572 patients with OHCA from the All-Japan Utstein Registry between 2016 and 2017. After we excluded 16,401 cases of initial shockable electrical rhythm and 12,115 patients who survived when paramedics arrived on-site, 222,056 patients with OHCA and an initial non-shockable rhythm were identified. Of these, patients aged <18 years and those with OHCA caused by cardiogenic origin, cancer, and external factors, including intoxication, trauma, accidental hypothermia, drowning, and anaphylaxis, were excluded, as they comprised separate patient subsets in terms of prognosis or cardiac arrest etiology. Finally, 58,901 adult patients with non-cardiogenic OHCA and an initial non-shockable rhythm were identified. After 47 cases with missing values for the minutes from the emergency medical service (EMS) call to hospital arrival time or EMS call to paramedics' site arrival time were deleted list-wise, data were subsequently classified into the training dataset (n=29,304, data from 2016) for the development of the machine learning model and the test dataset (n=29,550, data from 2017) for internal validation.

The primary outcome in this study was poor outcome at 1 month. A poor outcome was defined as CPC of 3-5. The secondary outcome was death at 1 month.

### Predictor Variables

Eleven prehospital variables were selected as outcome predictors of OHCA. These prehospital variables were sex, age, presence of prehospital physician, event witness, bystander cardiopulmonary resuscitation (CPR), initial electrical rhythm, prehospital defibrillation, prehospital adrenaline administration, return of spontaneous circulation (ROSC), EMS call to hospital arrival time, and EMS call to paramedics' site arrival time.

### Training of the machine learning model

Using the training dataset, we trained and developed an XGBoost machine learning model as a classifier for outcome prediction. In the training process, a 10-fold cross-validation was performed. The training data were split into 10 stratified subsets. Nine subsets (90% of training data) were used to train the model, and the remaining subset (10% of training data) was used for validation. These training and validation processes were repeated 10 times with each subset used once as a validation dataset, allowing us to obtain 10 estimates of predictive accuracy, which were averaged to obtain a single estimate. Thus, we avoided overfitting the model and searched for hyperparameters to obtain the best accuracy for outcome prediction. The weighting of rare outcomes by the ratio of the number of minor classes to the majority class was also used to control the outcome imbalance of the data.

### Internal validation of the machine learning model

The performance of the developed machine learning model was validated using the test dataset. We measured the area under the receiver oper-

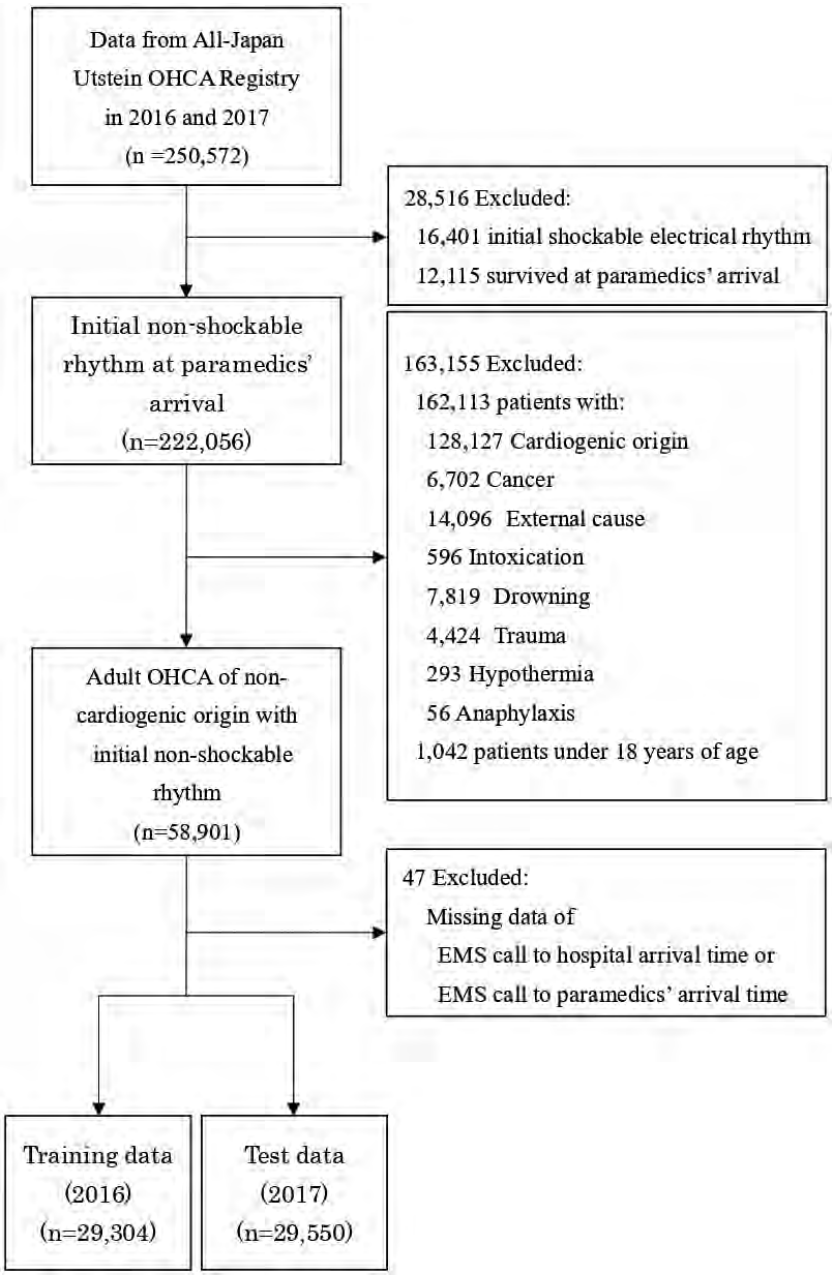



**Figure 1** Flow diagram of patient inclusion
OHCA: Out-of-hospital Cardiac Arrest, EMS: Emergency Medical Service.

ating characteristic curve (AUROC), area under the precision-recall curve (AUPR), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy as performance indicators. The feature importance for developing the XGBoost model was computed as the normalized total reduction of the criterion brought about by the feature, which is known as the Gini importance.

**Statistical analysis and library for machine learning**

Scikit-learn (version 0.21.3) with Python was employed for model development. Statistical analyses of the characteristics of the cohorts were performed using SciPy (version 1.4.1) and Python (version 3.7.4, in Anaconda 2019.10). Continuous variables are reported as means and standard deviations, and categorical variables are reported as counts and percentages. A t-test was used to compare the means between the two samples. A chi-square test was used to compare the frequencies. All tests were two-sided, and the significance level was set at 5% ($p<0.05$).

## Results

### Characteristics of study participants

The main characteristics of included patients with non-cardiogenic OHCA and an initial non-shockable rhythm are shown in Table 1. The mean age of the patients was 77.2 years, and 53.6% were men. The initial electrical rhythm comprised of 30.8% pulseless electrical activity and 69.2% asystole. Event witness and bystander CPR were observed in 46.1% and 25.0% of all cases, respectively. When comparing the training and test datasets, significantly lower rates of bystander-performed CPR were observed in the test dataset than those in the training dataset (23.6% vs. 26.3%, respectively). In contrast, adrenaline was administered more frequently at the prehospital scene in the test dataset (22.6%) than that in the training dataset (20.9%). The minutes from the EMS call to paramedics site arrival time was statistically different between these two cohorts; however, the absolute value of the difference was quite low.

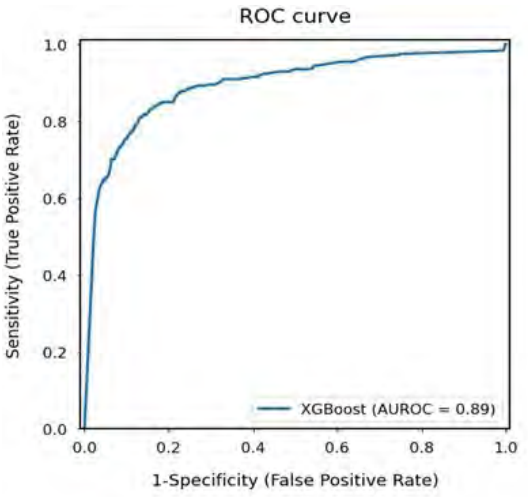
### Performance of the developed machine learning model

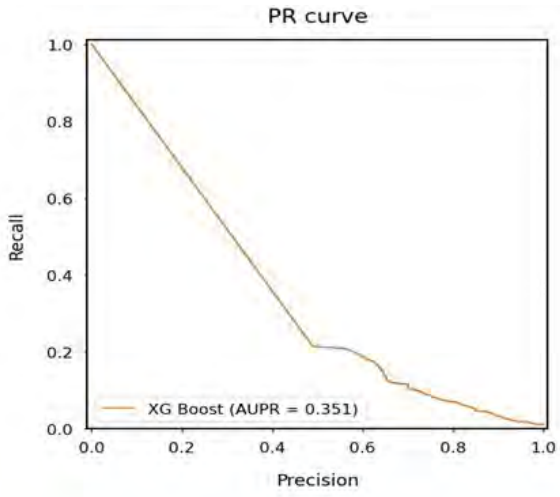
Figure 2 shows the ROC curve; PR curve; confusion matrix; and evaluation measures such as sensitivity, specificity, PPV, NPV, accuracy, AUROC, and AUPR values obtained in the test set model validation for the primary outcome. For the predic-

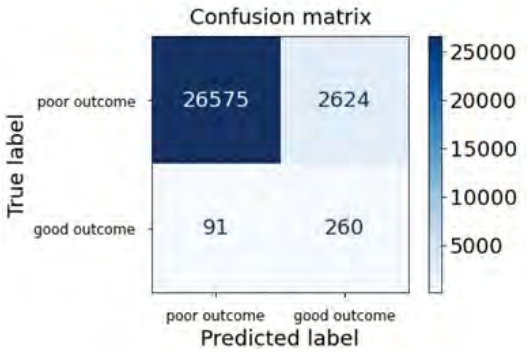
**Table 1** Characteristics of study participants

| Variable | All (n=58,854) | Training data (n=29,304) | Test data (n=29,550) | P value |
|---|---|---|---|---|
| Age (years) | 77.2 [14.8] | 77.1 [14.9] | 77.4 [14.7] | 0.27 |
| Sex (men) | 31,544 (53.6%) | 15,699 (53.5%) | 15,845 (53.6%) | 0.91 |
| Event witness | 27,150 (46.1%) | 13,567 (46.2%) | 13,583 (46.0%) | 0.42 |
| Bystander CPR | 14,690 (25.0%) | 7,730 (26.3%) | 6,960 (23.6%) | <0.01 |
| Initial electrical rhythm | | | | 0.76 |
| Pulseless electrical activity | 18,154 (30.8%) | 9,056 (30.9%) | 9,098 (30.8%) | |
| Asystole | 40,700 (69.2%) | 20,248 (69.0%) | 20,452 (69.2%) | |
| Defibrillation | 1,671 (2.8%) | 849 (2.9%) | 822 (2.8%) | 0.47 |
| Prehospital ROSC | 6,400 (10.9%) | 3,137 (10.7%) | 3,263 (11.0%) | 0.19 |
| Prehospital physician | 2,029 (3.4%) | 1,046 (3.5%) | 983 (3.3%) | 0.11 |
| Adrenaline administration | 12,816 (21.8%) | 6,126 (20.9%) | 6,690 (22.6%) | <0.01 |
| EMS call to hospital arrival time (minutes) | 33.9 [13.0] | 33.9 [13.3] | 33.9 [12.7] | 0.62 |
| EMS call to paramedics' site arrival time (minutes) | 9.2 [4.4] | 9.1 [4.6] | 9.2 [4.2] | 0.01 |
| Outcomes | | | | |
| Poor outcome at 1 month | 58,142 (98.8%) | 28,943 (98.8%) | 29,199 (98.8%) | 0.62 |
| Death at 1 month | 56,259 (95.6%) | 28,006 (95.6%) | 28,253 (95.6%) | 0.81 |

Categorical variables are presented as n (%). Continuous variables are presented as the mean [standard deviation]. CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; EMS, emergency medical service.




| Statistical measures | Value | 95% CI |
|---|---|---|
| Sensitivity | 91.4% | 90.7 - 91.4 |
| Specificity | 74.1% | 69.2 - 78.6 |
| Positive Predictive Value | 99.7% | 99.6 - 99.7 |
| Negative Predictive Value | 9.0% | 8.4 - 9.6 |
| Accuracy | 90.8% | 90.5 - 91.2 |
| AUROC | 0.89 | 0.87 - 0.92 |
| AUPR | 0.35 | 0.32 - 0.38 |

**Figure 2** ROC curve, confusion matrix, and statistical measures of performance of the machine learning model to predict primary outcome
AUROC: Area Under the Receiver Operating Characteristic Curve, AUPR: Area Under the Precision Recall, CI: Confidence Interval.

tion of death or survival with poor neurological function at 1 month, the developed machine learning model demonstrated a favorably high AUROC value of 0.89 (95% confidence interval [CI]: 0.87–0.92). In contrast, the AUPR was relatively low at 0.35 (95% CI: 0.32–0.38). The sensitivity and specificity were 91.4% and 74.1%, respectively. It also showed a high PPV (99.7%). The accuracy of the validation was 90.8%.

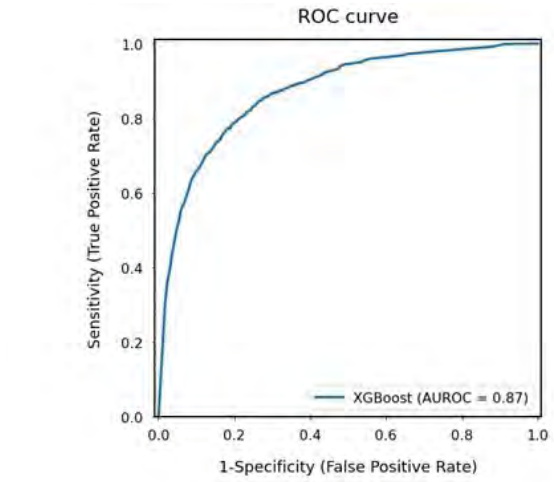
Figure 3 shows the ROC curve; PR curve; confusion matrix; and evaluation measures such as sensitivity, specificity, PPV, NPV, accuracy, AUROC, and AUPR values obtained in the test set model validation for the secondary outcome. For the prediction of death at 1 month, the developed machine learning model demonstrated a favorably high AUROC value of 0.87 (95% CI: 0.86–0.88). In contrast, the AUPR was relatively low at 0.38 (95% CI: 0.37–0.40). The sensitivity and specificity were 83.5% and 74.9%, respectively. It also showed a high PPV (98.6%). The accuracy of the validation was 83.1%.

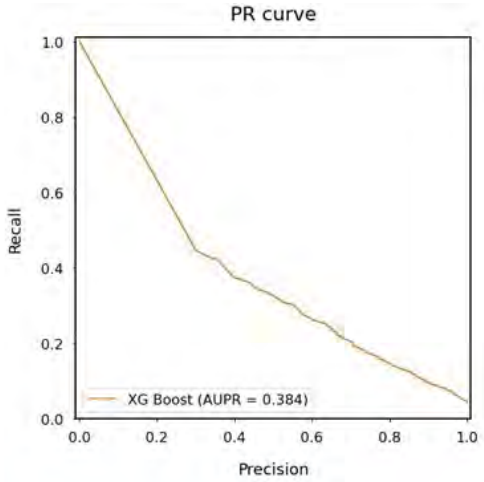
### Evaluation of feature importance

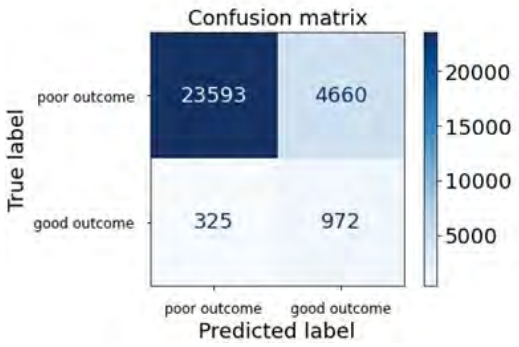
Figure 4 shows the feature importance for developing the machine learning model. The essential feature to develop the model was the prehospital ROSC. The second and third most important features were adrenaline administration and initial rhythm for the primary outcome, and initial rhythm and event witness for the secondary outcome. However, they were much less decisive features than the prehospital ROSC.

## Discussion

To the best of our knowledge, this study is the first to develop and internally validate a machine learning-based outcome prediction model targeting the population of patients with non-cardiogenic OHCA and an initial non-shockable rhythm. Our purpose was to assess the predictive performance




| Statistical measures | Value | 95% CI |
|---|---|---|
| Sensitivity | 83.5% | 83.1 - 83.9 |
| Specificity | 74.9% | 72.5 - 77.3 |
| Positive Predictive Value | 98.6% | 98.5 - 98.8 |
| Negative Predictive Value | 17.3% | 16.7 - 17.9 |
| Accuracy | 83.1% | 82.7 - 83.6 |
| AUROC | 0.87 | 0.86 - 0.88 |
| AUPR | 0.38 | 0.37 - 0.40 |

**Figure 3** ROC curve, confusion matrix, and statistical measures of performance of the machine learning model to predict secondary outcome
AUROC: Area Under the Receiver Operating Characteristic Curve, AUPR: Area Under the Precision Recall, CI: Confidence Interval.

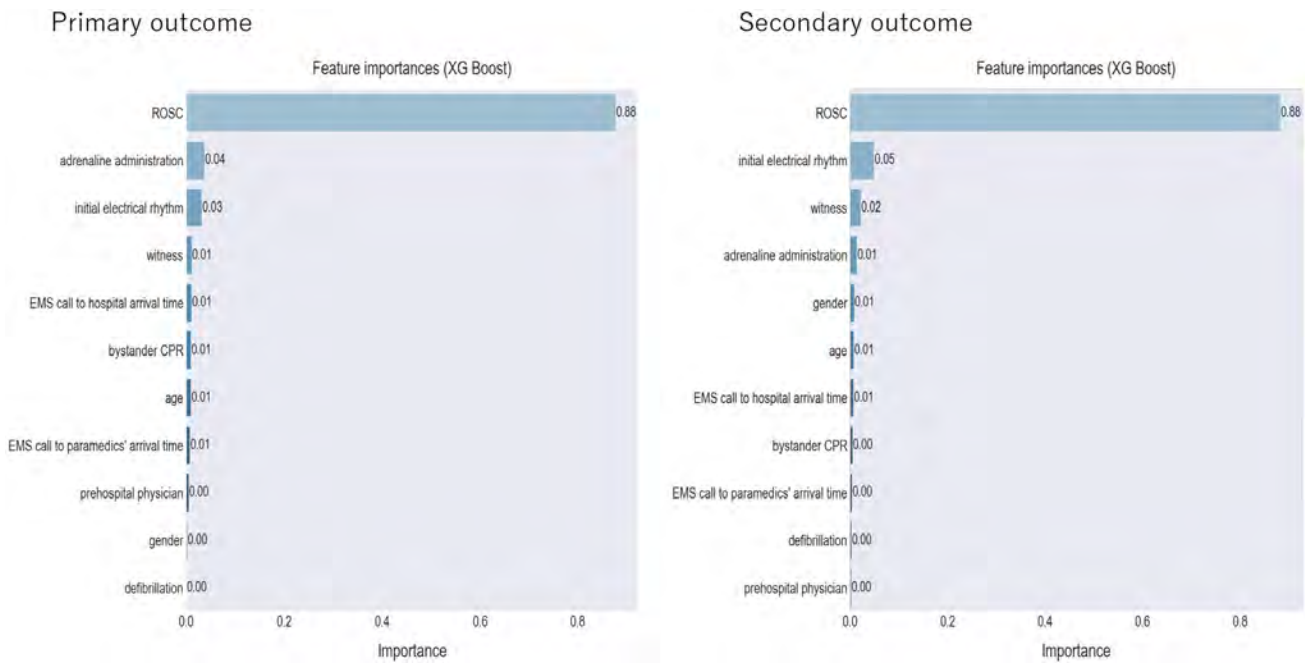



**Figure 4** Feature importance of the model variables
ROSC: Return of Spontaneous Circulation, EMS: Emergency Medical Service, CPR: Cardiopulmonary Resuscitation.

of the machine learning in OHCA and its possibility of use in the clinical setting in the future. In summary, our machine learning model, developed using 11 prehospital variables from the All-Japan Utstein Registry, showed a favorable prognostic performance in predicting a poor outcome of OHCA at 1 month, with a high PPV of 98.6%, encouraging the possibility of the model being used to decide the termination of resuscitation for patients with non-cardiogenic OHCA and a non-shockable rhythm.

Individuals under 18 years of age and those with OHCA caused by cardiogenic origin, cancer, and external factors were excluded from the study population. This selection of the OHCA population for the study was considered in order to include adult patients with endogenous cardiac arrest, whose cases often involve an uncertainty about the decision to interrupt resuscitation in the emergency department. In addition, this study did not include patients who were not in cardiac arrest at the time of EMS contact, even if they experienced cardiac arrest with an initial non-shockable rhythm. These patients were not included because they had a high chance of being resuscitated; thus, clinicians were not deciding on the early termination of resuscitation (TOR). Thus, we carefully selected patients for inclusion in the study in view of the usability of the prediction model to determine TOR in clinical practice.

Many efforts have been made to create specific rules for the TOR without relying solely on the clinician's judgment. Various TOR rules have been developed and validated[6-10]. A very high PPV is required for the use of TOR rules, owing to their ethical aspects. Similar to other TOR rules, our machine learning model showed a very high PPV of 99.7% in the internal validation. Although the question remains whether this value of 99.7% is sufficient for resuscitation interruption, it is considered reasonable to judge futility based on a percentage of expected therapeutic effect of 1% or less in Europe and the United States[11]. Therefore, the results of our study may provide a basis for using this machine learning prediction model for clinical use. However, it is necessary to consider not only whether the patient is alive or dead but also whether the family is present when resuscitation is interrupted and other aspects involving the time of death diagnosis. Ultimately, TOR rules should be carefully introduced into the emergency medical system based on the public's ethical viewpoints.

The feature with the greatest importance for our machine learning model development in the primary and secondary outcome was ROSC. Unfortunately, the results showed that OHCA cases with initial non-shockable rhythms that were not successfully resuscitated in prehospital settings resulted in poor outcomes. The second contributing feature in the primary outcome was prehospital adrenaline administration, although it made a very small contribution to the development of the prognostic model compared with the feature of prehospital ROSC. Although none of the previous TOR rules included prehospital adrenaline administration, early adrenaline administration in patients with OHCA and a non-shockable rhythm has been reported to increase ROSC rates[11-14]. Therefore, early administration of adrenaline is likely to be an important prognostic factor. Similarly, in the secondary outcome, initial electrical rhythm and witness were related to poor outcomes following ROSC. Thus, these prognostic features derived from our machine leaning models are consistent with historically proven, general understanding of clinicians and researchers that no prehospital ROSC, no prehospital adrenaline administration, no witnesses, and a non-shockable rhythm on the initial electrical rhythm are associated with a poor prognosis

Our machine learning model requires 11 predictive variables, more than the number of other TOR rules. Other TOR rules have only three to five criteria; therefore, it is easier to decide the discontinuation of resuscitation in the field because of simplicity. However, it is possible for our model to overcome this limitation by using technologies such as speech recognition or optical character recognition.

Our study has a strength in the use of a nationwide database. Previous studies on OHCAs used datasets restricted to specific regional areas[15, 16], and access to medical facilities, population density, and patient characteristics (such as underlying diseases) may differ between urban and rural areas. This difference may have a strong influence on the outcome of patients with OHCA. The nationwide Utstein database used in this study eliminates these regional differences. Additionally, another strength of our study is not only to review the importance of poor outcome prognostic factors

have been reported using machine learning but also to indicate a possibility that we can put the result to clinical use using some devices such as applications.

Our study had several limitations. First, this study was based only on data from Japanese patients for model training and validation. Therefore, the results cannot be generalized to countries with different emergency care systems. External validation using datasets from other communities or countries is also required. Second, listwise deletion of cases with missing data was performed during the data-cleaning process, which can decrease the sample size and cause bias in the parameter estimates. However, other methods to deal with missing data, such as multiple imputations, also cause bias. Moreover, some studies have used the same database and also treated missing data with listwise deletion[17]. Third, the dataset used for the current study was a bit old, precluding the guarantee of similar performance in future cases. However, the sample size is large, and remarkable innovation in the diagnostic or treatment process of OHCA resuscitation has not occurred in recent years. Thus, there is no substantial reason that influences model performance. Nevertheless, it is better that the machine learning model should be hopefully re-assessed and re-validated using the new dataset. Especially, when the model is intended to use in the clinical setting, the model should be validated using the new and external dataset.

In conclusion, we developed a favorable machine learning model to predict the prognosis of non-cardiogenic OHCA with an initial non-shockable rhythm using only prehospital information. Although the model should be externally validated in the future, this study has demonstrated the potential of a machine learning-based outcome prediction model in facilitating TOR decision-making for non-cardiogenic OHCA with an initial non-shockable rhythm.

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## Author contributions

SK and YH analyzed and interpreted the patient data regarding out-of-hospital cardiac arrest with an initial non-shockable rhythm. All authors have read and approved the final manuscript.

## Conflicts of interest statement

YH is the Chief Executive Officer, MedPop Co. Ltd. None of the other authors declare no conflict of interest.

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Original Articles

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# Fcα Receptor Type I and Its Association with Atherosclerosis Development

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***Objectives***: Atherosclerosis is a chronic inflammatory disease characterized by lipid accumulation and local inflammation, which are regulated by the immune system. The immunological aspects of this disease are unclear. Immunoglobulin A regulates many cell responses through interactions with Fc*α* receptor type I (Fc*α*RI). Anti-Fc*α*RI antibody inhibits activating receptors by inducing an inhibitory immunoreceptor tyrosine-based activation motif configuration. However, the role of Fc*α*RI in atherosclerosis development is unclear. Here, we investigated the utility of Fc*α*RI targeting to induce inhibitory immunoreceptor tyrosine-based activation motif signaling in atherosclerosis treatment.

***Materials***: ApoE$^{-/-}$ transgenic mice expressing the Fc*α*RIR209L/FcR*γ* chimeric protein (Fc*α*RIR209L/FcR*γ*ApoE$^{-/-}$ mice) were generated. We prepared an Fc*α*RIR209L/FcR*γ* transfectant (I3D) from a mouse macrophage cell line (RAW264.7).

***Methods***: Anti-Fc*α*RI or control antibody was used to investigate a high-fat-diet-induced Fc*α*RIR209L/FcR*γ*ApoE$^{-/-}$ mouse model of atherosclerosis. The antibody was also used to assess macrophage foam cell formation via Oil Red O staining and mitogen-activated protein kinase signaling via immunoblotting in the Fc*α*RIR209L/FcR*γ*-expressing RAW264.7 macrophage cell line I3D.

***Results***: Targeting of monovalent Fc*α*RI induced inhibitory effects in the Fc*α*RIR209L/FcR*γ*ApoE$^{-/-}$ mouse model of atherosclerosis by inhibiting macrophage infiltration. Fc*α*RI targeting using the anti-Fc*α*RI antibody also reduced mitogen-activated protein kinase signaling and foam cell formation, leading to decreased interleukin (IL)-1b and monocyte chemoattractant protein (MCP)-1.

***Conclusions***: We demonstrated that targeting monovalent Fc*α*RI suppresses atherosclerosis development. These findings can support the future clinical exploration of Fc*α*RI targeting for atherosclerosis treatment.

***Key words***: atherosclerosis, oxidized low-density lipoprotein, mitogen-activated protein kinase signaling, macrophage foam cell, Fc*α*RI

## Introduction

Atherosclerosis is a chronic disease with a multifactorial etiology that ultimately leads to the development of rupture-prone plaques and atherothrombotic events[1)]. Clinical trials and animal experiments have supported the notion that advanced plaques share common properties including augmented lipid-rich necrotic core and macrophage accumulation[2)]. Macrophages often accumulate in various regions of vulnerable plaques and are the main source of cytokines[3)]. During the last decade, oxidized low-density lipoprotein (ox-LDL) and its interactions with monocytes/macrophages were considered the primary atherogenic components in dyslipidemia[4)]. The concentration of ox-LDL is markedly elevated in atherosclerotic lesions and reaches cytotoxic levels and subsequent inflammatory events[3)]. Mitogen-activated protein kinase (MAPK)-induced phosphorylation events play important roles in macrophage

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migration in plaques[5]. Intracellular MAPK signaling cascades are involved in the pathogenesis of cardiac and vascular diseases. In macrophages, an interaction between CD36 and ox-LDL induces the phosphorylation of Lyn, one of several Src-family tyrosine kinases in immune cells, and subsequent activation of extracellular signal-regulated kinase (ERK) and p38 mediates the uptake of ox-LDL[6], leading to inhibition of MAPK signaling and attenuation of foam cell formation[7]. Ox-LDL induces vascular smooth muscle cell proliferation and inflammation with the foam cell formation[8]. Since MAPK signaling pathway affects foam cell aggregation and inflammatory responses, inhibition of nuclear factor-$\kappa$B and MAPK signaling attenuates atherosclerosis[9].

Fc*α* receptor type I (Fc*α*RI; CD89) is the only Fc receptor specific to immunoglobulin A (IgA) expressed on myeloid cells, including macrophages, monocytes, dendritic cells, Kupffer cells, neutrophils, and eosinophils[10]. Fc*α*RI is expressed in the presence or absence of a physical association with the Fc$\gamma$R adaptor, which contains an immunoreceptor tyrosine-based activation motif (ITAM). Fc*α*RI, a unique member of the FcR family, exerts a dual role in immune cell inhibition and activation[10, 11]. It is known that an inhibitory signal is generated when monomeric IgA (mIgA) binds to two Fc*α*RI, while an active signal is required the binding of IgA-immune complexes to Fc*α*RI. Serum IgA is generated mainly as a monomeric form (about 85% to 90% of total serum IgA) by bone marrow plasma cells. Therefore, we think that an inhibitory signal of Fc*α*RI is dominant at least in circulation. An inhibitory ITAM (ITAMi) configuration induced by monovalent targeting of Fc*α*RI (anti-Fc*α*RI Fab fragment) initiates the recruitment of Src homology domain 2-containing protein-tyrosine phosphatase-1 (SHP-1), which has inhibitory potential[12]. This step leads to the deactivation of the inflammatory reaction, thereby preventing autoimmune processes. Previous studies demonstrated the involvement of Fc$\gamma$R signal activation through the ITAM-containing Fc$\gamma$R adaptor in diseases[5, 13]. The anti-Fc*α*RI fragment antigen-binding (Fab) region negatively regulates the magnitude of the innate immune response and has been used as an anti-inflammatory drug to treat kidney diseases[14]. Furthermore, the inhibitory signal induced by anti-Fc*α*RI Fab in the Fc*α*RIR209L/FcR$\gamma$ chimeric receptor is more potent than that in wild-type Fc*α*RI, which is expressed in the presence or absence of a physical association with the Fc$\gamma$R adaptor.

Fc*α*RI targeting can halt disease progression and lupus activation by selectively inhibiting cytokine production, leukocyte recruitment, and renal inflammation[15]. Fc*α*RI-mediated inhibition can suppress several inflammatory diseases in mice, including asthma and glomerulonephritis. Intravenous mIgA and anti-Fc*α*R monovalent antibodies are promising tools for immunotherapy[16]. In this study, we aimed to evaluate whether Fc*α*RI targeting can prevent atherosclerosis.

## Materials and Methods

### Animals

The mice were bred and maintained in the mouse facilities of the Research Institute for Diseases of Old Age (Juntendo University School of Medicine, Tokyo, Japan). All experiments were conducted in accordance with national guidelines and were approved by a local ethics committee (Juntendo University School of Medicine Animal Experiment Committee; the approval number is 270258).

### Production of the construct, generation of Fc*α*RIR209L/FcR$\gamma$ transgenic (Tg) ApoE$^{-/-}$ mice, and preparation of Fc*α*RIR209L/FcR$\gamma$ transfectant

A construct encoding human Fc*α*RIR209L/FcR$\gamma$-FLAG was obtained by inserting an 1165-bp cDNA fragment into the EcoRI site of a CAG promoter containing $\beta$-actin (UniTeck, Kashiwa, Japan). The transgenic mouse contained human Fc*α*RIR209L/FcR$\gamma$-FLAG cDNA obtained via the polymerase chain reaction of tail DNA using the transgene-specific primers 5′-GGGTCATTAGTTCATAGCC-3′ and 5′-GGCATATGATACACTTGAT-3′. To determine whether inhibitory Fc*α*RI diminishes the progression of atherosclerosis, Fc*α*RIR209L/FcR$\gamma$ transgenic (Tg) ApoE$^{-/-}$ mice were generated. All mice used in this study were bred and housed under strictly controlled specific pathogen-free conditions. We prepared Fc*α*RIR209L/FcR$\gamma$ transfectant (I3D) cells from a mouse macrophage cell line (RAW264.7) using a Cell Line Optimization Nucleofector Kit (Lonza, Basel, Switzerland) (Figure 1).

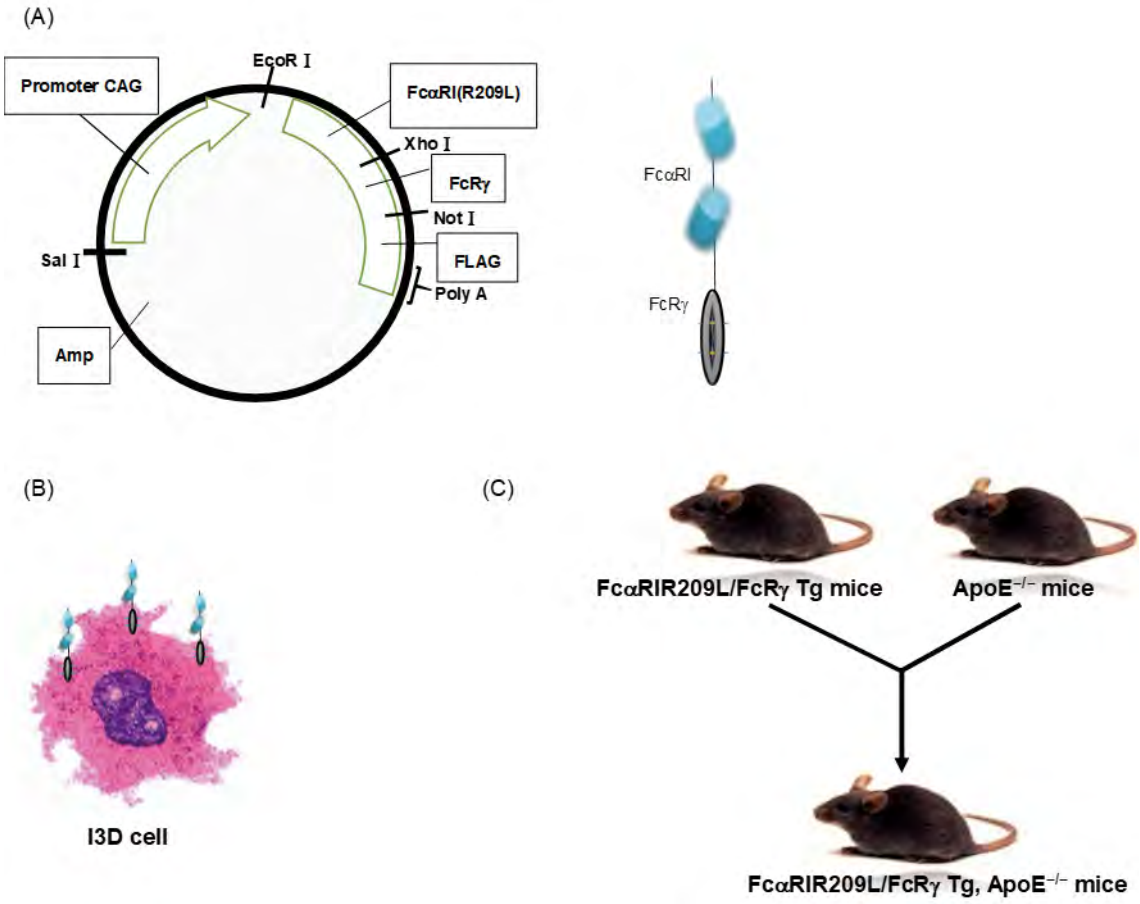


**Figure 1** Generation of a mouse macrophage transfectant expressing high levels of Fc*α*RIR209L/FcR*γ* (I3D cells), Fc*α*RIR209L/FcR*γ* transgenic (Tg) mice, and Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mice
The transgene consists of cDNA encoding human Fc*α*RIR209L/FcR*γ*-FLAG and construct containing the mouse *β*-actin promoter (A). Simplified schematic of Fc*α*RIR209L/FcR*α*. RAW264.7 macrophages (I3D cells) express high levels of human-28 CD89 on the cell surface (B). Tg mice expressing Fc*α*RI/FcR*γ* were bred on a C57BL/6J background and then crossed with ApoE$^{-/-}$ mice bred on a C57BL/6J background (C).

### Cell culture

RAW264.7 macrophages were cultured in Glutamax (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C with 5% $CO_2$ in a humidified incubator. Stable transfectants were selected by adding geneticin (1 mg/m; Sigma Aldrich, St. Louis, MO, USA).

### Animal study protocol

Ten male Fc*α*RIR209L/FcR*γ* transgenic (Tg) ApoE$^{-/-}$ mice (12-week-old) with a C57BL/6J background were used. The animals were fed a diet containing 15% cocoa butter and 0.25% cholesterol, which was obtained from the Animal Center of Juntendo University. After anesthesia (40 mg/kg pentobarbital sodium intraperitoneally), a constrictive silastic tube (0.30 mm), inserted via the caudal vein, was used to elicit plaque formation[17]. The mice were divided into two groups (n = 5 per group): group 1, Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mice were administered 20 μg of control Fab in 200 μL of saline once daily via the caudal vein for three months; group 2, Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mice were administered 20 μg of A77 (anti-Fc*α*RI antibody) Fab in 200 μL of saline once daily via the caudal vein for three months. Serum samples and aorta tissues were collected at the end of the study.

### IgGs and antibodies

A BALB/c-derived (IgG1) mouse monoclonal antibody (Ab) specific for Fc*α*RI (clones A77 or A59)[18] was used as the Fab fragment. Mouse IgG (Jackson Laboratories, Bar Harbor, ME, USA); rabbit anti-phospho ERK MAPK; p38 and c-Jun

N-terminal kinase (JNK) antibodies (Cell Signaling Technology, Danvers, MA, USA); and rat anti-mouse F4/80Ab (AbD Serotec, Oxford, UK) were used.

**Foam cell formation and Oil Red O staining**

I3D cells ($1 \times 10^6$ cells/well) were seeded into 6-well plates and stimulated with ox-LDL (100 μg/mL for 24 h; Yiyuan, Guangzhou, China) in the presence or absence of A77 Fab (100 μg/mL for 12 h). Subsequently, the cells were washed with phosphate-buffered saline (PBS) and stained with Oil Red O (Sigma Aldrich Chemicals). Stained (red) foam cells were imaged under a microscope at 40× magnification.

**Western blot analysis**

I3D cells were preincubated with A77, A59, or control (Ctrl) Fab (100 $\mu$g/mL) for 12 h. The cells were then stimulated with ox-LDL (100 $\mu$g/mL) for 20 min, and phosphorylation of ERK, P38, and JNK was assessed using western blotting. Briefly, cultured cells were washed twice with ice-cold PBS and solubilized by incubation at 4°C for 10 min in lysis buffer (50 mM HEPES [pH 7.4], 0.3% Triton X-100, 50 mM NaF, 50 mM NaCl, 1 mM $Na_3VO_4$, 30 mM $Na_4P_2O_7$, 50 U/mL aprotinin, and 10 mg/mL leupeptin). The protein concentration of the soluble extracts was determined using a protein assay kit (Bio-Rad, Hercules, CA, USA). The collected samples were mixed with a sample buffer (312.5 mmol/L Tris-HCl [pH 6.8], 10% sodium dodecyl sulfate, 50% glycerol, 10% 2-mercaptoethanol, and 0.025% bromophenol blue), heated at 95°C for 5 min before electrophoresis, resolved via sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 10% acrylamide gel, and transferred to polyvinylidene difluoride membranes. The blots were analyzed as described previously[10].

**Immunohistochemical staining**

For light microscopy, the sections of mouse aorta tissues were sectioned at 3 $\mu$m, paraffin-embedded, and stained using the periodic acid–Schiff reagent. For immunohistochemical staining, frozen mouse aorta tissues were sectioned at 3 $\mu$m, fixed in −20°C acetone, and blocked by incubation in a blocking solution (PBS [pH 7.2] containing 2.0% bovine serum albumin, 2% fetal calf serum, and 0.2% fish gelatin at room temperature) for 60 min. Histological features were graded, and F4/80+ cells were counted blindly. A minimum of 10 equatorially sectioned aortas were assessed per animal. The results are expressed as the number of cells per high-power field, which was quantified using a KS-400 version 4.0 image analysis system (KS-400; Carl Zeiss Vision, Oberkochen, Germany).

**Enzyme-linked immunosorbent assay (ELISA)**

Blood samples were collected from each mouse from the retro-orbital venous plexus under general anesthesia by inhalation of ether at the end of the study. Interleukin (IL)-1b and monocyte chemoattractant protein (MCP)-1 levels were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

**Data presentation**

All experiments were repeated more than three times, and representative results are shown. Data are expressed as the mean ± 2 standard error. Statistical analyses were performed using the Student's unpaired *t*-test (specifically, for immunoblotting determination, we compared the results with those of each respective control) and analysis of variance. $p < 0.05$ was considered to indicate statistical significance.

## Results

**Monovalent targeting of Fc*α*RI decreases ox-LDL-induced foam cell formation in Fc*α*RIR209L/FcR*γ* (I3D) cells**

Fc*α*RI-FcR*γ* ITAMi function can be triggered in the absence of co-aggregation. Therefore, we predicted that monovalent targeting, in addition to inhibiting co-expressed ITAM-bearing receptors, affects the responses of receptors involved in different signaling pathways[11]. We analyzed the effect of anti-Fc*α*RI Fab A77 pretreatment on the foaming response of Fc*α*RIR209L/FcR*γ* (I3D) to ox-LDL. Oil Red O staining showed that A77 Fab, but not the Ctrl Fab, markedly inhibited ox-LDL-induced foam cell formation (Figure 2).

**Ox-LDL-mediated MAPK signaling in Fc*α*RIR209L/FcR*γ* (I3D) cells**

Next, we analyzed the effect of anti-Fc*α*RI (A77

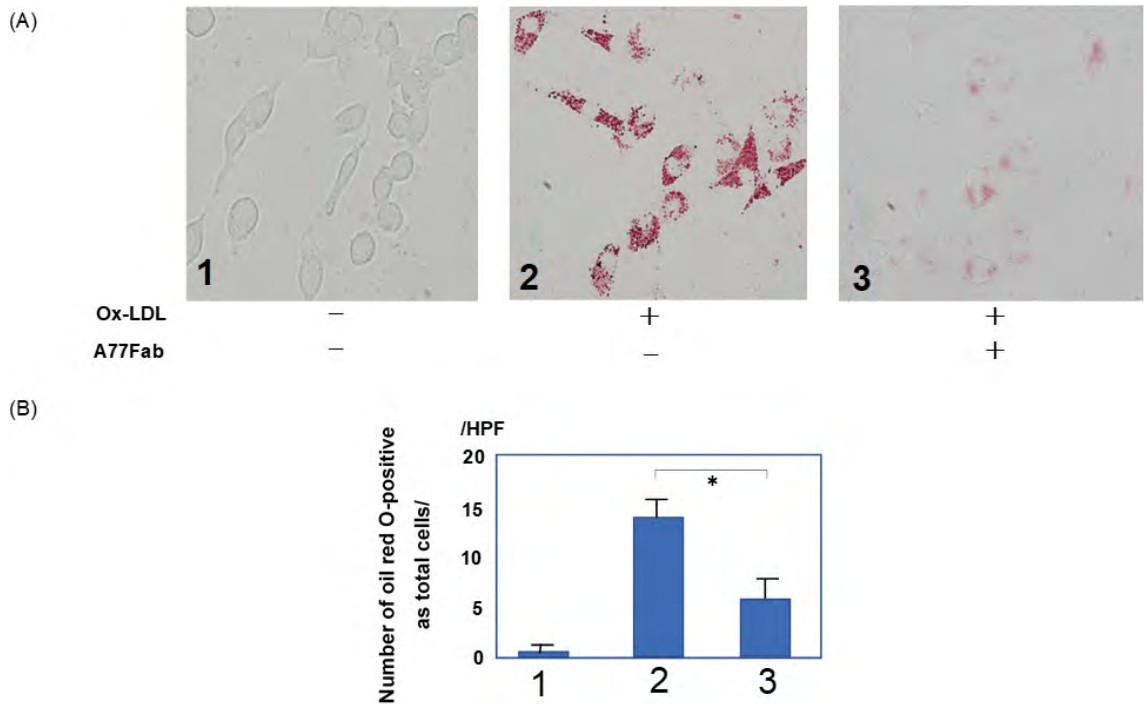


**Figure 2** Monovalent targeting of Fc*α*RI decreases foam cell formation in Fc*α*RIR209L/FcR*γ* (I3D) cells
I3D cells were stimulated with oxidized low-density lipoprotein (ox-LDL) in the presence or absence of A77 Fab (A). Oil Red O staining indicated the presence of foam cells. I3D cells were stimulated with ox-LDL (100 μg/mL for 24 h) in the presence or absence of A77 Fab (100 μg/mL for 12 h). Stained (red) foam cells were imaged under a microscope at 40× magnification. In I3D cells stimulated with ox-LDL with A77 Fab, the number of macrophage foam cells was significantly reduced, as demonstrated by Oil Red O staining. The number of Oil Red O-positive cells was expressed as the percentage of total cells (B). Results were obtained from three independent experiments ($p < 0.05$). HPF, high-power field.

Fab) pretreatment on MAPK in response to ox-LDL in I3D cells. Key events in ox-LDL-mediated signaling, such as JNK, p38, and p42–p44 ERK MAPK phosphorylation, as evaluated by immunoblotting using phospho-specific antibodies, are shown in Figure 2. Phosphorylation was strongly inhibited in I3D cells after preincubation with A77 Fab but not after incubation with Ctrl Fab (Figure 3).

### Oil Red O staining of the aorta of wild-type ApoE-deficient/Fc*α*RIR209L/FcR*γ* phenotype mice fed a high-fat diet for three months

Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mice were administered 20 μg of Ctrl Fab in 200 μL of saline once daily via the caudal vein for three months; in group 2, Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mice were administered 20 μg of Ctrl Fab in 200 μL of saline once daily via the caudal vein for three months. Staining the mouse aortas using Oil Red O showed that staining levels were lower in the A77 Fab treatment group than in the Ctrl Fab treatment group (Figure 4).

### Fc*α*RI targeting reduces leukocyte infiltration in A77 Fab-treated mice

To determine whether monovalent targeting of anti-Fc*α*R has therapeutic implications for high-fat diet-induced atherosclerosis, we analyzed the effect of A77 Fab treatment in a high-fat-diet-induced Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mouse model of atherosclerosis. Control antibody-treated animals showed high CD11b+/F4/80+ macrophage infiltration into the aortic tissues (Figure 4). However, A77 Fab-treated mice showed decreased infiltration of aortic tissues by CD11b+/F4/80+ macrophages compared to that in control antibody-treated animals. Thus, A77 Fab treatment showed marked efficacy against atherosclerosis induced by a high-fat diet in Fc*α*RIR209L/FcR*γ* Tg ApoE$^{-/-}$ mice (Figure 5).

### Fc*α*RI monomeric targeting blocks serum cytokine and chemokine production stimulated in atherosclerosis

To examine whether increased aortic macro-

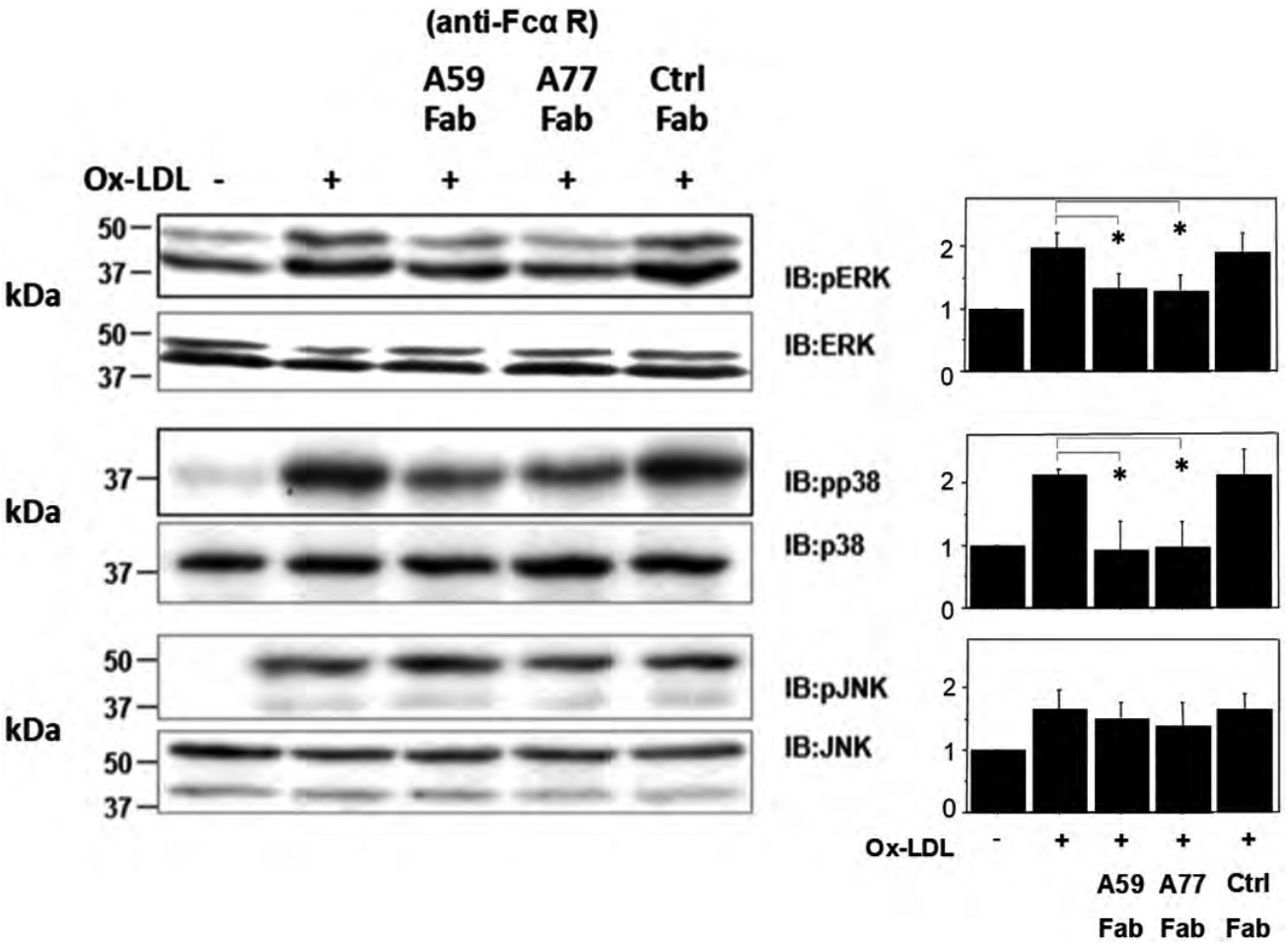


**Figure 3** Oxidized low-density lipoprotein (Ox-LDL)-mediated mitogen-activated protein kinase (MAPK) in Fc*α*RIR209L/FcR$\gamma$ (I3D) cells

I3D cells were preincubated with A77 Fab, A77 Fab, or Ctrl Fab (100 $\mu$g/mL for 12 h). The cells were then stimulated with ox-LDL (100 $\mu$g/mL) for 20 min, and phosphorylation of extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) was assessed. Western blot analysis showed that ox-LDL-induced MAPK activation was strongly abolished by A77 Fab treatment in I3D cells. Re-probing with JNK, p38, and p42-p44 ERK MAPK is shown as controls for equal loading. Results were obtained from three independent experiments ($p < 0.05$).

phage infiltration in Fc*α*RIR209L/FcR$\gamma$ ApoE$^{-/-}$ mice was correlated with serum cytokine and chemokine levels, we performed ELISA using serum isolated from the affected mice. At the end of the study, treatment with the control antibody significantly increased IL-1$\beta$ and MCP-1 secretion. In contrast, the A77 Fab treatment decreased IL-1$\beta$ and MCP-1 levels (Figure 5).

## Discussion

In a previous study, RAW264.7 cells were stimulated with lipopolysaccharide or ox-LDL to mimic the development of atherosclerosis[7]. To assess the involvement of Fc*α*RI/FcR$\gamma$ in the inhibitory process, we generated a chimeric receptor plasmid by fusing the extracellular and R209L transmembrane domains of Fc*α*RI to the intracytoplasmic tail of human FcR$\gamma$[14]. We also generated transfectants expressing Fc*α*RIR209L associated with FcR$\gamma$ (I3D) in RAW 264.7 macrophages[14]. Several previous studies demonstrated that inhibitory signaling by myeloid Fc*α*RI is a promising anti-inflammatory candidate for treating inflammatory diseases[10]. However, data supporting its inhibitory effects on atherosclerosis are lacking.

Although the presence of anti-ox-LDL IgG has been well-documented in clinical and animal studies, the role of Fc$\gamma$Rs in the progression of atherosclerosis remains unclear. The role of activating Fc$\gamma$R in the progression of atherosclerosis using apoE-Fc$\gamma$-chain double-knockout mice was examined[19,20]. In apoE knockout mice, arterial

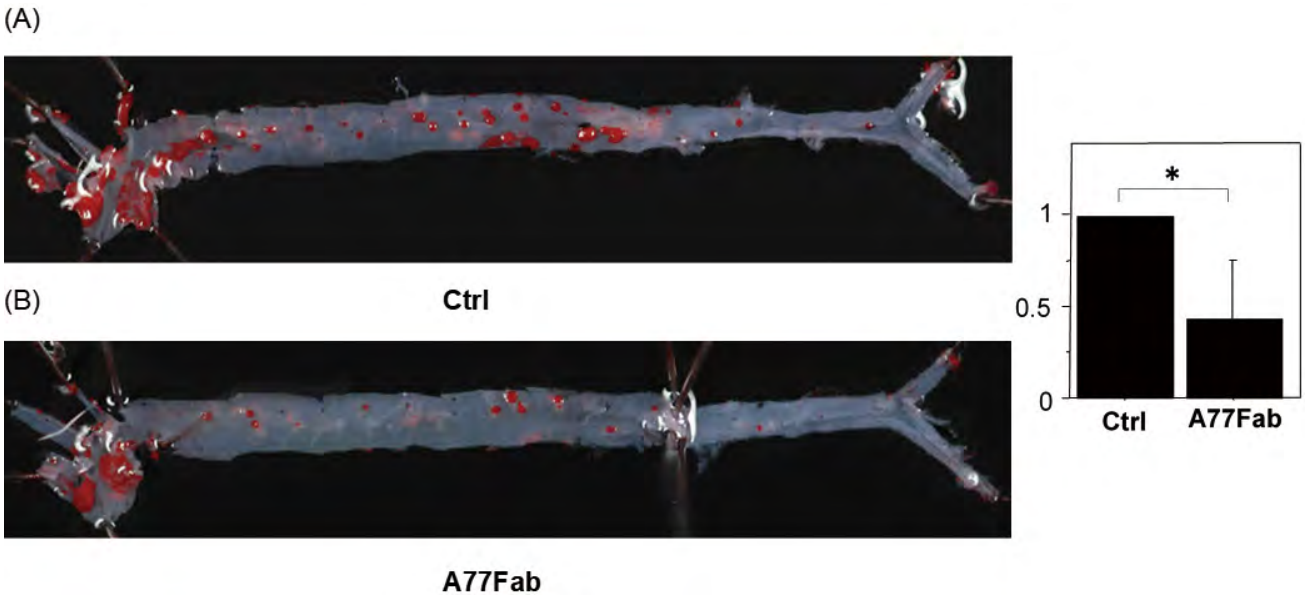


**Figure 4** Oil red O staining of the aorta of wild-type ApoE-deficient/Fc*α*RIR209L/FcR*γ* phenotype mice fed a high-fat diet for three months
Wild-type ApoE-deficient/Fc*α*RIR209L/FcR*γ* mice were generated, fed a high-fat diet for three months, and injected with A77 Fab or PBS Ctrl. Staining the mouse aortas with Oil Red O showed that the expression of A77 Fab (A) was lower than that of PBS Ctrl (B). Results were obtained from three independent experiments ($p < 0.05$).

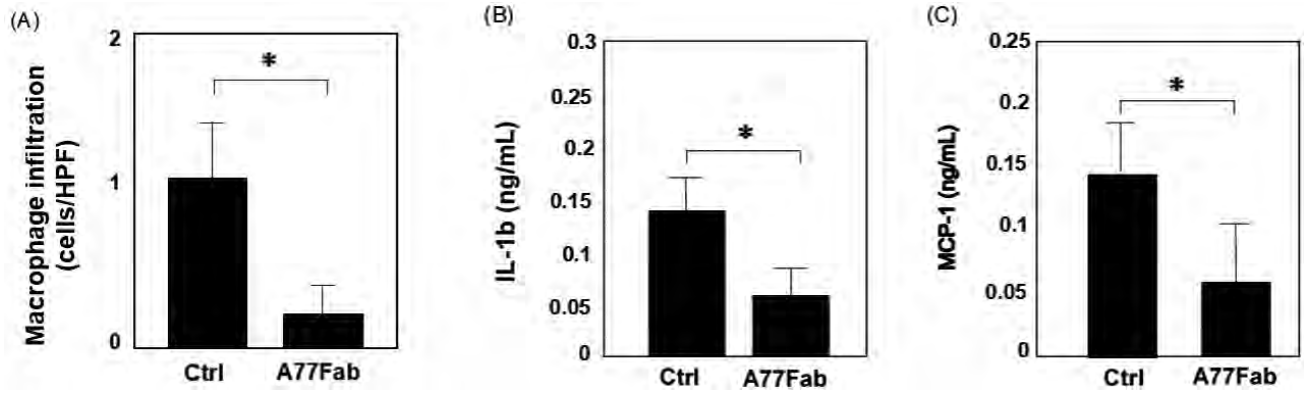


**Figure 5** Fc*α*RI targeting reduces leukocyte infiltration and blocks serum cytokine and chemokine production stimulated in an atherosclerosis model
Immunohistological analysis of aortic sections from each animal group using anti-mouse F4/80 Ab. The number of infiltrating macrophages is shown (A). Serum interleukin (IL)-1*β* (B) and monocyte chemoattractant protein-1 (MCP-1) (C) production in each group was measured using an enzyme-linked immunosorbent assay (ELISA). Anti-Fc*α*RI Fab-injected group showed lower protein production compared to the control Fab-injected group ($p < 0.05$). HPF, high-power field.

lesion formation was significantly decreased in apoE-Fc*γ*-chain double-knockout mice.

We also conducted an additional *in vivo* experiment using a Tg mouse with an ApoE-deficient/Fc*α*RIR209L/FcR*γ* phenotype under high-fat diet feeding, which exhibited severe atherosclerotic lesions. The results showed that monovalent targeting of Fc*α*RI in Tg mice with the ApoE-deficient/Fc*α*RIR209L/FcR*γ* phenotype significantly diminished aortic lesions by an inhibitory ITAM (ITAMi) configuration induced using monovalent targeting of Fc*α*RI through the inhibition of macrophage infiltration at the aortic lesion and decreased IL-1*β* and MCP-1 levels.

A previous report assessed atherosclerotic lesions of apoE-inhibitory Fc*γ*RIIb double-knockout mice (apoE-Fc*γ*RIIb (-/-))[21], and contrary to their hypothesis, when compared with the apoE single knockout mice, arterial lesions were significantly decreased in apoE-Fc*γ*RII (-/-) mice. Chimeric

mice generated by transplanting apoE-Fc*γ*RIIb (-/-) marrow into apoE single knockout mice also developed smaller lesions. Macrophages from Fc*γ*RIIb (-/-) mice produced more IL-1*β* and MCP-1. The mechanisms of this discrepancy remain unknown.

We observed that monovalent targeting of Fc*α*RI was inhibited in an *in vitro* model of ox-LDL-induced foam cell formation. Ox-LDL-induced foam cell formation was markedly decreased in the A77 Fab- or A57 Fab-treated groups compared with that in the Ctrl Fab-treated group. The expression of phosphorylated ERK and p38 was also decreased in the A77 Fab- and A57 Fab-treated groups compared with that in the Ctrl Fab-treated group. Interestingly, phosphorylated JNK expression did not significantly differ between the A77 Fab- or A57 Fab-treated groups and the Ctrl Fab-treated group after stimulation with ox-LDL in Fc*α*RIR209L/FcR*γ* chimeric receptor transfectant macrophages. In line with these findings, inhibitory signaling by myeloid Fc*α*RI significantly decreased the phosphorylation levels of MAPK during atherosclerosis development. The anti-atherogenic properties of inhibitory signaling by myeloid Fc*α*RI observed in I3D cells may be explained by its inhibitory effects on monocyte adhesion, oxidative stress, and the inflammatory response mediated by the ox-LDL/MAPK (ERK1/2/p38) signaling pathway, which was independent of JNK in macrophages.

A previous study demonstrated that IL-1*β* is upstream of the disrupted intestinal barrier function in a mouse model of Kawasaki disease vasculitis, which showed IgA vasculitis development and cardiac inflammation following genetic and pharmacological inhibition of IL-1*β* signaling[22]. Targeting mucosal barrier dysfunction and the IL-1*β* pathway may also apply to other IgA-related diseases, including IgA vasculitis, IgA nephropathy, and atherosclerosis. Elevated levels of circulating secretory IgA may promote atherosclerosis in Kawasaki disease.

Our study had some limitations. First, our *in vivo* physiological data are not sufficient. Unfortunately, this study did not check serum levels of cholesterol and ox-LDL in the mice. In our future study, we should check them and evaluate this point. However, we carefully reviewed optimal animal models in which the inhibitory effect of myeloid Fc*α*RI in atherosclerosis development has been confirmed. Second, although inhibitory signaling by myeloid Fc*α*RI showed multifunctional potential *in vitro* in both our and previous studies, the contribution of SH2-containing phosphatase SHP-1 recruitment should be evaluated. SHP-1 should be immunoprecipitated to demonstrate this association, and FcR*γ* co-immunoprecipitates in macrophages following treatment with anti-Fc*α*RI Fab should be examined.

We demonstrated that monovalent targeting of Fc*α*RI suppresses atherosclerosis development. These results indicate that the inhibitory signals of Fc*α*RI require FcR*γ* single association. Fc*α*RI is a complex receptor, and the balance in Fc*α*RI targeting is associated with the development of atherosclerosis. These results demonstrate the potential of inhibitory signaling by myeloid Fc*α*RI as a therapeutic approach for atherosclerosis.

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The authors received no financial support for the research.

## Author contributions

All authors have made substantial contributions to the manuscript. The details are follows: YD: Conceptualization. Methodology. Formal analysis. Investigation. Data curation. Visualization. Project administration. Writing – original draft. Writing – review and editing. YK: Resources. Writing – editing. Supervision. RM: Resources. Writing – editing. Supervision. YS: Conceptualization. Methodology. Data curation. Writing – review and editing. Supervision. All authors approved the final version of the manuscript to be submitted.

## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Study Protocols

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# Clinical Research on the Safety Evaluation of Platelet-rich Plasma Treatment in Oral Diseases: A Study Protocol

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***Background***: Platelet-rich plasma (PRP) is a biological product obtained from autologous blood that contains growth factors, promoting the healing and regeneration of human tissues. Several oral diseases require surgical intervention, producing residual wounds that undergo a healing process, accompanied by pain, swelling, superinfections, and bone remodeling. This protocol study aims to evaluate the safety of PRP use for the following dental procedures: post-extraction socket healing, periodontal tissue regeneration, maxillary sinus floor elevation, tooth transplantation, and intentional tooth replantation.

***Methods***: Ten patients will be enrolled and subjected to the required treatment with the addition of PRP, after appropriate hematological and biochemical evaluations. The participants will then be subjected to an observation period of 4 weeks to monitor adverse events through clinical observation. Secondary outcomes will regard pain, and clinical evolution of the treated site. Among these, presence of infection, swelling, wound healing, stability of the transplanted tooth.

***Discussion***: Safety of medical procedures represents the first requirement for their introduction in routine practice. A careful evaluation of clinical response during follow-up period and registration of adverse effects is fundamental for safety confirmation and subsequent use of PRP for the proposed dental procedures.

***Trial registration***: Japan Registry of Clinical Trials (https://jrct.niph.go.jp/, registry number: jRCTc030190273, jRCTc030190274, jRCTc030190275, jRCTc030190276, jRCTc030190277; Date of registration: 31 March 2020).

***Key words***: platelet-rich plasma, clinical research, safety, dental treatment

## Background

### Introduction to the trial

Clinical research on the safety evaluation of platelet-rich plasma (PRP) use in oral procedures: wound healing after tooth extraction, maxillary sinus floor elevation surgery, tooth transplantation, intentional tooth replantation, and periodontal tissue regeneration.

### Background and rationale

Platelet-rich plasma (PRP) is derived from venous blood and has been proposed for several clinical applications[1, 2]. The use of platelet concentrate is at the center of a recent academic debate, therefore new clinical studies are needed to prove its efficacy[3].

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In particular, its activity has been demonstrated for the treatment of chronic wounds and tissue repair[4]. The most diffused technique for obtaining PRP is to use autologous blood (from the patients themselves) and centrifugation to separate red blood cells from plasma and white blood cells. The obtained highly concentrated platelets contain several growth factors, including platelet-derived growth factor, epithelial growth factor, and fibroblast growth factor, as well as some other molecules involved in regenerative processes[5,6].

Its use has been proposed for the treatment of chronic ulcers[7], osteoarthritis, and degenerative diseases[8], demonstrating promising results in terms of promoting healing, pain reduction, and functional improvement.

The oral cavity comprises soft and hard tissues that may require surgical interventions for the treatment of tooth-related or periodontium-related diseases to replace missing teeth. These procedures produce wounds that are characterized by typical inflammatory reactions that lead to more or less rapid healing with different outcomes, including a certain rate of bone remodeling. The use of PRP for dental procedures has been proposed by several authors[9] to enhance soft tissue healing and bone regeneration. Furthermore, it has been debated if the use of PRP can improve the prevention and treatment of medication-related osteonecrosis of the jaws[10]. The reported results vary depending on the type of procedure, method of PRP preparation, observation period, and evaluated outcomes[11–15]. Furthermore, little evidence is available regarding the safety (infection risk, systemic complications, and enhanced inflammation) of PRP use in dentistry.

This study aims to present a protocol for safety evaluation of PRP use in the following dental procedures: 1) post-extraction sockets, 2) periodontal regenerative therapy, 3) maxillary sinus lift, 4) tooth transplantation, and 5) tooth replantation. The rationale and description of the procedures are briefly described as follows:

*Wound healing of the post-extraction socket*

Dental extractions are the most common surgical procedures in the oral cavity. Such a procedure can be more or less traumatic, and the post-extraction socket is often left uncovered when flap closure is not feasible. The healing process comprises a series of complex changes involving hard and soft tissues referred to as "socket healing." In this process, the following three sequential phases can be identified: inflammatory, proliferative, and modeling/remodeling[16]. Considering the biological activity of PRP, the hypothesis is that its application in post-extraction sockets may accelerate and improve the healing process.

*Periodontal tissue regeneration*

Periodontal disease is considered as the most common infection worldwide[17]. Its pathogenic process leads to the progressive destruction of tooth-supporting tissues. The treatment of periodontitis involves the adoption of well-established protocols of oral hygiene and surgical and non-surgical interventions. Tissue destruction caused by periodontitis is often irreversible; nevertheless, some specific conditions, if properly treated, can be regenerated[18]. Tissue engineering technologies are fundamental for this purpose, and continuous research exists on materials that can promote the regeneration of tissues surrounding the teeth[19–21].

*Maxillary sinus floor elevation*

Another widely diffused procedure nowadays is the dental implant rehabilitation of missing teeth. To perform this intervention, a sufficient amount of bone is required. In some cases, the close proximity of adjacent anatomical structures can limit the possibility of dental implant insertion. The maxillary sinus represents one of these, and sometimes, procedures of sinus floor elevation are needed before dental implant placement. Therefore, different techniques and materials have been proposed for the sinus lift, including various types of bone substitutes[22–26].

As part of these well-standardized and predictable procedures, some others have not been sufficiently experimented and are currently considered uninsured treatments. Among these, the tooth transplantation and intentional tooth replantation are becoming more and more investigated.

*Tooth transplantation*

Tooth transplantation (or tooth grafting) is a method that comprises transferring a tooth from another part to an area where a tooth has been lost due to dental caries or periodontal disease. In

general, wisdom teeth are often used because they have less impact on occlusion[27]. The 10-year survival rate of the transplanted tooth was reported to be 73.6%[28, 29].

*Intentional tooth replantation*

Dislocated teeth due to trauma or other reasons can be relocated to their original position through replantation. Tooth grafting is expected to last for several years after surgery, allowing for natural function.

The use of PRP in these and other oral procedures has been proposed by several authors, showing promising results[8]. Nevertheless, there is still a concern regarding the appropriateness of providing regenerative medicine as a medical treatment, since the evaluation method and the method of provision have not been sufficiently verified. Additionally, the benefit to the patient must outweigh the risk when regenerative medicine is used as a treatment[30, 31].

## Methods/Design

### Study design

This single-arm open-label study is ongoing and is being conducted from March 2019 to March 2024 at the Department of Dental Surgery, Juntendo University Hospital, Juntendo, Japan. The study protocol was approved by the Tokyo Medical and Dental University Specially Certified Committee for Regenerative Medicine (committee number: NA8140003, approval number: RM2018-008, RM2018-09, RM2018-010, RM2018-011, RM2018-012) and has been registered in the Japan Registry of Clinical Trials (https://jrct.niph.go.jp/, registry number: jRCTc030190273, jRCTc030190274, jRCTc 030190275, jRCTc030190276, jRCTc030190277).

### Eligibility criteria

A list of common and treatment-specific inclusion criteria is established. In particular, the common inclusion criteria are as follows:

1. A good systemic condition without chronic or acute diseases.
2. Number of platelets above 1x10E5/μL;
3. Aged ≥20 years;
4. Signed informed consent.

Treatment-specific requirements for enrollment in one of the protocols of the study (1–5) are as follows:

1. Having a wisdom tooth requiring extraction.
2. Having a periodontal pocket of >5 mm at baseline examination with an intrabony defect of ≥5 mm depth and ≥2 mm width at the interproximal site, as observed using radiographs. The mobility of experimental tooth has to not exceed grade 2, with availability of keratinized gingiva, good oral hygiene and the tooth must not require surgical/restorative/root canal treatment within 36 weeks after PRP transplantation.
3. Having a missing tooth in the maxillary posterior region requiring dental implant rehabilitation and sinus floor augmentation.
4. Having a molar tooth that needs to be extracted and having a wisdom tooth that can be extracted.
5. Having a fracture of the dental root for which replantation is a viable treatment option and no abscess at the root is present.

The exclusion criteria are defined as follows:

1. Patients suspected of or having a history of complicated malignant tumors;
2. Presence of or a history of abnormal gingival proliferation;
3. Presence of anti-coagulant or anti-platelet medications or bleeding disorders;
4. Pregnancy, breastfeeding or intention of pregnancy;
5. Alcoholism or drug dependence
6. Presence of hepatitis C virus (HCV) antibody, hepatitis B surface (HBs) antigen, adult T-cell leukemia virus-associated antigen virus antibody, or human immunodeficiency virus (HIV) antibody

### Planned sample size

Recruitment of 10 patients (two per treatment) is planned.

### Study procedures

All patients will be provided with a consent document approved by an authorized committee for regenerative medicine. Every oral and written explanation will be provided, and written voluntary consent will be obtained from the participants. Patients satisfying the inclusion criteria will be

subjected to a hematological examination:

1. White blood cell count, white blood cell fractions (neutrophils, eosinophils, basophils, and lymphocytes), red blood cell count, hematocrit, hemoglobin, and platelet count.
2. Blood biochemical tests: Aspartate aminotransferase (glutamic-oxaloacetic transaminase), alanine aminotransaminase (glutamic-pyruvic transaminase), total protein, and creatinine.
3. Viral tests: HCV antibody, HBs antigen, and HIV antibody.

Observation and investigation items are summarized in Table 1.

After enrollment, on the day of treatment, 26 mL of blood will be collected from the participants' mid-elbow vein using a syringe containing anticoagulant (anticoagulant citrate dextrose solution) as a blood sample for PRP preparation[32]. Approximately 1 mL of the collected peripheral blood will be used to produce PRP at the Juntendo University cell culture and processing facility. A visual test and inspection of the foreign matter in the fabricated PRP will be performed. Furthermore, the platelet concentration ratio will be counted in whole blood and PRP will be prepared before centrifugation using an automated hematology analyzer. A sterility test will be performed on gelatinized PRP to verify its sterility.

Approximately one-tenth of the volume of 2% $CaCl_2$ and, if necessary, autologous thrombin will be added to 1 mL of PRP to gel it immediately before use.

Simultaneously with PRP preparation, the appropriate dental procedure will be performed, and PRP gel will be used as follows:

1. After tooth extraction, the post-extraction socket will be filled with PRP and, if possible, the flaps will be approximated using a resorbable surgical suture.
2. In the periodontal flap surgery, after open-flap debridement, PRP will be applied on the tooth surface involved in bone defects, and the flap will be approximated using a resorbable suture.
3. For sinus lift, after the elevation of the flap and access to the sinus will be obtained using a piezoelectric instrument. After that, the sinus membrane will be elevated and PRP mixed with the bone graft will be applied. The mucoperiosteal flap will be sutured then.
4. In tooth transplantation and replantation, gelatinized PRP will be administered in the tooth

**Table 1** Observation, investigation, and evaluation items

| Item | Description |
|---|---|
| Patient background | Date of obtaining consent, sex, date of birth, name of the causative disease, complications and their severity, preexisting medical conditions, presence of infectious diseases, history of dental surgery, and concomitant treatment. |
| Vital signs | Blood pressure (maximum, minimum); heart rate; and body temperature |
| Clinical examination | Subjective symptoms |
| Hematological examination | White blood cell count; white blood cell fraction (neutrophils, eosinophils, basophils, lymphocytes); red blood cell count; Hct; Hb; and platelet count |
| Blood biochemical tests | AST (GOT), ALT (GTP), T-P, and CRE |
| Adverse events | The presence/absence, timing, resolution, extent, treatment, severity assessment, and relevance to the cell product in question of any adverse events observed during the study. |
| Concomitant medications | Type, dose, and timing of concomitant medications used during the study. |
| Pain | Interviews about the onset time of pain, type and degree of pain, duration of analgesia medications, and degree of pain control, rapidity, and persis-tence. |
| Sterility test | Sterility test using a portion of gelatinized PRP for transplantation. |
| Intraoral photography | Intraoral photography before and after treatment using a digital camera. |
| Dental radiography | Assessment of infection signs. |
| Platelet concentration ratio | Platelet count ($x10^5/\mu L$), red blood cell count ($x10^4/\mu L$), and white blood cell count ($x10^3/\mu L$) in whole blood and prepared PRP before centrifugation using automated hematology analyzer. |

Hct, hematocrit; Hb, hemoglobin; AST (GOT), aspartate aminotransferase (glutamic-oxaloacetic transaminase); ALT (GPT), alanine aminotransaminase (glutamic-pyruvic transaminase); T-P, total protein; CRE, creatinine

graft site, and the grafted tooth will be implanted. The transplanted or replanted tooth will be temporarily fixed to the tooth adjacent to the dental resin.

After the treatment, the participants will be evaluated for safety for 4 weeks and will be followed up for 11 months (48 weeks).

**Primary endpoint: Safety evaluation**

Adverse effects will be evaluated in terms of presence/absence, timing, resolution, extent, treatment, and severity. Adverse events will be evaluated during the 4-week period of transplantation by clinical examination of subjective and objective symptoms and encoded according to the Common Terminology Criteria for Adverse Events[33]; an adverse event of grade 3 or higher is suspected to be related to the provision of regenerative medicine, and a consideration of whether or not to continue enrollment in the study will be undertaken. Furthermore, the enrollment of the second case will be suspended until the safety evaluation of the first case is completed.

*Secondary endpoints*

1. Interviews for postoperative pain. Changes in postoperative pain will be evaluated during the observational period (4 weeks) using a 10-point numerical rating scale. Interviews will be conducted to determine the pain onset time, type, and degree; duration of analgesic medication; and degree of pain control, rapidity, and persistence.
2. Clinical and radiologic healing will be evaluated using intraoral photography, endoral radiography, and cone-beam computed tomography. The following items will be evaluated for each treatment:
   - Presence of infection;
   - Swelling;
   - Wound healing;
   - Stability of the transplanted tooth.
3. Correlation between the platelet enrichment rate of PRP and outcome.

## Discussion

The use of PRP can represent a breakthrough in regenerative dentistry because of its relatively simple and inexpensive extraction and application procedures. Its potential fields of application include several branches of dentistry, such as extractions, dental implant rehabilitation, periodontal treatment, and transplantation. The results of this study will provide additional data regarding the safety of PRP use for dental procedures and provide accurate data to both clinicians and patients.

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## Author contributions

Conceptualization, MT; methodology, YM; software, YM; validation, KW; formal analysis, MT; investigation, MT, SN, MH, TT, and MS; resources, MT; data curation, KW and HY; writing—original draft preparation, MT; writing—review and editing, MT; visualization, YM; supervision, MT; project administration, YM and KW. All authors read and approved the final manuscript.

## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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Case Reports

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# Young Adult Case of Fontan-associated Liver Disease with Hepatocellular Carcinoma During the Transition from Pediatric to Internal Medicine Care and Follow-up

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In recent years, the outcomes of the Fontan procedure have been good, but Fontan-associated liver disease (FALD), which causes congestive hepatopathy due to elevated central venous pressure (CVP), has become a serious problem when considering patients' long-term prognosis. A 28-year-old woman with Emanuel syndrome was admitted to our hospital for the treatment of hepatocellular carcinoma (HCC). She was diagnosed with pulmonary atresia and underwent a bidirectional pulmonary artery shunt at the age of 1 year and 10 months and the Fontan procedure at 4 years of age. Blood tests showed an increase in γ-glutamyltransferase in her early 20s and a marked increase in alfa-fetoprotein levels at age 27 years. She was diagnosed as having HCC in the S7 region by contrast-enhanced computed tomography and underwent hepatectomy. There were no serious adverse events, and the patient has survived 18 months after surgery without recurrence. In this report, the optimal time for the transition from the pediatrics department to adult healthcare units is also discussed, along with the management system for FALD in our hospital.

***Key words***: Fontan-associated liver disease, Fontan circulation, liver fibrosis, hepatocellular carcinoma, transitional care

## Introduction

Though advances in pediatric care have saved many lives, an increasing number of patients are coming of age with coexisting chronic diseases. At the later stages of Fontan surgery for complex congenital heart malformations, it has become apparent that patients present with cardiovascular, as well as hepatic-gastrointestinal, complications[1)]. Currently, Fontan-associated liver disease (FALD) caused by congestive hepatopathy, which is characterized by high central venous pressure (CVP) in the Fontan circulation compared with the normal heart, is one of the most severe problems[2, 3)]. Not only liver fibrosis, but also cirrhosis, localized nodular hyperplasia, hepatic adenoma, and hepatocellular carcinoma (HCC), which are defined as FALD, have been reported in the remote period after the Fontan operation[4, 5)]. In 1990, a case of HCC associated with cardiac cirrhosis was first recognized by Ho et al.[6)]. Fifteen years later, Ghaferi et al. reported the second case with HCC after the Fontan procedure[7)]. In the last 7–8 years, the incidence of HCC after Fontan surgery has become

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widely known, and the number of publications on this topic has been increasing.

At present, approximately 400 Fontan procedures are performed annually in Japan, and more than 1,000 cases are performed annually in the United States. Over the past several decades, various improvements in surgical techniques and perioperative management have significantly reduced perioperative and early postoperative mortality[8], and the average life expectancy of patients with underlying serious congenital heart disease has improved from 17 years in 2000 to 25 years in 2010[9, 10]. Consequently, liver management for FALD surveillance is important based on this background, and transitional care from pediatrics to internal medicine is gradually recognized these days. In order to build smooth transitional care for FALD surveillance, it is necessary to establish a coordinated care system for cardiovascular and liver diseases from the early postoperative period. A case of FALD in a young adult with HCC that was successfully resected due to early detection of the tumor is reported, along with a discussion of the management system for FALD in our hospital.

## Case presentation

A 28-year-old woman who had been born at a gestational age of 41 weeks and 5 days weighing 2,416 grams had cyanosis and a heart murmur, and the patient was transferred to our hospital on day 5. Echocardiography showed pulmonary atresia with an intact ventricular septum, total anomalous pulmonary venous return type IIa, atrial septal defect, patent ductus arteriosus, absent right superior vena cava, and persistent left superior vena cava. Based on these clinical symptoms, chromosomal analysis was performed and showed Emanuel syndrome [47XX, dic(22)(q11.2)]. Her history of heart surgery was as follows: balloon dilation of the ductus arteriosus at 2 months of age; left-sided systemic to pulmonary shunt (Blalock-Taussig shunt) and modified Brock's operation at 4 months of age; bidirectional cavo-pulmonary shunt, division of the PDA, and division of the left-sided Blalock-Taussig shunt at 1 year and 10 months of age; and the Fontan procedure using a 16-mm Gore-Tex extracardiac conduit at age 4 years and 2 months.

Since the patient remained asymptomatic for more than 20 years thereafter, her follow-up was conducted only by pediatric cardiologists. When she was over 20 years of age, at the time that pediatric cardiologists have widely recognized the development of HCC after the Fontan procedure, the course and prognosis of cases developing FALD and HCC were carefully explained to her family, and it was recommended that she visit the Department of Gastroenterology for adults. Her family was initially hesitant to have her seen at the Departments of Cardiology and Gastroenterology for adults because of her immature personality due to the chromosomal anomaly. Finally, her family decided that she would visit the Departments of Cardiology and Gastroenterology for adults when she was 27 years old. As soon as she was transferred to the Gastroenterology Department, progression of FALD was suspected on echosonography, and then contrast-enhanced computed tomography (CT) performed as the first screening showed a contrast-enhanced mass lesion in the S7 area of the liver (Figure 1). In addition, an elevated serum alfa-fetoprotein (AFP) level was found (772 ng/mL), strongly suggesting hepatocellular carcinoma.

On admission, she was in good general condition (New York Heart Association functional classification: NYHA I). The laboratory findings were as follows: hemoglobin 15.8 g/dL, platelet count 14.2 ×109/L, alanine aminotransferase 35 (13-55) U/L, γ-glutamyltransferase 212 (8-90) U/L, total bilirubin 1.85 (0.1-0.6) mg/dL, total protein 8.0 (5.3-7.2) g/dL, and the hepatic fibrosis marker Mac-2 binding protein glycosylation isomer (M2BPGi)

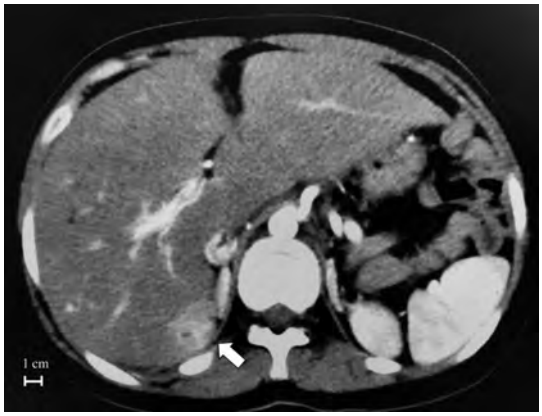


**Figure 1** Contrast-enhanced CT of the abdomen. A mass with contrast effect is seen in the S7 area of the right lobe of the liver (arrow), which was suspected to be hepatocellular carcinoma. The maximum diameter of the tumor is 27 mm.

0.43 (0.00–0.99; expressed as a cutoff index). Preoperative cardiac catheterization was performed, and CVP was 14 mmHg, which was the same as that at 5 years of age (Figure 2). Surgical treatment was performed, and the tumor was completely removed. The surface of the liver was irregular and plastic, and part of the tumor protruded outside the liver (Figure 3). The histopathological findings of liver tissue obtained at operation confirmed poorly differentiated hepatocellular carcinoma (Figure 4). Her postoperative course was uneventful, with no major perioperative circulatory disturbances, resulting in her discharge on the 8th postoperative day. Eighteen months have passed since the surgery, and no re-elevation of AFP levels has been observed.

## Discussion

Until 1990, the classic Fontan procedure was performed to directly connect the right atrium to the right pulmonary artery (PA) by closing the atrial-septal defect. After 2000, an extracardiac conduit insertion connecting the inferior vena cava to the right PA was performed. With the increasing number of long-term survivors after Fontan surgery, the problem of FALD has become evident. In patients with Fontan circulation, the complication rates of liver fibrosis, liver cirrhosis, hepatocellular carcinoma, gastroesophageal varices, and protein-losing gastroenteropathy after Fontan surgery were reported to be 0.85–58.3%, 20.5–23.0%, 1.2–9.8%, 19.2–33.3%, and 3.7–24.0%, respectively[11–16]. In addition, the estimated annual incidence of HCC is 1.5–5.0%[2]. In a report from one of the earliest centers to perform the Fontan procedure in Japan, HCC was diagnosed at a median age of 32.5 years (range: 20.6–46.1 years), and the median time from the Fontan procedure to diagnosis was 21.3 years (3.7–31.2 years)[12]. This is similar to a report from Western countries, in which the mean age at HCC diagnosis was 30.0 ± 9.4 years, and the mean interval was 21.6 ± 7.4 years[17]. Generally, the long-term course of Emanuel syndrome, characterized by multiple congenital anomalies and craniofacial dysmorphism, has not been well understood, since reports about patients are mainly from infancy and early childhood[18]. Of note, the relationship between carcinogenesis and Emanuel syndrome may also be unclear. In the present case, HCC was observed 24

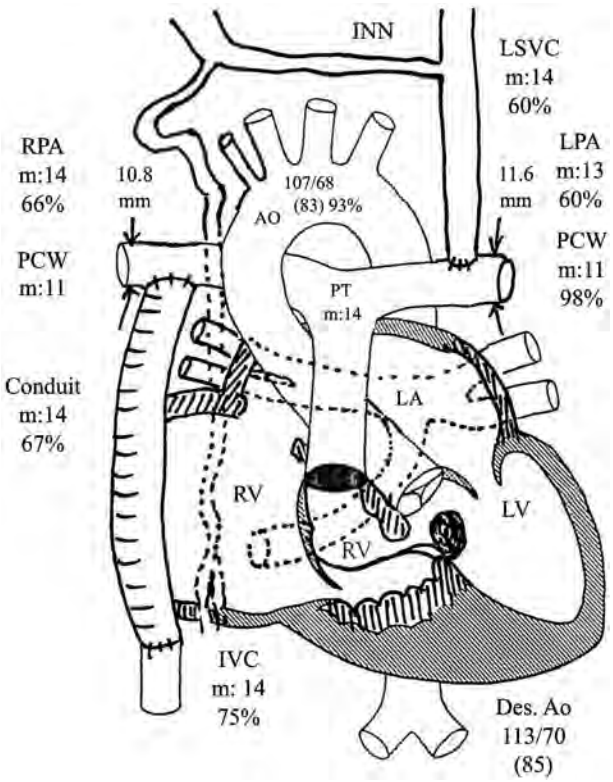


**Figure 2** Findings of preoperative cardiac catheterization at 28 years of age
Cardiac index = 3.08 L/min/m², pulmonary blood flow/systemic blood flow ratio (Qp/Qs) =1.02, pulmonary vessel resistance = 0.94–1.42 Um² (Wood units), pulmonary artery index = 152.
INN: innominate vein, LSVC: left superior vena cava, LPA: left pulmonary artery, PCW: pulmonary capillary wedge pressure, RPA: right pulmonary artery, PT: pulmonary trunk, LA: left atrium, RV: right ventricle, LV: left ventricle, Des. Ao: descending aorta, m: mean pressure value (mmHg).

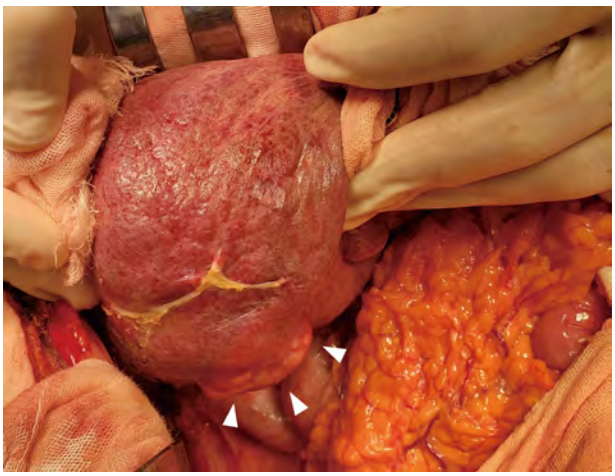

**Figure 3** Appearance of the liver during surgery
The hepatic surface is irregular and plastic, and part of the liver protrudes outside the liver. The location of the tumor is indicated by a triangle.

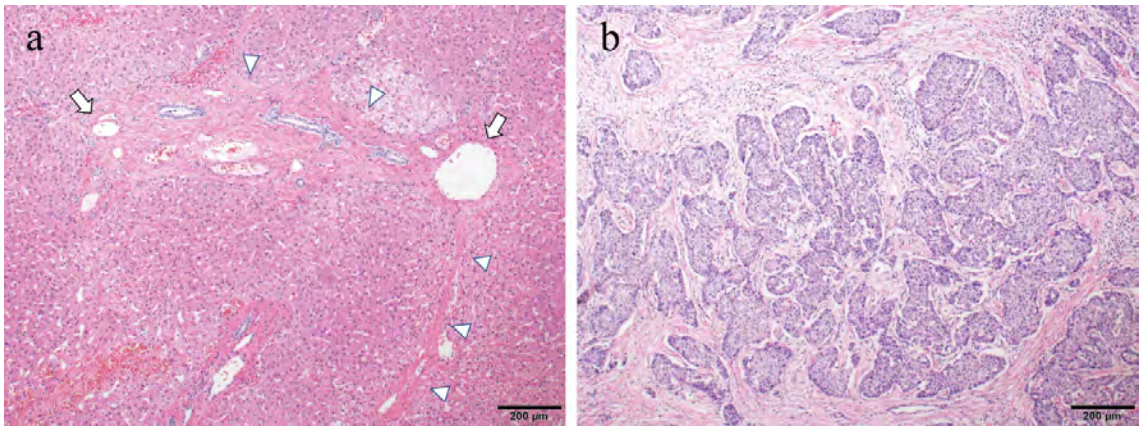


**Figure 4** a. Pathological findings of hematoxylin and eosin staining (non-tumor site). The portal vein area is fibrotic and enlarged (arrow), and bridging fibrosis is partially observed (F3) (triangle), indicating a pre-cirrhotic state.
b. Pathological findings of hematoxylin and eosin staining (tumor site). The histopathological diagnosis of poorly differentiated hepatocellular carcinoma is confirmed.

years after Fontan surgery, which is a common period for the diagnosis of HCC after Fontan surgery, suggesting the postoperative period after Fontan surgery may have influenced the pathogenesis of HCC.

Transitional care can be classified into three categories: (1) complete transition from pediatrics to internal medicine; (2) pediatrics continues to treat congenital diseases and disorders, while internal medicine takes over for health problems specific to adulthood; and (3) pediatrics alone continues to treat patients when no appropriate adult care department is available[19]. Currently, adult departments are usually specialized, and multiple referrals should be made in cases of multisystem syndromes or complications. In the present case, transitional care was practiced according to pattern (2). Regardless of the optimal transition time from the age of 10 to the early 20s, bridging to specialists in adult departments at an appropriate time according to the natural course of the disease and family needs would be considered a possible form of transitional care. A nationwide survey was conducted to determine the epidemiology of FALD from 2021 to 2022 by a research group of the Japanese Ministry of Health, Labour and Welfare, which will provide the basic data for the creation of a transitional care for FALD surveillance protocol in the future (https://mhlw-grants.niph.go.jp/project/147343, accessed on 27 Jan 2023). Since no official guidelines have yet been provided, the management system for postoperative Fontan patients at our institution has been developed (Figure 5). Persistently elevated AFP is well known to be a risk biomarker for the development of HCC in patients without a background of the Fontan procedure[20]. In patients who underwent the Fontan procedure, AFP levels can be measured together with routine blood tests and are likely to be useful as screening for HCC. However, approximately one-quarter of post-Fontan patients diagnosed with HCC showed normal AFP values, retrospectively, suggesting the importance of imaging analysis[17]. Especially in patients with mental retardation, as in the present case, the CT scan may require sedation and thus may not be easily performed. However, based on previous reports[2, 12, 17], CT or magnetic resonance imaging should be recommended to screen for HCC in patients more than 10 years after the procedure.

Pediatric cardiologists and thoracic surgeons need to be aware that FALD is the precursor of HCC in the Fontan circulation as general knowledge and should inform patients and their families of these risks before the operation. In addition, to monitor carcinogenesis, regular check-ups of tumor markers such as AFP combined with imaging analysis would be helpful even in childhood. Further studies should focus on creating a protocol for liver surveillance after Fontan surgery.

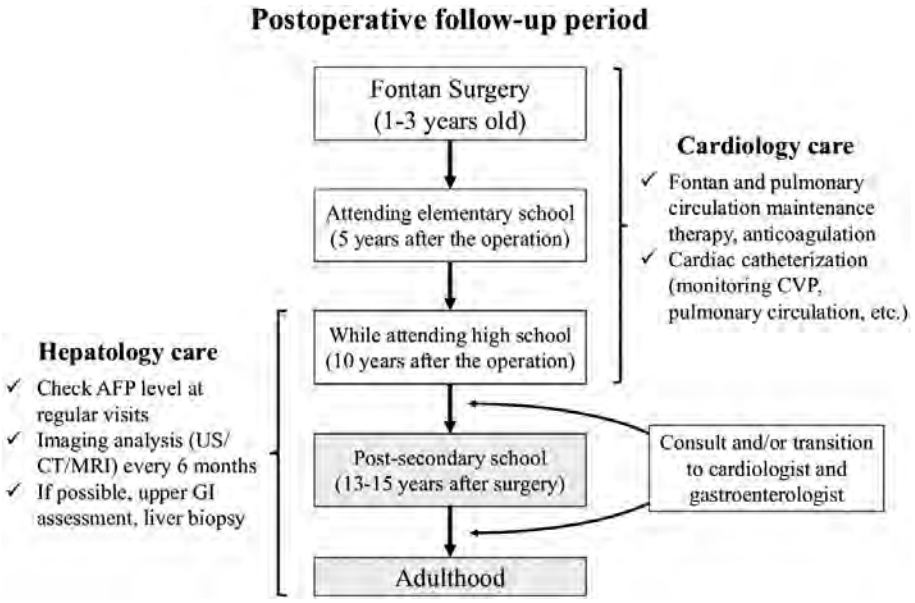


**Figure 5** Transitional care and FALD-HCC surveillance
CVP: central venous pressure, AFP: alfa-fetoprotein, US: ultrasonography, CT: computed tomography, MRI: magnetic resonance imaging, GI: gastrointestinal.

## Conclusion

A young adult patient with FALD-related HCC who was successfully treated by surgical treatment was described. After the Fontan procedure, it is necessary to provide continuous postoperative care considering complications such as FALD. Thus, it is crucial to build a transitional care system that smoothly bridges the gap from childhood to adulthood.

## Informed consent to participate

The patient's guardians provided written informed consent for publication of the case details and analysis.

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## Author contributions

HF and MS wrote the manuscript and created the figure. KS, SM, AU, and MM treated the patient during hospitalization and conducted follow-up at the outpatient clinic. KT and TS supervised the study and revised the manuscript. All authors approved the manuscript prior to submission.

## Conflicts of interest statement

The authors have no conflicts of interest to disclose.

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Publication List

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# Publications from Juntendo University Graduate School of Medicine, 2021 [1/6]

## Anatomy and Life Structure

〈Original Articles〉


＊ 1) Kawasaki Y, Hosoyamada Y, Miyaki T, Yamaguchi J, Kakuta S, Sakai T, Ichimura K: Three-dimensional architecture of glomerular endothelial cells revealed by FIB-SEM tomography. Front Cell Dev Biol, 2021; 9: 653472.

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### Infection Control Science

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### Diagnostics and Therapeutics of Intractable Diseases

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## 編 集 後 記

近年、AIを活用した医学論文の執筆や編集の支援が注目されています。医学論文の執筆は、多くの時間と労力がかかるだけでなく、正確さと科学的な信頼性が求められます。AI編集の導入により、論文の構成や文体の向上、文法や表現のチェックなど、多くの面で効率化と品質向上が期待できます。これらは、執筆者や編集者にとって大きなメリットです（時には情報が多すぎて困惑することもありますが・・・）。

しかしながら、まだAIは万能ではありません。一般的な情報でも間違った結果を示すことがあります。一般的なものであれば間違いに気づきやすいですが、高度な医学情報に対しては判断が難しく、見逃されてしまう可能性があります。また、AIを活用した医学論文の執筆や編集の支援には、まだ多くの課題が残っています。例えば、AIが医学の専門知識や倫理観を十分に理解していない場合や、著作権や出版倫理に関する問題を引き起こす可能性があることが指摘されています。

ともあれAIを使わない手はありません。AI利用の流れは今後も止まることはなく、むしろ加速していくでしょう。重要なのは、いかにAIを使いこなしていくかということです。AIはあくまでツールであり、最終的には我々の専門知識と判断力に頼らなければなりません。今後、査読・編集はAIの恩恵を受けられるのか、それともAIによって更なる困難に直面するのか？明日はどっちだ？！

（編集後記の後記：この文章もほぼAIにて作成しました。800文字と指定したのに637文字でした。）

落合　匠

医療法人社団 愛友会 介護老人保健施設 三郷ケアセンター

イラスト作者より

花屋でちょっと変わった色のバラを見つけました。多分人工的に交配を繰り返してできた種類なのでしょうが、とてもモダンで美しいです。花瓶に入れずに植物画のように描いてみました。（宮道明子）

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抄　録

順天堂醫事雑誌
2023. 69(3), 280

# 骨格筋疾患および心疾患に対するリアノジン受容体（RyR）阻害薬の開発

松川紘之，村山　尚

順天堂大学医学部薬理学講座

リアノジン受容体(RyR)は，骨格筋や心筋の筋小胞体に存在する細胞内カルシウム遊離チャネルで，興奮収縮連関に中心的な役割を担っている．RyR の遺伝子変異や翻訳後修飾はチャネルの過剰活性化を引き起こし，さまざまな骨格筋疾患や心疾患の原因となる．現在，これらの疾患のほとんどに対して特異的な治療法は存在しない．最近，RyR 関連筋疾患の治療薬候補を同定するためのハイスループットスクリーニング(HTS)アッセイが開発された．これらのアッセイは，動物モデルにおいても効果を示す新規 RyR 阻害剤化合物を開発することに成功した．本総説では，HTS アッセイの最近の進歩に焦点を当て，これらの有望なアプローチの概略と将来的な展望について議論する．

**キーワード**：リアノジン受容体，カルシウムチャネル，創薬，ハイスループットスクリーニング

この抄録は，順天堂醫事雑誌 69 巻 3 号，p180–187，2023 掲載の『Development of Ryanodine Receptor（RyR）Inhibitors for Skeletal Muscle and Heart Diseases』の和文抄録です．

抄　録

順天堂醫事雑誌
2023. 69(3), 281

# 順天堂医院における新型コロナウイルス感染症を契機としたオンライン診療の実際

桑　鶴　良　平

順天堂大学大学院 医学研究科 放射線診断学講座

新型コロナウイルス感染症が2020年3月中旬より本邦で爆発的に増加し，それに伴い易感染性の患者や感染により重症化リスクのある患者など，一部の外来患者の当院(順天堂医院)受診が困難になった．一方で，そのような疾患の患者こそ継続診療が必要であり電話再診で代替したが，一定間隔内での患者の状況把握を行い投薬継続などの判断と指示が必要であるため，何らかの形での診療が必要という判断に至った．当院では従来からオンライン診療を施行していたが，限られた疾患においてのみで施行しており経験としては乏しかった．本邦における新型コロナ感染症の急速な拡大により，2020年4月10日に厚生労働省からオンライン診療の時限的・特例的措置取り扱いの事務連絡があり，それに従い当院でも2020年5月よりオンライン診療の利用を拡大し，来院できない外来患者に対する投薬治療が途切れないように配慮した．これにより，対面での外来患者数は減少したものの，オンライン診療により外来患者は当院を受診し，投薬も受けることが可能であった．また，待ち時間の少ない時間帯に検査を受けてその結果をオンライン診療で共有するという試みも開始した．本稿では，この経緯について及び本邦におけるオンライン診療の指針やその診療報酬体系，オンライン診療のメリット，デメリットについて述べる．

**キーワード**：新型コロナウイルス感染症，オンライン診療，オンライン受診勧奨，診療前相談

この抄録は，順天堂醫事雑誌69巻3号，p197–202，2023掲載の『The Practice of Online Medical Care at Juntendo Hospital in Response to the Coronavirus Pandemic』の和文抄録です．

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(3) 医学会の正会員として 1 年以上の経歴を有し、医学会費を完納している者

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(2) 所属長の推薦書
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| 12 月末 | 2 月 |

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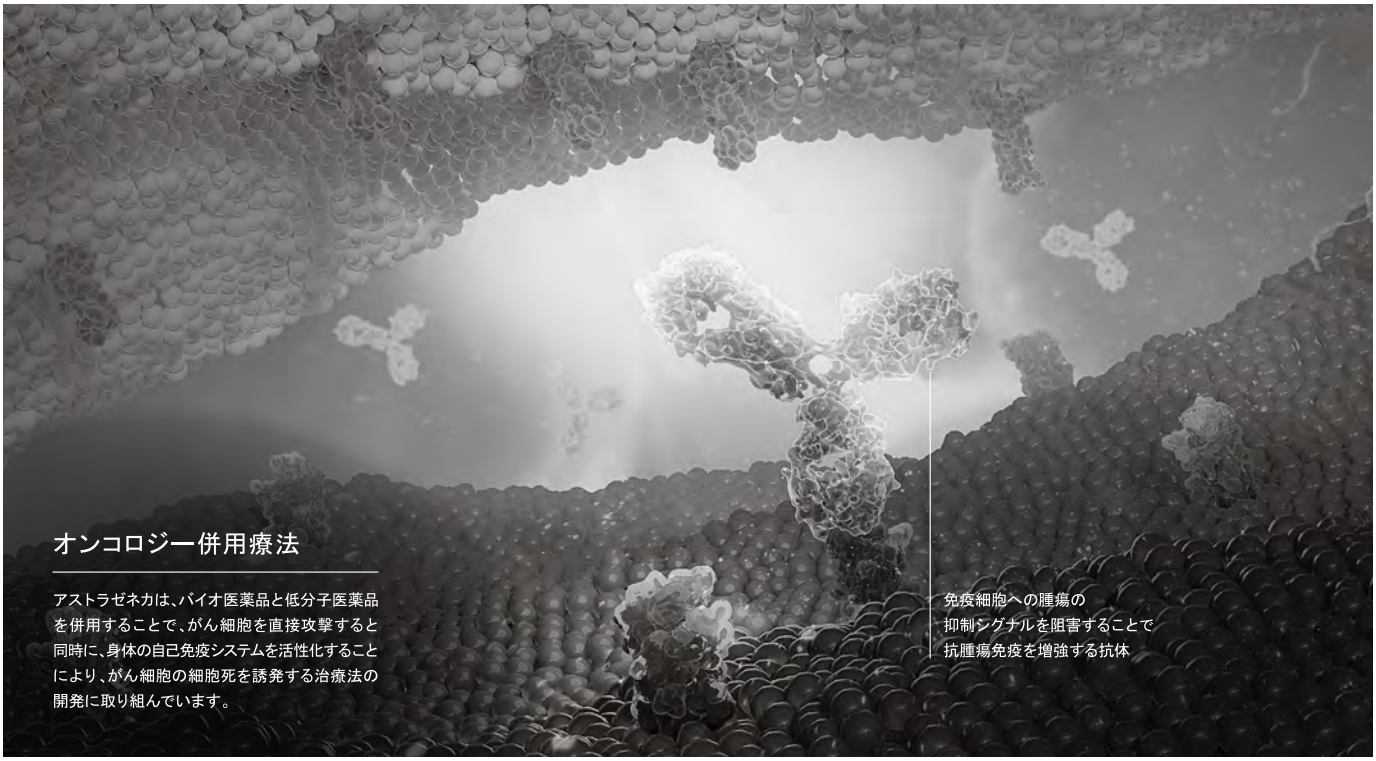


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