Figure 8. Structures of neutral, monosialyl, and disialyl PA-oligosaccharides in iPSCs, iPSC-CM, and heart cells. Glucose units (GU) were calculated from the peak elution times for the ODS column in Figure 5, 6 and 7, and the amide column (data not shown). Average mass (Mass) calculated from the *m/z* values of $[M+Na]^+$ or $[M+H]^+$ ion for neutral, $[M-H]^-$ ion for monosialyl, and $[M-H]^-$ & $[M+Na-2H]^-$ ions for disialyl PA-oligosaccharides.
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iPSC-CMs (959A2-1 CM: 77.4%, 959C1-1 CM: 60.0% and 956F-1 CM: 65.1%), and lowest in the Heart (46.9%). The quantity of monofucosylated, difucosylated, and other types of *N*-glycans were greater in the iPSC-CMs and Heart (Figure 8, 9).

Sialyl *N*-glycans increased with cardiomyogenic differentiation

The quantity of monosialyl *N*-glycans (MS) calculated from the total volume of M1–M23 increased in iPSC-CMs (959A2-1 CM: 6.4%, 959C1-1 CM: 15.7% and 956F-1 CM: 10.5%) and Heart (19%) and were low in iPSCs (959A2-1: 0.5%, 959C1-1: 0.7% and 956F-1: 1.1%). The disialyl *N*-glycans (DS; D1–D12) yielded a similar pattern. The quantity of asialyl *N*-glycans (AS; N1–N17) decreased in iPSC-CMs (959A2-1 CM: 89.2%, 959C1-1 CM: 79.4% and 956F-1 CM: 81.7%) and Heart (55.3%) in comparison to the iPSCs (959A2-1: 96.9%, 959C1-1: 98.1% and 956F-1: 95.8%) (Figure 9, 10).

Rarely expressed *N*-glycans

The sialic acids identified in this study were either *N*-acetyl neuraminic acid (NeuAc) or *N*-glycolyl neuraminic acid (NeuGc). The quantity of monosialyl and disialyl *N*-glycans containing only NeuAc (A, A/A) was lowest in iPSCs (959A2-1: 2.5%, 959C1-1: 1.7% and 956F-1: 3.7%) and similar in iPSC-CMs (959A2-1 CM: 10.6%, 959C1-1 CM: 21% and 956F-1 CM: 18%) and the Heart (8%). The quantity of monosialyl and disialyl *N*-glycans containing only NeuGc (G, G/G) was markedly higher in the Heart (32.8%) than in iPSCs (959A2-1: 0.6%, 959C1-1: 0.1% and 956F-1: 0.5%) or iPSC-CMs (959A2-1 CM: 0%, 959C1-1 CM: 0% and 956F-1 CM: 0%) (Figure 10a).

Expression of glycosyl transferase, ST3Gal-III, ST3Gal-IV, ST6Gal-I, and CMAH in the iPSCs, iPSC-CMs, and Heart was assessed by RT-PCR to explore the glycan structures responsible for the differences between groups. The Heart expressed high levels of CMAH (0.91±0.13/GAPDH); levels in the iPSCs and iPSC-CMs were markedly lower (iPSCs: 959A2-1 0.011±0.0065/GAPDH, 959C1-1 0.013±0.0070/GAPDH, 956F-1 0.0045±0.0042/GAPDH, $P<0.05$; iPSC-CM: 959A2-1 CM 0.21±0.16/GAPDH, 959C1-1 CM 0.19±0.04, 956F-1 CM 0.45±0.31, $P<0.05$). Expression of ST3Gal-III was significantly higher in the Heart (0.98±0.13/GAPDH) than in iPSCs (959A2-1: 0.21±0.05/GAPDH, 959C1-1: 0.18±0.07/GAPDH, 956F-1: 0.27±0.05/GAPDH) and iPSC-CMs (959A2-1 CM: 0.40±0.10/GAPDH, 959C1-1 CM: 0.35±0.09/GAPDH, 956F-1 CM: 0.66±0.18); expression of ST3Gal-IV did not differ between groups. ST6Gal-I expression was significantly higher in iPSC-CMs (959A2-1 CM: 1.87±0.41/GAPDH, 959C1-1 CM: 1.95±0.22/GAPDH, 956F-1 CM: 3.08±1.27/GAPDH) than in iPSCs (959A2-1: 0.51±0.18/GAPDH, 959C1-1: 0.40±0.09/GAPDH, 956F-1: 0.62±0.29/GAPDH) and the Heart (1.04±0.13/GAPDH) (Figure 10b).

Discussion

Sixty-eight different *N*-glycans were isolated from iPSCs, iPSC-CMs, and the Heart. The structures of 60 *N*-glycans were identified, based on their HPLC elution peaks (Figure 8, Table S1-S5). Each preparation contained a combination of neutral, monosialyl, and disialyl *N*-glycans.

The molar ratios of high-mannose, monofucosylated, and difucosylated *N*-glycans were substantially different between groups (Figure 9), although no clear differences in the abundance of these glycans were found. The decrease in high-mannose *N*-glycans and increase of fucosylated *N*-glycans in iPSC-CMs versus iPSCs is consistent with a previous report on a comparison of ESC derived cardiomyocytes to undifferentiated ESCs [18]. Generally, all *N*-glycans are synthesized from the high-mannose type by a large array of sequentially and competitively acting biosynthetic enzymes located throughout the endoplasmic reticulum and Golgi apparatus [26], indicating that the high-mannose type of *N*-glycans could be categorized as a marker of immaturity. In this study, the high-mannose *N*-glycans were highest in the immature iPSC and lowest in the Heart, or mature tissue; thus, the quantity of high-mannose-type *N*-glycans might be an indicator of maturity in iPSC-derivatives and the iPSC-CMs in our protocol may still be immature in comparison to cardiac tissue.

Clear differences in glycan abundance were observed, such as hybrid and complex types represented by N9-1, N9-3, N15, N16, M1, M2-1, M2-2, M7, M8, M10, M12, M13, M14-1, M14-2, M17, M18, M20-2, D6 and D9 in iPSC-CMs, M2-3, M3, M4, M9, M11-1, M11-2, M20-1, M21, D1, D2, D3, D5-1, D5-2, D10-2 and D11 in Heart and N14 and M15 in iPSCs; these may also be indicators of maturation stage. In addition, expression of monosialyl and disialyl *N*-glycans in iPSC-CMs fell between the levels observed in the iPSCs and Heart, as were the molar ratios, indicating that the iPSC-CMs may still be immature stage. While many *N*-glycolyl neuraminic acid (NeuGc) structures were detected in the Heart, iPSCs and iPSC-CMs did not contain NeuGc in their sialyl structures, except for D8. Moreover, the molar ratio of NeuAc was low in iPSCs and iPSC-CMs. This finding is one of the clearest differences between iPSCs or iPSC-CMs and Heart cells.

The proposed spectra-based composition of the D8 glycans in iPSCs was [(Hexose)5(HexNAc)5(NeuGc)2(PA)1], indicating that it contains NeuGc. However, D8 might be quite a rare exception because transcript levels of CMAH, which catalyzes the conversion of NeuAc to NeuGc, was quite low in iPSCs in comparison to the Heart. This data suggests that during the process of reprograming, iPSCs suppress or eliminate CMAH activity. We conclude that iPSCs contain less sialic acid (especially NeuGc) and high-mannose structures are abundant in the *N*-glycans. In contrast, heart cells produce numerous sialyl-*N*-glycans, especially NeuGc. Transcript levels of CMAH tended to increase in iPSC-CMs relative to iPSCs, suggesting cardiomyogenic differentiation may induce expression of CMAH. If the iPSC-CMs could be matured more closely to the Heart by some additional methods of culture, the quantity of high mannose type of *N*-glycans might decrease more closely to the Heart, and might produce *N*-glycans containing NeuGc, followed by the expression of CMAH.

A terminal NeuGc, the Hanganutziu-Deicher (H-D) epitope [27], is widely distributed in the animal kingdom with the exception of humans and chickens. Expression of NeuGc is controlled by CMAH activity. Irie et al. [28] and Chou et al. [29] cloned the cDNA for human CMAH and reported that the *N*-terminal truncation of human CMAH is caused by deletion of Exon 6, a 92-base pair segment in the genomic DNA. Expression of this truncation in the heart eliminates NeuGc in sialyl

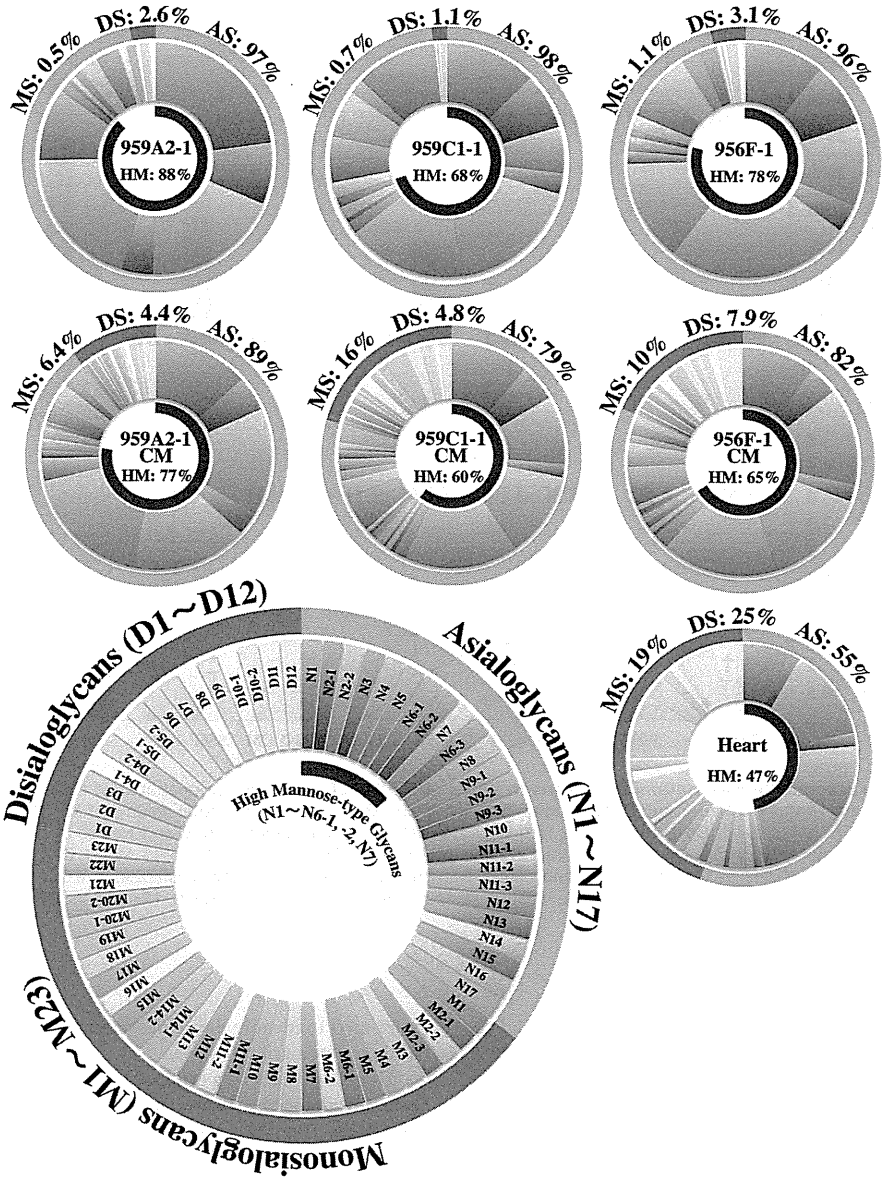

Figure 9. Relative quantities of neutral, monosialyl, and disialyl PA-oligosaccharides in iPSCs, iPSC-CM, and heart cells. Relative quantities of each glycan, calculated from the peak area in Figure 5, 6 and 7 vs. total *N*-glycan content in each cell, were expressed in the doughnut charts. Relative quantities of the asialoglycans, the monosialoglycans and the disialoglycans were showed outside of the charts, and relative quantities of the high mannose type glycans were showed inside of the charts. Asialoglycan (AS): the total volume of N1-N17; Monosialoglycan (MS): the total volume of M1-M23; Disialoglycan (DS): the total volume of D1-D12, High mannose-type glycan (HM): the total volume of N1–N6-1, N6-2, N7.
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structures. If human iPSCs or iPSC-CMs do not express CMAH in the same way as murine iPSCs or iPSC-CMs, there may be no difference between human iPSCs, iPSC-CMs, and the human Heart. Further study on human iPSC-CM will be needed to completely understand the features of the sialyl acid of *N*-glycans.

It was reported that human iPSCs produced α2,6sialyl glycans but did not contain α2,3sialyl structures, in contrast to human fibroblast, the origin of iPSCs, which produced α2,3sialyl but not α2,6sialyl structures [30,31]. The murine iPSCs in this study contained α2,3sialyl structures in NeuAc, M5, M23, D4-1, D10-1 and D12, and the iPSC-CMs produced α2,3 and α2,6sialyl structures in NeuAc. These differences may be due to variations between species, because mouse Heart cells also contained α2,3 and α2,6sialyl structures in NeuGc. Further studies are needed to characterize the glycome shift in the production and differentiation of iPSCs.

Type I Lactose structures were not detected, although over 98% of glycans in each cell were accounted for in this study. The *N*-glycans of N9-3, M8, M12, M17, and M23, which were identified after α-galactosidase digestion, contained Galα1-6Gal, not only in

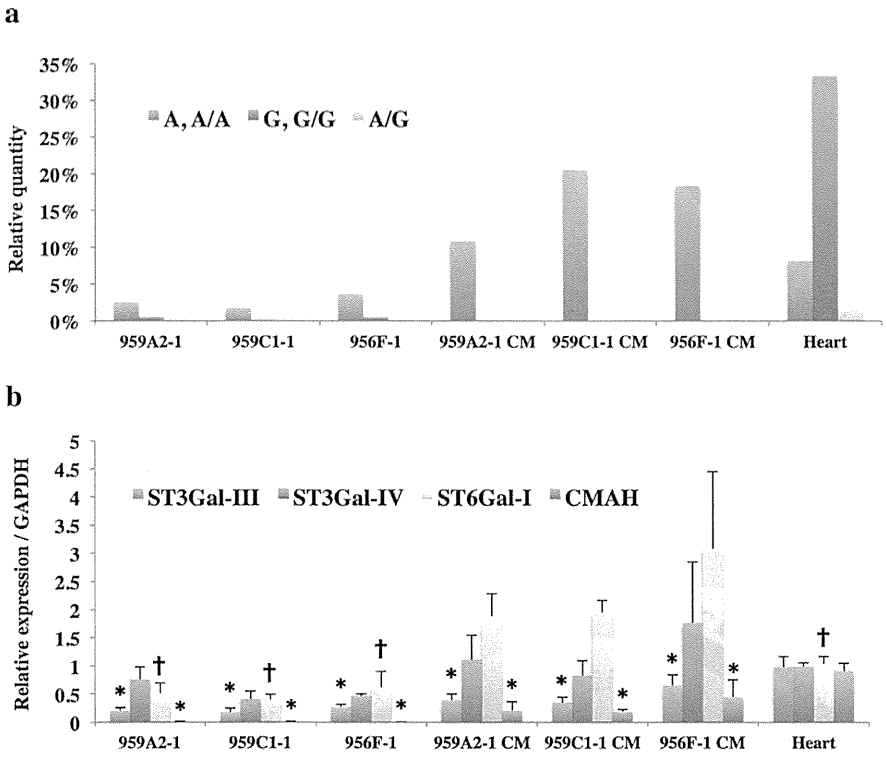

Figure 10. Rarely expressed NeuGc-containing glycans in iPSCs and iPSC-CMs. (a) Relative quantities of NeuAc- and NeuGc-containing glycans; Monosialoglycans containing NeuAc and Disialoglycans containing two NeuAc (A, A/A): the total volume of M1, M2-1, M2-2, M5-M8, M10-M14, M16-M19, M20-2, M21-M23, D4-1, D4-2, D6, D7, D9, D10-1, D12, Disialoglycan containing NeuAc and NeuGc (A/G): D11, Monosialoglycan containing NeuGc and Disialoglycan containing two NeuGc (G, G/G): the total volume of M2-3, M3, M4, M9, M15, M20-1, D1-D3, D5-1, D5-2, D8, D10-2. (b) Transcript expression of ST3Gal-III, ST3Gal-IV, ST6Gal-I, and CMAH; Transcript expression of glycosyltransferases in iPSCs, iPSC-CM, and heart cells was analyzed by real-time PCR. Results are expressed as the mean ± standard deviation. *$P<0.05$ vs. Heart, †$P<0.05$ vs. iPSC-CM (all of the 959A2-1 CM, 959C1-1 CM and 956F-1 CM).
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the neutral glycans but also in the monosialyl *N*-glycans of the iPSC-CM preparation. The same structure was not found in iPSCs, but only one structure, M23, was present in Heart cells. Therefore, in iPSC-CMs, Galα1-6Gal enzyme activity appears to be up-regulated in comparison to wild-type myocardium, although enzyme activity was not assessed by RT-PCR because of the limited availability of genetic sequence data.

The D8 was identified in all of three iPSC lines and not in the iPSC-CMs and Heart. This structure, unfortunately not identified in this study, may be useful as markers of undifferentiated iPSCs in the same way as well-known pluripotency biomarkers such as stage-specific embryonic antigens (SSEA)-3, SSEA-4 (glycosphingolipids) [32].

Previous MALDI-TOF/MS and MS/MS studies concluded that many kinds of *N*-glycans are found in organs and cells. The number of detected *N*-glycans is attributed to the sensitivity of the MS and HPLC methods employed. That is, MS data are sensitive and can be rapidly obtained, but a glycan structure is identified based only on the calculated molecular weight. Therefore, discriminating between isomeric structures is difficult. On the other hand, it thus appears that the accuracy of the data presented here using HPLC mapping in conjunction with a MALDI-TOF technique provides much more detailed information. Our data were used to identify the representative features of each *N*-glycan in these three cell types.

There may be a concern that the heart tissue used in this study contains connective tissues, vessels or nerves other than cardiomyocytes. Therefore, some of the *N*-glycans detected from the Heart sample might be derived from the tissues other than cardiomyocytes. However, heart is majority composed by cardiomyocytes, and furthermore, even if a small amount of *N*-glycans derived from connective tissues were contaminated in the Heart sample, the main evidences in this study, such as the proportion of the high-mannose type *N*-glycans, the ratio of the active sialyltransferase genes, the existence of NeuGc, and the uncommonness of Galα1-6 Gal, are essentially not affected.

In summary, murine iPSCs were rich in high-mannose type *N*-glycans but very poor in sialyl type *N*-glycans. Murine heart tissue contained a relatively low volume of high-mannose glycans, but was very rich in neuraminic acid, especially NeuGc type sialyl structures. Under these conditions, the volume of each type of glycan was similar for iPSC-CMs and iPSCs. That is, they were rich in high-mannose and relatively poor in sialyl type *N*-glycans by volume. In addition, most of the sialyl structures of the iPSC-CMs were different from those of the Heart, and the iPSC-CMs expressed no NeuGc. Moreover, the iPSC-CMs produced several unique glycans with the Galα1-6Gal structure. These results

provide important data that can be useful in future clinical iPSC studies.

It is quite important to investigate the meaning of *N*-glycans transitions during the cardiomyogenic differentiation presented in this study, for deeply understanding the relationship between the *N*-glycan expression and cardiomyogenic differentiation. Knock-out or knock-down of the genes related to cardiomyogenic differentiation or glycosylation may be useful for such purpose. However, the *N*-glycan signature in the cell surface is determined by a variety of the genes. Knock-out or knock-down of a single gene related to cardiomyogenic differentiation would alter an array of gene expressions, such as sarcomere proteins, transcriptional factors, or cell surface proteins, all of which would affect the signature of *N*-glycans in the cell surface. Therefore, the data interpretation for relationship between expression of a single gene and *N*-glycan signature would be difficult. Some different experimental approach may be needed to investigate the meaning of change in *N*-glycan expression during cardiomyogenic differentiation.

Supporting Information

Table S1 Structures and relative quantities of neutral (Table S1, S2) PA-oligosaccharides derived from iPSC, iPSC-CM, and heart cells. a. Glucose units (GU) were calculated from the peak elution times of the peaks obtained from the ODS column in Figure 5, 6, 7 and the Amide column (data not shown). b. Average mass calculated from the *m/z* values of $[M+Na]^+$ or $[M+H]^+$ ion for neutral, $[M-H]^-$ ion for mono-sialyl, and $[M-H]^-$ & $[M+Na-2H]^-$ ions for di-sialyl PA-oligosaccharides. c. PA-oligosaccharide structures. d. mol% was calculated from the peak area versus total *N*-glycan content in each cell
(TIFF)

Table S2 Structures and relative quantities of neutral (Table S1, S2) PA-oligosaccharides derived from iPSC, iPSC-CM, and heart cells.
(TIFF)

Table S3 Structures and relative quantities of mono-sialyl (Table S3, S4) PA-oligosaccharides derived from iPSC, iPSC-CM, and heart cells.
(TIFF)

Table S4 Structures and relative quantities of mono-sialyl (Table S3, S4) PA-oligosaccharides derived from iPSC, iPSC-CM, and heart cells.
(TIFF)

Table S5 Structures and relative quantities of disialyl PA-oligosaccharides derived from iPSC, iPSC-CM, and heart cells.
(TIFF)

Video S1
(MP4)

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Author Contributions

Conceived and designed the experiments: TK S. Miyagawa S. Miyagawa JL YS. Performed the experiments: TK AY NK AK EI AM HE KT. Analyzed the data: TK S. Miyagawa YS. Contributed reagents/materials/analysis tools: TK AY JL. Wrote the paper: TK S. Miyagawa YS SF. Obtained permission for use of cell line: S. Miyagawa AS YS.

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Comparison of 5-Year Survival After Acute Myocardial Infarction Using Angiotensin-Converting Enzyme Inhibitor Versus Angiotensin II Receptor Blocker

Masahiko Hara, MD[a], Yasuhiko Sakata, MD, PhD[a,b,c,*], Daisaku Nakatani, MD, PhD[a], Shinichiro Suna, MD, PhD[a], Masaya Usami, MD, PhD[a], Sen Matsumoto, MD, PhD[a], Toshifumi Sugitani, MSc[d], Masami Nishino, MD, PhD[e], Hiroshi Sato, MD, PhD[f], Tetsuhisa Kitamura, MD, MSc, DrPH[g], Shinsuke Nanto, MD, PhD[a,b], Toshimitsu Hamasaki, PhD[d], Masatsugu Hori, MD, PhD[h], and Issei Komuro, MD, PhD[i], on behalf of the OACIS Investigators

Few studies have investigated whether angiotensin II receptor blocker (ARB) is a practical alternative to angiotensin-converting enzyme inhibitor (ACEI) for long-term use after acute myocardial infarction (AMI) in real-world practice in the percutaneous coronary intervention era. We compared 5-year survival benefits of ACEI and ARB in patients with AMI registered in the Osaka Acute Coronary Insufficiency Study. Study subjects were divided into 3 groups: ACEI (n = 4,425), ARB (n = 2,158), or patients without either drug (n = 2,442). A total of 661 deaths were recorded. Cox regression analysis revealed that treatment with either ACEI or ARB was associated with reduced 5-year mortality (adjusted hazard ratio [HR] 0.70, 95% confidence interval [CI] 0.58 to 0.83, p <0.001 and HR 0.79, 95% CI 0.64 to 0.98, p = 0.03, respectively). However, Kaplan-Meier estimates and Cox regression analyses based on propensity score revealed that ACEI was associated with better survival than ARB from 2 to 5 years after survival discharge (adjusted HR 0.53, 95% CI 0.38 to 0.74, p <0.001). These findings were confirmed in a propensity score–matched population. In conclusion, treatment with ACEI was associated with better 5-year survival after AMI. **(Am J Cardiol 2014;114:1–8)**

Angiotensin-converting enzyme inhibitor (ACEI) was the first clinically approved renin-angiotensin-aldosterone system (RAS) inhibitor, and much evidence presented in the 1990s and early 2000s have demonstrated the effectiveness of ACEI for improving cardiovascular disease–related morbidity and mortality.[1–5] Angiotensin II receptor blocker (ARB) has also been examined clinically for cardiovascular disease treatment.[6–10] Based on the results of 2 randomized clinical trials (RCTs) such as Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan (OPTIMAAL) and the Valsartan in Acute Myocardial Infarction (VALIANT), which examined clinical impacts of ARB after acute myocardial infarction (AMI), the international guidelines recommend that ACEI should be used as the first-line treatment after AMI and that ARB should be considered in patients who are intolerant to ACEI therapy.[6,7,11,12] We investigated whether ACEI and ARB had comparable long-term benefits in a large cohort of post-AMI patients registered in the Osaka Acute Coronary Insufficiency Study (OACIS).[13,14]

Methods

The OACIS is a prospective, multicenter, observational study enrolling consecutive patients with AMI at 25 collaborating hospitals in the Osaka region of Japan.[13,14] The OACIS is registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) in Japan (ID: UMIN000004575). Details of OACIS are described elsewhere (Supplementary Material).[13,14]

The diagnosis of AMI was based on the World Health Organization criteria,[15] which required 2 of the following 3 criteria to be met: (1) clinical history of central chest pressure, pain, or tightness lasting ≥30 minutes; (2) ST-segment elevation >0.1 mV in at least 1 standard or 2 precordial leads; and (3) an increase in serum creatine phosphokinase concentration of more than twice the normal laboratory value. Research cardiologists and trained research nurses recorded data concerning sociodemographic variables, medical

Departments of [a]Cardiovascular Medicine, [b]Advanced Cardiovascular Therapeutics, and [d]Biomedical Statistics and [g]Division of Environmental Medicine and Population Sciences, Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Suita, Japan; [c]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [e]Division of Cardiology, Osaka Rosai Hospital, Sakai, Japan; [f]School of Human Welfare Studies, Kwansei Gakuin University, Nishinomiya, Japan; [h]Osaka Prefectural Hospital Organization, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; and [i]Department of Cardiovascular Medicine, The University of Tokyo Graduate School of Medicine, Tokyo, Japan. Manuscript received February 22, 2014; revised manuscript received and accepted March 25, 2014.

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See page 7 for disclosure information.

*Corresponding author: Tel: (+81) 22-717-7152; fax: (+81) 22-717-7156.

E-mail address: sakatayk@cardio.med.tohoku.ac.jp (Y. Sakata).

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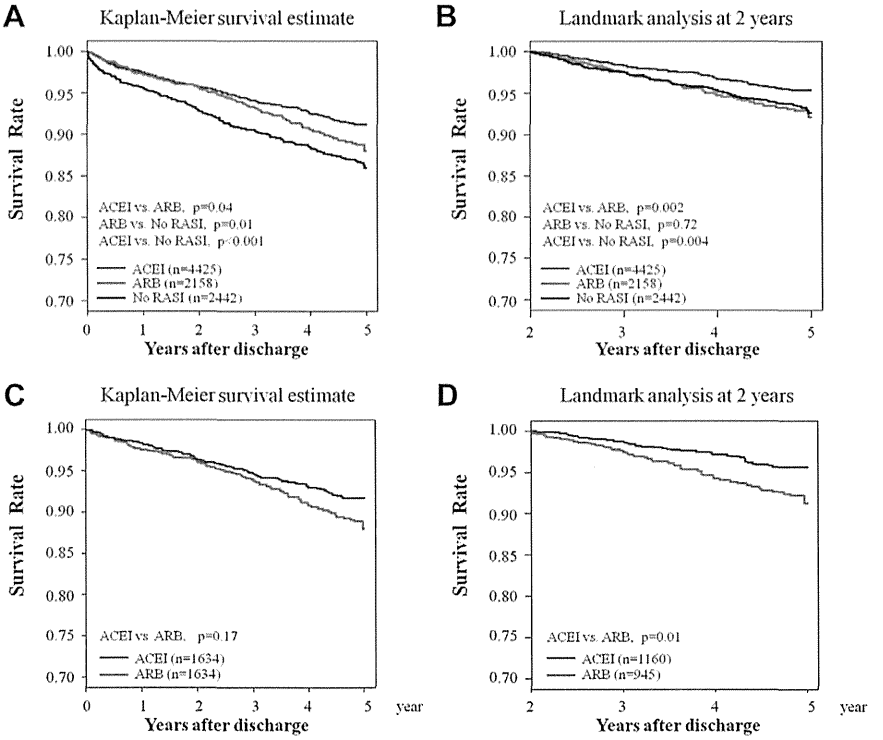

Figure 1. Kaplan-Meier survival estimates and landmark analysis results after survival discharge for AMI in the entire study population *(A* and *B)* and the PS-matched samples *(C* and *D)*. RASI = renin-angiotensin-aldosterone system inhibitor.

history, therapeutic procedures, and clinical events during the patient's hospital stay. The present study protocol complied with the Declaration of Helsinki and was approved by the institutional ethical committee of each participating institution. All study candidates were informed about data collection and blood sampling, and written informed consents were obtained.

A flowchart of patient selection is presented in Supplementary Figure 1. A total of 4,425 patients treated with ACEI at discharge, 2,158 with ARB, and 2,442 prescribed neither ACEI nor ARB (no RAS inhibitor) were enrolled in the present study. A direct comparison of survival benefit was performed between patients treated with ACEI and those with ARB at discharge. For the inverse probability of treatment weighting (IPTW) method using propensity score (PS), 5,563 eligible patients without missing data for Cox regression analysis were selected (3,784 and 1,779 patients with ACEI or ARB at discharge, respectively). For PS-matched analysis, 3,268 patients (1,634 in each treatment group) were selected and analyzed.

The primary end point was all-cause death, and the secondary end points were heart failure hospitalization and nonfatal re-myocardial infarction. For patients discharged alive, follow-up clinical data were obtained for 5 years. Categorical variables were compared by chi-square tests, and continuous variables were compared by the Kruskal-Wallis test for 3-group comparison (ACEI, ARB, and no RAS inhibitor) and Wilcoxon rank sum test for 2-group comparison (ACEI and ARB). The annual trend in the prescription rate of ACEI or ARB was assessed by the Cochran-Armitage trend test (Supplementary Figure 2). The Kaplan-Meier method was used to estimate event rates, and the differences were assessed by the log-rank test. Landmark analysis of the primary end point was also performed 2 years after survival discharge. Specifically, survival estimates were calculated in patients without any adverse events 2 years after survival discharge, because the Kaplan-Meier survival estimates for the ACEI and ARB treatment groups appeared to differentiate at this time point (Figure 1).

Inter- and intra-class drug differences in survival benefit were compared by age and sex-adjusted Cox regression analyses, and the hazard ratio (HR) and 95% confidence intervals (CI) were calculated using data obtained from the 2,442 patients without RAS inhibitors as a reference (Supplementary Figure 1). To reduce potential confounding effects due to patient background variability in the direct comparison between ACEI and ARB, the PS method was used in combination with Cox regression modeling. PS was defined as the probability of treatment assignment conditional on the measured baseline covariates. The inverse probability of treatment weighting method based on the PS was used to reduce confounding in time-to-event observational data.[16] To confirm the robustness of the inverse probability of treatment weighting results, we also performed PS matching with a caliper width of 0.001.[16] For the estimation of PS, we used a logistic regression model in which the treatment status (ACEI or ARB) was regressed on the following baseline characteristics: age, gender, body mass index, diabetes, hypertension, dyslipidemia, smoking, previous myocardial infarction, ST elevation myocardial infarction, Killip's classification, reperfusion therapy, and prescription of β blockers, calcium channel blockers, statins, diuretics, and antiplatelet agents.

Table 1
Demographics and clinical characteristics of the study population by treatment group

Parameter	No RASI (n = 2442)	ACEI (n = 4425)	ARB (n = 2158)	p-Value (Total)	p-Value (ACEI vs ARB)
Age (years)	67 (59–75)	65 (57–73)	67 (59–75)	<0.001	<0.001
Men	73.6%	77.9%	74.3%	<0.001	0.001
Body mass index (kg/m^2)	23.0 (21.0–25.2)	23.5 (21.5–25.7)	23.9 (21.6–26.0)	<0.001	0.001
ST-elevation myocardial infarction	82.3%	86.8%	83.7%	<0.001	<0.001
Diabetes mellitus	34.7%	32.6%	34.0%	0.19	0.27
Hypertension	49.4%	59.3%	70.3%	<0.001	<0.001
Dyslipidemia	40.6%	44.8%	46.5%	<0.001	0.19
Smoking	59.3%	66.0%	61.5%	<0.001	<0.001
Previous myocardial infarction	13.6%	11.9%	10.8%	0.02	0.18
KILLIP class				<0.001	0.01
1	79.5%	85.4%	84.2%		
2	9.1%	8.4%	7.4%		
3	4.1%	3.3%	4.4%		
4	7.3%	2.9%	4.0%		
Emergent coronary angiography	92.7%	95.3%	96.2%	<0.001	0.10
Target Lesion				<0.001	0.22
Left main	3.1%	0.9%	1.3%		
Left anterior descending artery	38.6%	47.9%	46.2%		
Right coronary artery	38.7%	34.9%	34.2%		
Left circumflex artery	16.3%	12.9%	14.8%		
Diagonal branch	3.0%	3.2%	3.4%		
Graft	0.4%	0.1%	0.1%		
Reperfusion therapy					
Percutaneous coronary intervention	80.4%	89.8%	93.2%	<0.001	<0.001
Thrombolysis	8.2%	7.1%	6.6%	0.12	0.49
Coronary artery bypass graft	6.6%	0.9%	1.4%	<0.001	0.07
Hemoglobin A1c (%)	6.0 (5.56.9)	5.9 (5.5–6.9)	6.0 (5.6–7.0)	0.01	0.001
Total cholesterol (mg/dL)	187 (158–218)	190 (164–220)	193 (166–224)	<0.001	0.02
Low-density lipoprotein cholesterol (mg/dL)	113 (87–139)	122 (99–148)	124 (101–149)	<0.001	0.57
High-density lipoprotein cholesterol (mg/dL)	45 (37–53)	44 (38–53)	44 (37–52)	0.78	0.49
Triglyceride (mg/dL)	91 (60–137)	94 (60–143)	99 (64–149)	<0.001	0.002
Estimated glomerular filtration rate (mL/min/1.73 m^2)	47.9 (33.8–61.8)	51.8 (41.2–64.5)	52.8 (40.9–65.4)	<0.001	0.35
Peak creatine phosphokinase (IU/L)	1701 (793–3400)	2025 (925–3801)	1793 (910–3503)	<0.001	0.02
Echocardiography data					
Left ventricular end-diastolic dimension (mm)	50.0 (46.0–54.0)	50.0 (46.5–54.0)	50.9 (47.0–55.0)	0.04	0.03
Left ventricular end-systolic dimension (mm)	34.0 (30.0–40.0)	34.0 (30.0–39.0)	34.0 (30.0–39.0)	0.051	0.04
Left ventricular ejection fraction (%)	52.6 (43.8–60.3)	53.5 (44.6–60.7)	55.6 (46.3–62.2)	<0.001	<0.001
Medication at discharge					
Beta-blocker	35.5%	48.8%	62.9%	<0.001	<0.001
Calcium channel blocker	26.0%	18.6%	19.7%	<0.001	0.27
Statin	29.7%	39.6%	57.0%	<0.001	<0.001
Antiplatelet	91.0%	98.1%	98.5%	<0.001	0.35
Diuretic	30.7%	26.9%	26.5%	<0.001	0.74
Follow-up duration (days)	1416 (345–1792)	1635 (707–1798)	1032 (343–1737)	<0.001	<0.001

Categorical variables are presented as number (percentage), and continuous variables are presented as the median (25-75 percentiles). Laboratory data were measured on admission.

Statistical significance was set as $p < 0.05$. All statistical analyses were performed using SAS, version 9.3 (SAS Institute Inc., Cary, North Carolina), or R software packages, version 2.15.1 (R Development Core Team, Vienna, Austria).

The corresponding author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Results

Patient characteristics based on treatment group are summarized in Table 1. Significant differences in nearly all background variables were detected among the ACEI, ARB, and no RAS inhibitor treatment groups. Notably, patients in the no RAS inhibitor group were less frequently treated with evidence-based medications. Between the ACEI and ARB treatment groups, patients who received ACEI had lower prescription rates for state-of-the-art medications at discharge, such as β blockers and statins, partly because these patients were likely registered in the earlier period of the OACIS registry (Supplementary Figure 2). In the PS-matched cohort, patient characteristics were well balanced (Supplementary Table 1).

Annual trends in the prescription rate of RAS inhibitors are shown in Supplementary Figure 2. The prescription rate

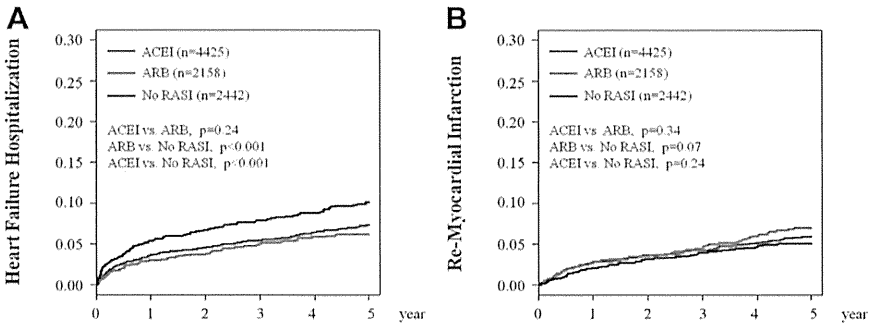

Figure 2. Cumulative event rates of heart failure hospitalization *(A)* and nonfatal re-myocardial infarction *(B)* in the 3 treatment groups during a 5-year follow-up period. RASI = renin-angiotensin-aldosterone system inhibitor.

of ARB had increased annually until 2007, whereas that of ACEI decreased. In 2010, approximately 80% of all study patients received RAS inhibitors at discharge. The types of ACEI and ARB prescribed at discharge are listed in Supplementary Table 2.

A total of 661 deaths (no RAS inhibitor, 231; ACEI, 293; and ARB, 137), 512 heart failure hospitalizations (no RAS inhibitor, 174; ACEI, 250; and ARB, 88), and 375 nonfatal re-myocardial infarctions (no RAS inhibitor, 85; ACEI, 200; and ARB, 90) were recorded during a median follow-up period of 3.9 years (median 1,426 days, interquartile range 402 to 1,794). Kaplan-Meier survival analysis demonstrated that both the ACEI and ARB groups had better 5-year mortality than the no RAS inhibitor group (Figure 1). Age and sex-adjusted Cox regression analysis revealed that both ACEI and ARB treatments were associated with reduced 5-year mortality compared with no RAS inhibitor treatment (adjusted HR 0.70, 95% CI 0.58 to 0.83, $p < 0.001$ for ACEI and adjusted HR 0.79, 95% CI 0.64 to 0.98, $p = 0.03$ for ARB, respectively). However, treatment with ACEI was associated with significantly lower 5-year mortality compared with that with ARB (Figure 1). Landmark analysis demonstrated that the superiority of ACEI with regard to long-term prognostic impact was only evident after 2 years of discharge. In addition, the survival estimate of the ARB group from 2 to 5 years after survival discharge was comparable to that of the no RAS inhibitor group (Figure 1). These observations were consistent with those obtained in the PS-matched cohort (Figure 1). In contrast to the survival rates, no significant differences in heart failure hospitalization or nonfatal re-myocardial infarction rates were detected between the ACEI and ARB groups (Figure 2).

Cox regression analysis in the PS-weighted sample revealed that the adjusted HRs of 2-year mortality in the ACEI group compared with the ARB group was 1.05 (95% CI 0.76 to 1.47, $p = 0.76$) in the first 2 years after survival discharge and 0.53 (95% CI 0.38 to 0.74, $p < 0.001$) from 2 to 5 years after survival discharge (Figure 3). These results are consistent with those obtained by the Cox regression analyses in the PS-matched sample. The adjusted HR of 2-year mortality was 1.17 (95% CI 0.77 to 1.76, $p = 0.46$) in the first 2 years after survival discharge and 0.56 (95% CI 0.34 to 0.91, $p = 0.02$) from 2 to 5 years (Figure 3). Subgroup analysis suggested that ACEI and ARB had generally comparable prognostic impacts for the 2 years after discharge, with the exception of the subgroups without hypertension (Figure 3), and that ACEI was associated with better survival from 2 to 5 years after discharge, except in patients aged $\leq$60 years (Figure 3). Intraclass drug comparisons revealed that both ACEI and ARB displayed similar effectiveness compared with the no RAS inhibitor patient group in the first 2 years after survival discharge (Figure 4). However, treatment with ACEI, but not with ARB, was associated with better mortality rates from 2 to 5 years after survival discharge in comparison with the no RAS inhibitor treatment group (Figure 4).

Discussion

We compared the long-term prognostic impacts of ACEI and ARB after AMI using a multicenter prospective observational registry database in Japan. The results primarily showed that treatment with either ACEI or ARB was associated with better 5-year survival compared with patients who did not receive either drug, confirming the clinical importance of RAS inhibition in post-AMI patients. However, our results further demonstrated that patients treated with ACEI had significantly lower long-term mortality compared with those treated with ARB from 2 to 5 years after AMI with the comparable prognostic impacts between ACEI and ARB in the first 2 years.

The present study is the first to compare the long-term prognostic impacts of ACEI and ARB in post-AMI patients in the contemporary percutaneous coronary intervention (PCI) era. The observation that prognostic impacts in the first 2 years after discharge of AMI were comparable between ACEI and ARB was consistent with the results derived from the OPTIMAAL and VALIANT RCTs,[6,7] which demonstrated comparable benefits between ACEI and ARB in post-AMI patients with relatively short follow-up periods. In contrast, we also demonstrated the better prognostic impact of ACEI beginning after 2 years of AMI onset, which was partly consistent with findings reported by Savarese et al.[17] In a meta-analysis of 26 RCTs comparing ACEI or ARB versus placebo in 108,212 patients at high cardiovascular risk without heart failure, they revealed that only ACEI, but not ARB, reduced the risk of all-cause death, whereas ACEI and ARB both reduced the risk of the composite outcome of cardiovascular death, myocardial infarction, and stroke.[17] We speculate that the mechanism for the superiority of ACEI over ARB treatment may be explained by a reduction in angiotensin II production and activation of the kallikrein-bradykinin system with ACEI treatment or prolonged elevation of angiotensin II levels

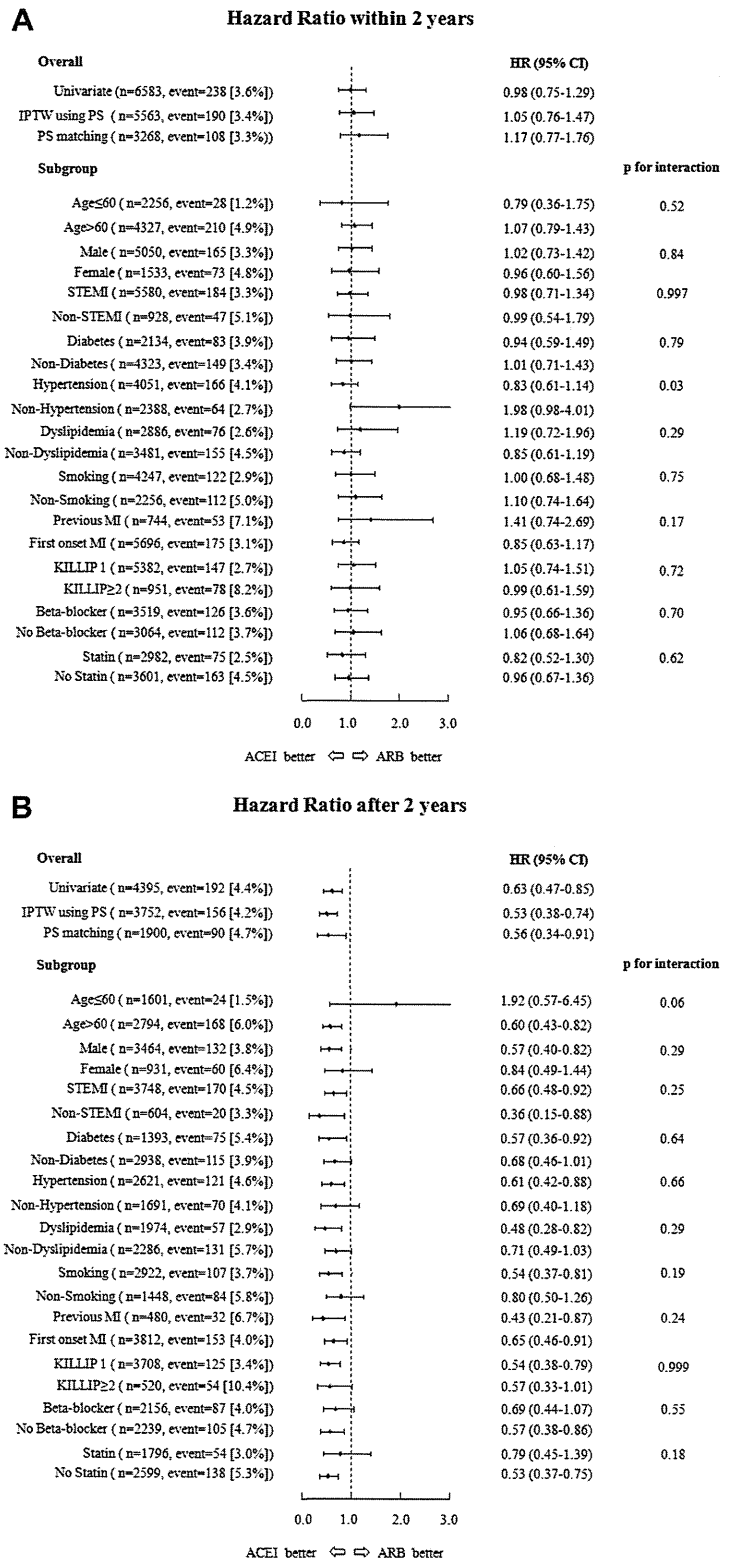

Figure 3. Comparison of survival benefits between ACEI and ARB treatment in the secondary prevention setting after AMI. HR for mortality during the first 2 years *(A)* and after 2 years after survival discharge *(B)*. IPTW = inverse probability of treatment weighting; STEMI = ST elevation myocardial infarction.

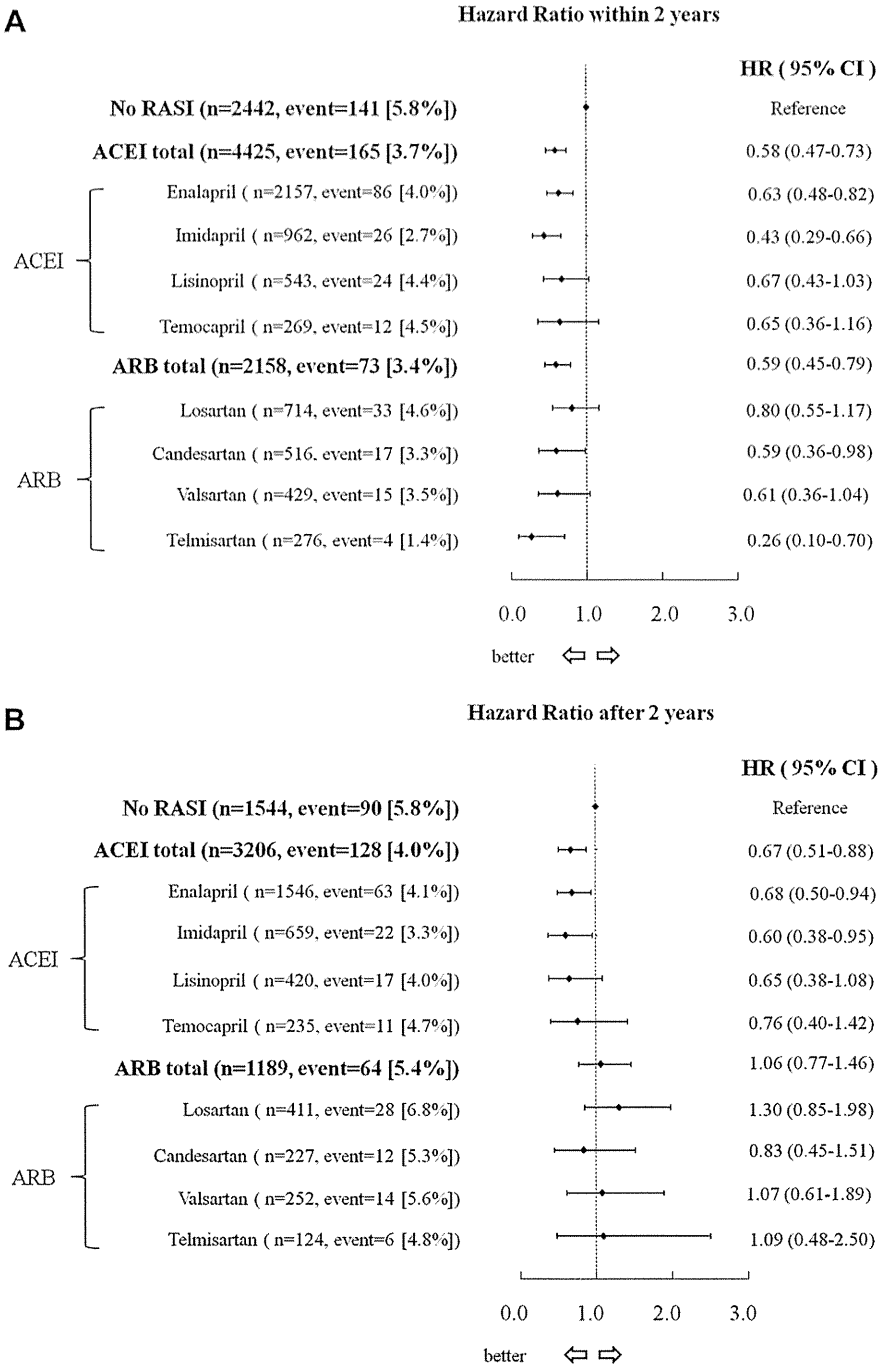

Figure 4. Inter- and intra-class drug differences of HR for mortality. HRs for mortality during the first 2 years *(A)* and after 2 years after survival discharge *(B)* were calculated using age and sex-adjusted Cox regression analysis. Intraclass drug differences were evaluated for the top 4 prescribed ACEI and ARB. RASI = renin-angiotensin-aldosterone system inhibitor.

and possible upregulation of angiotensin type 1 receptor with ARB treatment.[18–20]

Another important finding of the present study was the confirmation of the clinical significance of RAS inhibition in post-AMI patients in the contemporary PCI era. Recently, cardiovascular mortality and morbidity have greatly improved along with the implementation of evidence-based therapies in patients with cardiovascular disease.[21–24] Notably, however, most evidence for the improvement of survival outcomes in post-AMI patients was derived in the prereperfusion and thrombolytic eras, in which patients had markedly higher mortality risks compared with the contemporary era. Accordingly, reevaluation of the effectiveness of cardioprotective medications may be warranted in the contemporary PCI era, because the mortality benefits of such medications may have changed along with the decrease of mortality risk.[25] Indeed, it

has recently been questioned whether β blockers remain useful for the full spectrum of post-AMI populations.[14,26] Here, it was confirmed that treatment with RAS inhibitors is still associated with improved long-term mortality in post-AMI patients in the real-world practice in the primary PCI era, as the estimated mortality rate throughout the 5-year period was only 10.7% (95% CI 9.9 to 11.6), which is significantly lower than that of post-AMI patients in the previous eras.[27]

The present study has several limitations that warrant mention. First, the survival benefits of ACEI or ARB were compared based on medications at discharge. In addition, the prescription dose, long-term adherence, discontinuation, incidence of adverse events, and drug information after discharge were not available in the present study. Second, as our study population was mainly composed of a single race (Japanese), our findings should be validated in different races and ethnic groups. Third, landmark analysis was performed based on the results of Figure 1 and has a retrospective nature. Finally, possible selection bias and unmeasured confounding factors may have influenced the study outcomes because of the inherent nature of observational registry. Paradoxically, however, the use of observational data collected in the real-world setting may have provided additional information that could not be obtained from RCTs for increasing the generalizability of findings.[25] In other words, although RCTs are more suitable for evaluating the impact of a target drug, they also require external validation.[25] The limited applicability of RCTs to real-world settings could be obviated by performing complementary observational studies.[25]

Disclosures

Dr. Komuro has received research grants and speaker's fees from Takeda Pharmaceutical Company, Astellas Pharma, DAIICHI SANKYO COMPANY, Boehringer Ingelheim, Novartis Pharma and Shionogi. No other authors have relationships with industry to disclose or financial associations that might pose a conflict of interest in connection with the submitted article.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.amjcard.2014.03.055.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Introduction of Point-of-Care Testing in Japanese Outpatient Clinics Is Associated With Improvement in Time in Therapeutic Range in Anticoagulant-Treated Patients

Yuji Okuyama, MD; Miyuki Matsuo, MD; Hiroshi Matsuo, MD; Yoshihide Sakaguchi, MD; Hiroyuki Takai, MD; Yasunori Horiguchi, MD; Teruhiko Ryomoto, MD; Seiji Adachi, MD; Toshio Amano, MD; Masaki Togawa, MD; Masaharu Masuda, MD; Hitoshi Minamiguchi, MD; Shinsuke Nanto, MD; Issei Komuro, MD; Yasushi Sakata, MD

Background: Warfarin reduces the risk of stroke in patients with atrial fibrillation, but requires a moderate-to-high time in therapeutic range (TTR). We hypothesized that point-of-care (POC) testing for prothrombin time-internationalized normalized ratio (PT-INR) could improve the TTR in patients receiving warfarin.

Methods and Results: Eight outpatient clinics that introduced POC testing for PT-INR participated in this study. We identified 148 consecutive patients who received warfarin for at least 12 months before and after the introduction of POC testing. We compared the TTR before and after the introduction of POC testing for each patient. TTR after the introduction of POC testing was significantly higher than that beforehand (51.9%±33.0% vs. 69.3%±26.3%; P<0.0001). The improvement in TTR was statistically significant in patients who had low TTR (<70%) before the introduction of POC testing. After the introduction of POC, the time spent above the target INR showed no significant change (3.7%±10.6% vs. 3.3%±6.3%, P=0.7322), while that spent below the target INR improved significantly (44.4%±34.4% vs. 27.4%±27.6%, P<0.0001).

Conclusions: The introduction of POC testing was associated with an improvement in TTR, mainly through a reduction in the time spent below the target INR. (*Circ J* 2014; **78:** 1342–1348)

Key Words: Outpatient clinic; Point-of-care testing; Prothrombin time-internationalized normalized ratio; Time in therapeutic range; Warfarin

Strong evidence from clinical trials has shown that warfarin reduces the risk of stroke and mortality in patients with atrial fibrillation (AF).[1] Warfarin, however, is often underused because it is believed to be associated with an increased risk of bleeding.[2–4] A long time in therapeutic range (TTR) is required for warfarin therapy to be maximally effective.[5] This reduces the risk of not only stroke and systemic embolism, but also bleeding.[6,7] Patients with optimal international normalized ratios (INR) experience less severe disability than those with sub-therapeutic INR, when affected by stroke or systemic embolism. In addition, when assessed at 30 days after admission, the prognosis of patients with optimal INR was found to be better than that of patients with sub-therapeutic INR.[8] Therefore, it is critical that the INR of patients receiving warfarin is maintained within the target therapeutic range. In patients with mechanical heart valve(s) and mitral stenosis, INR should also be maintained within the target therapeutic range.[9] This is a major challenge, however, in actual clinical practice. Although the TTR of AF patients managed at specialized cardiology centers in Japan has been reported to be 64%, that of patients attending outpatient clinics has not yet been investigated.[10] In Japan, many elderly patients are followed up at outpatient clin-

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Advanced Cardiovascular Therapeutics and Department of Cardiology (Y.O., S.N.), Department of Cardiovascular Medicine (Y.O., M. Masuda, H. Minamiguchi, S.N., I.K., Y. Sakata), Osaka University Graduate School of Medicine, Suita; Matsuo Medical Clinic, Yao (M. Matsuo, H. Matsuo); Sakaguchi Medical Office, Osaka (Y. Sakaguchi); Takai Clinic, Kawachinagano (H.T.); Horiguchi Cardiovascular Clinic, Ikeda (Y.H.); Ryomoto Cardiovascular Clinic, Moriguchi (T.R.); Adachi Clinic, Suita (S.A.); Amanonaika Clinic, Ikeda (T.A.); Togawa Clinic, Suita (M.T.); and Department of Cardiovascular Medicine, Tokyo University Graduate School of Medicine, Tokyo (I.K.), Japan
Mailing address: Yuji Okuyama, MD, Advanced Cardiovascular Therapeutics and Department of Cardiology, Osaka University Graduate School of Medicine, 2-11 Yamada-oka, Suita 565-0871, Japan. E-mail: yujicardiology@gmail.com

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ics, and an important disadvantage of TTR control in these clinics is that blood samples for PT-INR measurement are sent to an offsite laboratory, and the results become available half a day or a full day after blood sampling. In 2007, CoaguChek®, a device used for point-of-care (POC) testing of PT-INR, was introduced in outpatient clinics of Japan; the use of this device was expected to increase the quality of warfarin therapy in terms of TTR, because POC testing could overcome the time delay in the availability of PT-INR test results.[11,12] In this study, we sought to test the hypothesis that the introduction of POC testing of PT-INR improved the TTR of patients visiting different outpatient clinics in Japan.

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Methods

Eight outpatient clinics located in the Osaka Prefecture in Japan participated in this retrospective observational study. The POC testing device, CoaguChek® (Roche Diagnostics), was made available to all the participating clinics >1 year before participation in this study. The study protocol was approved by the ethics committee of Osaka University Hospital. Written informed consent was waived because the analyzing center was blinded to the pre-existing data, as stipulated by the Japanese epidemiological study guidelines.

Subjects

We enrolled patients who received warfarin for the treatment of AF, heart valve replacement, as well as mitral stenosis, and who were followed up at any of the participating clinics between 28 June 2010 and 31 May 2012. Patients were enrolled only if they had received warfarin for more than 15 months at the time of introducing the POC testing device at the respective clinic. This was necessary because we intended to compare the data on the TTR for >1 year before and 1 year after implementation of the POC testing device. We reviewed patient charts and collected data on patient profile, concomitant antiplatelet drug use, and PT-INR measurements. PT-INR measurements were made every 1 or 2 months for at least 1 year before and 1 year after the introduction of the POC testing device. During follow-up, warfarin dosage for patients with non-valvular AF was adjusted according to the Japanese Guidelines for Pharmacotherapy of Atrial Fibrillation to maintain INR at 2.0–3.0 and 1.6–2.6 for patients aged <70 years and ≥70 years, respectively.[10] Warfarin dosage for patients with mechanical heart valve(s) and mitral stenosis was also adjusted according to the Guidelines to maintain INR at 2.0–3.0.[10] For the present study, we used the PT-INR results obtained at least 3 months after the initiation of warfarin. Patients who had an interval >100 days between 2 PT-INR measurements were excluded because the calculated TTR in such cases may not accurately reflect the quality of warfarin control.

Study Protocol

We analyzed the data with software designed specifically for the study (Medi-Skette, Tokyo, Japan). Successive PT-INR of each patient were entered into the computer program, and the TTR was calculated. The software program automatically drew successive demarcations between any 2 consecutive PT-INR obtained during the observation period and calculated the percentage of the total time within the preset therapeutic range over the specified period. The therapeutic range of the INR was set as described in the previous section. We defined TTR before the introduction of the POC testing device (TTR_{Before}), as the TTR obtained for the period between the first visit to the outpatient clinic for PT-INR evaluation and the last visit within the year before the POC testing device was introduced at that clinic. TTR after the introduction of the POC testing device (TTR_{After}) was defined as TTR obtained in the time period between the introduction of the device at a clinic and the last visit for PT-INR evaluation within 1 year after the introduction of the POC testing device. We also defined the time periods "time spent under therapeutic range (TUTR)" and "time spent over therapeutic range (TOTR)" to investigate reasons for change in TTR.

We first compared factors related to TTR_{Before} with those for TTR_{After}, as well as the TUTR and TOTR before and after the introduction of POC testing. We then analyzed the following factors with the potential to influence a change in TTR: TTR_{Before}, age, gender, history of concomitant antiplatelet drug use, $CHADS_2$ score,[13] and HAS-BLED (hypertension, abnormal renal/liver function, stroke, bleeding history or predisposition, labile INR, elderly, drugs/alcohol concomitantly) score.[14]

Table 1. Clinical Patient Characteristics

	All patients (n=109)
Age (years)	72.6±9.5
Men (n=57)	71.5±9.7
Women (n=52)	73.9±9.3
Indication for warfarin	
AF	80
Mechanical heart valve	25
Mitral stenosis	4
Warfarin dose at introduction of POC testing (mg/day)	2.6±1.0
AF	2.6±1.0
Mechanical heart valve	2.6±1.2
Mitral stenosis	2.6±1.3
Mean TTR_{Before} of all patients (%)	51.9±33.0
AF	55.2±32.1
Mechanical heart valve	43.3±33.8
Mitral stenosis	41.4±42.6
$CHADS_2$ score of AF patients	
0–1	20
2	19
3–6	41
HAS-BLED score of all patients	
0–2	83
3–9	22

Data given as mean±SD or n. Four patients did not have data for HAS-BLED score.
AF, atrial fibrillation; POC, point of care; TTR_{Before}, time in therapeutic range before the introduction of POC testing.

Statistical Analysis

All data are expressed as mean±SD. We used a 2-tailed paired t-test to assess the TTR change before and after the introduction of the POC testing device, and 1-way analysis of variance with Bonferroni post-hoc test to compare the TTR of 3 or more groups. We used the Tukey range test to compare TTR between clinics. $P<0.05$ was regarded as statistically significant.

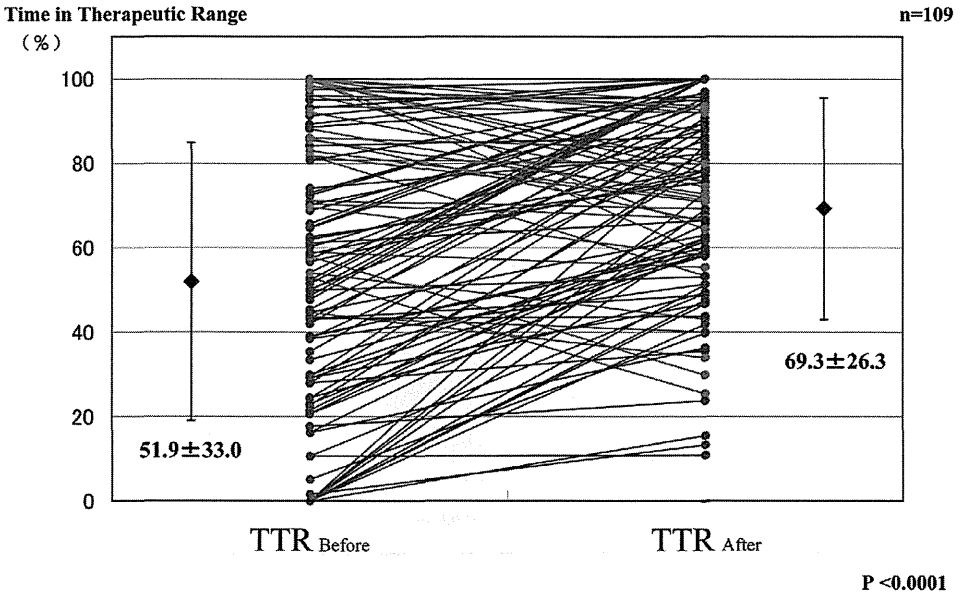

Figure 1. Improvement in time in therapeutic range (TTR). TTR after the introduction of a point-of-care testing device (TTR_{After}) was much higher than that before its introduction (TTR_{Before}). Each line indicates the TTR change in individual patients. Blue line, improvement in TTR; red line, deterioration of TTR. Error bars, mean±SD.

Results

Patient Clinical Characteristics

We identified 148 patients who were treated with warfarin. Thirty-nine of these 148 patients were excluded because they had an interval >100 days between 2 PT-INR measurements. Thus, data from 109 patients were included in the analysis: 80 patients received warfarin for AF; 25 patients for heart valve replacement; and 4 patients for mitral stenosis (**Table 1**). The mean interval between PT-INR measurements was 37.7±9.9 days. The mean patient age was 72.6±9.5 years (range, 45–97 years), and the mean age of the male (n=57) and female (n=52) patients was 71.5±9.7 years and 73.9±9.3 years, respectively. The INR range used for the calculation of TTR was maintained at 2.0–3.0 for patients with non-valvular AF who became older than 70 years of age during the study period. The mean $CHADS_2$ score in patients with non-valvular AF was 2.4±1.3 (median, 2), and the mean warfarin dose given at the first visit during the observational period was 2.6±1.0 mg/day (range, 0.75–5.5 mg/day). In all, 23 warfarin-treated patients were given antiplatelet drugs concomitantly (21.1%), and over the 2-year study period, 5 cases of symptomatic brain infarction and 1 case of brain hemorrhage were recorded. Bleeding associated with gastric cancer was reported in 1 other patient.

Change in TTR

TTR_{Before} was 51.9%±33.0%; TTR_{After} was significantly higher: 69.3%±26.3% (P<0.0001; **Figure 1**). Improvements in TTR were observed in patients with AF (55.2%±32.1% to 71.8%±25.1%, P<0.0001) and mechanical heart valve (43.3%±33.8% to 62.5%±27.1%, P=0.0013), but only a tendency for improvement was noted in patients with mitral stenosis (41.4%±42.6% to 62.7%±42.0%, P=0.3319).

TOTR was low, at 3.7%±10.6% and 3.3%±6.3%, respectively, both before and after the introduction of POC testing (P=0.7322). In contrast, TUTR decreased after the introduction of POC testing (44.4%±34.4% vs. 27.4%±27.6%; P<0.0001; **Figure 2**). The mean interval between PT-INR measurements before the introduction of POC testing was greater than that after its introduction (42.7±14.4 and 35.2±10.2 days, respectively; P<0.0001).

Factors Influencing Improvement of TTR

A recent study reported that TTR <40% could be associated with a tendency toward worse outcome in stroke incidence.[15] In that study, only warfarin-treated patients with TTR >70% had significantly fewer strokes than those not treated with warfarin.[15] To assess the effects of an improvement in TTR, we divided the present patients into the following 3 groups according to TTR_{Before}: those with TTR ≤40%; those with TTR 41–70%; and those with TTR ≥71% (**Figure 3**); the average TTR_{Before} in the 3 different groups was 15.8%±14.4% (n=40), 55.0%±8.9% (n=34), and 90.2%±9.2% (n=35), respectively. TTR of the 2 groups with low TTR_{Before} (≤40%, 41–70%) improved significantly after the introduction of POC testing (51.0%±26.1% and 75.4%±24.1%, respectively; both P≤0.0001), while that of the group with the highest TTR_{Before} (≥71%) decreased significantly (84.3%±13.4%, P=0.0299).

The patients were classified into 3 groups according to $CHADS_2$ score 0–1 (n=20), 2 (n=19), and 3–6 (n=41) to examine the relationship between the risk of stroke and improvement in TTR. TTR_{After} improved in patients with $CHADS_2$ score 0–1 (38.0%±34.5% to 58.1%±31.0%, P=0.0019) and those with $CHADS_2$ score 3–6 (63.1%±29.9% to 79.5%±19.7%, P=0.0007). TTR_{After} in patients with $CHADS_2$ score 2 showed a tendency for improvement, but statistical significance was not reached (56.1%±28.2% to 69.3%±23.1%, P=0.0572).

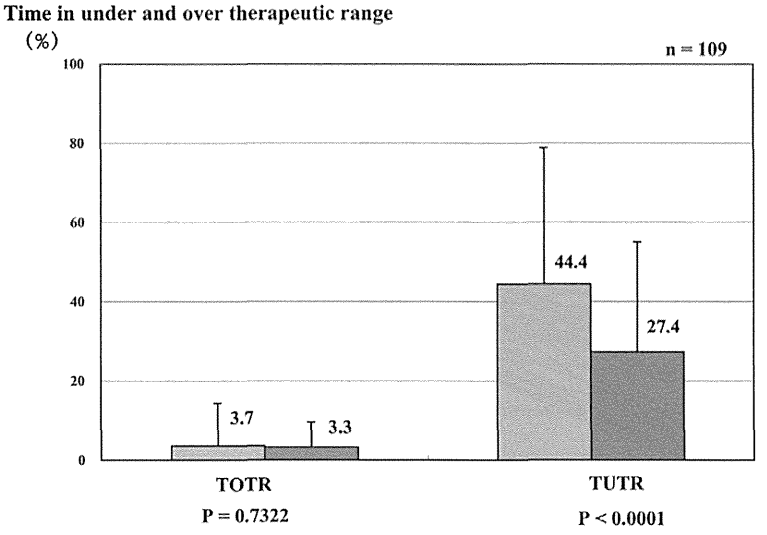

Figure 2. The time spent over the therapeutic range (TOTR) remained low after the introduction of point-of-care (POC) testing, but the time spent under the therapeutic range (TUTR) decreased. Column and bar, mean and SD, respectively Yellow, before the introduction of POC testing; green, after the introduction of POC testing.

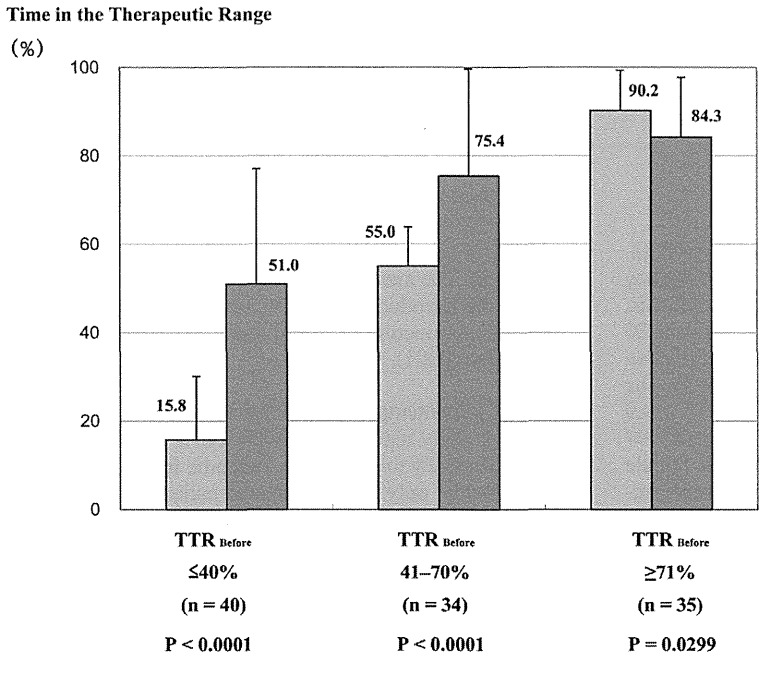

Figure 3. Effect of time in therapeutic range (TTR) before the introduction of point-of-care (POC) testing (TTR_{Before}) on change in TTR after the introduction of POC testing. In patients with TTR_{Before} ≤40% and 41–70%, TTR improved significantly, whereas in patients with TTR_{Before} ≥71% it decreased significantly. Column and bar, mean and SD, respectively Yellow, before the introduction of POC testing; green, after the introduction of POC testing.

HAS-BLED score is a predictor of major bleeding in patients receiving warfarin, and HAS-BLED score ≥3 is thought to indicate a high bleeding risk.[14] Improvements in TTR were observed in patients with HAS-BLED score 0–2 (n=83; these patients improved from 50.0%±33.4% to 68.1%±27.1%; P<0.0001). The change in TTR in patients with HAS-BLED score ≥3 (n=22), however, showed the same trend but did not reach statistical significance (58.2%±33.7% vs. 71.1%±24.2%, P=0.0551).

Improvements in TTR were consistently observed in both men and women enrolled in the present study (52.9%±29.2% to 70.9%±25.2%, P<0.0001; 50.9%±36.9% to 67.5%±27.5%, P=0.0002, respectively). In addition, similar levels of improvement in TTR were noted in patients treated with or without antiplatelet drugs (45.0%±34.9% to 60.4%±24.3%, P=0.0334; 53.8%±32.4% to 71.7%±26.4%, P<0.0001). There was no difference in TTR_{Before} between patients aged <70 years (49.0%±32.6%) and ≥70 years (53.4%±33.3%, P=0.514). Improvement in TTR was significant in all the subgroups of age (<70 years: 49.0%±32.6% to 60.8%±27.5%, P=0.0126; ≥70 years: 53.4%±33.3% to 73.5%±24.8%, P<0.0001). Calculation of TTR using the target ranges of 1.6–2.6, 2–3, and 1.6–3.0 indicated consistent improvement in TTR associated with POC testing in all subgroups of age.

Table 2. Difference in TTR Between Clinics

Clinic no.	Patients (n)	TTR_{Before} (%) (mean±SD)	TTR_{After} (%) (mean±SD)	P-value
1	7	73.6±29.5	83.9±8.1	0.4441
2	5	62.6±26.6	84.2±16.6	0.1474
3	19	65.7±24.0	79.3±21.5	0.0656
4	22	48.7±33.1	76.3±18.8	0.0002
5	2	21.9±30.9	20.1±28.4	NA
6	12	75.6±14.8	87.2±13.1	0.0596
7	36	38.5±34.2	56.7±26.6	0.0003
8	6	29.4±34.2	38.5±28.4	0.2820

Statistical comparison was not performed for the data from clinic 5 because the number of the patients was not sufficient for comparison.
NA, not assessed; POC, point of care; TTR_{Before}, time in therapeutic range before the introduction of POC testing; TTR_{After}, time in therapeutic range after the introduction of POC testing.

There were statistically significant differences in TTR_{Before} between clinics 3 and 7, and between clinics 6 and 7 (**Table 2**; Tukey range test, $P<0.05$). A strong tendency for improvement in TTR was observed at all of the clinics except for clinic 5. Statistically significant improvement in TTR was noted at 2 clinics (Nos. 4 and 7).

Discussion

Major Findings

The major findings are as follows: (1) overall, TTR_{After} was considerably higher than TTR_{Before}; this improvement could mainly be ascribed to a decrease in the TUTR; (2) improvement in TTR was consistent, independent of gender, age, and antiplatelet drug use; (3) improvement in TTR was significant in patients who had $CHADS_2$ score 0–1 or 3–6, and HAS-BLED score ≤2; and (4) only patients with high TTR before the introduction of the POC testing device had significant but small decreases in TTR.

Advent of POC Testing

The POC testing devices have been developed in recent years in order to provide quick results for the monitoring of INR in patients receiving oral anticoagulants. Before the advent of POC, general practitioners were required to wait for half a day or even a full day for INR results. Because POC testing devices enable quick measurement of INR, adjustments to warfarin dose can be made in a timely manner. Several studies comparing the INR results obtained with POC testing devices and conventional laboratory methods have shown that the former provide accurate test results.[11,12]

High TTR

Good INR control is critical to improvement in patient outcome.[16] In substudies of SPORTIF III and V, 3,587 patients with AF treated with warfarin were divided into 3 groups according to TTR (<60%, 60–75%, >75%).[7] The rates of stroke or systemic embolic events, as well as of bleeding, were higher among patients with poor INR control (<60%) than among those with moderate (60–75%) or good INR control (>75%). A substudy of ACTIVE W, which originally compared the effect of a dual antiplatelet regimen and warfarin on the prevention of stroke and systemic embolism, also showed that patients who were treated at centers that had mean TTR above the study median of 65% had a marked benefit against stroke and total vascular events.[17] That study also showed that warfarin therapy was effective only when TTR reached the lower limit of 58% (this value was based on a population-average model). Nevertheless, in community medical practices, INR control within the therapeutic range is typically achieved only in approximately 50% of measurements.[18] Okumura et al reported that the average TTR at 5 Japanese centers for cardiovascular disease was 64%.[10] These findings indicate the need for the establishment of strategies to improve TTR in actual clinical practice.

Several studies have shown that clinical practices are important determinants of TTR.[19] The introduction of anticoagulation clinics[20] and computer-assisted decision-support tools can improve TTR;[21] in contrast, patient education is important for improvements in TTR and for the outcome of warfarin therapy, because poor adherence is potentially a major source of poor anticoagulation control.[22,23] The present study clearly showed that the use of a POC testing device in an outpatient clinic was associated with improvement in TTR. Although the present study was not designed to ascertain why the introduction of POC in outpatient clinics may be associated with significant increases in TTR, there are some plausible explanations for the improvement in TTR observed in this study. First, it was easy for clinicians to adjust a patient's warfarin dosage because PT-INR could be measured on site. Before the introduction of the POC testing devices, clinicians had to adjust the dose after the patient had already left the clinic because the PT-INR result would not be available until 1 day after blood sampling. We do not have information regarding the frequency of dose adjustments and the final dose of warfarin. The frequency of dose adjustments, however, could be increased because the interval between PT-INR measurements was shortened by approximately 10 days after the introduction of POC testing devices. Rose et al showed that longer time between INR monitoring, and the failure to recheck INR promptly after out-of-range values are recorded, closely relate to poor INR control.[24,25] Therefore, a shorter interval between INR measurements in the present study could have facilitated the improvement in INR control. Second, the quality of warfarin therapy is, to some extent, dependent on a patient's understanding of the need for this treatment as well as of the importance of maintaining PT-INR within the therapeutic range.[19] Education programs have an important role in improving clinical outcome. One possible reason for this is the improvement in treatment adherence.[22] The use of POC testing devices could facilitate the education of patients on these issues on-site and thereby could contribute to good adherence to

therapy. Further studies are needed to elucidate the effect of the introduction of POC testing devices on patient understanding and adherence to therapy.

In community medical practice, PT-INR often does not reach the therapeutic range, which is reflected by TUTR. Possible reasons for prolonged TUTR may be clinician or patient concerns regarding the bleeding complications associated with excessive warfarin therapy.[18] Recent clinical trials, however, reported that warfarin therapy did not increase the risk of intracranial bleeding until PT-INR exceeded 3.5–4.0 and that the risk of intracranial hemorrhage in patients with PT-INR 2.0–3.0 was not higher than that in patients with lower PT-INR.[8,26] In the present study, significant improvement in TTR was observed because of a decrease in TUTR, without any increase in TOTR. Therefore, POC testing devices may help maximize the preventive effect of warfarin in patients affected by the risk of stroke and systemic thromboembolism while also minimizing the increase in the risk of bleeding complications.

Only patients with high TTR (≥71%) before the introduction of POC testing had significant but small decreases in TTR (**Figure 3**; 90.2% to 84.3%). The reason for the decrease in TTR is not known, but both TTR_{Before} and TTR_{After} were high enough to prevent stroke and systemic embolization.

New anticoagulants such as dabigatran are known to be effective in the prevention of stroke, and some of the novel therapies have been shown to be equally effective or better than warfarin.[27–29] Although new anticoagulants have favorable pharmacologic profiles overall, there are several disadvantages to the use of novel therapies, including the cost of individual treatment. TTR in patients receiving warfarin in the recent studies on new anticoagulants ranged from 55% to 64.4%.[27–29] Therefore, warfarin therapy that achieves TTR >70% could be similar or even superior to almost all the new anticoagulants for the prevention of stroke and systemic thromboembolism. In addition, warfarin is cost-effective and therefore an economical choice.

Study Limitations

The present findings should be interpreted in the light of some limitations. First, the study design was retrospective in nature, and it is not known whether the present results can be generalized to all outpatient clinics. It is possible that the clinicians at the clinics where POC was introduced were more enthusiastic about anticoagulant therapy than those at other clinics. This enthusiasm could have affected the results. Second, given that the number of subjects was relatively small, we were unable to examine how the use of POC testing devices affected the risk of bleeding or thromboembolism. Nevertheless, TTR is a strong and consistent predictor of bleeding and thromboembolism, and improvement in TTR without an increase in TOTR may reduce the risk of both bleeding and thromboembolism. We did not observe improvement of TTR in several subgroups, such as in patients with $CHADS_2$ score 2 and HAS-BLED score ≥3, probably because the subject group was relatively small. Finally, it is possible that the TTR in the present patients improved over time.[10,30] Okumura et al reported that TTR increased slightly, although not significantly, during the second year of treatment in a 2-year observation period.[10] In addition, Rose et al showed that TTR during an "experienced" period of warfarin therapy was higher than that during the "inception" period.[30] The improvements observed in the present study, however, were statistically significant, and we ensured that there was an interval of at least 3 months between the initiation of warfarin therapy and TTR measurements recorded in this study. Therefore, a change in TTR over time alone could not explain the improvement of TTR that we observed.

Conclusions

The introduction of POC was associated with improvements in TTR, mainly through a reduction in the TUTR of INR. The widespread use of POC testing devices for the measurement of INR may improve the management of patients receiving warfarin.

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Disclosures

No other conflicts of interest are reported.

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