Influence of Lifestyle-related Factors on Circadian Onset Patterns of Acute Myocardial Infarction: a Prospective Observational Study in Japan

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Influence of Lifestyle-related Factors on Circadian Onset Patterns of Acute Myocardial Infarction: a Prospective Observational Study in Japan

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Abstract

Objective: The onset of acute myocardial infarction (AMI) shows characteristic circadian variations involving a definite morning peak and less-defined nighttime peak. However, the factors influencing circadian patterns of AMI onset and their influence on morning and nighttime peaks have not been fully elucidated.

Design, Setting, and Participants: An analysis of patients registered between 1998 and 2008 in the Osaka Acute Coronary Insufficiency Study, which is a prospective, multicenter observational study of AMI patients in the Osaka region of Japan. The present study included 7755 consecutive patients with a known time of AMI onset.

Main Outcomes and Measures: A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI had uniform, unimodal, or bimodal distribution, and the likelihood ratio test was then used to select the best circadian pattern among them. The hierarchical likelihood ratio test was used to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier

method was used to estimate survival curves of one-year mortality according to AMI onset time.

Results: The overall population had a bimodal circadian pattern of AMI onset characterized by a high and sharp morning peak and a lower and less-defined nighttime peak (bimodal: p<0.001). Although several lifestyle-related factors had a statistically significant association with the circadian patterns of AMI onset, serum triglyceride levels had the most prominent impact on circadian patterns of AMI onset. Patients with triglyceride ≥150 mg/dl on admission had only one morning peak in the circadian pattern of AMI onset during the weekdays, with no peaks detected on weekends, whereas all other subgroups had two peaks throughout the week.

Conclusions: The circadian pattern of AMI onset was characterized by bimodality. Notably, several lifestyle-related factors, particularly serum triglyceride levels, had a strong relation with the circadian pattern of AMI onset.

Strengths and limitations of this study

- We comprehensively analyzed the circadian patterns of AMI onset in a large, multicenter cohort of patients in relation to patient characteristics, lifestyle factors, and day of the week.
- A mixture of two von Mises distributions revealed that the circadian pattern of AMI onset exhibited bimodality.
- Several lifestyle-related factors were shown to influence circadian patterns of AMI onset, depending on the day of the week. In particular, it was demonstrated that elevated serum triglyceride levels on admission accentuated morning peak of AMI onset during the weekdays.
- Subjects were limited to those who were hospitalized for AMI.
- Laboratory data were evaluated on admission.

Introduction

Onset patterns of acute myocardial infarction (AMI) exhibit circadian variation that is characterized by an increased frequency in the morning and a secondary peak incidence at nighttime.[1] Several studies have confirmed that AMI onset exhibits a bimodal circadian pattern, with peaks occurring in the morning hours [2-4] and nighttime hours.[1,4-7] However, it is not well understood what factors, particularly among lifestyle-related factors, influence the circadian patterns of AMI. Moreover, although these patterns appear to vary according to the day of the week,[8] it is unclear how circadian patterns of AMI onset vary throughout the week, particularly, in association with socioeconomic factors.

Because AMI and subsequent ischemic heart failure are the leading causes of death in both developed and developing countries, primary prevention of AMI is a major healthcare issue worldwide. Accordingly, identifying potential factors influencing the circadian pattern of AMI may help in the clinical management of patients for preventing the onset of AMI.

In the present study, we comprehensively analyzed the circadian patterns of AMI onset in a large, multicenter cohort of patients in relation to patient characteristics, lifestyle factors, and day of the week.

Methods

OACIS registry and study subjects

The OACIS is a prospective, multicenter observational study collecting demographic, procedural, biological, and outcome data as well as blood samples from AMI patients hospitalized at 25 collaborating hospitals from the Osaka region of Japan (UMIN-Clinical Trial Registry ID: UMIN000004575).[9-10] A diagnosis of AMI was made if the patient fulfilled at least two of the following three criteria; 1) history of central chest pressure, pain, or tightness lasting 30 min, (2) typical electrocardiographic changes (i.e., ST-segment elevation ≥ 0.1 mV in 1 standard limb lead or 2 precordial leads, ST-segment depression ≥ 0.1 mV in 2 leads, abnormal Q waves, or T-wave inversion in 2 leads), and (3) an increase in serum creatine kinase levels of two times the upper normal limit in each hospital. All the collaborating hospitals were encouraged to enroll consecutive patients with AMI.

We prospectively collected data by research cardiologists and trained research nurses using a specific reporting form, and the following variables were

extracted from the OACIS registry database: age, gender, working status, body mass index (BMI), coronary risk factors (diabetes, hypertension, dyslipidemia, smoking, drinking, previous myocardial infarction, multivessle disease, and collateral circulation), clinical presentation on admission (KILLIP classification, initial TIMI flow, and ST-elevation myocardial infarction), coronary angiography data, reperfusion therapy, laboratory data on admission (hemoglobin A1c, total cholesterol, low- and high-density lipoprotein cholesterol, triglyceride, and estimated glomerular filtration rate) and medications at discharge (RAS inhibitors, beta-blocker, calcium channel blocker, statin, anti-platelet agent, and diuretics). Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl, hemoglobin A1c ≥6.5% or a history of antidiabetic therapy. Hypertension was defined as a history of systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or antihypertensive therapy. Dyslipidemia was defined as fasting total cholesterol ≥220 mg/dl, low-density lipoprotein cholesterol ≥140 mg/dl, high-density lipoprotein cholesterol ≤40 mg/dl, fasting triglycerides ≥150 mg/dl, or antilipidemial therapy.

The study protocol has been approved by the ethics committee of each participating hospital. All in-hospital data were obtained after written informed

consent and were then transmitted to the data collection center at the Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan for processing and analysis. The corresponding authors had full access and validated to all data in the study.

In the present study, we analyzed 7755 AMI patients whose time of AMI onset was definitely identified among the 8603 consecutive patients registered in the OACIS registry between 1998 and 2008. Patients' baseline characteristics are presented in Table 1.

Statistical analysis

Continuous variables were summarized as quartiles and were compared by the Wilcoxon rank sum test for two-group comparisons, and the Kruskal-Wallis test for four-group comparisons. Categorical variables were presented as number and percentage, and were compared by the chi-square test. A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI had uniform (no peak), unimodal (one peak), or bimodal distribution (two peaks), and the likelihood ratio test was then used to select the best circadian pattern among them.[11] In addition, the hierarchical likelihood ratio test was assessed to identify

factors affecting the circadian patterns of AMI onset. The Kaplan-Meier method was used to estimate survival curves of one-year mortality according to AMI onset time (morning [6:00-11:59 h], afternoon [12:00-17:59 h], evening [18:00-23:59 h], and nighttime [0:00-5:59 h]). The log-rank test was used to compare survival curves between the groups, and the Cox proportional hazards regression model was used to estimate hazard ratios and 95% confidence intervals (CI). To reduce potential confounding effects due to patient background variability in the comparison between the afternoon-onset and other groups, a stratified Cox proportional hazards regression model was used, in which the potential confounding variables were included into the model as stratification factors. Statistical significance was set as $p<0.05$. All statistical analyses were performed using an in-house validated Fortran program or SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Bimodal circadian patterns of AMI onset in the overall population

The daily patterns of AMI onset in our cohort of 7755 patients were first analyzed using the likelihood ratio test (Figure 1). In the overall population, AMI

onset clearly exhibited a circadian pattern consisting of two peaks (bimodal: p<0.001): a primary peak at 9:01 h (95%CI: 8:53-9:08 h) and a secondary peak at 20:11 h (95%CI: 19:48-20:34 h). The primary peak was more clearly defined than the secondary peak in both the circular and columnar histograms (Figure 1A and 1B, respectively).

Likelihood ratio test analysis revealed that the peak time of AMI onset varied according to the day of the week (Figure 2). For example, the primary peak onset time was earliest on Monday (8:24 h [95%CI: 8:04-8:44 h]) and latest on Sunday (9:44 h [95%CI: 9:22-10:06 h]). On Tuesday, patients exhibited a circadian pattern of AMI onset characterized by late primary (9:28 h [95%CI: 9:06-9:51 h]) and secondary peak onset times (21:13 h [95%CI: 20:40-21:46 h]), whereas earlier peak onset times (8:43 h [95%CI: 8:15-9:10 h], and 19:09 h [95%CI: 18:23-19:55 h]) were detected on Thursday. Notably, the evening peak was higher, and sharper than the morning peak on Saturday (Figure 2).

Factors affecting circadian patterns of AMI onset

Hierarchical likelihood ratio analysis revealed that serum TG levels on admission, smoking, age, drinking, blood glucose levels on admission, gender, and working

status had a statistically significant influence on the circadian pattern of AMI onset, whereas several other known risk factors for AMI, including HDL- and LDL-cholesterol, Hba1c, hypertension, diabetes and dyslipidemia were not related to the observed patterns (Figure 3, eTable1).

Among the positively associated factors, serum TG levels on admission had the greatest impact on the circadian pattern of AMI onset. Although the likelihood ratio test demonstrated that patients with admission serum TG levels of ≥150 mg/dl had the two characteristic peaks during the day, the peak pattern clearly differed from the other subpopulation groups. In patients with admission serum TG levels of ≥150 mg/d, both peaks occurred in the morning and nearly overlapped (8:18 h and 8:47 h) (Figure 3A). Therefore, the subpopulation with admission TG levels ≥ 150 mg/dl was considered to have a high frequency of AMI onset only in the morning.

The baseline characteristics and laboratory data of patients with serum TG levels of ≥ 150 and <150 mg/dl on admission are shown in eTable2. In the subpopulation with higher TG levels, the circadian patterns of AMI onset was characterized by a large, sharp peak in the morning from Monday to Friday, but no peaks were detected on Saturday and Sunday (bimodal: p=0.32 and p=0.133,

respectively) (eFigure 1). In contrast, patients with admission serum TG levels of <150 mg/dl had onset peaks that occurred in the morning and evening consistently throughout the week (eFigure 1).

A likelihood ratio test demonstrated that other all subpopulations had two AMI onset peaks during the day: one in the morning and the other in the evening (Figure 3, eTable 1). The subpopulations that were grouped according to smoking habit, age < 65 years old, male gender, and active employment had a circadian pattern of AMI onset with a sharper primary peak and less-defined sharp secondary peak compared to the other subpopulations (Figure 3B,C,F,G, eTable1), although the peak heights were similar between the subpopulations, with the exception of the smokers/nonsmokers subpopulations. The primary AMI onset peak in the subpopulation of smokers was higher than that among nonsmokers, whereas the secondary peaks were similar. Drinkers had a circadian pattern of AMI onset that was characterized by a lower and less sharp peak in the morning, and a higher, sharper and later peak in the evening (9:00 h [95%CI: 8:48-9:13 h], 20:54 h [95%CI: 20:29-21:20 h]) compared to nondrinkers (9:03 h [95%CI: 8:53-9:14 h], 19:27 h [95%CI: 18:50-20:04 h]) (Figure 3D, eTable1). The subpopulation with admission blood glucose ≥140 mg/dl exhibited

a circadian pattern of AMI onset with a higher and sharper primary peak and a less-defined secondary peak compared to the subpopulation of AMI patients with blood glucose <140 mg/dl on admission (Figure 3E).

One-year mortality according to onset time of AMI

One-year mortality were compared among four patient subpopulations that were grouped according to the time range of AMI onset. The baseline characteristics and laboratory data for the four groups are presented in eTable 3. A total of 753 deaths were recorded during a median follow-up period of 365 days. Kaplan-Meier survival analysis demonstrated that the afternoon-onset (12:00-17:59 h) group had worse 1-year mortality than the other 3 groups (log rank test, p=0.032) (Figure 4A). Univariable Cox regression analysis revealed that the HR of one-year mortality in the afternoon-onset group as compared to the other three groups was 1.20 (95%CI 1.02-1.40, p=0.030, Figure 4B). This result did not change after stratification with potential confounding factors that showed a different trend between the afternoon-onset group and other three groups (Figure 4B).

Discussion

In the present study, we confirmed that AMI onset exhibits a circadian pattern characterized by bimodality, with a definite morning peak and a less-defined evening peak. Notably, several lifestyle-related factors were associated with variation in the circadian pattern of AMI onset. In particular, serum TG levels on admission for AMI were associated with a unique pattern of AMI onset that is characterized by augmented unimodal peaks on weekday mornings, suggesting that individual lifestyle may affect the onset pattern of AMI.

Bimodal pattern of AMI onset: morning and nighttime peaks

AMI onset in our large patient cohort generally followed a circadian pattern that was characterized by a high and sharp morning peak and a lower and less-defined sharp nighttime peak (Figure 1), a finding that is consistent with the results of previous investigations.[1-7] Interestingly, the time of two peaks shifted in a synchronous fashion during the weekdays; the secondary peaks generally occurred around 11 to 12 h after the morning on Monday through Friday (Figure 2). For example, AMI onset exhibited early morning and nighttime peaks on

Monday and Thursday, whereas that on Tuesday was most frequent in the late morning and nighttime. Although this finding is partly consistent with the observation of Peters et al.,[5] who reported that a secondary peak in AMI onset occurs 11 to 12 h after waking, the present study firstly demonstrated that this synchrony was present on the weekdays, but absent on the weekends.

Several physiological processes are considered to contribute to the bimodal pattern of AMI onset. For example, Stergiou et al.[12] demonstrated that the two-peak diurnal variation in stroke onset occurs in parallel with variation in blood pressure, pulse rate, and physical activity. Thus, the bimodality of blood pressure and heart rate [13-14] is the most likely explanation for the circadian patterns of AMI onset observed in the present study. A greater morning surge of blood pressure and heart rate[13] may explain why the nighttime peak of AMI onset was lower and less-defined than the morning peak. In addition, increased blood viscosity[15] and thrombogenicity due to morning hypercoagulability[16] and hypofibrinolysis[17] also likely increased the frequency of AMI onset in the morning. It is also possible that external factors, such as physical exertion and mental stress, could be triggers for the morning onset of AMI.[18] In the present study, the younger (<65 years old), working, male, and smoker subpopulations had a sharp

morning peak of AMI onset compared with the elderly, nonworking, female, and nonsmoking subpopulations (Figure 3B,C,F,G). The sharpness of the morning peak might be related to increased susceptibility to physical and mental stresses in these subpopulations, who are more likely to start activities or go to work soon after waking up. Similarly, the sharp and early morning peak of AMI onset that was detected on Monday may be due to the increased physical and mental stress that is associated with the first morning of the week (Figure 2). We also found that the morning peak occurred latest on Sunday (Figure 2). Together, these findings strongly suggest that mental and physical stress may act as a trigger for the morning onset of AMI.

Although many reports have examined the primary peak of AMI onset, relatively little attention has been paid to the secondary peak. We demonstrated that drinkers had a higher, sharper, and later nighttime peak of AMI onset than nondrinkers (Figure 3D). Moreover, the nighttime peak on Saturday was the highest and sharpest amongst the seven days of the week (Figure 2). This observation may be explained by the fact that people might be likely to consume alcohol and engage in social activities on Saturday night in Japan. Thus, these evening activities can result in increased sympathetic nerve activity and

therefore may have contributed to the increased frequency of AMI onset at night. Taken together, our findings suggest that both the morning and nighttime peaks of AMI onset are influenced by physiological and socioeconomic factors.

Impacts of lifestyle-related factors on circadian patterns of AMI onset

Most previous studies on circadian pattern of AMI onset have only considered gender, age, working status as potential factors affecting the circadian patterns of AMI onset.[1,4-6] Here, we additionally incorporated laboratory data, disease, and other socioeconomic factors into our analyses and found that several lifestyle-related factors, including admission serum TG and blood glucose levels, age, gender, working status, and smoking and drinking habits had statistically significant influences on the circadian pattern of AMI onset. Among these factors, elevated serum TG levels (≥150 mg/dl) on admission had the largest influence on AMI onset, as demonstrated by the absence of an evening peak in AMI onset compared to all other subgroups (Figure 3). Furthermore, the morning peak was not observed on the weekend in patients with admission TG of ≥150 mg/dl (eFigure 1). Fasting hypertriglyceridemia and postprandial hyperlipidemia, which is characterized by postprandial accumulation of TG-rich lipoproteins and their

partially hydrolyzed products, are both closely related to the development of atherosclerotic cardiovascular diseases.[19-21] Several studies have also reported that elevated serum TG levels are associated with an increased risk of myocardial infarction.[22-23] To our knowledge, however, this is the first study demonstrating an association between serum TG levels on admission and circadian patterns of AMI onset. Although LDL/HDL levels are considered to be closely associated with the development of atherosclerosis, LDL/HDL levels were not associated with onset patterns of AMI in the present study. Thus, these findings suggest for the first time that TG may strongly influence the circadian patterns of AMI onset.

Hypertriglyceridemia is associated with increased thrombogenecity,[24-25] which is reportedly associated with increased plasminogen activator inhibitor-1 (PAI-1)[26-28] and factor VII coagulant activities,[29-30] and viscosity.[31] These three factors have also been reported to affect the development of MI.[32-34] Moreover, hypertriglyceridemia is also related to endothelium dysfunction,[35-36] which contributes to the pathogenesis of coronary artery disease.[37] In healthy subjects, serum TG levels also exhibit circadian variation with a peak around 3 AM.[38] Thus, it is conceivable that patients with hypertriglycemia have further augmented TG

levels and are therefore exposed to increased thrombogenecity and endothelium dysfunction in the early morning hours before dawn, which may explain the accentuated morning peak of AMI onset in patients with admission TG ≥150 mg/dl. In addition, it is reported that high plasma PA1-1 levels and excessive surges in morning blood pressure are independently and additively associated with increased risk of stroke in older hypertensive patients,[39] supporting our observation of a higher morning risk of AMI onset in the subpopulation with admission hypertriglyceridemia.

Our subpopulation analyses also revealed that the circadian patterns of AMI onset in patients with admission TG levels of ≥150 mg/dl had a sharp morning peak during weekdays, whereas no such peak was detected on Saturday or Sunday. This observation strongly suggests that increased thrombogenicity and endothelium dysfunction was a factor, but not the trigger, for the morning onset of AMI in our study cohort. Thus, we speculate that the accentuated morning peak of AMI onset in patients with admission TG ≥150 mg/dl may be due to the combination of the following three factors: 1) increased hypercoagulability, hypofibrinolysis, viscosity, and endothelium dysfunction resulting from elevated serum TG levels, 2) increased risk of a morning surge of

blood pressure and heart rate, and 3) mental and physical stresses.

One-year mortality according to AMI onset time

In the present study, patients with an afternoon onset of AMI had the worst one-year mortality. Bae et al.[40] reported that patients with evening-onset AMI had the worst one-year mortality in association with poor baseline clinical characteristics. However, the baseline clinical characteristics were comparable among the four onset-time groups in our study cohort. Moreover, even after stratification for potential confounding variables, the results did not change, suggesting that the increased prognostic risk of AMI in the afternoon-onset group was not simply explained by differences in baseline characteristics.

Limitations

A few limitations of the present study warrant mention. First, this was an analysis of prospective observational study and the results may have therefore been influenced by potential confounding factors, even after adjustment for baseline clinical and angiographic characteristics. Thus, caution is needed when interpreting the data and making generalizations to other cohorts. Second, the

laboratory findings, including serum TG levels, were evaluated on admission. Therefore, we could not exclude the influence of food consumption, making interpretation of the data difficult. However, our results also demonstrated that serum TG levels were not likely the final trigger for AMI onset, as patients with TG ≥150 mg/dl on admission did not exhibit a morning peak of AMI onset on the weekend. In patients with hypertriglycemia, hypercoagulability, hypofibrinolysis, viscosity and endothelium dysfunction are generally increased during the early morning hours before dawn,[26-31,35-36,38] resuling in enhanced susceptibility to AMI onset. Thus, under such conditions, it is conceivable that increased sympathetic activity, which was further enhanced in association with mental, physical, and/or other factors, could be the final trigger for AMI onset on weekday mornings in patients with TG ≥150 mg/dl on admission. Based on these findings, the influence of meal intake on the morning peak of AMI onset in the population with TG ≥150 mg/dl may be minimal, if not negligible.

Conclusions

In our large cohort of consecutive AMI patients, the circadian pattern of AMI onset exhibited bimodality and was shown to be influenced by several

lifestyle-related factors. Among these factors, increased serum TG levels on admission had the most marked affect on circadian variation, which was characterized by an increased morning risk of AMI onset during weekdays in this subpopulation. Although confirmation in other cohorts is required, this finding may help to identify the underlying triggers and substrates of AMI onset and help suggest preventive measures of AMI.

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RE and YKS (Yasuhiko Sakata) participated in study concept and design. DN, SS, MU, SM and MH participated in acquisition of data. RE, YKS, SY and TH participated in analysis and interpretation of the data. YKS, TK, HS, SH, YSS (Yasushi Sakata), SY, MH and TH participated in drafting and critical revision of the manuscript for important intellectual content. RE and TH participated in statistical analysis. YKS, HS, SN, MH and IK obtained funding.

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Competing interests

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.

Patient consent

Obtained.

Ethics approval

The study protocol has been approved by the ethics committee of each participating hospital.

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Appendix

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Contributorship Statement

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Disclosure

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.

Data Sharing Statement

All OACIS investigators can access to the published and unpublished data.

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Table 1. Demographics and clinical characteristics of the study population

	N=7755
Patients	
Age (years)	66 (57-74)
Male (%)	5872 (75.7)
Job (%)	3364 (48.2)
BMI (kg/m^2)	23.4 (21.4-25.7)
Cardiovascular risk factors	
Smoker (%)	4865 (63.9)
Drinker (%)	3321 (45.3)
Diabetes (%)	2586 (33.4)
Hypertension (%)	4424 (58.9)
Dyslipidemia (%)	3259 (44.1)
Previous MI (%)	983 (13.0)
Angina pectoris (%)	1737 (23.4)
Multivessel disease (%)	2790 (38.4)
Collateral circulation (%)	2576 (35.7)
Clinical presentation	
Onset admission time<24h(%)	6804 (89.1)
Killip ≥ Ⅱ (%)	1331 (18.0)
Initial TIMI ≤ Ⅱ (%)	4759 (68.4)
STEMI (%)	6567 (86.0)
Labortory data on admission	
Blood glucose level (mg/dl)	152 (122-209)
HDL cholesterol (mg/dl)	44 (37-53)
LDL cholesterol (mg/dl)	121 (99-147)
Triglycerides (mg/dl)	92 (58-142)
HbA1c (%)	5.9 (5.5-6.9)
Peak CK (IU/l)	2147 (1069-4006)
eGFR (ml/min/1.73 m^2)	64.5 (49.2-80.9)

Localization of MI	
LAD	3050 (41.7)
RCA	2447 (33.4)
LCX	998 (13.6)
LMT	164 (2.2)

BMI = body mass index; MI = myocardial infarction; STEMI = ST-elevation myocardial infarction; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglyceride ; HbA1c = hemoglobin A1c; CK = creatinine phosphokinase; eGFR = estimated glomerular filtration rate; LAD = left anterior descending artery; LCX = left circumflex artery; LMT = left main trunk; RCA = right coronary artery. Categorical variables are presented as number (percentage), and continuous variables are presented as quartile. Laboratory data were measured on admission. Smoker was defined as patients with smoking history, and drinker was defined as active drinker. Number (percentage) of Localization of MI was calculated out of 7319 patients who underwent coronary angiography.

Figure legends

Figure 1. Circadian pattern of AMI onset in the overall population.

A circadian pattern of AMI onset in the overall population was clearly observed in a circular plot (A) and histogram (B). The solid line corresponds to the fitted von Mises distribution, and the dots with error bars are the estimated peak onset times and 95% confidence intervals (CI), respectively.

Figure 2. Circadian pattern of AMI onset according to day of the week.

Circadian patterns of AMI onset based on the day of the week are shown. The estimated peak onset time and 95%CIs are shown below each circular plot.

* P values from the likelihood ratio (LR) test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal.

Figure 3. Circadian pattern of AMI onset based on lifestyle-related factors.

(A) Circular plots of the circadian pattern of AMI onset in the subpopulation with TG levels ≥150 and <150 mg/dl, and the circular plot of the corresponding fitted von Mises distributions for each subgroup are shown. (B)-(M) Circular plots of

the fitted von Mises distributions of each subgroup based on smoking habit, age, drinking habit, BG levels, gender, and working status, LDL levels, HDL levels, Hba1c levels, Hypertension, Diabetes and Dyslipidemia.

* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.

† P values from the hierarchical LR test to examine whether each factor affected the circadian pattern of AMI onset.

Figure 4 . One-year mortality according to the onset time of AMI onset

(A) One-year mortality among the four subgroups based on AMI onset time.

(B) Hazard ratios (HRs) for one-year mortality in the afternoon-onset group versus the other three onset-time groups.

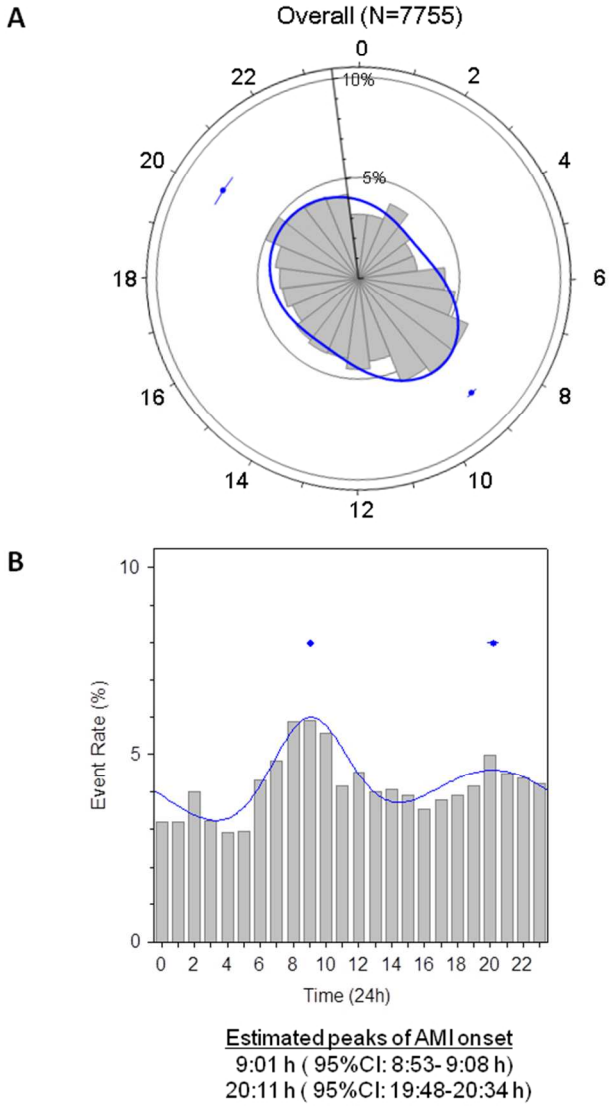
Kaplan-Meier survival curves of one-year mortality among the four AMI onset time subgroups (A). A p value from the log-rank test was used to examine difference in the Kaplan-Meier curves. HR and 95%CI, and p value for the overall population was calculated using univariable Cox regression analysis. The HRs and 95%CIs, and p values for the individual potential confounding variables were calculated using stratified Cox regression analysis, in which the variables

were included into the model as stratification factors (B).

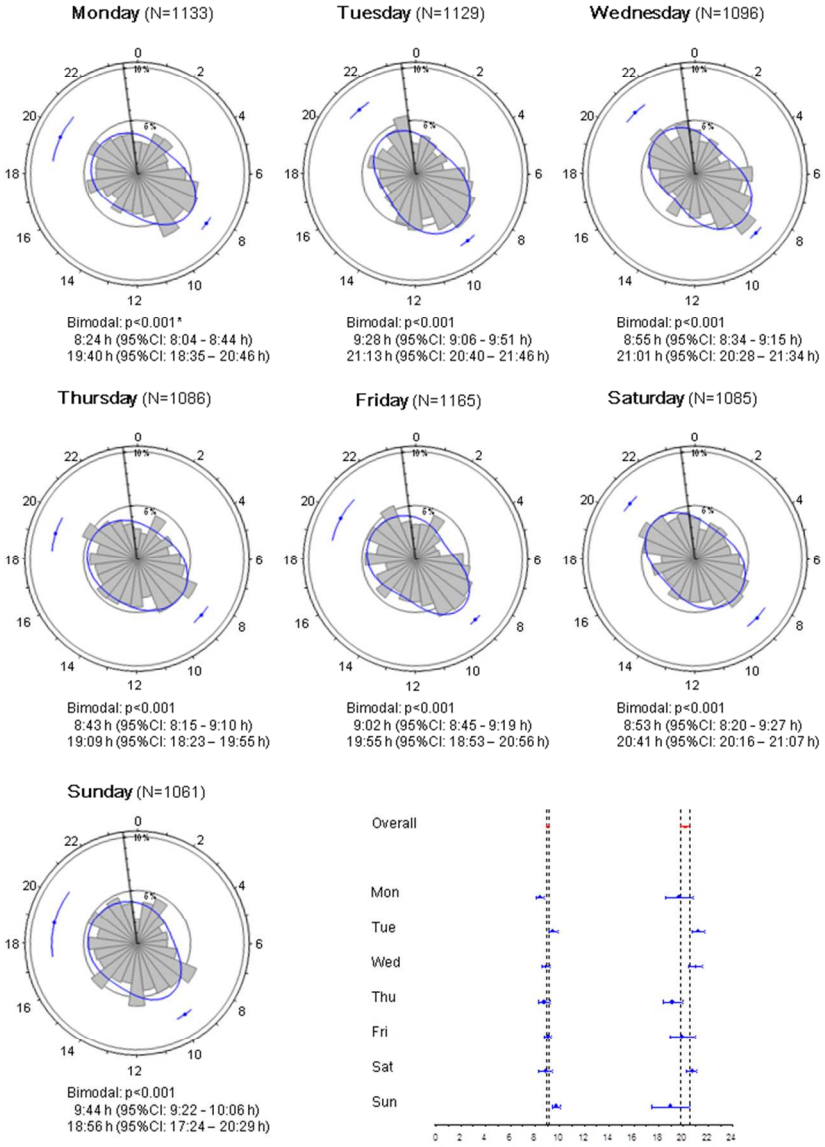
eFigure 1. Circadian pattern of AMI onset according to day of the week in the subpopulation with admission serum TG ≥150 and <150 mg/dl.

Circular plots of the fitted von Mises distributions for the subpopulation with admission serum TG ≥150 and <150mg/dl according to the day of the week are shown.

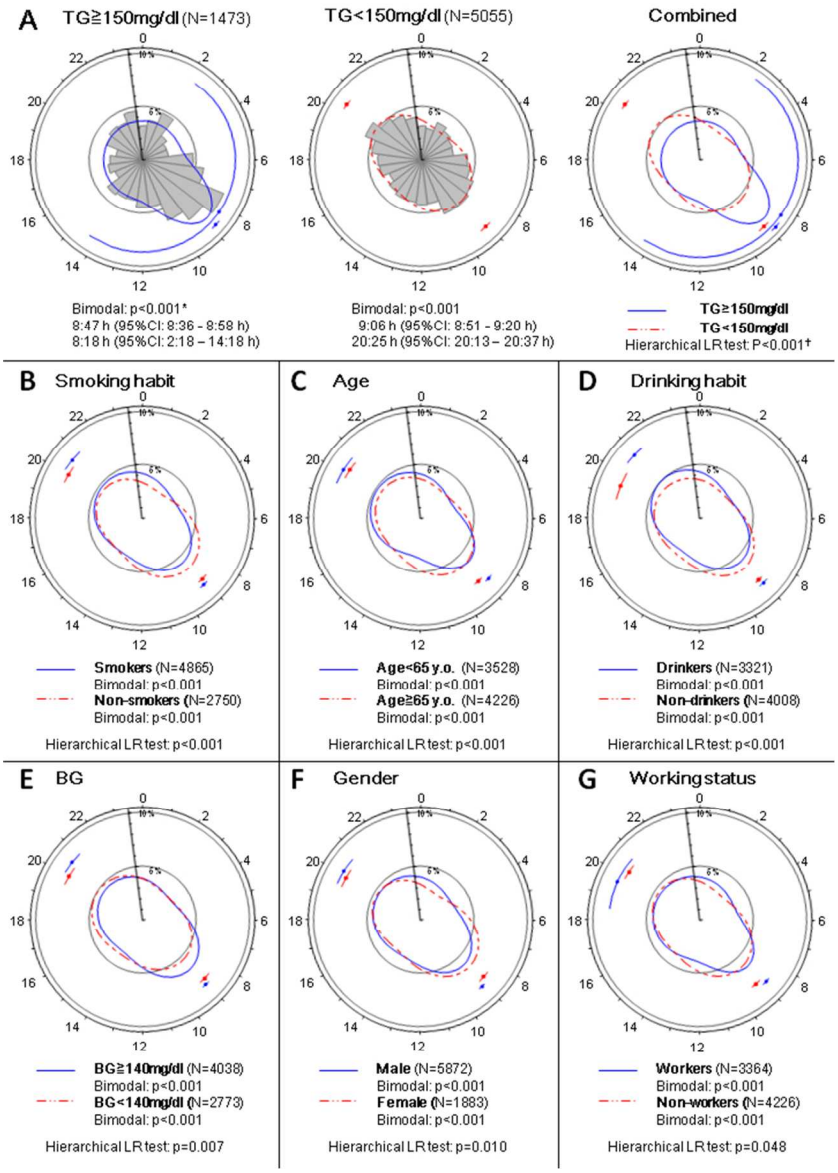
* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.



A circadian pattern of AMI onset in the overall population was clearly observed in a circular plot (A) and histogram (B). The solid line corresponds to the fitted von Mises distribution, and the dots with error bars are the estimated peak onset times and 95% confidence intervals (CI), respectively.
190x275mm (96 x 96 DPI)



Circadian patterns of AMI onset based on the day of the week are shown. The estimated peak onset time and 95%CIs are shown below each circular plot.
* P values from the likelihood ratio (LR) test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal.
190x275mm (96 x 96 DPI)



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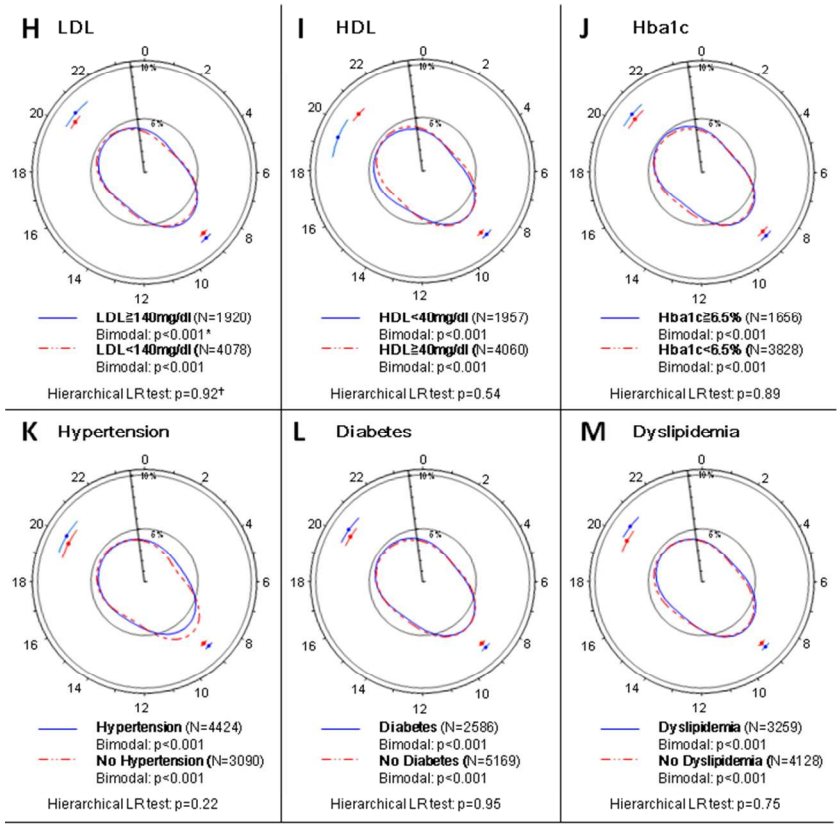
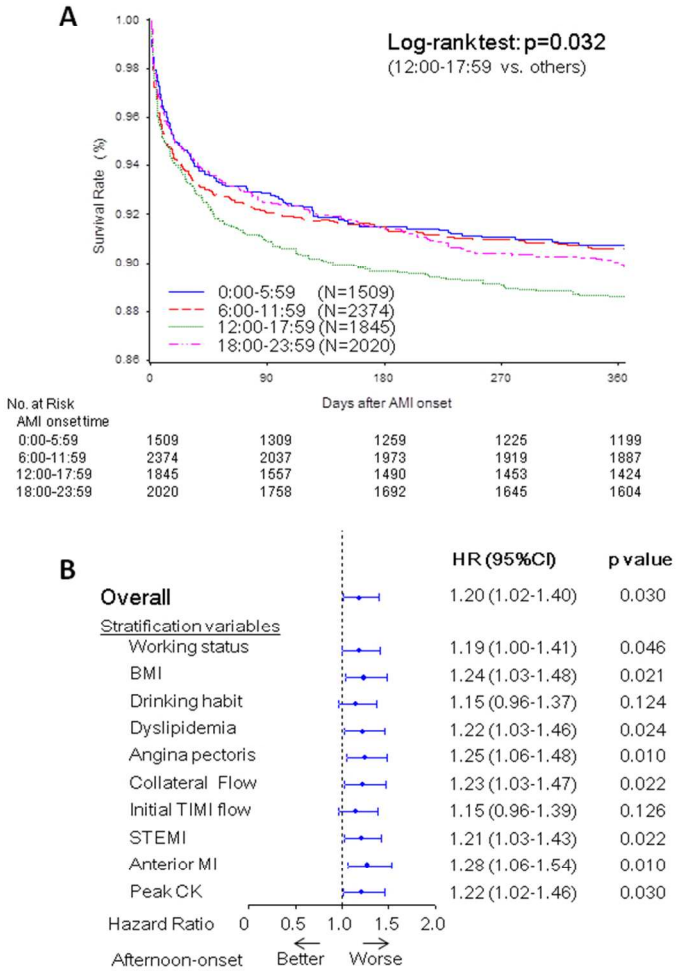


(A) Circular plots of the circadian pattern of AMI onset in the subpopulation with TG levels ≥150 and <150 mg/dl, and the circular plot of the corresponding fitted von Mises distributions for each subgroup are shown. (B)-(M) Circular plots of the fitted von Mises distributions of each subgroup based on smoking habit, age, drinking habit, BG levels, gender, working status, LDL levels, HDL levels, Hba1c, Hypertension, Diabetes and Dyslipidemia.

* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.

† P values from the hierarchical LR test to examine whether each factor affected the circadian pattern of AMI onset.

190x275mm (96 x 96 DPI)



Kaplan-Meier survival curves of one-year mortality among the four AMI onset time subgroups (A). A p value from the log-rank test was used to examine difference in the Kaplan-Meier curves. HR and 95%CI, and p value for the overall population was calculated using univariable Cox regression analysis. The HRs and 95%CIs, and p values for the individual potential confounding variables were calculated using stratified Cox regression analysis, in which the variables were included into the model as stratification factors (B).
190x275mm (96 x 96 DPI)

eTable 1. Estimated time of peak AMI onset according to subgroup

Subgroup	Estimated time of primary peak onset (95%CI)	Estimated time of second peak onset (95%CI)
TG		
≥150 mg/dl	8:47 h (8:36 - 8:58 h)	8:18 h (2:18 - 14:18 h)
<150 mg/dl	9:06 h (8:51 - 9:20 h)	20:25 h (20:13 - 20:37 h)
Smoking habit		
Yes	9:07 h (8:57 - 9:18 h)	20:37 h (20:09 - 21:04 h)
No	8:59 h (8:45 - 9:12 h)	20:01 h (19:38 - 20:24 h)
Age		
<65 y.o.	8:45 h (8:36 - 8:55 h)	20:07 h (19:30 - 20:44 h)
≥65 y.o.	9:09 h (8:57 - 9:20 h)	20:16 h (19:52 - 20:40 h)
Drinking habit		
Yes	9:00 h (8:48 - 9:13 h)	20:54 h (20:29 - 21:20 h)
No	9:03 h (8:53 - 9:14 h)	19:27 h (18:50 - 20:04 h)
BG		
≥140 mg/dl	9:03 h (8:53 - 9:12 h)	20:35 h (20:06 - 21:04 h)
<140 mg/dl	8:53 h (8:37 - 9:09 h)	20:03 h (19:38 - 20:27 h)
Gender		
Male	9:09 h (9:00 - 9:17 h)	20:09 h (19:34 - 20:45 h)
Female	8:47 h (8:31 - 9:04 h)	19:56 h (19:31 - 20:21 h)
Working status		
Yes	8:51 h (8:41 - 9:01 h)	19:37 h (18:28 - 20:47 h)
No	9:14 h (9:02 - 9:28 h)	20:15 h (19:55 - 20:36 h)
LDL		
≥ 140mg/dl	9:11 h (8:57 - 9:24 h)	20:36 h (19:39 – 21:34 h)
< 140mg/dl	9:04 h (8:53 - 9:15 h)	20:20 h (19:58 – 20:42 h)
HDL		
< 40mg/dl	8:59 h (8:44 - 9:13 h)	19:26 h (18:35 – 20:17 h)
≥ 40mg/dl	9:07 h (8:56 - 9:18 h)	20:45 h (20:23 – 21:07 h)
HbA1c		
≥6.5%	8:59 h (8:43 - 9:16 h)	20:37 h (20:02 – 21:13 h)
<6.5%	9:03 h (8:51 - 9:14 h)	20:16 h (19:49 – 20:42 h)
Hypertension		
Yes	8:59 h (8:48 - 9:10 h)	20:03 h (19:17 - 20:48 h)
No	9:04 h (8:54 - 9:14 h)	19:48 h (19:06 – 20:30 h)

Diabetes		
Yes	9:03 h (8:50 - 9:16 h)	20:22 h (19:45 – 21:00 h)
No	9:03 h (8:53 - 9:13 h)	20:09 h (19:42 – 20:36 h)
Dyslipidemia		
Yes	9:01 h (8:49 - 9:13 h)	20:31 h (19:59 – 21:02 h)
No	9:02 h (8:52 - 9:13 h)	19:55 h (19:24 – 20:26 h)

The estimated primary and secondary peak onsets and 95%CI of each subgroup are shown.

eTable 2. Baseline characteristics in patients based on triglyceride levels at admission

	Triglycerides ≥150 mg/dl (N=1473)	Triglycerides <150 mg/dl (N=5055)	p value
Patients			
Age (years)	60 (53-68)	67 (59-75)	<0.001
Male (%)	1233 (83.7)	3725 (73.7)	<0.001
Job (%)	823 (62.3)	2083 (45.3)	<0.001
BMI (kg/m^2)	24.7 (22.6-27.0)	23.1 (21.1-25.3)	<0.001
Cardiovascular risk factors			
Smoker (%)	1058 (72.4)	3028 (60.7)	<0.001
Drinker (%)	710 (50.4)	2104 (43.5)	<0.001
Diabetes (%)	581 (39.4)	1612 (31.9)	<0.001
Hypertension (%)	863 (59.9)	2954 (60.0)	0.90
Dyslipidemia (%)	956 (67.2)	1917 (39.2)	<0.001
Previous MI (%)	176 (12.1)	638 (12.9)	0.45
Angina pectoris (%)	316 (22.0)	1131 (23.1)	0.37
Multivessel disease (%)	500 (35.6)	1910 (39.8)	0.005
Collateral circulation (%)	506 (36.4)	1728 (36.2)	0.92
Clinical presentation			
Onset admission time<24h(%)	1304 (89.0)	4444 (89.1)	0.93
Killip ≥Ⅱ (%)	190 (13.4)	877 (18.1)	<0.001
Initial TIMI ≤Ⅱ(%)	919 (67.7)	3135 (68.1)	0.75
STEMI (%)	1250 (85.6)	4305 (86.1)	0.60
Labortory data on admission			
Blood glucose (mg/dl)	159 (126-220)	148 (121-203)	<0.001
HDL cholesterol (mg/dl)	41 (36-49)	45 (38-54)	<0.001
LDL cholesterol (mg/dl)	130 (104-157)	120 (98-145)	<0.001
Triglycerides (mg/dl)	199 (169-261)	77 (51-105)	<0.001
HbA1c (%)	6.1 (5.6-7.4)	5.9 (5.5-6.8)	<0.001
Peak CK (IU/l)	2097 (1062-3910)	2187 (1083.5-4019.5)	0.28
eGFR (ml/min/1.73 m^2)	65.7 (51.7-80.1)	64.6 (49.3-81.9)	0.21
Localization of MI			
LAD	570 (40.3)	2051 (42.5)	0.153
RCA	512 (36.2)	1601 (33.2)	
LCA	215 (15.2)	652 (13.5)	
LMT	23 (1.6)	102 (2.1)	

Categorical variables are presented as number (percentage), and continuous variables are presented as

quartiles.

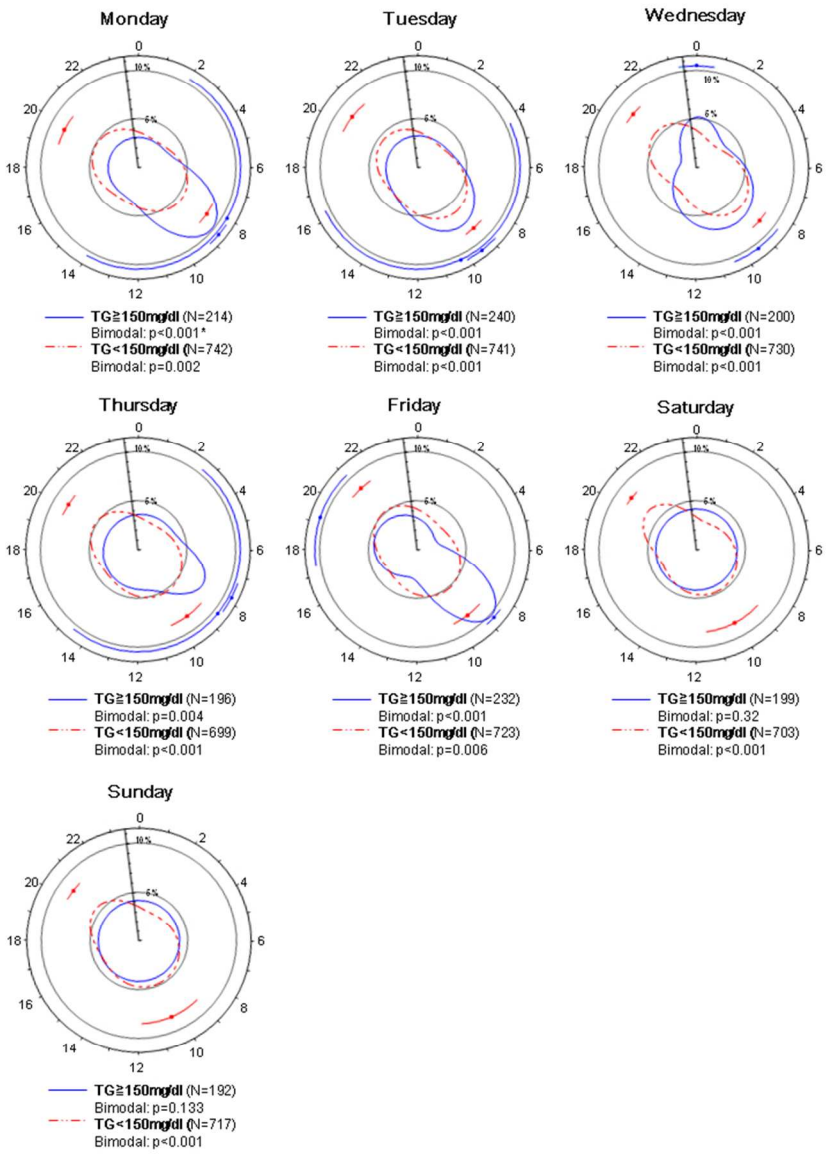
Categorical variables were compared by the chi-square test, and continuous variables were compared by the Wilcoxon rank sum test for two-group comparisons.

eTable 3. Baseline characteristics among groups based on time of AMI onset

	0:00-5:59 h (N=1509)	6:00-11:59 h (N=2374)	12:00-17:59 h (N=1845)	18:00-23:59 h (N=2020)	p value
Patients					
Age (years)	66 (57-74)	66 (58-74)	66 (57-74)	65 (57-73)	0.077
Male (%)	1166 (77.2)	1752 (73.7)	1412 (76.5)	1542 (76.2)	0.050
Employed (%)	656 (48.9)	1023 (47.7)	785 (47.0)	900 (49.2)	0.55
BMI (kg/m^2)	23.4 (21.5-25.8)	23.4 (21.3-25.6)	23.6 (21.5-25.7)	23.4 (21.4-25.7)	0.44
Cardiovascular risk factors					
Smoker (%)	974 (65.6)	1417 (60.8)	1166 (64.3)	1308 (65.8)	0.002
Drinker (%)	687 (47.9)	970 (43.3)	737 (42.4)	927 (48.4)	<0.001
Diabetes (%)	520 (34.4)	784 (33.0)	612 (33.2)	670 (33.1)	0.80
Hypertension (%)	891 (60.4)	1326 (57.8)	1057 (59.1)	1150 (58.8)	0.47
Dyslipidemia (%)	636 (44.3)	1026 (45.3)	757 (43.0)	840 (43.7)	0.50
Previous MI (%)	177 (12.0)	291 (12.6)	230 (12.9)	285 (14.5)	0.140
Angina pectoris (%)	339 (23.4)	521 (22.9)	400 (22.7)	477 (24.7)	0.43
Multivessel disease (%)	533 (37.6)	828 (37.2)	663 (38.6)	766 (40.5)	0.151
Collateral circulation (%)	517 (36.7)	750 (33.8)	636 (37.2)	673 (35.8)	0.129
Clinical presentation					
Onset admission time <24 h (%)	1348 (90.8)	2077 (88.8)	1622 (89.2)	1757 (87.9)	0.057
Killip ≥ Ⅱ (%)	276 (19.0)	377 (16.6)	316 (18.0)	362 (18.8)	0.198
Initial TIMI ≤ Ⅱ (%)	906 (66.9)	1457 (68.0)	1186 (71.6)	1210 (67.2)	0.014
STEMI (%)	1258 (84.6)	2024 (86.5)	1588 (87.3)	1697 (85.2)	0.092
Anterior MI (%)	672 (46.9)	987 (43.9)	703 (40.5)	852 (44.9)	0.002
Labortory data on admission					
Blood glucose level (mg/dl)	151 (123 - 206)	155 (124 - 213)	149 (122 - 203)	149 (120 - 210)	0.033
HDL cholesterol (mg/dl)	44 (38-53)	44 (38-53)	44 (37-53)	44 (38-53)	0.42
LDL cholesterol (mg/dl)	123 (101-149)	123 (99-148)	121 (99-146)	119 (97-147)	0.171
Triglycerides (mg/dl)	91(57-141)	100 (63-155)	91.5 (58-140)	87 (54-132)	<0.001
HbA1c (%)	5.9 (5.5 - 7.0)	6.0 (5.5 - 6.9)	5.9 (5.5 - 6.8)	5.9 (5.5 - 7.0)	0.95
Peak CK (IU/l)	2137 (1089-3934.5)	2111 (1027-4144)	2104 (1095-3894)	2274 (1070-3985)	0.92
Treatment					
Reperfusion (%)	1349 (89.6)	2113 (89.2)	1645 (89.4)	1791 (88.8)	0.88
PCI (%)	1316 (87.2)	2068 (87.1)	1601 (86.8)	1749 (86.6)	0.94

Drugs at discharge					
β blockers (%)	677 (44.9)	964 (40.6)	752 (40.8)	836 (41.4)	0.043
RAS inhibitors (%)	1038 (68.8)	1594 (67.1)	1240 (67.2)	1386 (68.6)	0.56
Statin (%)	580 (38.4)	859 (36.2)	611 (33.1)	690 (34.2)	0.007
Diuretics (%)	387 (25.7)	556 (23.4)	416 (22.6)	529 (26.2)	0.024
Ca blockers (%)	335 (22.2)	493 (20.8)	333 (18.1)	398 (19.7)	0.020
Antiplatelet (%)	1359 (90.1)	2124 (89.5)	1624 (88.0)	1814 (89.8)	0.197

Categorical variables are presented as number (percentage), and continuous variables are presented as quartiles. Categorical variables were compared by the chi-square test, and continuous variables were compared by the Kruskal-Wallis test for four-group comparisons.



Circular plots of the fitted von Mises distributions for the subpopulation with admission serum TG ≥150 and <150mg/dl according to the day of the week are shown.
* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.
190x275mm (96 x 96 DPI)

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Association of Lifestyle-related Factors with Circadian Onset Patterns of Acute Myocardial Infarction: a Prospective Observational Study in Japan

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Association of Lifestyle-related Factors with Circadian Onset Patterns of Acute Myocardial Infarction: a Prospective Observational Study in Japan

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Abstract

Objective: The onset of acute myocardial infarction (AMI) shows characteristic circadian variations involving a definite morning peak and less-defined nighttime peak. However, the factors influencing circadian patterns of AMI onset and their influence on morning and nighttime peaks have not been fully elucidated.

Design, Setting, and Participants: An analysis of patients registered between 1998 and 2008 in the Osaka Acute Coronary Insufficiency Study, which is a prospective, multicenter observational study of AMI patients in the Osaka region of Japan. The present study included 7755 consecutive patients with a known time of AMI onset.

Main Outcomes and Measures: A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI had uniform, unimodal, or bimodal distribution, and the likelihood ratio test was then used to select the best circadian pattern among them. The hierarchical likelihood ratio test was used to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier

method was used to estimate survival curves of one-year mortality according to AMI onset time.

Results: The overall population had a bimodal circadian pattern of AMI onset characterized by a high and sharp morning peak and a lower and less-defined nighttime peak (bimodal: p<0.001). Although several lifestyle-related factors had a statistically significant association with the circadian patterns of AMI onset, serum triglyceride levels had the most prominent association with circadian patterns of AMI onset. Patients with triglyceride ≥150 mg/dl on admission had only one morning peak in the circadian pattern of AMI onset during the weekdays, with no peaks detected on weekends, whereas all other subgroups had two peaks throughout the week.

Conclusions: The circadian pattern of AMI onset was characterized by bimodality. Notably, several lifestyle-related factors, particularly serum triglyceride levels, had a strong relation with the circadian pattern of AMI onset.

Strengths and limitations of this study

- We comprehensively analyzed the circadian patterns of AMI onset in a large, multicenter cohort of patients in relation to patient characteristics, lifestyle factors, and day of the week.
- A mixture of two von Mises distributions revealed that the circadian pattern of AMI onset exhibited bimodality.
- Several lifestyle-related factors were shown to be associated with circadian patterns of AMI onset, depending on the day of the week. In particular, it was demonstrated that elevated serum triglyceride levels on admission accentuated morning peak of AMI onset during the weekdays.
- Subjects were limited to those who were hospitalized for AMI.
- Laboratory data were evaluated on admission.

Introduction

Onset patterns of acute myocardial infarction (AMI) exhibit circadian variation that is characterized by an increased frequency in the morning and a secondary peak incidence at nighttime.[1] Several studies have confirmed that AMI onset exhibits a bimodal circadian pattern, with peaks occurring in the morning hours [2-4] and nighttime hours.[1,4-7] However, it is not well understood what factors, particularly among lifestyle-related factors, influence the circadian patterns of AMI. Moreover, although these patterns appear to vary according to the day of the week,[8] it is unclear how circadian patterns of AMI onset vary throughout the week, particularly, in association with socioeconomic factors.

Because AMI and subsequent ischemic heart failure are the leading causes of death in both developed and developing countries, primary prevention of AMI is a major healthcare issue worldwide. Accordingly, identifying potential factors influencing the circadian pattern of AMI may help in the clinical management of patients for preventing the onset of AMI.

In the present study, we comprehensively analyzed the circadian patterns of AMI onset in a large, multicenter cohort of patients in relation to patient characteristics, lifestyle factors, and day of the week.

Methods

OACIS registry and study subjects

The OACIS is a prospective, multicenter observational study collecting demographic, procedural, biological, and outcome data as well as blood samples from AMI patients hospitalized at 25 collaborating hospitals from the Osaka region of Japan (UMIN-Clinical Trial Registry ID: UMIN000004575).[9-10] A diagnosis of AMI was made if the patient fulfilled at least two of the following three criteria; 1) history of central chest pressure, pain, or tightness lasting 30 min, (2) typical electrocardiographic changes (i.e., ST-segment elevation ≥ 0.1 mV in 1 standard limb lead or 2 precordial leads, ST-segment depression ≥ 0.1 mV in 2 leads, abnormal Q waves, or T-wave inversion in 2 leads), and (3) an increase in serum creatine kinase levels of two times the upper normal limit in each hospital. All the collaborating hospitals were encouraged to enroll consecutive patients with AMI.

We prospectively collected data by research cardiologists and trained research nurses using a specific reporting form, and the following variables were

extracted from the OACIS registry database: age, gender, working status, body mass index (BMI), coronary risk factors (diabetes, hypertension, dyslipidemia, smoking, drinking, previous myocardial infarction, multi-vessel disease, and collateral circulation), clinical presentation on admission (KILLIP classification, initial TIMI flow, and ST-elevation myocardial infarction), coronary angiography data, reperfusion therapy, laboratory data on admission (hemoglobin A1c, total cholesterol, low- and high-density lipoprotein cholesterol, triglyceride, and estimated glomerular filtration rate) and medications at discharge (RAS inhibitors, beta-blocker, calcium channel blocker, statin, anti-platelet agent, and diuretics). Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl, hemoglobin A1c ≥6.5% or a history of anti-diabetic therapy. Hypertension was defined as a history of systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or antihypertensive therapy. Dyslipidemia was defined as fasting total cholesterol ≥220 mg/dl, low-density lipoprotein cholesterol ≥140 mg/dl, high-density lipoprotein cholesterol ≤40 mg/dl, fasting triglycerides ≥150 mg/dl, or lipid-lowering therapy.

The study protocol has been approved by the ethics committee of each participating hospital. All in-hospital data were obtained after written informed

consent and were then transmitted to the data collection center at the Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan for processing and analysis. The corresponding authors had full access and validated to all data in the study.

In the present study, we analyzed 7755 AMI patients whose time of AMI onset was definitely identified among the 8603 consecutive patients registered in the OACIS registry between 1998 and 2008. Patients' baseline characteristics are presented in Table 1.

Statistical analysis

Continuous variables were summarized as quartiles and were compared by the Wilcoxon rank sum test for two-group comparisons, and the Kruskal-Wallis test for four-group comparisons. Categorical variables were presented as number and percentage, and were compared by the chi-square test. A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI onset had uniform (no peak), unimodal (one peak), or bimodal distribution (two peaks), and the likelihood ratio test was then used to select the best circadian pattern among them.[11] In addition, the hierarchical likelihood ratio test was

assessed to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier method was used to estimate survival curves of one-year mortality according to AMI onset time (morning [6:00-11:59 h], afternoon [12:00-17:59 h], evening [18:00-23:59 h], and nighttime [0:00-5:59 h]). The log-rank test was used to compare survival curves between the groups, and the Cox proportional hazards regression model was used to estimate hazard ratios and 95% confidence intervals (CI). To reduce potential confounding effects due to patient background variability in the comparison between the afternoon-onset and other groups, a stratified Cox proportional hazards regression model was used, in which the potential confounding variables were included into the model as stratification factors. Cosinor-analysis was used to estimate the amplitude of serum TG levels on admission according to AMI onset time. Then, F-test for the existence of a rhythm (amplitude) was used to examine whether the amplitude of serum TG levels on admission in patients with AMI had circadian variation or not. Statistical significance was set as $p<0.05$. All statistical analyses were performed using an in-house validated Fortran program or SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Bimodal circadian patterns of AMI onset in the overall population

The daily patterns of AMI onset in our cohort of 7755 patients were first analyzed using the likelihood ratio test (Figure 1). In the overall population, AMI onset clearly exhibited a circadian pattern consisting of two peaks (bimodal: $p<0.001$): a primary peak at 9:01 h (95%CI: 8:53-9:08 h) and a secondary peak at 20:11 h (95%CI: 19:48-20:34 h). The primary peak was more clearly defined than the secondary peak in both the circular and columnar histograms (Figure 1A and 1B, respectively).

Likelihood ratio test analysis revealed that the peak time of AMI onset varied according to the day of the week (Figure 2). For example, the primary peak onset time was earliest on Monday (8:24 h [95%CI: 8:04-8:44 h]) and latest on Sunday (9:44 h [95%CI: 9:22-10:06 h]). On Tuesday, patients exhibited a circadian pattern of AMI onset characterized by late primary (9:28 h [95%CI: 9:06-9:51 h]) and secondary peak onset times (21:13 h [95%CI: 20:40-21:46 h]), whereas earlier peak onset times (8:43 h [95%CI: 8:15-9:10 h], and 19:09 h [95%CI: 18:23-19:55 h]) were detected on Thursday. Notably, the evening peak was higher, and sharper than the morning peak on Saturday (Figure 2).

Factors affecting circadian patterns of AMI onset

Hierarchical likelihood ratio analysis revealed that serum TG levels on admission, smoking, age, drinking, blood glucose levels on admission, gender, and working status had a statistically significant association with the circadian pattern of AMI onset, whereas several other known risk factors for AMI, including HDL- and LDL-cholesterol, Hba1c, hypertension, diabetes and dyslipidemia were not related to the observed patterns (Figure 3, eTable 1).

Among the positively associated factors, serum TG levels on admission had the greatest association with the circadian pattern of AMI onset. Although the likelihood ratio test demonstrated that patients with admission serum TG levels of ≥150 mg/dl (N=1473) had the two characteristic peaks during the day, the peak pattern clearly differed from the other subpopulation groups. In patients with admission serum TG levels of ≥150 mg/d, both peaks occurred in the morning and nearly overlapped (8:18 h and 8:47 h) (Figure 3A). Therefore, the subpopulation with admission TG levels ≥ 150 mg/dl was considered to have a high frequency of AMI onset only in the morning.

The baseline characteristics and laboratory data of patients with serum

TG levels of ≥ 150 and <150 mg/dl on admission are shown in eTable 2. In the subpopulation with higher TG levels, the circadian patterns of AMI onset was characterized by a large, sharp peak in the morning from Monday to Friday, but no peaks were detected on Saturday and Sunday (bimodal: p=0.32 and p=0.133, respectively) (eFigure 1). In contrast, patients with admission serum TG levels of <150 mg/dl (N=5055) had onset peaks that occurred in the morning and evening consistently throughout the week (eFigure 1).

A likelihood ratio test demonstrated that other all subpopulations had two AMI onset peaks during the day: one in the morning and the other in the evening (Figure 3, eTable 1). The subpopulations that were grouped according to smoking habit, age < 65 years old, male gender, and active employment had a circadian pattern of AMI onset with a sharper primary peak and less-defined sharp secondary peak compared to the other subpopulations (Figure 3B,C,F,G, eTable1), although the peak heights were similar between the subpopulations, with the exception of the smokers/nonsmokers subpopulations. The primary AMI onset peak in the subpopulation of smokers was higher than that among nonsmokers, whereas the secondary peaks were similar. Drinkers had a circadian pattern of AMI onset that was characterized by a lower and less sharp

peak in the morning, and a higher, sharper and later peak in the evening (9:00 h [95%CI: 8:48-9:13 h], 20:54 h [95%CI: 20:29-21:20 h]) compared to nondrinkers (9:03 h [95%CI: 8:53-9:14 h], 19:27 h [95%CI: 18:50-20:04 h]) (Figure 3D, eTable1). The subpopulation with admission blood glucose ≥140 mg/dl exhibited a circadian pattern of AMI onset with a higher and sharper primary peak and a less-defined secondary peak compared to the subpopulation of AMI patients with blood glucose <140 mg/dl on admission (Figure 3E).

One-year mortality according to onset time of AMI

One-year mortality was compared among four patient subpopulations that were grouped according to the time range of AMI onset. The baseline characteristics and laboratory data for the four groups are presented in eTable 3. A total of 753 deaths were recorded during a median follow-up period of 365 days. Kaplan-Meier survival analysis demonstrated that the afternoon-onset (12:00-17:59 h) group had worse 1-year mortality than the other 3 groups (log rank test, p=0.032) (Figure 4A). In the subgroup of patients with ST-elevation myocardial infarction (STEMI), the result was similar (log rank test, p=0.007). Univariable Cox regression analysis revealed that the HR of 1-year mortality in

the afternoon-onset group as compared to the other three groups was 1.20 (95%CI 1.02-1.40, p=0.030, Figure 4B). This result did not generally change after stratification with potential confounding factors that showed a different trend between the afternoon-onset group and other three groups (Figure 4B).

Discussion

In the present study, we confirmed that AMI onset exhibits a circadian pattern characterized by bimodality, with a definite morning peak and a less-defined evening peak. Notably, several lifestyle-related factors were associated with variation in the circadian pattern of AMI onset. In particular, serum TG levels on admission for AMI were associated with a unique pattern of AMI onset that is characterized by augmented unimodal peaks on weekday mornings, suggesting that individual lifestyle may affect the onset pattern of AMI.

Bimodal pattern of AMI onset: morning and nighttime peaks

AMI onset in our large patient cohort generally followed a circadian pattern that was characterized by a high and sharp morning peak and a lower and less-defined sharp nighttime peak (Figure 1), a finding that is consistent with the

results of previous investigations.[1-7] Interestingly, the time of two peaks shifted in a synchronous fashion during the weekdays; the secondary peaks generally occurred around 11 to 12 h after the morning peaks on Monday through Friday (Figure 2). For example, AMI onset exhibited early morning and nighttime peaks on Monday and Thursday, whereas that on Tuesday exhibited late morning and nighttime peaks. Although this finding is partly consistent with the observation of Peters et al.,[5] who reported that a secondary peak in AMI onset occurs 11 to 12 h after waking, the present study firstly demonstrated that this synchrony was present on the weekdays, but absent on the weekends.

Several physiological processes are considered to contribute to the bimodal pattern of AMI onset. For example, Stergiou et al.[12] demonstrated that the two-peak diurnal variation in stroke onset occurs in parallel with variation in blood pressure, pulse rate, and physical activity. Thus, the bimodality of blood pressure and heart rate [13-14] is the most likely explanation for the circadian patterns of AMI onset observed in the present study. A greater morning surge of blood pressure and heart rate[13] may explain why the nighttime peak of AMI onset was lower and less-defined than the morning peak. In addition, increased blood viscosity[15] and thrombogenicity due to morning hypercoagulability[16] and

hypofibrinolysis[17] also likely increased the frequency of AMI onset in the morning. It is also possible that external factors, such as physical exertion and mental stress, could be triggers for the morning onset of AMI.[18] In the present study, the younger (<65 years old), working, male, and smoker subpopulations had a sharp morning peak of AMI onset compared with the elderly, nonworking, female, and nonsmoking subpopulations (Figure 3B,C,F,G). The sharpness of the morning peak might be related to increased susceptibility to physical and mental stresses in these subpopulations, in which they are more likely to start activities or go to work soon after waking up. Similarly, the sharp and early morning peak of AMI onset that was detected on Monday may be due to the increased physical and mental stress that is associated with the first morning of the week (Figure 2). We also found that the morning peak occurred latest on Sunday (Figure 2). Together, these findings strongly suggest that mental and physical activity and/or stress may act as a trigger for the morning onset of AMI.

Although many reports have examined the primary peak of AMI onset, relatively little attention has been paid to the secondary peak. We demonstrated that drinkers had a higher, sharper, and later nighttime peak of AMI onset than nondrinkers (Figure 3D). Moreover, the nighttime peak on Saturday was the

highest and sharpest amongst the seven days of the week (Figure 2). This observation may be explained by the fact that people might likely consume alcohol and engage in social activities on Saturday night in Japan. Thus, these evening activities can result in increased sympathetic nerve activity and therefore may have contributed to the increased frequency of AMI onset at night. Taken together, our findings suggest that both the morning and nighttime peaks of AMI onset are influenced by physiological and socioeconomic factors.

Associations of lifestyle-related factors with circadian patterns of AMI onset

Many previous studies on circadian pattern of AMI onset considered gender, age, working status as potential factors affecting the circadian patterns of AMI onset.[1,4-6] Here, we additionally incorporated laboratory data, disease, and other socioeconomic factors into our analyses and found that several lifestyle-related factors, including admission serum TG and blood glucose levels, age, gender, working status, and smoking and drinking habits had statistically significant associations with the circadian pattern of AMI onset. Among these factors, elevated serum TG levels (≥150 mg/dl) on admission had the largest

associations with circadian patterns of AMI onset, while the amplitude of serum TG levels on admission in patients with AMI didn't have circadian variation (p=0.52) (eFigure 2).

There are several evidences to support our findings. First, fasting hypertriglyceridemia and postprandial hyperlipidemia, which is characterized by postprandial accumulation of TG-rich lipoproteins and their partially hydrolyzed products, are both closely related to the development of atherosclerotic cardiovascular diseases.[19-21] Several studies have also reported that elevated serum TG levels are associated with an increased risk of myocardial infarction.[22-23] Hypertriglyceridemia is associated with increased thrombogenecity,[24-25] which is reportedly associated with increased plasminogen activator inhibitor-1 (PAI-1)[26-28] and factor VII coagulant activities,[29-30] and viscosity.[31] These three factors have also been reported to affect the development of MI.[32-34] Moreover, hypertriglyceridemia is also related to endothelium dysfunction,[35-36] which contributes to the pathogenesis of coronary artery disease.[37] In healthy subjects, serum TG levels also exhibit circadian variation with a peak around 3 AM.[38] Thus, it is conceivable that patients with hypertriglycemia have further augmented TG levels and are therefore exposed

to increased thrombogenecity and endothelium dysfunction in the early morning hours before dawn, which may explain the accentuated morning peak of AMI onset in patients with admission TG ≥150 mg/dl. Finally, it is reported that high plasma PAI-1 levels and excessive surges in morning blood pressure are independently and additively associated with increased risk of stroke in older hypertensive patients.[39] Thus, these lines of evidence strongly support our observation of a higher morning risk of AMI onset in the subpopulation with admission hypertriglyceridemia.

Altered circadian patterns of AMI onset in patients with increased TG levels on admission.

To our knowledge, this is the first study to demonstrate an association between admission serum TG levels and circadian patterns of AMI onset, as characterized by a lack of an evening peak in AMI onset in the subgroup of serum TG levels on admission ≥150 mg/dl compared to all other subgroups (Figure 3). While LDL/HDL levels are considered to be closely associated with the development of atherosclerosis, LDL/HDL levels were not associated with onset patterns of AMI in the present study. Although the precise mechanisms for

the altered circadian patterns of AMI onset in patients with increased admission serum TG levels are unclear, increased serum TG might have influenced peripheral clocks residing in various tissues throughout the body, disrupting circadian patterns of AMI onset. Indeed, recent studies have shown that energy metabolism is an important modulator of peripheral circadian clock in cardiovascular tissues.[40-41]

Our subpopulation analyses also revealed that the circadian patterns of AMI onset in patients with admission TG levels of ≥150 mg/dl had a sharp morning peak during weekdays, whereas no such peak was detected on Saturday or Sunday. This observation strongly suggests that increased thrombogenicity and endothelium dysfunction was a factor, but not the trigger, for the morning onset of AMI in our study cohort. Thus, it is conceivable that the accentuated morning peak of AMI onset in patients with admission TG ≥150 mg/dl may be due to the combination of the following three factors: 1) increased hypercoagulability, hypofibrinolysis, viscosity, and endothelium dysfunction resulting from elevated serum TG levels, 2) increased risk of a morning surge of blood pressure and heart rate, and 3) mental and physical stresses.

One-year mortality according to AMI onset time

The association between AMI onset time and mortality is controversial. For example, Manfredini et al.[42] reported that patients with a morning onset of AMI are characterized by higher fatal outcome, independent of site and size of infarction, while Bae et al.[43] reported that patients with an evening-onset AMI had the worst one-year mortality in association with poor baseline clinical characteristics. On the other hand, Holmes et al.[44] observed no significant association between circadian patterns of onset time and in-hospital mortality in patients with ST-elevation myocardial infarction (STEMI) after adjusting for clinical risk factors.

In the present study, patients with an afternoon onset of AMI had the worst 1-year mortality (Figure 4A). However, the baseline clinical characteristics were comparable among the four onset-time groups in our study cohort. Indeed, stratification for potential confounding variables did not generally change the results, suggesting that the increased prognostic risk of AMI in the afternoon-onset group was not simply explained by differences in baseline characteristics in the present study (Figure 4B). Anyway, the patient background and physiological circadian rhythms might complexly interact with each other

and affect mortality after AMI, which could lead to these different results among the studies and difficulty in interpreting the results. Further investigations are required to clarify the association of mortality after AMI and onset time.

Limitations

A few limitations of the present study warrant mention. First, this was an analysis of prospective observational study and the results may have therefore been influenced by potential confounding factors, even after adjustment for baseline clinical and angiographic characteristics. Thus, caution is needed when interpreting the data and making generalizations to other cohorts. Second, the laboratory findings, including serum TG levels, were evaluated on admission. Therefore, we could not exclude the influence of food consumption and circadian variation of several factors, particularly serum TG levels, making interpretation of the data difficult. However, our results also demonstrated that serum TG levels were not likely the final trigger for AMI onset, as patients with TG ≥150 mg/dl on admission did not exhibit a morning peak of AMI onset on the weekend. In patients with hypertriglycemia, hypercoagulability, hypofibrinolysis, viscosity and endothelium dysfunction are generally increased during the early morning hours

before dawn,[26-31,35-36,38] resulting in enhanced susceptibility to AMI onset. Thus, under such conditions, it is conceivable that increased sympathetic activity, which was further enhanced in association with mental, physical, and/or other factors, could be the final trigger for AMI onset on weekday mornings in patients with TG ≥150 mg/dl on admission. Based on these findings, the influence of meal intake and circadian variation of serum TG levels on the morning peak of AMI onset in the population with TG ≥150 mg/dl may be minimal, if not negligible.

Conclusions

In our large cohort of consecutive AMI patients, the circadian pattern of AMI onset exhibited bimodality and was shown to be associated with several lifestyle-related factors. Among these factors, increased serum TG levels on admission had the most marked association with circadian variation, which was characterized by an increased morning risk of AMI onset during weekdays in this subpopulation. Our findings may help to identify the underlying triggers and substrates of AMI onset and help suggest preventive measures of AMI. However, cautions are warranted to interpret our results and confirmation in other cohorts

is required.

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RE and YKS (Yasuhiko Sakata) participated in study concept and design. DN, SS, MU, SM and MH participated in acquisition of data. RE, YKS, SY and TH participated in analysis and interpretation of the data. YKS, TK, HS, SH, YSS (Yasushi Sakata), SY, MH and TH participated in drafting and critical revision of the manuscript for important intellectual content. RE and TH participated in statistical analysis. YKS, HS, SN, MH and IK obtained funding.

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Competing interests

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.

Patient consent

Obtained.

Ethics approval

The study protocol has been approved by the ethics committee of each participating hospital.

Data sharing statement

No additional data available.

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Table 1. Demographics and clinical characteristics of the study population

	N=7755
Patients	
Age (years)	66 (57-74)
Male (%)	5872 (75.7)
Job (%)	3364 (48.2)
BMI (kg/m^2)	23.4 (21.4-25.7)
Cardiovascular risk factors	
Smoker (%)	4865 (63.9)
Drinker (%)	3321 (45.3)
Diabetes (%)	2586 (33.4)
Hypertension (%)	4424 (58.9)
Dyslipidemia (%)	3259 (44.1)
Previous MI (%)	983 (13.0)
Angina pectoris (%)	1737 (23.4)
Multivessel disease (%)	2790 (38.4)
Collateral circulation (%)	2576 (35.7)
Clinical presentation	
Onset admission time<24h(%)	6804 (89.1)
Killip ≥Ⅱ (%)	1331 (18.0)
Initial TIMI ≤Ⅱ(%)	4759 (68.4)
STEMI (%)	6567 (86.0)
Labortory data on admission	
Blood glucose level (mg/dl)	152 (122-209)
HDL cholesterol (mg/dl)	44 (37-53)
LDL cholesterol (mg/dl)	121 (99-147)
Triglycerides (mg/dl)	92 (58-142)
HbA1c (%)	5.9 (5.5-6.9)
Peak CK (IU/l)	2147 (1069-4006)
eGFR (ml/min/1.73 m^2)	64.5 (49.2-80.9)
Localization of MI	
LAD	3050 (41.7)
RCA	2447 (33.4)
LCX	998 (13.6)

LMT	164 (2.2)

BMI = body mass index; MI = myocardial infarction; STEMI = ST-elevation myocardial infarction; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglyceride ; HbA1c = hemoglobin A1c; CK = creatinine phosphokinase; eGFR = estimated glomerular filtration rate; LAD = left anterior descending artery; LCX = left circumflex artery; LMT = left main trunk; RCA = right coronary artery. Categorical variables are presented as number (percentage), and continuous variables are presented as quartile. Laboratory data were measured on admission. Smoker was defined as patients with smoking history, and drinker was defined as active drinker. Number (percentage) of Localization of MI was calculated out of 7319 patients who underwent coronary angiography.

Figure legends

Figure 1. Circadian pattern of AMI onset in the overall population.

A circadian pattern of AMI onset in the overall population was clearly observed in a circular plot (A) and histogram (B). The solid line corresponds to the fitted von Mises distribution, and the dots with error bars are the estimated peak onset times and 95% confidence intervals (CI), respectively.

Figure 2. Circadian pattern of AMI onset according to day of the week.

Circadian patterns of AMI onset based on the day of the week are shown. The estimated peak onset time and 95%CIs are shown below each circular plot.

* P values from the likelihood ratio (LR) test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal.

Figure 3. Circadian pattern of AMI onset based on lifestyle-related factors.

(A) Circular plots of the circadian pattern of AMI onset in the subpopulation with TG levels ≥150 and <150 mg/dl, and the circular plot of the corresponding fitted von Mises distributions for each subgroup are shown. (B)-(M) Circular plots of

the fitted von Mises distributions of each subgroup based on smoking habit, age, drinking habit, BG levels, gender, and working status, LDL levels, HDL levels, Hba1c levels, Hypertension, Diabetes and Dyslipidemia.

* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.

† P values from the hierarchical LR test to examine whether each factor affected the circadian pattern of AMI onset.

Figure 4 . One-year mortality according to the onset time of AMI onset

(A) One-year mortality among the four subgroups based on AMI onset time.

(B) Hazard ratios (HRs) for one-year mortality in the afternoon-onset group versus the other three onset-time groups.

Kaplan-Meier survival curves of one-year mortality among the four AMI onset time subgroups (A). A p value from the log-rank test was used to examine difference in the Kaplan-Meier curves. HR and 95%CI, and p value for the overall population was calculated using univariable Cox regression analysis. The HRs and 95%CIs, and p values for the individual potential confounding variables were calculated using stratified Cox regression analysis, in which the variables

were included into the model as stratification factors (B).

eFigure 1. Circadian pattern of AMI onset according to day of the week in the subpopulation with admission serum TG ≥150 and <150 mg/dl.

Circular plots of the fitted von Mises distributions for the subpopulation with admission serum TG ≥150 and <150mg/dl according to the day of the week are shown.

* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.

eFigure2. Circadian distribution of serum TG levels on admission according to AMI onset time.

Circadian distribution of serum TG levels on admission based on AMI onset time is shown. Red solid line represents the model of circadian distribution of serum TG levels on admission fitted by Cosinor-analysis, and blue dot line represents no circadian change.

* P-value from F-test for the existence of a rhythm (amplitude) to examine whether the amplitude of serum TG levels on admission in patients with AMI had

circadian variation or not.

Association of Lifestyle-related Factors with Circadian Onset Patterns of Acute Myocardial Infarction: a Prospective Observational Study in Japan

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Abstract

Objective: The onset of acute myocardial infarction (AMI) shows characteristic circadian variations involving a definite morning peak and less-defined nighttime peak. However, the factors influencing circadian patterns of AMI onset and their influence on morning and nighttime peaks have not been fully elucidated.

Design, Setting, and Participants: An analysis of patients registered between 1998 and 2008 in the Osaka Acute Coronary Insufficiency Study, which is a prospective, multicenter observational study of AMI patients in the Osaka region of Japan. The present study included 7755 consecutive patients with a known time of AMI onset.

Main Outcomes and Measures: A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI had uniform, unimodal, or bimodal distribution, and the likelihood ratio test was then used to select the best circadian pattern among them. The hierarchical likelihood ratio test was used to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier

method was used to estimate survival curves of one-year mortality according to AMI onset time.

Results: The overall population had a bimodal circadian pattern of AMI onset characterized by a high and sharp morning peak and a lower and less-defined nighttime peak (bimodal: p<0.001). Although several lifestyle-related factors had a statistically significant association with the circadian patterns of AMI onset, serum triglyceride levels had the most prominent association with circadian patterns of AMI onset. Patients with triglyceride ≥150 mg/dl on admission had only one morning peak in the circadian pattern of AMI onset during the weekdays, with no peaks detected on weekends, whereas all other subgroups had two peaks throughout the week.

Conclusions: The circadian pattern of AMI onset was characterized by bimodality. Notably, several lifestyle-related factors, particularly serum triglyceride levels, had a strong relation with the circadian pattern of AMI onset.

Strengths and limitations of this study

- We comprehensively analyzed the circadian patterns of AMI onset in a large, multicenter cohort of patients in relation to patient characteristics, lifestyle factors, and day of the week.
- A mixture of two von Mises distributions revealed that the circadian pattern of AMI onset exhibited bimodality.
- Several lifestyle-related factors were shown to be associated with circadian patterns of AMI onset, depending on the day of the week. In particular, it was demonstrated that elevated serum triglyceride levels on admission accentuated morning peak of AMI onset during the weekdays.
- Subjects were limited to those who were hospitalized for AMI.
- Laboratory data were evaluated on admission.

Introduction

Onset patterns of acute myocardial infarction (AMI) exhibit circadian variation that is characterized by an increased frequency in the morning and a secondary peak incidence at nighttime.[1] Several studies have confirmed that AMI onset exhibits a bimodal circadian pattern, with peaks occurring in the morning hours [2-4] and nighttime hours.[1,4-7] However, it is not well understood what factors, particularly among lifestyle-related factors, influence the circadian patterns of AMI. Moreover, although these patterns appear to vary according to the day of the week,[8] it is unclear how circadian patterns of AMI onset vary throughout the week, particularly, in association with socioeconomic factors.

Because AMI and subsequent ischemic heart failure are the leading causes of death in both developed and developing countries, primary prevention of AMI is a major healthcare issue worldwide. Accordingly, identifying potential factors influencing the circadian pattern of AMI may help in the clinical management of patients for preventing the onset of AMI.

In the present study, we comprehensively analyzed the circadian patterns of AMI onset in a large, multicenter cohort of patients in relation to patient characteristics, lifestyle factors, and day of the week.

Methods

OACIS registry and study subjects

The OACIS is a prospective, multicenter observational study collecting demographic, procedural, biological, and outcome data as well as blood samples from AMI patients hospitalized at 25 collaborating hospitals from the Osaka region of Japan (UMIN-Clinical Trial Registry ID: UMIN000004575).[9-10] A diagnosis of AMI was made if the patient fulfilled at least two of the following three criteria; 1) history of central chest pressure, pain, or tightness lasting 30 min, (2) typical electrocardiographic changes (i.e., ST-segment elevation ≥ 0.1 mV in 1 standard limb lead or 2 precordial leads, ST-segment depression ≥ 0.1 mV in 2 leads, abnormal Q waves, or T-wave inversion in 2 leads), and (3) an increase in serum creatine kinase levels of two times the upper normal limit in each hospital. All the collaborating hospitals were encouraged to enroll consecutive patients with AMI.

We prospectively collected data by research cardiologists and trained research nurses using a specific reporting form, and the following variables were

extracted from the OACIS registry database: age, gender, working status, body mass index (BMI), coronary risk factors (diabetes, hypertension, dyslipidemia, smoking, drinking, previous myocardial infarction, multi-vessel disease, and collateral circulation), clinical presentation on admission (KILLIP classification, initial TIMI flow, and ST-elevation myocardial infarction), coronary angiography data, reperfusion therapy, laboratory data on admission (hemoglobin A1c, total cholesterol, low- and high-density lipoprotein cholesterol, triglyceride, and estimated glomerular filtration rate) and medications at discharge (RAS inhibitors, beta-blocker, calcium channel blocker, statin, anti-platelet agent, and diuretics). Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl, hemoglobin A1c ≥6.5% or a history of anti-diabetic therapy. Hypertension was defined as a history of systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or antihypertensive therapy. Dyslipidemia was defined as fasting total cholesterol ≥220 mg/dl, low-density lipoprotein cholesterol ≥140 mg/dl, high-density lipoprotein cholesterol ≤40 mg/dl, fasting triglycerides ≥150 mg/dl, or lipid-lowering therapy.

The study protocol has been approved by the ethics committee of each participating hospital. All in-hospital data were obtained after written informed

consent and were then transmitted to the data collection center at the Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan for processing and analysis. The corresponding authors had full access and validated to all data in the study.

In the present study, we analyzed 7755 AMI patients whose time of AMI onset was definitely identified among the 8603 consecutive patients registered in the OACIS registry between 1998 and 2008. Patients' baseline characteristics are presented in Table 1.

Statistical analysis

Continuous variables were summarized as quartiles and were compared by the Wilcoxon rank sum test for two-group comparisons, and the Kruskal-Wallis test for four-group comparisons. Categorical variables were presented as number and percentage, and were compared by the chi-square test. A mixture of two von Mises distributions was used to examine whether a circadian pattern of AMI onset had uniform (no peak), unimodal (one peak), or bimodal distribution (two peaks), and the likelihood ratio test was then used to select the best circadian pattern among them.[11] In addition, the hierarchical likelihood ratio test was

assessed to identify factors affecting the circadian patterns of AMI onset. The Kaplan-Meier method was used to estimate survival curves of one-year mortality according to AMI onset time (morning [6:00-11:59 h], afternoon [12:00-17:59 h], evening [18:00-23:59 h], and nighttime [0:00-5:59 h]). The log-rank test was used to compare survival curves between the groups, and the Cox proportional hazards regression model was used to estimate hazard ratios and 95% confidence intervals (CI). To reduce potential confounding effects due to patient background variability in the comparison between the afternoon-onset and other groups, a stratified Cox proportional hazards regression model was used, in which the potential confounding variables were included into the model as stratification factors. Cosinor-analysis was used to estimate the amplitude of serum TG levels on admission according to AMI onset time. Then, F-test for the existence of a rhythm (amplitude) was used to examine whether the amplitude of serum TG levels on admission in patients with AMI had circadian variation or not. Statistical significance was set as $p<0.05$. All statistical analyses were performed using an in-house validated Fortran program or SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Bimodal circadian patterns of AMI onset in the overall population

The daily patterns of AMI onset in our cohort of 7755 patients were first analyzed using the likelihood ratio test (Figure 1). In the overall population, AMI onset clearly exhibited a circadian pattern consisting of two peaks (bimodal: $p<0.001$): a primary peak at 9:01 h (95%CI: 8:53-9:08 h) and a secondary peak at 20:11 h (95%CI: 19:48-20:34 h). The primary peak was more clearly defined than the secondary peak in both the circular and columnar histograms (Figure 1A and 1B, respectively).

Likelihood ratio test analysis revealed that the peak time of AMI onset varied according to the day of the week (Figure 2). For example, the primary peak onset time was earliest on Monday (8:24 h [95%CI: 8:04-8:44 h]) and latest on Sunday (9:44 h [95%CI: 9:22-10:06 h]). On Tuesday, patients exhibited a circadian pattern of AMI onset characterized by late primary (9:28 h [95%CI: 9:06-9:51 h]) and secondary peak onset times (21:13 h [95%CI: 20:40-21:46 h]), whereas earlier peak onset times (8:43 h [95%CI: 8:15-9:10 h], and 19:09 h [95%CI: 18:23-19:55 h]) were detected on Thursday. Notably, the evening peak was higher, and sharper than the morning peak on Saturday (Figure 2).

Factors affecting circadian patterns of AMI onset

Hierarchical likelihood ratio analysis revealed that serum TG levels on admission, smoking, age, drinking, blood glucose levels on admission, gender, and working status had a statistically significant association with the circadian pattern of AMI onset, whereas several other known risk factors for AMI, including HDL- and LDL-cholesterol, Hba1c, hypertension, diabetes and dyslipidemia were not related to the observed patterns (Figure 3, eTable 1).

Among the positively associated factors, serum TG levels on admission had the greatest association with the circadian pattern of AMI onset. Although the likelihood ratio test demonstrated that patients with admission serum TG levels of ≥150 mg/dl (N=1473) had the two characteristic peaks during the day, the peak pattern clearly differed from the other subpopulation groups. In patients with admission serum TG levels of ≥150 mg/d, both peaks occurred in the morning and nearly overlapped (8:18 h and 8:47 h) (Figure 3A). Therefore, the subpopulation with admission TG levels ≥ 150 mg/dl was considered to have a high frequency of AMI onset only in the morning.

The baseline characteristics and laboratory data of patients with serum

TG levels of ≥ 150 and <150 mg/dl on admission are shown in eTable 2. In the subpopulation with higher TG levels, the circadian patterns of AMI onset was characterized by a large, sharp peak in the morning from Monday to Friday, but no peaks were detected on Saturday and Sunday (bimodal: p=0.32 and p=0.133, respectively) (eFigure 1). In contrast, patients with admission serum TG levels of <150 mg/dl (N=5055) had onset peaks that occurred in the morning and evening consistently throughout the week (eFigure 1).

A likelihood ratio test demonstrated that other all subpopulations had two AMI onset peaks during the day: one in the morning and the other in the evening (Figure 3, eTable 1). The subpopulations that were grouped according to smoking habit, age < 65 years old, male gender, and active employment had a circadian pattern of AMI onset with a sharper primary peak and less-defined sharp secondary peak compared to the other subpopulations (Figure 3B,C,F,G, eTable1), although the peak heights were similar between the subpopulations, with the exception of the smokers/nonsmokers subpopulations. The primary AMI onset peak in the subpopulation of smokers was higher than that among nonsmokers, whereas the secondary peaks were similar. Drinkers had a circadian pattern of AMI onset that was characterized by a lower and less sharp

peak in the morning, and a higher, sharper and later peak in the evening (9:00 h [95%CI: 8:48-9:13 h], 20:54 h [95%CI: 20:29-21:20 h]) compared to nondrinkers (9:03 h [95%CI: 8:53-9:14 h], 19:27 h [95%CI: 18:50-20:04 h]) (Figure 3D, eTable1). The subpopulation with admission blood glucose ≥140 mg/dl exhibited a circadian pattern of AMI onset with a higher and sharper primary peak and a less-defined secondary peak compared to the subpopulation of AMI patients with blood glucose <140 mg/dl on admission (Figure 3E).

One-year mortality according to onset time of AMI

One-year mortality was compared among four patient subpopulations that were grouped according to the time range of AMI onset. The baseline characteristics and laboratory data for the four groups are presented in eTable 3. A total of 753 deaths were recorded during a median follow-up period of 365 days. Kaplan-Meier survival analysis demonstrated that the afternoon-onset (12:00-17:59 h) group had worse 1-year mortality than the other 3 groups (log rank test, p=0.032) (Figure 4A). In the subgroup of patients with ST-elevation myocardial infarction (STEMI), the result was similar (log rank test, p=0.007). Univariable Cox regression analysis revealed that the HR of 1-year mortality in

the afternoon-onset group as compared to the other three groups was 1.20 (95%CI 1.02-1.40, p=0.030, Figure 4B). This result did not generally change after stratification with potential confounding factors that showed a different trend between the afternoon-onset group and other three groups (Figure 4B).

Discussion

In the present study, we confirmed that AMI onset exhibits a circadian pattern characterized by bimodality, with a definite morning peak and a less-defined evening peak. Notably, several lifestyle-related factors were associated with variation in the circadian pattern of AMI onset. In particular, serum TG levels on admission for AMI were associated with a unique pattern of AMI onset that is characterized by augmented unimodal peaks on weekday mornings, suggesting that individual lifestyle may affect the onset pattern of AMI.

Bimodal pattern of AMI onset: morning and nighttime peaks

AMI onset in our large patient cohort generally followed a circadian pattern that was characterized by a high and sharp morning peak and a lower and less-defined sharp nighttime peak (Figure 1), a finding that is consistent with the

results of previous investigations.[1-7] Interestingly, the time of two peaks shifted in a synchronous fashion during the weekdays; the secondary peaks generally occurred around 11 to 12 h after the morning peaks on Monday through Friday (Figure 2). For example, AMI onset exhibited early morning and nighttime peaks on Monday and Thursday, whereas that on Tuesday exhibited late morning and nighttime peaks. Although this finding is partly consistent with the observation of Peters et al.,[5] who reported that a secondary peak in AMI onset occurs 11 to 12 h after waking, the present study firstly demonstrated that this synchrony was present on the weekdays, but absent on the weekends.

Several physiological processes are considered to contribute to the bimodal pattern of AMI onset. For example, Stergiou et al.[12] demonstrated that the two-peak diurnal variation in stroke onset occurs in parallel with variation in blood pressure, pulse rate, and physical activity. Thus, the bimodality of blood pressure and heart rate [13-14] is the most likely explanation for the circadian patterns of AMI onset observed in the present study. A greater morning surge of blood pressure and heart rate[13] may explain why the nighttime peak of AMI onset was lower and less-defined than the morning peak. In addition, increased blood viscosity[15] and thrombogenicity due to morning hypercoagulability[16] and

hypofibrinolysis[17] also likely increased the frequency of AMI onset in the morning. It is also possible that external factors, such as physical exertion and mental stress, could be triggers for the morning onset of AMI.[18] In the present study, the younger (<65 years old), working, male, and smoker subpopulations had a sharp morning peak of AMI onset compared with the elderly, nonworking, female, and nonsmoking subpopulations (Figure 3B,C,F,G). The sharpness of the morning peak might be related to increased susceptibility to physical and mental stresses in these subpopulations, in which they are more likely to start activities or go to work soon after waking up. Similarly, the sharp and early morning peak of AMI onset that was detected on Monday may be due to the increased physical and mental stress that is associated with the first morning of the week (Figure 2). We also found that the morning peak occurred latest on Sunday (Figure 2). Together, these findings strongly suggest that mental and physical activity and/or stress may act as a trigger for the morning onset of AMI.

Although many reports have examined the primary peak of AMI onset, relatively little attention has been paid to the secondary peak. We demonstrated that drinkers had a higher, sharper, and later nighttime peak of AMI onset than nondrinkers (Figure 3D). Moreover, the nighttime peak on Saturday was the

highest and sharpest amongst the seven days of the week (Figure 2). This observation may be explained by the fact that people might likely consume alcohol and engage in social activities on Saturday night in Japan. Thus, these evening activities can result in increased sympathetic nerve activity and therefore may have contributed to the increased frequency of AMI onset at night. Taken together, our findings suggest that both the morning and nighttime peaks of AMI onset are influenced by physiological and socioeconomic factors.

Associations of lifestyle-related factors with circadian patterns of AMI onset

Many previous studies on circadian pattern of AMI onset considered gender, age, working status as potential factors affecting the circadian patterns of AMI onset.[1,4-6] Here, we additionally incorporated laboratory data, disease, and other socioeconomic factors into our analyses and found that several lifestyle-related factors, including admission serum TG and blood glucose levels, age, gender, working status, and smoking and drinking habits had statistically significant associations with the circadian pattern of AMI onset. Among these factors, elevated serum TG levels (≥150 mg/dl) on admission had the largest

associations with circadian patterns of AMI onset, while the amplitude of serum TG levels on admission in patients with AMI didn't have circadian variation (p=0.52) (eFigure 2).

There are several evidences to support our findings. First, fasting hypertriglyceridemia and postprandial hyperlipidemia, which is characterized by postprandial accumulation of TG-rich lipoproteins and their partially hydrolyzed products, are both closely related to the development of atherosclerotic cardiovascular diseases.[19-21] Several studies have also reported that elevated serum TG levels are associated with an increased risk of myocardial infarction.[22-23] Hypertriglyceridemia is associated with increased thrombogenecity,[24-25] which is reportedly associated with increased plasminogen activator inhibitor-1 (PAI-1)[26-28] and factor VII coagulant activities,[29-30] and viscosity.[31] These three factors have also been reported to affect the development of MI.[32-34] Moreover, hypertriglyceridemia is also related to endothelium dysfunction,[35-36] which contributes to the pathogenesis of coronary artery disease.[37] In healthy subjects, serum TG levels also exhibit circadian variation with a peak around 3 AM.[38] Thus, it is conceivable that patients with hypertriglycemia have further augmented TG levels and are therefore exposed

to increased thrombogenecity and endothelium dysfunction in the early morning hours before dawn, which may explain the accentuated morning peak of AMI onset in patients with admission TG ≥150 mg/dl. Finally, it is reported that high plasma PAI-1 levels and excessive surges in morning blood pressure are independently and additively associated with increased risk of stroke in older hypertensive patients.[39] Thus, these lines of evidence strongly support our observation of a higher morning risk of AMI onset in the subpopulation with admission hypertriglyceridemia.

Altered circadian patterns of AMI onset in patients with increased TG levels on admission.

To our knowledge, this is the first study to demonstrate an association between admission serum TG levels and circadian patterns of AMI onset, as characterized by a lack of an evening peak in AMI onset in the subgroup of serum TG levels on admission ≥150 mg/dl compared to all other subgroups (Figure 3). While LDL/HDL levels are considered to be closely associated with the development of atherosclerosis, LDL/HDL levels were not associated with onset patterns of AMI in the present study. Although the precise mechanisms for

the altered circadian patterns of AMI onset in patients with increased admission serum TG levels are unclear, increased serum TG might have influenced peripheral clocks residing in various tissues throughout the body, disrupting circadian patterns of AMI onset. Indeed, recent studies have shown that energy metabolism is an important modulator of peripheral circadian clock in cardiovascular tissues.[40-41]

Our subpopulation analyses also revealed that the circadian patterns of AMI onset in patients with admission TG levels of ≥150 mg/dl had a sharp morning peak during weekdays, whereas no such peak was detected on Saturday or Sunday. This observation strongly suggests that increased thrombogenicity and endothelium dysfunction was a factor, but not the trigger, for the morning onset of AMI in our study cohort. Thus, it is conceivable that the accentuated morning peak of AMI onset in patients with admission TG ≥150 mg/dl may be due to the combination of the following three factors: 1) increased hypercoagulability, hypofibrinolysis, viscosity, and endothelium dysfunction resulting from elevated serum TG levels, 2) increased risk of a morning surge of blood pressure and heart rate, and 3) mental and physical stresses.

One-year mortality according to AMI onset time

The association between AMI onset time and mortality is controversial. For example, Manfredini et al.[42] reported that patients with a morning onset of AMI are characterized by higher fatal outcome, independent of site and size of infarction, while Bae et al.[43] reported that patients with an evening-onset AMI had the worst one-year mortality in association with poor baseline clinical characteristics. On the other hand, Holmes et al.[44] observed no significant association between circadian patterns of onset time and in-hospital mortality in patients with ST-elevation myocardial infarction (STEMI) after adjusting for clinical risk factors.

In the present study, patients with an afternoon onset of AMI had the worst 1-year mortality (Figure 4A). However, the baseline clinical characteristics were comparable among the four onset-time groups in our study cohort. Indeed, stratification for potential confounding variables did not generally change the results, suggesting that the increased prognostic risk of AMI in the afternoon-onset group was not simply explained by differences in baseline characteristics in the present study (Figure 4B). Anyway, the patient background and physiological circadian rhythms might complexly interact with each other

and affect mortality after AMI, which could lead to these different results among the studies and difficulty in interpreting the results. Further investigations are required to clarify the association of mortality after AMI and onset time.

Limitations

A few limitations of the present study warrant mention. First, this was an analysis of prospective observational study and the results may have therefore been influenced by potential confounding factors, even after adjustment for baseline clinical and angiographic characteristics. Thus, caution is needed when interpreting the data and making generalizations to other cohorts. Second, the laboratory findings, including serum TG levels, were evaluated on admission. Therefore, we could not exclude the influence of food consumption and circadian variation of several factors, particularly serum TG levels, making interpretation of the data difficult. However, our results also demonstrated that serum TG levels were not likely the final trigger for AMI onset, as patients with TG ≥150 mg/dl on admission did not exhibit a morning peak of AMI onset on the weekend. In patients with hypertriglycemia, hypercoagulability, hypofibrinolysis, viscosity and endothelium dysfunction are generally increased during the early morning hours

before dawn,[26-31,35-36,38] resulting in enhanced susceptibility to AMI onset. Thus, under such conditions, it is conceivable that increased sympathetic activity, which was further enhanced in association with mental, physical, and/or other factors, could be the final trigger for AMI onset on weekday mornings in patients with TG ≥150 mg/dl on admission. Based on these findings, the influence of meal intake and circadian variation of serum TG levels on the morning peak of AMI onset in the population with TG ≥150 mg/dl may be minimal, if not negligible.

Conclusions

In our large cohort of consecutive AMI patients, the circadian pattern of AMI onset exhibited bimodality and was shown to be associated with several lifestyle-related factors. Among these factors, increased serum TG levels on admission had the most marked association with circadian variation, which was characterized by an increased morning risk of AMI onset during weekdays in this subpopulation. Our findings may help to identify the underlying triggers and substrates of AMI onset and help suggest preventive measures of AMI. However, cautions are warranted to interpret our results and confirmation in other cohorts

is required.

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Competing interests

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Patient consent

Obtained.

Ethics approval

The study protocol has been approved by the ethics committee of each participating hospital.

Data sharing statement

No additional data available.

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Table 1. Demographics and clinical characteristics of the study population

	N=7755
Patients	
Age (years)	66 (57-74)
Male (%)	5872 (75.7)
Job (%)	3364 (48.2)
BMI (kg/m^2)	23.4 (21.4-25.7)
Cardiovascular risk factors	
Smoker (%)	4865 (63.9)
Drinker (%)	3321 (45.3)
Diabetes (%)	2586 (33.4)
Hypertension (%)	4424 (58.9)
Dyslipidemia (%)	3259 (44.1)
Previous MI (%)	983 (13.0)
Angina pectoris (%)	1737 (23.4)
Multivessel disease (%)	2790 (38.4)
Collateral circulation (%)	2576 (35.7)
Clinical presentation	
Onset admission time<24h(%)	6804 (89.1)
Killip ≥Ⅱ (%)	1331 (18.0)
Initial TIMI ≤Ⅱ(%)	4759 (68.4)
STEMI (%)	6567 (86.0)
Labortory data on admission	
Blood glucose level (mg/dl)	152 (122-209)
HDL cholesterol (mg/dl)	44 (37-53)
LDL cholesterol (mg/dl)	121 (99-147)
Triglycerides (mg/dl)	92 (58-142)
HbA1c (%)	5.9 (5.5-6.9)
Peak CK (IU/l)	2147 (1069-4006)
eGFR (ml/min/1.73 m^2)	64.5 (49.2-80.9)
Localization of MI	
LAD	3050 (41.7)
RCA	2447 (33.4)
LCX	998 (13.6)

LMT	164 (2.2)

BMI = body mass index; MI = myocardial infarction; STEMI = ST-elevation myocardial infarction; HDL = high density lipoprotein; LDL = low density lipoprotein; TG = triglyceride ; HbA1c = hemoglobin A1c; CK = creatinine phosphokinase; eGFR = estimated glomerular filtration rate; LAD = left anterior descending artery; LCX = left circumflex artery; LMT = left main trunk; RCA = right coronary artery. Categorical variables are presented as number (percentage), and continuous variables are presented as quartile. Laboratory data were measured on admission. Smoker was defined as patients with smoking history, and drinker was defined as active drinker. Number (percentage) of Localization of MI was calculated out of 7319 patients who underwent coronary angiography.

Figure legends

Figure 1. Circadian pattern of AMI onset in the overall population.

A circadian pattern of AMI onset in the overall population was clearly observed in a circular plot (A) and histogram (B). The solid line corresponds to the fitted von Mises distribution, and the dots with error bars are the estimated peak onset times and 95% confidence intervals (CI), respectively.

Figure 2. Circadian pattern of AMI onset according to day of the week.

Circadian patterns of AMI onset based on the day of the week are shown. The estimated peak onset time and 95%CIs are shown below each circular plot.

* P values from the likelihood ratio (LR) test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal.

Figure 3. Circadian pattern of AMI onset based on lifestyle-related factors.

(A) Circular plots of the circadian pattern of AMI onset in the subpopulation with TG levels ≥150 and <150 mg/dl, and the circular plot of the corresponding fitted von Mises distributions for each subgroup are shown. (B)-(M) Circular plots of

the fitted von Mises distributions of each subgroup based on smoking habit, age, drinking habit, BG levels, gender, and working status, LDL levels, HDL levels, Hba1c levels, Hypertension, Diabetes and Dyslipidemia.

* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.

† P values from the hierarchical LR test to examine whether each factor affected the circadian pattern of AMI onset.

Figure 4 . One-year mortality according to the onset time of AMI onset

(A) One-year mortality among the four subgroups based on AMI onset time.

(B) Hazard ratios (HRs) for one-year mortality in the afternoon-onset group versus the other three onset-time groups.

Kaplan-Meier survival curves of one-year mortality among the four AMI onset time subgroups (A). A p value from the log-rank test was used to examine difference in the Kaplan-Meier curves. HR and 95%CI, and p value for the overall population was calculated using univariable Cox regression analysis. The HRs and 95%CIs, and p values for the individual potential confounding variables were calculated using stratified Cox regression analysis, in which the variables

were included into the model as stratification factors (B).

eFigure 1. Circadian pattern of AMI onset according to day of the week in the subpopulation with admission serum TG ≥150 and <150 mg/dl.

Circular plots of the fitted von Mises distributions for the subpopulation with admission serum TG ≥150 and <150mg/dl according to the day of the week are shown.

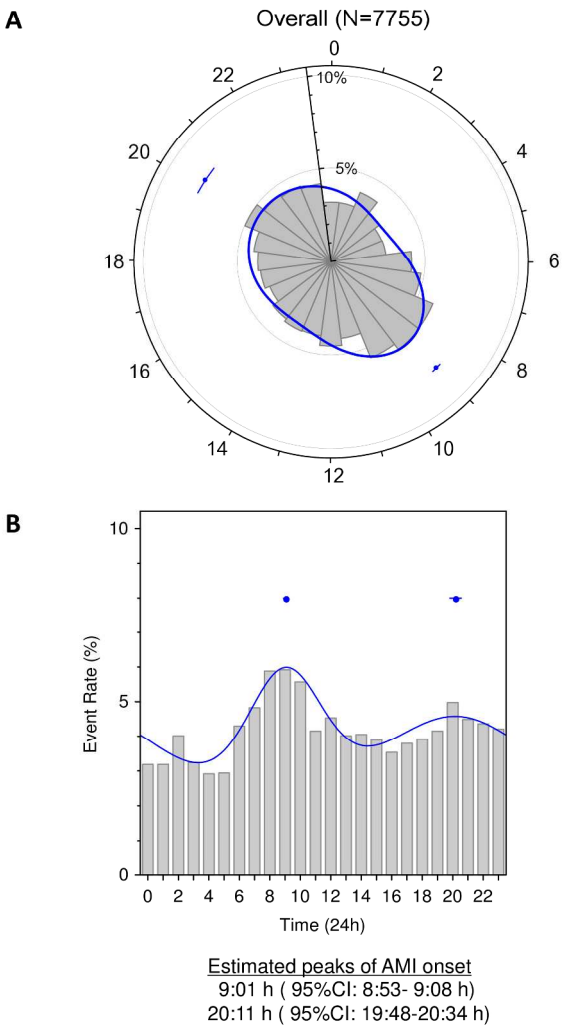
* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.

eFigure2. Circadian distribution of serum TG levels on admission according to AMI onset time.

Circadian distribution of serum TG levels on admission based on AMI onset time is shown. Red solid line represents the model of circadian distribution of serum TG levels on admission fitted by Cosinor-analysis, and blue dot line represents no circadian change.

* P-value from F-test for the existence of a rhythm (amplitude) to examine whether the amplitude of serum TG levels on admission in patients with AMI had

circadian variation or not.



Circadian pattern of AMI onset in the overall population /
A circadian pattern of AMI onset in the overall population was clearly observed in a circular plot (A) and histogram (B). The solid line corresponds to the fitted von Mises distribution, and the dots with error bars are the estimated peak onset times and 95% confidence intervals (CI), respectively.
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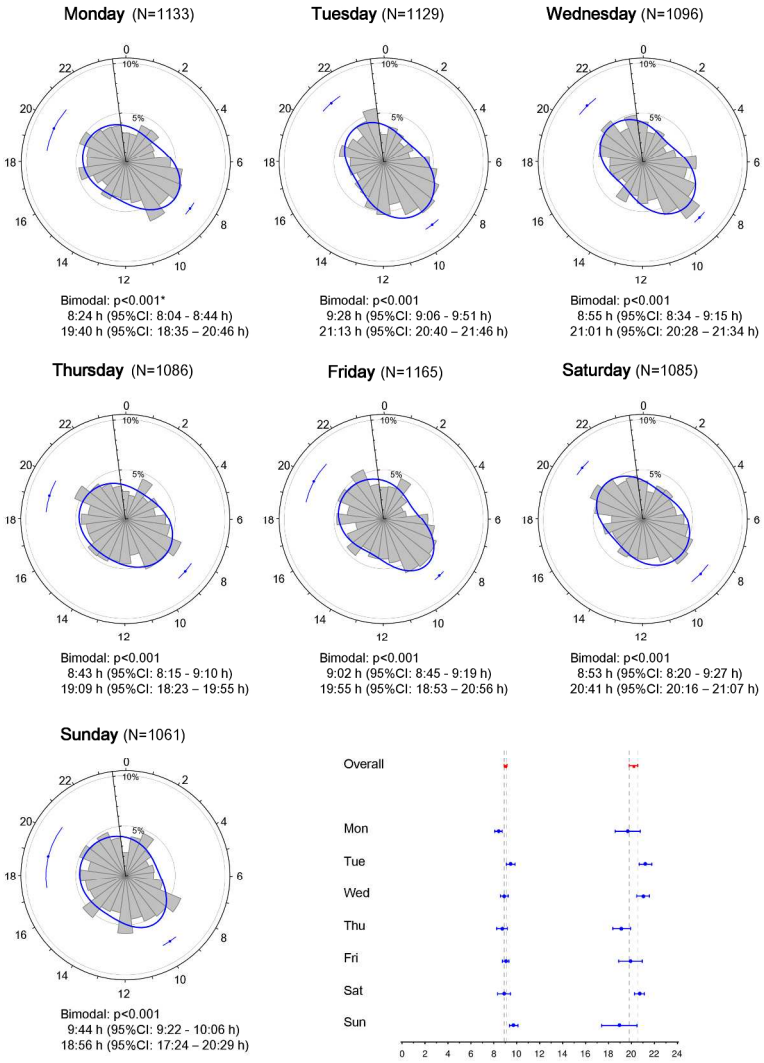

Circadian pattern of AMI onset according to day of the week / Circadian patterns of AMI onset based on the day of the week are shown. The estimated peak onset time and 95%CIs are shown below each circular plot. * P values from the likelihood ratio (LR) test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal.
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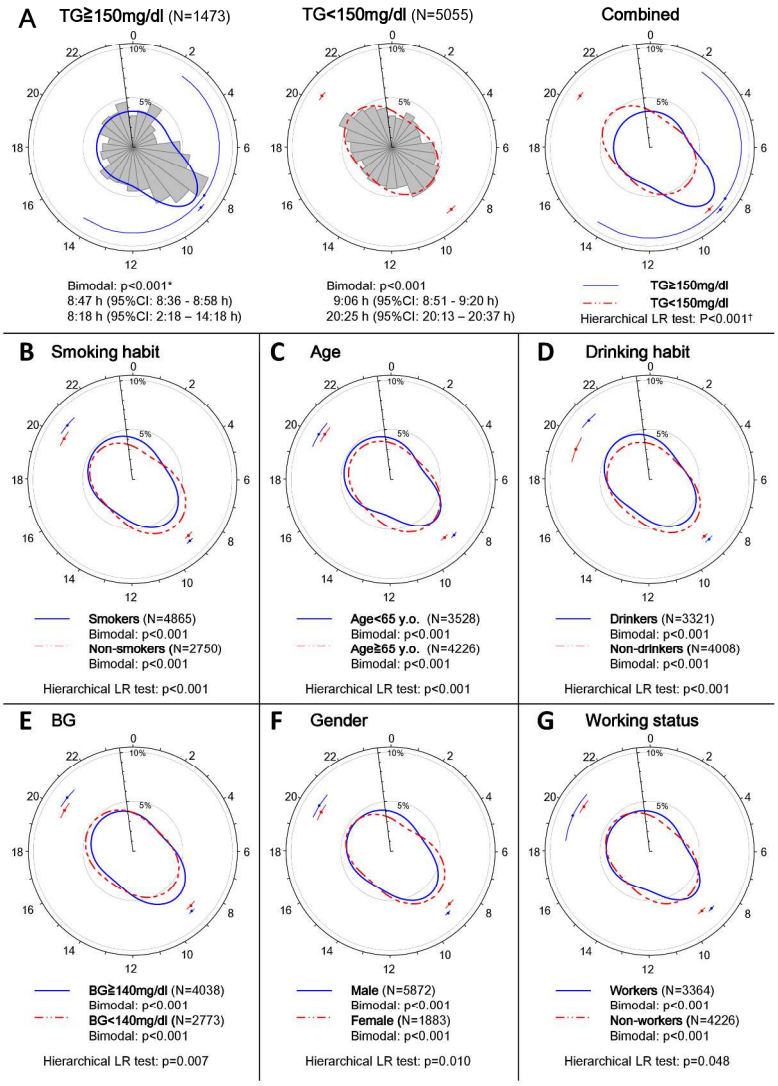

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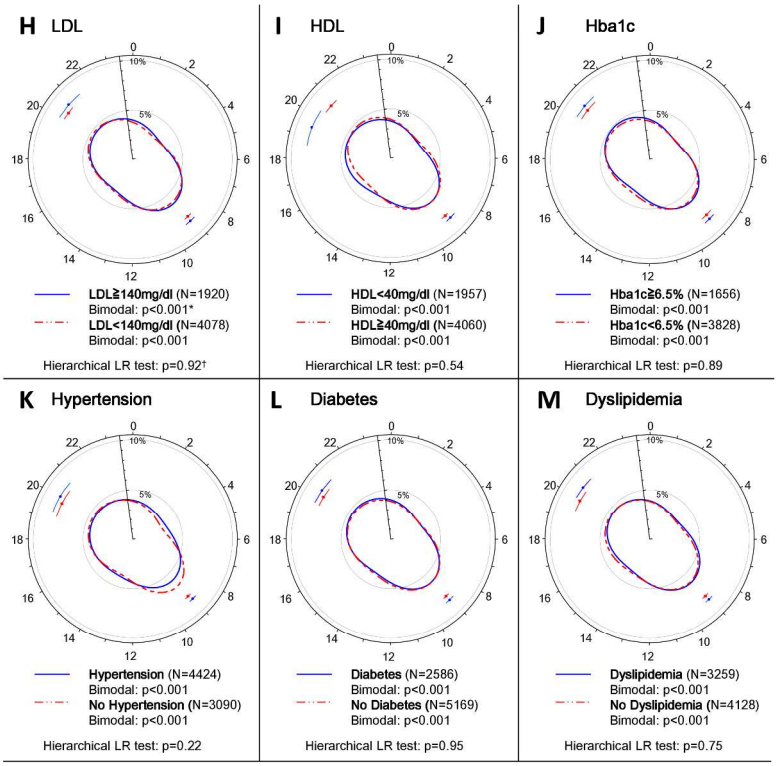

Circadian pattern of AMI onset based on lifestyle-related factors / (A) Circular plots of the circadian pattern of AMI onset in the subpopulation with TG levels ≥150 and <150 mg/dl, and the circular plot of the corresponding fitted von Mises distributions for each subgroup are shown. (B)-(M) Circular plots of the fitted von Mises distributions of each subgroup based on smoking habit, age, drinking habit, BG levels, gender, and working status, LDL levels, HDL levels, Hba1c levels, Hypertension, Diabetes and Dyslipidemia.
* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.
† P values from the hierarchical LR test to examine whether each factor affected the circadian pattern of AMI onset.
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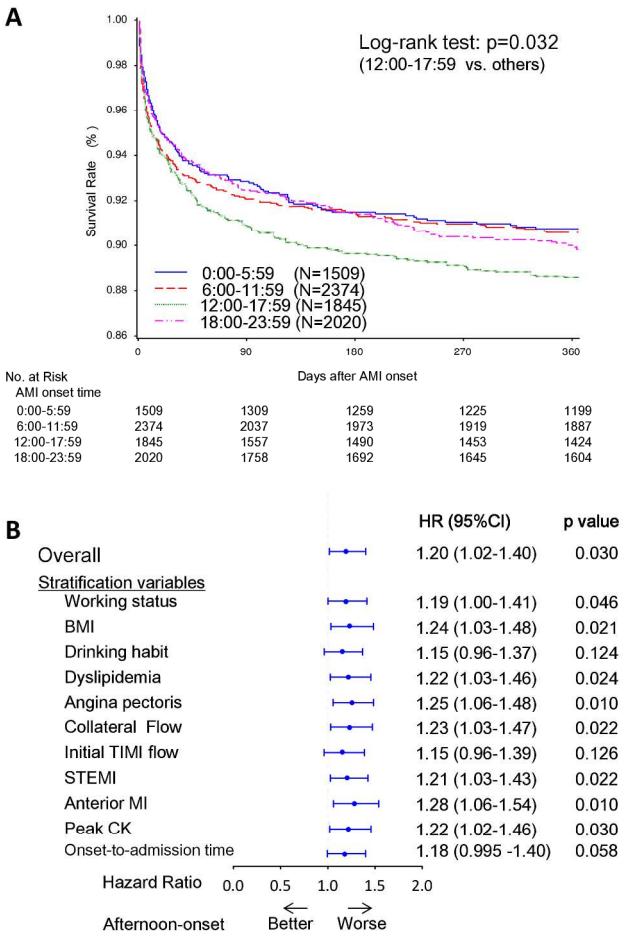

One-year mortality according to the onset time of AMI onset

(A) One-year mortality among the four subgroups based on AMI onset time. (B) Hazard ratios (HRs) for one-year mortality in the afternoon-onset group versus the other three onset-time groups. / Kaplan-Meier survival curves of one-year mortality among the four AMI onset time subgroups (A). A p value from the log-rank test was used to examine difference in the Kaplan-Meier curves. HR and 95%CI, and p value for the overall population was calculated using univariable Cox regression analysis. The HRs and 95%CIs, and p values for the individual potential confounding variables were calculated using stratified Cox regression analysis, in which the variables were included into the model as stratification factors (B).

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eTable 1. Estimated time of peak AMI onset according to subgroup

Subgroup	Estimated time of primary peak onset (95%CI)	Estimated time of second peak onset (95%CI)
TG		
≥150 mg/dl*	8:47 h (8:36 - 8:58 h) 8:18 h (2:18 - 14:18 h)	----------
<150 mg/dl	9:06 h (8:51 - 9:20 h)	20:25 h (20:13 - 20:37 h)
Smoking habit		
Yes	9:07 h (8:57 - 9:18 h)	20:37 h (20:09 - 21:04 h)
No	8:59 h (8:45 - 9:12 h)	20:01 h (19:38 - 20:24 h)
Age		
<65 y.o.	8:45 h (8:36 - 8:55 h)	20:07 h (19:30 - 20:44 h)
≥65 y.o.	9:09 h (8:57 - 9:20 h)	20:16 h (19:52 - 20:40 h)
Drinking habit		
Yes	9:00 h (8:48 - 9:13 h)	20:54 h (20:29 - 21:20 h)
No	9:03 h (8:53 - 9:14 h)	19:27 h (18:50 - 20:04 h)
BG		
≥140 mg/dl	9:03 h (8:53 - 9:12 h)	20:35 h (20:06 - 21:04 h)
<140 mg/dl	8:53 h (8:37 - 9:09 h)	20:03 h (19:38 - 20:27 h)
Gender		
Male	9:09 h (9:00 - 9:17 h)	20:09 h (19:34 - 20:45 h)
Female	8:47 h (8:31 - 9:04 h)	19:56 h (19:31 - 20:21 h)
Working status		
Yes	8:51 h (8:41 - 9:01 h)	19:37 h (18:28 - 20:47 h)
No	9:14 h (9:02 - 9:28 h)	20:15 h (19:55 - 20:36 h)
LDL		
≥ 140mg/dl	9:11 h (8:57 - 9:24 h)	20:36 h (19:39 – 21:34 h)
< 140mg/dl	9:04 h (8:53 - 9:15 h)	20:20 h (19:58 – 20:42 h)
HDL		
< 40mg/dl	8:59 h (8:44 - 9:13 h)	19:26 h (18:35 – 20:17 h)
≥ 40mg/dl	9:07 h (8:56 - 9:18 h)	20:45 h (20:23 – 21:07 h)
HbA1c		
≥6.5%	8:59 h (8:43 - 9:16 h)	20:37 h (20:02 – 21:13 h)
<6.5%	9:03 h (8:51 - 9:14 h)	20:16 h (19:49 – 20:42 h)
Hypertension		
Yes	8:59 h (8:48 - 9:10 h)	20:03 h (19:17 - 20:48 h)

No	9:04 h (8:54 - 9:14 h)	19:48 h (19:06 – 20:30 h)
Diabetes		
Yes	9:03 h (8:50 - 9:16 h)	20:22 h (19:45 – 21:00 h)
No	9:03 h (8:53 - 9:13 h)	20:09 h (19:42 – 20:36 h)
Dyslipidemia		
Yes	9:01 h (8:49 - 9:13 h)	20:31 h (19:59 – 21:02 h)
No	9:02 h (8:52 - 9:13 h)	19:55 h (19:24 – 20:26 h)

The estimated primary and secondary peak onsets and 95%CI of each subgroup are shown.

* In patients with admission serum TG levels of ≥150mg/dl, both peaks occurred in the morning and nearly overlapped. Therefore, this subpopulation was considered to have only morning peak of AMI onset.

eTable 2. Baseline characteristics in patients based on triglyceride levels at admission

	Triglycerides ≥150 mg/dl (N=1473)	Triglycerides <150 mg/dl (N=5055)	p value
Patients			
Age (years)	60 (53-68)	67 (59-75)	<0.001
Male (%)	1233 (83.7)	3725 (73.7)	<0.001
Job (%)	823 (62.3)	2083 (45.3)	<0.001
BMI (kg/m^2)	24.7 (22.6-27.0)	23.1 (21.1-25.3)	<0.001
Cardiovascular risk factors			
Smoker (%)	1058 (72.4)	3028 (60.7)	<0.001
Drinker (%)	710 (50.4)	2104 (43.5)	<0.001
Diabetes (%)	581 (39.4)	1612 (31.9)	<0.001
Hypertension (%)	863 (59.9)	2954 (60.0)	0.90
Dyslipidemia (%)	956 (67.2)	1917 (39.2)	<0.001
Previous MI (%)	176 (12.1)	638 (12.9)	0.45
Angina pectoris (%)	316 (22.0)	1131 (23.1)	0.37
Multivessel disease (%)	500 (35.6)	1910 (39.8)	0.005
Collateral circulation (%)	506 (36.4)	1728 (36.2)	0.92
Clinical presentation			
Onset admission time<24h(%)	1304 (89.0)	4444 (89.1)	0.93
Killip ≥Ⅱ (%)	190 (13.4)	877 (18.1)	<0.001
Initial TIMI ≤Ⅱ(%)	919 (67.7)	3135 (68.1)	0.75
STEMI (%)	1250 (85.6)	4305 (86.1)	0.60
Labortory data on admission			
Blood glucose (mg/dl)	159 (126-220)	148 (121-203)	<0.001
HDL cholesterol (mg/dl)	41 (36-49)	45 (38-54)	<0.001
LDL cholesterol (mg/dl)	130 (104-157)	120 (98-145)	<0.001
Triglycerides (mg/dl)	199 (169-261)	77 (51-105)	<0.001
HbA1c (%)	6.1 (5.6-7.4)	5.9 (5.5-6.8)	<0.001
Peak CK (IU/l)	2097 (1062-3910)	2187 (1083.5-4019.5)	0.28
eGFR (ml/min/1.73 m^2)	65.7 (51.7-80.1)	64.6 (49.3-81.9)	0.21
Localization of MI			
LAD	570 (40.3)	2051 (42.5)	0.153
RCA	512 (36.2)	1601 (33.2)	
LCA	215 (15.2)	652 (13.5)	
LMT	23 (1.6)	102 (2.1)	

Categorical variables are presented as number (percentage), and continuous variables are presented as

quartiles.

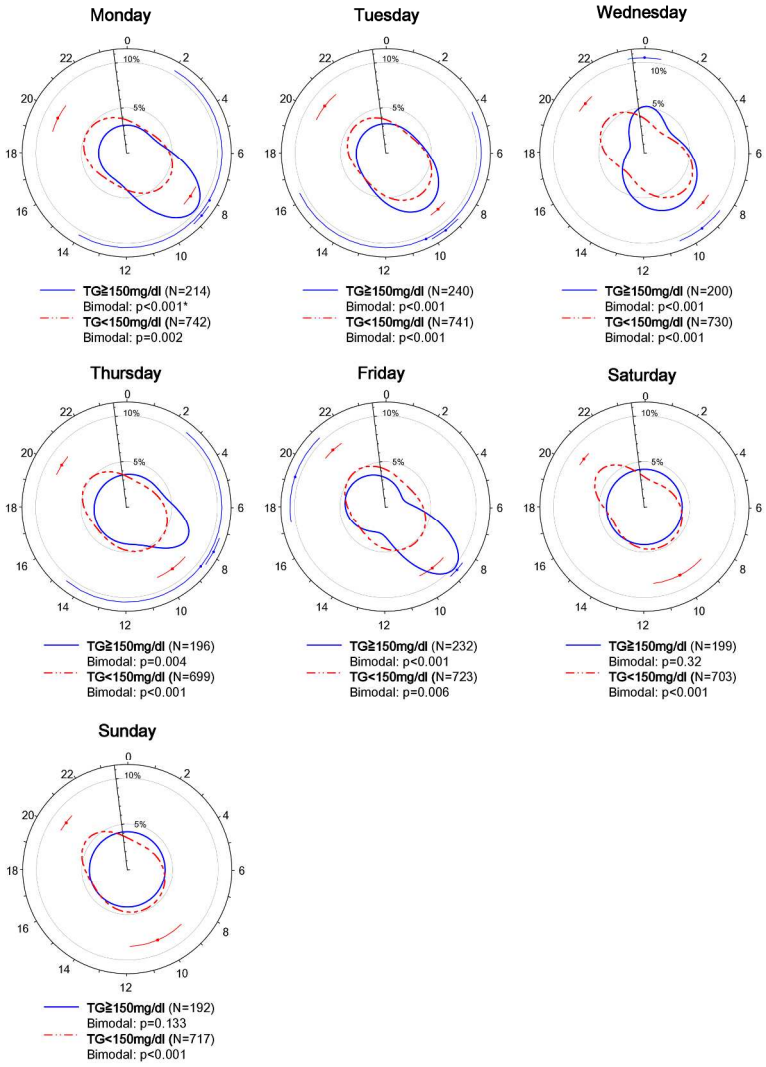
Categorical variables were compared by the chi-square test, and continuous variables were compared by the Wilcoxon rank sum test for two-group comparisons.

eTable 3. Baseline characteristics among groups based on time of AMI onset

	0:00-5:59 h (N=1509)	6:00-11:59 h (N=2374)	12:00-17:59 h (N=1845)	18:00-23:59 h (N=2020)	p value
Patients					
Age (years)	66 (57-74)	66 (58-74)	66 (57-74)	65 (57-73)	0.077
Male (%)	1166 (77.2)	1752 (73.7)	1412 (76.5)	1542 (76.2)	0.050
Employed (%)	656 (48.9)	1023 (47.7)	785 (47.0)	900 (49.2)	0.55
BMI (kg/m^2)	23.4 (21.5-25.8)	23.4 (21.3-25.6)	23.6 (21.5-25.7)	23.4 (21.4-25.7)	0.44
Cardiovascular risk factors					
Smoker (%)	974 (65.6)	1417 (60.8)	1166 (64.3)	1308 (65.8)	0.002
Drinker (%)	687 (47.9)	970 (43.3)	737 (42.4)	927 (48.4)	<0.001
Diabetes (%)	520 (34.4)	784 (33.0)	612 (33.2)	670 (33.1)	0.80
Hypertension (%)	891 (60.4)	1326 (57.8)	1057 (59.1)	1150 (58.8)	0.47
Dyslipidemia (%)	636 (44.3)	1026 (45.3)	757 (43.0)	840 (43.7)	0.50
Previous MI (%)	177 (12.0)	291 (12.6)	230 (12.9)	285 (14.5)	0.140
Angina pectoris (%)	339 (23.4)	521 (22.9)	400 (22.7)	477 (24.7)	0.43
Multivessel disease (%)	533 (37.6)	828 (37.2)	663 (38.6)	766 (40.5)	0.151
Collateral circulation (%)	517 (36.7)	750 (33.8)	636 (37.2)	673 (35.8)	0.129
Clinical presentation					
Onset-to-admission time (hour)	4.7 (1.7 - 10.5)	3.0 (1.3 - 6.7)	2.7 (1.2 - 7.5)	3.0 (1.3 - 14.8)	<0.001
Killip ≥ Ⅱ (%)	276 (19.0)	377 (16.6)	316 (18.0)	362 (18.8)	0.198
Initial TIMI ≤ Ⅱ (%)	906 (66.9)	1457 (68.0)	1186 (71.6)	1210 (67.2)	0.014
STEMI (%)	1258 (84.6)	2024 (86.5)	1588 (87.3)	1697 (85.2)	0.092
Anterior MI (%)	672 (46.9)	987 (43.9)	703 (40.5)	852 (44.9)	0.002
Labortory data on admission					
Blood glucose level (mg/dl)	151 (123 - 206)	155 (124 - 213)	149 (122 - 203)	149 (120 - 210)	0.033
HDL cholesterol (mg/dl)	44 (38-53)	44 (38-53)	44 (37-53)	44 (38-53)	0.42
LDL cholesterol (mg/dl)	123 (101-149)	123 (99-148)	121 (99-146)	119 (97-147)	0.171
Triglycerides (mg/dl)	91(57-141)	100 (63-155)	91.5 (58-140)	87 (54-132)	<0.001
HbA1c (%)	5.9 (5.5 - 7.0)	6.0 (5.5 - 6.9)	5.9 (5.5 - 6.8)	5.9 (5.5 - 7.0)	0.95
Peak CK (IU/l)	2137 (1089-3934.5)	2111 (1027-4144)	2104 (1095-3894)	2274 (1070-3985)	0.92
Treatment					
Reperfusion (%)	1349 (89.6)	2113 (89.2)	1645 (89.4)	1791 (88.8)	0.88
PCI (%)	1316 (87.2)	2068 (87.1)	1601 (86.8)	1749 (86.6)	0.94

Drugs at discharge					
β blockers (%)	677 (44.9)	964 (40.6)	752 (40.8)	836 (41.4)	0.043
RAS inhibitors (%)	1038 (68.8)	1594 (67.1)	1240 (67.2)	1386 (68.6)	0.56
Statin (%)	580 (38.4)	859 (36.2)	611 (33.1)	690 (34.2)	0.007
Diuretics (%)	387 (25.7)	556 (23.4)	416 (22.6)	529 (26.2)	0.024
Ca blockers (%)	335 (22.2)	493 (20.8)	333 (18.1)	398 (19.7)	0.020
Antiplatelet (%)	1359 (90.1)	2124 (89.5)	1624 (88.0)	1814 (89.8)	0.197

Categorical variables are presented as number (percentage), and continuous variables are presented as quartiles. Categorical variables were compared by the chi-square test, and continuous variables were compared by the Kruskal-Wallis test for four-group comparisons.



Circadian pattern of AMI onset according to day of the week in the subpopulation with admission serum TG ≥150 and <150 mg/dl / Circular plots of the fitted von Mises distributions for the subpopulation with admission serum TG ≥150 and <150mg/dl according to the day of the week are shown.
* P values from the LR test to examine whether the circadian pattern of AMI onset was uniform, unimodal, or bimodal in each subgroup.
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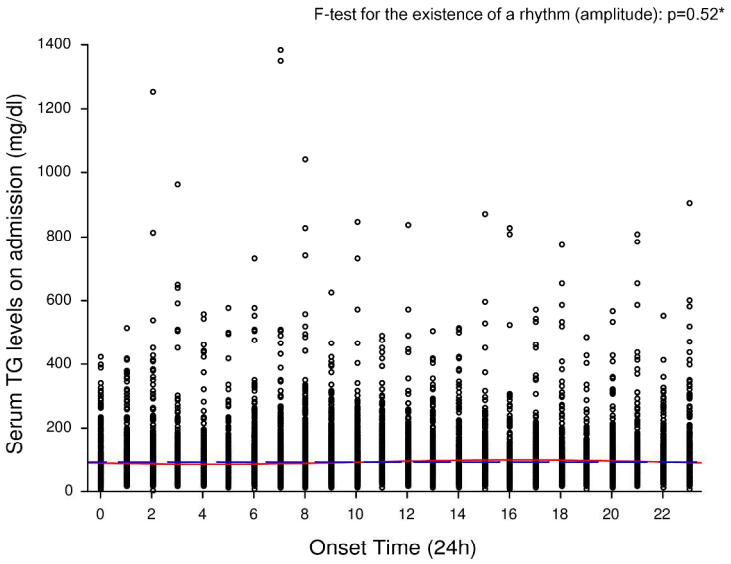

Circadian distribution of serum TG levels on admission according to AMI onset time / Circadian distribution of serum TG levels on admission based on AMI onset time is shown. Red solid line represents the model of circadian distribution of serum TG levels on admission fitted by Cosinor-analysis, and blue dot line represents no circadian change.

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