The plasma levels of free melatonin is not associated with cardiovascular events after acute myocardial infarction

Jingwei Li
Chinese PLA General Hospital https://orcid.org/0000-0001-6746-1889
Qiang Ma
Chinese PLA General Hospital
Zhi-Wei Liu
Chinese PLA General Hospital
Jie Liu
Chinese PLA General Hospital
Shun-Ying Hu
Chinese PLA General Hospital
Jing Jing
Chinese PLA General Hospital
Yun-Dai Chen (cydplagh@sina.com)
https://orcid.org/0000-0002-5853-2865

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Abstract

Background Lower circulating levels of total melatonin is associated with adverse cardiovascular (CV) events in acute myocardial infarction (AMI) patients. Free melatonin is easier to measure in clinical practice compared with total melatonin. Whether free melatonin is associated with follow-up CV events in AMI patients has not been determined yet. Methods A total of 732 consecutive AMI patients treated with percutaneous coronary intervention between January 2013 and January 2015 participated in the study. Blood samples were collected as fast samples on the first morning after admission. The plasma levels of free melatonin were determined using non-extraction radioimmunoassays. The cox regression was used to explore the association between circulating melatonin and endpoints. The median follow-up was 31.6 months. Results Patients with high melatonin levels were more likely to be younger and to have poorer blood lipid control. Multivariate cox-regression analyses (adjusted for confounding variables) showed that one unit increase in log-transformed melatonin was not associated with increased risks of major adverse CV events (MACE, composite of cardiovascular death, myocardial infarction, stroke and heart failure, hazard ratio [HR], 1.74; 95% confidence interval [CI] 0.94 to 3.21; p =0.078). Conclusions Higher free melatonin levels on the onset of AMI is not associated with MACE in AMI patients, independent of established conventional risk factors.

Background

Melatonin is the metabolite of tryptophan, which is an important regulator of the body's internal time-keeping system. It participates in major physiological processes including the sleep wake cycle, pubertal development and seasonal adaptation[1]. Melatonin is mainly synthesized in the pineal gland, while is also produced by retina, gastrointestinal tract, skin, lymphocytes, platelets and bone marrow[2]. Plasma melatonin is mainly produced during the night while keeps at low levels during the day, following circadian rhythm[3]. Melatonin receptors have been identified in the human coronary arteries, aorta and left ventricles[4], and melatonin participates in a variety of cardiovascular (CV) pathophysiological processes including anti-inflammatory, antioxidant, anti-hypertensive and possibly as an antilipidemic function[5].

In the past decade, accumulating evidence has demonstrated that melatonin might serve as a potential prognostic factor in myocardial infarction (MI). Nocturnal total melatonin levels decreased after acute MI (AMI)[6], and lower nocturnal total melatonin concentrations after AMI are associated more left ventricular remodeling[7] and heart failure or cardiac deaths in 6-month follow-up[8]. Moreover in women with increased BMI, lower nocturnal total melatonin secretion is associated with higher risks of MI[9]. Two major method exists to detect melatonin in clinical practice. The nonextraction melatonin method mainly detect free melatonin while the methanol-based extraction method detect both bound and free melatonin[10]. Compared with total melatonin measurement, free melatonin is easier to measure in clinical practice both as it does not need extraction which is hard to chieve in clinical practice and also it require much less sample volumes (100uL vs. 500uL). Whether free melatonin can provide prognostic information in patients with AMI has not been determined yet. The circulating levels of free melatonin

and its relations to clinical characteristics, cardiac injury biomarkers, and adverse cardiovascular events in AMI patients were studied in this research.

Methods

Study population

A total of 732 consecutive patients with diagnosed AMI, consisting of ST-segment elevation (STEMI) and non–ST-segment elevation myocardial infarction (NSTEMI), admitted to Department of Cardiology, People's Liberation Army General Hospital (PLAGH) between January 2013 and January 2015 were included. The study protocol was approved by the local research ethics committee (PLAGH). The definition of AMI is the presence of a positive cardiac troponin T or creatine kinase MB test, typical chest pain lasting at least 20 min, and electrocardiograph changes indicative of ischemia (ST segment elevation or depression). The PCI operation and postoperative medication (including aspirin, clopidogrel, β-blockers, statins, angiotensin-converting-enzyme inhibitors or angiotensin II receptor blockers and so on) were conducted according to the current standard guidelines[11, 12]. Patients with autoimmune diseases, collagen tissue diseases, drug addiction, patients receiving immunosuppressive treatment, taking sedatives, antiepileptic drugs, tricyclic antidepressants or any medication known to influence melatonin metabolism, psychiatric sleeping disorders, shift workers, and subjects with jet-lag syndrome were excluded from this study. Written consents were gotten from all patients. The median follow-up time is 31.6 months. This study was registered on clinicaltrial (clinicaltrials.gov, NCT03230630).

Outcome events and follow-up

Demographic, clinical, and biochemical data were obtained from electronic medical record system. The primary endpoint was major adverse cardiovascular events (MACE) including cardiovascular death, myocardial infarction, stroke and heart failure. Cardiovascular death is defined as death in the presence of ACS, significant cardiac arrhythmia, or refractory congestive heart failure. Hospitalization for heart failure was defined as a hospital readmission for which heart failure was the primary reason. Stroke is defined as persistent central nervous system deficit, usually with confirmatory CT imaging. The endpoints were obtained from outpatient follow-up, in-hospital clinical records of the re-hospitalized patients or by contacting each patient or their relatives individually.

Biochemical measurements

The blood were collected as fast samples in an EDTA-tube between 5:30 a.m. and 06:00 a.m. on the first day after admission. We collect blood samples accompanied with the patient's routine fast morning blood collection. The patients were gently awakened from sleep just before the blood collecting with only

bed lamp on. Lights were turned off at 10:00 p.m. and on at 06:00 a.m. in all the wards. The plasma was frozen at -80°C until further analysis. The plasma melatonin levels were determined using a nonextraction radioimmunoassay (RIA) kit (Cat: BA R-3300, LDN, Germany) on XH6080 Radioimmuno-detector (Xi'an Nuclear Instrument Factory, China) according to the manufacturer's instructions. The detection limit for melatonin was 2.3 pg/mL. Total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, fast plasma glucose (FPG), cardiac troponin T (cTNT), myoglobin, MB isoenzyme of creatine kinase (CK-MB), NT-pro-brain natriuretic peptide (NT-proBNP), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT) and creatinine were measured on autoanalyzer (Cobas C and E system, Roche, Swiss). Haemostatic profile, including thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen were measured on the STA-R Evolution® device (Diagnostica Stago®, Asnières sur Seine, France). We collected the first biochemical test results after admission. All biochemical measurements were performed by investigators who were blinded to patients' characteristics and outcomes.

Echocardiographic measurements

Echocardiography was performed during hospital stay. Transthoracic two-dimensional and M-mode echocardiographic data were obtained. Doppler recordings of mitral inflow were also performed by placing a 2.5 mm sample volume at the tip of the mitral valve leaflets and recording the pulsed wave Doppler signal using VIVID 7 system (GE, USA). Peak velocity of early (E) and atrial (A) diastolic filling were measured and the E/A ratio calculated. Left ventricular ejection fraction (LVEF), left ventricular systolic volumes (LVESV) and left ventricular end-diastolic volumes (LVEDV) were calculated as previously reported[13]. All analyses were performed by an experienced operator blinded to melatonin values and clinical parameters of patients.

Statistical analysis

Analyses were conducted using SPSS 13.0 software (IBM, USA). Patients were divided into tertiles based on the values of plasma melatonin levels. Normality of continuous variables was determined by the Kolmogorov-Smirnov test. Normally distributed continuous variables were presented as mean with standard deviation (SD), variables with skewed distribution as median with interquartile range, and categorical variables as number with percentages. Baseline characteristics were compared across plasma melatonin tertiles using ANOVA test for normal distribution and Kruskal–Wallis for asymmetric distribution and chi-square test for discrete variables. Trend analysis were conducted using trend test from generalized linear model for continuous variables and Cochran-Armitage trend test for discrete variables. The plasma melatonin levels were left-skewed, and therefore, log-transformed (natural logarithms) prior to inference testing.

Hazard ratios (HR) and 95% confidence intervals (CI) for endpoints were calculated using Cox proportional hazard analyses by melatonin tertiles (using the first tertile as reference). For log-transformed melatonin as a continuous variable, the HR (with 95% CI) for endpoints were calculated per unit increment of log melatonin. In addition to the variables that resulted significant ($p < 0.05$) from univariable Cox proportional hazards model using the forward variable selection method, some other factors biologically shown to be associated with death in AMI patients were also included into the multivariable model[14]. Besides presenting a crude model, variables considered for multivariate model 1 included age, sex, for model 2 age, sex, smoking, body mass index, creatinine, triglyceride, peak CK-MB, peak NT-proBNP, GGT, type 2 diabetes and previous myocardial infarction and types of myocardial infarction (STEMI, NSTEMI). All reported *p* values are based on two-tailed tests with <0.05 considered as statistical significance.

Results

Baseline data

The mean age of 732 study participants was 58.7 ± 10.6 years; 20.2% were female. The distribution of plasma melatonin is left-skewed (Supp. Figure 1). The median plasma melatonin levels were 27.47 (interquartile range, 13.82-53.62) pg/ml. There was no significant difference between male and female patients (28.29 (14.18-53.68) pg/ml for the male versus 24.56 (12.53-52.29) pg/ml for the female; P=0.37), between diabetic and non-diabetic patients (24.76 (11.66-49.00) pg/ml for the diabetic versus 28.58 (14.86-54.94) pg/ml for the non-diabetic; P=0.06), between hypertensive and non-hypertensive patients (25.15 (13.83-54.84) pg/ml for the hypertensive versus 28.56 (13.71-50.97) pg/ml for the non-hypertensive; P=0.77) and between STEMI and non-STEMI patients (28.56 (13.92-53.31) pg/ml for STEMI versus 24.56 (12.22-53.68) pg/ml for NSTEMI; P=0.46).

Association of plasma melatonin with and biochemical factors cardiovascular and non-cardiovascular events

Lower age, fibrinogen, higher total cholesterol, triglyceride were more frequently found in patients with higher melatonin levels (All P<0.05). Over a median follow-up of 31.6 month, 46 cases of cardiovascular death, 17 cases of non-cardiovascular death, 11 cases of recurrent MI, 16 cases of stroke and 19 cases of hospitalization for heart failure occurred in this population.

Kaplan-Meier survival analysis was carried out to compare the difference in survival rate in AMI patients according to tertiles of plasma melatonin. The results showed that higher melatonin was not associated with composite CV outcomes (Figure 1). Treating log melatonin as a continuous variable that 1 unit

increase of log melatonin was not associated CV outcomes (HR: 1.74; 95% CI: 0.94-3.21; p =0.078), and patients in the highest tertile of plasma melatonin levels (≥41.50 pg/mL) were not associated with composite CV events (HR: 1.79; 95% CI: 0.99-3.25; p =0.054) compared with patients in the lowest tertile (< 19.03 pg/mL). Subgroup indicate there is a possible interaction between baseline pro-BNP and melatonin as to composite CV outcomes.

Discussion

This study demonstrates that increased plasma free melatonin levels are not associated with cardiovascular outcomes. There exists a possible interaction between pro-BNP and melatonin.

In cell and small animal MI model studies with no comorbidities and comedications, melatonin could attenuate post-MI injury by breaking the cycle of mitochondrial impairment and ROS generation[15]. Melatonin could up-regulate autophagy, decrease apoptosis and modulate mitochondrial integrity and biogenesis thus alleviating post-infarction cardiac remodeling and dysfunction[16]. However it's also reported physiological melatonin concentrations are important in reducing the I/R-induced myocyte damage, while additional pharmacological concentrations did not add to the beneficial effect [17]. In a large animal closed-chest porcine model, melatonin usage immediately prior to reperfusion failed to reduce MI size[18], suggesting complex cardioprotection with melatonin in different MI models[19]. In addition to experimental studies in animal models, a recent clinical randomized trial has found intravenous and intracoronary usage of melatonin is not associated with a reduction in infarct size in STEMI patients and has an unfavourable effect on the ventricular volumes and LVEF evolution[20, 21], however in subgroups of timely treated patients (shorter symptoms onset to balloon time) the administration of melatonin is associated with a significant reduction in the infarct size[22]. In another study, oral melatonin started on the night following primary PCI and continued daily during the hospitalization had conflicting results on enzymatic markers of MI injury following STEMI[23]. These results suggest that melatonin may be effective only in certain kinds of MI or within a specific period of time.

Nocturnal plasma total melatonin levels decreased in AMI patients compared with controls without cardiovascular diseases[6]. The AMI patients who experienced adverse events during 6 month follow-up had significantly lower nocturnal total melatonin levels than patients without events in this study[6]. And a recent published study has reported that lower uric melatonin secretion was significantly associated with a higher risk of MI[9]. In our study both STEMI and non-STEMI patients were included, and diabetic and cancer patients were not excluded, while in the previous study only STEMI patients without diabetes and cancers were included. Moreover the plasma samples were obtained between 5:30 and 6:00 a.m. on the first day after admission in our study while serum samples were collected at 2:00 a.m in theirs. Until now no study has reported the changes of melatonin levels over time in AMI patients.

It should be pointed out that the detection methods is different between our study and previous ones. We used a nonextraction melatonin detection method, while previous studies used methanol-based

extraction method. The nonextraction melatonin method mainly detect free melatonin while the methanol-based extraction method detect both bound and free melatonin[10]. It's reported in amniotic fluid there was no significant difference between melatonin concentration measured by non-extraction versus extraction method in early pregnancy women while in late pregnancy the melatonin concentration was significantly lower when measured by non-extraction method than when by extraction one[24]. Future studies are needed to clarify this by using both two methods at the same time in the AMI population.

Limitations

We only used the non-extraction but not the extraction method to detect plasma melatonin, as mentioned above the results may vary greatly between the two methods. We did not measure the nocturnal levels of melatonin at different times at night, it is possible that at 5:30 a.m., the levels of melatonin do not coincide with its peak. Moreover it's a single center study with a Chinese population, future studies is need to clarify the results in different populations. The study is observational and the authors cannot exclude the possibility of residual confounding.

Conclusion

The current study shows that free circulating melatonin levels is associated with risks of cardiovascular death, and is associated with decreasing risks of non-cardiovascular death in AMI patients. In summary, the current study shows that free circulating melatonin levels is not associated with risks of cardiovascular outcomes in AMI patients.

Abbreviations

AMI: acute myocardial infarction; CV: cardiovascular; LVEF: left ventricular ejection fraction; MACE: major adverse cardiovascular events; NSTEMI: non–ST-segment elevation myocardial infarction; STEMI: ST-segment elevation myocardial infarction.

Declarations

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None.

Authors' contributions

All authors have read and approved the manuscript. JWL was responsible for the conception and design of the study. ZWL and QM analyzed the data. SYH wrote the manuscript. JL contributed to the discussion and reviewed/edited the manuscript. JJ analyzed the data and contributed to the discussion. YDC reviewed/edited the manuscript and gave the final approval for the manuscript.

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Availability of data and materials

All relevant data is in the manuscript. The datasets used are available from the corresponding author upon a reasonable request.

Ethics approval and consent to participate

The study was performed according to good clinical practice and in compliance with the Helsinki declaration. An individual written consent was obtained from each patient. The study was approved by the local Ethics committee (People's Liberation Army General Hospital).

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Patients' characteristics according to plasma free melatonin tertiles

	Total	T1	T2	T3	
pg/ml		<18.03	18.03-41.5	>41.5	*p* trend
N	732	244	246	242	-
Age (y)	58.67±11.54	60.52±12.05	58.04±10.71	57.47±11.63	0.004
Male, n (%)	584（79.8）	185（75.8）	208（84.6）	191（78.9）	0.051
BMI	25.60±3.58	25.36±3.47	25.28±3.29	26.17±3.90	0.012
SBP (mmHg)	127.39±20.52	126.92±21.00	127.57±20.06	127.67±20.57	0.693
DBP (mmHg)	74.43±12.06	73.53±12.14	74.23±11.78	75.52±12.23	0.072
Current smoker, n (%)	319（43.6）	105（43.0）	102（41.5）	112（46.3）	0.472
Ex-smoker, n (%)	90（12.3）	29（11.9）	32（13.0）	29（12.0）	0.973
Hypertension, n (%)	406（55.5）	133（54.5）	141（57.3）	132（54.5）	0.992
Type 2 diabetes, n (%)	246（33.6）	93（38.1）	74（30.1）	79（32.6）	0.201
Previous MI, n (%)	82（11.2）	31（12.7）	31（12.6）	20（8.3）	0.121
STEMI, n (%)	584（79.8）	195（79.9）	192（78.0）	197（81.4）	0.685
Medications, n (%)					
Aspirin	720（98.4）	240（98.4）	243（98.8）	237（97.9）	0.712
ACEI/ARB	654（89.3）	222（91.0）	223（90.7）	209（86.4）	0.099
β-blocker	642（87.7）	219（89.8）	215（87.4）	208（86.0）	0.202
Clopidogrel	710（97.0）	232（95.1）	243（98.8）	235（97.1）	0.189
Nitrate	658（89.9）	215（88.1）	225（91.5）	218（90.1）	0.470
Statin	721（98.5）	239（98.0）	244（99.2）	238（98.3）	0.718

Data are presented as mean ± SD, median (interquartile range) or numbers (percentages). ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; MI, myocardial infarction; SBP, systolic blood pressure; STEMI, ST-elevation myocardial infarction.

Table 2. Baseline laboratory and echocardiographic parameters by plasma free melatonin tertiles and their correlations with plasma melatonin.

	Total	T1	T2	T3	P trend
pg/ml		<18.03	18.03-41.50	>41.50	
FPG (mmol/L)	7.33±3.27	7.59±3.26	7.21±3.50	7.17±3.01	0.169
Lipid profile					
Total cholesterol (mmol/L)	4.12±1.07	4.02±1.03	4.05±1.15	4.31±1.00	0.004
Triglyceride (mmol/L)	1.58±0.88	1.40±0.70	1.44±0.65	1.90±1.12	<0.001
HDL cholesterol (mmol/L)	1.04±0.29	1.06±0.29	1.04±0.28	1.01±0.30	0.100
LDL cholesterol (mmol/L)	2.55±0.91	2.48±0.87	2.51±1.01	2.64±0.82	0.059
Coagulation profile					
TT (sec)	16.40 (15.80-17.43)	16.50 (15.60-17.50)	16.20 (15.80-17.40)	16.50 (15.80-17.48)	0.401
PT (sec)	13.40 (12.90-14.10)	13.60 (13.00-14.20)	13.40 (12.90-13.95)	13.30 (12.70-14.08)	0.103
APTT (sec)	37.60 (34.60-41.18)	37.75 (34.48-41.10)	37.80 (34.68-41.50)	37.40 (34.23-40.85)	
Fibrinogen (g/L)	3.78±1.16	3.69±1.10	3.71±1.10	3.94±1.26	0.026
D-dimer (ug/mL)	0.32 (0.25-0.55)	0.34 (0.26-0.57)	0.32 (0.22-0.46)	0.34 (0.24-0.65)	0.108
Myocardial injury profile					
Peak CK-MB (ng/mL)	8.21 (1.94-90.94)	10.92 (2.12-114.40)	7.45 (1.99-86.05)	5.35 (1.85-81.83)	0.150
Peak cTNT (ng/mL)	0.54 (0.05-3.07)	0.73 (0.05-4.25)	0.55 (0.05-2.54)	0.46 (0.06-2.98)	0.885
Peak Myoglobin (ng/mL)	53.91 (29.88-274.40)	64.90 (31.67-437.60)	50.72 (29.23-191.68)	47.93 (29.41-217.00)	0.410
Peak NT-proBNP (pg/mL)	1016.50 (364.98-2354.25)	1143.00 (495.80-2547.00)	942.15 (355.40-2143.00)	974.00 (332.70-2424.50)	0.353
Hepatic and renal profile					
GGT (U/L)	31.90 (21.38-54.75)	31.00 (19.50-50.33)	29.85 (21.25-47.55)	39.00 (23.78-67.13)	0.066
ALT (U/L)	30.70 (19.80-52.78)	30.80 (19.73-52.43)	29.30 (19.60-52.13)	33.45 (20.25-54.35)	0.425
AST (U/L)	29.10 (18.90-63.20)	30.85 (19.25-67.30)	27.40 (18.30-56.58)	29.10 (19.80-65.60)	0.805
Creatinine (umol/L)	77.80 (68.20-	78.20 (68.25-	77.30 (67.80-	79.10 (68.30-	0.135

	91.10)	91.05)	88.70)	92.70)	
Echocardiography profile					
E/A ratio	0.96±0.47	0.97±0.43	0.94±0.47	0.98±0.52	0.776
LVEF (%)	51.95±8.48	52.34±7.91	51.82±8.28	51.73±9.16	0.478
LVEDV (mL)	93.53±31.18	90.47±28.49	93.54±29.44	96.11±34.83	0.080
LVESV (mL)	56.70±30.20	57.14±30.73	55.91±27.64	57.14±32.35	0.999

Data are presented as mean ± SD or median (interquartile range). A, maximum late transmitral velocity in diastole; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; DBP, diastolic blood pressure; CK-MB,MB isoenzyme of creatine kinase; cTNT, cardiac troponin T; E, maximum early transmitral velocity in diastole; FPG, fasting plasma glucose; GGT, g-glutamyl transferase; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LV, left ventricular; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVESV, LV end-systolic volume; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; PT, prothrombin time; SBP, systolic blood pressure; TT, thrombin time.

Table 3. Multivariable Cox Proportional Hazards Models for predicting composite CV outcomes by tertiles of free melatonin

		Unadjusted		Model 1		Model 2	
	No. of events	HR (95% CI)	p Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Composite CV outcomes							
Low	23/244	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Median	28/246	1.23 (0.71-2.13)	0.465	1.68 (0.96-2.95)	0.070	1.43 (0.76-2.68)	0.266
High	34/242	1.44 (0.85-2.45)	0.177	1.87 (1.10-3.20)	0.021	1.79 (0.99-3.25)	0.054
1 unit increase of log Mel	85/732	1.43 (0.84-2.43)	0.184	1.80 (1.07-3.01)	0.026	1.74 (0.94-3.21)	0.078

CI, confidence interval; HR, hazard ratio;

Model 1 adjusted for age and sex.

Model 2 adjusted for age, sex, smoking, body mass index, creatinine, triglyceride, peak CK-MB, peak NT-proBNP, type 2 diabetes and previous myocardial infarction.

Supplemental Figure Legend

Supp Figure 1. Plasma levels of free melatonin was left-skewed in AMI patients.

Figures

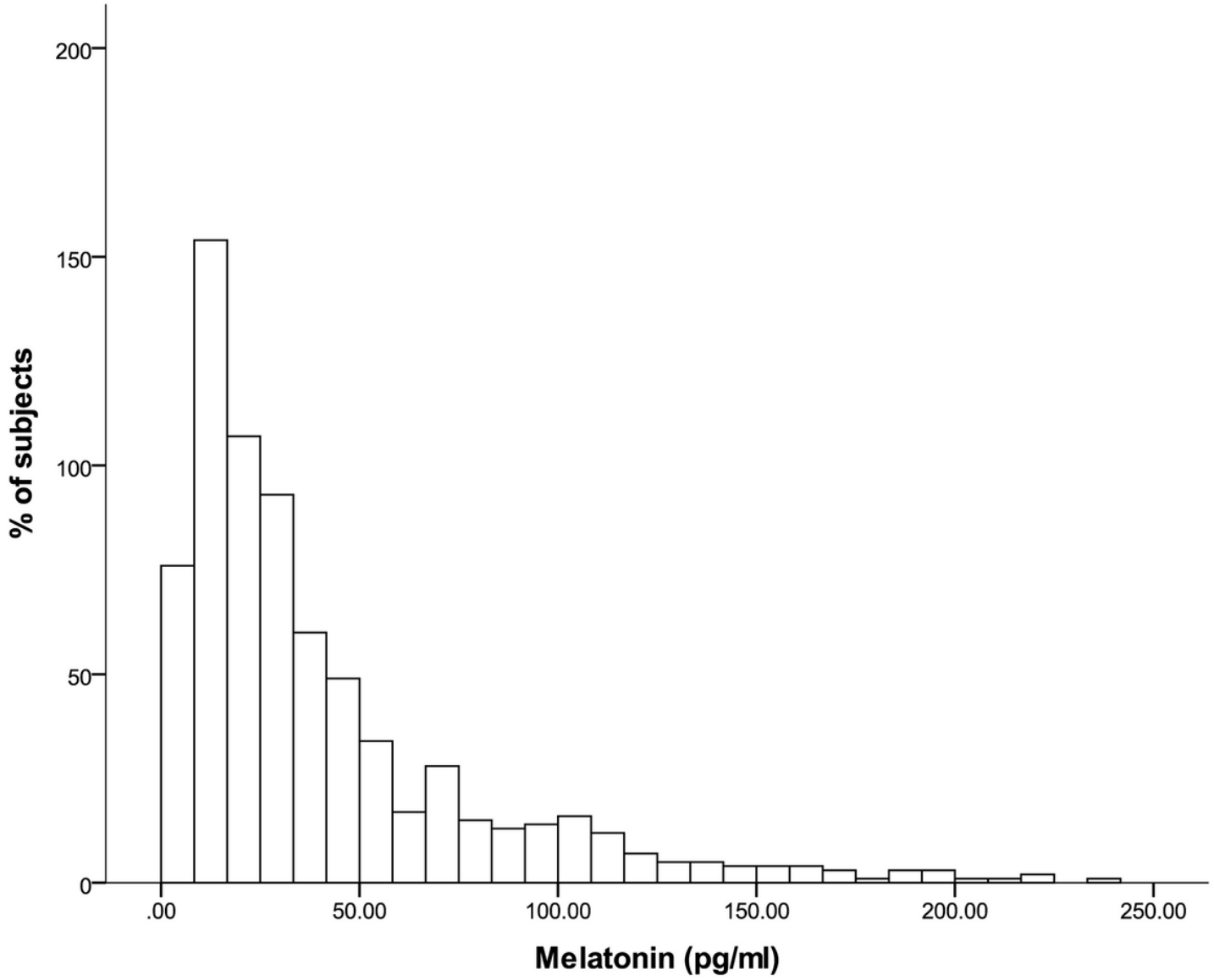

Figure 1

Results of Kaplan–Meier analysis of cumulative event-free rates in AMI patients.

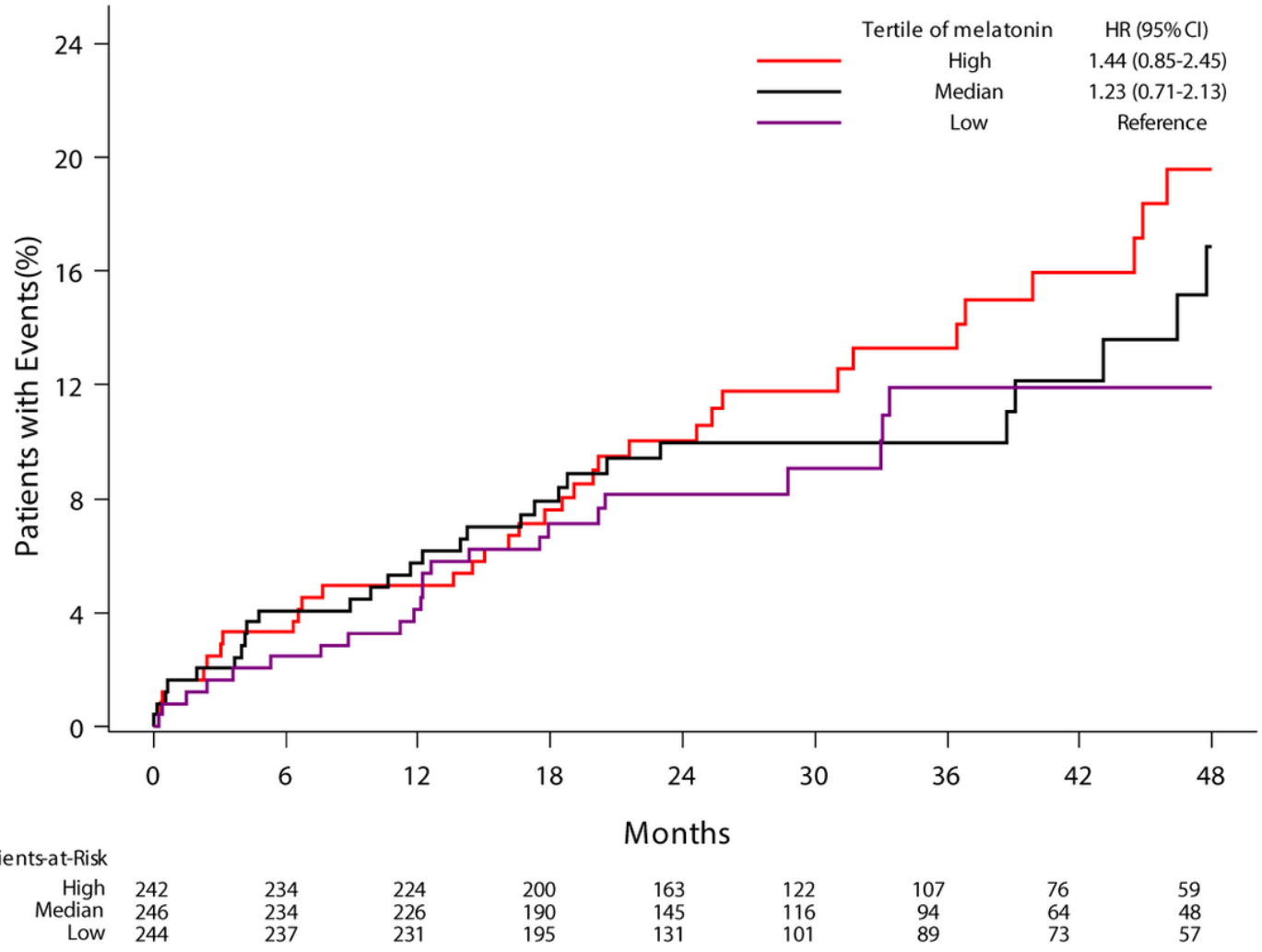

Figure 2

Subgroup analysis of free melatonin and MACE

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppFigure1.jpeg
- STROBEchecklistcohort.docx