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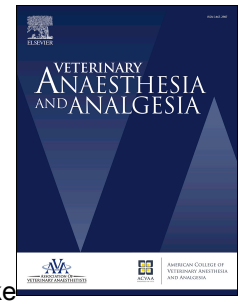

A clinical study to evaluate the cardiopulmonary characteristics of two different anaesthetic protocols (tiletamine/zolazepam +/- medetomidine) and to evaluate their suitability for the immobilisation of healthy chimpanzees

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Title page

'A clinical study to evaluate the cardiopulmonary characteristics of two different anaesthetic protocols (tiletamine/zolazepam +/- medetomidine) and to evaluate their suitability for the immobilisation of healthy chimpanzees'

Victoria Strong[a,b,1], Torsten Möller[c,1], Ann-Sofie Tillman[d], Stefan Träff[d,e], Louise Guevara[c], Mike Martin[f], Sharon Redrobe[b], Kate White[a,2]

a School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK

b Twycross Zoo, Atherstone, UK

c Kolmården Wildlife Park, Kolmården, Sweden

d Diagnostic Centre, Vrinnevi Hospital, Norrköping, Sweden

e Heart Centre, University of Linköping, Sweden

f Willows Referrals, Solihull, UK

Correspondence: Prof. Kate L White, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Loughborough, Leics LE12 5RD, UK.

E-mail: Kate.White@nottingham.ac.uk

Telephone +44 (0)1159516096

Running head: medetomidine and chimpanzee anaesthesia

Authors' contributions:

VS: study conception, design, data collection, analysis and interpretation, preparation of manuscript; TM: study design, data collection and management, preparation of manuscript; KW: study design, data analysis, preparation of manuscript; AST, ST:

data collection and interpretation, manuscript revisions; LG: data collection, manuscript revisions; MM: study design, data interpretation, manuscript revisions; SR: study design, manuscript revisions

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The authors declare no conflict of interest

Abstract

Objective To characterise the cardiopulmonary characteristics of two different anaesthetic protocols (tiletamine/zolazepam +/- medetomidine) and their suitability for the immobilisation of healthy chimpanzees undergoing cardiac assessment.

Study design Prospective, clinical, longitudinal study

Animals Six chimpanzees (*Pan troglodytes*) aged 4 - 16 years weighing 19.5 - 78.5 kg were anaesthetized on two occasions

Methods Anaesthesia was induced with tiletamine/zolazepam (TZ) (3-4 mg kg^{-1}) or tiletamine/zolazepam (2 mg kg^{-1}) and medetomidine (0.02 mg kg^{-1}) (TZM) via blow dart (IM) and maintained with intermittent boluses of ketamine (IV) or zolazepam/tiletamine (IM) as required. The overall quality of the anaesthesia was quantified based on scores given for: quality of induction, degree of muscle relaxation and ease of intubation. The time to achieve a light plane of anaesthesia, number of supplemental boluses needed and recovery characteristics were also recorded. Chimpanzees were continuously monitored and heart rate (HR), pulse rate (PR), respiratory rate (*f*R) oxygen saturation of heamoglobin (SpO_2), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), rectal temperature, mucous membrane colour and capillary refill time recorded. During the first procedure (TZ) animals underwent a 12 channel ECG, hematology, biochemistry and cardiac biomarker assessment to rule out the presence of pre-existing cardiovascular disease. A detailed echocardiographic examination was carried out by the same blinded observer during both procedures. Data were compared using Student's paired t-test or Wilcoxon rank tests as appropriate.

Results There was a significant difference for the area under the curves between anaesthetic protocols for HR, SAP, MAP and *f*R. No significant differences in the echocardiographic

measurements were evident. Quality of anaesthesia was significantly better with TZM and no additional boluses were required. The TZ protocol required multiple supplemental boluses.

Conclusions and clinical relevance Both combinations are suitable for immobilization and cardiovascular evaluation of healthy chimpanzees. Further work is required to evaluate the effect of medetomidine in cardiovascular disease.

Keywords chimpanzee, *Pan troglodytes*, medetomidine, ketamine, tiletamine-zolazepam

Introduction

The topic of cardiovascular disease among chimpanzee (*Pan troglodytes*), gorilla (*Gorilla gorilla gorilla)*, orangutans (*Pongo pygmaeus*) and bonobos (*Pan paniscus*) has featured heavily in the zoological literature in recent years (Strong et al. 2016) being identified as a major cause of mortality across all the great ape taxa. Affected animals often do not display clinical signs, presenting only as cases of sudden death (Lammey et al. 2008). For this reason, pro-active screening is key to identifying affected animals early in the disease process, but the size and dangerous nature of the animals necessitates that cardiac assessments are usually performed under anaesthesia (Cerveny & Sleeman 2014). A small number of (mostly American) zoos are undertaking conscious echocardiographic evaluation in trained animals, but the restrictions of scanning an animal, through mesh or bars for safety, severely restricts the probe angles and positions and thus the diagnostic potential.

The alpha-2-agonist medetomidine, is frequently incorporated with ketamine or tiletamine/zolazepam, because it contributes to rapid safe induction; stable, and reversible immobilization, excellent muscle relaxation and smooth recoveries. Medetomidine binds to alpha-adrenergic receptors in the peripheral vasculature, causing vasoconstriction and an increased systemic vascular resistance. The initial effect is an increase in blood pressure, but the baroreceptor response typically causes slowing of the heart and a reduced cardiac output (Khan et al. 1999). In great apes, medetomidine use remains controversial given anecdotal reports that it impedes meaningful echocardiographic interpretation and, may contribute to increased perioperative mortality in those animals with pre-existing heart disease (Brainard 2016).

The aim of this study was to compare the effects of two different anaesthetic protocols (tiletamine/zolazepam +/- medetomidine) and evaluate their suitability for the immobilisation of healthy chimpanzees undergoing cardiac assessment.

Materials and methods

This work was carried out at Kolmården Wildlife Park, Sweden. Ethical approval was granted by the University of Nottingham's ethics committee (1692 160222).

Animals

Six chimpanzees, two males and four females from a mixed age group (n=25) were studied. They were housed in an indoor/outdoor enclosure with free access to drinking water, a mixed diet of vegetables, fruit, branches and pellets. The animals were being moved to another collection and required two anaesthetics, one for pre-export health assessment and one for transport on the day of export.

The study was carried out as a prospective, clinical, longitudinal study. Food, but not water, was withheld for > 6 hours prior anaesthesia.

Induction of anaesthesia

For the first procedure estimated body weights were used and all chimpanzees were offered midazolam orally followed by IM tiletamine-zolazepam 3-4 mg kg^{-1} (Zoletil 100 Vet; Virbac, France;) (TZ). For the second procedure, 8 weeks later, a combination of IM tiletamine-zolazepam (2 mg kg^{-1}) and medetomidine (0.02 mg kg^{-1}) (Zalopine; Orion Pharma, Finland) (TZM) was used (without oral midazolam). The animals' pre-darting (post-midazolam for TZ individuals that did accept midazolam) activity level and demeanour at induction were scored (ranging from 1 to 4 Appendix A) using a basic descriptive scale. The time taken to achieve a light plane of anaesthesia (defined as recumbency, muscle relaxation, and absence of voluntary movements) after darting was recorded in minutes.

Maintenance of anaesthesia

After induction of anaesthesia, animals were weighed and oxygen was delivered via an endotracheal tube (Teleflex Medical, Ireland), or if tracheal intubation was difficult via nasal tube (Mediplast AB, Sweden). Supplemental doses of ketamine (Ketaminol vet; Intervet, The Netherlands) +/- tiletamine-zolazepam were administered IM/IV as required (Table 1).

Monitoring of anaesthesia

Continuous monitoring commenced once the animals were on the examination table and the following parameters were recorded at 5-minute intervals: respiratory rate (f_R), eye position, palpebral reflex, jaw tone, muscle relaxation, spontaneous movement, heart rate (HR) (via auscultation) and pulse rate (PR) (via palpation of the femoral artery). Arterial oxygen saturation (SpO_2%) was measured using a pulse oximeter (WM 2160; Viamed Ltd, UK) with the probe placed on the lip/tongue. Blood pressures were measured using a non-invasive oscillometric device (BMG 4907; AEG, Germany) with a cuff width approximately 40% of the circumference, placed on the arm. An echocardiographic examination (Vivid q ultrasound system; GE Healthcare, UK) was performed as per guidelines issued for European zoos (available from: https://twycrosszoo.org/wp-content/uploads/2018/05/C2-Standardised-echo-protocol.pdf with the following echocardiographic measurements collected: left atrial diameter (LA), aortic root diameter (Ao), interventricular septum thickness in diastole (IVSd), left ventricular end diastolic diameter (LVIDd), left ventricular end systolic diameter (LVIDs), left ventricular posterior wall thickness (LVPW), ejection fraction (EF; by Simpson's biplane), left ventricular outflow tract diameter (LVOT), peak flow velocity at the pulmonic valve (Vel_{PV}) and aortic valve (Vel_{AV}), tissue (E and A wave) velocities (E' vel., A' vel.). Routine health assessment was performed during both procedures including haematology and biochemistry. During TZ, a 12-channel electrocardiogram (CardioExpress

SL6, Spacelabs Healthcare Ltd., United Kingdom); blood sampling for serum cardiac biomarkers and serological screening and comparative intra-palpebral tuberculin testing was undertaken. Additional medical/surgical interventions were also carried out as required for each animal.

The overall quality of the anaesthetic was quantified based upon the combined scores for: quality of induction (1: excellent to 4: poor); degree of muscle relaxation (1: excellent to 4: poor) and; ease of intubation (1: easy to 4: extremely difficult). The plane of anaesthesia and any supplemental doses was also scored. Descriptions of all scoring criteria used are provided in Appendix A.

At the end of the procedure, animals were moved to their enclosure (TZ) or transport crate (TZM). The medetomidine was antagonised by IM administration of atipamezole (Antisedan vet; Orion Pharma Animal Health, Sweden) at five times the dose of the medetomidine. All animals were positioned in lateral recumbency, administered oxygen and monitored closely throughout recovery.

Statistical Analyses

Statistical tests were performed using GraphPrism 7 (GraphPad Software; CA, USA). The normality assumptions were tested with Shapiro-Wilk test. Parametric data were compared using paired Students t-tests, with Wilcoxon matched pairs test for non-parametric data. Data are presented as mean (± SD) or median (range). Differences were considered statistically significant when $p < 0.05$.

Results

Preanaesthetic aggression and high activity levels (scores 3 - 4) were common for both procedures. All animals were found to be in good overall health. Cardiac auscultation, ECG and cardiac biomarker results were within normal ranges.

Induction and Maintenance of anaesthesia

For the first procedure two of the animals took the complete midazolam dose offered, two animals took 80% and 90% respectively and two chimps refused the dose completely. Oral midazolam was not offered for the second procedure. There was no significant difference in the time taken to achieve a light plane of anaesthesia between the two protocols, however, the quality of anaesthetic induction was significantly better for TZM (p = 0.0312). For TZ, the plane of anaesthesia achieved was scored as being light, whereas for TZM it was rated as surgical. The median number of supplemental doses required during the TZ procedure was 3.5 (2-7); no additional anaesthetic agents were administered during TZM. For one induction (Chimpanzee 2, procedure 1) the dart did not fully discharge. This animal required two further doses of ketamine (105 mg).

Cardiopulmonary parameters

The time to echocardiographic examination from induction of anaesthesia was 40 (23-75) minutes and was not significantly different between groups (p = 0.05).

Data relating to all haemodynamic and respiratory parameters for both anaesthetic protocols are displayed in Table 1. Data were compared (AUC) between protocols over the time course of the anaesthetic. The area under the curve for SAP, MAP, HR, and fR for the TZM anaesthetic protocol was significantly different to the TZ protocol. TZM was associated with significantly lower heart and pulse rates as well as lower systolic and mean arterial blood pressure when compared with the TZ protocol. One period of hypotension (defined as MAP

below 70 mmHg) occurred in one chimp (Chimp 3) during TZM lasting 15 minutes with a nadir of 48 mmHg. Blood pressure measurements obtained with TZ exceeded normal published ranges (Erickson & Olsen 1985). Arterial oxygen saturation was lower for TZM compared to TZ.

Echocardiography

None of the differences in echocardiographic measurements between the two protocols were statistically significant.

Duration

Anaesthetic duration for TZ (75 ± 13 minutes) was not significantly longer than TZM (64 ± 14 minutes).

Anaesthetic recovery

Following TZ, five of the six recoveries were rated as excellent (score 1 out of 4) and one rated as poor (score 3 out of 4). All TZM recovery scores were rated as excellent (score 1 out of 4).

Discussion

TZM was associated with a significantly lower heart rate and mean arterial blood pressure when compared to TZ. Bradycardia and hypotension are well recognised effects of medetomidine in other species. In healthy animals these changes are generally no cause for concern and, in fact, the comparatively higher blood pressures observed following TZ are

more concerning and potentially detrimental (Wongprasartsuk & Sear 2003). They reflect an increase in sympathetic drive/tone; which is a feature of dissociative anaesthesia but might also be expected due to the comparatively lighter plane of anaesthesia. Such increased sympathetic drive has the potential to increase myocardial oxygen demand and increase the risk of cardiac arrhythmias. In contrast, alpha-2-agonists prevent catecholamine-induced arrhythmias (Hayashi et al. 1991). Given that chimpanzee cardiac deaths have been attributed to terminal arrhythmias (Lammey 2008) these factors should be considered.

Echocardiography parameters

In this study, medetomidine in combination with tiletamine/zolazepam was not associated with a significant decrease in cardiac output and function. Echocardiographic parameters for TZM anaesthesia in this study were similar to the those for adult chimpanzees during tiletamine-zolazepam anaesthesia in another study (Sleeper et al. 2014). Specifically, these findings therefore contradict the conclusion drawn by Napier et al. (2013), who compared echocardiographic and blood pressure measurements of seven gorillas during three stages of an anaesthetic protocol and concluded medetomidine caused a significant reduction in ejection fraction and increased left ventricular dimension, thereby recommending caution. Notwithstanding this conclusion, five of the seven gorillas did have evidence of cardiovascular disease and given that the diseased heart is likely to respond differently to anaesthesia compared to a healthy heart these findings may not be widely representative and might explain the differences in conclusions drawn by this and our study. Differences might also be attributable to a different study population (gorillas versus chimpanzees), study design (ketamine and medetomidine) drug dose range (medetomidine 0.05 – 0.07 mg kg^{-1}) in an all-male group (Napier et al. 2013).

Anaesthetic quality

TZM produced a smoother induction and improved muscle relaxation, easier endotracheal intubation and a surgical plane of anaesthesia without supplemental drug administration. Particularly when working with dangerous animals, the anaesthetic plane is an important clinical and safety consideration. With TZ, the chimpanzees exhibited a greater degree of muscle tension and reflex activity prolonging some of the diagnostics. A clinician unfamiliar with TZ anaesthesia might conclude the animal is inadequately anaesthetised and administer unnecessary supplemental doses. Following this study, it was the opinion of the authors that TZ resulted in the immobilization of an animal, suitable for example: for moving it into another enclosure or hospital facility or; in the hands of a confident/experienced anaesthetist, a non-invasive health assessment, but not a surgical plane of anaesthesia.

From the echocardiographer's perspective adequate muscle relaxation also aids image acquisition, and this was subjectively reported to be easier during TZM anaesthesia.

Clinical implications

Whilst several great apes housed in North American zoos have been trained for conscious echocardiographic and/or blood pressure assessment, this is not commonplace and views obtained even in a well-trained animal are still often of limited diagnostic quality.

The Great Ape Heart Project (GAHP; based at Zoo Atlanta, USA) recommend that great apes of unknown cardiovascular disease status and those undergoing cardiac assessment should not be anaesthetised using alpha-2-agonists based on the concern these drugs impede meaningful interpretation of echocardiographic assessment and may be associated with an

increased risk of mortality. This study provides insufficient evidence of such effects to substantiate such advice and therefore no advice relating to the use of specific drugs for great apes undergoing routine cardiac assessment is currently issued by the European vet advisors.

Limitations of the study

The clinical nature of this study meant that there were several variables that could not be controlled. Firstly, induction agents were administered based upon estimated rather than actual bodyweights but good accuracy (mean 5% over/under-estimation) meant this effect was likely minimal. It was also not possible to control the need for additional boli and total drug doses. Furthermore, data recording did not start immediately after drug administration; and there is a possibility of changes such as the initial hypertension following TZM was missed. Although oral midazolam was offered prior to TZ it was taken with variable success, and not offered prior to TZM in view of the poor uptake and limited effects. Oral midazolam has been shown to induce sedation and anxiolysis in humans although a recent Cochrane review found no high-quality evidence to suggest midazolam was more or less effective than placebo or other medications (Conway et al. 2016). Training animals to accept hand injection rather than being darted can significantly reduce stress at the time of induction of anaesthesia.

Conclusions

Both TZ and TZM combinations are suitable for immobilization and cardiovascular evaluation of healthy chimpanzees, although TZM offers additional benefits. Further work is required to evaluate the effects of medetomidine in animals with cardiovascular disease.

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Appendix A: Table showing scoring system used for rating anaesthetic characteristics

Characteristic	Score	Description
Pre-anaesthetic activity level	**1**	None; very still
	2	Low; small amount of activity, e.g. moving to elsewhere in enclosure to avoid aim of dart gun
	3	Moderate; moving around enclosure to numerous locations prior to the successful administration of the induction agent
	4	High; constant moving around enclosure to numerous locations, resulting in significant prolongation of the time required to administer induction agent
Pre-anaesthetic demeanour	**1**	Depressed; collapsed, reduced or lack of awareness of external stimuli, e.g. sick or sedated animal
	2	Alert; aware of stimuli but reduced response compared with anticipated level of response, relaxed
	3	Apprehensive; responsive to stimuli, taking avoiding action e.g. hiding in corner of enclosure or behind another individual
	4	Aggressive; hyper-responsive to stimuli, displaying violent activity e.g. to the veterinarian administering induction agent
Quality of induction	***1***	Excellent; rapid, calm and smooth induction, recumbency and adequate and safe level of anaesthesia achieved
	2	Good; slightly prolonged but smooth induction, recumbency and adequate and safe level of anaesthesia achieved but some movement/response to stimulus remains e.g. able to be safely moved from enclosure but requires additional anaesthesia for intubation
	3	Fair; recumbency but not adequate/safe level of anaesthesia achieved, still has sluggish responses to stimuli and requires additional anaesthetic agents to be administered before can be safely handled
	4	Poor; inadequate sedative effect achieved, very responsive to stimuli, requires administration of additional anaesthetic agents before observers can enter enclosure
Degree of muscle relaxation	***1***	Excellent; trunk and limb muscle relaxed, no muscle tone or twitching
	2	Good; muscles relaxed, occasional mild muscle twitching in small focal regions of body, e.g. eyes, lips, hands, feet
	3	Fair; small amount of muscle tone, sustained or repeated muscle twitching of larger muscle bodies, e.g. whole limb movement
	4	Poor; muscles rigid, severe muscle twitching of multiple large muscle groups e.g. trunk and/or limbs
Depth of anaesthesia	***1***	Mild sedation; slight sedation, still able to move around without ataxia but at slower speed than usual
	2	Heavy sedation; responds to stimuli e.g. sound/touch with purposeful movements, safe level of anaesthesia for handling of e.g. non-dangerous animals
	3	Light anaesthesia; safe level of anaesthesia for handling dangerous animals, non-responsive to stimuli e.g. sound/touch, responsive to painful stimuli, most reflexes still present
	4	Surgical anaesthesia; non-responsive to painful stimuli, appropriate level of anaesthesia for e.g. surgical intervention, most reflexes are absent
	5	Excessively deep anaesthesia; excessive depression of central nervous system results in reduced ability to maintain normal functions e.g. respiration, animal at risk of anaesthetic death
Ease of intubation	***1***	Easy; coughing/gagging absent or minimal
	2	Moderate; small to moderate amount of reflex coughing/gagging but intubation achieved without need for additional (top-up) anaesthesia
	3	Difficult; pronounced coughing, intubation requires additional anaesthetic agents to be administered
	4	Extremely difficult; severe swallowing, coughing or gagging, intubation requires additional anaesthetic agents to be administered or is unsuccessful
Anaesthetic recovery	***1***	Excellent; smooth, non-vocal without paddling or uncoordinated movement
	2	Good; some minor paddling/excitation of short duration but no vocalisation or uncoordinated movement
	3	Fair; some vocalisation, paddling or uncoordinated movement but of short duration and easily calmed
	4	Poor; vocalisation, paddling or uncoordinated movement of moderate to severe duration and intensity

Table 1 Characteristics of and measured data from six chimpanzees, anaesthetized on two occasions with tiletamine and zolazepam (TZ) (3 - 4 mg kg^{-1}), or zolazepam (2 mg kg^{-1}) and medetomidine (0.02 mg kg^{-1}) (TZM). Atipamezole was administered at the end of the TZM procedure.

Parameter	**Chimp 1**		**Chimp 2**		**Chimp 3**		**Chimp 4**		**Chimp 5**		**Chimp 6**		**ALL**	
Sex	M		F		M		F		F		F		-	
Age (years)	16		15		5		16		14		4		15 (4-16)	
Induction protocol	TZ	TZM	TZ	TZM	TZ	TZM	TZ	TZM	TZ	TZM	TZ	TZM	TZ	TZM
Time to light anaesthesia (minutes)	1.5	5	21	4	4	5	9	5	4	4	5	3	4.5	4.3
Duration of anaesthesia[1] (minutes)	64	77	88	48	83	53	90	89	67	59	57	58	75	64
Number of supplemental doses [2]	3	0	2	0	7	0	5	0	3	0	4	0	4	0
Total mg of supplemental drugs (K or TZ) [2]	140K	0	55K	0	175K	0	280K,60TZ	0	3150K	0	80K,20TZ	0	-	0
HR (beats $minute^{-1}$)	92±12	57±4	79±11	67±6	100±11	59±5	79±3	64±3	90±3	79±8	80±8	68±9	87±3	62±6*
SAP (mmHg)	171±15	136±24	121±19	121±22	-	98±16	141±25	106±18	155±24	134±12	-	109±19	150±23	116±22*
DAP (mmHg)	104±15	79±16	91±19	78±14	-	55±15	86±2	74±5	82±9	69±4	-	64±12	92±15	69±15
MAP (mmHg)	128±14	98±10	101±16	91±5	-	69±15	104±3	85±3	106±6	91±4	-	79±13	111±10	85±11**
SpO_2 (%)	98±3	-	98±2	96±5	-	97±6	98±1	93±4	97±1	89±5	-	-	98±1	93±4****
f_R (breaths $minute^{-1}$)	15	13	13	15	23	13	17	18	17	16	29	26	20	16*

A_O (mm)	21	23	20	22	17	17	22	27	22	22	17	18	20	22
LA (mm)	35	37	33	25	23	23	37	35	30	35	22	25	30	30
IVSd (mm)	9	11	8	8	-	6	9	10	8	11	7	-	8	9
LVIDd (mm)	49	48	41	42	-	31	46	44	42	38	40	-	44	41
LVIDs (mm)	28	30	29	29	-	20	29	30	26	25	26	-	28	27
LVPW (mm)	8	10	7	6	-	6	9	9	8	6	6	-	8	6
EF (%)	55	55	60	50	50	64	55	-	58	55	60	54	56	56

[1]Duration of anaesthesia: from administration of induction agent to spontaneous waking (TZ) or reversal (TZM) was purposefully manipulated for logistic/safety reasons

[2]Supplemental boluses of ketamine (K) or tiletamine-zolazepam (TZ) or both were administered at the discretion of the same anaesthetist to improve the plane of anaesthesia.

HR, f_R, NIBP and SpO_2 are given as mean ± SD, with age given as median (range) and these were calculated from all measurements taken during anaesthesia.

HR, heart rate; f_R, respiratory rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; SpO_2, oxygen saturation of haemoglobin; Ao, aortic root diameter; LA, left atrial diameter; IVSd, interventricular septum thickness in diastole; LVIDd left ventricular end diastolic diameter; LVIDs, left ventricular end systolic diameter; LVPW, left ventricular posterior wall thickness; EF, ejection fraction.

** $p \leq 0.05$** $p \leq 0.01$; **** $p \leq 0.0001$ denotes statistical differences between the area under the curves of TZ and TZM groups*