EuroIntervention 2020;16:e518-e522 published online June 2019 published online e-edition August 2020

A novel percutaneous closed chest swine model of ischaemic mitral regurgitation guided by contrast echocardiography

Ouafa Hamza, MD; Attila Kiss, PhD; Anne-Margarethe Kramer, MSc; Katharina Elisabeth Tillmann, MSc; Bruno K. Podesser*, MD

Ludwig Boltzmann Cluster for Cardiovascular Research at the Centre for Biomedical Research, Medical University of Vienna, Vienna, Austria

This paper also includes supplementary data published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-19-00095

Introduction

Alternative approaches for mitral valve repair are constantly being developed. Even though the safety of these devices has been extensively assessed in healthy animals before approval for first-in-man studies, often the efficacy of these new devices has hardly been challenged *in vivo* in the setting of mitral regurgitation (MR). Therefore, reliable and reproducible preclinical animal models of MR are crucial to allow the safety assessment and efficacy evaluation of these novel approaches.

Material and methods

Eight young female Landrace domestic pigs (60±12 kg) were used. A control group with age, sex and weight-matched animals was constituted after the six-week follow-up period (n=7). The experimental protocol was approved by the local authority ethics committee.

To obtain an ischaemic MR, a localised posteromedial papillary muscle (PMPM) myocardial infarction (MI) involving the underlying inferior wall was achieved. Contrast echocardiography was used as a PMPM vascularisation mapping tool. The obtuse marginal (OM) branches irrigating the PMPM were identified by selectively injecting regular radiographic contrast agent through an over-the-wire (OTW) balloon. Echocardiography allowed detection of any contrast enhancement of the area of interest. To achieve MI, 1 to 2 ml of pure ethanol was slowly injected into each target branch through the OTW balloon after inflation. The balloon was left in place for 15 minutes, then deflated **(Figure 1)**.

Six weeks post MI, the animals were sacrificed and the hearts harvested. Mitral valve diameters were measured and compared to data from human patients from the literature[1–5].

Results

All the animals survived the acute phase. One animal was euthanised at two weeks after an acute pulmonary oedema.

Table 1 summarises the echocardiographic findings. A semi-quantitative method was used to evaluate and monitor MR severity and evolution. A moderate ischaemic MR was obtained at the end of the follow-up period. Parallel to MR development,

**Corresponding author: Ludwig Boltzmann Cluster for Cardiovascular Research at the Centre for Biomedical Research, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria. E-mail: bruno.podesser@meduniwien.ac.at*

SUBMITTED ON 25/01/2019 - REVISION RECEIVED ON 15/05/2019 - ACCEPTED ON 20/06/2019

DOI: 10.4244/EIJ-D-19-00095

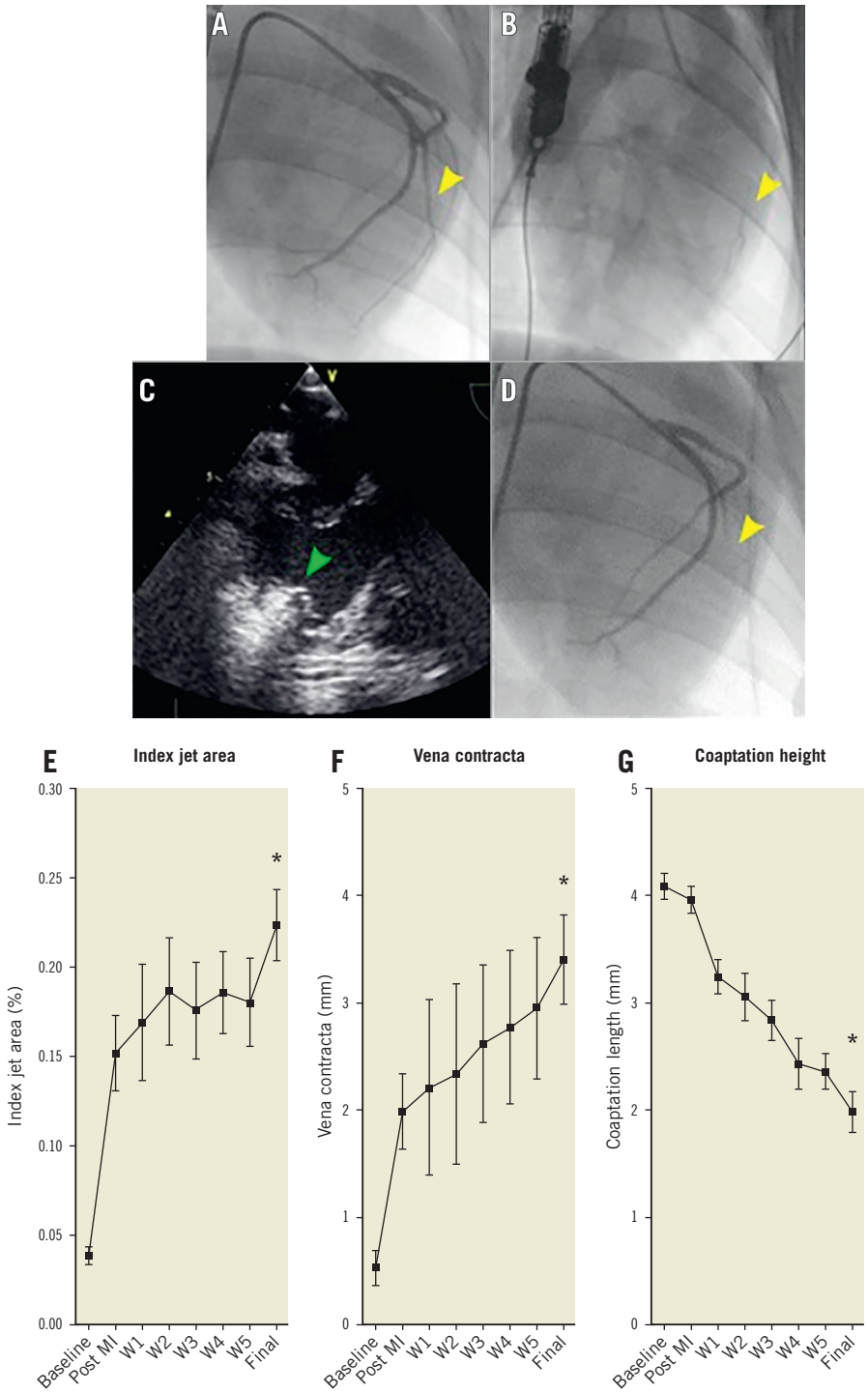

Figure 1. *Ischaemic MR induction procedure supported by contrast echocardiography and its evolution. Representative baseline LCA angiogram (A). Injection of contrast media in the obtuse marginal (OM) branch (yellow arrow) through an OTW balloon (B) and corresponding contrast enhancement on the echocardiography (green arrow) (C). Result after injection of ethanol (D). Temporal evolution of index jet area (E), vena contracta (F) and coaptation height (G). * p<0.001 ANOVA.*

valve tethering evolved progressively as reflected by the significantly increased tenting height and area concordant with an ischaemic MR **(Figure 2)**.

As expected, the echo values of the mitral annulus diameter were slightly higher than the ones obtained *post mortem* and showed a range of values close to the human data **(Table 2)**. Post-mortem measurement of the anterior leaflet in the pigs showed similar lengths to those of human patients, as opposed to the posterior leaflet which was found to have a longer length in pigs.

Table 1. Comparison of echocardiographic parameters between baseline, final examination at six weeks and published closed (W. Shi et al[6]) and open (X. Zeng et al[7]) chest model.

	Baseline	6 weeks	*p*-value	W. Shi et al[6]	X. Zeng et al[7]
EF, %	63±3	47±3	<0.001	33.2±2.2	43.8±3.4
EDD, mm	50.31±4.58	62.12±3.93	<0.001	–	–
ESD, mm	29.86±2.96	42.53±5.78	<0.001	–	–
EDV, ml	84.71±8.53	209.71±7.68	<0.001	159±39	72.7±13.9
ESV, ml	38.14±8.65	95.42±21.61	<0.001	99±24	41.0±9.3
LAS, cm^2	7.46±0.48	14.87±1.5	<0.001	8.74±3.21	–
LAV, ml	14.57±1.05	58±16.86	<0.001	–	–
Jet surface indexed to LA surface, %	3.85±1.21	23.9±2.74	<0.001	27±3	–
Vena contracta, mm	0.53±0.15	3.49±0.27	<0.001	–	5.6±1
Tenting area, cm^2	0.71±0.09	2.63±0.7	<0.001	3.2±0.4	–
Tenting height, mm	3.27±0.45	12.03±3.18	<0.001	–	–

Values are expressed as mean±SD. EDD: end-diastolic diameter; EF: ejection fraction; ESD: end-systolic diameter; LAS: left atrium surface; LAV: left atrium volume

Discussion (Supplementary Appendix 1)

We established for the first time a closed chest pig model of ischaemic MR guided by contrast echocardiography. Echo mapping of the PMPM vasculature allowed achieving a controlled and reproducible MI of the PMPM with consistent development of a significant MR in all animals.

Our model did not rely exclusively on fluoroscopic guidance while identifying the irrigating branches as in the previous ethanol injection model described by W. Shi et al[6]. Intraoperative contrast echocardiography support allowed more reliable identification of target branches.

Pig hearts are widely accepted for preclinical studies given their close similarities to human hearts. In our model, mitral annular diameter values were in a range close to the values measured in MR patients. Therefore, this model can easily accommodate mitral devices designed for humans.

Limitations

Our study was limited by the growth rate of the animals and resulted in a short follow-up period which did not allow the development of a higher MR grade. A longer follow-up period might be necessary to observe a more severe MR.

Table 2. Comparison between mitral valve dimensions in pigs after six weeks of MR (post-mortem measurements and echocardiographic data in bold) and patients with MR (values from the literature[1-5]).

Author	Year	Subjects	n	Intercommissural diameter, mm	Anteroposterior diameter, mm	Mitral valve anterior leaflet length (A2), mm	Mitral valve posterior leaflet length (P2), mm	Mitral annulus circumference, mm
K. Owais et al[1]	2016	Ischaemic MR	36	42.2±9.4	29.6±7.9	–	–	–
S. Kovalova et al[2]	2011	Ischaemic MR	35	40.31±7.38	33.44±8.14	–	–	125.2±22.68
M. Shanks et al[3]	2010	Pre TAVR with MR	43	39.7±4.9	31.6±4.4	24.1±3.9	12.2±3.2	125.1±10.6
R. De Simone et al[4]	2006	Ischaemic MR	30	54.3±3.1	32.2±3.3	–	–	–
S. Kaji et al[5]	2005	Chronic ischaemic MR	15	46±6	35±5	–	–	–
Total	–		159	44.5±6	32.37±2.02	24.1±3.9	12.2±3.2	125.2±0.07
Pigs post-mortem data	–	Ischaemic MR in pigs	**7**	**38.8±3.7**	**27.2±0.7**	**26.6±1.49**	**16±1.41**	**126.6±7.96**
Pigs echo data	–	Ischaemic MR in pigs	**7**	**45.45±2.01**	**39.45±1.27**	**33.94±1.33**	**12.37±1.18**	–

Values are expressed as mean±SD.

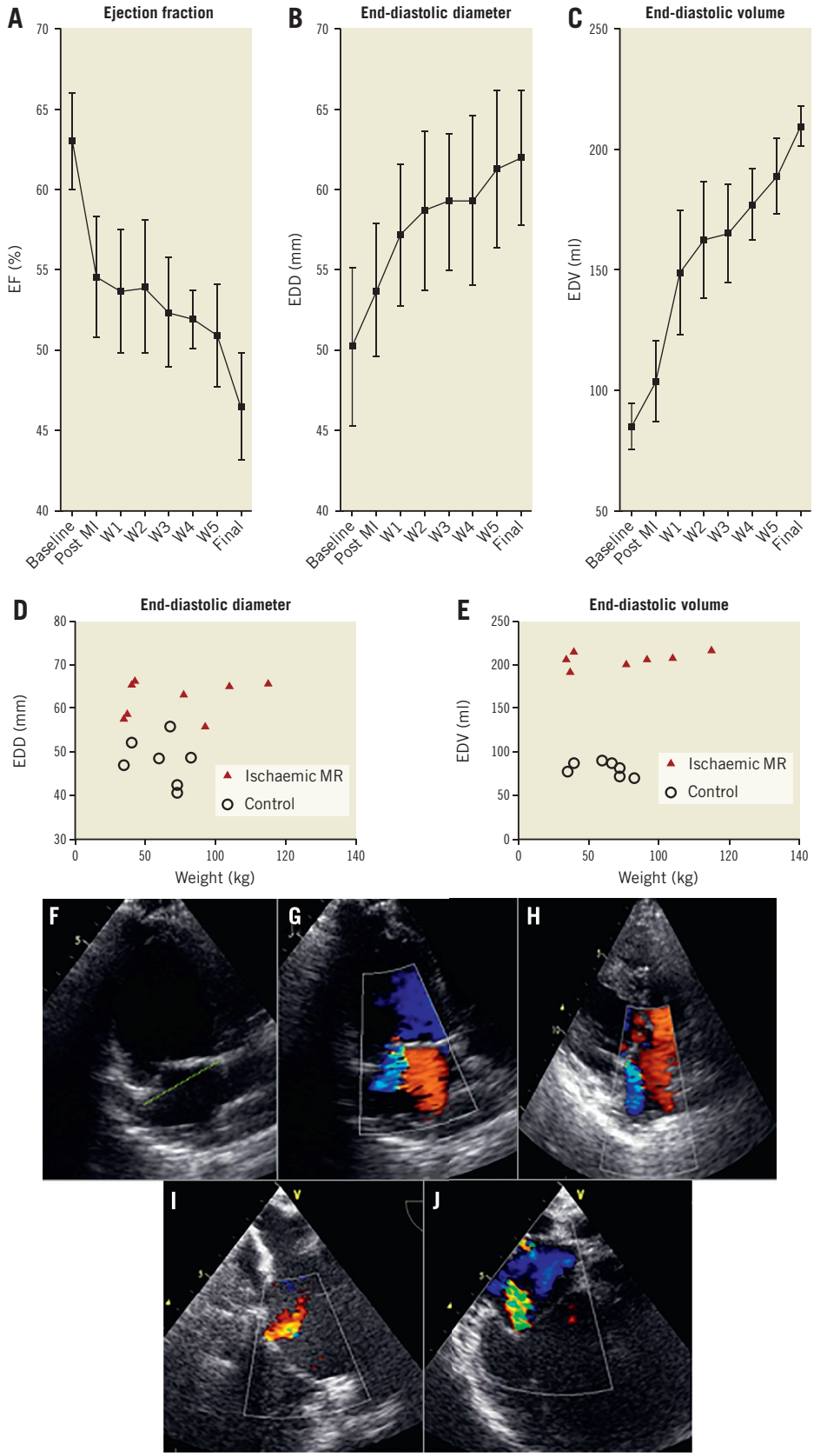

Figure 2. *Echocardiographic follow-up of ischaemic MR and LV function and dimensions. Temporal evolution of ejection fraction (A), LV end-diastolic diameter (B) and volume (C). Distribution of LV end-diastolic diameter (D) and volume (E) in mitral regurgitation pigs (red triangles) and age, sex and weight-matched animals (empty circles). Representative echocardiograms of the tenting below the annular plane (yellow dotted line) (F). Representative TTE colour Doppler of MR after six weeks (G & H) and TEE (I & J).*

Conclusion

We established a novel ischaemic MR model in pigs using contrast echocardiography guidance. This model could be a suitable platform for testing new mitral valve devices.

Impact on daily practice

Transcatheter alternatives for ischaemic MR are the optimal solution for surgical high-risk patients. We provide a reproducible model of ischaemic MR which can serve as a relevant platform to test transcatheter therapy efficacy before first-in-man trials.

Funding

The study was supported by a grant from the Ludwig Boltzmann Society (REM2017-20).

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Supplementary data

Supplementary Appendix 1. Discussion.

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Supplementary data

Supplementary Appendix 1. Discussion

In the present study, we established for the first time a closed chest pig model of ischaemic MR guided by contrast echocardiography. The model is inspired by the echo-guided alcohol septal ablation technique performed in hypertrophic obstructive cardiomyopathy patients. Echo mapping of the PMPM vasculature allowed achieving a controlled and reproducible MI of the PMPM with a consistent development of a significant MR in all animals.

Open chest large animal models of MR are the most commonly available. Although presenting the advantage of direct visualisation of the site of arterial occlusion, these models lack the precision that transcatheter methods can offer to identify the vascularisation of a certain targeted region. Furthermore, the higher risk of infection and adhesions make it not an attractive option for surgical device testing.

Other models of coronary artery occlusion used microbeads, coils and (for some) electrical injuries to achieve arterial occlusion; however, these materials have the disadvantage of being expensive, can dislodge and can present some deployment complications. On the other hand, ethanol intracoronary infusion is a safe, reproducible and cheap method to induce myocardial infarction and subsequently MR.

Our model did not rely exclusively on fluoroscopic guidance while identifying the irrigating branches as the previous ethanol injection model as described by Shi et al[6] did. Intraoperative contrast echocardiography support allowed reduction of the amount of contrast media injected as well as more reliably identifying target branches. One of the challenges in producing an ischaemic MR is the anatomical variability of the circumflex branches and the PMPM vascularisation. Therefore, contrast echocardiography is more suitable to map the irrigating branches of the region of interest.

After six weeks, the animals subjected to MI developed a moderate ischaemic MR, according to the American Society of Echocardiography (ASE) recommendations, with a reduced LVEF of around 45% with a maximum decrease of 21% observed immediately after ethanol injection. As a consequence, both LV and LA enlarged progressively.

In their model, Shi et al[6] describe an indexed jet surface which was slightly greater (27±3 vs 23.9±2.74) compared to our model. However, MR severity assessment by surface jet measurements is biased by haemodynamic conditions as well as LA compliance. Our animals showed a larger LA, which could also have contributed to MR underestimation. Zeng et al[7] published an open chest model with ischaemic MR in sheep by systematically ligating the 2nd and 3rd OM branches. The vena contracta measured in their animals was higher, and the underlying infarct was larger as the sheep have a left coronary dominance. The EF estimated in their study of around 43% would probably reflect a lower EF after elimination of the MR.

The infarct of the PMPM resulted in volume overload and interpapillary desynchrony and ultimately resulted in the development of ischaemic MR. Over time, the post-infarct LV enlargement resulted in mitral annulus remodelling as shown by a significant increase in mitral annulus diameters. The ratio anterior mitral leaflet length to septo-lateral diameter (L/D) decreased, reflecting the inadequacy of the mitral valve leaflets to close properly, as confirmed by the development of MR and the diminished coaptation height.

Similar to the echocardiographic findings in patients with an ischaemic MR, our closed chest model presented leaflet tethering as shown by the increased tenting area and heights over the follow-up period. To obtain an ischaemic MR, the extent of the MI is not as crucial as the localisation. In fact, an extensive LV MI with no PM involvement may not necessarily result in an ischaemic MR. Rather it is the desynchrony and the geometric distortion involving the PM that explain the higher incidence of ischaemic MR in inferior MI patients.

Pig hearts are widely accepted for preclinical studies given their close similarities to human

hearts. Right dominance, rare collateral anastomosis in a healthy state as well as similar myocardium healing characteristics make the swine a good basis for human cardiovascular disease modelling. In our model, mitral annular diameter values were in a range close to the values measured in MR patients. Therefore, this model can easily accommodate mitral valve devices designed for humans.

Although sheep present some advantages compared to the pig in terms of weight gain and ease of handling, the ruminant digestive system represents a major obstacle in acquiring echocardiographic data and limits the quality of the ultrasound windows available. Besides, our experience with sheep has shown that this animal has a more delicate respiratory system, which is more sensitive under general anaesthesia.

MR severity assessment revealed itself to be more challenging than in human patients. During the study we had to limit our assessment to a semiquantitative method. A more comprehensive approach including the PISA method would have been more accurate in evaluating the MR severity. Unfortunately, acquiring perfect images in lightly sedated animals is challenging. To overcome this limitation, one could train the pigs prior to the experiments to be handled for the echocardiography. Despite being a time-consuming method, this would have allowed a non-biased and more thorough MR evaluation.

Transcatheter alternatives for ischaemic mitral regurgitation are the optimal solution for surgical high-risk patients. We provide a reproducible model of ischaemic MR which can serve as a relevant platform to test the efficacy of transcatheter therapies before first-in-man trials.