Trimethylamine-N-oxide versus echocardiographic, biochemical and histopathological indices of heart failure in patients with severe aortic stenosis: Rationale and design of the prospective, observational TASTE study

Aleksandra Gąsecka¹,²*, Łukasz Rzepa¹*, Michał Konwerski¹, Magdalena Zawadzka¹, Karol Wysocki¹, Monika Budnik¹, Paweł Czub³, Radosław Wilimski³, Mateusz Wondolkowski³, Joanna Wilczyńska-Burlikowska¹, Piotr Scisło¹, Marek Konop⁴, Zenon Huczek¹, Janusz Kochman¹, Janusz Kochanowski¹, Grzegorz Opolski¹, Krzysztof J. Filipiak¹, Marcin Ufnal⁵, Agnieszka Kaplons-Cieślicka¹

¹¹st Chair and Department of Cardiology, Medical University of Warsaw, Poland
²Laboratory of Experimental Clinical Chemistry, Academic Medical Center, Amsterdam University Medical Centers, The Netherlands
³Department of Cardiac Surgery, Medical University of Warsaw, Poland
⁴Department of Clinical Sciences, Maria Skłodowska-Curie Medical Academy, Warsaw, Poland
⁵Department of Experimental Physiology and Pathophysiology, Laboratory of Center for Preclinical Research, Medical University of Warsaw, Poland

Background

Trimethylamine N-oxide (TMAO) has recently gained increasing scientific interest in the field of cardiovascular disease. An association between elevated plasma concentration of TMAO and an increased prevalence of diabetes, atherosclerosis and ischemic heart disease was observed [1, 2]. TMAO originates from the liver, which oxidizes trimethylamine (TMA), TMAO precursor. TMA is produced by conversion of L-carnitine, betaine and choline by intestinal symbiotic bacteria [3]. The rich source of the nutrients includes red meat, eggs and cheese. Recently, several studies have shown that TMA is deleterious for the circulatory system, and that TMAO may be a surrogate marker only [3, 4]. Nevertheless, an interventional study conducted by Gawrys-Kopczyska et al. [5] reported that TMAO applied to heart failure (HF) rats reduced mortality, which was associated with diuretic, natriuretic and hypotensive effects [5, 6]. Hence, unlike its precursor — TMA, TMAO might exert some favourable effects on the cardiovascular system. However, the role of TMAO in the pathogenesis of human diseases remains to be defined.

Aortic stenosis (AS) is the most common organic valvular heart disease, affecting approximately 7.6 million people over 75 years of age in North America and Europe alone [7]. The prevalence of AS is expected to increase due to an ageing population [8]. In the course of AS, aortic valve orifice gradually narrows, leading to chronic left ventricular (LV) pressure overload, LV hypertrophy and fibrosis [9]. Once AS symptoms...
(including HF symptoms, syncope, and/or angina) occur, if the patient is left untreated, the annual mortality oscillates around 25%, with an average survival of 2 to 3 years [10]. Hence, symptomatic patients with severe AS should promptly undergo either surgical aortic valve replacement (SAVR) or transcatheter aortic valve implantation (TAVI) [11]. The choice of the method of treatment (SAVR vs. TAVI) is mainly determined by the patients’ individual risk of surgery, as assessed by the Heart Team [11]. SAVR is preferred in patients under 75 years old and with low perioperative risk, while TAVI is recommended in high-risk patients not suitable for SAVR, as assessed by the Heart Team [12]. Noteworthy, adverse LV remodeling due to AS is partially reversible after interventional treatment [13, 14]. However, in some patients, LV remodeling with LV dysfunction (mainly diastolic, but also systolic in patients with a long history of severe AS) and HF symptoms persists after intervention [14]. The course of reversible remodeling might be evaluated using circulating markers of cardiac fibrosis such as matrix metalloproteinase 2 and 9 (MMP-9, MMP-2), collagen I C-terminal telopeptide (CITP) and galectin-3 (gal-3) [15–17]. Uremic toxins such as indoxyl sulphate (IS) were also shown to exacerbate fibrosis and proliferation of cardiomyocytes [18, 19].

To some extent, LV pressure overload in patients with AS may resemble hydrostatic pressure affecting deep-sea marine animals [20, 21]. Based on the fact that TMAO plays a protective role in marine animals, it was hypothesized herein, that TMAO might play a role in protection of the heart against pressure overload in patients with AS. The primary aim of the present study is to investigate the association between serum and urine TMAO concentrations, and (i) echocardiographic, (ii) biochemical and (iii) histopathological indices of HF in patients with severe AS referred for SAVR or TAVI. The secondary aim of the study is to evaluate the relationship between baseline TMAO concentrations and changes in clinical status, echocardiographic and biochemical parameters after interventional treatment of severe AS.

**Methods**

**Study design**

The TASTE (TMAO in severe Aortic STenosis: association with Echocardiographic, biochemical and histopathological indices of heart failure) study is a prospective, observational study. The recruitment phase started in January 2019 and its duration was expected to be 24 months, however, due to the coronavirus disease 2019 (COVID-19) pandemic, it has been prolonged for another 24 months.

**Selection of participants**

Inclusion and exclusion criteria are listed in Table 1. Patients are enrolled among those (i) aged from 18 to 99 years, (ii) admitted to the department of cardiology or cardiac surgery in a tertiary referral, university hospital due to severe AS, and (iii) qualified for treatment with either SAVR or TAVI by the Heart Team. Patients with HF due to causes other than AS, patients with coexisting significant aortic regurgitation and those who underwent myocardial infarction within the last 3 months or coronary revascularization within the last month are excluded from the study. Since TMAO is excreted by the urinary tract, patients with chronic kidney disease (CKD) with estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m², calculated using the Modification of Diet in Renal Disease (MDRD) formula, are excluded as well. Finally, because the intestinal metabolism of TMAO is affected by the state of gastrointestinal tract and its microbiota, patients with acute or chronic gastrointestinal diseases, autoimmune disease, treated with antibiotics within the last 2 months or taking dietary supplements within the last 7 days are excluded from the study. All patients provide written informed consent.

**Study schedule**

The study schedule is presented in Figure 1. Screening and eligibility check are performed after qualification for treatment (TAVI or SAVR). All patients screened are registered in the screening log. Patients not eligible for enrollment are registered as a screen failure with a reason for failure and not followed-up.

Patients enrolled in the study are evaluated at 4 time points: (i) prior to SAVR or TAVI, (ii) 5–7 days after the procedure (before hospital discharge) (follow-up visit 1), (iii) 1 month after the procedure (follow-up visit 2), and (iv) 6 months after the procedure (follow-up visit 3). At each visit, data regarding medical history and concomitant pharmacotherapy are collected, a thorough physical examination is conducted, transthoracic echocardiogram (TTE) is performed, and blood and urine samples are collected for laboratory tests.

**Laboratory tests**

Venous blood is collected to EDTA tubes to prepare plasma and to clotting activator tubes.
Table 1. Eligibility criteria, laboratory and non-biochemical parameters assessed in the TASTE study.

| Complete blood count | Heart failure | Inflammatory markers |
|----------------------|---------------|----------------------|
| NT-proBNP | C-reactive protein |

| Fibrosis | Renal function | Glucose and lipid metabolism |
|----------|---------------|-----------------------------|
| MMP-2 | Creatinine | Fasting glucose$^1$ |
| MMP-9 | eGFR$^2$ | Lipid profile$^1$ |
| CIPT | Urea in blood |
| Gal-3 |

| Hepatic function | Metabolites | Other parameters |
|------------------|-------------|------------------|
| ALT | TMAO$^3$ | Potassium and sodium$^1$ |
| AST | TMA$^3$ | Thyroid-stimulating hormone$^1$ |
| | IS$^2$ | Fibrinogen$^1$ |

| Main echocardiographic parameters | Left heart catheterization (in patients undergoing TAVI) | Exclusion criteria |
|-----------------------------------|---------------------------------------------------|------------------|
| E/e' (assessment of LV filling pressures) | End-diastolic and end-systolic LV and aortic pressures | Heart failure etiology other than AS |
| e' (lateral and septal) | | Chronic intestinal disease |
| S' (LV) | Age between 18 and 99 years | Coexisting, hemodynamically significant aortic regurgitation |
| LV GLS | Informed consent to participate in the study | Myocardial infarction within the last 3 months |
| LAVI | Severe AS, defined as AVA < 1.0 cm$^2$ | Coronary revascularization within the last month or planned during TAVI or SAVR |
| LV EDV | or indexed AVA < 0.6 cm$^2$/m$^2$ | Chronic kidney disease with eGFR < 45 mL/min/1.73 m$^2$ |
| LV ESV | as calculated by the continuity equation on transthoracic echocardiography, regardless of transvalvular gradient, with or without coexisting symptoms of heart failure | Acute gastrointestinal disease within the last month |
| RV and RA dimensions | Qualification for SAVR or TAVI by the Heart Team in accordance with European Society of Cardiology guidelines [11] | Active neoplastic disease |
| S' (RV) | | Chronic inflammatory disease |
| LAVI | | Autoimmune disease |
| TAPSE | | Antibiotic therapy within the last 2 months |
| TRV and estimated SPAP | | Dietary supplements within the last 7 days |

| Histopathological study (in patients undergoing SAVR) | Severity of myocardial fibrosis |
|------------------------------------------------------|--------------------------------|

$^1$Parameters assessed only at enrolment.

$^2$eGFR is calculated based on serum creatinine using the Modification of Diet in Renal Disease (MDRD) formula.

$^3$Concentrations of these parameters will be measured both in serum and urine.

ALT — alanine transaminase; AS — aortic stenosis; AST — aspartate aminotransferase; AVA — aortic valve area; C1TP — collagen I C-terminal telopeptide; eGFR — estimated glomerular filtration rate; Gal-3 — galectin-3; IS — indoxyl sulfate; MMP-2, MMP-9 — matrix metalloproteinase 2 and 9; NT-proBNP — N-terminal prohormone of B-type natriuretic peptide; TMAO — trimethylamine-N-oxide; TMA — trimethylamine; LV — left ventricular; e' — early diastolic mitral annular velocity (lateral and septal); S' — systolic mitral annular velocity; GLS — global longitudinal strain; LAVI — left atrial volume index; EDV — end-diastolic volume; ESV — end-systolic volume; RV — right ventricular; RA — right atrial; SAVR — surgical aortic valve replacement; SPAP — systolic pulmonary artery pressure; TAPSE — tricuspid annular plane systolic; TAVI — transcatheter aortic valve implantation; TRV — tricuspid regurgitation velocity

to prepare serum. Urine is collected to sterile urine cups. For standard laboratory tests, blood is transferred to the accredited hospital laboratory. For TMAO analysis, EDTA-anticoagulated blood is centrifuged for 15 min at 2,000 g within 60 min after blood collection to prepare plasma [22]. Both plasma and urine are aliquoted and stored in a freezer at −80°C until analyzed. The analysis is performed in the Center of Preclinical Research and Technology, Medical University of Warsaw. All collected samples are coded with a unique number and will be analyzed in one block by operators blinded to patient data. Concentrations of TMAO in plasma and urine will be measured using a Waters Acquity Ultra Performance Liquid Chromatograph coupled with a Waters TQ-S Triple-Quadrupole Mass Spectrometer. The mass spectrometer will be operated in the multiple-reaction monitoring-positive electrospray ionization mode, as previously described [23].
Echocardiographic examination

Each patient undergoes TTE at four prespecified time points. Echocardiographic examination includes evaluation of: (i) LV end-diastolic and end-systolic dimensions and volumes, LV wall thickness and LV mass index, (ii) LV systolic function, including assessment of LV ejection fraction (EF; using biplane Simpson’s method) and global longitudinal strain, (iii) LV diastolic function (using tissue Doppler imaging [TDI]) and estimated left atrial pressure, (iv) left atrial dimension and indexed volume, (v) right ventricular size and systolic function (tricuspid annular plane systolic excursion, S’ from TDI), right atrial size, (vi) maximal and mean aortic gradients, aortic valve area (AVA), indexed AVA, indexed LV stroke volume, (vii) presence and severity of aortic regurgitation or — after intervention — paravalvular leaks, (viii) function of other heart valves, and (ix) probability of pulmonary hypertension. At follow-up visit 2 (7 days after the procedure), echocardiographic assessment may not include all the above parameters due to limited visualization.

Severe AS is defined as AVA < 1.0 cm² or indexed AVA < 0.6 cm²/m² as calculated by the continuity equation on TTE. The study includes both patients with high-gradient severe AS, and those with low-flow, low-gradient severe AS, regardless of LVEF. In patients with low-flow, low-gradient AS and reduced LVEF to differentiate between true severe AS and pseudo-severe AS, dobutamine stress echocardiography is typically performed, and in patients with low-flow, low-gradient AS and preserved LVEF — computed tomography with assessment of aortic valve calcium score, as recommended by the European Society of Cardiology (ESC) guidelines [11].

Histopathological evaluation

In patients undergoing SAVR, biopsy of interventricular septum is performed during surgery (a specimen of ~2 mm in diameter) for histopathological evaluation of myocardial fibrosis. The material is temporarily stored in 5% solution of formalin and transferred to the Department of Physiology and Experimental Pathophysiolo, Medical University of Warsaw for histopathological evaluation. All collected samples are coded with a unique number and will be analyzed in one block by operators blinded to patient data. Microscopic examination of each sample will include assessment of myocardial morphology and interstitial fibrosis or inflammation. Only samples containing at least 50% of the cardiac muscle tissue will be examined.

Left heart catheterization

In patients undergoing TAVI, before and after prosthesis implantation end-diastolic and end-systolic LV and aortic pressures are measured.

Endpoints

The primary endpoint of this study is the association between serum and urine TMAO concentrations and (i) echocardiographic, (ii) biochemical and (iii) histopathological indices of HF. This will be assessed by (i) comparing clinical, echocardiographic, biochemical and histopathological variables in patients with TMAO concentrations above and below median, and (ii) analyzing correlations between TMAO concentrations and those parameters (for continuous variables). TMAO concentrations will also be correlated with LV pressures measured invasively during TAVI. The secondary endpoints include relationship between baseline TMAO
concentrations and post-treatment clinical status, echocardiographic and biochemical parameters in 6-month follow-up. This will include assessment whether TMAO is an independent predictor of clinical and/or echocardiographic improvement at 6 months. Clinical improvement will be defined as an improvement of at least 1 New York Heart Association class. In addition, changes in serum and urine TMAO, TMA and IS concentrations after the procedure will be analyzed in relation to other biochemical and echocardiographic changes. Clinical end-points, including all-cause death, HF death, HF hospitalizations and other cardiovascular hospitalizations will be recorded.

Sample size

Because insufficient data are available to assess the association between the concentration of TMAO and severity of echocardiographic, biochemical and histopathological features of HF in patients with severe AS, the calculation was based on two previous studies [24, 25]. Required sample size was calculated by a power test at a significance level of 0.05 with the following assumptions (i) the expected correlation coefficient R 0.35, (ii) nominal test power 0.8, (iii) p-value considered significant 0.05. Based on this sample size estimation, a total of 62 patients should be enrolled in the trial. Assuming that up to 15% of patients may be potentially lost to follow-up, we estimated that 70 patients should be enrolled in the trial. As of January 2021, 26 patients have been included in the study.

Legal considerations

The study protocol was approved by the Bioethical Committee of the Medical University of Warsaw, and registered in the ClinicalTrials database (NCT04406805). The study is conducted according to good clinical practice, the ethical principles described in the Declaration of Helsinki, the requirements of the European Medicines Agency and local legal and regulatory requirements. Data storage is conducted in compliance with local data protection laws. Authorities may request access to the study documentation in case of an inspection or audit. Documentation can be copied during inspection or audit only in case the identity of the participant has been made unrecognizable.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics, version 24.0 (IBM). Categorical variables will be presented as number and percentage and compared using the χ² test. The Shapiro-Wilk test will be used to assess normal distribution of continuous variables. Continuous variables will be presented as mean and standard deviation or median with interquartile range and compared using an unpaired t-test or the Mann-Whitney U test. The Pearson or Spearman correlation coefficient will be used to analyze a correlation between serum and urine TMAO concentration and continuous echocardiographic, biochemical and histopathological variables. The analyses will also include a comparison of patient groups with TMAO concentrations above and below the median, and patients with and without echocardiographic and clinical improvement at 6 months. The Cox regression model will be used to determine the prognostic ability of TMAO to predict echocardiographic, clinical and biochemical improvement at 6 months. Mortality and other adverse events will be reported descriptively. A p-value below 0.05 will be considered significant.

Discussion

The TASTE study is the first clinical study to evaluate the association between concentrations of TMAO and TMA and the severity of HF in patients with AS in an investigator-blinded way [22]. Recently, it has been shown that TMA, but not TMAO reduced rat cardiomyocytes viability, likely due to its disturbing effect on proteins, whereas a concomitant treatment with TMAO protected cardiomyocytes against the deleterious effect of TMA. Therefore, TMA but not TMAO seems to be a toxin and a marker of cardiovascular risk [22]. This study is expected to shed a light on the respective roles of TMA and TMAO in cardiovascular disease in a human model of pressure overload (AS). Noteworthy, the study offers an opportunity to assess the correlation of TMA and TMAO with the histopathology of human heart tissue. The state-of-the-art methods to analyze TMAO and TMA will account for the reliability of results.

In this study, other promising markers of cardiac fibrosis will also be measured, such as MMP-9, MMP-2, CITP, gal-3 and IS. Indoxyl sulphate is considered to be a molecule linking CKD and HF, involved in the pathogenesis of the cardiorenal syndrome (CRS) [18, 26]. CRS is a condition characterized by kidney and HF, where the failure of one organ may induce dysfunction of the other, thus further accelerating the progressive failure of both organs [27]. Given the fact that AS and CKD often coexist, and that over half of patients have an improvement in eGFR after interventional treatment.
of severe AS, assessment of IS concentrations in this study offers a unique possibility to investigate its role in the development of CRS in patients with severe AS [18, 28, 29].

TASTE is expected to determine whether TMAO and/or TMA reflect the severity of HF in AS patients, or predict clinical and echocardiographic improvement after interventional treatment of AS. This could be yet another step to understand the role of TMAO and TMA in cardiovascular disease [30]. If the association between TMAO and the severity of HF is confirmed, TASTE might provide a basis for future studies aimed to develop new methods for cardiac muscle protection by increasing the concentration of TMAO, for example by diet or supplements rich in TMAO precursors, and to diminish the detrimental effect of LV pressure overload on LV structure and function in patients with AS or arterial hypertension. Altogether, TMAO may be the key to discover new, breakthrough ways to prevent and/or treat HF. The findings of this study might potentially change the present paradigm of TMAO as a cardiovascular risk marker and trigger a debate on the protective effects of TMAO.

Funding
This research was funded by National Science Center, Poland, grant number 2018/31/B/NZ5/00038 and UMO-2020/37/B/NZ5/00366.

Conflict of interest: None declared

References
1. Ufnal M, Zadlo A, Ostaszewski R. TMAO: A small molecule of great expectations. Nutrition. 2015; 31(11-12): 1317–1323, doi: 10.1016/j.nut.2015.05.006.
2. Lever M, George PM, Slow S, et al. Betaine and trimethylamine-n-oxide as predictors of cardiovascular outcomes show different patterns in diabetes mellitus: an observational study. PLoS One. 2014; 9(12): e114969, doi: 10.1371/journal.pone.0114969, indexed in Pubmed: 25493436.
3. Jaworska K, Bielinska K, Gawrys-Kopczynska M, et al. TMA (trimethylamine), but not its oxide TMAO (trimethylamine-oxide), exerts haemodynamic effects: implications for interpretation of cardiovascular actions of gut microbiome. Cardiovasc Res. 2019; 115(14): 1948–1949, doi: 10.1093/cvr/cvz231, indexed in Pubmed: 31504256.
4. Jaworska K, Konop M, Hutach T, et al. Trimethylamine but not trimethylamine oxide increases with age in rat plasma and affects smooth muscle cells viability. J Gerontol A Biol Sci Med Sci. 2020; 75(7): 1276–1283, doi: 10.1093/gerona/glz181, indexed in Pubmed: 31411319.
5. Gawrys-Kopczynska M, Konop M, Maksymiuk K, et al. TMAO, a seafood-derived molecule, produces diuresis and reduces mortality in heart failure rats. Elife. 2020; 9, doi: 10.7554/eLife.5028, indexed in Pubmed: 32510330.
6. Huc T, Drapala A, Gawrys M, et al. Chronic, low-dose TMAO treatment reduces diastolic dysfunction and heart fibrosis in hypertensive rats. Am J Physiol Heart Circ Physiol. 2018; 315(6): H1805–H1820, doi: 10.1152/ajpheart.00536.2018, indexed in Pubmed: 30266149.
7. Osnabrugge RJ, Mylotte D, Head SJ, et al. Aortic stenosis in the elderly: disease prevalence and number of candidates for transcatheter aortic valve replacement: a meta-analysis and modeling study. J Am Coll Cardiol. 2013; 62(11): 1002–1012, doi: 10.1016/j.jacc.2013.05.015, indexed in Pubmed: 23727214.
8. Jung B, Baron G, Butchart EG, et al. A prospective survey of patients with valvular heart disease in Europe: The Euro Heart Survey on Valvular Heart Disease. Eur Heart J. 2003; 24(13): 1231–1243, doi: 10.1016/s0195-668x(03)00201-x, indexed in Pubmed: 12831818.
9. Dweck MR, Boon NA, Newby DE. Calcific aortic stenosis: a disease of the valve and the myocardium. J Am Coll Cardiol. 2012; 60(19): 1854–1863, doi: 10.1016/j.jacc.2012.02.093, indexed in Pubmed: 22982541.
10. Otto CM, Lind BK, Kitzman DW, et al. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. N Engl J Med. 1999; 341(3): 142–147, doi: 10.1056/NEJM199907013410302, indexed in Pubmed: 10403851.
11. Baumgartner H, Falk V, Bax JJ, et al. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. Eur Heart J. 2017; 38(36): 2739–2794, doi: 10.1093/eurheartj/ehx391, indexed in Pubmed: 28886619.
12. Joseph J, Naqvi SY, Giri J, et al. Aortic stenosis: pathophysiology, diagnosis, and therapy. Am J Med. 2017; 130(3): 253–263, doi: 10.1016/j.amjmed.2016.10.005, indexed in Pubmed: 27810479.
13. Treibel TA, Kozor R, Schofield R, et al. Reverse myocardial remodeling following valve replacement in patients with aortic stenosis. J Am Coll Cardiol. 2018; 71(8): 860–871, doi: 10.1016/j.jacc.2017.12.035, indexed in Pubmed: 29471937.
14. Kayama S, Aratake S, Sawamura S, et al. Medium and long-term prognosis of transcatheter aortic valve implantation from the perspective of left ventricular diastolic function. Cardiol J. 2019; 26(1): 29–35, doi: 10.5603/CJ.a2018.0005, indexed in Pubmed: 29507210.
15. Bjorndal JL, Neverdal NO, Vengen OA, et al. Alterations in circulating activin A, GDF-15, TGF-beta and MMP-2,-3, and -9 during one year of left ventricular reverse remodelling in patients operated for severe aortic stenosis. Eur J Heart Fail. 2008; 10(12): 1201–1207, doi: 10.1016/j.ejheart.2008.09.010, indexed in Pubmed: 18996047.
16. Kupari M, Laine M, Turto H, et al. Circulating collagen metabolites, myocardial fibrosis and heart failure in aortic valve stenosis. J Heart Valve Dis. 2013; 22(2): 166–176, indexed in Pubmed: 23798204.
17. Zhong X, Qian X, Chen G, et al. The role of galectin-3 in heart failure and cardiovascular disease. Clin Exp Pharmacol Physiol. 2019; 46(3): 197–203, doi: 10.1111/1440-1681.13048, indexed in Pubmed: 30725458.
18. Fu Q, Cao L, Li H, et al. Cardiorenal syndrome: pathophysiological mechanism, preclinical models, novel contributors and potential therapies. Chin Med J (Engl). 2014; 127(16): 3011–3018, indexed in Pubmed: 25131243.
19. Lekawianjijit S, Kompa AR, Manabe M, et al. Chronic kidney disease-induced cardiac fibrosis is ameliorated by reducing circulating levels of a non-dialysable uremic toxin, indoxyl sulphate. PLoS One. 2012; 7(7): e41281, doi: 10.1371/journal.pone.0041281, indexed in Pubmed: 22829936.
20. Organ CL, Otsuka H, Bluschan S, et al. Choline diet and its gut microbe-derived metabolite, trimethylamine N-oxide, exacerbate pressure overload-induced heart failure. Circ Heart Fail. 2016; 9(1): e002314, doi: 10.1161/CIRCHEARTFAILURE.115.002314, indexed in Pubmed: 26699388.

21. Ufnal M, Jazwiec R, Dudlew M, et al. Trimethylamine-N-oxide: a carnitine-derived metabolite that prolongs the hypertensive effect of angiotensin II in rats. Can J Cardiol. 2014; 30(12): 1700–1705, doi: 10.1016/j.cjca.2014.09.010, indexed in Pubmed: 25475471.

22. Jaworska K, Hering D, Mosieniak G, et al. TMA, A Forgotten Uremic Toxin, but Not TMAO, Is Involved in Cardiovascular Pathology. Toxins (Basel). 2019; 11(9), doi: 10.3390/toxins11090490, indexed in Pubmed: 31454905.

23. Jaworska K, Huc T, Samborowska E, et al. Hypertension in rats is associated with an increased permeability of the colon to TMA, a gut bacteria metabolite. PLoS One. 2017; 12(12): e0189310, doi: 10.1371/journal.pone.0189310, indexed in Pubmed: 29236735.

24. Yancey PH, Siebenaller JF. Co-evolution of proteins and solutions: protein adaptation versus cytoprotective micromolecules and their roles in marine organisms. J Exp Biol. 2015; 218(Pt 12): 1880–1886, doi: 10.1242/jeb.143355, indexed in Pubmed: 26085665.

25. Burg MB, Ferraris JD. Intracellular organic osmolytes: function and regulation. J Biol Chem. 2008; 283(12): 7309–7313, doi: 10.1074/jbc.R700042200, indexed in Pubmed: 18256030.

26. Konopelski P, Ufnal M. Indoles: gut bacteria metabolites of tryptophan with pharmacotherapeutic potential. Curr Drug Metab. 2018; 19(10): 883–890, doi: 10.2174/138920021966618100427164731, indexed in Pubmed: 29708069.

27. Lekawanvijit S, Krum H. Cardiorenal syndrome: acute kidney injury secondary to cardiovascular disease and role of protein-bound uraemic toxins. J Physiol. 2014; 592(18): 3969–3983, doi: 10.1113/jphysiol.2014.273078, indexed in Pubmed: 24907399.

28. Reuillard A, Garrouste C, Pereira B, et al. Evolution of chronic kidney disease after surgical aortic valve replacement or transcatheter aortic valve implantation. Arch Cardiovasc Dis. 2019; 112(3): 162–170, doi: 10.1016/j.acvd.2018.10.003, indexed in Pubmed: 30655226.

29. Vavilis G, Bäck M, Occhino G, et al. Kidney dysfunction and the risk of developing aortic stenosis. J Am Coll Cardiol. 2019; 73(3): 305–314, doi: 10.1016/j.jacc.2018.10.068, indexed in Pubmed: 30678761.

30. Winther SA, Ølgaard JC, Tofte N, et al. Utility of plasma concentration of trimethylamine n-oxide in predicting cardiovascular and renal complications in individuals with type 1 diabetes. Diabetes Care. 2019; 42(8): 1512–1520, doi: 10.2337/dc19-0048, indexed in Pubmed: 31123156.