We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 3

Matrix Metalloproteinases MMP-3 and MMP-9 as Predictors of In-Stent Restenosis

Pavlina Kusnierova and Leos Pleva

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69589

Abstract

The present study aimed to demonstrate the use of matrix metalloproteinases (MMP-2, MMP-3 and MMP-9) as possible additional biochemical predictors of in-stent restenosis (ISR) and determined their reference intervals in adults with respect to gender and age. We included 111 consecutive patients treated at the cathlab of the University Hospital Ostrava, Czech Republic, with ISR within 12 months after implantation of a bare-metal stent. The control group consisted of 111 matched patients with identical main demographic and clinical risk factors. To set the reference intervals for MMPs, we measured the blood concentrations of these analytes in a group of healthy volunteers (N = 180) with an average age of 40–50 years. The enzyme concentrations were measured by immunosorbent assay. Statistical analysis was performed using IBM SPSS Statistics version 22 and MedCalc Version 14.12. We found that increased levels of MMP-3 and 9 were associated with a significant increase in ISR risk. The MMP-9 cut-off value for ISR risk prediction was determined to be ≥64.8 ng/mL. We suppose that screening of these biochemical parameters might be helpful to a more detailed risk stratification of patients after percutaneous coronary interventions, who would be able to benefit from implantation of drug-eluting stents.

Keywords: adult, age dependence, enzyme-linked immunosorbent assay, gender dependence, in-stent restenosis, matrix metalloproteinases

1. Introduction

Matrix metalloproteinases (MMPs) are members of a family of zinc-dependent proteolytic enzymes. The main function of MMPs is to degrade the various components of the extracellular matrix (ECM) and participate as regulators of extracellular tissue signalling networks. MMPs

have broad substrate specificity and contribute to the homeostasis of many tissues and participate in several physiological processes, such as bone remodelling, angiogenesis, immunity and wound healing. MMPs are produced by activated inflammatory cells (neutrophils and macrophages) and wound cells (epithelial cells, fibroblasts and vascular endothelial cells). MMP activity is tightly regulated at the level of transcription, pro-peptide activation and inhibition by tissue inhibitors of MMPs (TIMPs).

The MMP process represents the procedure of pathologic changes in the tissues in atherosclerosis, arterial remodelling and myocardial repair following infarction [1]. A mechanical damage causes expression of matrix metalloproteinases (MMP), and it is proved that they may take part in the pathogenesis of in-stent restenosis (ISR), when undergoing coronary stent implantation by percutaneous coronary intervention [2–5].

Effectiveness of percutaneous coronary intervention (PCI) has remarkably become better after coronary stent implantation. Thanks to the early obstacles of plane balloon angioplasty that could be managed and in case the elastic recoil and constrictive remodelling has been applied, it comes to the reduction of the frequency of restenosis after PCI. Although these improvements have been implemented, a new complication has occurred in-stent restenosis (ISR) caused by neointimal hyperplasia. The clinical probability of occurrence of ISR after bare-metal stent implantation is about 20–35% [6, 7]. Additional decline of ISR occurrence to 5–10% has been caused by the usage of drug-eluting stents [6, 7]. Unfortunately, long-term dual antiplatelet therapy needs to be applied and is connected with the threat of late and very late stent thrombosis. The known predictors of ISR include patient-, vessel- and procedure-related agents [6, 7]. Because of not decreasing amount of patients going through PCI, there were strives to find other predisposing factors that would lead to more focused treatment.

2. Aims

We focused on matrix metalloproteinases (MMP-2, MMP-3 and MMP-9) as possible additional biochemical predictors of in-stent restenosis (ISR) after coronary artery bare-metal stenting and determined their reference intervals in adults with respect to gender and age.

3. Patient group and methods

Note that, 111 patients were recruited from the University Hospital Ostrava, Czech Republic affected by ISR within 12 months after implantation of a bare-metal stent. The control group (n = 111) with absent ISR 12 months after implantation of a bare-metal stent coincided with patients treated at our cathlab with identical main demographic and clinical risk factors (sex, age, diabetes mellitus and implanted stent diameter ±0.5 mm).

To set the reference intervals for MMP-2, MMP-3 and MMP-9, we measured the blood concentrations of these analytes in a group of 180 healthy adult volunteers attending the Blood

Centre of the University Hospital Ostrava. We measured MMP concentrations in heparin plasma. Plasma samples from each patient and healthy volunteers were stored at −80°C for 2–3 months after centrifugation at 2500 × *g* at 4°C for 6 min and in 2 aliquots of 2 mL. The measurement of enzyme concentrations was performed by immunosorbent assay (ELISA) (BioVendor-Laboratorni Medicina a.s., Brno, Czech Republic).

Selective coronarography was determined in a Siemens Axiom device (Forchheim, Germany) in a common way from the radial approach with 5 F diagnostic catheters and the contrast medium Iomeron 400. Quantitative coronary angiography was executed, and percent diameter stenosis (%DS) was figured out. ISR was observed as a diameter stenosis ≥50% in the stented segment.

Multi-slice CT coronarography was assessed in all patients in the Siemens Somatom Definition AS + device (Forchheim, Germany), a single-source CT scanner in a 128-slice configuration. The maximum intensity projection (MIP) reconstructions and automatic software vessel analysis were applied for assessment of the lumen. Homogeneous enhancement (from the visual point of view, similar to the CT attenuation in the reference vessels) inside the stent lumen was reported to be normal or without any relation to ISR [8].

Statistical analysis was performed using IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA). Measured biochemical parameters were categorized according to reference values, which were calculated using MedCalc Version 14.12 (origin) [9, 10]. Parametric and non-parametric statistical methods were applied, as appropriate, using NCSS 2007 (origin) for calculations and R software for graphical displays. Potential outliers were evaluated by Cook's distance and partitioning of the data according to Harris and Boyd [11]. There were linkages between measurands, which were assessed using Spearman's rank correlation (r_s) or Pearson's product-moment correlation coefficients (r_p), respectively. What is more, correlation testing, the age dependence of MMP-2 and MMP-3 was examined using piecewise polynomial models (multiphase models) [12].

Continuous variables with non-normal distribution are presented as the median and range (minimum−maximum) and were compared using the non-parametric Mann-Whitney U test. Categorical variables were compared by the chi-square test. The difference between measured biochemical parameters of the study and control groups was analysed by the chi-square test. The assessment of potential consequences of other features on the association observed between the levels of individual markers and ISR was applied multiple logistic regression. A forward stepwise method was used to establish the most notable risk values for ISR. Diabetes mellitus and other possible confounding variables have been taken into consideration. The stating of the optimal cut-off values of biochemical criteria was applied the receiver operating characteristic (ROC) analysis in order to prognosticate in-stent restenosis.

The studied protocol complied with the declaration of Helsinki and was approved by the Ethics Committee of the University Hospital Ostrava, Czech Republic. Written informed consent was obtained from each participant.

4. Results

There have not been found any remarkable differences in the patients with ISR and the control group, as far as the main demographic factors (age, gender and body mass index) or clinical risk factors (diabetes mellitus) concerned. Same ranges of coronary disease (multi-vessel disease, acute coronary syndromes) and similar lesion characteristics (complex lesion B2/C and length and diameter (±0.5 mm) of implanted stents) have been proved in the groups. What is more, in both groups, mostly similar biochemical criteria have been found (creatinine, glucose, triglycerides, High-density lipoprotein (HDL) and hsCRP). Nevertheless, the ISR group showed particularly lower total cholesterol (p = 0.016) and Low-density lipoprotein (LDL) (p = 0.001), and notably higher NT-pro-BNP (p = 0.010), in comparison to the treatment group. **Table 1** shows the summary of plasma levels of matrix metalloproteinases in all groups [13].

To set the reference intervals for MMP-2, MMP-3 and MMP-9, we measured the blood concentration in a group of healthy volunteers with an average age of 40–50 years. Plasma MMP-2 levels in these population showed statistically significant age dependence (r_p = 0.171; p = 0.02; r_s = 0.161; p = 0.034). Therefore, we divided the tested file into two groups: ≤49 and >50 years. The medians of these age groups were significantly different from each other (p = 0.001; z = 3.15). MMP-3 plasma concentrations were found to be significantly correlated with age (r_p = 0.355; p = 0.00007; r_s = 0,308; p = 0.0006) as well as with gender ($p \leq 0.001$; $z > 3.0$). Because of this, there were created two groups of the probands, according to the age ≤47 and >47 years. The dependence of the measured values on gender showed to be crucial. Since, only a small amount of men in the group of ≤47 years took part in the survey, the reference range has not been taken into account for this group. No statistically significant changes could be observed in plasma MMP-9 levels in relation to age, but there was found a link with gender (p = 0.014; z = 2.39). Because of the fact that the z-value was lower than 3 and the data performed a non-Gaussian distribution, the distribution in separate age-dependence subgroups was figured out. The reference ranges are depictured in **Table 2**. **Table 3** presents an outline of the results [14].

Furthermore, we compared the levels of plasma of matrix metalloproteinases in ISR patients with the normal reference values of these markers in the healthy population.

The correlation between MMP-3, MMP-9 and the incidence of ISR with respect to diabetes mellitus was studied using a logistic regression analysis. It was shown that MMP-3 (OR: 1.013, 95% CI: 1.004–1.023, p = 0.005) and MMP-9 (OR: 1.014, 95% CI: 1.008–1.020, $p < 0.0001$) are significantly associated with the occurrence of ISR (**Table 4**).

Methods	In-stent restenosis	Controls
MMP-2 (ng/mL)	173.76 (122.18–248.40)	163.58 (113.57–247.85)
MMP-3 (ng/mL)	43.37 (26.66–72.74)	31.48 (23.14–53.16)
MMP-9 (ng/mL)	88.40 (53.80–131.7)	44.90 (25.56–79.90)

Note: Data are given as median (lower and higher quartiles).

Table 1. Plasma levels of measured parameters of all patients and matched controls [13].

Analyte	*N*	Age (year)	Gender	2.5th	90thconfidence interval	97.5th	90th confidence interval
MMP-2 (ng/mL)	180	35–62	Both	110.0	97.1–124.1	590.9	520.8–670.0
	111	35–49	Both	98.3	83.0–115.2	466.9	417.9–519.1
	62	50-62	Both	160.8	144.8–179.0	550.2	456.9–666.7
MMP-3 (ng/mL)	120	34–61	Both	12.49	11.20–13.86	39.37	36.19–42.72
	38	34–47	Female	11.11	9.24–13.15	29.91	26.49–33.53
	14[a]		Male	–	–	–	–
	22	48–61	Female	12.76	11.11–14.69	31.14	25.41–38.40
	46		Male	18.03	15.84–20.38	42.04	38.00–46.33
MMP-9 (ng/mL)	116	16–61	Both	27.63	24.55–30.99	103.06	92.85–114.06
	58	20–61	Female	24.83	20.93–29.25	98.82	84.28–115.27
	58	19–55	Male	33.37	29.47–37.77	108.67	93.01–126.8

[a] Reference interval cannot be calculated due to the small number of data.

Table 2. The gender- and age-dependent reference intervals of MMP-2, MMP-3 and MMP-9 (μg/l)—2.5th, 97.5th centile values and their 90th confidence intervals according to parametric statistical methods [14].

Analyte	Age dependence	Gender dependence
MMP-2	Yes	No
MMP-3	Yes	Yes
MMP-9	No	Yes

Table 3. Summary of results as regards age- and gender-dependence of MMP-2, MMP-3 and MMP-9 [14].

Analyte	OR	95% CI for OR	*p*
MMP-2	1.001	0.999–1.004	0.326
MMP-3	1.013	1.004–1.023	0.005
MMP-9	1.014	1.008–1.020	<0.0001

Note: OR is associated with a single-unit increase in the value of the parameter.

Table 4. Logistic regression analysis (separately for each parameter with adjustment for diabetes mellitus (DM)) [13].

The result of this study is also the finding that the decreased concentration of MMP-9 below the lower limit of the reference interval is associated with a significant reduction in the incidence of ISR (OR: 0.265, 95% CI: 0.121–0.582, $p = 0.001$). On the other hand, the increased concentration of MMP-9 above the upper limit of the reference interval is associated with an increase in the incidence of ISR (OR: 2.685; 95% CI: 1.344–5.366; $p = 0.005$). At the same time, the increased concentration of MMP-3 above the upper limit of the reference interval

is linked with an increased incidence of ISR (OR: 2.502; 95% CI: 1.441–4.344; $p = 0.001$). In this case, a multivariate logistic regression analysis was also used. A step-by-step method was used to select the most important categorized parameters that are predictive of ISR, with DM modifications and individual categorized parameters. It was found that MMP-9 is the most important parameter in terms of ISR prediction (OR: 0.322; 95% CI: 0.122–0.854; $p = 0.023$), its decrease below the lower limit of the reference interval is connected with a decrease in ISR.

Based on receiver operating characteristic (ROC) analysis, the plasma abundance of MMP-9 may be considered the most suitable parameter for use in ISR risk prediction (**Table 5** and **Figure 1**). The following cut-off values for prediction of ISR were determined: MMP-9 ≥ 64.8 ng/mL with sensitivity 65.8 and specificity 65.8% (**Table 6**).

Analyte	AUC
MMP-2	0.525
MMP-3	0.605
MMP-9	0.715

Note: Parameters with AUC > 0.7 or > 0.75 are suitable for the in-stent restenosis prediction.

Table 5. ROC analysis; area under the curve (AUC) of all parameters [13].

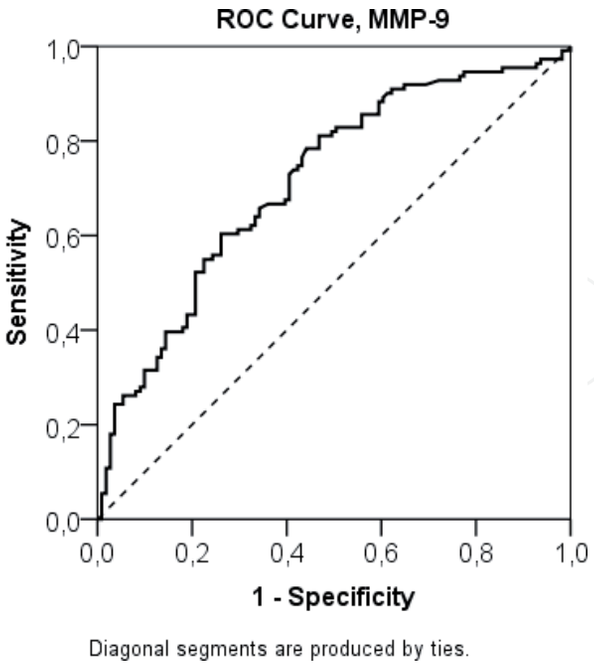

Figure 1. ROC curve of MMP-9 with AUC >0.7 is suitable for the ISR prediction [13].

Analyte	MMP-9	
AUC	0.715	
Cut-off value	≥64.8 (ng/mL)	
Sensitivity	65.8%	56.2–74.5%
Specificity	65.8%	56.2–74.5%
PPV	65.8%	56.2–74.5%
NPV	65.8%	56.2–74.5%
False positivities	34.2%	25.5–43.8%
False negativities	34.2%	25.5–43.8%

Table 6. Cut-off values of MMP-9 for ISR prediction [13].

5. Discussion

MMPs are a growing family of endopeptidases, they currently have at least 24 members, although some of them have not been well understood. Most of the MMPs contain a pro-peptide, a catalytic domain, a linker peptide and a hemopexin domain. MMPs are synthesized and secreted as latent pro-enzymes, containing a Zn^{2+}-binding active site and require Ca^{2+} for activation. MMPs are categorized by their structure and substrates into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and others. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are classified as a gelatinases, which degrade both collagens and gelatins [1].

MMP-2 and MMP-9 play an essential role in angiogenesis and arteriogenesis, two processes are critical to restoration of tissue perfusion after ischemia. MMP-2 expression is increased in tissue ischemia, but the responsible mechanisms remain unknown.

Expression and activity of MMPs are regulated at different levels: gene expression, proenzyme activation and enzyme secretion are inhibited specifically by tissue inhibitors of MMPs (TIMPs) and non-specifically alpha-2 macroglobulin. There is an increased expression of the genes encoding these proteins. Mutations of these genes are likely to affect the degree of expression, mRNA stability and the properties of the particular proteins, which may influence the process etiopathogenesis.

MMP-2 and MMP-9 are the most widely studied MMPs in blood vessel. MMP-3 (stromelysin-1) is included among stromelysins and is digested a wide range of extracellular matrix molecules and participated in proMMPs proteolysis. MMP-3 overexpression significantly reduces smooth muscle cells migration and inhibits neointimal formation in arterialized vein grafts. Ogata et al. reported that MMP-3 regulates vascular smooth muscle cells migration via MMP-9 activation. MMP-3 is already known as an activator of pro-MMP-9. Combination of MMP-3 and MMP-9 is required and efficient in neointimal hyperplasia [15–17].

Based on several published studies describing the discrepancies in MMP concentrations between serum and plasma [18–31], we measured MMP concentrations in heparin plasma. To set the reference intervals for MMP-2, MMP-3 and MMP-9, we measured the blood concentrations of these analytes in a group of healthy volunteers with an average age of 40–50 years. We found that plasma MMP-2 concentrations depend on the proband age (healthy volunteers). Lower MMP-2 concentrations were found in subjects <50 years of age and the concentration increased with age. However, no similar data were reported for a group of healthy volunteers.

The concentration of MMP-3 was statistically significant correlated with age and sex as well. The lower plasma concentrations of MMP-3 have been demonstrated in probes <47 years. MMP-3 concentrations in women were lower in both age groups than that in men.

Normal levels for MMP-3 determined by a one-step sandwich ELISA method with reagents provided by Dr Jaspar, Biosource Europe S. A., Belgium were described in 96 healthy controls (46 females and 50 males) in the study of Ribbens et al. [32, 33]. Our results showed higher plasma MMP-3 concentration in both women and men, as compared to Ribbens et al.'s data. The cause of this difference seems to be the use of different reagents.

Plasma MMP-9 concentrations are associated with difference in respect to gender. Our findings indicate lower values in women and higher in men. The publication of Lizasa et al. [34] also evaluated a group of healthy volunteers (n = 138). They used the plasma samples without any further specification of primary material and one-step sandwich enzyme immunoassay kit (Fuji Chemical Industries, Toyama, Japan) for their determination. Their results showed a common range of plasma MMP-9 concentration lower than our data and independent of gender or age. They also failed to mention the age variance of their healthy volunteers. It is found from other published studies that the measured concentrations of not only MMP-9 but also of other MMPs in blood are strongly influenced by the sampling procedure and by the type of used anticoagulant agent [18–31]. These reasons may stand for the found differences. Such differences in methodology could underlie the observed discrepancy between these studies.

These normal values were used for comparison of patients with in-stent restenosis after PCI and matched controls.

Percutaneous coronary intervention (PCI) is a rapid transduction of the coronary artery in most cases by means of a balloon catheter. Concomitant implantation of the coronary stent, which prevents the re-narrowing of the coronary artery, has reached a significant improvement in PCI. However, a new complication occurs: stent restenosis (ISR), developing from neointimal hyperplasia, which the basic element, is the transformation of smooth muscle cells from the contractile to the proliferative phenotype. The process of restenosis is initiated by endothelial denudation and deep vascular damage that starts adhesion and platelet activation. Activated platelets exclude growth factors that release smooth muscle cells from growth inhibition and induce their proliferation and subsequent migration from the media into the intimate. Proliferation of smooth muscle cells continues after the platelet phase. Activated smooth muscle cells themselves exclude growth factors that can affect the surrounding cells and help to maintain proliferation and migration. The second fundamental element of restenosis is the production and secretion of extracellular matrix by smooth muscle cells that migrated to the injured intimal zone. A complete neointimal layer can be observed already 2

weeks after implantation of the stent. Maximum restenosis is observed after 3–6 months and remains relatively stable after 1 year [6, 7].

To exclude ISR was used Multi-slice coronarography (MS-CT) coronarography due to non-invasiveness in the control group. Note that, 64- and more-slice MS-CT have almost 99% sensitivities and specificities for the detection of coronary artery stenoses in the native coronary arteries.

Visualization of the lumen inside the metal stents of the coronary artery by MS-CT is more demanding than the assessment of the native coronary artery with respect to the material used for stent manufacturing [35].

Sun [36] and Kumbhani [37] in their study described the advantages of MS-CT in detecting stent coronary restenosis (nearly 1400 and 1500 evaluated stents).

The sensitivity and specificity of MS-CT in detection of ISR were 90 and 91%, with positive and negative predictive values of 68 and 98%, respectively [36, 37].

In this study, the results of ISR patients were compared with those of the control group. Increased plasma concentrations of MMP-3 and MMP-9 were found to be significantly associated with a significant increase in ISR risk. The MMP-9 cut-off value for ISR risk prediction was determined to be ≥64.8 ng/ml. At the same time, no correlation between the MMP-2 values and the ISR occurrence has been demonstrated.

6. Conclusions

Age- and gender-specific reference intervals for heparin-plasma MMP-2, MMP-3 and MMP-9 were established based on a cohort of healthy subjects. Transference studies suggest that these intervals established by enzyme-linked immunosorbent assay are not comparable to published data mainly because of different type of used anticoagulant agent. In addition to this, each laboratory should have these reference intervals checked for its own population according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

At the same time, a statistically significant correlation has been demonstrated between increased MMP-3 and MMP-9 concentrations and an increased risk of ISR. For predicting the risk of ISR, the concentration of ≥ 64.8 ng/ml MMP-9 was determined. No correlation was demonstrated between MMP-2 concentration and ISR occurrence.

We suppose that screening of these biochemical parameters might be helpful to a more detailed risk stratification of patients after percutaneous coronary interventions, who would be able to benefit from implantation of drug-eluting stents.

Acknowledgements

This study was supported by Ministry of Health, Czech Republic—conceptual development of research organization—FNOs/2013 and by the project of European Social Fund and the State Budget of the Czech Republic, registration number CZ.1.07/2.3.00/20.0040.

Conflict of interest

The authors state that there are no conflicts of interest regarding the publication of this chapter.

Author details

Pavlina Kusnierova[1,2]* and Leos Pleva[3,4]

*Address all correspondence to: pavlina.kusnierova@fno.cz

1 Department of Biomedical Sciences, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

2 Department of Clinical Biochemistry, Institute of Laboratory Medicine, University Hospital Ostrava, Ostrava, Czech Republic

3 Department of Cardiovascular Diseases, University Hospital Ostrava, Ostrava, Czech Republic

4 Department of Clinical Studies, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

References

[1] Chen Q, Jin M, Yang F, Zhu J, Xiao Q, Zhang L. Matrix metalloproteinases: Inflammatory regulators of cell behaviors in vascular formation and remodeling. Mediators of Inflammation. 2013;**2013**:928315, 1-14. DOI: http://dx.doi.org/10.1155/2013/928315

[2] Ge J, Shen C, Liang C, Chen L, Qian J, Chen H. Elevated matrix metalloproteinase expression after stent implantation in associated with restenosis. International Journal of Cardiology. 2006;**112**(1):85-90

[3] Jones GT, Tarr GP, Philips LV, Wilkins GT, van Rija AM, Wiliams MJA. Active matrix metalloproteinases 3 and 9 are independently associated with coronary artery in-stent restenosis. Atherosclerosis. 2009;**207**(2):603-607

[4] Katsaros KM, Kastl SP, Zorn G, Maurer G, Wojta J, Huber K, Christ G, Speidl WS. Increased restenosis rate after implantation of drug-eluting stents in patients with elevated serum activity of matrix metalloproteinase 2 and 9. JACC: Cardiovascular Interventions. 2010;**3**(1):90-97

[5] Tarr GP, Williams GT, Wilkins VHT, Chenb LV, Phillips AM, van Rij AM, Jones GT. Intra-individual changes of active matrix metalloproteinase-9 are associated with clinical in-stent restenosis of bare metal stents. Cardiology. 2013;**124**:28-35

[6] Kim MS, Dean LS. In-stent restenosis. Cardiovascular Therapeutics. 2011;**29**(3):190-198

[7] Byrne RA, Joner M, Massberg S, Kastrati A. Restenosis in bare metal and drug-eluting stents. In: Escaned J, Serruys PW, editors. Coronary Stenosis, Imaging, Structure Anfphysiolohy. 1st ed. Toulouse, France: Europa Edition; 2010. pp. 475-496

[8] Sun Z, Davidson R, Lin CH. Multi-detector row CT angiography in the assessment of coronary in-stent restenosis: A systematic review. European Journal of Radiology. 2009;**69**(3):489-495

[9] Solberg HE. International Federation of Clinical Chemistry (IFCC). Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values (EPTRV) and International Committee for Standardization in Haematology (ICSH), Standing Committee on Reference Values. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. Journal of Clinical Chemistry and Clinical Biochemistry. 1987;**25**:645-656

[10] Horowitz GL, Altaie S, Boyd JC, Ceriotti F, Garg U, Horn P, Pesce A, Sine HE, Zakowski J. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. CLSI D

[11] Harris EK, Boyd JC. On dividing reference data into subgroups to produce separate reference ranges. Clinical Chemistry. 1990;**36**(2):265-270

[12] Seber GAF, Wild CJ. Nonlinear regression. New York: John Wiley & Sons; 1989. DOI: ISBN 0471617601

[13] Pleva L, Kusnierova P, Plevova P, Zapletalova J, Karpisek M, Faldynova L, Kovarova P, Kukla P. Increased levels of MMP-3, MMP-9 and MPO represent predictors of in-stent restenosis, while increased levels of ADMA, LCAT, ApoE and ApoD predict bare metal stent patency Biomedical Paper Palacký University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic. 2015;**159**(4):586-594. DOI: 10.5507/bp.2015.037. Epub 2015 Sep 3

[14] Kusnierova P, Vsiansky F, Pleva L, Plevova P, Safarcik K, Svagera Z. Reference intervals of plasma matrix metalloproteinases 2, 3, and 9 and serum asymmetric dimethylarginine levels. Scandinavian Journal of Clinical and Laboratory Investigation. 2015;**75**(6):508-513

[15] Kallenbach K, Salcher R, Heim A, Karck M, Mignatti P, Haverich A. Inhibition of smooth muscle cell migration and neointima formation in vein grafts by overexpression of matrix metalloproteinase-3. Journal of Vascular Surgery. 2009;**49**(3):750-758

[16] Ogata Y, Enghild JJ, Nagase H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. The Journal of Biological Chemistry. 1992;**267**(6):3581-3584

[17] Johnson JL, Dwivedi A, Somerville M, et al. Matrix metalloproteinase (MMP)-3 activates MMP-9 mediated vascular smooth muscle cell migration and neointima formation in mice. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;**31**(9):35-44

[18] Mannello F. Effects of blood collection methods on gelatin zymography of matrix metalloproteinases. Clinical Chemistry. 2003;**49**:339-340

[19] Kodama S, Iwata K, Iwata H, Yamashita K, Hayakawa T. Rapid one-step sandwich enzyme immunoassay for tissue inhibitor of metalloproteinases: An application for rheumatoid arthritis in serum and plasma. Journal of Immunological Methods. 1990;**127**:103-108

[20] Jung K, Nowak L, Lein M, Henke W, Schnorr D, Loening SA. Role of specimen collection in preanalytical variation of metalloproteinases and their inhibitors in blood. Clinical Chemistry. 1996;**46**:2043-2045

[21] Lein M, Nowak L, Jung K, Koenig F, Liuchtinghagen R, Schnorr D, Loening SA. Analytical aspects regarding the measurement of metalloproteinases and their inhibitors in blood. Clinical Biochemistry. 1997;**30**:491-496

[22] Jung K, Laube C, Lein M, Lichtinghagen R, Tschesche H, Schnorr D, Loening SA. Kind of sample as preanalytical determinant of matrix metalloproteinases 2 and 9 and tissue inhibitor of metalloproteinase 2 in blood. Clinical Chemistry. 1998;**44**:1060-1062

[23] Jung K, Lein M, Laube C, Lichtinghagen R. Blood specimen collection methods influence the concentration and the diagnostic validity of matrix metalloproteinase 9 in blood. Clinica Chimica Acta. 2001;**314**:241-244

[24] Jung K, Lein M, Roemer A, Lichtinghagen R. Circulating gelatinase B (MMP-9): The impact of preanalytical step of blood collection. Matrix Biology. 2002;**21**:381-382

[25] Alby C, Abdesselam OB, Foglietti MJ, Beaudeux JL. Preanalytical aspects regarding the measurement of metalloproteinase-9 and tissue inhibitor or metalloproteinase-1 in blood. Clinica Chimica Acta. 2002;**325**:183-186

[26] Mannello F, Luchetti F, Canonico B, Papa S. Effect of anticoagulants and cell separation media as preanalytical determinants on zymographic analysis of plasma matrix metalloproteinases. Clinical Chemistry. 2003;**49**:1956-1957

[27] John M, Jung K. Pre-analytical conditions for the assessment of circulating MMP-9 and TIMP-1: Consideration of pitfalls. The European Respiratory Journal. 2005;**26**:364-366

[28] Jung K, Meisser A, Bischof P. Blood sampling as critical preanalytical determinant to use circulating MMP and TIMP as surrogate markers for pathological processes. International Journal of Cancer. 2005;**116**:1000-1001

[29] Jung K, Gerlach RF, Tanus-Santos JE. Preanalytical pitfalls of blood sampling to measure true circulating matrix metalloproteinase 9 and tissue inhibitors of matrix metalloproteinases. Clinica Chimica Acta. 2006;**373**:180-181

[30] Jung K. Sample processing and its preanalytical impact on the measurement of circulating matrix metalloproteinases. Clinical Chemistry and Laboratory Medicine. 2006; **44**:500-502

[31] Jung K. Impact of blood sampling on circulating tissue inhibitors of metalloproteinases. Clinical Cancer Research. 2006;**12**:2648

[32] Ribbens C, Martiny Porras M, Franchimont N, Kaiser MJ, Jaspar JM, Damas P, Houssiau FA, Malaise MG. Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: Relationship with synovitis and steroid treatment. Annals of the Rheumatic Diseases. 2002;**61**:161-166

[33] Brennan FM, Browne KA, Green PA, Jaspar JM, Maini RN, Feldmann M. Reduction of serum matrix metalloproteinase 3 in rheumatoid arthritis patients following anti-tumor necrosis factor-a (cA2) therapy. British Journal of Rheumatology. 1997;**36**:643-650

[34] Lizasa T, Fujisawa T, Suzuki M, Motohashi S, Yasufuku K, Yasukawa T, Baba M, Shiba M. Elevated levels of circulating plasma matrix metalloproteinase 9 in non-small cell lung cancer patients. Clinical Cancer Research. 1999;**5**:149-153

[35] Rixe J, Achenbach S, Ropers D, Baum U, Kuettner A, Ropers U, Bautz W, Daniel WG, Anders K. Assessment of coronary artery stent restenosis by 64-slice multi-detector computed tomography. European Heart Journal. 2006;**27**:2567-2572

[36] Sun Z, Marzouq A, Almutairi D. Diagnostic accuracy of 64 multislice CT angiography in the assessment of coronary in-stent restenosis: A meta-analysis. European Journal of Radiology. 2010;**73**:266-273

[37] Kumbhani DJ, Ingelmo CP, Schoenhagen P, Curtin RJ, Flamm SD, Desai MY. Meta-analysis of diagnostic efficacy of 64-slice computed tomography in the evaluation of coronary in-stent restenosis. American Journal of Cardiology. 2009;**103**:1675-1681

IntechOpen