Inflammatory markers in psoriasis

Doctoral (Ph.D.) theses

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2019

Abbreviations

ADMA: asymmetric dimethylarginine
ACEI: angiotensin converting enzyme inhibitor
AMI: acute myocardial infarction
APACHE II: acute physiology and chronic health evaluation II
DBP: diastolic blood pressure
DDAH: dimethylarginine dimethyl aminohydrolase
ELISA: enzyme linked immunosorbent assay
HPLC: high performance liquid chromatography
Hs-CRP: high-sensitivity C reactive protein
ICU: intensive care unit
IQR: interquartile range
L-arg: L-arginine
LC/MS: liquid chromatography / mass spectrometry
MDA: alondialdehyde
NO: nitric oxide
NOS: nitric oxide synthase
PTC: procalcitonin
RAAS: renin-angiotensin-aldosterone system
SAPS II: simplified acute physiology score II
SBP: systolic blood pressure
SD: standard deviation
SEM: standard error of mean
SOFA: Sequential organ failure assessment
WBC: white blood cell count
LDH: lactate dehydrogenase

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I. Introduction

Cardiovascular diseases are the most common diseases worldwide. They are responsible for one third of global deaths and they are the leading cause of disability, too. The usage of different levels of prevention in combination with effective risk assessment improved these statistical data. Risk assessment based on classic risk factors has recently been supported with several new markers, such as asymmetric dimethylarginine (ADMA) which is an endogenous competitive inhibitor of nitric oxide synthase (NOS). Approximately 15% of the generated ADMA is excreted through the renal system. The remaining amount is degraded by the dimethylarginine dimethyl aminohydrolase (DDAH) enzyme, which is impaired by oxidative stress.

Moreover, ADMA has been shown capable of uncoupling electron transport between L-Arg and NOS resulting in production of reactive oxygen species. Accordingly, ADMA can be a useful marker and mediator of oxidative stress. Compared to healthy controls elevated ADMA concentrations were found in patients suffering from hypertension, coronary artery disease (CAD), heart failure, stroke, obesity, diabetes mellitus, kidney injury and even inflammatory bowel diseases, which are of high public health significance. Correlation between intima media thickness and ADMA concentrations was also demonstrated

Previous studies showed that ADMA is a suitable indicator of endothelial dysfunction, which is held to be the previous state of atherosclerosis. Several researches found positive correlation between higher levels of ADMA and coronary artery disease onset, or progression of existing coronary disease. According to a study involving 3000 patients, asymmetric dimethylarginine is an independent risk factor of cardiovascular mortality in patients with coronary artery disease.

Moreover, NO pathway and ADMA seems to play an important role in the pathophysiology of sepsis. Sepsis syndrome remains one of the most challenging healthcare issues worldwide. The prominently high mortality rate (approximately 30%) and costs of care (22,000 USD/case) makes it a remarkable disease. Septic shock belongs to the leading causes of death in intensive care units, even nowadays. Early diagnosis and goal-directed therapy are essential for favorable outcome. Beside of the clinical signs and symptoms, laboratory parameters are essential in proper decision-making. Monitoring the septic process is an essential part of successful therapy; therefore, biomarkers with predictive capacity would be of utmost importance, which are unfortunately not available in the current management of sepsis. NO is a crucial mediator in the inflammatory activation process. During sepsis, NO overproduction plays a major role in the development of hemodynamic instability and organ failures. Due to uncoupling of NOS, excessive production of free radicals, particularly peroxynitrite, leads to oxidative cell injury.

Previous studies have shown that NOS inhibitors like endogenous dimethylarginines take considerable role in sepsis by modulating NO related biochemical pathways.

II. Aims

II.1. ADMA and sepsis

We aimed to investigate the changes of ADMA levels and its connection with routine laboratory parameters and scores in septic patients.

II.2. ADMA levels and CO2 treatment

We aimed to investigate the effect of CO2 therapy on ADMA levels in hypertensive patients. Furthermore we aimed to find connection between the changes of ADMA levels and antihypertensive drugs.

II.3. ADMA reference range

We aimed to determine the reference range of plasma ADMA in healthy adults by performing a systematic review and a meta-analysis.

III. Materials and Methods

II.1. ADMA and sepsis

The present study was performed at our multidisciplinary adult ICU from January 2015 to April 2015. Severe septic patients were enrolled and followed- up for 5 days. Severe sepsis was defined as recommended by the current consensus and guidelines. Inclusion criteria were survival of intensive care unit (ICU) stay, sepsis-induced organ dysfunction, hypoperfusion abnormalities or hypotension and procalcitonin levels of >2 ng/ml. The first sample was obtained within 24 hours after clinical diagnosis and further samples were taken on the 3rd and 5th days, respectively. Sequential organ failure assessment (SOFA) scoring system was used to describe organ dysfunctions. The study protocol was approved by the Regional Ethics Committee of University of Pécs (permission No.: 4327.316- 2900/KK15/2011.) in accordance with the 2008 Helsinki declaration. Informed written consent was obtained from every patient. Exclusion criteria were history of chronic kidney disease, acute myocardial infarction (AMI), stroke, likelihood risk of death due to primary disease and withdrawal of consent. Age- and gender-matched patients with similar medical history were recruited for control group. Only one sample was obtained from the control patients. Daily routine parameters (white blood cells (WBC), high-sensitivity Creactive protein (hs-CRP), procalcitonin (PCT), lactate

dehydrogenase (LDH), creatinine, urea, etc.) were measured in our university clinical laboratory on automated analyzers by manufacturer's protocol. Clinical data, like organ dysfunction parameters (blood pressure, urine output, drugs, etc.), were registered daily. For the assessment of disease severity and mortality prediction, the simplified acute physiology score II (SAPS II), the acute physiology and chronic health evaluation II (APACHE II) and the SOFA scores were calculated. Patients surviving ICU were considered to be survivors. Blood samples were taken using both from septic and control patients besides the daily blood collection for routine laboratory tests. After sample preparation, L-arginine, ADMA and SDMA levels were determined by liquid chromatography-tandem mass spectrometry method described by Martens-Lobenhoffer et al. Statistical analysis was performed by IBM SPSS Statistics for Windows Version 22 (IBM Corp., New York, NY, USA).

II.2. ADMA levels and CO2 treatment

The present study has been performed at our ISO 9001 accredited Cardiology Rehabilitation Inpatient Unit from April 2016 to November 2016. Non-smoker, abstinent, hypertensive patients with an ejection fraction over 55% were enrolled. Patients who had previously received CO2 therapy were excluded. Moreover, patients who had suffered from myocardial infarction, stroke or undergone open surgery less than a year before the study were also excluded. Additionally, individuals diagnosed with cancer or kidney injury were also excluded. To monitor the changes of plasma ADMA concentrations, blood samples were obtained one hour before and 1 hour, 24 hours and 3 weeks after the first CO2 treatment, respectively. The patients received three transcutaneous CO2 treatments per week for 3 weeks. CO2 gas was administered for 35 minutes in a plastic bag sealed at mid-thoracic level, as previously described by Fabry et al. Clinical data (medical history, age, weight, height, drugs, ejection fraction, laboratory data, etc.), were registered by the same investigator, respectively. Healthy individuals were recruited for the control group. Controls did not undergo CO2 treatment. Only one sample was obtained from controls. Informed written consent was obtained from every patient. The study protocol was approved by the Regional Ethics Committee of University of Pécs, Pécs, Hungary (Permission No.: 5919.), in accordance with the 2008 Helsinki declaration. Plasma ADMA concentrations were determined by ELISA. Statistical analysis was performed by IBM SPSS Statistics for Windows Version 22 (IBM Corp., New York, NY, USA).

II.3. ADMA reference range

On June 30th 2016 a comprehensive literature search was performed in Medline and Web of Science using the following keywords: “asymmetric dimethylarginine” AND “healthy” NOT “animal”. Literature search and managing of references were performed using “End- Note X7 software”.

To be included in full text evaluation records had to: 1, report plasma ADMA concentrations; 2, report ADMA concentrations of healthy individuals; 3, report the method of ADMA analysis; 4, report20 patients. Review articles, meta-analyses and measurement methodical studies, were excluded. To analyze the data of an adult population papers reporting ADMA concentrations from individuals under the age of 18 were dropped. Furthermore, due to well-known endocrinological changes, studies investigating pregnant individuals were excluded. After full text evaluation, to be included in quantitative analysis articles had to: 1, report ADMA values measured by either ELISA or HPLC; 2, report ADMA concentrations numerically; 3, state and/or indicate in “patients characteristics” that the controls are healthy individuals; 4, refer or report the method of ADMA measurement in detail. Before statistical analysis another detailed review was performed to reveal diseases (e.g. hypertension, diabetes, obesity, etc.) and sample origin. Papers reporting any unhealthy individuals or other samples than plasma (e.g. serum, urine, cell cultures, ect.) were excluded from the final database. The final database contained the following parameters: name of the first author, publication date, ADMA levels in μmol/L, number of participants, age of participants, gender distribution, the applied method, percentage of smokers, country and region of the study, SBP, DBP and BMI. Continuous variables were recorded as mean + standard deviation (SD) or standard error of mean (SEM) or median + interquartile range (IQR).

Normal approximation was used for the meta-analysis, both for the mean and for the reference interval. Reference interval was calculated as mean ± 1.96SD (under the assumption of normality, this has a coverage of 95%). Confidence interval for the endpoints of the reference interval was also calculated with normal approximation. Both fixed effects and random effects models were estimated, but due to the extreme heterogeneity, only the results of the random effects models are presented. The models were estimated using restricted maximum likelihood. Effects of moderator variables were studied with standard meta-regression approach. All calculations were performed under R statistical program package version 3.3.2.

IV. Results

II.1. ADMA and sepsis

In the present study 17 severe septic patients and 16 age-, gender- and medical history- matched control individuals were enrolled. Demographic data are represented in Table 1.

	Control group	**Septic patients**
Number of patients	16	17
Mean age, years (± SD)	61 (13)	66 (15)
Male, n (%)	9 (56)	8 (47)
Hypertension, n (%)	13 (81)	14 (82)
Pulmonary disease, n (%)	6 (38)	8 (47)
Diabetes Type 2, n (%)	4 (24)	4 (24)

Table 1 Demographic data of the involved patients

Among 17 septic patients 12 suffered from septic shock, the remaining five patients developed sepsis-induced organ dysfunctions. characteristics of the involved patients are shown in Table 2.

	Septic patients
Type of admission	
surgical, n (%)	11 (65)
non-surgical, n (%)	6 (35)
Length of ICU stay, days (IQR)	7 (3-8)
SOFA score, (IQR)	7 (5-9)
SAPS II score, (IQR)	39 (34-51)
APACHE II score, (IQR)	16 (12-23)

Table 2 Clinical characteristics of septic patients. Median data and IQR are presented.

The baseline of controls and follow-ups of septic ADMA, SDMA, L-arginine, ADMA/SDMA ratio and L-arginine/ADMA ratio are shown on our figures (Fig. 1 and Fig. 2). Both hs-CRP and PCT showed decreasing tendency during the follow-up period. Hs-CRP and PCT levels decreased significantly from day 1 to day 3 ($p<0.005$), further decrease was found from day 3 to day 5 ($p<0.005$). There were no significant differences in ADMA concentrations between controls and septic patients on the first follow-up day. However, ADMA was significantly higher in septic patients on day 3 ($p=0.001$) and day 5 ($p=0.003$) compared to controls. During

the follow-up ADMA increased significantly from day 1 to day 3 (p=0.003), afterwards on day 5 ADMA decreased but remained significantly higher than on day 1 (p=0.027). Patients suffering from more than three organ failures had significantly higher ADMA concentrations compared to patients with less than three organ failures (0.573 vs. 0.425 mmol/L, p=0.018). Regarding the first follow-up day higher ADMA concentrations were found in patients with SOFA >10 compared to SOFA <10 patients (0.542 vs. 0.307 mmol/L, p=0.004). ADMA levels correlated well with SOFA scores (0.587, p=0.013). On the first follow-up day lower L-arginine concentrations were measured in septic patients compared to controls (p<0.001). However, a significant elevation was found from day 1 to day 3 in sepsis, (p<0.001), afterwards L-arginine levels decreased significantly from day 3 to day 5 (p=0.004). Control patients had significantly higher L-arginine/ADMA ratio than septic patients on day 1 (p<0.001), afterwards the ratio showed an increasing non-significant tendency. Finally on day 5 it decreased and remained significantly lower compared to controls (p=0.007). We found significantly increased L-arginine/ADMA ratio from day 1 to day 3 (p=0.005). Moreover on day 5 L-arginine/ADMA ratio decreased significantly compared to day 3 (p=0.023). Patients with sepsis-induced hypoperfusion showed significantly elevated L-arginine/ADMA ratios (p=0.015).

Control patients had significantly higher ADMA/SDMA ratio than septic patients on day 1 (p<0.001). Regarding the septic patients significantly increased ADMA/SDMA ratio was found from day 1 to day 3 (p=0.015), afterwards the ratio levels decreased significantly from day 3 to day 5 (p=0.036).

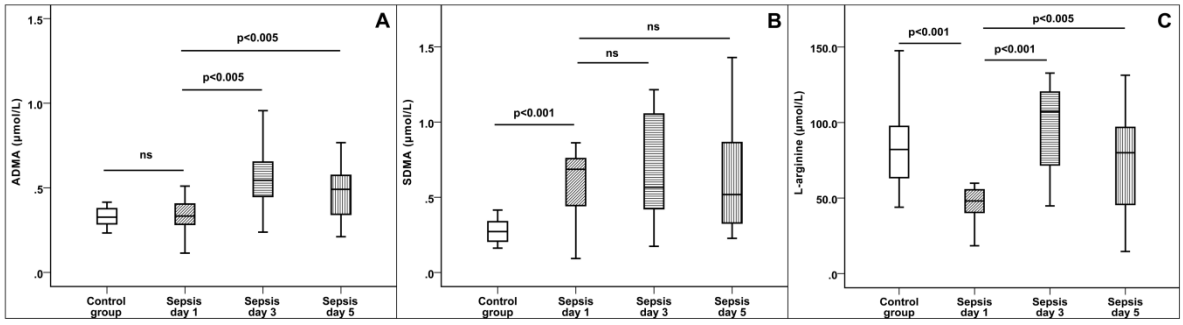

Fig. 1 Monitoring of endogenous dimethylarginines (A, B) and L-arginine (C) in sepsis (ns: non-significant)

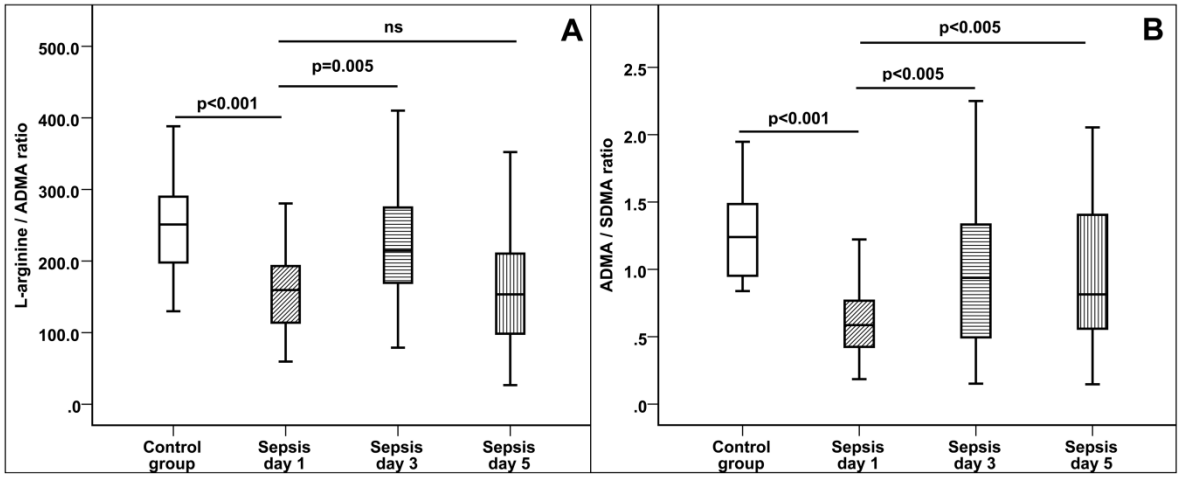

Fig. 2 L-arginine/ADMA ratio (A) and ADMA/SDMA ratio (B) in sepsis (ns: non-significant)

II.2. ADMA levels and CO2 treatment

We enrolled 47 patients and 30 controls. Clinical characteristics of the subjects are shown in Table 3.

	Control group (n=30)	Patient group (n=47)	P-value
Mean age, years	28 ± 8.4	67±12.7	<0.001
Male, n (%)	13 (43)	20 (43)	0.808
Diabetes Type 2, n (%)	-	18 (38)	-
CABG, n (%)	-	10 (21)	-
MI, n (%)	-	8 (17)	-
BMI, kg/m^2	25.2 ± 3.8	29.1 ± 4.7	0.706
WBC count, G/L	6.53 ± 1.81	6.12 ± 1.36	0.238
ADMA 0, µmol/L	0.35 ± 0.07	0.43 ± 0.03	0.018
ADMA 1h, µmol/L	-	0.38 ± 0.02	-
ADMA 24h, µmol/L	-	0.41 ± 0.03	-
ADMA 3w, µmol/L	-	0.40 ± 0.08	-

Table 3. Clinical characteristics of the participants. CABG: coronary artery bypass surgery, MI: myocardial infarction, BMI: body mass index, WBC: white blood cell count, EF: ejection fraction, ADMA: asymmetric dimethylarginine; Mean ± SD values are presented.

Baseline plasma ADMA concentrations were significantly higher in the patients compared to the controls (0.41 µmol/l vs. 0.35 µmol/l; p=0.018). Patients suffering from diabetes mellitus had significantly higher baseline plasma ADMA concentrations compared to non-diabetic

patients (0.47 μmol/l vs. 0.37 μmol/l; p=0.038). Relatively weak but significant positive correlation was found between baseline ADMA levels and age (p=0.011, r=0.392). ADMA levels decreased significantly one hour after the first CO2 treatment compared to the baseline concentrations (p=0.003; Figure 3).

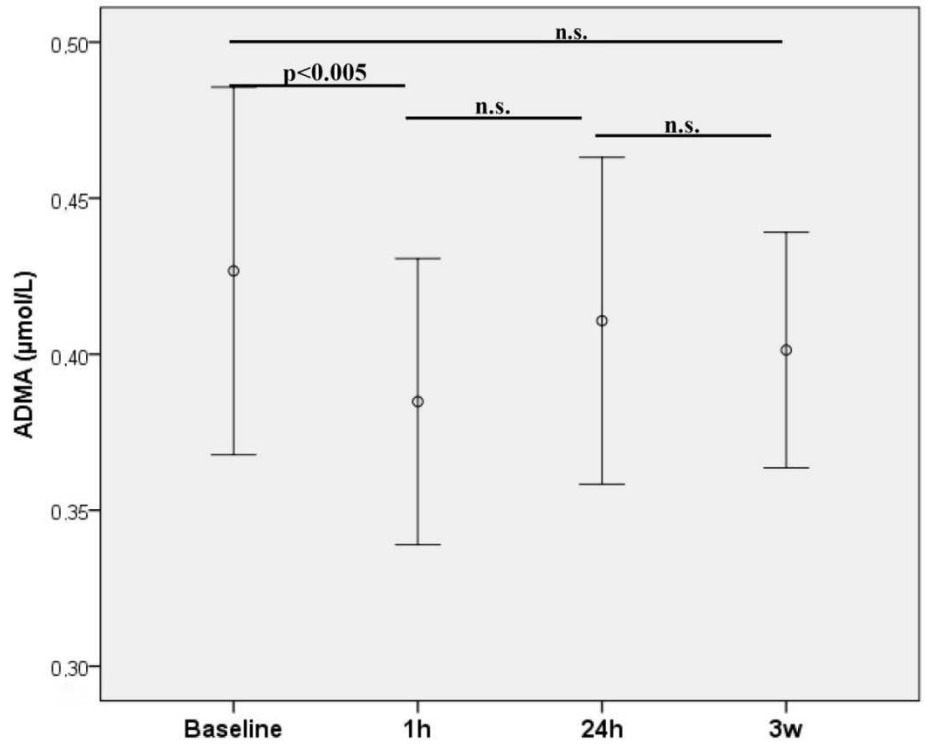

Figure 3. Plasma ADMA levels (mean and SD) of the involved controls and follow-ups of the patients. ADMA: asymmetric dimethylarginine,

Twenty-four hours after the treatment, ADMA levels increased approximately to the baseline. Comparing ADMA concentrations measured 1 and 24 hours after the first CO2 treatment, a modest but statistically not significant increase was observed. After receiving 9 CO2 treatments in an interval of 3 weeks, ADMA levels were found to be modestly lower than the baseline (p=0.210). We investigated the effects of baseline medication (angiotensin converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers, beta-receptor blockers, diuretics, antidiabetics, antiplatelet therapy, proton pump inhibitors, H2-receptor blockers) on the lowering of ADMA levels that was demonstrated one hour after the first CO2 treatment. Significantly greater reduction was only found among patients in whom ACEIs were administered (p=0.019). Other medications showed no effects on ADMA levels.

II.3. ADMA reference range

Using the method discussed above 914 citations were identified on June 30th 2016. After dropping duplicates 642 abstracts were reviewed. After abstract screening, 183 records were included in full text evaluation. . Detailed reasons of exclusions are given in Fig 4.

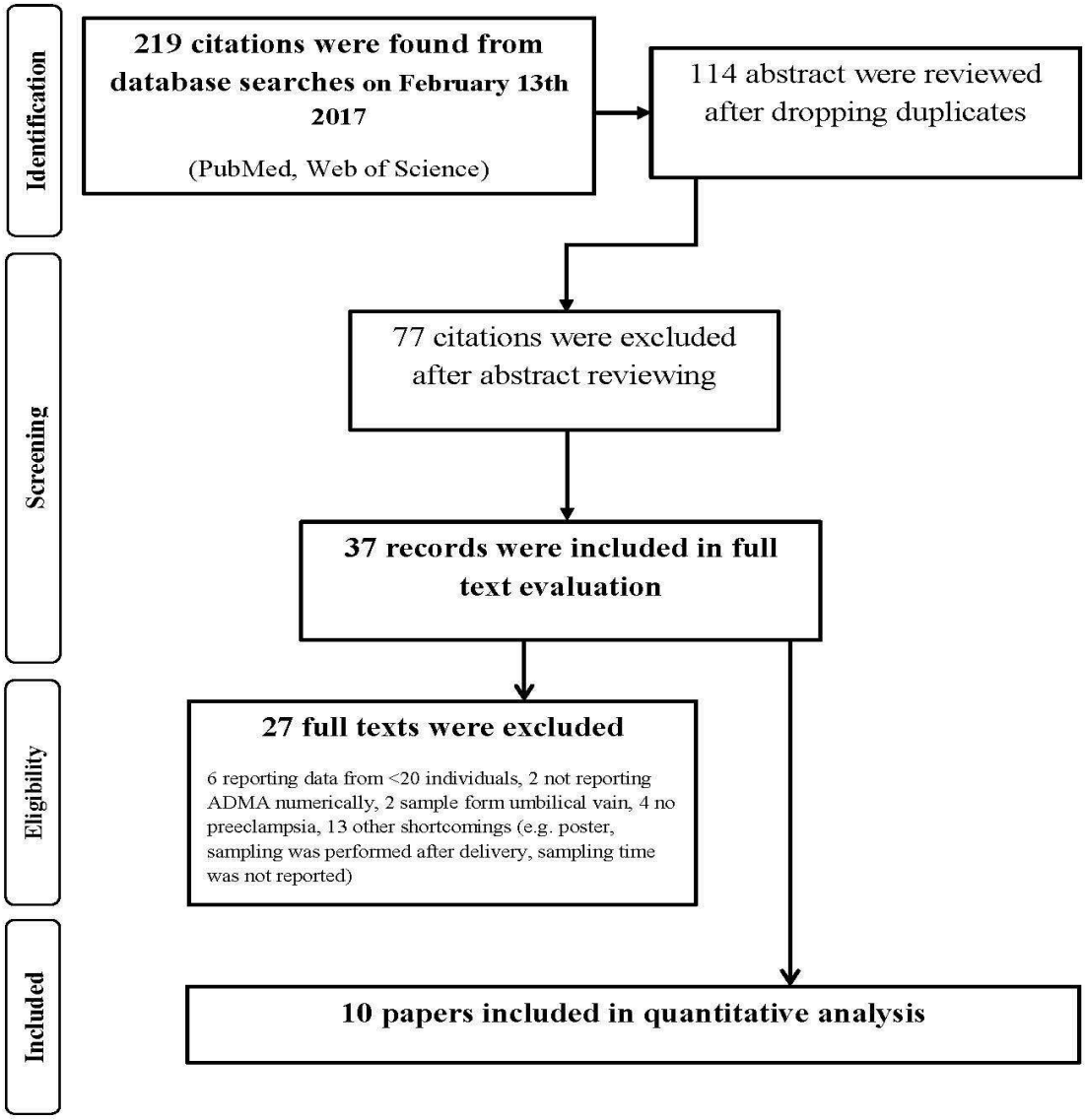

Figure 4. Study flow diagram.

Eventually, 66 studies were included in the quantitative analysis (24 using ELISA and 44 using HPLC). The total number of healthy individuals identified was 5528 (3178 men and 2350 women, 41.6 ± 16.9 years old). The majority of the studies involved in our article were undertaken in Europe (HPLC: 78.7%, ELISA: 93.1%). Due to the character of this meta-analysis most of the involved articles are case-control studies. 42 studies using HPLC were

included in the statistical analysis. 36 of these articles referred to a HPLC method using fluorescence detection, the remaining 6 studies used LC/MS detection to measure ADMA concentrations. 24 studies using ELISA were involved in the quantitative analysis. 11 used ELISA kits purchased from DLD Diagnostika GmbH (Hamburg, Germany), 12 used ELISA kits purchased from Immundiagnostik (Bensheim, Germany) the remaining 1 referred to a method developed by Schulze et al. The reference range of ADMA (in μmol/l) was 0.34 (0.29±0.38)± 1.10 (0.85±1.35) with a mean of 0.71 (0.57±0.85) (n = 4093) measured by HPLC and 0.25 (0.18±0.31)± 0.92 (0.76±1.09) with a mean of 0.57 (0.48±0.66) (n = 1435) by ELISA. Detailed results for each subgroup are given in. Overall, with both methods combined, ADMA had a reference range of 0.30 (0.27±0.34)± 1.03 (0.87±1.20) (mean: 0.66 [0.56±0.75]).

V. Discussion

II.1. ADMA and sepsis

Several studies investigated plasma dimethylarginine levels is sepsis. However, in some studies more than 24 hours elapsed between sepsis diagnosis and sample collection, consequently initial stage of sepsis was missed, while others were only limited to two samples. Only two relevant studies investigated L-arginine, ADMA and SDMA levels together. One of these used an inaccurate sampling protocol, taking blood samples at the onset of sepsis and between the 2nd and 4th day. Another study missed to report the changes of L-arginine and L-arginine/ADMA ratio, thereby lacking the information of NO bioavailability. The precise regulation of NO system is essential to survive sepsis. Insufficient production of NO impairs the activity of antimicrobial system. However, uncontrolled NO production can lead to uncontrollable hypotension and massive oxidative stress, which can result in organ failure and death. These findings delineate the effects of L-arginine and dimethylarginines on NO system during sepsis. ADMA levels are considered to be associated with sepsis survival due to its significant role in vascular reactivity, microcirculation and organ perfusion. This association was confirmed even in this few-patient population by revealing significant correlation between ADMA levels and SOFA score measured and calculated on day 1. Regarding organ dysfunctions, SDMA seem to be an early marker of acute kidney injury as described in 3.2 which can be explained by its urinary excretion. The initially decreased L-arginine levels showed on Fig. 1 are in line with literature explained by massive catabolic state and reduced „de novo” L-arginine production. The causes of increased L-arginine levels during the course of sepsis are controversial. As described in subsection 3.1 and shown on Fig. 1, blood levels of L-arginine and ADMA

elevating significantly from day 1 to day 3 which is corresponding with the so called „L-arginine paradox". Namely, ADMA levels are high enough to impair the function of eNOS enzyme making it unable to convert L-arginine to NO despite the sufficient amount of L-arginine. Afterwards both L-arginine and ADMA show non-significant decreasing tendencies, but remain still significantly higher than on the onset of sepsis. This can be explained by the efficient treatment which is indicated by the decreasing tendencies of hs-CRP and PCT and high survival rate. L-arginine/ADMA ratio is considered as an indicator of NO bioavailability. The crucial importance of NO levels in sepsis is proved by several studies. Regarding the onset of sepsis we found decreased L-arginine/ADMA ratio which can indicate reduced endothelial NO production due to reduced availability of L-arginine to NOS. The elevation of L-arginine/ADMA ratio experienced from day 1 to day 3 can be once again dedicated to the „L-arginine paradox". Interestingly comparing L-arginine/ADMA ratio calculated on day 1 to day 5 showed no significant differences. The results provided by Brenner et al. showed similar trends in changes of L-arginine/ADMA ratio, however with a 2-day right shift in time. Due to the high survival rate and decreasing tendencies of PCT and hs-CRP L-arginine/ADMA ratio showed on Fig. 2 could be a survival pattern. In addition ADMA/SDMA ratio shows an increasing trend from day 1 to day 3 and from day 3 to day 5 which is very similar to the time course of survival patients demonstrated by Iapichino et al.

II.2. ADMA levels and CO2 treatment

The observed baseline elevation in ADMA concentration is probably due to the ongoing disease. Young individuals were enrolled as controls to rule out possible undiscovered diseases that could alter ADMA concentrations. In line with literature data, elevated ADMA levels were found in patients suffering from diabetes mellitus and positive correlation was found between ADMA and age. To the best of our knowledge, this is the first study investigating the effect of CO2 treatment on ADMA levels. The prompt decrease of ADMA levels demonstrated 1 hour after CO2 treatment indicates that the treatment had beneficial effects on the NO pathway, possibly due to vasodilation. Thus, ADMA levels can represent the current vascular state. Such rapid changes have already been reported previously while monitoring ADMA levels during on-pump and off-pump cardiac surgery. The authors related the changes of ADMA concentrations to the intensity of inflammation and oxidative stress. After the initial decrease of ADMA concentrations, we observed an increase within 24 hours after the CO2 treatment, which may be due to a rebound effect explained by the subsiding of the vasodilator effect. In a recent study,

Bolevich et al. have proved that CO2 is “a universal inhibitor of oxidative stress”. Reduced oxidative stress results in an increased efficiency of DDAH leading to decreased ADMA levels. In line with these findings, long-term CO2 treatment can decrease the production of reactive oxygen species, which is indicated by the decreasing tendency of ADMA observed after receiving 9 CO2 treatments within 3 weeks. Besides presenting the changes of ADMA levels after CO2 treatment, significantly greater short-term ADMA reduction was found among 25 patients in whom ACEIs were administered. Only a few studies have investigated the relation between ADMA and ACEIs. Veresh et al. showed that ADMA activates the renin-angiotensin-aldosterone system (RAAS), which leads to vasoconstriction and increased oxidative stress. Moreover, Ito et al. found reduced ADMA levels among hypertensive patients after being treated with ACEIs. Napoli et al. investigated the influence of zofenopril on oxidative stress. ADMA and malondialdehyde (MDA) were used to monitor the oxidative changes. After administering zofenopril, both ADMA and MDA levels decreased. The authors suggest that zofenopril alters the NO pathway and reduces oxidative stress. Moreover, ACEIs have been shown to stimulate NO production through their “bradykinin-sparing” property. Interestingly, in the present study, baseline ADMA levels were not shown to be significantly lower in patients receiving ACEIs compared to those without ACEIs. However, according to our results and previous findings, ACE inhibition is not only protective against oxidative stress, but it can also improve vascular reactivity demonstrated by the short-term changes of ADMA after CO2 treatment.

II.3. ADMA reference range

The results of this study are based on the plasma ADMA levels of 5528 apparently healthy individuals, which is eligible to calculate a proper reference interval. Most of the studies provided narrow mean ADMA concentrations with low standard deviation, which indicates that the involved subjects had similar ADMA levels. Seemingly, the number of participants suffering from ADMA altering conditions was low. Moreover, according to the meta-regression, age had no significant influence on ADMA levels, which can indicate that the involved individuals were indeed healthy. The study identification and selection method used in this manuscript were sufficient to enroll a population, which is appropriate for analysis. The aim of our study was to provide a proper reference interval for plasma ADMA. However, due to the high heterogeneity the interpretation of results can be challenging. The practical usage of the relatively wide reference interval determined by this meta-analysis is limited. This can be

explained by pre-analytical errors, the usage of different laboratory equipment and evaluation softwares, especially in the case of studies using HPLC. Some studies using HPLC provided noticeably higher ADMA levels. In the case of the study published by Zincir et al., the correct separation of asymmetric dimethylarginine from symmetric dimethylarginine can be doubted, however, ADMA and SDMA levels are presented separately. Turkcuoglu et al. obtained nearly double plasma ADMA concentration as referred to in the methodological instructions of the kit that they have used; nevertheless, the possible reason for this is not discussed by the Authors. Despite the fact that, these studies might have been biased by some methodological errors, they were not excluded because they were found eligible according to the criteria stated in materials and methods. Interestingly, in the case of studies using ELISA, high heterogeneity persisted even in testing studies using ELISA kits developed by the same manufacturer. Previous comparative studies found ELISA to overestimate plasma ADMA concentrations compared to HPLC.

In 2007 Horowitz et al. published a non-systematic overview, which included the plasma and serum ADMA levels of 2371 healthy individuals. Taking into account that the focus of this study is on the ADMA measurement methodological considerations, the comparability of mean plasma ADMA levels provided by this paper and the current meta-analysis is limited. To the best of our knowledge, this is the first systematic review and meta-analysis investigating plasma ADMA levels in healthy individuals. Interestingly, the available data have shown opposite results regarding the plasma ADMA concentrations measured by HPLC and ELISA. Our study points out how analytical differences can result in almost incomparable results in the case of ADMA levels determined by HPLC and ELISA methods. Nevertheless, the results of this meta-analysis bring up several methodological questions connected to ADMA measurement, which could be answered by prominent researchers of the field.

VI. Theses

1. ADMA and sepsis

We monitored the changes of L-arginine, ADMA and SDMA during sepsis(first, third and fifth day).

We achieved to identify an L-arginine/ADMA “survival pattern” during sepsis. Namely, L-arginine/ADMA ratio increased significantly from day 1 to day 3 and decreased significantly from day 3 to day 5.

2. ADMA levels and CO_2 treatment

We monitored the short term (1h and 24h) and long term (3week) changes of ADMA levels after CO_2 treatment.

Significantly lower ADMA levels were found 1h after the CO2 treatment compared to the baseline values.

Significantly greater AMA reduction was found among patients in whom ACEIs were administered.

3. ADMA reference range

We performed a comprehensive literature search in Medline and Web of Science using the following keywords: “asymmetric dimethylarginine” AND “healthy” NOT “animal” in 2016.

We determined the plasma ADMA reference interval for healthy adults for both ELISA and HPLC measurement techniques

We pointed out that analytical differences can result in almost incomparable results in the case of ADMA levels determined by HPLC and ELISA methods.

VII. Publications

Publications related to the Theses

1. Balázs Németh, István Kiss, Iván Péter, Zénó Ajtay, Ádám Németh, László Márk, Attila Csorba, Tamás Kőszegi, Diána Mühl, Péter Kustán.
   Monitoring of L-arginine and Endogenous Dimethylarginines in Survivor Septic Patients – A Pilot Study. **In vivo** 30:(5) pp. 663-669 (2016) (Q2; **IF=0.953**)
2. Péter Iván, Jagicza Anna, Ajtay Zénó, Kiss István, Németh Balázs.
   A psoriasis és az oxidatív stressz. **Orvosi Hetilap** 157:(45) pp. 1781-1785. (2016) (Q3; **IF=0.349**)
3. Németh Balázs, Kustán Péter, Németh Ádám, Lenkey Zsófia, Cziráki Attila, Kiss István, Sulyok Endre, Ajtay Zénó.
   Aszimmetrikus dimetilarginin: a cardiovascularis betegségek prediktora? **Orvosi Hetilap** 157:(13) pp. 483-487. (2016) (Q3; **IF=0.349**)
4. Balázs Németh, István Kiss, Tímea Jencsik, Iván Péter, Zita Kreska, Tamás Kőszegi, Attila Miseta, Péter Kustán, Zénó Ajtay.
   Angiotensin converting enzyme inhibition improves the effectiveness of transcutaneous carbon dioxide treatment. **In vivo** 31:(3) pp. 425-428 (2017) (Q2, IF=**1.116**)
5. Balázs Németh, Zénó Ajtay, László Hejjel, Tamás Ferenci, Edit Murányi, István Kiss.
   The issue of plasma asymmetric dimethylarginine reference range – a systematic review and meta-analysis. **PLOS ONE** 12: (5) p. e0177493. (2017) (Q1, **IF= 2.766**)

Publications not related to the Theses

Kustán Péter, Horváth-Szalai Zoltán, Németh Balázs, Török Csaba, Ragán Dániel, Kőszegi Tamás, Mühl Diána.A szepszis diagnózisa napjainkban. **Magyar Epidemiológia** 12:(1-2) pp.59-66. (2016)

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VIII. Acknowledgement

Firstly, I would like to express my sincere gratitude to my supervisors and mentors Prof. Dr. István Kiss and Dr. Zénó Ajtay. for the continuous support of my Ph.D study and related research, for his patience, motivation, and immense knowledge. Besides my mentors, I would like to thank Dr. Péter Kustán, Attila Csorba and Prof. Dr. Tamás Kőszegi for their invaluable help in the laboratory measurements. Furthermore I have to express my special thanks to the nurses of the Heart Institute and the Zsigmondy Vilmos Spa Hospital for their help in sample collection. Last but not the least, I would like to thank my family for supporting me spiritually throughout writing this thesis and my my life in general.

My work was supported by the ÚNKP-17- 3-III and ÚNKP-18- 3-III New National Excellence Program of The Ministry of Human Capacities.