Translational Oncology 14 (2021) 100890

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Original research

Plasma thiobarbituric acid reactive substances predicts survival in chemotherapy naïve patients with metastatic urothelial carcinoma

Jan Slopovsky [a,b,*], Jarmila Kucharska [c], Jana Obertova [a,b], Michal Mego [a,b,d], Katarina Kalavska [a,d], Silvia Cingelova [b], Daniela Svetlovska [a,b], Anna Gvozdjakova [c], Samuel Furka [e], Patrik Palacka [a,b]

[a] 2nd Department of Oncology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[b] National Cancer Institute, Bratislava, Slovakia
[c] Pharmacobiochemical Laboratory of the 3rd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[d] Translational Research Unit, Faculty of Medicine, Comenius University, Bratislava, Slovakia
[e] Department of Physical and Theoretical Chemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 4 July 2020
Received in revised form 10 September 2020
Accepted 10 September 2020

ABSTRACT

Oxidative stress plays a significant role in development and progression of cancer, including urothelial carcinomas. TBARS (Thiobarbituric acid reactive substances) represents a marker of oxidative stress increased in various diseases. In this prospective study, we tested the hypothesis of plasma TBARS concentration and correlation with survival in chemotherapy naïve MUC (metastatic urothelial carcinoma) patients. Most of subjects ($N = 65$) were treated with gemcitabine and cisplatin (GC) chemotherapy. Performance status ECOG ≥ 2 had 11 patients, visceral metastases were present in 43. Based upon the mean of plasma TBARS, subjects were dichotomized into low and high groups. Progression-free survival (PFS), overall survival (OS) and their 95% CI were estimated by Kaplan-Meier method and compared by log-rank test. At median follow-up of 9.6 months, 65 patients experienced progression and 64 died. Subjects with low TBARS had significantly better PFS (HR 0.51) and OS (HR 0.44) opposed to high TBARS. Patients with low TBARS had significantly higher rate of neutropenia G4 and less liver involvement. High TBARS correlated with BMI above 30 kg/m^2. Performance status and plasma TBARS were proven to be independent predictors of PFS and OS. In this study, high TBARS in MUC patients were associated with poor survival, likely due to more aggressive disease activity as reflected in increased liver involvement. Therefore, this biomarker could be used in clinical practice for early identification of patients with worse prognosis, better patient stratification, and treatment decision making.

Introduction

Bladder cancer is the most common malignancy of the urinary tract and due to its poor prognosis and highest recurrence rates compared to any cancer, it imposes a great public hazard and substantial health-care burden [1]. Worldwide, it is the 9th most common cancer with crude incidence of 20.4/100000 in men [2]. Transitional cell subtype is seen in 90% of all cases, the rest 10% is comprised of squamous-cell carcinoma and adenocarcinoma. In general, transitional cell carcinoma is classified as non-muscle invasive bladder cancer (NMIBC) stage pTa or pT1 and muscle-infiltrating bladder cancer (MIBC) stage pT2 or more. Approximately 20–30% of patients at the time of diagnosis present with MIBC and with or later develop metastasis, currently treated mostly by a standard gemcitabine-cisplatin chemotherapeutic regimen [3].

Urothelial cancer development is a multifactorial process ranging from genetic to environmental stimuli like tobacco smoke, heavy metals and other xenobiotics [4]. Some of these factors, *e.g.* smoking, are able to activate immune system cells and therefore induce chronic inflammation, which promotes the development of oxidative stress [5].

Oxidative stress is an imbalance between the production and elimination of reactive oxygen species (ROS). ROS are responsible for alteration of macromolecules. To prevent oxidative damage, cells possess various antioxidant defense mechanisms such as superoxide dismutase (SOD) and catalase.

Various mechanisms have the ability to affect ROS formation, such as aberrant metabolism of cancer cells, activation of oncogenes, mitochondrial dysfunction or loss of p53 function [6]. Chronic irritation or inflammation, infections or cytokines and growth factors also increase ROS formation in

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; GC, gemcitabine + cisplatin; MDA, malondialdehyde; MIBC, muscle-infiltrating bladder carcinoma; MUC, metastatic urothelial carcinoma; OS, overall survival; PFS, progression-free survival; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, Thiobarbituric acid reactive substances.

* Corresponding author at: National Cancer Institute, Klenova 1, 831 01 Bratislava, Slovakia.
E-mail address: jan.slopovsky@nou.sk. (J. Slopovsky).

http://dx.doi.org/10.1016/j.tranon.2020.100890

cancerous tissues [7]. Low levels of ROS can have beneficial effects on cell processes such as pathogen killing or tissue repair [8]. However, increased levels of ROS can lead to damage of DNA, lipids, and proteins, which can result in tissue oxidative damage, death or subsequent cancer development [9]. Oxidative stress effects on cancer initiation, promotion, and progression are well-known [10]. High levels of ROS are cytotoxic, but during the course of carcinogenesis, cancer cell are able to develop mechanisms to evade cell death caused by ROS and subsequently, these mechanisms help cancer cells to develop resistance to treatment and to increase their survival in hypoxic environments [11].

Lipids are major components of cell membranes and play an important role in cell membrane stabilization and signal transduction [12]. Lipid peroxidation defined as oxidative deterioration of polyunsaturated fatty acids (PUFAs), is a process, which can damage DNA and help in cancer development [13]. Reactive aldehydes such as 4-hydroxy-2-nonenal, acrolein, and malondialdehyde (MDA), can affect cell proliferation through formation of DNA-DNA or DNA-protein crosslinks, which can result in replication errors, mutations and genomic instability, if not repaired before DNA replication process [14]. MDA is the end-product of lipid peroxidation of PUFAs and is used in the TBARS (Thiobarbituric acid reactive substances) assay as a good indicator of oxidative stress. In healthy population the physiologic values of TBARS are equal to or less than 4.5 μmol.L^{-1}.

The objective of this prospective study was to explore the prognostic value of TBARS measured before first-line chemotherapy in MUC patients. We hypothesized that elevated TBARS levels before the initiation of systemic treatment may affect the activity of the disease, as well as alter the effectivity of chemotherapy used in this setting. Finding new potential biomarkers is helpful for better prognostic assessment before initiation of treatment. Biomarkers should be easily measurable and overall easily assessed. Such new biomarkers would help clinicians to better stratify patients before treatment and/or offer them early clinical trial inclusion. There is a lack of prognostic biomarkers in MUC patients.

Patients and methods

This study was approved by the Ethical Committee of the National Cancer Institute (NCI) in Bratislava, Slovakia and carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Study patient population

From May 2010 to April 2014, 72 consecutive MUC patients from National Cancer Institute (NCI) in Bratislava were prospectively enrolled into this study after informed consent signing. Pathologic, clinical, and radiologic data were collected by physicians into electronic data files and their accuracy was validated for each patient by an independent investigator.

Patients were eligible if they had histologically proven urothelial carcinoma of bladder, renal pelvis, ureter or urethra, and additionally, creatinine clearance of more than 30 mL/min, hepatic function SGOT less than 1.17 U and serum bilirubin less or equal to 25.7 mol/L, adequate bone marrow function described as leucocytes 3,5 × 10 [9]/L and platelet count 100 × 10 [9]/L, hemoglobin above 90 g/dL. Patients with prior systemic therapy, history of prior malignancy except basal cell or squamous cell carcinoma of the skin, unresolved bacterial infection, severe cardiovascular disease (American Heart Association class III or IV) or pregnant were ineligible.

Final data cutoff was October 4, 2019. At the time of final analysis, 65 (90%) of patients progressed and 64 patients (89%) died. Of all patients included in this study, 58 (81%) had bladder cancer and 9 (12%) upper genitourinary tract cancers. Most of the patients were men 57 (79%). Performance status according to Eastern Cooperative Oncology Group (ECOG) ≥ 2 had 11 (15%) patients and visceral metastases were present in 43 (60%) patients. Baseline characteristics are shown in Table 1.

Table 1
Baseline characteristics of patients.

		N (%)
Study population		72 (100%)
Age	Median (range)	66 (39–84)
Men		57 (79)
Progression		65 (90)
Death		64 (89)
Primary tumor site	Bladder	58 (80)
	Renal Pelvis	9 (12.5)
Histology type	Urothelial carcinoma	72 (100)
Chemotherapy	Gemcitabine/Cisplatin	65 (90)
	Gemcitabine/Carboplatin	6 (8)
	Dose dense MVAC	1 (2)
Performance status	ECOG ≥2	11 (15)
Visceral metastasis present	Lungs	25 (35)
	Liver	18 (25)
Progression-free survival (months)	Median (range)	5.42 (0.26–114.54)
Overall survival (months)	Median (range)	9.6 (0.26–114.54)

MVAC: methotrexate, vinblastine sulfate, doxorubicin hydrochloride, cisplatin; ECOG: Eastern Cooperative Oncology Group.

The most common type of chemotherapy administered in concordance with good clinical practice were cisplatin (70 mg per m^2 day 1) and gemcitabine (1000 mg per m^2 days 1 and 8, new course on day 22) in 65 patients (90%) and carboplatin AUC 5 (day 1) and gemcitabine (1000 mg per m^2 days 1 and 8, new course on day 22) in 6 patients (8%). Dose adjustments were adopted according to toxicities or other relevant medical conditions.

Plasma isolation

Peripheral blood samples (12 mL) were collected from all participants enrolled into this study. Samples were collected into Vacutainer® EDTA Blood Collection Tubes (BD Biosciences, Franklin Lakes, NJ, USA) at baseline in the morning on day 0 or day 1 before the first dose of the first line chemotherapy. All plasma samples were frozen down to −80 °C and stored for maximum 28 days. Median retention time of frozen samples was 21 days (range 14–28). Median samples analyzed per one TBARS assay experiment was 5 (range 2–7). Patient blood samples were centrifuged at 1000*g* for 10 min at room temperature within 2 h of venipuncture. To avoid cellular contamination, plasma was carefully harvested and centrifuged again at 1000*g* for 10 min at room temperature. The cell-free plasma samples were cryopreserved at −80 °C and further processed in Pharmacobiochemical Laboratory of the 3rd Department of Internal Medicine, Faculty of Medicine, Comenius University (FMCU) in Bratislava, Slovakia.

Measurement of TBARS

For oxidative stress assessment the TBARS assay was used (OxiSelect TBARS Assay Kit). TBARS were determined from plasma. Briefly, 100 μL of plasma was mixed with 1 mL of 0.67% thiobarbituric acid (TBA), 1 mL 20% trichloroacetic acid, and 1.5 mL 0.04% butylated hydroxytoluene (BHT) in test tubes. The mixtures were incubated in a boiling water bath for 20 min. After cooling to room temperature, the reaction mixture was centrifuged at 4000*g* for 10 minutes and the absorbance of the supernatant was measured at 532 nm using the same UV–visible Spectrophotometer. The concentrations of TBARS were calculated using MDA as a reference standard [15].

Statistical analysis

Based on the mean of TBARS of 6.06 μmol.L^{-1}, patients were dichotomized into low <6.06 μmol.L^{-1} (N = 35) and high ≥6.06 μmol.L^{-1} (N = 37) groups. Progression-free-survival (PFS) and overall survival (OS) were calculated from the initiation of the first-line chemotherapy administration to progression or death, and subsequently were calculated with their

proper 95% CI by the Kaplan-Meier method and compared with log-rank test. A multivariate Cox proportional hazards analysis was used to evaluate prognostic parameters with respect to the risk of death or progression. For statistical assessment NCSS 2019 software was employed [16].

Results

Association between TBARS and patient/tumor characteristics

The patient characteristics and their proper associations are shown in Table 2. Subjects with neutropenia grade 4 (G4) after chemotherapy, had significantly decreased levels of TBARS measured in blood plasma (4.70 *vs.* 6.16 μmol.L^{-1}, $P = 0.02$). Patients with liver metastasis had significantly higher TBARS levels in plasma compared to patients without liver metastasis (6.60 *vs.* 5.88 μmol.L^{-1}, $P = 0.03$). Patients with TBARS above mean had significantly higher BMI (7.07 *vs.* 5.0 μmol.L^{-1}, $P = 0.02$). No other associations between TBARS and characteristics of patients or tumor were found.

Table 2
Association between TBARS and patient/tumor characteristics.

	N	Mean	Median	SD	SEM	*P* value
TBARS (μmol.L^{-1})	72	6.06	6.15	1.28	0.15	NA
ECOG						
0	22	5.63	5.36	1.03	0.26	0.10
1	39	6.13	6.16	1.29	0.20	
2	9	6.50	6.84	1.42	0.41	
3	2	7.61	7.61	1.78	0.88	
Toxicity						
Anemia G3						
No	59	6.07	6.16	1.28	0.17	0.66
Yes	13	6.01	5.38	1.29	0.36	
Anemia G4	NA	NA	NA	NA	NA	NA
Thrombocytopenia G3						
No	69	6.09	5.86	1.27	0.15	0.21
Yes	3	7.07	6.43	1.17	0.73	
Thrombocytopenia G4						
No	68	6.01	5.99	1.26	0.15	0.16
Yes	4	6.96	6.82	1.43	0.63	
Neutropenia G3						
No	50	6.11	6.16	1.33	0.18	0.64
Yes	22	5.96	5.73	1.17	0.27	
Neutropenia G4						
No	67	6163	6.16	1.22	0.15	0.02*
Yes	5	4,70	4.26	1.31	0.55	
Alopecia G3						
No	65	6.10	6.16	1.28	0.16	0.38
Yes	7	5.68	5.3	1.25	0.48	
Metastasis sites						
Lymph nodes						
N0	4	6.34	6.26	1.11	0.64	0.57
N+	68	6.05	6.15	1.29	0.16	
Skeletal						
Absent	48	5.95	5.64	1.35	0.18	0.26
Present	24	6.29	6.32	1.10	0.26	
Pulmonary						
Absent	47	6.08	6.16	1.16	0.19	0.51
Present	25	6.02	5.6	1.49	0.26	
Liver						
Absent	54	5.88	5.64	1.25	0.17	0.03*
Present	18	6.60	6.47	1.22	0.29	
Lungs and liver (either)						
Absent	38	6.03	6.01	1.24	0.21	0.94
Present	34	6.10	6.15	1.33	0.22	
Lungs and liver (both)						
Absent	62	6.05	6.15	1.30	0.16	0.62
Present	9	6.30	6.56	1.13	0.43	
Peritoneum						
Absent	60	6.16	6.16	1.32	0.16	0.16
Present	12	5.58	5.62	0.94	0.37	
BMI						
TBARS <6.06 μmol.L^{-1}	35	5.00	5.23	0.62	0.13	<0.0001*
TBARS ≥6.06 μmol.L^{-1}	37	7.07	6.77	0.84	0.12	

TBARS: Thiobarbituric acid reactive substances; ECOG: Eastern Cooperative Oncology Group; SD: standard deviation; SEM: standard error of mean; NA: not applicable.
* means statistically significant.

Prognostic values of TBARS on progression-free survival and overall survival

The median follow-up was 9.6 months (range: 0.26–114.54), the median PFS was 5.42 months (range 0.26–114.54), and the median OS was 9.6 months (range 0.26–114.54). Patients with TBARS levels <6.06 μmol.L^{-1} had a median progression-free survival of 7.7 *vs.* 4.3 months compared to patients with TBARS levels ≥6.06 μmol.L^{-1}, HR 0.51; 95% CI 0.31–0.84; $P = 0.006$ (Fig. 1A and Table 3). Statistically significant decrease in overall survival was observed in population of patients with increased levels of lipid peroxidation measured by the TBARS assay, HR 0.44, 95% CI 0.27–0.74; $P = 0.0009$ (Fig. 1B and Table 4), median survival time for patients with TBARS levels <6.06 μmol.L^{-1} was 13.1 months, whereas median survival of the group of patients with TBARS levels ≥6.06 μmol.L^{-1} was 6.9 months.

Multivariate Cox proportional hazards analysis for independent prognostic factors assessment

TBARS and ECOG were proven to be independent prognostic factors for OS and PFS, respectively, with hazard ratios for TBARS of HR 1.70, CI 1.00–2.90; 2.00, $P = 0.04$ for PFS and HR 2.00, CI 1.16–3.46; $P = 0.01$ for OS. For ECOG HR: 4.42, CI 2.05–9.52; $P = 0.0001$ for PFS and HR: 5.78, CI 2.42–13.84; P = 0.0001 for OS (Table 5 and Table 6).

Discussion

Oxidative stress is induced by an imbalance between pro- and antioxidative mechanisms. ROS have the ability to affect membrane bilayers and cause lipid peroxidation of polyunsaturated fatty acids (PUFAs) leading to formation of molecules such as MDA, hexanal, 4-hydroxynonenal, which have the ability to locally react with macromolecules leading to alteration of their functions. If ROS levels are increased enough and antioxidant mechanisms become overwhelmed, ROS irreversibly damage DNA, lipids, proteins, which in turn leads to genetic and epigenetic alterations that drive tumorigenesis. Signaling pathways such as the epidermal growth factor receptor signaling pathway, nuclear factor erythroid 2-related factor 2, the mitogen-activated protein kinases MAPK/ERK, phosphatidylinositol-3-kinase, phospholipase C, and protein kinase C, p53 are altered by oxidative stress [17].

As was shown in previous studies conducted on this topic, oxidative stress plays a major role in cancer development and subsequently in cancer progression and dissemination [9,18]. Moderate levels of oxidative stress stimulate progression and dissemination of tumors however, intrinsically increased levels of ROS in cancer cells, which are necessary for maintaining their proliferation, makes them also more susceptible to ROS induced death, when further increasing the levels of ROS above their threshold. This finding can be exploited in cancer treatment because some cytotoxic drugs, such as cisplatin was recently shown not only to kill cancer cells through formation of DNA adducts, but also through increase in ROS formation and lipid peroxidation, which alters enzymes and structural proteins, and directs a cell-to-cell apoptotic pathway [19].

One of the main issues with chemotherapeutic treatments is its toxicity. Cisplatin, a gold standard in MUC patient treatment, expresses its toxicity through generation of DNA adducts but partly also through oxidative stress. In this study, subjects with neutropenia grade 4 after platinum-derived combined chemotherapy had lower levels of TBARS measured in plasma compared to patients without neutropenia grade 4 toxicity. This finding might be explained by reduced neutrophil numbers, which are significant oxidative stress producers in a human body [20].

In this prospective study, MUC patients with high baseline levels of TBARS had significantly shortened PFS and OS (Fig. 1A and B). This finding can be explained by an increased activity of this malignant disease

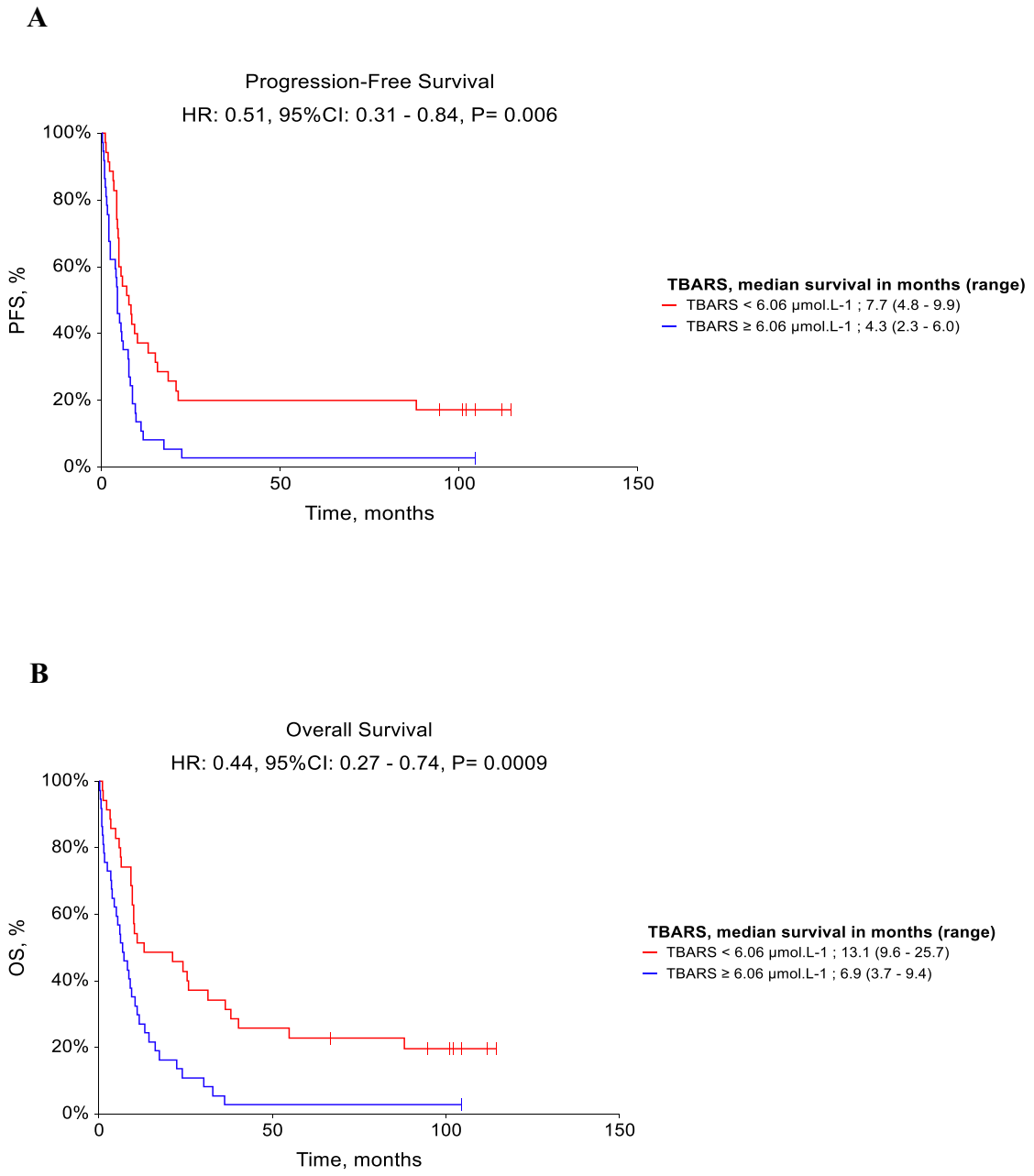

Fig. 1. A Kaplan-Meier survival analysis for progression-free survival and TBARS B Kaplan-Meier survival analysis for overall survival and TBARS *TBARS: Thiobarbituric acid reactive substances.*

expressed in the increased levels of TBARS. As it was shown in some trials, presence of visceral metastases is associated with negative prognosis, either combined (liver and lung) or as single site metastases [21,22]. In this prospective study, there was a trend toward worse prognosis in patients with presence of lung and liver involvement, however presence of visceral metastases was not shown to be an independent prognostic factor and did not significantly affect OS and PFS. The lack of statistical significance is probably due to insufficient number of subjects enrolled into this trial.

However, in this study population, a significant association between TBARS levels and presence of liver involvement was found (Table 2). To

Table 3
Univariate analysis for progression-free survival (PFS).

Variable	N	Median	95% CI low	95% CI High	HR	95% CI Low	95% CI High	P value
TBARS ($\mu mol.L^{-1}$)	72							
$\geq 6.06\ \mu mol.L^{-1}$	37	4.3	2.3	6.0	0.51	0.31	0.84	0.006*
$< 6.06\ \mu mol.L^{-1}$	35	7.7	4.8	9.9				

TBARS: Thiobarbituric acid reactive substances.
* means statistically significant.

Table 4
Univariate analysis for overall survival (OS).

Variable	N	Median	95% CI low	95% CI high	HR	95% CI low	95% CI high	P value
TBARS (μmol.L^{-1})	72							
≥6.06 μmol.L^{-1}	37	6.9	3.7	9.4	0.44	0.27	0.74	0.0009*
<6.06 μmol.L^{-1}	35	13.1	9.6	25.7				

TBARS: Thiobarbituric acid reactive substances.
* means statistically significant.

our best knowledge, this is the first study which associated TBARS with liver metastasis and this finding further supports the prognostic value of increased TBARS levels. Plasma TBARS determination method applied in this study has some limitations, mostly in the term of the lack of specificity. Factors, as increased body mass index (BMI) may confound the results. In this trial, association between increased BMI and increased TBARS levels (Table 2) did not affect the prognostic value of TBARS when adjusting multivariate analysis for BMI (Table 5 and Table 6). TBARS concentrations in plasma were reported to modestly be increased with age and certain vitamin supplementations [23]. However, age of patients enrolled in this study was approximately homogeneous and most of the patients were men.

In conclusion, based on data obtained in this study, chemotherapy naïve MUC patients with increased TBARS had worse prognosis in terms of shorter PFS and OS, respectively. Measurement of plasma TBARS at baseline might be useful for early identification of patients who are candidates for different treatment options within or out of clinical trials. The authors speculate that therapeutic targeting of signaling pathways involved in regulations of oxidative stress could contribute to increase in effectivity and/or reduce the toxicity of current platinum-based treatments in metastatic urothelial carcinomas.

Table 5
Multivariate analysis for progression-free survival adjusted for body mass index (BMI) above 30 kg/m^2.

Variable	N	HR	95% CI Low	95% CI High	*P*-value
TBARS (μmol.L^{-1})	**72**	NA	NA	NA	NA
≥6.06 μmol.L^{-1}	**37**	1.70	1.00	2.90	**0.04***
<6.06 μmol.L^{-1}	**35**				
ECOG	**72**	NA	NA	NA	**NA**
2 and more	**11**	4.42	2.05	9.52	**0.0001***
0–1	**61**				
Visceral metastases (liver and lungs)	**72**	NA	NA	NA	**NA**
Present	**9**	1.33	0.60	2.92	**0.47**
Absent	**63**				
Obesity	**72**	NA	NA	NA	**NA**
BMI above 30 kg/m^2	**10**	0.87	0.42	1.80	**0.71**
BMI below 30 kg/m^2	**62**				

TBARS: Thiobarbituric acid reactive substances; ECOG: Eastern Cooperative Oncology Group; NA: not applicable.
* means statistically significant.

Table 6
Multivariate analysis for overall survival adjusted for body mass index (BMI) above 30 kg/m^2.

Variable	N	HR	95% CI low	95% CI high	P value
TBARS (μmol.L^{-1})	**72**	NA	NA	NA	NA
≥6.06 μmol.L^{-1}	**37**	2.00	1.16	3.46	**0.01***
<6.06 μmol.L^{-1}	**35**				
ECOG	**72**	NA	NA	NA	**NA**
2 and more	**11**	5.78	2.42	13.84	**0.0001***
0–1	**61**				
Visceral metastases (lungs and liver)	**72**	NA	NA	NA	**NA**
Present	**9**	1.39	0.59	3.24	**0.45**
Absent	**63**				
Obesity	**72**	NA	NA	NA	**NA**
BMI above 30 kg/m^2	**10**	1.11	0.52	2.36	**0.70**
BMI below 30 kg/m^2	**62**				

TBARS: Thiobarbituric acid reactive substances; ECOG: Eastern Cooperative Oncology Group; NA: not applicable.
* means statistically significant.

CRediT authorship contribution statement

Slopovsky, J: Data accuracy validation, statistical analysis, manuscript writing; approved the final version of the manuscript to be published.

Kucharska, J: Performed analysis of TBARS and calculated obtained data; approved the final version of the manuscript to be published.

Obertova, J: Enrollment of patients, data collection; approved the final version of the manuscript to be published.

Mego, M: Enrollment of patients, manuscript reviewing; approved the final version of the manuscript to be published.

Kalavska, K: Performed sample collection and processing; approved the final version of the manuscript to be published.

Cingelova, S: Statistical analysis; approved the final version of the manuscript to be published.

Svetlovska, D: Data accuracy processing and validation; approved the final version of the manuscript to be published.

Gvozdjakova, A: Rewieved manuscript; approved the final version of the manuscript to be published.

Furka, S: Data accuracy processing and validation; approved the final version of the manuscript to be published.

Palacka, P: Study design, enrollment of patients, data collection and processing, statistical analysis, manuscript writing and reviewing; approved the final version of the manuscript to be published.

Funding sources

This study was supported by Scienific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and Slovak Academy of Sciences : VEGA 1/0614/12 and OncoReSearch, Slovak Republic.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Meszarosova K, Duratna M, Pekova Z, and Stetkova A for technical assistance.

References

[1] Wong MCS, Fung FDH, Leung C, Cheung WWL, Goggins WB, Ng CF. The global epidemiology of bladder cancer: A joinpoint regression analysis of its incidence and mortality

trends and projection. *Sci Rep*. 2018;8(1). doi:https://doi.org/10.1038/s41598-018-19199-z
[2] Bellmunt J, Orsola A, Leow JJ, Wiegel T, De Santis M, Horwich & A. Bladder cancer: ESMO Practice Guidelines for diagnosis, treatment and follow-up † on behalf of the ESMO Guidelines Working Group * incidence and epidemiology. 2014. doi:https://doi.org/10.1093/annonc/mdu223
[3] A. Stenzl, J.A. Witjes, Compérat E, et al, *Guidelines on Bladder Cancer Muscle-Invasive and Metastatic*. https://uroweb.org/wp-content/uploads/07_Bladder-Cancer_LR-Feb-17th.pdf 2012. (Accessed 3 March 2019).
[4] S. Letaiová, A. Medveová, A. Ovíková, et al., Bladder cancer, a review of the environmental risk factors, Environ Heal A Glob Access Sci Source. 11 (SUPPL.1) (2012) S11, https://doi.org/10.1186/1476-069X-11-S1-S11.
[5] Islam MO, Bacchetti T, Ferretti G. Alterations of Antioxidant Enzymes and Biomarkers of Nitro-oxidative Stress in Tissues of Bladder Cancer. Karihtala P, ed. *Oxid Med Cell Longev*. 2019;2019:2730896. doi:https://doi.org/10.1155/2019/2730896
[6] M. Brandon, P. Baldi, D.C. Wallace, Mitochondrial mutations in cancer, Oncogene. 25 (34) (2006) 4647–4662, https://doi.org/10.1038/sj.onc.1209607.
[7] Y.S. Bae, J.Y. Sung, O.S. Kim, et al., Platelet-derived growth factor-induced H2O2 production requires the activation of phosphatidylinositol 3-kinase, J. Biol. Chem. 275 (14) (2000) 10527–10531, https://doi.org/10.1074/jbc.275.14.10527.
[8] Patel R, Rinker L, Peng J, Chilian WM. Reactive Oxygen Species: The Good and the Bad. In: *Reactive Oxygen Species (ROS) in Living Cells*. InTech; 2018. doi:https://doi.org/10.5772/intechopen.71547
[9] S. Reuter, S.C. Gupta, M.M. Chaturvedi, B.B. Aggarwal, Oxidative stress, inflammation, and cancer: how are they linked? Free Radic. Biol. Med. 49 (11) (2010) 1603–1616, https://doi.org/10.1016/j.freeradbiomed.2010.09.006.
[10] M. Sødring, G. Gunnes, J.E. Paulsen, Spontaneous initiation, promotion and progression of colorectal cancer in the novel A/J Min/+ mouse, Int. J. Cancer 138 (8) (2016) 1936–1946, https://doi.org/10.1002/ijc.29928.
[11] L. Postovit, C. Widmann, P. Huang, S.B. Gibson, Harnessing oxidative stress as an innovative target for cancer therapy, Oxidative Med. Cell. Longev. 2018 (2018)https://doi.org/10.1155/2018/6135739.
[12] Casares D, Escribá P V., Rosselló CA. Membrane lipid composition: Effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. *Int J Mol Sci*. 2019;20(9). doi:https://doi.org/10.3390/ijms20092167
[13] E. Sawicka, E.M. Kratz, B. Szymańska, et al., Preliminary study on selected markers of oxidative stress, inflammation and angiogenesis in patients with bladder cancer, Pathol Oncol Res. (2019)https://doi.org/10.1007/s12253-019-00620-5.
[14] J.E. Klaunig, L.M. Kamendulis, The role of oxidative stress in carcinogenesis, Annu. Rev. Pharmacol. Toxicol. 44 (1) (2004) 239–267, https://doi.org/10.1146/annurev.pharmtox.44.101802.121851.
[15] D.R. Janero, B. Burghardt, Thiobarbituric acid-reactive malondialdehyde formation during superoxide-dependent, iron-catalyzed lipid peroxidation: influence of peroxidation conditions, Lipids 24 (2) (1989) 125–131, https://doi.org/10.1007/BF02535249.
[16] NCSS, Statistical Software (2019) 2019.
[17] L. Huo, C.W. Li, T.H. Huang, et al., Activation of keap1/nrf2 signaling pathway by nuclear epidermal growth factor receptor in cancer cells, Am. J. Transl. Res. 6 (6) (2014) 649–663.
[18] S. Toyokuni, K. Okamoto, J. Yodoi, H. Hiai, Persistent oxidative stress in cancer, FEBS Lett. 358 (1) (1995) 1–3, https://doi.org/10.1016/0014-5793(94)01368-b.
[19] G. Barrera, Oxidative stress and lipid peroxidation products in cancer progression and therapy, ISRN Oncol. 2012 (2012)https://doi.org/10.5402/2012/137289.
[20] Dogantekin A, Gurel A, Ustundag B, Ilhan S, Elkiran ET. Oxidative stress and antioxidant parameters in neutropenic patients secondary to chemotherapy. *Pakistan J Med Sci*. 2016;32(2):309–313. doi:10.12669/pjms.322.9200
[21] D.F. Bajorin, P.M. Dodd, M. Mazumdar, et al., Long-term survival in metastatic transitional-cell carcinoma and prognostic factors predicting outcome of therapy, J. Clin. Oncol. 17 (10) (1999) 3173–3181, https://doi.org/10.1200/JCO.1999.17.10.3173.
[22] F. Dong, Y. Shen, F. Gao, et al., Prognostic value of site-specific metastases and therapeutic roles of surgery for patients with metastatic bladder cancer: A population-based study, Cancer Manag. Res. 9 (2017) 611–626, https://doi.org/10.2147/CMAR.S148856.
[23] A.G. Rumley, M. Woodward, A. Rumley, J. Rumley, G.D.O. Lowe, Plasma lipid peroxides: relationships to cardiovascular risk factors and prevalent cardiovascular disease, Q. J. Med. 97 (2004) 809–816, https://doi.org/10.1093/qjmed/hch130.