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REVIEW

Serum metallothionein – a potential oncomarker?

Horakova Z, Starek I

Clinic of Otorhinolaryngology and Neck Surgery, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic. **zuzana.horakova@fnol.cz**

ABSTRACT
INTRODUCTION: Metallothionein's (MT) overexpression has been demonstrated immunohistochemically in neoplastic cells of many tumour types. Its elevation above the physiological level has been confirmed in circulation of their hosts. The results of studies dealing with the topic have been summarized to verify if this marker can be applied in the current oncologic practise.
METHODS: The Pubmed and Google Scholar medical databases were reviewed for full-text articles focused on MT blood (plasma / serum) levels in patients with malignant tumours.
RESULTS: In our review, after a precise selection, we included 8 prospective randomized trials encompassing 561 blood samples taken from patients with a large histopathological spectrum of malignancies. In general, significant differences in blood MT levels between oncological patients and healthy subjects were confirmed. No particular value of the MT level has been demonstrated to be unequivocally predictive of oncologic disease
CONCLUSION: The results of our review suggest that although the elevation of MT in blood serum in patients with solid malignancy can be regarded as a promising tumour marker, the recommendations of its applicability in clinical practice require to be derived from further research on extended cohorts of patients *(Tab. 1, Fig. 1, Ref. 49)*. Text in PDF *www.elis.sk*
KEY WORDS: metallothionein, tumour marker, solid tumour.

Introduction

A tumour marker is defined as a parameter capable of indicating the presence or progression of a tumour with sufficient sensitivity and specificity. Clinical applications of oncomarkers can be divided into screening, diagnosis, prognosis, prediction of the therapeutic response and monitoring of tumour's biological activity (1).

For some malignancies, nonspecific markers (SCCAg, CEA, CYFRA 21–1 etc) are being used in the above-mentioned applications, while the use of specific markers (e.g., PSA) is rare. For other tumours, including the head and neck squamous cell carcinoma (SCC), the former show small effectiveness and the latter have not been identified so far (2, 3).

Metallothionein (MT) belongs to the superfamily of metal-binding proteins present in the cells of all living organisms (4). In low concentrations, this protein can be detected in some body fluids, e.g., in plasma, bile, urine, and saliva (5).

MT is involved in many metabolic pathways (4). Its synthesis and consequently, its plasmatic concentrations are controlled by various factors, primarily by metal ions, glucocorticoids, acute phase proteins, inflammatory cytokines and stress mediators (6, 7, 8).

It is generally accepted that MT plays a very important role in heavy metal detoxification, pooling and regulation of copper and zinc ions, with the latter being essential in regulating the activity of DNA repair enzymes. Moreover, MT acts as a free radical scavenger protecting cell structures against acute inflammation and associated oxidative stress. (9, 10, 11, 12)

The serum MT concentration in healthy individuals may be influenced by many physiological factors (age, sex, food supplement intake, physical exercises), as well as environmental and geographical circumstances (13, 14).

Moreover, increased blood levels of this protein have been demonstrated under various pathologic conditions (e.g., inflammation, injury, cardiovascular or endocrine disease, hepatopathy) (15).

Until now, four MT isoforms expressed in human cells have been identified, namely MT1 (subtypes A, B, E, F, G, H, L, M, X), MT2 (subtypes A, B), MT3, and MT4. Particular isoforms which are similar to each other in their physical and chemical characteristics are produced in different proportions in various tissues and herein degraded at different ratios. MT1 and MT2 are ubiquitous whereas the minorities MT3 and MT4 were detected in human brain and stratified squamous epithelium of the skin, oesophagus, and tongue, respectively (Miles). MT3 and MT4 are present constantly and independently from signals of induction by pathologic alterations, as opposed to MT1 and MT2, the expression of which is stimulated by many various stimuli (*vide supra*) (16).

Clinic of Otorhinolaryngology and Neck Surgery, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic

Address for correspondence: Z. Horáková, KORLHK, FN Olomouc, IP Pavlova 6, CZ-779 00 Olomouc, Czech Republic.
Phone: +420776240518

Tab. 1. Set of so far published studies focused on serum MT level.

Author	Tumour location	Oncologic patients	Control group	Statistical results	Value of MT–oncologic patients (min–max; mean (m); standard deviation (SD); median) [uM]	Value of MT–control group (min–max; mean (m); standard deviation (SD); median) [uM]
Adam., 2008 (27)	Various solid tumours	80	10	Significantly elevated in patients	min–max 1.5–3.5	m 0.72; SD 0.09
Adam. 2010 (28)	Breast cancer	10	5	Significantly elevated in patients		
Fabrik. 2007 (29)	Breast cancer	15	no	???		
Fabrik. 2008 (30)	Head and neck cancer	3	no	???		
Gumulec. 2012 (31)	Prostate cancer	80	53	Significantly elevated in patients	min–max 0.7–0.9 *(relative values)	min–max 0.1–0.3
Horakova. 2008 (32)	Oropharyngeal cancer	73	58	Significantly elevated in patients	m 2.73; SD 1.19	m 0.50; SD 0.20
Krejcova. 2012 (33)	Head and neck cancer	145	58	Significantly elevated in patients	min–max 1.08–6.39	min–max 0.17–0.90; m 0.51
Krizkova. 2010 (34)	Paediatric solid tumours	38	58	Significantly elevated in patients	m 3.4 SD 0.8	m 0.5; SD 0.2
Krizkova. 2011 (35)	Prostate cancer	46	6	Significantly elevated in patients	m 2.12; median 2.07	min–max 0.55–0.88
Kruseova 2013 (36)	Paediatric tumours	172	Previous study of the authors	MT was related to early disease recurrence	m 2.67; SD 0.5	m 0.57; SD 0.2
Petrlova. 2006 (37)	Breast cancer, malignant melanoma, colon cancer	27	Previous study of the authors	Significantly elevated in oncologic patients		
Tariba. 2015 (13)	Testicular tumours	25	22	Significantly elevated in patients		
Tariba. 2016 (26)	Ttumours	25	no	???	m 0.545 [mgmL]	m 0.412 [mg/mL]

MT is suggested to be involved also in cancerogenesis (Si). However, relevant specific mechanisms have not been elucidated as yet (17). Woo demonstrated MT activity mainly in the early phase of that process (17); Pastuszewski and Ioachim assumed that MT overexpression is associated with oncogenous dedifferentiation of cells elicited by reexpression of primarily suppressed oncoproteins (18, 19).

In tumorous tissues, MT was found to be overexpressed not only in neoplastic cells but also in associated stromal fibroblasts, which supports the theory of remodelling of the microenvironment by tumoral cells, while thereby promoting their local and nodal metastatic spread (20).

Immunohistochemical MT expression in tumorous tissues was tested in numerous studies. In 2014, Gumulec published a large meta-analysis of 77 studies on MT expression comprising 4,631 various solid malignancies. As opposed to the correspondent adjacent healthy tissues, the increase in MT expression was found in all but hepatic tumours. The differences were significantly increased, primarily in head and neck, mammary, ovarian, uterine and prostate carcinomas (21). Moreover, some recent studies have demonstrated significant correlations of MT overexpression with tumour grade (22), metastatic spread (23), prognosis and radiochemo-resistance in breast cancer (24).

Previous studies tested the expression of complex MT in neoplastic tissues without having analysed its particular isoforms (25). These were identified in recent trials, while demonstrating the prevalence of MT1, lower prevalence of MT2 and only sporadic expression of MT3 and MT4.

Thus, a question can be raised as to whether the serum MT concentration could reflect the presence and biological activity of tumours and consequently act as an efficient tumour marker. Up to now, only a limited number of papers dealing with that topic has been published. We therefore review their conclusions to delineate further conceivable investigation in this perhaps promising field of oncology.

Materials and methods

The search was performed using the PubMed (Medline 1968 to November 2018) and Google Scholar, and subsequently, in bibliographies of identified references. Following keywords were used: metallothionein, serum or plasma or blood circulation, tumour or neoplasm or malignancy or cancer or carcinoma. The language was not a restricting selection criterion. Full-text articles were included only. If duplicated data were found in subsequent studies published by the same author(s), only the more comprehensive were taken into consideration. Following information was extracted from the studies: (1) blood MT level in patients with malignant tumours and set of healthy controls, (2) tumour location and histology.

Altogether, 13 studies were found to be analysing serum MT concentrations in oncologic patients. Four studies were subsequently excluded as they dealt mainly with the methodology of MT blood analysis without providing sufficient relevant clinical and/or histopathological data. Identical sets of patients were reported in two consecutive papers by Tariba (13, 26) and therefore,

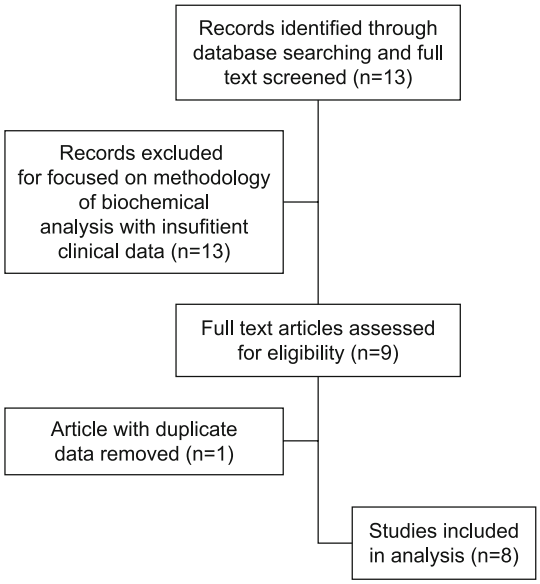

Fig. 1. Flow diagram of literature search and study selection process.

only those referred in the latter were included (26). Eventually, eight randomized prospective studies were involved in our analysis. The process of literature reviewing is illustrated in a flow diagram (Fig. 1) and details of the finally included studies are summarized in Table 1.

Results

The total of patients enclosed was 579, of whom 561 were treated for histologically verified solid malignancies (351 adults and 210 children) and 18 for leukemia. In all patients, blood sampling preceded the therapy (Tab. 1). With 218 cases, head and neck cancer (HNC) was the prevailing entity (30, 32, 33), followed by 126 cases of prostate carcinoma (31, 35) and 25 cases of testicular carcinoma (13). Remaining cases were of other histological types and locations (28, 35, 36, 37) (Tab. 1).

All 8 analysed studies compared serum MT concentrations in oncological cases to those of control groups comprised of non-oncologic healthy volunteers. The results including the values of MT concentration and statistical significance between the two groups are summarized in Table 1. The results of most of the cited studies confirmed a significant elevation of MT serum level in oncological patients.

In the majority of trials (Tab. 1), solely means and standard deviations of the MT concentration were reported: the former varied from 2.12 to 3.4 uM in oncologic and from 0.5 to 0.72 uM in control groups.

In other two (28, 33) analyses comprising a total of 225 oncologic patients, only value ranges were available: the MT levels in oncologic and control groups varied greatly from 1.08 to 6.39 uM and from 0.17 to 0.9 uM, respectively.

Discussion

Many trials dealt with immunohistochemical MT expression in samples taken from a broad histopathologic spectrum of neoplasms. Their results were summarized by Gumulec (21), who confirmed MT expression in tumours to be higher than in adjacent healthy tumour-free tissues (38). In their recent comprehensive literature review, Si pointed out the association between immunohistochemical MT overexpression in some tumours and their prognosis, chemo-/ radio- resistance, stage and grade (38).

On the contrary, only a few studies dealt with blood MT levels in oncologic patients. All the eight analysed trials demonstrated that serum MT concentrations were significantly higher in oncologic patients than those in healthy controls (Tab. 1), including the two largest ones analysing 218 head and neck cancers (32, 33).

Six studies (Tab. 1) reported MT level means and medians with low standard deviations indicating a relative homogeneity of results. In other two studies dealing with various solid malignancies (28) and head and neck carcinomas (33), large ranges of individual blood MT levels were reported. However, neither means, nor medians and standard deviations were available, making farther comments impossible.

Our review demonstrated that in oncologic patients, the ranges of blood MT concentration values were wider than in healthy individuals. We suggest that this may reflect the specificity of particular oncopathological groups, as well as induced biologic response and individual variability of the hosts. Our opinion is further supported by the results of two other studies, in which the variabilities of serum MT concentrations in control groups were generally low, while not exceeding 1.0 uM, (32, 33).

Considering the great variability of significantly elevated blood MT concentrations in oncologic patients and homogeneity of generally low MT levels in healthy probands, we suggest that physiological factors (such as sex and age) and environmental conditions have no substantial impact on MT levels in the former group. Our opinion seems to be supported by a study (31) analysing the impact of hypertension, hyperlipidaemia, ischaemic heart disease and duodenal ulcer on serum MT levels in patients diagnosed with solid malignancies. The comorbidities had no effect on concentrations of this protein. The result concerts with another trial (36) confirming no association between the serum MT concentration and inflammation blood markers in oncologic patients (36).

A relatively great interindividual variability of the MT level in oncologic cases may be explained by histopathologic diversity of tested tumours and by individual biological response of the host organism.

The relations between clinical and histopathological characteristics of tumours, tissue MT overexpression and blood MT concentrations were tested in the studies as follows.

Krizkova found that in detection of a prostate carcinoma, the combination of MT and PSA was superior to the latter marker alone; the MT level showed high specificity and markedly reduced the high false positivity of PSA (35). Surprisingly, Gumulec found an inverse relation between blood MT level, Gleason histopathological score and prognosis in patients with malignancy

(31). Tariba confirmed an association between MT levels and clinical stages of testicular cancer (26). Krejcova tested blood MT levels in patients with squamous cell head and neck carcinomas. The average values for particular tumour locations (larynx, oropharynx, hypopharynx, oral and nasal cavity) varied and showed a slight tendency to increase with more advanced T stage and presence of nodal metastases. The lowest MT serum concentration in oncologic group was 1.09 uM, while in healthy individuals, the level did not exceed 0.9 uM. The author consequently suggested the 1-uM level as the cut-off value with that level showing 100% sensitivity and specificity (33). Kruseova analyzed MT blood concentrations in 865 samples of 172 paediatric patients with solid malignancies treated with chemotherapy. Cases with persistent or early recurrent disease showed that their MT concentrations at the beginning and during the treatment were significantly lower than in those achieving long-term remissions. The results suggested that in the latter group, the therapy applied was more efficient due to escalated oxidative stress and cell damage. In patients in long-term remission (follow-up ranging from 18 to 39 months), a decrease in MT levels lasted for 2 months after the treatment had been finished (36).

Other studies failed to find any association between the blood MT concentrations and tumour characteristics. No relation of MT with the clinical stage or histological grade was found in oropharyngeal SCC (32) and solid paediatric malignancies (36). Moreover, MT blood concentration showed no predictiveness for the response to chemotherapy in prostatic cancer (35).

Krizkova performed a large literature review focused on tissue expression of particular MT isoforms, their ratio and prognostic significance in various types of malignancies. She concluded that the majority of them show upregulation of MT1, MT2 and rarely also MT3. Expression patterns of particular MT isoforms varied in various types of cancers and had no prognostic relevance (39). The only two exceptions were studies on breast cancer in which increased tissue MT3 portended a poor outcome (40, 41).

So far, no study identifying particular blood MT isoforms in oncologic patients has been performed. Consequently, it is not clear whether they would have diagnostic, predictive o prognostic relevance. As suggested by some authors, an analysis of the oncologically relevant MT isoforms might also contribute to better understanding of their role in cancerogenesis (17, 18, 19).

For quantitative analysis of blood MT levels, various methods can be used. In all the reviewed trials, an electrochemical voltametric detection (Brdicka reaction) of complex MT was applied (13, 28, 29, 30, 37, 39, 42). This very sensitive method of identifying MT on zeptomol level is based on differential pulse voltametric analysis of the SH group, which is the fraction dominating its structure. The MT concentration values are subsequently calculated from the specific catalytic peak of voltammogram. The capability of voltametric reaction to discriminate particular MT isoforms is intensively tested now (28, 44).

As for the blood MT analysis, other authors used radioimmunoassay (RIA) (45, 46) or enzyme-linked immunoassay (ELISA) (47, 48, 49) which showed the capacity to detect the human serum MT, the levels of which reach the order of uM. Unfortunately, the methods are largely limited by current absence of standardized specific antibodies necessary for MT detection.

As none of these analytical methods are routinely used, the precise assessment of the clinical relevance of MT serum concentrations has not been performed yet.

Conclusions

The systematic literature review comprised 8 up to now published studies in which relevant clinical data were available. It revealed that in comparison to healthy probands, means and ranges of blood MT concentrations in patients diagnosed with a broad histopathologic spectrum of solid malignancies are significantly higher with a suggested cut-off value of concentration of 1uM for oncologic patients.

However, the prognostic and predictive relevance of blood MT levels in clinical oncology remains unclear. Consequently, further research is necessary. It should be performed on large cohorts of patients stratified into specific oncological groups, with sufficient follow-ups. For the purpose of blood MT analysis, reliable and standardized laboratory methods must be applied. As the Brdicka reaction is not available in standard clinical labs, the alternative in form of ELISA and RIA should be preferred. All these methods are capable of detecting particular ontologically relevant MT isoforms, the analysis of which seems to be essential for further research in this field of clinical oncology.

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