Significant correlation of matrix metalloproteinase and macrophage colony-stimulating factor serum concentrations in patients with head and neck cancer

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Serum matrix metalloproteinases (MMPs) and the macrophage colony-stimulating factor (M-CSF) are of potential interest as serum tumor markers in various malignancies. There is still a lack of reliable tumor markers in patients with squamous cell carcinoma of the head and neck (SCCHN). Therefore, the tumor marker potential of MMPs and M-CSF was investigated in these malignancies.

Serum of 59 patients suffering from SCCHN and of 59 healthy volunteers was obtained. The concentration of MMP-3, MMP-8, MMP-9, and M-CSF was determined by sandwich enzyme immunoassays. The MMP-3, -8, -9, as well as the M-CSF serum concentrations were significantly elevated in the patient group, compared to the healthy controls ($p<0.001$, $p<0.05$, $p<0.001$, $p=0.002$). There was significant correlation between the M-CSF and the MMP-3 serum concentration ($p<0.0001$), and between the M-CSF and the MMP-8 serum concentration ($p=0.005$). A significant correlation with the tumor stage was found only for MMP-8. MMP and M-CSF serum concentrations are of potential interest as serum tumor markers in SCCHN.

Key words: MMP, M-CSF, serum tumor marker, squamous cell carcinoma, head and neck

We are still in need for suitable tumor markers for patients suffering from squamous cell carcinomas of the head and neck (SCCHN). Unlike for e.g. prostate or breast cancer, no reliable tumor marker exists for SCCHN, up to now. The factors responsible for prognosis and response to therapy seem to be complex and, thus a combination of many prognostic and predictive factors will be probably necessary to assess prognosis and response to therapy in head and neck cancer. The prognosis of patients with SCCHN did not improve significantly during the last two decades despite several new therapy approaches. Therefore, tumor makers are crucial for finding the appropriate therapy concept. SCCHN are considered aggressive tumors with early locoregional metastatic spread, which is known as a strong prognostic factor by itself.

More than 20 different members of matrix metalloproteinases (MMPs) are known. They are subdivided into collagenases, gelatinases, stromelysins, stomelysin-like MMPs, membrane-type MMPs, and new MMPs [12]. MMPs are zinc-dependent endopeptidases and capable of degrading extracellular matrix, including the basement membrane. Physiologically, they play a role in tissue remodelling processes like e.g. embryogenesis or angiogenesis. But they are also considered to be involved in the process of tumor invasion and metastatic spread. Therefore, a high MMP serum concentration implicates a high invasive potential of the tumor. The importance of MMPs in SCCHN has been shown previously [6, 18].

The macrophage colony stimulating factor (M-CSF) is also known as CSF-1. It is a glycoprotein that stimulates the proliferation and differentiation of cells in the hematopoietic pathway [4]. But M-CSF has also many effects on mature cells, for example stimulation of monocytes to produce interferon and tumor necrosis factor [17]. Due to the macrophage activation, including phagocytosis and enzyme production, M-CSF is an important factor for the immune

system concerning the defence against infections and malignancies. The M-CSF serum concentration has prognostic value in patients with gynaecologic malignancies [11, 13, 14, 15]. Also, in patients with SCCHN, the M-CSF serum concentration is of potential interest as a tumor marker [9]. In this study we have investigated the role of MMPs and M-CSF in patients with SCCHN.

Patients and methods

Patients. A group of patients suffering from SCCHN, which was previously investigated for M-CSF serum concentrations [9] was investigated for MMP-3, -8, and -9 serum concentrations. These matrix metalloproteinases seem to be of special interest in head and neck cancer [6]. Serum of 59 patients was obtained after histopathological confirmation of the disease, but prior to the start of treatment. All patients were diagnosed with SCCHN between 1998 and 2000 in the Department of Otorhinolaryngology, Head and Neck Surgery, University of Marburg, Germany. The gender ratio was 53 male to 6 female patients. The mean age at tumor diagnosis was 59.1 years, with a range of 37 to 81 years. Twenty-seven patients had a squamous cell carcinoma of the larynx, 17 were oropharyngeal cancers, and 15 tumors were located in the hypopharynx. Table 1 shows the distribution of the tumors according to the TNM classification. According to the UICC staging (1993), 14 patients (23.7%) had a stage I disease, 8 (13.6%) were in stage II, 9 (15.3%) patients had a stage III disease, and 28 (47.5%) were already of a stage IV. In detail, sixteen (27.1%) patients had a T1 tumor, 19 (32.2%) suffered from a T2 carcinoma, 13 (22.1%) were diagnosed with a T3 tumor, and 11 (18.6%) were classified as T4 tumor. Thirty patients (50.8%) presented with a N0 status of the neck, 3 (5.1%) patients had an N1 neck, 25 an N2 neck, and only 1 (1.7%) patient presented with an N3 status of the neck. Three patients had already distant metastases at the time of tumor diagnosis. According to the histopathologic grading, the majority of the tumors was moderately differentiated (n=34; 57.6%), 5 (8.5%) were well differentiated, and 20 (33.9%) of the carcinomas were poorly differentiated.

As control group, serum from 59 healthy volunteers (42 (71.2%) male, 17 (28.8%) female) was obtained. The age range was from 19 to 86 years, with a mean of 37.9 (SD $\pm$ 25.3) years.

Methods. The sera were stored at –80C until evaluation for M-CSF and MMP serum concentrations. For determination of the M-CSF serum concentrations a quantitative sandwich-enzyme immunoassay (Quantikine, Human-M-CSF-Immunoassay, RND Systems, Wiesbaden, Germany) was used according to the manufacturer's instructions. Briefly, the serum of the patients, and of healthy individuals, as well as standard serum samples were applied to microtitre wells, which were precoated with a monoclonal M-CSF specific antibody. After washing procedures, an enzyme-linked polyclonal antibody directed against M-CSF was added. After washing, visualization was performed by adding an appropriate color solution. The color development was proportional to the amount of M-CSF bound to the wells. The optical density was measured at 570 nm with a microtitreplate reader. The detection limit for M-CSF was given at 9 pg/ml, without any significant cross reactivity or interference.

Table 1. Distribution of 59 patients suffering from squamous cell carcinomas of the head and neck according to the TNM classification, UICC 1993

	T1	T2	T3	T4	
N0	14	8	6	2	M0
N0	–	–	–	–	M1
N1	–	3	–	–	M0
N1	–	–	–	–	M1
N2	2	8	4	8	M0
N2	–	–	2	1	M1
N3	–	–	1	–	M0
N3	–	–	–	–	M1

For MMP measurements, a two-site sandwich enzyme immunoassay (Biotack MMP, human, ELISA system, Amersham Pharmacia Biotech Europe, Freiburg, Germany) was used. The serum probes for patients and controls were incubated in precoated microtitre plates with an corresponding MMP antibody. The MMPs bound to the wells were detected by a peroxidase-labeled Fab' antibody. Visualization was performed by adding a color substrate, which was read at 450 nm, as described previously [6]. The assays for MMP-3, -8, and -9 recognize the total MMP in the serum, including the precursor form, the active form, and the MMPs complexed with TIMP (tissue inhibitors of metalloproteinases). The sensitivity for MMP-3 is given at 2.35 ng/ml, for MMP-8 at 0.032 ng/ml, and for MMP-9 at 0.6 ng/ml.

Statistical analysis. The statistical package SPSS/PC (StatSoft Inc. Tulsa, Okla., USA) was used for all analysis. The Mann-Whitney-U test was performed for the comparison of groups. The statistical significance of differences was evaluated by χ^2 test. The correlation coefficient according to Spearman was used for correlation analysis. Statistical significance was assumed at $p<0.05$.

Results

Double measurements with the sandwich enzyme immunoassays revealed reproducible results for the detection of MMP serum concentrations, as well as for M-CSF serum concentrations in all 59 patient and control sera. For MMP-3, the serum concentrations in the patient group ran-

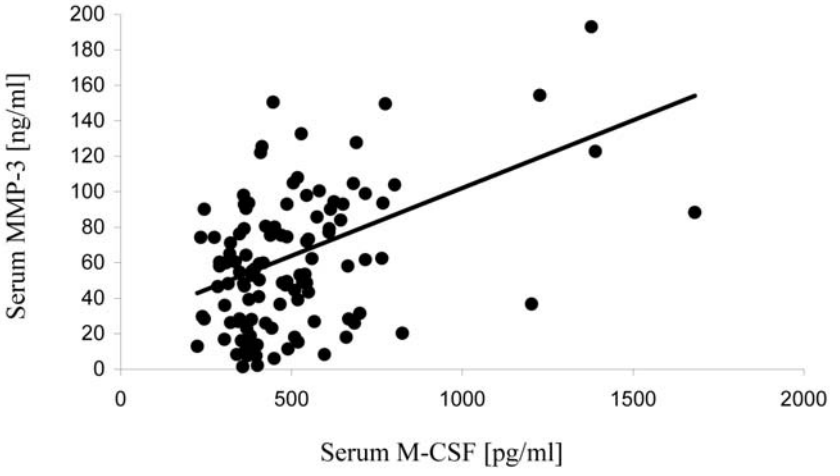

Figure 1. Significant correlation of the M-CSF serum concentration and the MMP-3 serum concentration in 59 patients with squamous cell carcinoma of the head and neck (r=0.3; p<0.0001).

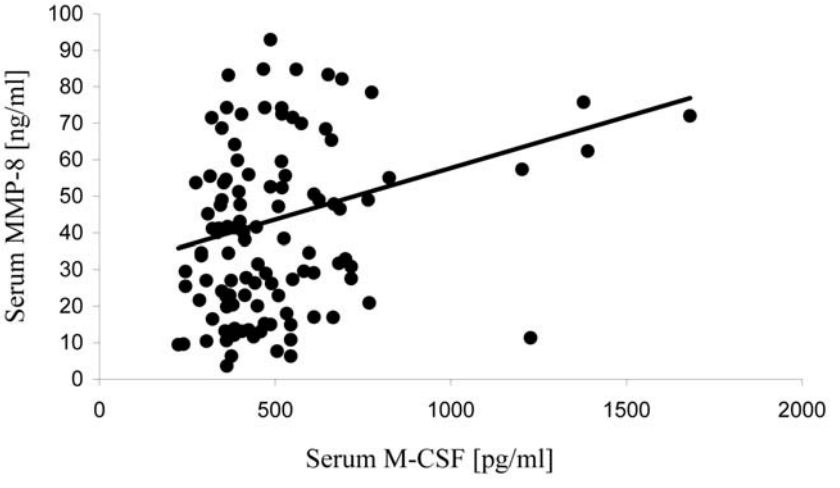

Figure 2. Significant correlation of the M-CSF serum concentration and the MMP-8 serum concentration in 59 patients with squamous cell carcinoma of the head and neck (r=0.3; p<0.005).

ged from 31.4 to 242.8 ng/ml. The mean was 94.7 ng/ml. In the control group, the MMP-3 serum concentrations ranged from 7.5 to 92.8 ng/ml. The mean was 44.3 ng/ml. The differences between the groups were statistically significant (p<0.001). The range of the MMP-8 serum concentrations in the patient group was from 7.7 to 106 ng/ml, with a mean serum concentration at 82.6 ng/ml. In the control group, the MMP-8 serum concentrations ranged from 3.6 to 90.1 ng/ml. The mean was 61.2 ng/ml. The differences between the groups were marginally significant (p<0.05). For MMP-9, the serum concentrations in the patient group ranged from 37.6 to 267.5 ng/ml, with a mean serum concentration at 79.8 ng/ml. In the control group, the MMP-9 serum concentrations ranged from 11.4 to 241.2 ng/ml. The mean serum concentration was at 46.3 ng/ml. The differences between the groups were statistically significant (p<0.001). Even, the M-CSF serum concentration was significantly elevated in the patient group, when compared to the control grup (p<0.002). The M-CSF serum concentration in the patient group ranged from 235 to 1681 pg/ml. The mean serum concentration was 565 pg/ml. In the control group, the M-CSF serum concentration ranged from 225 to 1203 pg/ml, with a mean serum concentration of 447 pg/ml.

For MMP-3 and MMP-9 serum concentrations no correlation with the TNM status or UICC stage of the patients was observed. For the MMP-8 serum concentrations, in the patient group a significant correlation with the T-status of the disease was seen (r=0.3, p=0.02). Also, a significant correlation of the MMP-8 serum concentrations with the N-status (r=0.4, p<0.0001), as well as with the UICC stage of disease (r=0.4, p=0.02) was found. For the M-CSF serum concentrations, no correlation with the TNM status or UICC stage was observed. The histopathological grading correlated significantly with the N-status of disease (r=0.5, p<0.0001) and with the UICC stage (r=0.5, p<0.0001), but no correlation with MMP-3, -8, -9, or M-CSF serum concentrations was seen. The M-CSF serum concentrations correlated significantly with the MMP-3 serum concentrations (r=0.3, p<0.0001) (Fig. 1) and with the MMP-8 serum concentrations (r=0.3, p<0.005) (Fig. 2).

In conclusion, the investigated MMPs -3, -8, and -9, as well as the M-CSF are significantly elevated in the serum of patients with SCCHN, compared to healthy controls. Only for MMP-8 serum concentrations a significant correlation with the stage of disease was found in these patients. A significant correlation of the potential tumor markers MMP-3 and MMP-8 with M-CSF in the serum of patients with SCCHN was seen.

Discussion

Tumor markers gain more and more importance in modern oncology, due to various new therapy options apart from surgery, with different chemo-/radiation therapy protocols. For patients with SCCHN, the prognosis did not improve significantly during the last two decades. This seems to be due to the narrow anatomical conditions in the head and neck region, making it difficult for surgical removing of

the tumor without leaving back single tumor cells. Another reason seems to be the aggressiveness of squamous cell carcinomas in the head and neck region with early locoregional metastatic spread. Additionally, patients suffering from SCCHN are often in a poor immunological competence, due to heavy tobacco and alcohol abuse. For patients with SCCHN no widely accepted tumor marker is available. The most investigated tumor markers for these malignancies are CEA (carcino-embryonic antigen) and SCC-AG (squamous cell carcinoma antigen), which could not be routinely established, because of their low sensitivity and specificity [2, 16]. We found interesting tumor marker potential of the monoclonal Ki-S1 in patients with squamous cell carcinoma of the hypopharynx [8]. Ki-S1 was found to be a prognostic marker by detecting the proliferative activity. And Ki-S1 was of predictive potential what response to chemotherapy is concerned, by targeting topoisomerase II alpha. But for Ki-S1 measurements, a biopsy has to be taken, and therefore Ki-S1 is not suitable as a follow-up marker. The serum Cyfra 21-1 concentration in patients with SCCHN is an accepted marker for clinical follow-up, since an increase indicates disease progression [3, 7]. But because the individual Cyfra 21-1 serum levels vary strongly among patients with SCCHN, it is not possible to find an appropriate cut-off level for these malignancies. Therefore, Cyfra 21-1 is not suitable as tumor marker for the diagnosis and prediction of response to therapy in patients with SCCHN. MMPs are known to have tumor marker potential in head and neck cancer [1, 5, 6, 10], and also for M-CSF there is evidence for its tumor marker potential in these malignancies [9]. Elevated serum concentrations of MMPs, especially in combination with elevated M-CSF serum concentrations could be of interest in the diagnosis and follow-up in patients with SCCHN, and thus they could have good tumor marker potential in SCCHN.

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