Introduction

Although the overall incidence and mortality of gastric cancer have dramatically declined over the last few decades, it remains a major health problem and the second leading cause of cancer deaths worldwide (1,2). Radical surgery is the most efficient treatment for operable cancer, but recurrences are common, being detected in approximately 60% of patients (3). Therefore, identifying of poor prognostic factors that may predict the tumor recurrence and prognosis of patients is an important for selection appropriate treatment protocols. Several clinicopathological parameters such as tumor size, histological type, tumor differentiation, depth of tumor invasion, regional lymph node involvement, distant metastasis and tumor stage, have been reported as important prognostic factors (4). Recently, new prognostic biological and molecular indicators have been documented including oncogenes, cell-cycle regulators, DNA repair genes, c-erbB-2 (5,6). Thus, understanding of the importance of these markers remains an important challenge in translational research.

Cytokeratin 18 (CK18) is an intermediate filament that released from cells into the circulation during both necrotic and apoptotic cell death. Caspase-cleaved CK18 (M30) and total CK18 (M65) are measured in the circulation by enzyme-linked immunoabsorbent assays (ELISA) (Figure 1). It is believed that to act as a quantitative biomarker of total cell death (8,9). Prognostic importance of both M30 and M65 assays have previously been evaluated and it has been shown that they may have an important prognostic and predictive biomarkers in several malignancies (10-15). Scott et al. indicated that these assays may be potential markers of tumor response to chemotherapy and were associated with increased risk of recurrence in gastrointestinal malignancy (16).

The anticancer activity of chemotherapeutic agents is directly associated with the induction of apoptosis in
tumors. Whilst the apoptotic pathway is complex, the intrinsic mitochondrial pathway is the predominant apoptotic pathway in cancer cells. Among the several cellular substrates of the capsases, members of the cytokeratin family, including CK18, contribute to cellular collapse and apoptosis. Most cytotoxic drugs induce apoptosis, which increase significantly 24 hours after chemotherapy (17). Therefore, apoptosis can be measured in serum by several biomarkers by which the efficacy of cytotoxic chemotherapy can be detected (11,18). de Haas et al. showed that both serum M30 and M65 levels were increased after chemotherapy. These assays could also reflect chemotherapy induced cell-death in patients with testicular cancer (19). Recently, we analyzed prognostic significance of the increase in the serum M30 and M65 values after chemotherapy in two studies for patients advanced-non-small cell lung cancer (NSCLC) and gastric cancer (20,21). In addition, we showed that increased serum M65 levels after chemotherapy could be predicted tumor response in advanced-gastric cancer patients (21). This article reviews the importance of CK18 for the efficacy of chemotherapy in patients with gastric cancer in the light of recent advances.

**Prognostic significance of CK18 in cancer**

The majority of chemotherapeutic agents kill tumor cells by some mechanisms including apoptosis and necrosis. Apoptosis may be an important mechanism for the evaluation of the efficacy of the anti-cancer therapy (7,22,23). Some biomarkers such as CK18 have been used for the detection of apoptosis of epithelial cells. M30 is a caspase-cleaved form of CK18 that it is released into circulation during apoptosis, whereas necrosis is supposed to release M65 that is an intact or total form of CK18 (8,9,24). Two sandwiched ELISA assays, M30 and M65, can be used to determine different circulating forms of CK18 in either plasma or serum, and have been proposed to be surrogate biomarkers of different mechanisms of cell death (8,25). The M30 ELISA assay uses the M5 antibody as a catcher and the M30 antibody to detect caspase-cleaved CK18 produced during the early stages of apoptosis (25). The M65 assay also detects cleaved fragments but uses a different detection antibody from M30, which does not distinguish between the full-length protein and its fragments. The M65 assay theoretically measures both caspase cleavage and cellular release of intact CK18 (8). Serum M65 and M30 levels were shown to be elevated in patients with different types of carcinoma (10,12,14,16). The measurement of caspase-cleaved or total CK18 from epithelial-derived tumors could be a simple, noninvasive way to monitor or predict tumor progression (26) and prognosis (10,12,26).

de Haas et al. showed that serum M30 level was an important prognostic factor in testicular cancer (19). In a study performed by Dive et al., they reported that the median M65 levels in patients with metastatic pancreas cancer were higher compared to patients with locally advanced or resectable pancreas cancer. Moreover, they found that M65 levels were associated with poor overall survival (OS) in the univariate analysis (27). Koelink et al. indicated that M65 levels were related to disease stage and tumor diameter in colorectal cancer patients (26). The caspase-cleaved/CK18 ratio, which decreased with tumor progression, was also predictive of disease-free survival (DFS), with a low ratio associated with worse DFS. In a study of patients with advanced gastric carcinoma, Yaman et al. found that both serum M30 and M65 levels were significantly increased in patients with advanced gastric cancer compared to control group (10). In addition, the patient population with higher M30 levels had significantly shorter median survival rates than the population with lower serum M30 levels, whereas there was no impact of serum M65 levels on survival. In contrast to their study, we showed that increased plasma levels of both M30 and M65 could predict progression-free survival (PFS) in patients with advanced-gastric cancer in our study (28).

Recently, Yildiz et al. analyzed the serum M30 and M65 levels in patients with epithelial ovarian cancer (EOC) (29). They found that the median M30 and M65 serum levels were significantly elevated in the EOC patients compared with the healthy controls. Furthermore, patients with higher M65 levels had shorter PFS, both M30 and M65...
serum levels were significantly higher for serous-type histology and increased M65 serum levels were associated with advanced disease and higher grade. M65 levels were higher for chemotherapy-resistant patients and in the multivariate analysis an elevated serum M65 level was found to be only significant independent prognostic factor (29).

Similarly, in our study including advanced-staged NSCLC, our findings demonstrated that serum 65 levels elevated in patients population compared to a healthy control group and increased M65 level could predict PFS (30). On the other hand, it found that there were discrepancies in other studies. Although M30 and M65 levels were detected to be increased in patients with breast cancer, pancreatic cancer and nasopharyngeal carcinoma compared to healthy control, their predictive and prognostic roles on survival were not proved (14,31,32). Selected trials evaluating prognostic significance of CK18 in solid tumors are summarized in Table 1. Changing of CK18 after chemotherapy in solid tumors

M30 and M65 levels have been previously found to be increased within 1 to 3 days after the chemotherapy in patients with breast and prostate cancer (13,33). However, de Haas et al. observed that most significant changes occurred 7 days after the chemotherapy in patients with testicular cancer, which may reflect the cumulative effect of the 5-day dosing regimen used in testicular cancer (19). Demiray et al. evaluated the serum M30 levels before and 24 and 48 hours after neoadjuvant chemotherapy in 42 patients with breast cancer (11). The authors found that the serum M30 levels increased significantly at 24 and 48 hours after chemotherapy and that this change was a predictor of the tumor response. Similar findings for M30 levels were reported for patients with breast cancer in Ulukaya et al.'s study (18).

We previously reported that the serum M30 and M65 levels were increased significantly after chemotherapy in patients with NSCLC (20). In addition, the elevated M30 values were an independent prognostic factor for both PFS and OS after chemotherapy. de Haas et al. showed that both serum M30 and M65 levels were significantly increased up to 7 days after chemotherapy in patients with testicular cancer (19). Furthermore, they found a significant decrease in the serum M30 and M65 levels during the first two weeks of chemotherapy compared to baseline values. Thus, the authors indicated that the overall decrease in M30 and M65 values may be indicative for response to treatment and reflect a decrease in tumor load because of chemotherapy-induced cell death.

Greystoke et al. explored the utility of serum total

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<tbody>
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<td>M30 level was an important prognostic factor</td>
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<td>Dive et al. (27)</td>
<td>Pancreatic cancer</td>
<td>103</td>
<td>M65 levels in metastatic patients were higher than locally advanced or resectable patients. Moreover, M65 levels were associated with poor OS</td>
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<tr>
<td>Yaman et al. (10)</td>
<td>Gastric cancer</td>
<td>38</td>
<td>Higher M30 levels was significantly related with shorter OS</td>
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<tr>
<td>Bilici et al. (28)</td>
<td>Gastric cancer</td>
<td>34</td>
<td>Increased plasma M30 and M65 levels were associated with poor PFS in advanced-gastric cancer patients</td>
</tr>
<tr>
<td>Yildiz et al. (29)</td>
<td>Ovarian cancer</td>
<td>56</td>
<td>Higher M65 levels was associated with poor PFS. Both M30 and M65 levels were significantly higher for serous-type histology. Moreover, M65 levels were higher for chemotherapy-resistant patients and an elevated M65 level was the only significant independent prognostic factor</td>
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<tr>
<td>Oven Ustaalioglu et al. (30)</td>
<td>Non-small cell lung cancer</td>
<td>32</td>
<td>Increased M65 level was related with poor PFS and could predict PFS</td>
</tr>
<tr>
<td>Tas et al. (31)</td>
<td>Breast cancer</td>
<td>80</td>
<td>Neither serum M30 nor serum M65 were significantly associated with survival</td>
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CK18, cytokeratin 18; OS, overall survival; PFS, progression-free survival.
CK18 (M65) and caspase cleaved CK18 (M30) as a pharmacodynamic biomarker in patients treated with chemotherapy for metastatic colorectal cancer (34). They showed that in patients with progressive disease on therapy repeated sampling revealed profiles with high pre-treatment and progressive upwardly after one cycle of chemotherapy.

**Prognostic and predictive significance of CK18 after chemotherapy for patients with gastric cancer**

Firstly, the serum M30 and M65 levels were analyzed in a study carried out by Yaman et al. in patients with advanced-gastric cancer (10). They showed that the serum M30 and M65 values were significantly higher in patient population compared with healthy control group. In addition, only M30 levels were associated with worse survival. Thereafter, we also evaluated plasma M30 and M65 levels for advanced-gastric cancer patients (28). We found that plasma 65 levels for patients with gastric cancer were significantly higher those of healthy controls. Furthermore, both elevated plasma M30 and M65 levels were related with worse PFS and OS, but this relationship could not be proved in the multivariate analysis.

In our recent study, the significance of changes in the serum M30 and M65 levels after chemotherapy in patients with advanced-gastric cancer (21). We showed that both serum M30 and M65 serum levels were significantly increased 48 hours after start of chemotherapy. A multivariate analysis showed that only the increase of M30 values was an independent prognostic indicator for PFS, but not for OS. Although the increased M65 value was an important prognostic marker for both PFS and OS in the univariate analysis, its’ prognostic significance could not be confirmed by multivariate analysis. Moreover, patients with increased both serum M30 and M65 values after chemotherapy had better objective response rate compared to patients with low M30 and M65 levels. However, only the changing of M65 after chemotherapy was significantly found to be an independent factor in predicting response to chemotherapy. In other words, patients who responded to chemotherapy had 1.4-fold higher increase in serum M65 values compared with the non-responder.

Previous studies showed that CK18 assays may be beneficial in early assessment of treatment-related tumor death and subsequent prediction of response to therapy (35,36). On the other hand, early both M30 and M65 changes during chemotherapy may not be helpful, because of overlap with host toxicity (37). We found significant changes of both serum M30 and M65 levels within 48 hours after the first chemotherapy cycle in patients with advanced-gastric cancer (21). This was in contrast to the results of Greystoke et al. (34), who did not show that any significant changes in the serum levels of both M30 and M65 within the first 48 hours, but they have indicated that for many patients there was a decrease in serum M65 levels, and to a lesser extent M30, from 1 week after chemotherapy. These findings were compatible with de Haas et al.’s study (19).

The effect of taxanes on mitotic catastrophe, characterized by the occurrence of aberrant mitosis has been demonstrated. Although mitotic catastrophe is not a type of cell death, it will result in cell death either by apoptosis or necrosis (38,39). Kramer et al. also reported that serum caspase-cleaved CK18 (M30) levels were increased during docetaxel treatment in patients with hormone refractory prostate cancer (33). Hence, the authors concluded that docetaxel induced apoptosis *in vivo*. The majority of patients with metastatic gastric cancer are treated with a combination chemotherapy including docetaxel. Therefore, significant changes in serum M30 and M65 levels for patients with advanced-gastric cancer receiving docetaxel are reasonable. There is an initial effect of chemotherapy on the population of cells that are chemo-sensitive leading to an initial reduction in overall tumour burden, with the later increases in circulating CK18 reflecting subsequent growth in the population of chemo-resistant cells. Therefore, both serum M30 and M65 levels could be used as surrogates of treatment response to monitor the development of chemoresistance and lead to early changes in therapy. Table 2 shows selected trials that analyzed prognostic and predictive significance of CK18 after chemotherapy for patients with gastric cancer and other cancer.

**Conclusions and future direction**

CK18 may modulate intracellular signaling and apoptosis via interactions with various related proteins. There is evidence to show that CK18 is involved in the invasive or growth properties of tumors. Caspase-cleaved CK18 (M30) and total CK18 (M65) are measured in the circulation by enzyme-linked immunoabsorbent assays (ELISA). The measurement of M30 or M65 from epithelial-derived tumors could be a simple, noninvasive way to monitor or predict tumor progression and prognosis. However, there are conflicting results *in vitro* and *in vivo*. These discrepancies may be due to differences among the
carcinoma types, therefore the precise roles of CK18 are currently unknown. But, particularly, in patients with gastric cancer, testicular cancer and colorectal cancer, serum M30 and/or M65 levels could be used as biomarkers to evaluate treatment response and they might guide in determining of the most appropriate combination chemotherapy regimen.

These assays may be useful for evaluating treatment effects and survival in patients with gastric cancer, but in combination with other cell death markers, their importance should be tested after multiple chemotherapy sessions in larger prospective studies with long follow-up time in future.

Acknowledgements

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References


Table 2

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<td>Demiray et al. (11)</td>
<td>Breast cancer</td>
<td>42</td>
<td>M30 levels increased significantly at 24 and 48 hours after chemotherapy and that this change was a predictor of the tumor response</td>
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<tr>
<td>Ulukaya et al. (18)</td>
<td>Breast cancer</td>
<td>37</td>
<td>M30 levels significantly increased after chemotherapy. The baseline M30 levels increased about 3-times in patients showing tumor regression and its measurement may help clinicians to predict the effectiveness of chemotherapy</td>
</tr>
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<td>Greystoke et al. (34)</td>
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