Transient Increase in Serum Thymidine Kinase 1 within One Week after Surgery of Patients with Carcinoma

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Abstract. *Aim: To investigate transient increases of thymidine kinase 1 in serum (STK1) after surgery of patients with carcinoma. Patients and Methods: STK1 was determined before and within one week after surgery of carcinoma patients (non-small cell lung (NSCLC), n=25, oesophagueal, n=12, cardial, n=4,) by a chemiluminescent dot blot assay using anti-TK1 IgY antibody. Results: The mean age of the patients with high STK1 values after surgery was 63.2±8.7 years, while that for patients with stable/low STK1 values was 54.8±11.8 years; this was significantly different at P<0.05. Sixty-one percent of the patients with high STK1 values after surgery exhibited declining red blood cell (35%) or increasing white blood cell/neutrophil cell (29%) counts. The high STK1 values after surgery correlated with a prolonged operation execution time in anaemic patients (3.43±0.90 h), as compared non-anaemic patients (2.72±0.79 h). The high STK1 values also correlated with extensive surgery programs (oseophageal/ cardial carcinoma, 3.31±0.70 h, versus NSCLC, 2.44±0.63 h). Conclusion: The transiently elevated STK1 values post-operation might be due to surgery-induced complications, such as anaemia and infection/inflammation, but also to operation execution times and age of patients. We suggest that STK1 should not be used within one week post-operation, but before surgery and after one month to avoid non-tumor-related increases in STK1, and thus misleading results.*

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Surgery induces physiological stress responses such as activation of immunological, inflammatory, metabolic and endocrine mediators (1, 2). Oxidative stress is also believed to be part of the surgical stress response (1), associated with myocardial injury, sepsis, pulmonary oedema, kidney and liver failure and increased mortality. Oxidative stress appears when reactive oxygen species exceed the capacity of the detoxification systems in the cells. In a study where laparoscopic (minimal invasive surgery) cholecystectomy patients were compared with patients receiving open cholecystectomy, adrenaline levels increased significantly in the open cholecystectomy patients, but not in the laparoscopy patients (2). The plasma glucose, interleukin-6 (IL-6), and C-reactive protein (CRP) were also higher after using open cholecystectomy, as compared to the laparoscopy. Thus, it seems that endocrine and inflammatory stress responses increase significantly when using a more extensive surgery.

Thymidine kinase 1 (STK1) is a reliable proliferation and prognostic tumour marker in a number of different types of malignancies (3-5). STK1 is also useful for monitoring the effect of tumour therapy (6-12). Unexpectedly, we found that the STK1 value increased transiently in about 40% of patients with gastric carcinoma during the first week after surgery, and then declined to values corresponding to those found in healthy persons one month post operation (13). In another study, we also found that STK1 increased in persons with inflammation and infection (12). Growth factors of different types (such as epidermal growth factor, transforming growth factor-∂, transforming growth factors, platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor-1, tumor necrosis factor) are also involved in healing and repair, associated with inflammation (14). An increase in STK1 may be expected since inflammation activates the immune system, leading to enhanced cell proliferation of the immunologically competent cells (growth factors bind to specific rectors on cell membranes and trigger a series of events culminating in cell proliferation). Thus, we suggest that the transient increases in STK1 observed might be due to surgery-induced

physiological reactions or medical complications. Differential increases in STK1 concentrations in relation to various surgical programs may be further supported by the lack of increase in STK1 found in bladder carcinoma patients receiving cystectomy by electron therapy (9). Electron therapy is more gentle than using the scalpel, which was probably the reason why no increases in the STK1 values within the first week post operation were found, but there was a rapid decrease in STK1 values for healthy persons.

In this study, we investigated possible factors leading to transient high STK1 values in patients during the first week post-operation. For this purpose, we chose patients with non-small cell lung carcinoma (NSCLC), oesophageal and cardial carcinomas, representing surgery of different extents. Although surgical complications may not be well defined, in this study, the presence of anaemia, infections and inflammations, which may result from temporal blood loss and injury of tissues due to the surgical procedure (14), were studied. We also investigated the ages of the patients, considering that older patients may be more stressed by surgery than younger patients.

Patients and Methods

Patients. Sera from pre- and postoperative patients with histologically diagnosed NSCLC (n=25) and oesophageal (n=12) and cardial (n= 4) carcinoma, 41 patients in total, were collected at the Department of Thoracic Surgery Oncology, Cancer Institute and Hospital, CAMS, Beijing, P.R. China, during 2006-2008. All patients received surgery. The lung carcinomas were a mixture of squamous/adenoma types, as well as a mixture of stages IA/IB and IIIA, and G3/G4 (stages: IA n=5, IB n=6, IIB n=2, IIIA n=7; grades: G2 n=1, G2/G3 n=4, G3 n=5, G3/G4 n=3, G4 n=6; 6 squamous, 11 adenoma, 2 squamous/adenoma type; 2 benign). The oesophageal carcinomas were of squamous type and consisted predominantly of stage III and G3 (I n=1, IIA n=3, IIB n=1, III n=6; grades: G2 n=1, G3 n=7, G3/G4 n=1, G4 n=1). The cardial carcinomas were of adenoma type of stages III/IV and G3/G4 (IIIA n=2, IV n=2; grades: G3 n=2, G3/G4 n=2). Sera from 95 healthy persons, *i.e.* without any known disease, were collected and used as controls. All serum samples were taken in non-heparinised tubes from patients and healthy individuals before breakfast, and then stored at –20˚C before the analysis of STK1. The study was conducted in accordance with the Helsinki Declaration of 1983 and with ethical permission from the Cancer Institute and Hospital, CAMS, Beijing, P. R. China.

Red blood cells (RBC),white blood cells (WBC) and neutrophils (NC). The RBC, WBC and NC values were determined in a routine manner. RBC values are expressed as n/l; the normal range of RBC values for men is 4.0-5.5/l and for women 3.5-5.5/l. WBC values are expressed as n/l and the NC values are expressed as a %; the normal range for WBC is 4.0-10.0/l and for NC 51-75%.

STK1 enhanced chemoluminescent (ECL) dot blot assay. The procedure of the ECL dot blot assay was carried out as described elsewere (11). Briefly, 3 μl of serum were directly applied onto a nitrocellulose membrane (Hyband-C, Amersham, Sweden). Anti-TK1 IgY antibody (SSTK Ltd, Shenzhen, P.R. China) was added and incubated at room temperature for 2 h. After incubation with a biotinylated secondary antibody (Jackson ImmunResearch Lab. Inc., PA, USA) for 40 min at room temperature, the membrane was incubated in TBS buffer with avidin-HRP-streptavidin (Amersham, Sweden), followed by addition of the substrate ECL (Amersham). The light intensity of the single spot on the membrane was detected in a CIS-1 imaging system (SSTK Ltd, Shenzhen, P. R. China). The value of the area under the receiver operating curve (ROC) for the STK1 assay was found to be 0.941 (11). The coefficient of variation (CV) of the dot blot assay was less then 10% (11).

The STK1 values were divided into three groups: high STK1, stable STK1 and low STK1. The high STK1 group was defined by an increase of more than 10% and the low STK1 group by a decrease of more than 10%, as compared to the STK1 values before the operation. The stable STK1 group was defined by changes in the STK1 values within ±10% of the preoperative value.

Statistical analysis. Statistical analysis was carried out using Student's *t*-test. A *p*-value <0.05 was considered as being statistically significant.

Results

In this study, we examined factors that could affect the levels of STK1, such as executive surgery time, loss of blood and appearance of infection/inflammation, reflected by changes in the number of RBC and WBC/NC, respectively, and by ages of the patients. Older patients may suffer more from surgery than younger patients, and thus, may exhibit more stress reactions that could influence the STK1 values. Serum samples from 31 carcinoma patients with high STK1 values were collected. As controls to the elevated STK1 values, serum samples from 10 carcinomas patients with stable or low STK1 values were used. Furthermore, sera from 95 healthy persons were analysed to show that STK1 increase in sera when healthy persons have tumours.

STK1 values. The mean STK1 values of the carcinoma patients before surgery (lung 4.6±6.7 pM, oesophageal 4.3±4.3 pM, cardial 4.3±3.2 pM) were significantly higher than these of healthy persons (1.3±0.7 pM) (p=0.01). In the group of patients with high STK1 values after surgery, the mean STK1 values of the various carcinoma groups were elevated (lung 6.5±4.7 pM, oesophageal 8.6±5.0 pM, cardial 10.1±5.2 pM), but not statistically significant different. The reason for this may be that the individual level of STK1 differed markedly between the patients, reducing the significance level. Therefore, we decided plot the individual values (Figure 1); in this figure we show serum samples with high, stable and low STK1 values.

STK1 in relation to RBC and WBC/NC. Thirty five percent (11/31) of the patients with high STK1 values at post-operative day 7 exhibited decreasing RBC values (*i.e.*

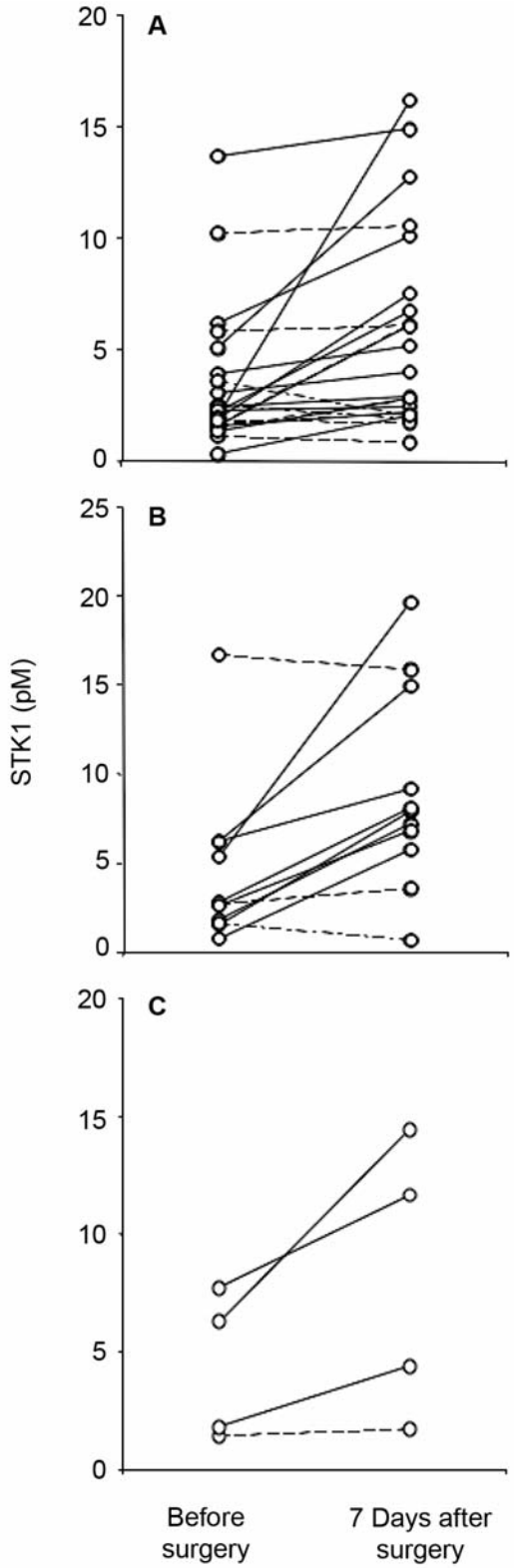

Figure 1. *STK1 values for individual patients with (A) non-small cell lung carcinoma, (B) oesophageal and (C) cardial carcinoma before and seven days after surgery. Solid line, STK1 increase; dotted line, STK1 stable or decrease.*

anaemia) starting at day 2 after operation (Table I); this decrease was statistical significantly (p=0.002). One patient with a decreasing RBC value had a stable STK1 value (STK1: 16.6 -> 15.8 pM; RBC: 3.35 -> 2.7/l). In another group of patients with high STK1 values, separated from the patients with anaemia, 26% (8/31) were found to have increased WBC and/or NC values (Table II). One of the patients also exhibited severe infection. All of the patients with stable or decreasing post-operative STK1 values exhibited normal WBC/NC values. Thus, of the patients with high STK1 values after surgery, in total 61% exhibited anaemia (35%) or abnormal WBC/NC (26%) values.

STK1 and operation time. Extensive operation may causes stress reactions or physiological complications in patients. However, there was no significant difference in the operation execution time between non-anaemia patients with high or stable/low STK1 values when patients were combined (data not shown). On the other hand, in patients with lung and oesephageal carcinomas exhibiting anaemia and high STK1 values, operation execution time was found to be significantly prolonged (3.43±0.90 h, n=10, p=0.05), as compared to patients without anaemia (2.44±0.63 h, n=18). Furthermore, for operation of oesephageal and cardial carcinoma patients, which recieve more extensive surgery, a significantly (p=0.018) prolonged mean operation execution time was found (3.31±0.70 h, n=9), as compared to lung carcinoma patients with less extensive surgery (2.44±0.63 h, n=11). Among the oesophageal and cardial carcinoma patients, 81.3% had a high STK1 value, while the corresponding value for the lung carcinoma patients was 64.0% (data not shown).

STK1 and ages. Patients with high STK1 values after surgery were significantly (p<0.05) older by 8.4 years than those with stable/low STK1 values after surgery (STK1 high: 63.2±8.7 years, n=29; STK1 stable/low: 54.8±11.8 years, n=12; STK1 high with anaemia 63.3±7 years, n=12).

Discussion

Serological TK1 has been used as a proliferation tumour marker for more than 25 years. Its activity has been used as a prognostic factor in lymphoma and leukaemia (15), and in some solid tumours, for example breast cancer (16). Recently, the concentration of TK1 in serum (STK1) was shown to be a marker for the prognosis of patients with of a number of types of both solid and blood cancer, and also for monitoring of tumour therapy (3-13). When a tumour is resected by surgery successfully, STK1 starts to decrease, with a half-life from weeks to months, depending on the tumour type (9, 13). On the other hand, when the patients are treated with chemotherapy or radiation, the STK1 value

Table I. *STK1 and red blood cell (RBC) before and after surgery of male and female of patients with lung and oesophageal carcinoma.*

	STK1 (pM)			RBC (x10^6/μl)			
Gender	Day 0	Day 7	*p*-Value	Day 0	Day 2	Day 7	*p*-Value
Men	4.8±4.2 n=7	8.1±4.5 n=7	0.185	4.2±0.4 n=7	3.8±0.2 n=7	3.3±0.4 n=7	0.002
Women	5.5±6.5 n=5	9.3±5.7 n=5	0.358	3.9±0.4 n=5	3.2±0.3 n=4	3.2±0.1 n=5	0.02

Normal range for man: 4.0-5.5x10^6/μl, for woman 3.5-5.5x10^6/μl. Values are mean±standard deviation. There was a significant decrease in the RBC count from day 0 to day 7 in both men and women.

increases during the first few days in those patients who respond to the therapy, and then declines (17). The transient increase in STK1 is due to disintegration of the tumour cells, and thus release of TK1 into the blood. However, in some patients receiving surgery, STK1 also increases within one week postoperatively (13). It is not very likely, however, that this increase in STK1 values is due to release of TK1 from the tumour cells, since the tumour has been removed by the surgery. STK1 also increases to some degree in healthy persons who are blood donors and in women during menstruation (8), indicating stimulation of production of new blood cells in the bone marrow. Inflammation and infection also increase the STK1 value to some degree (8, 12).

In this study, 61% of the patients with high STK1 values, increased STK1 correlated to changes in the blood status (RBC, anaemia) and activation of the immunological system (WBS/NC), factors recently shown to influence the level of STK1 (8, 12). It is likely that the anaemia was due to loss of blood during the surgery, since the patients received 200 to 400 ml of blood during the operations. The high STK1 values after operation also correlated with the degree of surgery extensiveness, shown by the fact that those patients with longer operation times also showed higher STK1 values. Furthermore, when comparing patients undergoing a more extensive type of surgery (*i.e.* these with oesophageal/cardial carcinoma) a higher frequency of patients with high STK1 values was found. These results are in agreement with results from a study on rats after partial hepatectomy. Liver cytosolic TK activity was found to peak at 36-48 hours, while the activity of AST, ALT and TK in the plasma peaked at 12-24 hours (18). The authors concluded that the elevated TK activity and the concentrations of AST and ALT in the plasma did not reflect regenerative growth of the liver, but were more likely due to injury of the liver. In another study in human patients recently, TK activity and protein kinase (PK) were significantly elevated at seven days after nephrectomy and then decreased continuously to normal values after 2 months (personal communication, Dr. B. Nisman, Depart. of Oncology and Urology, Hadassah University Hospital, Jerusalem, Israel, 2008). They concluded that the increase in TK activity and PK values reflected proliferative activities of cells involved in the wound-healing processes.

Table II. *STK1 in relation to white blood cell (WBC) and neutrophil (NC) counts of lung and oesophageal carcinoma patients before and after surgery.*

	STK1 (pM)		Blood values, day 7	
Patient no.	Day 0	Day 7	WBC (x10^3/μl)	NC (%)
5	1.3	2.1	10.2	78
153	1.5	7.9	17.0	80
241	2.8	8.0	14.3	77
252	3.9	5.1	14.3	77
282	5.0	12.7	11.1	74
295	1.3	2.8	13.6	56
240	5.3	19.6	9.0	77
289	2.0	16.2	7.8	41

Normal range of WBC: 4.0-10.0x10^3/μl, NC: 51-71%.

The reasons for the elevated WBC/NC values found here could be due to beginning of infection or inflammation. Infection appears from time to time in connection with surgery. In this study, only some of the patients with high STK1 values exhibited elevated RBC or WBC/NC values. Abnormal WBC/NC values could also have other explanations rather than infection/inflammation. However, these persons did not have higher WBC/NC values before the operation, thus likely excluding other illness. High STK1 values also correlated with age, *i.e.* patients that were significantly older also had higher STK1 values, indicating that older patients who go through an operation may exhibit more biological stress reactions or heal surgery-induced wounds less efficiently than younger patients.

The mechanism(s) for the transient increases in the STK1 values after surgery is not clear, but could be due to activation of biological defense systems (immunological, wound healing, stimulation of RBC production and growth factors, *etc*), which are linked to cell proliferation, and thus reflected by an elevation of the STK1 proliferation

biomarker. However, further investigations are needed to clarify these mechanisms. Whatever the reason for the elevated STK1 shortly after surgery, it could not be due to any release from the tumour, since the tumour was removed.

We conclude that the use of STK1 as a biomarker for monitoring the outcome of tumour therapy should not be used during the first week after surgery in order to avoid transient non-tumour-related increases in the STK1 value. Instead, we suggest that STK1 is determined just before and one month after the surgery to receive the full benefit of this biomarker in clinical use. The physiological changes observed in this study just after surgery may also influence the utility of other biomarkers in use today.

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