The role of thymidine kinase in cancer diseases

Ondrej Topolcan† & Lubos Holubec Jr
Charles University Prague, Medical Faculty in Pilsen, Department of Nuclear Medicine, Faculty Hospital Pilsen, 13 Edvnda Benes, 305 99 Pilsen, Czech Republic

Thymidine kinase 1 (TK 1-fetal) is a cell cycle-dependent marker that increases dramatically during the S-phase of the cell cycle. In this review, the authors discuss serum levels of thymidine kinase in a variety of neoplasias. Determination of thymidine kinase helps to monitor the follow-up of solid tumours and haematological malignancies as well as indicating the efficacy of adjuvant and palliative chemotherapy. Elevated levels of thymidine kinase must always be interpreted together with a detailed knowledge of the patient’s condition because nonspecific elevations of serum levels (inflammatory and autoimmune diseases) must be excluded.

Keywords: breast cancer, colorectal cancer, follow-up, haematological malignancies, lung cancer, radioenzymatic assay, therapy monitoring, thymidine kinase


1. Introduction

Laboratory diagnosis currently represents an integral part of patient care. A doctor expects of the laboratory examination either confirmation of his/her hypothesis, determination of further examining procedures or help with monitoring the course of the disease. Laboratory methods are also used for the choice and monitoring of treatment. However, quite often the monitored parameters do not comply with any of the above-mentioned requirements, but they still have an irreplaceable position in routine practice. These are the kinds of parameters that determine the activity or, better, the aggressive nature of the disease. Another possibility is in their prognostic value, particularly with regard to disease-free interval, overall survival and life quality. Cellular enzyme thymidine kinase, determining cellular proliferation in connection with the inception, development and treatment of the disease, belongs to such a group of parameters. It is for this reason that this overview article is devoted to thymidine kinase.

2. Characterization of thymidine kinase

The incorporation of thymidine in DNA was demonstrated around 1950 [1]. Somewhat later, it was shown that this incorporation was preceded by phosphorylation, and around 1960 the enzyme responsible was purified and characterised. It was shown that higher organisms have two isoenzymes that are chemically very different, TK1 and TK2. The former was first found in fetal tissue, the second was more abundant in adult tissue, and initially they were termed fetal and adult thymidine kinase. Soon it was shown that TK1 was present in the cytoplasm only in anticipation of cell division (cell cycle dependent), whereas TK2 was located in mitochondria and is cell cycle independent [2,3]. In 1973 Hopkins et al. dealt with enzymatic activity of thymidine kinase (TK) and its role in growing and regenerating livers from rats [4]. Also, Fukui analysed factors regulating thymidine kinase in regenerating liver in rats after heptectomy [5].

De novo thymidine phosphate synthesis is normally catalysed by thymidilate synthetase from deoxyuridine monophosphate in the presence of folic acid and
The role of thymidine kinase in cancer diseases

Thymidine kinase (EC 2.7.1.21) is a cytosolic enzyme in the one-step pyrimidine salvage pathway catalysing the transfer of the terminal phosphate from ATP to 5'-hydroxyl group of thymidine (dThd) to produce dThdMP, which is then incorporated into DNA in the presence of a divalent cation such as Mg^{2+}. Several TK isoenzymes are found in a given eukaryotic cell [2,6].

The enzyme appears to play the primary role in regulating intracellular thymidine pools throughout the cell cycle. There are two thymidine (dThd) kinases in human cells. The first isoenzyme, the cytosolic, S-phase-specific (fetal, TK1), is found only in proliferating cells, with production restricted to the S-phase of the cell cycle that is associated with cell division. The activity of TK1 increases markedly after the G1-S transition and then declines rapidly in G2. TK1 is a homodimer or homotetramer with a 24-kDa monomer subunit [7-9]. TK1 can phosphorylate analogues with modifications at the N-3 or C-5 position of the pyrimidine ring and the 3' position of the ribose; it also phosphorylates clinically important nucleoside analogues, including 5-fluoro-2'-deoxythymidine and 3'-azido-2',3'-dideoxycytidine (AZT, azidothymidine) [9,10]. The second isofrom, the mitochondrial (adult, TK2), is cell cycle independent. The mitochondrial thymidine kinase TK2 can also phosphorylate thymidine and deoxyuridine. The concentration of TK2 in tissues is not correlated with proliferation. TK2 plays an essential role in the synthesis of mitochondrial DNA precursors and is involved in certain forms of mitochondrial diseases but not in diseases related to cell proliferation [12]. The substrate specificity of TK2 is different from that of TK1, for example thymidine analogues such as AZT are phosphorylated at a much lower efficiency by TK2 than by TK1 [9,10].

In human cells, the gene for TK1 is located on chromosome 17 and the gene for TK2 on chromosome 16 [13]. Information about the properties of various forms of the dThd kinases is extensive, and diverging observations have been reported as to the molecular mass and electrophoretic behaviour and kinetic properties of these two enzymes at different stages of purification. However, human TK1 has recently been purified to homogeneity from HeLa cells, and the enzyme was a tetramer of 24-kDa polypeptides. The gene has been cloned, and its cell cycle regulation has been studied in detail [14].

The role of the TK1 promoter in regulation has been reviewed, and the mechanism for cell-cycle-dependent degradation of TK in most G2-M cells has been determined [15]. This is a feature that is of particular importance for the assay described here. Serum TK has been determined by the use of a commercially available, highly sensitive 125I-deoxyuridine radioenzyme assay (TK REA). This assay can provide prognostic information in malignancies, and in some cases can aid in the choice of therapy [5]. The assay has disadvantages in that it uses 125I, is relatively complex and partly measures the activity of TK2. In most cases the latter is not a major concern because TK1 is the predominant form in serum, but in certain instances, particularly when cellular extracts are assayed, false-positive results may occur. Although immunochemical methods have been reported, a highly sensitive but convenient approach for routine measurement of serum TK1 in haematological malignancies has not been forthcoming [16-19].

The transcriptional rate of the TK1 gene has been shown to increase many times following growth stimulation of non-proliferating cells and during the cell cycle [20]. Kim and Lee have identified a 20-bp region in the promoter of the human thymidine kinase gene containing an inverted CCAAT motif responsible for transcriptional activation of the TK1 gene [21,22]. Others have demonstrated that thymidine kinase activity is also regulated by post-transcriptional and post-translational mechanisms such as the half-life of TK1 mRNA and TK1 protein [8,23,24]. In CLL cells, Kristensen et al. have found expression of high levels of TK1-mRNA without concomitant expression of any active enzyme, indicating defective regulation at the post-transcriptional level [25]. Kauﬀman and Kelly have presented strong evidence for a regulatory mechanism at the post-translational level. Thus, the stability of the TK1 protein in HeLa cells is apparently controlled by a 40-amino-acid sequence in the carboxyl terminus of TK1, signalling speciﬁc elimination of the TK1 protein at mitosis [26]. Deletions or mutations in this part of the TK1 gene not only abolish the cell-cycle-speciﬁc degradation, but also allow TK1 to be expressed even in G0 phase [27]. Post-translational modiﬁcations may also participate in the regulation of TK1 activity, at least in HL60 cells, where the ﬂuctuation of thymidine kinase activity was related to the extent of phosphorylation of serine residues of the TK1 protein [28]. Together, these ﬁndings reveal a highly complicated regulation of TK1 expression at the transcriptional, post-transcriptional, translational and post-translational levels. The reason for such a meticulous control may, at the moment, seem obscure. The precise function of TK1 has not yet been elucidated and many TK1-deﬁcient cells apparently grow normally in culture. On the other hand, it seems likely that the control is important for the precise ﬁne-tuning of the pool size of dITP, being an allstERIC regulator of the mammalian ribonucleotide reductase [29]. Indeed, there have been several reports on increased mutagenesis due to deoxynucleoside triphosphate pool imbalances or to the lack of thymidine kinase [30,31].

The level of thymidine kinase in serum or plasma is so low that the measurement is best based on the enzymatic activity. In commercial assays, this is done by incubation of a serum sample with a substrate analogue. The most common commercially available technique is the radioenzymoanalytical method using radiolabeled deoxyuridine in which a methyl group in thymidine has been replaced with radioactive iodine. This substrate is accepted well by the enzyme (thymidine kinase in human biological ﬂuids). The monophosphate
of iododeoxyuridine is adsorbed on aluminium oxide that is suspended in the incubation medium. After decantation and washing, the radioactivity of the aluminium oxide gives a measurement of the amount of thymidine kinase in the sample [32].

A newly developed non-isotopic technique uses another thymidine analogue, for example bromo-deoxyuridine or AZT-related compounds, 3'-amino-2',3'-deoxythymidine. The only so far commercially available kit is the kit for the LIAISON instrument (DiaSorin, Italy) [33]. The source of this method is ELISA, as described by Öhrvik et al., so we have used part of the publication in this overview article [2].

The TK ELISA described by Öhrvik et al. is a non-radiometric method specifically to determine TK1 activity [2]. This was achieved by designing a two-step strategy. In the first step, TK1 present in the patient sample phosphorylates AZT to the corresponding 5'-monophosphate (AZTMP). In the second step, a competitive ELISA measures the amount of AZTMP by the use of specific goat antibodies against AZTMP and HRP-labelled AZTMP. Hydrogen peroxide was introduced to stop the TK enzyme and eliminate DTE. DTE or other sulphhydryl reagents are necessary for optimum serum TK activity, but they interfere strongly with the peroxidase activity [34]. Here the authors describe a convenient and easy method of separating the enzyme substrate and the product formed by the use of an antibody specific to the 5'-monophosphate product. A crucial point is the choice of substrate and the pyrimidine 5'-monophosphate to which the antibody is directed. An unnatural substrate is preferred because the assay relies on indirect quantification of a nucleotide. In addition, the antibodies would have to be very specific and bind selectively to the product in the presence of large amounts of substrate without exhibiting significant crossreactivity to endogenous substances. AZT and its monophosphate derivative, AZTMP, are ideal candidates against which antibodies could be raised. Highly specific antibodies against AZT and AZTMP have been generated previously and used in clinical settings to determine the concentrations of these compounds in serum and extracts from tissues and cells [35,36]. More importantly, the substrate specificity of TK1 differs from that of TK2; AZT is much less efficiently phosphorylated by TK2 than TK1 [9]. Furthermore, AZTMP constitutes a stable metabolite in blood, serum and cell extracts and is not degraded or phosphorylated further at a significant rate [37,38].

Another non-isotopic method, which is still at the stage of evaluation, even in our centre, is TK, the DiviTum® assay of the Biovica Company (Sweden). To compare it with other kits, the basic characteristics of the kit are presented according to the instructions and the paper at the ISOBM conference [39]. As a substrate for TK, the DiviTum® assay uses bromo-deoxyuridine, which is phosphorylated to its monophosphate. To immobilize and remove the monophosphate produced from the solution, it is phosphorylated further to the tri-phosphate by kinases present in the reaction solution. The tri-phosphate is immobilized by DNA synthesis, in which a polyA strand covalently bound to the microtitre plate acts well as a template, reverse transcriptase as the catalyst and oligonucleotide as the primer. After the TK activity incubation is completed, the plate is washed and incubated with an anti-bromodeoxyuridine-antibody-conjugated to alkaline phosphatase followed by a second wash. The alkaline phosphatase thus bound corresponds to the TK activity in the sample. This amount of alkaline phosphatase is evaluated using a chromogenic substrate, p-nitrophenyl phosphate (pNPP, optionally a fluorogenic substrate can be used). In summary, the novel patent applied procedure avoids product feedback inhibition, uses saturated substrate concentration and has been found to give at least a 10 times increased sensitivity for detecting a growing tumour mass. A series of standards included in the microtitre plate simplify evaluation of results and ensure a high reproducibility over time.

The TK activity found is given as nanograms per litre. This does not imply that the actual amount of enzyme in the sample corresponds to this number, but that the activity of the enzyme in the sample is the same as that of a reference sample containing this quantity of recombinant thymidine kinase [40]. In all methods this means measuring enzymatic activity, but in spite of this all manufacturers give different enzyme units per litre (IU/l) and the data concerning the time interval during which the measured amount of thymidine kinase materialised is missing. In our opinion, the unit normally used should be IU per litre per hour. This parameter was apparently given by the Immunotech Company (Prague, Czech Republic) in the transitional period, around 2000 (IU = 1.2 \times 10^{-12} \text{mol/s}). At present, no company supplying commercially either isotopic or non-isotopic kits gives this parameter in the instructions.

Thymidine kinase has been determined in tissue samples after extraction of the tissue. No standard method for the extraction or for the assay has been developed. The results indicate that there is a relationship between tissue thymidine kinase in tumour tissue and the malignant character of the tumour, but no practical use for the determination has been found. Antibodies against thymidine kinase are available for immunohistochemical detection. The frequency of positivity in a tumour tissue sample reflects the rate of proliferation, but this is not a standard procedure to assess the malignancy of tumours [41-43].

The results in the control group of healthy people and in people with inflammatory diseases, benign breast and colorectal tumours are summarized in Table 1. The table shows that considerably increased TK serum levels are found in viral infections, mainly in herpes simplex and zoster, autoimmune disease and benign granulomatous diseases (e.g., sarcoidosis, chronic hepatitis) and particularly in pernicious anemia [44-46].
The role of thymidine kinase in cancer diseases

Table 1. The levels of thymidine kinase (IU/l) in the control group of healthy people and in people with inflammatory diseases, benign breast and benign gastrointestinal tumours.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Median</th>
<th>Min. – Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy probands</td>
<td>100</td>
<td>4</td>
<td>1 – 6</td>
</tr>
<tr>
<td>Patients with bacterial</td>
<td>100</td>
<td>10</td>
<td>4 – 15</td>
</tr>
<tr>
<td>infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with viral infection</td>
<td>100</td>
<td>54</td>
<td>12 – 164</td>
</tr>
<tr>
<td>Patients with autoimmune</td>
<td>40</td>
<td>28</td>
<td>2 – 56</td>
</tr>
<tr>
<td>disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign tumours of breast</td>
<td>40</td>
<td>9</td>
<td>0 – 30</td>
</tr>
<tr>
<td>Benign tumours of GIT</td>
<td>20</td>
<td>10</td>
<td>0 – 12</td>
</tr>
</tbody>
</table>

GIT: Gastrointestinal tract.

Table 2. TK levels (IU/l) and sensitivity at 95% specificity for the prediction of progression 9, 6 and 3 months before the clinical manifestation of relapse and at the time of clinical manifestation (time zero) in patients with breast tumour.

<table>
<thead>
<tr>
<th>Time</th>
<th>TK median</th>
<th>TK min. – max.</th>
<th>TK sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3</td>
<td>2 – 11</td>
<td>5</td>
</tr>
<tr>
<td>3 months</td>
<td>11</td>
<td>4 – 23</td>
<td>34</td>
</tr>
<tr>
<td>6 months</td>
<td>18</td>
<td>5 – 24</td>
<td>47</td>
</tr>
<tr>
<td>9 months</td>
<td>24</td>
<td>17 – 46</td>
<td>69</td>
</tr>
</tbody>
</table>

TK: Thymidine kinase

3. Thymidine kinase in breast cancer

Invasive breast carcinoma (BC) is the most frequent carcinoma and the second commonest cause of death from malignant disease among women in the western world. Disease-free interval (DFI) and overall survival (OS) have been obtained with the extensive use of adjuvant systemic therapies. The possibility of having strong prognostic and/or predictive markers is of the utmost importance for clinicians in order to identify patients at higher risk of relapse and to select the most appropriate systemic treatment for an individual patient. Prognostic factors are those that predict the risk of recurrence or of death from BC independently of treatment. Predictive factors are those that distinguish between patients who are more or less likely to respond to a given therapy. However, the distinction between the prognostic and predictive values of each marker is not straightforward. The retrospective nature of the great majority of these studies may jeopardize their results. Hundreds of papers evaluating several prognostic and predictive factors have been published in the last 30 years. The prognostic factors are tumour (T) size, lymph node (N) status, hormone receptor (HR) status, histological grade and age; as predictive factors: HR status and human epidermal growth factor receptor-2 (HER2) status for endocrine and trastuzumab therapy, respectively [42,43]. Tumour cell proliferation has been investigated in breast cancer for its association with neoplastic growth, progression and metastatic potential; this article gives an overview of the knowledge gathered on tumour cell proliferative markers in the past decade, with a critical assessment of their prognostic and/or predictive value [47,48].

High levels of TK have been reported in BC patients [49-51]. For the prognostic role of TK there is only one prospective study of 290 N-negative and N-positive BC patients in whom high TK activity correlated with high grade and PgR-negativity (PgR: progesterone receptor) and predicted a worse relapse-free survival (RFS) in the pre/peri-menopausal subset and a worse OS in the post-menopausal subgroup [42]. The predictive value of TK was retrospectively evaluated in two studies, of which one analysed 1692 BC patients [42,52]. In both studies, high levels were correlated with large T size and HR-negativity or PgR-negativity [42,52]. In the first, all N-positive patients received CT with a regimen containing fluorouracil (CMF or CAF [cyclophosphamide, doxorubicin and 5-fluorouracil]) and no endocrine therapy [42]. High levels of TK were predictive of worse RFS and OS independently of the treatment, as though the overexpression of this enzyme could allow tumour cells to escape the effects of fluorouracil and methotrexate. In the second study, high TK was predictive of better disease-specific survival (DSS) and distant relapse-free interval in N-negative patients treated with anthracycline-based CT (FAC or FEC) in comparison with those who were untreated [52].

In our centre it was discovered that the preoperative average TK value in the group of 400 patients with breast carcinoma was significantly higher (p < 0.05) than in healthy people, but TK sensitivity was 35% at 95% specificity [43,53,54]. It was also demonstrated that remission values differ significantly from the progressive values detected in 90 patients during the first progression, and that preoperative values do not correlate with DFI, but with OS. A significant finding, which is documented in Table 2, is the increase in TK sensitivity at 3 – 6 months before progression, when TK sensitivity is higher than CA 15-3 sensitivity and considerably higher than carcinoembryonic antigen (CEA) sensitivity. Simultaneous assessment of CA 15-3 and TK will improve considerably the early detection of tumour disease relapse [53]. The results herein are in accordance with the publications of O’Neill et al. [49]. He reported that TK levels in the serum of breast cancer patients demonstrated a statistically significant positive correlation with the cancer stage. They also demonstrated that total tumour TK levels were significantly higher in breast cancer patients who subsequently had a recurrence than in those who did not. It appears, therefore, that TK is a potentially useful marker in the management of breast cancer. Serial measurement of TK levels, particularly TK1, in serum from breast tumours, may make it possible to predict recurrences of breast cancer.
rates of $67\%$ and $10\%$, respectively in stage or a distant stage, with resultant less-desirable survival is $90\%$. Most CRC cases are diagnosed at either a regional stage (39%), when the survival rate is 90%. Few CRC cases are cancer deaths in both men and women. Few CRC cases are ranked third in the world for estimated new cancer cases and third for estimated cancer deaths in both men and women. Few CRC cases are diagnosed at a localised stage (39%), when the survival rate is 90%. Most CRC cases are diagnosed at either a regional stage or a distant stage, with resultant less-desirable survival rates of $67\%$ and $10\%$, respectively.

Table 3. TK levels (IU/l) and sensitivity at 95% specificity for the prediction of progression 9, 6 and 3 months before the clinical manifestation of relapse and at the time of clinical manifestation (time zero) in patients with colorectal tumour.

<table>
<thead>
<tr>
<th></th>
<th>9 months</th>
<th>6 months</th>
<th>3 months</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK median</td>
<td>3.3</td>
<td>13</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>TK min. – max.</td>
<td>2 – 9</td>
<td>8 – 27</td>
<td>12 – 34</td>
<td>17 – 56</td>
</tr>
<tr>
<td>TK sensitivity (%)</td>
<td>14</td>
<td>29</td>
<td>48</td>
<td>60</td>
</tr>
</tbody>
</table>

TK: Thymidine kinase.

4. Thymidine kinase in colorectal cancer

Colorectal cancer (CRC) death rates continue to increase in both men and women, yet the disease ranks third in the world for estimated new cancer cases and third for estimated cancer deaths in both men and women. Few CRC cases are diagnosed at a localised stage (39%), when the survival rate is 90%. Most CRC cases are diagnosed at either a regional stage or a distant stage, with resultant less-desirable survival rates of $67\%$ and $10\%$, respectively.

At our centre, thymidine kinase is systematically assessed in colorectal carcinoma before surgery, immediately after surgery and during follow-up [44-46,57-60]. Preoperative levels of thymidine kinase were increased compared with normal values, but this elevation was not significant as pathological values were found mainly in little differentiated carcinomas and in patients at an advanced stage. Immediately after surgery a significant increase in TK levels in all postoperative conditions was registered, irrespective of the disease aetiology (benign or malignant tumour). This increase in TK levels was absent only in small-scale surgical procedures, for example hernia, and it was therefore presumed that the increase is connected with the postoperative regeneration of tissue, not with the aetiology of the basic disease. Remission values were no different from normal levels [45,46]. In the disease progression the increase in thymidine kinase levels preceded the clinical manifestation of the tumour disease relapse by 3 – 6 months, and the sensitivity of thymidine kinase was from 30 to 60%, which was higher than CEA and CA 19-9 sensitivity at the same intervals before progression (see Table 3) [44,46].

Apart from this monitoring, the authors found in the literature only three papers dealing with TK serum levels and one paper dealing with TK assessment in cellular cytosol in connection with colorectal carcinoma [61-64]. For example, Thomas et al. have measured levels of TK in the serum of patients with asymptomatic colorectal adenomas, asymptomatic colorectal carcinoma and patients known to have hepatic metastases from colorectal tumours. Enzyme levels have been compared with an age-matched group of asymptomatic people with no evidence of colorectal neoplasia at screening colonoscopy. TK activity in patients with metastatic disease and in patients with adenomas was significantly higher than in the normal controls. However, TK activity in patients with asymptomatic cancer was not significantly different from the control group [62].

5. Thymidine kinase in lung cancer

Several reports concerning possible serological markers for lung cancer have been published. Although serum tumour markers such as CEA, CA 125, TPA (tissue polypeptide antigen) and Monototal are widely used in clinical practice of non-small cell lung cancer (NSCLC), their predictive role for the response to anticancer treatment is still controversial [65-68]. Vascular endothelial growth factor (VEGF) showed no association with the prognosis of lung cancer [69]. Cyfra 21-1 or CA 125 tend to imply a negative prognosis. Cyfra 21-1 and CA 125 together imply the worst prognosis [70]. In the study of Li et al., the authors examined the concentration of TK1 in serum (STK1) in 250 preoperative non-small cell lung cancer patients (NSCLC), including 188 non-metastatic (group M0) and 62 metastatic patients (group M1). Serum from 16 healthy individuals was used as a control. The concentration of STK1 in preoperative NSCLC patients was significantly higher than STK1 in healthy individuals ($p < 0.0001$). In group M0 preoperative, STK1 concentration was significantly higher in patients of tumour size T2 as compared with tumour size T1 ($p = 0.042$), and in T3 – T4 as compared with T1 – T2 ($p = 0.01$). No significant difference in STK1 concentration between patients of tumour stage I and tumour stage II ($p = 0.057$) was found, but a significantly higher STK1 concentration was found in patients of stage III, as compared with stages I – II ($p = 0.025$). No significant differences of STK1 concentration were found in patients of group M1 concerning tumour size or tumour stage, or between patients with adenocarcinomas (ACs) and squamous cell carcinomas (SCCs). The authors also studied the changes of STK1 concentration individually 1 month after surgery in metastatic subjects (group M1, $n = 19$) and in tumour-free subjects (group M0, $n = 38$). In the M0 group, the concentration of STK1 1 month after surgery had declined significantly, by 45%, when compared with concentrations of STK1 preoperative ($p < 0.001$). In the group M1, however, no significant decrease in STK1 concentrations was found 1 month after the operation. The authors conclude that STK1 has a prognostic value and is a reliable marker for monitoring the response to surgery of NSCLC patients [71]. Similar results were presented in our pilot study on 34 patients with NSCLC, where a correlation of preoperative values with DFI and OS was also demonstrated [72]. Mao et al. examined concentration of S-TK1 in serum in 120 breast cancer patients at the time of surgery and in 67 patients 3 months after surgery. The results of the study indicate that the S-TK1 concentration is higher in patients developing distant metastatic disease without adenomas was significantly higher than in the normal controls. However, TK activity in patients with asymptomatic cancer was not significantly different from the control group [62].
6. Thymidine kinase in gynaecological cancer

In the western world, gynaecological cancers represent approximately ~ 15% of all cancers in women and are responsible for ~ 10% of all cancer deaths. The most common are endometrial cancers and cancers of the ovary and uterine cervix. However, ovarian cancer has the highest mortality rates [74].

At present there are no effective methods to screen for ovarian cancer in asymptomatic women. The best available marker for epithelial cancer is the mucin, CA 125 [75]. Over the last 15 years, significant progress has been made in understanding the potential and the limitations of a CA 125 assay. More than 2000 papers concerning laboratory and clinical studies of CA 125 have been published in the field of gynaecological carcinomas. CA 125 is most consistently elevated in epithelial ovarian cancer, but it can also be detected in some other gynaecological cancers. The best established application of the CA 125 assay is in monitoring ovarian cancer. Rising values of CA 125 during subsequent chemotherapy have been associated with a progressive disease in more than 90% of cases. In spite of these data, the regular use of CA 125 in routine clinical practice is very often omitted [76].

Owing to the low sensitivity of CA 125, further tumour markers are evaluated, especially for use in prognosis and monitoring of the effect of chemotherapy. Thymidine kinase was also considered in our evaluation. In this study, a comparison was made of diagnostic accuracy of the imaging methods (ultrasonography, CT, PET/CT) and tumour markers for early detection of relapse of tumour during follow-up of ovarian carcinoma patients. With a sensitivity of 72% at 95% specificity, thymidine kinase was the second best tumour marker for early detection of relapse. The sensitivity of CA 125 was 84%. Owing to the lead time of thymidine kinase (3 – 6 months), a combination of both tumour markers seems to be optimal for the follow-up of patients with ovarian carcinoma [77].

Fujiwaki et al. examined TK1 gene expression by RT-PCR and related it to gene expression of TS, TP and DPD in 69 samples from epithelial ovarian cancer, 8 low-malignant-potential tumours, 16 benign ovarian tumours and 34 normal ovaries. Also, cytosolic and serum TK activities were determined by radioenzymatic assay. In epithelial ovarian cancer, TK1 gene expression correlated with cytosolic and serum TK activities, and patients with high TK1 gene expression had a significantly poorer survival than those with low TK1 gene expression [78]. Hallek et al. also investigated serum levels of TK in patients with advanced ovarian cancer. These authors proved elevated levels of TK in patients with advanced ovarian cancer and a strong correlation between serum levels of TK and CA 125 [79]. Look et al. also found increased thymidine kinase and thymidylate synthase activities in human epithelial ovarian carcinoma [80].

In patients with cervical carcinoma, Fujiwaki et al. determined the clinical value of thymidine kinase. TK mRNA expression was upregulated in invasive cervical carcinoma and the serum TK level was also significantly higher in patients with invasive carcinoma than in normal women and patients with carcinoma in situ. In patients with invasive cervical carcinoma, the serum TK level significantly correlated with TK mRNA expression. Multivariate analysis showed serum TK level to be an independent prognostic factor [81].

7. Thymidine kinase in haematological diseases

The main feature of haematological diseases is cellular proliferation; because of this, TK finds a large application in this area. In Table 4, thymidine kinase sensitivity for haematological diseases is summarised in the way it was demonstrated in the group of 355 patients [46].

Chronic lymphocytic leukaemia (CLL) is often considered to be an indolent disease; it can have an extremely variable course, with life expectancy in patients ranging from as long as that in a healthy age-matched normal population to a median of 1.5 years. Owing to this variability, in the past few years several clinical and haematological parameters have been evaluated as possible indicators of prognosis [82]. One such indicator is the level of TK. TK is a cellular enzyme involved in a salvage pathway of DNA synthesis, and its level is directly correlated with the proliferative activity of tumour cells [83,84]. In normal cells, TK activity is present only for a short period in the early S-phase, while its activity is much higher in abnormally growing cells [85]. Several studies in patients with lymphoproliferative diseases have shown the prognostic value of this enzyme. For example, in patients with non-Hodgkin’s lymphoma and multiple myeloma, the level of TK correlates with the grade of malignancy, the stage of disease and the length of survival [86-88]. In CLL, the TK level correlates not only with the RAI stage, but also with the disease status, which in turn has allowed a distinction to be made between aggressive and indolent disease [89]. Studies also showed that the serum TK level can be an independent predictor of the duration of the progression-free interval in CLL and it can add information to the definition of smouldering and non-smouldering in early stage CLL [90,91]. It remains unclear, however, whether the TK level can be used to predict response and length of survival in patients with CLL. In their study, Raimondo et al. measured the TK level in 188 patients with active or advanced CLL treated with fludarabine, correlated the serum TK level with other presenting features, and assessed the TK level. Serum TK levels were elevated in 92% of the patients, and the levels proved to associate with previous treatment, stage of disease and other tumour-burden related features (i.e., white blood cell counts, absolute lymphocyte count, bone marrow cellularity). The levels were
also directly associated with indicators of tumour cell turnover (i.e., β-2-microglobulin [β2MG] and lactate dehydrogenase levels). What was of particular importance was that the authors found that the TK level was a significant prognostic indicator of both response to treatment and survival. Specifically, 83% of patients with a TK level of < 10 U/l responded (complete and partial response) to treatment with fludarabine, whereas only 45% of patients with a TK level of > 10 U/l responded to the treatment (p < 0.01). This difference was maintained when untreated and previously treated patients were analysed separately, and in patients divided according to the Binet stage. Of further importance is the fact that the median survival rate in patients with a TK level of < 10 U/l was 65%, as opposed to a rate of 22% in patients with a TK level > 10 U/l.

The authors conclude that serum TK level in CLL patients provides useful prognostic information regarding both response to therapy and length of survival and should be used in planning appropriate therapy. In particular, patients with a TK level of > 10 U/l have a poor prognosis and should be considered for aggressive treatment [92].

The study of Hallek et al. aimed to assess the prognostic value of S-TK in 122 previously untreated patients with Binet stage A CLL (mean age ± s.d., 58.7 ± 8.5 years). In univariate analyses, 18 of the 22 parameters investigated predicted progression-free survival (PFS). In a stepwise multiple regression analysis, only three parameters provided independent prognostic information on PFS: S-TK > 7.1 U/l; the concentration of lymphadenopathy; and a white blood cell (WBC) count > 75,000/µl. When added to the classification of smouldering versus non-smouldering CLL, S-TK levels separated into two groups within the group of non-smouldering stage A patients: patients with S-TK values > 7.1 U/l had a median PFS of 8 months, whereas patients with S-TK values ≤ 7.1 U/l expected a much longer PFS (49 months; p < 0.001), similar to smouldering CLL (42 months). The results demonstrate that S-TK is a prognostic parameter that adds independent prognostic information to the definitions of smouldering and non-smouldering CLL in Binet stage A [91].

Our experience of using thymidine kinase in leukaemia in children at our centre is now presented. In the laboratory, TK serum levels in 38 children with acute leukaemia (34 lymphoblastic, 4 myeloblastic) were determined using radio-receptor analysis (RRA, Immunotech, Prague, Czech Republic). All patients included in this study had had TK examined before the start of the treatment and at least twice during the follow-up. Serum levels are helpful in predicting relapse during follow-up, but it is necessary to note that they did not correlate with prognosis in our group of patients during the time of the initial diagnosis of acute leukaemia.

The results showed that TK serum levels at the time of diagnosis were extremely high (78 – 5826 U/l, median value 403 U/l, normal < 8 U/l), whereas in remission TK serum levels were much lower (5 – 80 U/l, median value 31 U/l). During relapse of acute leukaemia (five cases), TK levels increased considerably to measurements between 120 and 800 U/l (median value 324 U/l). The study showed that the elevation of TK serum levels during follow-up was a helpful marker for the recognition of an early stage of relapse and in some cases occurred as early as 1 month before the appearance of clinical signs. Sensitivity in this case was 87%, thus TK serum levels seem to be a very good parameter during follow-up because of their acceptable sensitivity, low cost ($4/sample) and the elimination of a requirement for screening of bone marrow samples. TK serum levels were helpful in predicting relapse during follow-up, but it is necessary to note that they did not correlate with the prognosis in our group of patients during the time of the initial diagnosis of acute leukaemia [93].

Several papers were devoted to the study of TK in lymphomas, particularly between 1980 and 1990. These papers demonstrated an increased level in serum TK, but correlation with the stage of the disease was missing. As documented in the studies mentioned below, TK is increased particularly in aggressive forms of these diseases.

Masaki et al. studied 67 patients with malignant lymphoma (one with Hodgkin’s lymphoma and 66 with non-Hodgkin’s lymphomas [NHL]) who did not manifest superficial lymphadenopathy. In such cases, to assess the growth of the tumour, the number of tumour cells is usually estimated from clinical parameters such as serum concentrations of soluble IL2 receptor (sIL2R) and deoxythymidine kinase (dTK), and β2MG and lactate dehydrogenase (LDH). Serum concentration of sIL2R was higher in stage IV than in stages I (p 0.000), II (p = 0.001) and III (p = 0.027). Serum concentration of dTK was higher in stage IV than in stages II (p = 0.046) and III (p = 0.018), and serum concentration of β2MG was higher in stage IV than in stage I (p = 0.046). However, the serum concentration of LDH did not correlate with the tumour stage. Extremely high levels of serum sIL2R and dTK can be observed in some patients with lymphomas that secrete certain cytokines and chemical mediators when secondary inflammation occurs [94].

NHL forms a heterogeneous group of diseases. Tumour markers may help to identify high-risk patients who might benefit from more aggressive therapy. Serum soluble CD27 (sCD27) and TK are potentially valuable markers.

### Table 4. Thymidine kinase sensitivities (IU/l) at 95% specificity in haematological malignancies.

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>N</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>56</td>
<td>62</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>Non-Hodgkin lymphomas</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>155</td>
<td>72</td>
</tr>
</tbody>
</table>
since sCD27 seems to be related to tumour load and TK to proliferation of malignant cells. The authors determined serum sCD27, TK, β2MG and lactic dehydrogenase levels at the time of diagnosis in 79 lymphoma patients and correlated these parameters with the stage of disease, the International Prognostic Index (IPI) score and survival. Receiver operator characteristic (ROC) curve analysis showed an excellent ability for sCD27 to discriminate between low- and high-stage disease (p < 0.001), especially in indolent lymphomas. In comparison with an aggressive NHL, sCD27, TK, β2MG and LDH did predict survival in the univariate analyses. However, lactic dehydrogenase was found to be the most independent prognostic factor in a multivariate Cox regression model. In indolent lymphomas, sCD27 proved to be a powerful marker to predict progression-free survival (p = 0.008). Taken together, the results of the ROC curve and survival analysis suggest that substitution of lactic dehydrogenase by sCD27 in the IPI may be considered for indolent lymphomas to enhance the prognostic value [95].

In the study of Poley et al., s-TK levels were determined by means of a radioenzyme assay (REA). In 95% of healthy controls (n = 97), S-TK values were < 8.5 U/l. In patients with monoclonal gammapathies of undetermined significance (MGUS) (n = 27) or polyclonal gammapathies (n = 45), the cutoff was 10.3 U/l or 25 U/l, respectively. With regard to monoclonal gammapathies, MGUS had lower S-TK than multiple myeloma (MM) patients (p < 0.05), and patients with stage I MM according to Durie and Salmon had S-TK levels significantly lower than those with more advanced stages (p < 0.01). There was a correlation between S-TK and the plasma cell labelling index (r = 0.56, p < 0.001). Patients with chronic lymphocytic leukaemia showed significantly higher S-TK levels in the RAI stages 3 and 4 than in stages 1 and 2 (p < 0.01). In cases of other malignant NHL in progression, sensitivities of S-TK were found to be: immunocytoma 36%, centrocytic/centroblastic-centrocytic lymphoma 54% and high-grade NHL 40% (cut-off defined on lymphomas in remission). S-TK levels varied in MM according to the course of disease and response to therapy, decreasing at remission and increasing again at relapse. Analogous variations were found in the other NHL. After 2 years, 83% of patients with a pretreatment S-TK of < 10 U/l and 47% of the patients with a S-TK of ≥ 10 U/l were still alive. S-TK proved to be a highly significant prognostic indicator for MM patients (log-rank and Wilcoxon: p < 0.0001). In the other NHL patients, patients with a S-TK level > 10 U/l had a median follow-up of only 7 months. NHL patients with lower S-TK levels did not even reach the median survival time (log-rank and Wilcoxon: p < 0.005). Our results suggest that the determination of S-TK may help to monitor the clinical course of NHL during therapy and predict the prognosis of NHL [96].

In haematological diseases, TK is often correlated with other markers. One example is the study of Fujiwara et al. [97]. In this study, he examined 35 patients with adult T-cell leukaemia (ATL). The types of ATL were acute (n = 15), lymphoma (n = 10) and 10 chronic (n = 10). The diagnosis of ATL was based on clinical features and haematological characteristics, serum antibodies to ATL-associated antigens, and monoclonal integration of human T-lymphotropic virus type 1 proviral DNA into leukaemic cells obtained from peripheral blood or lymph node cells of the patients. The clinical subtypes of ATL were determined according to the criteria of the Lymphoma Study Group (LSG) of Japan [40]. All patients with acute and lymphoma types and one with a chronic type were treated with an OPEC/MPEC regimen. The concentrations of TK, sIL-2R and LDH were determined. Forty-three percent (15/35) of the ATL patients were positive for serum neuron-specific enolase (NSE). Serum NSE values of acute and lymphoma types were significantly higher than that of the chronic type. When the authors analysed the relationships between serum NSE values and the other three serum markers of ATL aggressiveness, the concentrations of TK, sIL-2R and LDH within the NSE-positive group were significantly higher than those of the negative group.

The significance of the assessment of thymidine kinase in myelodysplastic syndrome (MDS) is mentioned in the following part of this paper. The aim of the study of Musto et al. was to evaluate the prognostic relevance of TK serum levels as a marker of proliferative activity, in MDSs [98]. S-TK levels were monitored in 90 patients affected by MDS (22 refractory anaemia [RA]; 17 RA with ring sideroblasts [RARS]; 21 RA with blast excess [RAEB]; 15 RAEB in transformation [RAEB-T]; and 15 chronic myelomonocytic leukaemia [CMMoL]). Mean S-TK levels (U/µl) measured at diagnosis were 11.9 ± 12.6 for RA, 11.4 ± 13.6 for RARS, 19.9 ± 28.4 for RAEB, 39.6 ± 34.3 for RAEB-T and 77.7 ± 69.7 for CMMoL (normal values < 5 U/µl). No correlation was found between initial S-TK values and other clinical or laboratory parameters. MDS patients with S-TK > 38 U/µl, a cutoff level selected by means of ROC statistical analysis, showed a significantly shorter survival than those with S-TK < 38 U/µl (8.2 versus 37.4 months, respectively; p < 0.0001).

Acute myeloid leukaemia (AML) occurred in 17/21 (81%) of patients with S-TK > 38 U/µl and 9/69 (13%) of those with lower levels at diagnosis (p < 0.0001), independently of the FAB subtype. High S-TK levels were also useful to predict evolution in AML during the course of the disease in patients with normal initial values. Multivariate analysis confirmed the independent prognostic value of S-TK on both overall survival and risk of acute transformation. Musto et al. concluded that S-TK may be an important prognostic factor in MDS, and that it correlated strongly with the development of AML [98].

8. Thymidine kinase for therapy control

The importance of levels of thymidine kinase in serum or cytosol for therapy control of haematological malignancies
has been mentioned earlier. There are only a few references on the field of use of thymidine kinase levels in serum or cytosol for therapy control in solid tumours. Borovansky et al. suggested that serum thymidine kinase levels might be used as a marker to follow the effect of melanoma therapy ([99]). Foekens et al. determined the activity levels of TS and TK in 257 primary breast tumours of patients who received tamoxifen as first-line systemic therapy after diagnosis of advanced disease. In conclusion, for patients with recurrent breast cancer, high tumour TK activity was a significant marker of a poor clinical outcome in tamoxifen therapy. Elevated tumour TS activity predicted a favourable outcome for 5-fluorouracil-containing polychemotherapy when applied after tumour progression on endocrine therapy ([100]).

He et al. developed poly/monoclonal antibodies against TK1, which proved useful for diagnostics in both serum and immunohistochemistry of cancer patients. They used these antibodies for immunohistochemical staining of tumour sections from 54 patients with ductal infiltrated breast carcinoma. In conclusion, the positivity of cytosolic TK1 staining correlated with higher staging and grading of the tumour ([101]). He et al. also confirmed the clinical significance of TK1 measurement in the serum of breast cancer patients ([102]). Zhang et al. proved the importance of TK1 as a proliferating marker for prognosis and monitoring the outcome of surgery of primary bladder carcinoma patients ([103]). In this study, the serum levels of thymidine kinase in the course of colorectal cancer chemotherapy have been examined. The TK levels were examined in 30 patients with colorectal cancer who had undergone adjuvant or palliative chemotherapy (CHT schemes). The condition for being included in the study was a minimum of three cycles of chemotherapy. TK was always assessed with radio-receptor analysis, before and after every chemotherapy cycle, together with other tumour markers. Of the monitored tumour markers, only TK changed typically in the course of chemotherapy. In adjuvant chemotherapy, it was mostly low at the beginning of the cycle and its values usually increased considerably at the end. On the other hand, in palliative chemotherapy the dynamics of TK varied depending mainly on the effect of the therapy. Other tumour markers showed non-standard behaviour and minimal correlation with TK changes. It has been proved that thymidine kinase is a suitable parameter for monitoring the effect of adjuvant and palliative chemotherapy in colorectal cancer ([104]).

9. Expert opinion

Thymidine kinase was most widely used clinically from 1980 to 2000 because assessing serum or cytosol TK levels continued the methods of incorporating thymidine kinase for monitoring proliferation activity of tissue used in basic research. Also, unlike the present situation, there was no problem with its being an isotopic method since immunoanalysis was then largely performed directly in isotopic centres. In the following years there was a considerable reduction in the clinical application of thymidine kinase because priority was given to the tumour markers connected with histological types of tumour (e.g., CA 19-9, CA 15-3) or tumour markers that are organ specific (e.g., prostatic-specific antigen), so the markers characteristic of proliferation continued to be used only in haematology. Here it kept its position, although there are much more widely used methods, such as PCR and flow cytometry. When thymidine kinase started to be used in the treatment of tumour diseases, its application in clinical practice was brought back in around 2003, and it has been used more widely since. Unlike the past situation, thymidine kinase is used at present as a secondary tumour marker for determining the aggressive nature of a tumour. Its advantageous lead time is also used for the early diagnosis of disease relapse or progression, both in haematological diseases and in solid tumours (breast cancer, colorectal cancer, NSCLC, gynaecological cancer). Apart from that, it is significant for monitoring the follow-up of the tumour and for monitoring therapy.

The present state of the problems of TK assessment and application was discussed at the 35th Meeting of the International Society for Oncodevelopmental Biology and Medicine, ISOBOM 2007, in Prague, Czech Republic, in Section 20, whose chairman was Ondrej Topolcan [42]. Methodological papers presented at this meeting give the prospect of a wide application of the methods for thymidine kinase assessment in basic research and routine practice. At present there are not only non-isotopic methods for the assessment of serum and cytosol levels of thymidine kinase enzymatic activity, but also methods that measure the amount (rather than the activity) of thymidine kinase present in biological material, and even methods for the immunohistochemical demonstration of thymidine kinase. Apart from that, initial experiments have been carried out with molecular-biological methods, making possible the assessment of thymidine kinase expression or biological activity. Clinical studies have shown that the prospect of thymidine kinase assessment in the next 5 years is mainly in the tumour problem area, but also, to a limited extent, in non-tumour diseases of a proliferative nature.

In the next 5 – 10 years it may be presumed that thymidine kinase will become part of the recommended procedures for follow-up in most haematological malignancies and in solid tumours. The reason for this will be the low cost and high efficiency of the result in combination with other markers. It appears to be a significant marker for the determination of ascites and pleural effusion aetiology, where it will most probably replace cytological examination.

The authors presume that the assessment of thymidine kinase will facilitate the evaluation of regeneration processes, for example after the surgery of liver metastases. At the moment its application in this area is at a very early stage. A new area of thymidine kinase application is in monitoring the effect of therapy, both of surgical treatment (demonstrating
The role of thymidine kinase in cancer diseases

residual tissue) and of oncological treatment (radiotherapy, chemotherapy, biological treatment). In the authors’ experience, the monitoring of thymidine kinase has a unique position in the evaluation of the effect of adjuvant therapy. In palliative treatment it represents an indispensable component in the panel of assessed tumour markers, where it makes possible a targeted choice and change in therapy. In contrast to the present situation, where the choice of therapy is governed by empirical schemes based mainly on the clinical picture and monitoring techniques, the situation in the future will have to change, particularly in connection with still more widely used methods of biological treatment. The empirical approach will be replaced with 'tailoring treatment', for which the basis will be the determination of tumour biological activity. The assessment of the proliferation degree with thymidine kinase will then play a significant role. It is difficult at the moment to predetermine the connection between diagnostic and therapeutic applications of thymidine kinase. No data about this kind of application are available. It is, however, certain that thymidine kinase will find its application even here, either in the choice or in the monitoring of this type of treatment.

Apart from this, immunohistochemical assessment of thymidine kinase as a proliferation marker, and thus a marker of tumour aggressiveness, will undoubtedly find a wide application. At the moment it is at the stage of checking optimal antibodies. TK will become yet another marker of cellular proliferation, along with the most commonly used immunohistochemical tumour markers such as Ki-67, PCNA, and others.

Thymidine kinase will still have its position in basic research, in the study of tumour disease aetiopathogenesis. Apart from tumour problem areas, thymidine kinase assessment will find its application in studying diseases of a proliferative nature (inflammatory and autoimmune diseases), both for studying aetiopathogenesis and for routine diagnosis, particularly differential diagnosis, aetiology and disease activity.

The authors consider that the application of thymidine kinase is closely connected with the development of laboratory methods and in clinical practice mainly with the existing recommended guidelines for follow-up and treatment of tumour diseases. This means, therefore, that the application of thymidine kinase will be closely connected with the introduction of personalised medicine methods into the system of medical care.

Declaration of interest

O Topolcan was supported by the research project VZ. MSM 0021620819 and L Holubec was supported by the grant IGA NR 9343-3.

Bibliography


24. Gross MK, Merrill GF. Thymidine kinase synthesis is repressed in nonreplicating muscle cells by a translational mechanism that does not affect the polysomal distribution of thymidine kinase mRNA. Proc Natl Acad Sci USA 1989;86(13):4987-91


27. Kauffman MG, Rose PA, Kelly TJ. Mutations in the thymidine kinase gene that allow expression of the enzyme in quiescent (G0) cells. Oncogene 1991;6(8):1427-35


The role of thymidine kinase in cancer diseases


microglobulin, and thymidine kinase.
Leuk Lymphoma 1995;18(1-2):87-92

Affiliation
Ondrej Topolcan† MD PhD & Lubos Holubec Jr MD PhD
†Author for correspondence
Charles University Prague, Medical Faculty in Pilsen, Department of Nuclear Medicine, Faculty Hospital Pilsen, 13 Edwarda Benese, 305 99 Pilsen, Czech Republic Tel: +420 377402948; Fax: +420 377402454; E-mail: topolcan@seznam.cz