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Influence of the selenium level on overall survival in lung cancer

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Highlights

- Serum Se level above 69 µg/L may be associated with improved outcomes of treatment for patients with I stage lung cancer .

- The optimization of Se levels in patients with stage I disease may improve overall survival.
- Serum Se levels in patients with stage I LuCa may be a valuable biomarker for the prognosis of lung cancer.

Abstract

Background

Although the results of studies in populations with low selenium status indicate an inverse correlation between body selenium levels and the risk of the lung cancer, the effect of this microelement on survival has not been studied.

Materials and Methods

We performned a prospective study of 302 patients diagnosed with lung cancer in Szczecin, Poland. Selenium concentration in serum was measured at the time of diagnosis and before treatment. All patients were followed for a maximum of 80 months or until death. Vital status was obtained from the Polish National Death Registry.

Results

Using Cox proportional hazard analysis, performed for all individuals with lung cancer, the hazard ratio (HR) for death from all causes was 1.25 (95% CI 0.86 to 1.83, P=0.99) for patients in the lowest tertile compared to those in the highest tertile of serum selenium levels. Among the patients with stage I disease this relationship was significant (HR-2.73; P = 0.01) for selenium level in tertile 1 (<57 µg/L) compared to tertile 3 (>69 µg/L, reference). The 80 months crude survival after diagnosis was 79.5% (95% CI: 68.5 – 92.4%) for individuals in the highest tertile and 58.1% (95% CI: 45.1 - 74.9%) for individuals in the lowest tertile with stage I lung cancer.

Conclusion

These results suggest that in patients undergoing treatment for stage I lung cancer, serum selenium levels at the time of diagnosis (>69 µg/L) may be associated with improved overall survival.

Abbreviations

HR - Hazard Ratio, LuCa - Lung Cancer, NSCLC - Non-Small cell Lung Cancer, TNM - Tumor Node and Metastasis, Se - selenium, ICP-MS - Inductively Coupled Plasma Mass Spectrometry, NPC – Nutritional Prevention of Cancer, NHANES - Nutritional Examination Survey

Keywords:

serum Se level, microelements and lung cancer, lung cancer prognosis, prognostic markers

Introduction

Lung cancer (LuCa) still remains the leading cause of death from all cancers. The average five-year survival rate in Europe is approximately 15% [1, 2]. Nevertheless, the five-year survival rate in early-stage, operable, non-small cell lung cancer (NSCLC) is approximately 50%–70% but drops below 5% for patients with metastatic disease [3]. Although TNM (Tumor Node and Metastasis) staging is the most frequently used clinical parameter for cancer prognosis, a precise prognosis of survival within the same staging groups requires more parameters. The determination of prognostic biomarkers is important for the early detection of recurrence and for the enrollment of the patients into different treatment regimens [4].

It has been reported that there is a close correlation between serum selenium (Se) levels and the risk of death, regardless of the cause [5]. As a necessary component for the function of selenoproteins, selenium plays both an enzymatic and structural function. The benefit of Se supplementation may differ between individuals and therefore may require different doses to obtain maximal benefit. The preventative effect of Se supplementation strongly depends on the baseline levels of Se and is beneficial only for individuals with a low serum Se

concentration. Very low serum selenium levels are associated with an increased risk of cancers [5, 6]. Too high a selenium concentration (selenosis) is also correlated to higher occurrence of some common diseases [6]. Previous studies have shown that low serum selenium is associated with higher risk of lung and laryngeal cancer morbidity, specifically in Poland [7]. To date, there have been no studies reported aimed at evaluating the role of low serum selenium concentration and lung cancer prognosis. In this study we evaluated the association between serum selenium levels at the time of diagnosis and outcome among a series of lung cancer patients.

Materials and Methods

Study participants

A total of 302 lung cancer patients were enrolled in the study after providing written informed consent. Newly diagnosed LuCa patients were enrolled in the study at the Department of Thoracic Surgery in Szczecin-Zdunowo Hospital between February 2010 and December 2011. This centre treats more than 90% of patients diagnosed with LuCa in the West Pomeranian region of Poland. All LuCa patient diagnoses were confirmed by histopathological examination after surgery. The study was conducted in accordance with the Declaration of Helsinki and all participants signed a written informed consent document prior to donating a blood sample for analysis. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin (number KB-0012/73/10). All patient blood samples were collected at the time of lung cancer diagnosis, but before the commencement of any treatment. Consenting patients were asked to fast for at least four hours prior to blood collection. A blood sample (10 cc) was obtained during the diagnostic workup and was collected into tubes certified for quantification of trace metals (Vacutainer® System, royal blue cap). Blood samples were taken between 8 am and 2 pm and were centrifuged within 30

to 120 minutes of collection to separate the serum from the cellular fraction. The serum samples were stored at -80°C until required for the selenium assay.

Measurement of selenium level

Serum selenium levels were quantified by inductively coupled mass spectrometry (ICP-MS NexION 350D, Perkin Elmer) using methane for reduction of polyatomic interferences. Calibration standards were prepared by dilution of 10 mg/l Multi-Element Calibration Standard 3 (PerkinElmer Pure Plus, PerkinElmer Life and Analytical Sciences, USA) with reagent blank consisting of 0.65% solution of nitric acid (Merck, Germany) and 0.002 % Triton X-100 (PerkinElmer, USA). Calibration curves were created using four different concentrations: 0.1 µg/l, 0.5 µg/l, 1 µg/l, 2 µg/l. Germanium (PerkinElmer Pure, PerkinElmer Life and Analytical Sciences, USA) was used as an internal standard and ClinChek® Plasma Control Level I (Recipe, Germany) was used as a reference material. Reference material was measured after each of the six samples. If the difference of the reference material measurements was greater than 5%, the entire series was repeated. Each sample was measured in duplicate from different analytical runs. Prior to analysis, all samples were centrifuged (4000g, 15 min) and the supernatant diluted 100 times with the reagent blank. Technical details, plasma operating settings and mass-spectrometer acquisition parameters are available on request.

Statistical analysis

We assigned patients to one of three categories of serum selenium levels (tertiles) based on the distribution of selenium levels in the entire study group. The highest tertile was selected as the reference level. We followed the study population from date of diagnosis until death or September 20th, 2016 with a total follow-up time for our study of 80 months. Death was established by linkage to the Polish Vital Statistics Registry. Subjects in the study were

linked to the vital statistics registry records using a unique eleven digit identification number (PESEL). Since the specific cause of death was not available only all-cause mortality was used in our analyses. We modeled the relationship between serum selenium levels on overall survival using a Cox proportional hazard analysis. The multivariate analysis was adjusted for age (continuous), sex, stage (I, II, III, IV), radiotherapy (yes/no) and chemotherapy (yes/no). Overall, 80-month survival according to selenium levels was demonstrated using Kaplan-Meier curves. All calculations were performed using R for Statistical Computing (version 3.2.0).

Results

The characteristics of the subjects included in this study are shown in Table 1. Mean age of diagnosis was 64 years (range 43 to 86 years). The majority of the patients were male (65%), 93% had a history of smoking, 42% were diagnosed with stage I disease, 25% received radiotherapy and 34% received chemotherapy. The overall 80 months survival was 40.1% for the entire cohort.

Table 1. Characteristics of the study group (n = 302).

	N	%
Sex		
Male	196	64.9
Female	106	35.1
Age, mean (range)	64.2 (43-86)	
Packyears, mean (range)	33.2 (0-232.8)	
Smoking status		
Yes	283	93.7
No	19	6.3
Stage		
I	129	42.7
II	75	24.8
III	79	26.2
IV	19	6.3
Radiotherapy		
Yes	78	25.8
No	224	74.2

Chemotherapy		
Yes	105	34.8
No	197	65.2
Histology		
Adenocarcinoma	136	45.0
Squamous cell carcinoma	124	41.1
Large cell carcinoma	21	7.0
Combined large cell - small cell carcinoma	5	1.7
Small cell carcinoma	3	1.0
Other	13	4.3

Median Se levels among all LuCa patients was 60.6 µg/L, interquartile range was 18.2 µg/L and the mean Se level was 61.6 µg/L (range 16.7 µg/L to 108.3 µg/L). The median, interquartile range, mean selenium levels (and range) according to age, sex, stage and treatment are presented in Table 2. No significant differences in the selenium concentration were observed for any of the subgroups. Crude 80-months survival by serum selenium tertiles were 26.4% (tertile 1), 48.2% (tertile 2) and 47.7% (tertile 3).

Table 2. Mean selenium levels by subgroups.

Subgroup	N	Median selenium level µg/L	Interquartile range µg/L	Mean selenium level (range) µg/L
All	302	60.6	18.2	61.6 (16.7-108.3)
Male	196	59.5	18.9	60.5 (16.7-92.7)
Female	106	63.7	17.1	63.8 (33.5-108.3)
Age				
<=60	104	61.1	20.2	61.6 (27.4-86.4)
>60	198	60.1	16.9	61.7 (16.7-108.3)
Smoking[a]				
Yes	283	60.1	18.2	61.3 (16.7-108.3)
No	19	66.3	16.1	67.4 (48.2-93.2)
Selenium				
Tertile 1	100	50.1	6.1	48.3 (16.7-55.1)
Tertile 2	99	60.2	6.5	60.8 (55.2-67.4)
Tertile 3	103	73.4	7.8	75.5 (67.4-108.3)
Radiotherapy				
Yes	78	59.6	20.4	60.7 (35.4-85.8)
No	224	60.7	17.5	62.0 (16.7-108.3)

Chemotherapy				
Yes	105	60.0	18.9	61.2 (35.4-92.7)
No	197	61.0	17.1	61.9 (16.7-108.3)
Stage				
I	129	64.6	16.7	64.2 (33.5-108.3)
II	75	58.4	15.5	59.8 (35.4-85.3)
III	79	57.2	21.3	59.6 (16.7-92.7)
IV	19	55.9	21.6	60.0 (42.8-84.2)

[a]Smoking includes current and past smokers

The hazard ratios (HR) and 95% confidence intervals in univariate Cox regression models and the multivariate HRs for various factors on overall survival is summarized in Table 3. In the univariate Cox analysis, sex (P=0.04), stage (P<0.01) and selenium levels (P<0.01, only for the lowest tertile) were all significant predictors of mortality. However, only the relationship between tumor stages II and IV (*P*<0.0001) remained significant in the multivariate model.

Table 3. Factors predicting mortality for patients with lung cancer.

	Univariate Cox Regression Models			**Multivariate Cox Regression Models**		
Risk factor	**Hazard ratio**	**95% CI**	***P* - value**	**Hazard ratio**	**95% CI**	***P* - value**
Age						
<=60	0.9	0.66-1.24	0.53	0.73	0.53-1.02	0.06
>60	1			1		
Sex						
Male	1.4	1.01-1.95	0.04	1.4	0.99-1.98	0.06
Female	1			1		
Stage						
I	1			1		
II	2.33	1.53-3.53	<0.01	2.01	1.29-3.12	<0.01
III	4.17	2.82-6.16	<0.01	3.7	2.36-5.82	<0.01[a]
IV	5.75	3.26-10.14	<0.01	5.43	2.95-10.00	<0.01
Radiotherapy						
Yes	1.96	1.42-2.68	<0.01[a]	1.32	0.92-1.88	0.13
No	1			1		
Chemotherapy						
Yes	1.52	1.12-2.07	<0.01[a]	1.04	0.75-1.45	0.81
No	1			1		

Smoker						
Yes	1.39	0.68-2.83	0.36	1.13	0.53-2.40	0.76
No	1			1		
Selenium						
Tertile1 (16.72-55.13)	1.64	1.14-2.37	<0.01	1.25	0.86-1.83	0.24
Tertile 2 (55.19-67.35)	1,00	0.68-1.48	0.99	1.1	0.74-1.64	0.63
Tertile 3 (67.36-108.27)	1			1		

[a] Proportional Hazard Requirement is not achieved

A similar analysis was performed separately for each clinical stage of LuCa (data and results shown only for stage I: n=129, for others available on request). Median Se levels among patients with stage I LuCa was 64.6 µg/L, interquartile range was 16.7 µg/L and mean Se level was 64.2 µg/L (range 33.5 µg/L to 108.3 µg/L, Table 4). Similar to the entire group, no significant differences were observed in selenium levels according to age, sex, stage and treatment. Crude 80-months survival rates by serum selenium tertiles were 58.1% (tertile 1), 54.7% (tertile 2) and 79.5% (tertile 3) for patients with stage I disease.

Table 4. Mean selenium levels in the stage I of LuCa.

Subgroup	N	Median selenium level µg/L	Interquartile range µg/L	Mean selenium level (range) µg/L
All	129	64.6	16.7	64.2 (33.5-108.3)
Male	80	64.3	16.9	63.3 (34.2-86.4)
Female	49	65.1	15.0	65.7 (33.5-108.3)
Age				
<=60	42	64.3	19.3	64.5 (34.2-86.4)
>60	87	64.8	15.1	64.1 (33.5-108.3)
Smoking[a]				
Yes	117	63.9	15.8	63.7 (33.5-108.3)
No	12	65.8	13.1	69.0 (51.9-93.2)
Selenium				
Tertile 1	43	53.2	7.4	50.8 (33.5-57.9)

Tertile 2	42	64.5	5.3	63.7 (57.9-68.9)
Tertile 3	44	75.0	7.6	77.8 (69.3-108.3)
Radiotherapy				
Yes	12	65.3	16.9	64.3 (40.8-83.1)
No	117	64.4	16.0	64.2 (33.5-108.3)
Chemotherapy				
Yes	26	62.4	17.9	64.5 (48.0-86.4)
No	103	64.8	15.8	64.1 (33.5-108.3)

[a]Smoking includes current and past smokers

In univariate Cox regression analysis, low selenium levels (tertile 1 vs 3) were a significant predictor of mortality (HR-2.45; P=0.03) and this relationship became more significant in the multivariate model (HR-2.73; $P = 0.01$). Hazard ratio for chemotherapy was also statistically significant in multivariate Cox regression model (HR-2.24; P=0.02) (Table 5).

Table 5. Factors predicting mortality for patients with stage I of lung cancer.

	Univariable Cox Regression Models			**Multivariable Cox Regression Models**		
Risk factor	**Hazard ratio**	**95% CI**	***P* -value**	**Hazard ratio**	**95% CI**	***P* -value**
Age						
<=60	1.09	0.58-2.03	0.8	0.81	0.41-1.58	0.53
>60	1			1		
Sex						
Male	1.76	0.90-3.43	0.1	1.74	0.84-3.60	0.14
Female	1			1		
Radiotherapy						
Yes	2.19	0.97-4.93	0.06	1.53	0.64-3.67	0.34
No	1			1		
Chemotherapy						
Yes	2.37	1.26-4.43	<0.01[a]	2.24	1.11-4.52	0.02
No	1			1		
Smoker						
Yes	1.33	0.41-4.31	0.63	0.93	0.26-3.27	0.91
No	1			1		
Selenium						
Tertile 1 (33.46-57.91)	2.45	1.10-5.46	0.03	2.73	1.21-6.11	0.01
Tertile 2 (57.92-68.86)	1.99	0.88-4.50	0.1	1.88	0.83-4.28	0.13

Tertile3 (69.29-108.27)	1			1		

[a] Proportional Hazard Requirement is not achieved

The Kaplan-Meier survival estimates according to the tertiles of serum selenium are presented graphically in Fig. A.1 for all cases and in Fig. A.2 for the subgroup of patients with stage I disease. The beneficial effect of selenium concentration on the survival was observed in the group of patients with stage I disease in the tertile with the highest serum selenium concentration >68 µg/L (tertile 3, Fig. A.2).

Discussion and Conclusions

The results of this study demonstrate that a low serum selenium concentration measured before treatment in I stage disease is associated with an increased risk of death. Individuals in the lowest tertile of selenium had an almost three-fold increased risk of death during the 80 months of follow-up (HR-2.73, P=0.01). This observation suggests that in populations with low-selenium levels (to which Polish population belongs) this may be a prognostic marker of overall survival for patients with lung cancer. More specifically, it appears that this relationship is limited to patients with I stage disease. The results of our previous study indicated that low selenium levels are a risk factor for lung cancer [7] and both a risk and prognosis factor for laryngeal cancer patients [8].

To our knowledge, there have been no reports specifically evaluating the role of low serum selenium concentration and lung cancer prognosis. Several studies have been published showing the association between selenium levels and lung cancer risk. Some suggesting a protective effect of higher selenium concentration against lung cancer [9-11], while others

failed to confirm these observations, and indeed showing either no association or an adverse effect [12-15].

Although there are no studies about lung cancer survival and Se levels, there have been association studies in kidney and colorectal cancers. In an earlier study by Psathakis *et al.* Se levels below 70ug/l were associated with poorer survival in patients with colorectal cancer [16], which is comparable to our study, where the cut-off Se level was 69 µg/L. Similarly, Meyer *et al.* concluded that selenium and Selenoprotein P concentrations in blood might have a prognostic value in renal cell cancer [17]. Interestingly, both of these studies were performed in patients from northern Germany, a geographically adjacent region to West Pomerania in Poland and came to similar conclusions as presented in the current study. Association studies have shown that there is a strong correlation between the geographical distribution of selenium in the soil and consequently agricultural crops and the amount of selenium consumed in the diet and its relationship with cancer mortality. Schrauzer *et al.* in a study comprising 27 countries evaluating the selenium content in the diet observed an inverse correlation between the amount of selenium intake and mortality caused by lung, colon, rectum, prostate, breast and ovarian cancers [18]. Results of the Nutritional Prevention of Cancer (NPC) trial suggested that selenium supplementation could reduce not only the incidence but also mortality due to cancer [19]. The treatment effect in this trial was limited to males and to those with lower baseline selenium levels (<121.6 ng/mL). It was found that in the group of participants, who were supplemented with selenium, cancer mortality was reduced by more than 40%. The results of meta-analysis of 49 prospective studies have also shown a reduction in cancer mortality by 45% in subjects with optimized selenium levels [20].

Notwithstanding, there are many trials that counteract the claims of benefit from selenium. For example, Bjelakovic *et al.* published a meta-analysis of 68 randomized trials including 23,606 participants and found no clear association between selenium supplementation and

overall mortality [21]. In a meta-analysis of 47 trials, Schwingshackl *et al.* observed an inverse trend between selenium supplementation and all-cause mortality (RR: 0.93), with no significant effect on cancer related mortality [22]. With respect to LuCa, Karp *et al.* in a randomized, double-blind, placebo-controlled, phase III trial in patients with stage I resected NSCLC found no benefit of selenium supplementation over placebo in the prevention of a second primary tumor. The authors suggest, however, that the beneficial effects of selenium might be more apparent in patients with low baseline selenium levels [23]. In another report, summarizing 16 epidemiological studies on selenium and lung cancer, Zhuo *et al.* as well noted that selenium may have protective effect against lung cancer, especially in the populations where overall selenium levels are low [24]. Vinceti *et al*., in a review suggested to rule out the hypothesis that low environmental selenium exposure might be associated with an increased cancer risk [25]. However, in different geographic regions serum selenium levels differ. Because of lower soil selenium levels in Europe compared to the United States, serum selenium levels differ at a population scale [26, 27]. The correlation between serum selenium concentration and its influence on overall survival observed in our study might be due to the low selenium levels to which the Polish population is exposed. The average concentration of Se in the Polish population is ~76 µg/l [7] and there are regions in Poland where the average concentration of selenium in blood plasma is as low as 50-55 µg/L [28]. Selenium deficiency is characterized by plasma serum concentration below 70 µg/L, which is quite rare in the United States and Canada [27]. According to the data from the United States published by the National Health and Nutritional Examination Survey (NHANES), the mean selenium concentration in adults was 136.7 µg/L [29].

The result of our previous study conducted on laryngeal cancer suggests that serum selenium levels above 70 µg/L are associated with better outcomes [8]. In the current study, the threshold serum Se levels above which there appears to be a beneficial effect on overall survival was similar - 69 µg/L. However, in a study of laryngeal cancer, a prognostic role of

low selenium status on mortality was observed among all laryngeal cancer stages and a stronger effect was observed among those with more advanced disease stages (stages III and IV). In this study, however, this effect was limited to patients with I stage lung cancer. We do not have an obvious explanation for these differences. It is possible, that in higher stage disease the influence of selenium is too weak to limit further adverse changes as a result of this type of cancer. On the other hand, the results of many *in vitro* and animal studies summarized by Chen *et al.* support the notion, that Se may be an anti-metastatic factor in addition to being a cancer preventative agent [30]. Studies performed by Jönsson-Videsäter *et al.* provided some evidence that sodium selenite is linked to the induction of apoptosis in lung cancer cell lines [31]. Therefore, it seems that our observation limited to Stage I disease requires confirmation in other population studies. The precise mechanism by which serum selenium affects survival is still unclear and the association between poor prognosis and low selenium levels remains controversial. Evans *et al.* proposed several mechanisms which may explain this relationship that include, antioxidative activity of Se and selenium dependent enzymes; stimulation of the immune response (including enhanced activity of cytotoxic lymphocytes and natural killer cells); and Se mediated increased efficacy and reduced toxicity of anticancer treatments [32].

The strength of our study includes the large number of patients from the same geographical region who were diagnosed in the same institution, accurate measurement of selenium from fasting blood samples as well as extensive experience in microelement measurement. Modifiers of selenium levels and the risk of lung cancer such as age and smoking status were also taken into consideration. There are several limitations to our study with the most important being that the selenium measurements were made only after the diagnosis of lung cancer, although before therapy. The presence of cancer may have affected serum Se levels and since its presence was measured only once and it is known that serum Se concentrations fluctuate, this could lead to bias. Arsenic (present in cigarettes) could also have affected Se

measurements as the two elements influence each others serum levels [ref]. However, the arsenic content of cigarettes is controlled by WHO guidelines and the influence was probably limited to a minimum.

Because the Polish vital statistics database does not include cause of death, we could only use all-cause mortality as an endpoint. We suspect, however, that the majority of the deaths in this study were probably caused by lung cancer. By using only all-cause mortality as an endpoint, we could not estimate the lung cancer specific mortality.

In summary, the present study suggests, that serum selenium levels in patients with stage I LuCa may be a valuable biomarker for disease prognosis. Higher serum levels of selenium (>69 µg/L) appear to be associated with an improved overall survival in patients with this disease. While this study was conducted in West Pomeranian region of Poland and the results most relevant to individuals from this particular geographic region, the prognostic role of selenium status on lung cancer mortality needs to be replicated in other independent studies, specifically in populations with low baseline selenium levels.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Fig. A.1 80-month overall survival by serum selenium levels in all enrolled LuCa patients.

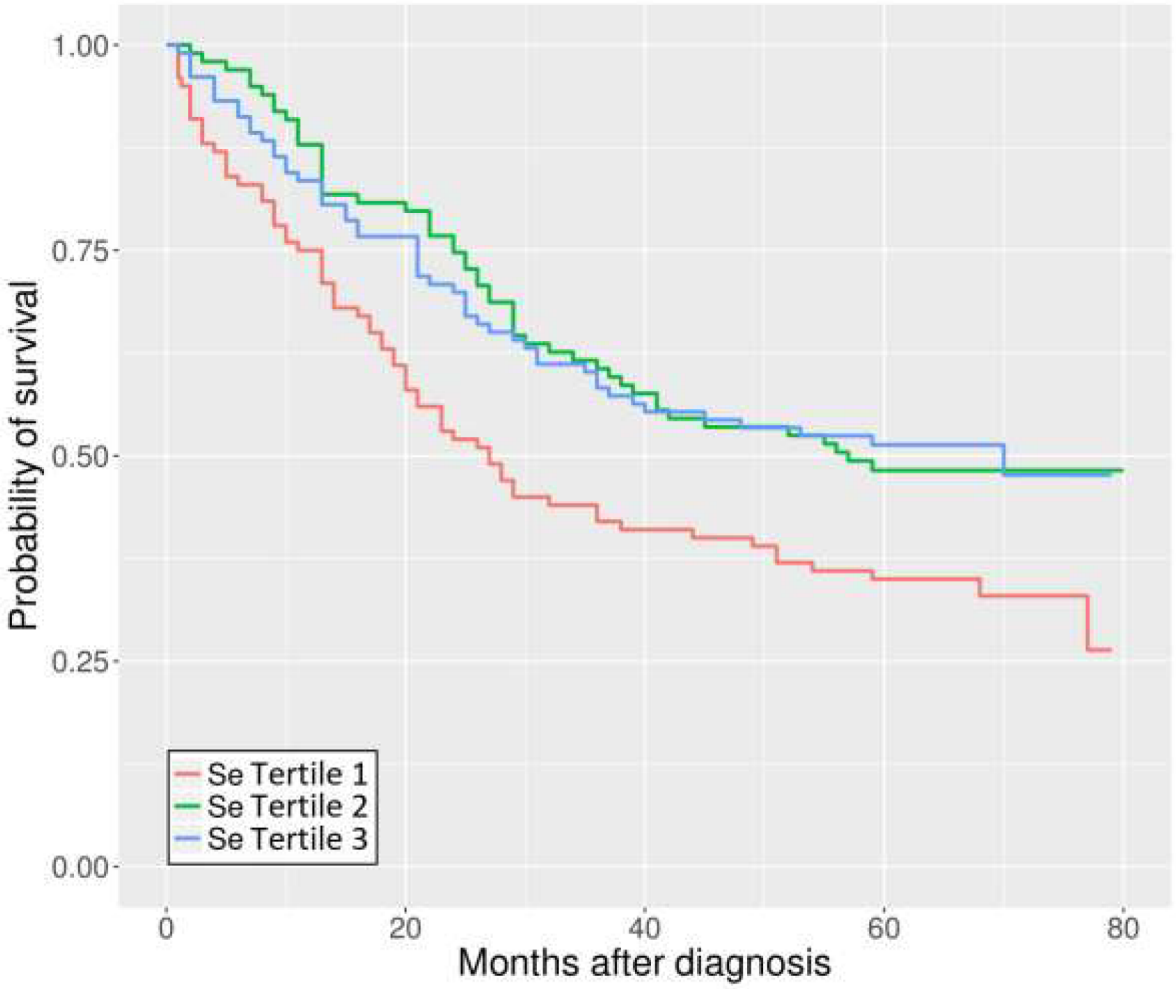

Fig. A.2 80-month overall survival by serum selenium levels for stage I LuCa patients.