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Pre-diagnostic Blood Selenium Status and Mortality Among Women with Breast Cancer

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Bachelor of Science

The Ohio State University

2019

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Abstract

Pre-diagnostic Blood Selenium Status and Mortality Among Women with Breast Cancer

By Bradley C. Frueh

Evidence from experimental studies support a possible association between higher selenium (Se) status and lower mortality risk among breast cancer survivors. However, human data are limited and mostly include dietary assessment of Se intake. Therefore, more studies are needed to understand this association especially in populations that may have low exposure to Se.

The associations of pre-diagnostic Se status [as measured by serum Se and selenoprotein P (SePP)] with overall and breast cancer-specific mortality were estimated using multivariable Cox proportional hazards regression among 2,205 breast cancer cases in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Potential effect modification by biologically plausible reproductive, lifestyle, and environmental factors was also investigated.

Over a mean follow-up period of 10.6 years (SD = 3.70), 496 deaths occurred of which 322 (64.9%) were due to breast cancer. Se was measured on average 4.48 years (SD = 2.71) and SePP was measured on average 4.75 years (SD = 2.64) before cancer diagnosis. None of the associations in the primary analysis were statistically significant. In stratified analyses, there was evidence of a potential effect modification by tumor stage, geographic region, body mass index, and smoking status. For breast cancer-specific mortality, the HRs per 1 SD increase in Se were 0.77 (95% CI: 0.61-0.96) for never smokers, 1.25 (95% CI: 0.97-1.60) for former smokers, and 1.32 (95% CI: 0.93-1.89) for current smokers ($p_{\text{interaction}} < 0.001$). For overall mortality, the HRs per 1 SD increase in Se were 0.82 (95% CI: 0.68-0.98) for never smokers, 1.15 (95% CI: 0.93-1.42) for former smokers, and 0.91 (95% CI: 0.70-1.19) for current smokers ($p_{\text{interaction}} = 0.006$).

Our results suggested that higher pre-diagnostic exposure to Se is not associated with lower risk for overall and breast cancer-specific mortality among breast cancer survivors. However, it is possible that this association is limited to never smokers and possibly other subgroups of breast cancer survivors.

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INTRODUCTION

Descriptive Epidemiology of Breast Cancer

Breast cancer is the leading cause of cancer-related death among women worldwide and the second leading cause of cancer-related death among women in the European Union[1,2]. The lowest rates of breast cancer are observed in Africa and Asia while some of the highest incidence rates are observed in the United States and Western Europe[1]. The high incidence of breast cancer can be attributed in part to an increase in cancer screenings but also in part to an increase in the prevalence of breast cancer risk factors such as obesity[2]. Breast cancer remains a leading cause of death despite declining mortality rates since the 1990s. Declines in breast cancer mortality have been attributed to several factors, including early screening, early diagnosis, improvements in treatment, and declines in hormone replacement therapy for menopause[2]. Mortality for breast cancer can vary considerably depending on geography and demographic characteristic. The highest mortality rates worldwide are observed in black women residing in the United States and the lowest rates are observed among Korean women[1].

Molecular Basis for Breast Cancer

Breast cancer is not a single entity but a heterogeneous disease with multiple tumor subtypes, each of which have their own associated risk factors for cancer incidence and survival. The four primary subtypes are: Luminal A, Luminal B, Basal-like (triple negative), and HER2-enriched[3]. These molecular subtypes are classified in large part by the presence or absence of specific hormone receptors and proteins. Hormone receptor positive subtypes, such as Luminal A and Luminal B, comprise approximately two thirds of all breast cancer cases and are hormonally driven[4]. Hormone receptor positive (HR+) tumors are defined by the presence of estrogen receptors (ER) or progesterone receptors (PR) which allow tumors to receive signals from one or both hormones[3,4]. Hormone receptor negative (HR-) tumors are defined by the absence of both hormone receptors. The effect of estrogen on the initiation and progression of cancer is well established and is thought to exert its carcinogenic effects in

two ways: (1) active signaling via estrogen receptors resulting in cell proliferation and (2) the metabolism of estrogen which results in oxidative DNA damage[5,6]. Hormone receptor positive subtypes, particularly Luminal A breast cancer, have the most favorable prognosis in part because of their susceptibility to hormone therapy. Similarly, Basal-like breast cancer has poorer prognostic outcomes due to an absence of hormone receptors and HER2 proteins required for hormone and targeted therapies to be effective[3].

Genetic mutations in oncogenes and tumor suppressor genes play a key role in tumor initiation and progression for breast cancer. Two tumor suppressor genes, *BRCA1* and *BRCA2*, are well known for their role in the pathogenesis of cancer and their strong associations with breast cancer incidence. Deficiencies in BRCA1 lead to disruptions in the cell cycle, genetic instability, and apoptosis while deficiencies in BRCA2 lead to disruptions in the repair of DNA double-strand breaks[7]. Despite their strong associations with breast cancer incidence, inheritable mutations of genes such as BRCA1/2 are attributable to less than 10% of breast cancers[4,7]. There are several other genes involved in the development and progression of cancer. For instance, overexpression of human epidermal growth factor receptor 2 (HER2) is involved in 20% of primary breast cancers and is associated with poorer clinical outcomes[7].

The development and progression of breast cancer parallels normal human cell development. Human cell growth and development is regulated by complex signaling pathways which allow cells to communicate with each other. Cancer cells disrupt the regulation of these complex signaling pathways and hijack them for their own development[8]. Two known pathways include the ER signaling pathway among ER+ breast cancers and the HER2 signaling pathway among HER2+ breast cancers, but many other pathways exist and interact with each other[8]. For instance the HER2 signaling pathway, activates multiple downstream signaling pathways through phosphorylation of tyrosine residues in the intracellular domain of HER2[8]. These downstream pathways are strongly associated with breast tumorigenesis[8].

However breast cancer is initiated, it begins as a single cancer cell. After inception, the first cancer cell needs to divide between 20 and 30 times before the mass becomes clinically evident[9].

Estimates of breast tumor doubling times are highly variable between studies and molecular subtypes[9]. While tumor doubling times do vary among subtypes, median tumor doubling times across all subtypes have been estimated between 45 to 260 days[9,10]. Assuming constant growth rates, it could take tumors as little as two years to two decade to grow to a detectable size.

Breast cancer originates in either the milk-producing lobules of the breast (lobular carcinoma) or in the ducts that transport milk (ductal carcinoma)[3]. Breast cancers that are diagnosed without spreading past their origin, either in the lobules or the ducts of the breast, are referred to as In Situ cancer while tumors that spread beyond their origin are referred to as invasive[3]. Lobular Carcinoma In Situ (LCIS) is believed to be a benign condition with an associated higher risk for breast cancer but without the potential to become invasive while Ductal Carcinoma In Situ (DCIS) is a precursor to invasive disease[3]. It is estimated that 81% of breast cancers are invasive and that 20-53% of individuals with untreated DCIS will progress towards invasive disease[3].

Risk Factors for Breast Cancer

The majority of breast cancers are associated with reproductive, environmental, and lifestyle factors. Two of the strongest non-modifiable risk factors for breast cancer are sex and age. Approximately 99% of all breast cancers occur in females and are most often diagnosed in women between the ages of 55 and 64[4,7]. As with most malignancies the risk of developing breast cancer increases with age. Genetic factors also play a role. Women with a first-degree relative with breast cancer have a higher risk for disease. When compared with women with no family history of breast cancer, the risk of breast cancer is approximately 1.5 times higher for women with one first-degree relative and as high as 4 times the risk for women with multiple relatives[3,4]. Other than sex, age, and family history the most important risk factors for breast cancer are hormonally mediated risk factors.

Risk factors that increase a women's exposure to estrogen over her lifetime increase the risk of breast cancer incidence. Reproductive risk factors such as early age of menarche, late age of menopause,

late age of first birth, low parity, hormone replacement therapy, and oral contraceptive use all increase breast cancer risk through prolonging exposure to estrogen. While reproductive risk factors are associated with a higher risk for hormone receptor positive breast cancer subtypes their associations may be heterogenous with other subtypes. For instance, a systematic review of eleven studies consistently found that compared to women who have never given birth, greater parity was associated with a lower risk of luminal A breast cancer; the odds of luminal A breast cancer in women with multiple pregnancies was consistently reported as roughly half the odds in nulliparous women[11]. However, the same systematic review found that greater parity was consistently associated with a higher risk for Basal-like tumors[11].

Several lifestyle risk factors were identified to be associated with risk for breast cancer, including: body mass index (BMI), physical activity, alcohol consumption, diet, and smoking. The association between breast cancer risk and high BMI differs by cancer subtype and menopausal status[12–16]. The Million Women Study in the United Kingdom (UK), a cohort study consisting of 1.2 million women and 45,037 breast cancer cases, compared women with BMIs ≥30 kg/m^2 to women in the normal BMI range (22.5-24.9 kg/m^2) and showed a 21% lower relative risk for breast cancer pre-menopause, (RR = 0.79, 95% CI: 0.68-0.92)[12]. The same large prospective cohort study observed a 29% higher relative risk for postmenopausal breast cancer (RR = 1.29, 95% CI: 1.22-1.36).

The literature on breast cancer risk and body fatness (defined by BMI, waist circumference, and waist-hip ratio) was reviewed by the 3rd World Cancer Research Fund/American Institute for Cancer Research Expert Report (WCRF/AICR, 2018). The report concluded that there was strong probable evidence of an association between greater body fatness in adulthood and a lower risk of pre-menopausal breast cancer[17]. The report also concluded there was strong convincing evidence of an associated higher risk between greater adult body fatness and post-menopausal breast cancer[17]. For young women between the ages of 18 and 30 years old, the report concluded there was strong probable evidence of an associated lower risk between greater BMI and both pre-menopausal and post-menopausal breast cancers[17].

Higher levels of physical activity are associated with lower risk for breast cancer regardless of type of physical activity, adiposity, or menopausal status[18,19]. A meta-analysis of 38 prospective cohort studies found the highest level of physical activity compared to the lowest level of physical activity was associated with a summary relative risk of 0.88 (95% CI: 0.85-0.90) for all breast cancers, 0.89 (95% CI: 0.83-0.95) for ER+/PR+ tumors, and 0.80 (95% CI: 0.69-0.92) for ER-/PR- tumors[18].

The WCRF/AICR, 2018 report reviewed the current literature on dietary risk factors for breast cancer. The report concluded that there was strong evidence to suggest alcoholic drinks were associated with higher risk for pre-menopausal breast cancer[17]. There was limited but suggestive evidence of a lower risk for pre-menopausal breast cancer for non-starchy vegetables (for ER- Tumors only), dairy products containing carotenoids, and diets high in calcium. For all other dietary factors included in the analysis the report stated there was limited evidence that was inconclusive of a higher or a lower risk for pre-menopausal breast cancer. For post-menopausal breast cancer, there was strong convincing evidence for an association with higher alcohol intake. There was limited but suggestive evidence of a lower risk for post-menopausal breast cancer for non-starchy vegetables (ER- tumors only), foods containing carotenoids, and diets high in calcium. For all other dietary factors included for review, the report stated there was limited and inconclusive evidence of higher or a lower risk for post-menopausal breast cancer[17].

The dietary factor most consistently associated with higher breast cancer risk is alcohol consumption[20,21]. Since the 1980s, numerous studies have reported higher breast cancer incidence in relation to alcohol consumption[4,21,22]. The association between alcohol and breast cancer incidence is present regardless of type of beverage consumed or menopausal status. A collaborative reanalysis of 53 epidemiologic studies conducted around the world, found that women who drank $\geq$ 45g per day of alcohol had a relative risk for breast cancer that was 46% higher than women who reported no daily alcohol use (RR = 1.46, 95% CI: 1.33 – 1.61). The higher risk associated with alcohol consumption was present in both smokers and nonsmokers and there was no statistical evidence of effect modification by race, education, family history, breast feeding, or use of exogenous hormones. Evidence of an increasing

dose response was present. The study reported an estimated 7% higher breast cancer risk for each 10g per day increase in alcohol consumption[22].

Previously, the association between smoking and breast cancer risk was controversial. A 2004 surgeon general report from the United States Surgeon General concluded there was no consistent evidence for an association for smoking and breast cancer risk. More recent evidence has suggested that smoking is associated with a higher risk for breast cancer. Evidence from the Canadian National Breast Screening Study (NBSS), a randomized controlled trial of breast cancer screening (n = 89,835), found that ever smokers and current smokers, compared to never smokers, had HRs of 1.08 (95% CI: 1.03-1.14) and 1.17 (95% CI: 1.10-1.25), respectively[23]. The European Prospective Investigation into Cancer and Nutrition (EPIC), found that ever smokers had a HR of 1.06 (95% CI: 1.01-1.10) compared to women who never actively smoked[24], while current and former smoking was not statistically significantly associated with breast cancer risk (HR = 1.05, 95% CI: 1.00-1.10 and HR = 1.06, 95% CI: 1.00-1.12, respectively). Additionally, the EPIC study was able to investigate the association of second hand smoke on breast cancer risk. Among women who had data on passive smoking (n = 183,608), the study found that women with passive exposure to cigarette smoke (in childhood, living with a smoker, or at work) had a higher risk of developing breast cancer (HR = 1.10, 95% CI: 1.01-1.22) compared to women who were never exposed to active or passive smoking[24].

Risk Factors of Survival After Breast Cancer Diagnosis

Survival outcomes among female breast cancer survivors, women who have been diagnosed with and received treatment for breast cancer, are associated with several non-modifiable factors. The most well-established prognostic factor for breast cancer survivors is tumor stage[25,26]. Breast cancers that are diagnosed at early stages (when a tumor has not spread far from its site of origin) are associated with 5-year survival rate that are between 80-90% in many countries[26]. For tumors that reach late stage disease (spreading to distant lymph nodes and organs), 5-year survival rates have been estimated as low as 24%[26]. Survival rates may also vary by molecular subtype due to differences in available treatments such as

hormone and targeted therapies[3,10]. A descriptive study conducted in the United States using Surveillance, Epidemiology, and End Results (SEER) cancer registry data, observed the highest 4-year survival rates for HR+ tumors (92.5% and 90.3% for Luminal A and Luminal B, respectively) and the lowest survival rates for HR- tumors (82.7% and 77.0% for HER2-enriched and Basal-like, respectively)[10]. In addition to stage, nodal status and tumor grade are also important risk factors for predicting long-term survival[25].

The WCRF/AICR, 2018 report reviewed current literature on several modifiable prognostic factors among breast cancer survivors. The report looked at three primary survival outcomes of interest, including: all-cause mortality, breast cancer-specific mortality, and second primary breast cancer (a new breast cancer occurring in the breast after treatment)[26]. The report concluded there was limited suggestive evidence of an associated lower risk of all-cause mortality for survivors who were exposed to physically activity, foods containing fiber, and foods containing soy before diagnosis as well as survivors exposed twelve or more months after diagnosis[26]. The report concluded that there was limited suggestive evidence of an associated higher risk of all-cause mortality for survivors who were exposed to greater body fatness, total fat, and saturated fatty acids before diagnosis as well as twelve or more months after diagnosis for body fatness only[26]. For breast cancer-specific mortality, the report concluded there was limited suggestive evidence of a lower risk for survivors exposed to physical activity prior to diagnosis and limited suggestive evidence of higher risk for survivors exposed to greater body fatness both before and twelve months or more months after diagnosis[26]. Lastly, the report concluded there was limited suggestive evidence of an associated higher risk of second primary breast cancer for survivors exposed to greater body fatness both before and twelve months or more after diagnosis[26]. Due to limitations in either the design or implementation of studies, the report concluded there was not strong evidence for an association between any of the potential modifiable prognostic factors reviewed and survival outcomes among cancer survivors[26]. There is a need for more research into potential modifiable prognostic factors among breast cancer survivors in order to make specific dietary and lifestyle recommendations for this growing vulnerable population.

Selenium as an Essential Microelement and its Role in Cancer Development and Progression

Selenium (Se) is a trace element and essential micronutrient that is naturally present in the environment and in a variety of common foods, including beef, eggs, milk, bread, and fish[27]. Se is best known as an antioxidant, anti-inflammatory agent, and a component for enzymes involved in the production of active thyroid hormone[28]. Se is involved in several pathways that may have anticarcinogenic properties[29]. Mechanisms observed from *in vitro* and animal studies found Se protection against DNA damage, induction of apoptosis, inhibition of cell proliferation, and angiogenesis[29]. Oxidized Se is toxic to cancer cells and detoxification of Se is required for breast cancer cell survival[30]. The best known biomarker for functional Se status is Selenoprotein P (SePP), a glycoprotein produced in the liver that transports hepatic Se in the blood and may indicate long term Se status[31,32].

The primary source of Se in the global food supply stems from erosion of rocks and minerals containing Se that are deposited into the soil[33]. Concentrations of Se and SePP in the blood vary considerably worldwide due to variations of Se in soil and in turn the food supply[33]. Compared to the U.S. population, European countries have low levels of Se in the environment as well as low Se intake from diet. According to the UK Reference Nutrient Intake (RNI), 75 µg per day for men and 60 µg per day for women are necessary to maximize synthesis of the selenoprotein glutathione peroxidase (GPx) and what is recommended to meet the needs of the population[28]. Daily Se intake varies among European countries but for most Western European countries daily intakes are well below recommended levels[28]. Lower dietary intake results in lower serum Se concentrations. Using data collected in the 1990s, mean serum Se concentrations in European countries were compared to the Nutritional Prevention of Cancer (NPC) Trial, a randomized trial conducted in the Eastern U.S, and found that European countries (France, Italy, Spain, Greece, Austria, Denmark, Germany, Sweden, Czech Republic, Poland, Hungary, Yugoslavia, Serbia, and the U.K) all had mean concentrations of serum Se within the lowest tertial found in the NPC trial[28]. In addition, all countries had mean concentrations below the 100 µg/L necessary to optimize GPx activity[28].

Selenium and Overall Cancer Risk

Epidemiology studies as far back as the 1970's have found inverse associations between Se intake and cancer incidence[28]. One nested-case control study, of 111 cases of cancer (18 lung, 16 breast, 11 prostate, 12 lymphoma/leukemia, 13 gastrointestinal, 43 unknown) and 210 matched controls from a cohort of 10,940 individuals with hypertension found that the odds of overall cancer incidence in the lowest quintile of pre-diagnostic Se was approximately twice the odds compared to the highest three quintiles combined (OR = 1.9, 95% CI: 1.1-3.3)[34]. Another prospective study conducted in the Netherlands in 1975-1978 (69 cancer death cases and 164 controls) found that risk of death from cancer in the lowest quintile of Se was approximately twice that of individuals in higher quintiles and failed to find statistical significance (RR = 1.9, 95% CI: 1.0-3.5). However, men in the lowest Se quintile had a risk of cancer that was 2.7 times higher than men in higher quintiles (RR = 2.7, 95% CI: 1.2-6.2)[35].

Recent epidemiologic studies on Se and overall cancer risk have been consistent with previous observations. A meta-analysis published in 2018, found an inverse association between high Se exposure and overall cancer risk. The analysis included 70 longitudinal studies (either cohorts or nested case-control studies) and calculated a pooled OR of 0.72 (95% CI: 0.55-0.93)[36].

Selenium and Breast Cancer Risk

While there is consistent evidence of an inverse association between Se exposure and overall cancer risk, the evidence for an association for breast cancer is mixed. A meta-analysis of 14 observational studies found higher Se exposure to be inversely associated with breast cancer risk (pooled OR = 0.88, 95% CI: 0.84-0.93)[37]. While evidence from another meta-analysis consisting of 8 observational studies found little association between Se concentrations and breast cancer risk (pooled OR = 1.09, 95% CI: 0.87-1.37)[36]. The 3rd expert report from the WCRF/AICR reviewed the present evidence for Se as a risk factor for breast cancer and concluded that there was inconclusive evidence for an association between Se and breast cancer incidence regardless of timing around menopause[17].

Selenium and Risk for Overall and Cancer-Specific Mortality

Observational studies investigating Se exposure and overall cancer mortality have observed inverse associations. A study conducted by Schrauzer and colleagues found that dietary intake of Se among 27 countries was inversely correlated with total age-adjusted cancer mortality (r: -0.4, p-value: 0.01)[38]. In a large prospective study of 13,887 adults from the National Health and Nutrition Examination Survey III (NHANES III), an investigation of serum Se levels and mortality outcomes was conducted and observed a HR of 0.83 (95% CI: 0.72-0.96) for overall mortality and a HR of 0.69 (95% CI: 0.53-0.90) for cancer mortality, comparing the highest tertile to the lowest tertile[39]. Another prospective study of 133,957 men and women from two large cohorts, the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS), did not find a statistically significant association between Se exposure and cancer-specific mortality. However, the study did report an inverse association between Se exposure and all-cause mortality, reporting a HR of 0.79 (95% CI: 0.71-0.88) and 0.79 (95% CI: 0.70-0.89) for women and men, respectively, when comparing the highest and the lowest Se quintiles[40].

Selenium and Survival Outcomes After Diagnosis of Breast and Other Cancers

Studies have been published investigating the association between selenium exposure and mortality among cancer survivors, with several studies reporting no association. A study among 3021 lung cancer patients, found a HR of 1.25 (95% CI: 0.86-1.83) for overall mortality, comparing the lowest tertile of serum Se to the highest tertile, among all lung cancer patients. The observed association was stronger among cases with stage I disease (HR = 2.73, 95% CI: 1.21-6.11)[41]. Another study of 784 men with prostate cancer in the Diet Cancer and Health cohort, found a statistically non-significant association between serum selenium concentrations and prostate cancer-specific mortality (HR = 0.93, 95% CI: 0.60-1.43)[42].

In terms of breast cancer-specific mortality, observation studies have found inverse associations for female breast cancer survivors with higher Se exposure[43–45]. In a study of 3,146 participants in the

Swedish Mammography Cohort (SMC), an inverse association was found between dietary Se intake and breast cancer mortality (HR = 0.69, 95% CI: 0.52-0.92), comparing the highest level of intake to the lowest[45]. A prospective study analyzed serum samples, collected after diagnosis but prior to treatment, from 546 breast cancer patients in the Szczecin region of Poland and observed a HR of 2.03 (95% CI: 1.12-3.65) for breast cancer-specific mortality, comparing the lowest quartile of serum Se to all other quartiles combined[44]. Finally, a nested case-control study within the Malmo Diet and Cancer Study, compared pre-diagnostic serum selenium concentrations among 1186 female breast cancer cases and 1186 controls, and reported an inverse association for both overall mortality and breast cancer-specific mortality, comparing women in the highest quartile of Se to women in the lowest quartile (HR = 0.63, 95% CI: 0.37-0.98 and HR = 0.60, 95% CI: 0.37-0.98, respectively)[43]. These studies on breast cancer mortality and Se were conducted on subjects from individual European countries. Results may not be representative of Western Europe as a whole and results may not be replicable in populations with higher Se intake such as the United States.

Randomized Clinical Trials of Selenium Supplementation

There have been at least two large supplementation trials involving Se, the Se and Vitamin E Cancer Trial (SELECT) and the Nutritional Prevention of Cancer (NPC) Trial, both of which were conducted in the United States[46,47]. The SELECT trial evaluated the efficacy of selenium, vitamin E, or both in preventing the incidence of prostate and other cancers among 35,533 men without disease. The SELECT trial found no statistically significant associations between treatment and prostate cancer incidence or any other cancer site[46]. The NPC trial, which was designed to evaluate the efficacy of selenium supplementation to prevent the recurrence of nonmelanoma skin cancer among 1312 cases residing in the Eastern United States, reported a statistically significant association between treatment and total cancer incidence as well as total cancer mortality (HR = 0.61, 95% CI: 0.46-0.82 and HR = 0.59, 95% CI: 0.39-0.87, respectively)[47]. Due to higher baseline serum Se in the United States population, these

trials were unable to assess the treatment efficacy of Se supplementation in populations with low concentrations of Se[46].

Conclusions

Tremendous progress has been made in recent decades in terms of breast cancer treatment and mortality outcomes. It is important to continue identifying potential prognostic factors for breast cancer to further extend the lifespan of breast cancer survivors. Several dietary and lifestyle risk factors have been investigated and continue to inform chemoprevention interventions and dietary guidelines. Existing evidence on Se for chemoprevention and improved survival show promise but more research is needed on populations with low Se status. Therefore, our study investigates whether higher pre-diagnostic serum Se and SePP concentrations are associated with lower overall and breast cancer specific mortality in Western European women diagnosed with breast cancer in the EPIC cohort.

METHODS

Study Population and Data Collection

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a large multicenter prospective cohort study designed to investigate associations between cancer, nutrition, genetics and other lifestyle and environmental risk factors. The rationale and methods for the EPIC study have been published previously[48,49]. Participating countries include Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. At study enrollment, between 1992 and 1998, standardized questionnaires on diet and lifestyle/personal history, blood samples, and anthropometric data were collected on most participants before disease diagnosis. Dietary questionnaires were designed and validated to capture usual diet and to be country specific to measure local eating habits. Serum samples from participating countries with the exception of Denmark were stored at the International Repository Agency for Research on Cancer (IARC). Samples at IARC were stored at -196ºC using liquid nitrogen while samples in Denmark were stored at -150ºC using nitrogen vapor. All

participants provided written informed consent. Ethical approval for the EPIC study was obtained through the institutional review boards of IARC and participating centers.

Cancer Incidence Follow-up

Incident breast cancer cases were identified through population cancer registries (Denmark, all centers in Italy except Naples, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) or by active follow-up (France, Germany, Naples). Active follow-up procedures included analyzing health insurance records, analyzing registry data, and contacts with participants and next-of-kin.

Vital Status Follow-up

Vital status was determined either through record linkage with regional and national mortality registries, Boards of Health, and death indices (Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) or through active follow-up to municipal registries, regional health departments, physicians, and hospitals (France and Germany). Censoring for complete follow-up varies by country and study center. For France, Germany, and Naples (Italy) the end of follow-up was considered last known contact and took place between June 2008 and December 2010. For all other countries censoring took place between December 2009 and December 2013. The 10th revision of the International Classification of Disease, Injuries, and Causes of Death (ICD-10) was used to code mortality and assigned based on underlying cause of death.

Case Ascertainment and Selection

Cancer data was coded using the ICD-10 and the second revision of the International Classification of Disease for Oncology. Incidence cases were selected from participants who developed female breast cancer (C50.0-50.9) after study enrollment and provided serum samples at baseline. The present study investigated associations among female breast cancer survivors with measured serum Se and SePP. Initial exclusions were made for prevalent cancers, missing information on date of censoring or diagnosis, and missing lifestyle/dietary information. Additionally, to prevent inclusion of extreme values

in the analysis, participants in the top and bottom 1% of the ratio of energy intake to recommended energy requirement (based on age, weight, height and sex) were excluded. 2208 participants with incidence breast cancer between recruitment and end of follow-up were identified. Among these cases, one participant was excluded for unknown vital status, one participant was excluded for In Situ Cancer (stage zero), and one was removed for having zero follow-up time after cancer diagnosis (diagnosed at death), resulting in 2205 eligible cases for analysis. The present analysis was based on data collected from all participating countries with the exception of Greece due to administrative data use issues.

Selenium and Selenoprotein P Measurements

Detailed information on the measurement of Se and SePP concentrations has been described previously[50,51]. Se levels were measured on 50 µl of plasma samples taken prior to disease diagnosis on 2,208 matched breast cancer case-control pairs for SePP and 1,781 for Se. Concentrations of total plasma Se were measured by using bench-top total reflection X-ray fluorescence spectrometer (Picofox S2; Bruker Nano GmbH). To quantify Se levels, a certified gallium solution (1000 mg/L; Sigma) with a defined concentration was equally added to each sample and used as reference. An internal serum stand was incorporated in each measurement as a positive control. To determine SePP concentrations, a colorimetric enzyme-linked immunoassay (Selenotestl ICI GmbH) was used. Coefficients of variation (CV) were determined by stablishing 3 controls which covered the upper, middle and lower part of the assay's working range (13.5–484.8 mg/L). These controls were included in the 16 separate analyses needed to assay all samples. For quality-control of intra-assay variability, case–control status was blinded for analysis and two serum samples of known Se and SePP concentrations were used in each analysis plate. The samples were measured in duplicate, and mean concentration values, SDs, and CVs were calculated. Duplicate samples with differences in CVs more than 10% were measured again to corroborate the results. The evaluation was performed with GraphPad Prism 6.01 by using a 4-parameter logistic function. The coefficient of variation was 7.3% for control 1 (SePP = 1.5 mg/L) and 7.2% for

control 2 (SePP = 8.6 mg/L). Due to technical issues with the Se assay, Se concentrations were not measures on 424 participants.

Covariates

The following *a priori* identified covariates were assessed as potential confounders: age at diagnosis (continuous), tumor stage (stage I, stage II, stage III, stage IV), estrogen receptor status (ER+, ER-), menopause status (premenopausal, postmenopausal), use of hormones for menopause (yes, no), age at menopause (continuous), age at menarche (age tertiles; <12 years old, ≥12 and <14 years old, and ≥14 years old), full term pregnancy status (ever/never), age of first full term pregnancy (continuous), number of all pregnancies (continuous), smoking status (never smoker, former smoker, current smoker), smoking intensity (never, current, cig/day, former, years since quitting), lifetime average daily alcohol intake (g/day), lifetime alcohol pattern (never drinkers, former light drinkers, former heavy drinkers, light drinkers, never heavy drinkers, periodically heavy drinkers, always heavy drinkers), body mass index (BMI, kg/m^2), physical activity index (average daily activity, including both recreational and household activity), and self-reported diabetes status at baseline (yes, no). These variables were chosen based on previously published evidence investigating their association with breast cancer incidence, survival, and serum selenium concentrations. Separate categories were created for missing values. Confounding was assessed by evaluating if there was a greater than 10% difference in hazard ratios (HRs) after including each covariate in the model. Age at diagnosis, tumor stage, estrogen receptor status, menopause status, age at menarche, smoking intensity, and body mass index (kg/m^2) were included in the final analysis.

A tumor stage harmonization procedure was conducted using tumor, nodes, and metastases (TNM) stage and a four-stage classification variable (localized, metastatic, metastatic regional, and metastatic distant) that was provided by study centers. First, tumor stage (I-IV) was assigned based on TNM staging for participants with available information (n = 1,318), then for the remaining participants (n = 887) based on stage categories (localized, metastatic, metastatic regional and metastatic distant).

Statistical analyses

The primary endpoint of the present study was death from breast cancer and death from any cause was used as a secondary endpoint. Age of first tumor diagnosis and age at death or censor were used as the two time interval points for participant follow-up time. To investigate the association between Se and SePP and breast cancer-specific death and overall mortality, two Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs): (1) a crude model stratified by country of diagnosis with adjustments made for age of first tumor diagnosis and tumor stage and (2) a multi-variable model stratified by country of diagnosis which controlled for age at diagnosis, tumor stage, estrogen receptor status, menopause status, age at menarche, smoking intensity, and BMI. The proportional hazards assumption was assessed using a combination of graphical methods, goodness of fit tests, and likelihood ratio tests. Graphical methods included estimating "log-log" survival curves and assessing parallelism. Correlations between Schoenfeld residuals in the Cox model were evaluated by goodness of fit tests. In addition, interaction between covariates and time dependent variables in the Cox model were evaluated using likelihood ratio tests. P_{trend} was calculated with the median value of each Se and SePP quintile as a continuous variable while adjusted for covariates.

The potential non-linear association between Se, SePP, and risk for mortality was investigated using restricted cubic splines fitted to a Cox proportional hazards model using the SAS macro "lgtphcurv9"[52]. Tests for non-linearity utilized the likelihood ratio test that compared nested models; one model with a linear term and another model with both a linear and cubic spline terms.

In a sensitivity analysis the effect of missing tumor stage information was assessed using several methods. First, participants with missing tumor stage were reclassified into a separate missing category and were adjusted for in the final model. Second, a sensitivity analysis was conducted by excluding participants with missing stage information and assessing how results were affected by the incomplete data. Finally, multiple imputations of missing stage values was conducted using SAS PROC MI. Information on multiple imputations for tumor stage have been published previously for colorectal cancer

and were adapted in the present study for breast cancer[53]. The multiple imputation model was based on available data for known covariates and assumed stage data were missing at random. Complete case analyses were conducted for models with Se as the exposure of interest (n =1781). The effects of missing Se data were assessed by reanalyzing SePP models only with participants who had Se data. Exclusion of cases with missing Se data did not substantially change results.

Subgroup analyses by categories of potentially biologically relevant effect modifiers were conducted. Potential effect modifiers included: length of follow-up (> 2 years and ≥ 5 years), age at diagnosis (< 50 years old and ≥ 50 years old), tumor stage (stages I-II and stages III-IV), estrogen receptor status (ER+ and ER-), menopause status (premenopausal and postmenopausal), BMI (< 25 kg/m^2 and ≥ 25 kg/m^2), smoking status (never, former, and current), and geographic region (northern, central, southern). Geographic regions was categorized as Northern (Denmark), Central (United Kingdom, the Netherlands, Germany, North of France), and Southern (South of France, Italy, Spain). Multi-variable-adjusted HRs and 95% CIs were reported per 1 SD increase in Se or SePP. Tests for statistical interaction were conducted by including in the models a cross-product of Se or SePP as a continuous variable and covariates of interest as categorical variables. P-values for interaction were calculated using likelihood ratio tests based on models with and without the interaction terms. All statistical tests were conducted using SAS version 9.4 (SAS Institute) and P-values of < 0.05 were considered statistically significant.

RESULTS

Characteristics of study participants

The distribution of selected baseline characteristics of breast cancer cases according to quintiles of Se are shown in Table 1. Over a mean follow-up period of 10.6 years (SD = 3.70), 496 deaths occurred of which 322 (64.9%) were due to breast cancer. Se was measured on average 4.48 years (SD = 2.71) and SePP was measured on average 4.75 years (SD = 2.64) before diagnosis.

Selenium and Mortality Among Breast Cancer Patients

The results of age and stage as well as multivariable adjusted Cox proportional hazards models for the association of Se and breast cancer-specific and overall mortality are shown in Table 1. None of the associations were statistically significant. For breast cancer-specific mortality, the multivariable adjusted HR comparing the fifth quintile to the first quintile was 1.29 (95% CI: 0.79-2.10, $P_{trend} = 0.826$). The HR per 1 SD increase in Se was 1.02 (95% CI: 0.89-1.16) and 1.06 (95% CI: 0.92-1.22; Table 3) when the analysis was restricted to complete breast cancer stage data. For overall mortality, the multivariable adjusted HR comparing the fifth quintile to the first quintile was 0.95 (95% CI: 0.65-1.38, $P_{trend} = 0.286$). The HR per 1 SD increase in Se was 0.94 (95% CI: 0.84-1.05) and 0.94 (95% CI: 0.83-1.06; Table 3) when the analysis was restricted to complete breast cancer stage data.

Selenoprotein P and Mortality Among Breast Cancer Patients

The results of age and stage as well as multivariable adjusted Cox proportional hazards models for the association of SePP and breast cancer-specific and overall mortality are show in Table 3. None of the associations were statistically significant. For breast cancer-specific mortality, the multivariable adjusted HR comparing the fifth quintile to the first quintile was 0.86 (95% CI: 0.59-1.26, , $P_{trend} = 0.923$). The HR per 1 SD increase in SePP was 1.01 (95% CI: 0.90-1.12) and 0.99 (95% CI: 0.88-1.11; Table 3) when the analysis was restricted to complete breast cancer stage data. The HR for overall mortality, comparing the fifth selenium quintile to the first, was 0.81 (95% CI: 0.60-1.08, $P_{trend} = 0.538$). For overall mortality, the HR per 1 SD increase in SePP was 0.99 (95% CI: 0.91-1.08) and 0.95 (95% CI: 0.87-1.05; Table 3) when the analysis was restricted to complete breast cancer stage data.

Stratified and sensitivity analyses

For Se there was evidence of statistically significant interaction between breast cancer-specific mortality and tumor stage ($p_{interaction} = 0.016$) as well as interaction with smoking status ($p_{interaction} < 0.001$). The HRs for tumor stage strata were 1.18 (95% CI: 0.98-1.43) for stages I-II and 0.90 (95% CI:

0.71-1.14) for stages III-IV. The HRs for smoking status strata were 0.77 (95% CI: 0.61-0.96) for never smokers, 1.25 (95% CI: 0.97-1.60) for former smokers, and 1.32 (95% CI: 0.93-1.89) for current smokers. Also, there was evidence of statistical interaction between overall mortality and age at diagnosis, smoking status, and geographic region (age at diagnosis $p_{\text{interaction}} = 0.047$, smoking status $p_{\text{interaction}} = 0.006$, geographic region $p_{\text{interaction}} = 0.008$). The HRs for age at diagnosis strata were 1.25 (95% CI: 0.58-2.69) for < 50 years old and 0.94 (95% CI: 0.84-1.06) for ≥ 50 years old. The HRs for smoking status strata were 0.82 (95% CI: 0.68-0.98) for never smokers, 1.15 (95% CI: 0.93-1.42) for former smokers, and 0.91 (95% CI: 0.70-1.19) current smokers. The HRs for strata by geographic region were 0.93 (95% CI: 0.80-1.07) for participants in the northern region, 1.10 (95% CI: 0.83-1.46) for participants in the central region, and 0.66 (95% CI: 0.42-1.05) for participants in the southern region.

For SePP there was evidence of statistically significant interaction between breast cancer-specific mortality BMI category and smoking status ($p_{\text{interaction}} = 0.004$). The HRs for BMI strata were 1.08 (95% CI: 91-1.29) for individuals with BMIs < 25 kg/m^2 and 0.94 (95% CI: 0.80-1.10) for individuals with BMIs ≥ 25 kg/m^2. The HRs for smoking status strata were 0.89 (95% CI: 0.74-1.06) for never smokers, 1.08 (95% CI: 0.89-1.31) for former smokers, and 1.06 (95% CI: 0.78-1.44) for current smokers. Additionally, there was evidence of statistical interaction between overall mortality and smoking status and geographic region (smoking status $p_{\text{interaction}} = 0.020$, geographic region $p_{\text{interaction}} = 0.048$). The HRs for smoking status strata were 0.86 (95% CI: 0.74-1.00) for never smokers, 1.08 (95% CI: 0.92-1.26) for former smokers, and 1.03 (95% CI: 0.83-1.28) for current smokers. The HRs for strata by geographic regions were 1.01 (95% CI: 0.88-1.15) for participants in the northern region, 0.95 (95% CI: 0.80-1.13) for participants in the central regions, and 0.77 (95% CI: 0.58-1.01) for participants in the southern regions. Sensitivity analyses conducted by including only complete breast cancer stage data or imputed data demonstrated that missing tumor stage data did not considerably change the study results.

DISCUSSION

This study is the second prospective investigation of the association between Se status and mortality among breast cancer patients that utilized pre-diagnostic serum Se and SePP. Results from this study suggest that there is not an association between high pre-diagnostic Se status and lower overall or breast cancer-specific mortality. However, it is possible that an association is limited to women who are never smokers for Se only. The discrepancy in results between Se and SePP may be explained by measurement error in Se biomarkers. It's possible that serum SePP status more accurately reflects the mortality risk associated with selenium because SePP is a better biomarker for functional Se status[31]. The discrepancy most likely could not be explained by the 424 observations missing data on serum selenium that were included in the SePP analysis as the results excluding these observations were highly similar to results in the primary analysis.

Evidence from biological and epidemiologic studies support a possible association between Se and survival after breast cancer diagnosis[54]. However, a systematic review of 11 randomized controlled trials (RCTs) published by Cochrane in 2018, reported that Se supplementation did not reduce overall cancer incidence (RR = 0.99, 95% CI: 0.86-1.14) or mortality (RR = 0.81, 95% CI: 0.49-1.32)[36]. While this experimental evidence suggests there is no association between Se status and mortality, the two largest randomized controlled trials were conducted in the U.S. population, which has optimal sources of Se, and may not reflect benefits in populations with suboptimal sources of Se[46,47]. The same systematic review examined 70 observational cohort studies and reported that Se exposure was associated with lower cancer incidence (summary OR = 0.72, 95% CI: 0.55-0.93) and lower cancer mortality (summary OR = 0.76, 95% CI: 0.59-0.97) when comparing the highest category of Se to the lowest. The discrepancy in findings from RCTs and observational studies may be explained by differences in baseline Se status as well as demographic characteristics between the study populations. The SELECT trial, which was designed to study prostate cancer, recruited men age 50 and older for African American males and age 55 and older for all other men. The NPC trial recruited both men and women and with an average age of 63 years old.

The discrepancy may also be due to differences in the source and form of Se used in RCTs and observational studies (high-dose supplementation vs. dietary intake of Se).

Three observational studies investigating associations between Se exposure and breast cancer mortality have been published. A study on 3,146 women with invasive breast cancer in the Swedish Mammography Cohort reported an inverse association between dietary Se intake and breast cancer-specific mortality when comparing the fourth quintile to the first (HR = 0.64, 95% CI: 0.48-0.84)[45]. An analysis of post-diagnostic serum Se samples from 546 invasive breast cancer patients in the Szczecin region of Poland reported a HR of 2.03 (95% CI: 1.12-3.65), when comparing the lowest quartile of serum Se to all other quartiles combined[44]. Finally, Swedish population-based cohort and observed that women, comparing the highest quartiles of pre-diagnostic serum Se to the lowest, had both lower overall mortality (HR = 0.63, 95% CI: 0.37-0.98) and breast cancer-specific mortality (HR = 0.60, 95% CI: 0.37-0.98)[43]. Even though our study did not find significant associations between pre-diagnostic serum selenium and mortality outcomes our results are consistent with previously published reports for breast cancer cases, and suggest a potential inverse association between high Se status as indicated by SePP concentrations prior to breast cancer diagnosis and mortality among breast cancer patients in Western European populations with sub-optimal exposure to Se.

Results from this study suggest that tumor stage (I-II, III-IV), geographic region (Northern, Central, Southern), BMI category (normal weight < 25 kg/m^2, overweight/obese ≥ 25 kg/m^2), and smoking status (never, former, current) may be potential effect modifiers for the association between Se status and overall or breast cancer specific mortality. There were substantial differences in associations by tumor stage for the association between Se and breast cancer-specific mortality ($p_{\text{interaction}} = 0.016$), substantial differences in associations by geographic region for the associations between both Se and SePP and overall mortality ($p_{\text{interaction}} = 0.008$, $p_{\text{interaction}} = 0.048$, respectively), substantial differences in associations by BMI category for the association between SePP and breast cancer-specific mortality ($p_{\text{interaction}} = 0.021$), and substantial differences in associations by smoking status for associations between both Se and

SePP and breast cancer-specific and overall mortality (Table 4). For all potential effect modifiers except for smoking status, stratified associations were not statistically significant (Table 4). For nonsmokers only, there were statistically significant inverse associations between higher serum Se concentrations and both breast cancer-specific and overall mortality (HR = 0.77, 95% CI: 0.61-0.96 and HR = 0.82, 95% CI: 0.68-0.98, respectively).

These potential effect modifiers may reflect biological phenomena relevant to breast cancer survivors. For instance, tobacco smoking continued after a cancer diagnosis has been associated with higher risk of treatment failure, increased toxicity from treatment, a higher incidence of secondary primary tumors, and shorter survival[55]. It is possible that any potential survival benefits from higher Se status could be negated by the deleterious effects of exposure to tobacco smoke and therefore significant inverse associations are only observed among nonsmokers. The relationship between BMI and mortality among cancer survivors is complex. Counter intuitively, a higher BMI has been associated with better survival outcomes among survivors, a phenomenon which has been referred to as the obesity paradox[56]. In contrast, some studies have found higher BMI to be associated with poorer survival outcomes, indicating that more research is needed to understand the mechanisms involved[57,58]. Multiple hypotheses have been proposed to explain the association with BMI and survival, including interactions with treatment efficacy through differences in the pharmacokinetics of cancer drugs by adiposity (cancer drugs are metabolized differently in fat tissue), differences in adverse events related to obesity, and cardiotoxicity as a results of both cancer treatment and obesity increasing risk for adverse cardiac events[58]. Potential effect modification by tumor stage could be explained by difference in prognostic risk profiles for breast cancer subtypes. Molecular subtypes of breast cancer can be thought of as separate diseases each with their own associated risk factors for survival, such as HR+ tumors due to their susceptibility to hormone therapy[3,59]. It is plausible that survival benefits of higher Se exposure may act through biological pathways that are only present in select breast cancer subtypes or subtypes are differentially impacted by the influence of these pathways. Finally, potential effect modification by geographic region could be explained by differences in

environmental sources of Se in the northern, central, and southern regions of Western Europe which results in different baseline levels of serum Se in geographic regions.

This study has several key strengths. A large prospective study design and serum samples collected prior to cancer diagnosis helps to establish temporality between exposures (Se and SePP) and survival outcomes. Breast cancer patients included in our study represent a population of female breast cancer patients with often sub-optimal Se status. The measurement of both Se and SePP in the study was beneficial as SePP is a more informative biomarker for Se status. Additionally our study was also able to control for several potential confounders, and addressed missing data values for tumor stage through sensitivity analysis and multiple imputation techniques.

There are several limitations in this study. First, we did not have information on breast cancer treatment. To address this issue, we conducted our analyses by stratifying on country of breast cancer diagnosis, adjusting for age of diagnosis, and adjusting for tumor stage as a proxy for treatment. We further estimated the effect of missing breast cancer stage data using validated methods. Due to geographical differences in Se content, results from this type of study may be difficult to generalize to a population with sufficient Se levels. Finally, we had unequal sample sizes for Se and SePP analyses due to missing data. As with other observational studies, there is the possibility for residual confounding despite controlling for covariates.

In summary, the findings from this study suggest that higher pre-diagnostic exposure to Se is not associated with improved survival among breast cancer patients. Additionally, results from this study provide some evidence to suggest that tumor stage, geographic region, BMI, and smoking status are potential effect modifiers for the association between Se status and overall or breast cancer specific mortality. Further research is necessary to replicate these findings in larger populations and to understand the mechanisms of action of Se metabolism in relation to tumor development and progression.

Public Health Implications

Invasive breast cancer incidence among women over 50 in developed nations has increased since 1975[3]. Death rates for breast cancer have steadily declined since the 1990's. With increasing breast cancer incidence and decreasing breast cancer death rates, breast cancer survivors are a rising demographic with 3.8 million cancer survivors in the United States alone as of January 2019[3]. Survival rates for breast cancer are high among developed nations. The 5-year survival rates for breast cancer in the United states are as high as 99% for localized tumors and 90% for all tumor stages combined[3]. Survival rates in Western Europe are not far behind the U.S. with estimated 5-year survival rates as high as 81%[60].

While survival rates for breast cancer in developed nations are high there is still much work to be done to further improve outcomes for breast cancer survivors. Studies that investigate associations of exposures and breast cancer-specific mortality are needed to help indicate what potential factors are involved in early deaths among breast cancer survivors. Just as risk factors for breast cancer help to inform interventions and guidelines for the prevention of cancer incidence, prognostic risk factors help to inform prevention initiatives for breast cancer survivors.

This study does not support an association between higher pre-diagnostic Se status and lower overall and breast cancer specific mortality. Nonsignificant associations are an important part of public health research. Publication bias, or the tendency to publish significant results, is a key reason why some fields have trouble replicating results. While a study that reports non-significant results is less likely to be published than an equivalent study reporting significant results, both studies provide evidence to support a hypothesis given that all potential design issues and biases were addressed. Additionally, publishing non-significant results, particularly detailed explanations of study design and methods, provides prospective researchers with potential reasons for the non-significant finds which in turn help principal investigators to design studies that are able to observe an association if one exists.

This study does not support our initial hypothesis that higher Se status is associated with lower mortality among breast cancer patients. However, we observed potential suggestions that some subgroups

of women with breast cancer (e.g., never smokers) may benefit from higher Se exposure. Future research should be done in larger populations in other setting with sub-optimal Se status and consider potential biologically plausible effects modifiers in their analyses. Should the further evidence support an inverse association between higher Se status and mortality among populations with low Se status, this study along with others may be used to justify randomized clinical trials of Se supplementation. While previous supplementation trials have been published and have largely found no benefit for Se supplementation for breast cancer risk or mortality, most of these trials were conducted in populations with optimal Se status. If future randomized controlled trials are conducted and find benefits for supplementation in populations with low Se status, the evidence could influence cancer prevention initiatives, nutritional guidelines, and public health policy. Possible policy implications could include changes to dietary recommendations for European countries.

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TABLES

Table 1. Baseline characteristics of breast cancer cases by quintiles of serum selenium concentrations, the EPIC study (N = 1,781)

Baseline characteristics	Selenium, µg/L				
	Quintile 1 <49.44 (N = 356)	**Quintile 2 49.44-57.3 (N = 355)**	**Quintile 3 57.4-65.9 (N = 356)**	**Quintile 4 66.0-78.0 (N = 357)**	**Quintile 5 >78.1 (N = 357)**
Blood selenium biomarkers					
Selenium, µg/L mean (SD),	42.1 (6.3)	53.5 (2.2)	61.5 (2.4)	71.4 (3.4)	97.9 (21.7)
Selenoprotein P µg/L, mean (SD)	4.2 (0.9)	4.6 (0.8)	4.9 (0.80)	5.2 (1.1)	6.3 (1.4)
Demographics and lifestyle					
Age at blood collection, yrs, mean (SD)	54.3 (7.6)	55.2 (8.0)	55.0 (7.9)	55.0 (7.7)	56.3 (6.2)
Age at breast cancer diagnosis, yrs, mean (SD)	58.6 (8.0)	59.7 (8.3)	59.6 (8.3)	59.8 (8.1)	60.6 (6.5)
BMI, kg/m^2, mean (SD)	25.2 (4.3)	25.2 (4.4)	25.4 (4.1)	25.1 (4.2)	25.3 (4.7)
Smoking status, n (%)*					
Never	185 (52.0)	201 (56.6)	198 (55.6)	200 (56.0)	181 (50.7)
Former	103 (28.9)	76 (21.4)	73 (20.5)	81 (22.7)	79 (22.1)
Current	62 (17.4)	69 (19.4)	76 (21.4)	70 (19.6)	88 (24.7)
Smoking intensity, n (%)*					
Never	148 (42)	180 (51)	182 (51)	184 (52)	162 (45)
Current, 1-15 cig/day	33 (9)	42 (12)	45 (13)	46 (13)	61 (17)
Current, 16-26+ cig/day	28 (8)	25 (7)	31 (9)	21 (6)	26 (7)
Former, quit ≤ 10 yrs	26 (7)	19 (5)	23 (6)	15 (4)	27 (8)
Former, quit 11-20 yrs	35 (10)	20 (6)	23 (6)	29 (8)	15 (4)
Former, quit 20+ yrs	40 (11)	31 (9)	23 (6)	34 (10)	32 (9)
Current, pipe/cigar/occas	38 (11)	23 (6)	17 (5)	18 (5)	21 (6)
Physical activity, n (%)*					
Inactive	80 (22)	62 (17)	92 (26)	73 (20)	59 (17)
Moderately inactive	139 (39)	129 (36)	119 (33)	127 (36)	122 (34)
Moderately active	83 (23)	91 (26)	81 (23)	82 (23)	85 (24)
Active	52 (15)	66 (19)	58 (16)	72 (20)	87 (24)
Reproductive characteristics					
Menopausal status at baseline, n (%)					
Premenopausal	94 (26)	80 (23)	83 (23)	68 (19)	46 (13)
Postmenopausal	262 (74)	275 (77)	273 (76)	289 (81)	311 (87)
Age at menopause, yrs, mean (SD)*	48.9 (5.4)	49.8 (4.3)	49.4 (5.4)	49.0 (4.8)	49.5 (4.6)
Age at menarche, yrs, mean (SD)*	13.0 (1.6)	13.2 (1.4)	13.1 (1.6)	13.2 (1.7)	13.4 (1.6)
Age at first full term pregnancy, yrs, mean (SD)*	25.4 (4.3)	25.6 (4.2)	25.4 (4.5)	25.3 (4.2)	24.3 (3.9)
Full term pregnancy ever, n (%)*					
Yes	301 (85)	295 (83)	306 (86)	286 (80)	287 (80)
No	50 (14)	44 (12)	37 (10)	46 (13)	47 (13)
Breast cancer characteristics at diagnosis					
Estrogen receptor status, n (%)					
ER+	278 (78)	287 (81)	281 (79)	283 (79)	270 (76)
ER-	78 (22)	68 (19)	75 (21)	74 (21)	87 (24)
Progesterone receptor status, n (%)*					
PR+	191 (66)	161 (63)	153 (65)	135 (64)	95 (61)
PR-	100 (34)	95 (37)	81 (35)	76 (36)	61 (39)
Tumor stage, n (%)*					
I-II	290 (81.5)	268 (75.5)	244 (68.5)	267 (74.8)	223 (62.5)
III-IV	35 (9.8)	39 (11.0)	49 (13.8)	48 (13.5)	100 (28.0)
Tumor grade, n (%)*					
Well-differentiated	35 (12)	39 (16)	37 (18)	21 (11)	11 (11)
Moderately differentiated	83 (29)	69 (29)	67 (32)	49 (26)	16 (16)
Poorly differentiated	51 (18)	60 (25)	45 (21)	48 (26)	21 (21)
Undifferentiated	2 (1)	0 (0)	1 (0.5)	0 (0)	0 (0)

Abbreviations: yrs, years; BMI, body mass index; cig, cigarette; ER, estrogen receptor; PR progesterone receptor; menopause status at baseline classified pre or post menopause with postmenopausal defined as either postmenopausal, perimenopausal or surgical menopause; *Missing values of categorical variables were classified as a separate category: smoking intensity (n = 58), physical activity (n = 22), full term pregnancy ever (n = 82), progesterone receptor (n = 633), breast cancer stage at diagnosis (n = 218), and tumor grade (n = 1126). Percentages may not add up to 100% in each category due to the fact that unknown values were not excluded from the frequency calculations.

Table 2. Baseline characteristics of breast cancer cases by quintiles of serum selenoprotein P concentrations, the EPIC study (N = 2,203)

Baseline characteristics	Selenoprotein P mg/L				
	Quintile 1 <4 (N = 448)	**Quintile 2 4.1-4.5 (N = 401)**	**Quintile 3 4.5-5.0 (N = 458)**	**Quintile 4 5.1-5.7 (N = 453)**	**Quintile 5 >5.7 (N = 443)**
Blood selenium biomarkers					
Selenium, µg/L mean (SD),	52.2 (16.3)	57.4 (12.9)	61.0 (14.0)	68.2 (18.0)	83.7 (26.2)
Selenoprotein P µg/L, mean (SD)	3.6 (0.4)	4.3 (0.1)	4.8 (0.14)	5.4 (0.2)	6.7 (1.1)
Demographics and lifestyle					
Age at blood collection, yrs, mean (SD)	52.9 (8.1)	53.6 (7.7)	54.5 (7.5)	55.3 (7.7)	56.7 (6.7)
Age at breast cancer diagnosis, yrs, mean (SD)	58.0 (8.2)	58.6 (7.8)	59.1 (7.9)	60.0 (8.1)	61.0 (6.9)
BMI, kg/m^2, mean (SD)	25.3 (4.9)	25.3 (4.3)	25.5 (4.3)	25.0 (4.2)	25.5 (4.6)
Smoking status, n (%)*					
Never	237 (52.9)	235 (58.6)	263 (57.4)	255 (56.3)	230 (51.9)
Former	118 (26.3)	83 (20.7)	105 (22.9)	94 (20.8)	98 (22.1)
Current	85 (19.0)	75 (18.7)	85 (18.6)	93 (20.5)	103 (23.3)
Smoking intensity, n (%)*					
Never	212 (47)	214 (53)	235 (51)	218 (48)	198 (45)
Current, 1-15 cig/day	50 (11)	51 (13)	50 (11)	54 (12)	65 (15)
Current, 16-26+ cig/day	33 (7)	23 (6)	35 (8)	37 (8)	35 (8)
Former, quit ≤ 10 yrs	28 (6)	25 (6)	28 (6)	31 (7)	28 (6)
Former, quit 11-20 yrs	38 (8)	18 (4)	36 (8)	33 (7)	26 (6)
Former, quit 20+ yrs	47 (10)	38 (9)	35 (8)	28 (6)	37 (8)
Current, pipe/cigar/occas	25 (6)	24 (6)	28 (6)	40 (9)	36 (8)
Physical activity, n (%)*					
Inactive	93 (21)	106 (26)	97 (21)	102 (23)	83 (19)
Moderately inactive	183 (41)	146 (36)	155 (34)	170 (38)	158 (36)
Moderately active	103 (23)	84 (21)	110 (24)	104 (23)	104 (23)
Active	66 (15)	62 (15)	91 (20)	74 (16)	90 (20)
Reproductive characteristics					
Menopausal status at baseline, n (%)					
Premenopausal	141 (31)	114 (28)	120 (26)	91 (20)	52 (12)
Postmenopausal	307 (69)	287 (72)	338 (74)	362 (80)	391 (88)
Age at menopause, yrs, mean (SD)*	49.1 (5.2)	49.5 (5.1)	49.5 (4.6)	49.4 (4.7)	49.2 (4.5)
Age at menarche, yrs, mean (SD)*	13.2 (1.6)	13.2 (1.6)	13.2 (1.5)	13.0 (1.5)	13.1 (1.6)
Age at first full term pregnancy, yrs, mean (SD)*	25.1 (4.0)	25.4 (4.1)	25.4 (4.7)	25.7 (4.2)	25.0 (4.0)
Full term pregnancy ever, n (%)*					
Yes	380 (85)	332 (83)	397 (87)	369 (81)	353 (80)
No	59 (13)	52 (13)	46 (10)	65 (14)	65 (14)
Breast cancer characteristics at diagnosis					
Estrogen receptor status, n (%)					
ER+	346 (77)	313 (78)	357 (78)	363 (80)	357 (81)
ER-	102 (23)	88 (22)	101 (22)	90 (20)	86 (19)
Progesterone receptor status, n (%)*					
PR+	197 (63)	210 (67)	230 (67)	213 (64)	176 (66)
PR-	116 (37)	102 (33)	114 (33)	119 (36)	92 (34)
Tumor stage, n (%)*					
I-II	321 (71.7)	314 (78.3)	352 (76.9)	347 (76.6)	319 (72.0)
III-IV	54 (12.1)	47 (11.7)	66 (14.4)	48 (10.6)	91 (20.5)
Tumor grade, n (%)*					
Well-differentiated	36 (13)	38 (13)	33 (10)	49 (16)	32 (14)
Moderately differentiated	104 (39)	90 (31)	110 (34)	89 (29)	63 (28)
Poorly differentiated	62 (23)	74 (26)	87 (27)	67 (22)	54 (24)
Undifferentiated	1 (0.4)	1 (0.3)	1 (0.3)	0 (0)	0 (0)

Abbreviations: yrs, years; BMI, body mass index; cig, cigarette; ER, estrogen receptor; PR progesterone receptor; menopause status at baseline classified pre or post menopause with postmenopausal defined as either postmenopausal, perimenopausal or surgical menopause. *Unknown values of categorical variables were classified as a separate category: smoking intensity (n = 64), physical activity (n = 22), full term pregnancy ever (n = 85), progesterone receptor (n = 636), breast cancer stage at diagnosis (n = 244), and tumor grade (n = 1212). Percentages may not add up to 100% in each category due to the fact that unknown values were not excluded from the frequency calculations.

Table 3. HRs and 95% CIs for overall and breast cancer-specific mortality according to quintiles of pre-diagnostic serum selenium and selenoprotein P among breast cancer patients in the EPIC study.

	Selenium, µg/L			Selenoprotein P, mg/L		
	Event/Total		**HR (95% CI)**	**Event/Total**		**HR (95% CI)**
Overall mortality						
Age and Stage-adjusted [a]						
Quintile 1	80/356	< 49.44	1.00 (ref)	114/448	≤ 4.0	1.00 (ref)
Quintile 2	85/355	49.44-57.3	1.14 (0.81-1.61)	75/401	4.1-4.5	0.77 (0.56-1.04)
Quintile 3	94/356	57.44-66.03	1.19 (0.84-1.68)	90/458	4.6-5.0	0.77 (0.57-1.03)
Quintile 4	76/357	66.04-78.11	0.95 (0.65-1.37)	106/453	5.1-5.7	0.95 (0.72-1.25)
Quintile 5	93/357	≥ 78.12	0.97 (0.67-1.40)	111/443	> 5.7	0.86 (0.65-1.15)
P_{trend} [b]			*0.318*			*0.810*
Per 1 SD [c]	428/1781		0.94 (0.84-1.06)	496/ 2203		0.99 (0.91-1.08)
Multivariable-adjusted [d]						
Quintile 1	80/356	< 49.44	1.00 (ref)	114/448	≤ 4.0	1.00 (ref)
Quintile 2	85/355	49.44-57.3	1.17 (0.83-1.65)	75/401	4.1-4.5	0.73 (0.54-1.00)
Quintile 3	94/356	57.44-66.03	1.19 (0.84-1.68)	90/458	4.6-5.0	0.76 (0.56-1.01)
Quintile 4	76/357	66.04-78.11	0.96 (0.66-1.40)	106/453	5.1-5.7	0.94 (0.71-1.24)
Quintile 5	93/357	≥ 78.12	0.95 (0.65-1.38)	111/443	> 5.7	0.81 (0.60-1.08)
P_{trend} [b]			*0.286*			*0.538*
Per 1 SD [c]	428/1781		0.94 (0.84-1.05)	496/ 2203		0.97 (0.89-1.06)
Breast cancer-specific mortality						
Age and Stage-adjusted [a]						
Quintile 1	47/356	< 49.44	1.00 (ref)	70/448	≤ 4.0	1.00 (ref)
Quintile 2	49/355	49.44-57.3	1.37 (0.87-2.16)	48/401	4.1-4.5	0.88 (0.60-1.29)
Quintile 3	65/356	57.44-66.03	1.63 (1.04-2.55)	62/458	4.6-5.0	0.87 (0.60-1.25)
Quintile 4	53/357	66.04-78.11	1.35 (0.83-2.19)	73/453	5.1-5.7	1.18 (0.83-1.66)
Quintile 5	60/357	≥ 78.12	1.26 (0.78-2.05)	69/443	> 5.7	0.91 (0.63-1.32)
P_{trend} [b]			*0.763*			*0.644*
Per 1 SD [c]	274/1781		1.02 (0.89-1.17)	322/2203		1.03 (0.92-1.14)
Multivariable-adjusted [d]						
Quintile 1	47/356	< 49.44	1.00 (ref)	70/448	≤ 4.0	1.00 (ref)
Quintile 2	49/355	49.44-57.3	1.45 (0.92-2.29)	48/401	4.1-4.5	0.88 (0.60-1.30)
Quintile 3	65/356	57.44-66.03	1.68 (1.07-2.64)	62/458	4.6-5.0	0.87 (0.60-1.25)
Quintile 4	53/357	66.04-78.11	1.39 (0.86-2.26)	73/453	5.1-5.7	1.19 (0.83-1.69)
Quintile 5	60/357	≥ 78.12	1.29 (0.79-2.10)	69/443	> 5.7	0.86 (0.59-1.26)
P_{trend} [b]			*0.826*			*0.923*
Per 1 SD [c]	274/1781		1.02 (0.89-1.16)	322 / 2203		1.01 (0.90-1.12)

[a] Adjusted for age at diagnosis and stage; stratified on country.

[b] P_{trend} was calculated using the median value of each Se of SePP quintiles as continuous variable, adjusted for variables in the corresponding models.

[c] 1 SD = 21.59 µg/L Se; 1 SD = 1.19 mg/L of SePP.

[d] Adjusted for age at diagnosis, stage, smoking status, physical activity, BMI, age at menarche, menopause status, and estrogen receptor status, stratified on country; menopause classified pre or post menopause with postmenopausal defined as either postmenopausal, perimenopausal, or surgical menopause; age at menarche was categorized by age tertiles.

Table 4. Multivariable-adjusted HRs and 95% CIs for an increment of 1 SD of selenium or selenoprotein P for breast cancer and overall mortality across strata of potential effect modifiers among breast cancer patients in the EPIC study.

Sensitivity Analysis/Stratifying Factors	Overall mortality			Breast cancer-specific mortality		
	Event/ Total	HR (95% CI)	*P-values*	Event/ Total	HR (95% CI)	*P-values*
Selenium						
All participants	428/1781	0.94 (0.84-1.05)	*0.286* [c]	274/1781	1.02 (0.89-1.16)	*0.826* [c]
Sensitivity analyses						
Complete tumor stage data	369/1563	0.94 (0.83-1.06)	*0.300* [c]	238/1563	1.06 (0.92-1.22)	*0.461* [c]
Imputed tumor Stage data	428/1781	0.94 (0.84-1.06)	*0.308* [c]	274/1781	1.02 (0.89-1.17)	*0.764* [c]
Follow-up, yrs						
≥ 2	383/1733	0.94 (0.83-1.06)	*0.318* [c]	236/1733	1.03 (0.89-1.19)	*0.695* [c]
≥ 5	269/1612	0.91 (0.78-1.05)	*0.197* [c]	138/1612	1.01 (0.84-1.22)	*0.933* [c]
Age at diagnosis, yrs						
< 50	44/191	1.25 (0.58-2.69)	*0.047* [b]	40/191	1.39 (0.59-3.28)	*0.080* [b]
≥ 50	384/1590	0.94 (0.84-1.06)		234/1590	1.02 (0.89-1.17)	
Tumor stage						
I-II	244/1292	0.94 (0.79-1.12)	*0.224* [b]	142/1292	1.18 (0.98-1.43)	*0.016* [b]
III-IV	125/271	0.92 (0.76-1.13)		96/271	0.90 (0.71-1.14)	
Estrogen receptor status						
ER +	315/1399	0.97 (0.85-1.11)	0.648 [b]	188/1399	1.04 (0.89-1.23)	*0.834* [b]
ER -	113/382	0.90 (0.69-1.17)		86/382	1.05 (0.78-1.40)	
Menopause status at baseline						
Premenopausal	73/371	1.26 (0.84-1.89)	*0.386* [b]	60/371	1.38 (0.89-2.14)	*0.663* [b]
Postmenopausal	355/1410	0.91 (0.80-1.03)		214/1410	0.99 (0.86-1.15)	
BMI, kg/m^2						
< 25	209/976	0.88 (0.74-1.05)	0.505 [b]	131/976	1.01 (0.82-1.24)	*0.348* [b]
≥ 25	219/805	0.97 (0.82-1.15)		143/805	1.00 (0.82-1.23)	
Smoking status*						
Never	216/965	0.82 (0.68-0.98)	*0.006* [b]	147/965	0.77 (0.61-0.96)	*<0.001* [b]
Former	107/412	1.15 (0.93-1.42)		73/412	1.25 (0.97-1.60)	
Current	93/365	0.91 (0.70-1.19)		45/365	1.32 (0.93-1.89)	
Geographic region						
Northern	163/605	0.93 (0.80-1.07)	*0.008* [b]	105/605	1.02 (0.86-1.21)	*0.076* [b]
Central	180/749	1.10 (0.83-1.46)		105/749	1.35 (0.94-1.94)	
Southern	85/427	0.66 (0.42-1.05)		64/427	0.89 (0.54-1.44)	
Selenoprotein P						
All participants	496/2203	0.97 (0.89-1.06)	*0.538* [c]	322/2203	1.01 (0.90-1.12)	*0.923* [c]
Sensitivity analyses						
Complete tumor stage data	432/1959	0.95 (0.87-1.05)	*0.336* [c]	284/1959	0.99 (0.88-1.11)	*0.843* [c]
Imputed tumor stage data	496/2203	0.97 (0.89-1.07)	*0.546* [c]	322/2203	1.01 (0.91-1.13)	*0.822* [c]
Follow-up, yrs						
≥ 2	441/2144	0.98 (0.89-1.08)	*0.709* [c]	276/2144	1.03 (0.92-1.16)	*0.598* [c]
≥ 5	308/1995	0.97 (0.86-1.09)	*0.597* [c]	163/1995	1.03 (0.88-1.21)	*0.695* [c]
Age at diagnosis. yrs						
< 50	61/264	0.92 (0.61-1.37)	0.288 [b]	54/264	0.91 (0.59-1.40)	*0.284* [b]
≥ 50	435/1939	0.99 (0.90-1.08)		268/1939	1.02 (0.91-1.14)	
Tumor Stage						
I-II	287/1653	0.95 (0.83-1.07)	0.515 [b]	170/1653	1.05 (0.90-1.23)	*0.114* [b]
III-IV	145/306	0.98 (0.83-1.16)		114/306	0.95 (0.78-1.15)	
Estrogen receptor status						
ER +	367/1736	1.02 (0.92-1.14)	*0.103* [b]	224/1736	1.07 (0.94-1.22)	*0.203* [b]
ER -	129/467	0.85 (0.68-1.05)		98/467	0.90 (0.70-1.16)	
Menopause status at baseline						
Premenopausal	98/518	1.02 (0.76-1.36)	*0.474* [b]	79/518	1.05 (0.75-1.46)	*0.098* [b]
Postmenopausal	398/1685	0.97 (0.88-1.07)		243/1685	1.00 (0.89-1.13)	
BMI, kg/m^2						
< 25	239/1209	0.96 (0.84-1.11)	*0.286* [b]	152/1209	1.08 (0.91-1.29)	0.021 [b]
≥ 25	257/994	0.97 (0.85-1.10)		170/994	0.94 (0.80-1.10)	
Smoking status*						
Never	255/1220	0.86 (0.74-1.00)	*0.020* [b]	172/1220	0.89 (0.74-1.06)	*0.004* [b]
Former	121/498	1.08 (0.92-1.26)		83/498	1.08 (0.89-1.31)	
Current	107/441	1.03 (0.83-1.28)		58/441	1.06 (0.78-1.44)	
Geographic Region						
Northern	163/605	1.01 (0.88-1.15)	*0.048* [b]	105/605	1.04 (0.89-1.22)	*0.500* [b]
Central	207/942	0.95 (0.80-1.13)		121/942	0.97 (0.77-1.22)	
Southern	126/656	0.77 (0.58-1.01)		96/656	0.88 (0.64-1.22)	

[a] Adjusted for age of diagnosis, tumor stage, estrogen receptor status, BMI, menopause status, age at menarche, and smoking intensity. Stratified on country.
[b] P for interaction (as estimated by likelihood ratio tests).
[c] Missing data were not included in the analysis.
[d] Unknown values of categorical variables were classified as a separate category: smoking status (n = 44).

APPENDIX

Supplemental Table 1. HRs and 95% CIs for overall and breast cancer-specific mortality according to quintiles of pre-diagnostic serum selenium and selenoprotein P among breast cancer cases and cases with Se measurements in the EPIC study.

	Selenium			Selenoprotein P			Selenoprotein P (limited to cases with Se)		
	Event/Total	**µg/L**	**HR (95% CI)**	**Event/Total**	**mg/L**	**HR (95% CI)**	**Event/Total**	**mg/L**	**HR (95% CI)**
Overall mortality									
Age and stage-adjusted [a]									
Quintile 1	80/356	< 49.44	1.00 (ref)	114/448	≤ 4.0	1.00 (ref)	93/333	≤ 4.0	1.00 (ref)
Quintile 2	85/355	49.44-57.3	1.14 (0.81-1.61)	75/401	4.1-4.5	0.77 (0.56-1.04)	64/304	4.1-4.5	0.76 (0.55-1.06)
Quintile 3	94/356	57.44-66.03	1.19 (0.84-1.68)	90/458	4.6-5.0	0.77 (0.57-1.03)	77/372	4.6-5.0	0.77 (0.56-1.06)
Quintile 4	76/357	66.04-78.11	0.95 (0.65-1.37)	106/453	5.1-5.7	0.95 (0.72-1.25)	88/370	5.1-5.7	0.89 (0.65-1.21)
Quintile 5	93/357	≥ 78.12	0.97 (0.67-1.40)	111/443	> 5.7	0.86 (0.65-1.15)	105/400	> 5.7	0.87 (0.64-1.18)
P_{Trend} [b]			*0.32*			*0.81*			*0.76*
Per 1 SD [c]	428/1781		0.94 (0.84-1.06)	496/ 2203		0.99 (0.91-1.08)	427/ 1779		0.99 (0.90-1.08)
Multivariable-adjusted [d]									
Quintile 1	80/356	< 49.44	1.00 (ref)	114/448	≤ 4.0	1.00 (ref)	93/333	≤ 4.0	1.00 (ref)
Quintile 2	85/355	49.44-57.3	1.17 (0.83-1.65)	75/401	4.1-4.5	0.73 (0.54-1.00)	64/304	4.1-4.5	0.72 (0.51-1.01)
Quintile 3	94/356	57.44-66.03	1.19 (0.84-1.68)	90/458	4.6-5.0	0.76 (0.56-1.01)	77/372	4.6-5.0	0.75 (0.54-1.03)
Quintile 4	76/357	66.04-78.11	0.96 (0.66-1.40)	106/453	5.1-5.7	0.94 (0.71-1.24)	88/370	5.1-5.7	0.87 (0.64-1.19)
Quintile 5	93/357	≥ 78.12	0.95 (0.65-1.38)	111/443	> 5.7	0.81 (0.60-1.08)	105/400	> 5.7	0.82 (0.60-1.11)
P_{Trend} [b]			*0.29*			*0.54*			*0.58*
Per 1 SD [c]	428/1781		0.94 (0.84-1.05)	496/ 2203		0.97 (0.89-1.06)	427/ 1779		0.97 (0.89-1.07)
Breast cancer-specific mortality									
Age and stage-adjusted [a]									
Quintile 1	47/356	< 49.44	1.00 (ref)	70/448	≤ 4.0	1.00 (ref)	59/333	≤ 4.0	1.00 (ref)
Quintile 2	49/355	49.44-57.3	1.37 (0.87-2.16)	48/401	4.1-4.5	0.88 (0.60-1.29)	40/304	4.1-4.5	0.87 (0.57-1.33)
Quintile 3	65/356	57.44-66.03	1.63 (1.04-2.55)	62/458	4.6-5.0	0.87 (0.60-1.25)	51/372	4.6-5.0	0.86 (0.58-1.29)
Quintile 4	53/357	66.04-78.11	1.35 (0.83-2.19)	73/453	5.1-5.7	1.18 (0.83-1.66)	57/370	5.1-5.7	1.08 (0.73-1.59)
Quintile 5	60/357	≥ 78.12	1.26 (0.78-2.05)	69/443	> 5.7	0.91 (0.63-1.32)	67/400	> 5.7	0.98 (0.66-1.45)
P_{Trend} *b*			*0.76*			*0.64*			*0.67*
Per 1 SD [c]	274/1781		1.02 (0.89-1.17)	322/2203		1.03 (0.92-1.14)	284/ 1779		1.03 (0.91-1.15)
Multivariable-adjusted [d]									
Quintile 1	47/356	< 49.44	1.00 (ref)	70/448	≤ 4.0	1.00 (ref)	59/333	≤ 4.0	1.00 (ref)
Quintile 2	49/355	49.44-57.3	1.45 (0.92-2.29)	48/401	4.1-4.5	0.88 (0.60-1.30)	40/304	4.1-4.5	0.86 (0.56-1.32)
Quintile 3	65/356	57.44-66.03	1.68 (1.07-2.64)	62/458	4.6-5.0	0.87 (0.60-1.25)	51/372	4.6-5.0	0.85 (0.56-1.27)
Quintile 4	53/357	66.04-78.11	1.39 (0.86-2.26)	73/453	5.1-5.7	1.19 (0.83-1.69)	57/370	5.1-5.7	1.07 (0.72-1.59)
Quintile 5	60/357	≥ 78.12	1.29 (0.79-2.10)	69/443	> 5.7	0.86 (0.59-1.26)	67/400	> 5.7	0.94 (0.63-1.40)
P_{Trend} [b]			*0.83*			*0.92*			*0.82*
Per 1 SD [c]	274/1781		1.02 (0.89-1.16)	322 / 2203		1.01 (0.90-1.12)	284/ 1779		1.01 (0.90-1.14)

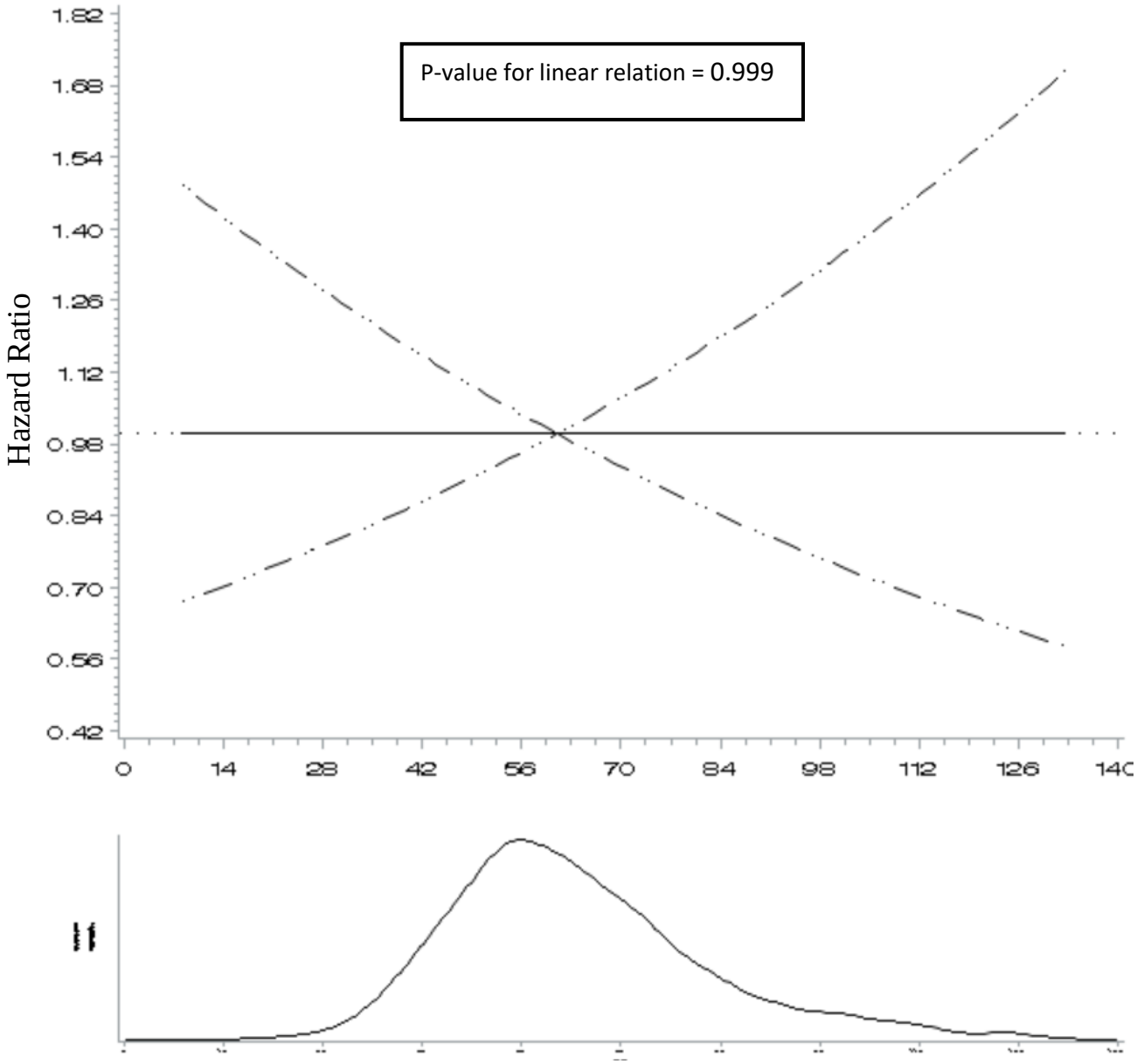
[a] Adjusted for age at diagnosis and stage; stratified on country.

[b] P_{trend} was calculated using the median value of each Se or SePP quintiles as continuous variables, adjusted for variables in corresponding models.

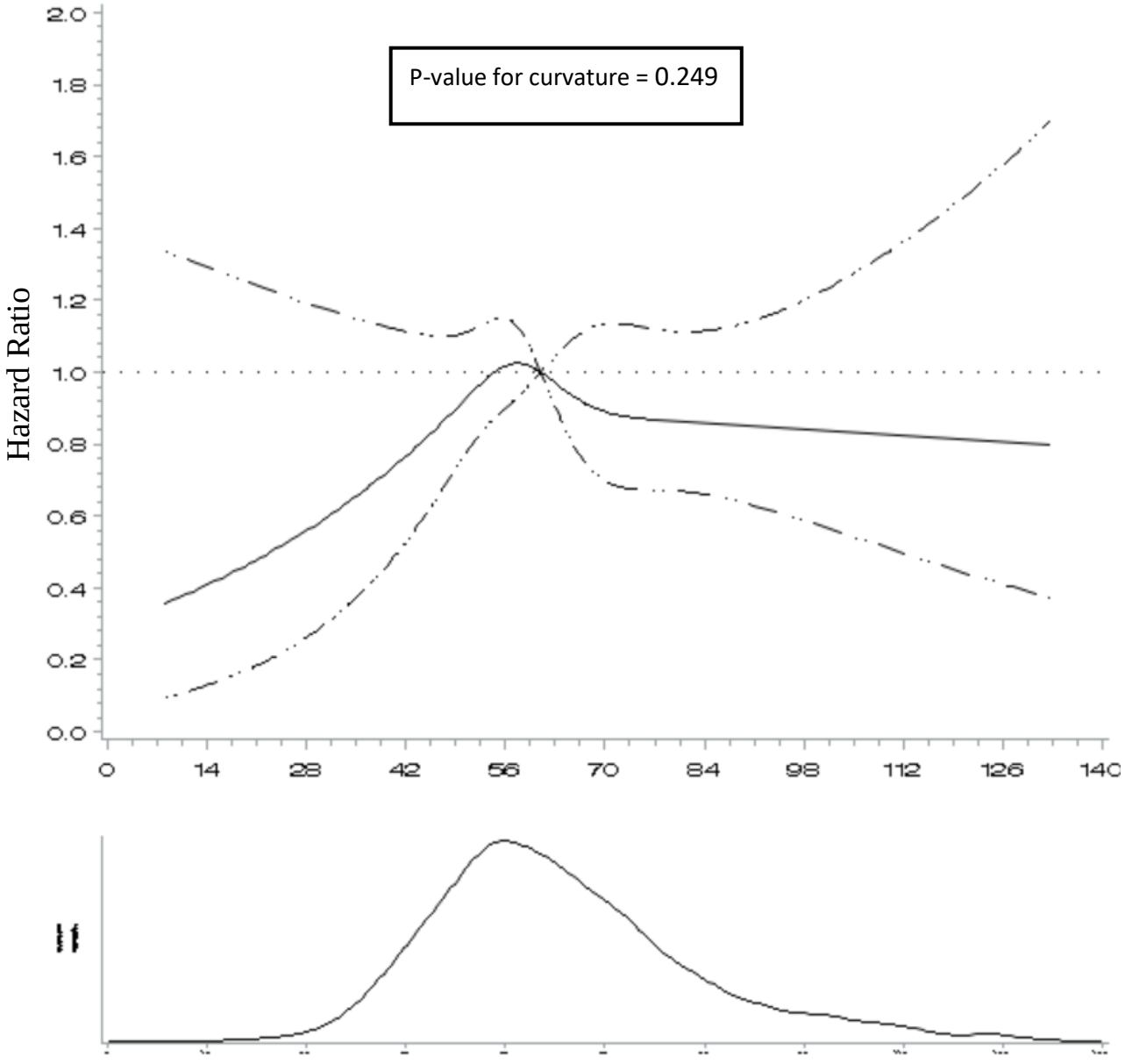
[c] 1 SD = 12.59 µg/L Se; 1 SD = 1.19 mg/L of SePP.

[d] Adjusted for age at diagnosis, smoking status, physical activity, BMI, age at menarche, menopause status, and estrogen receptor status; stratified on country; menopause status classified pre or post menopause with postmenopausal defined as either postmenopausal, perimenopausal, or surgical menopause; age at menarche was categorized as a categorical variable with age tertiles and an unknown/missing category.

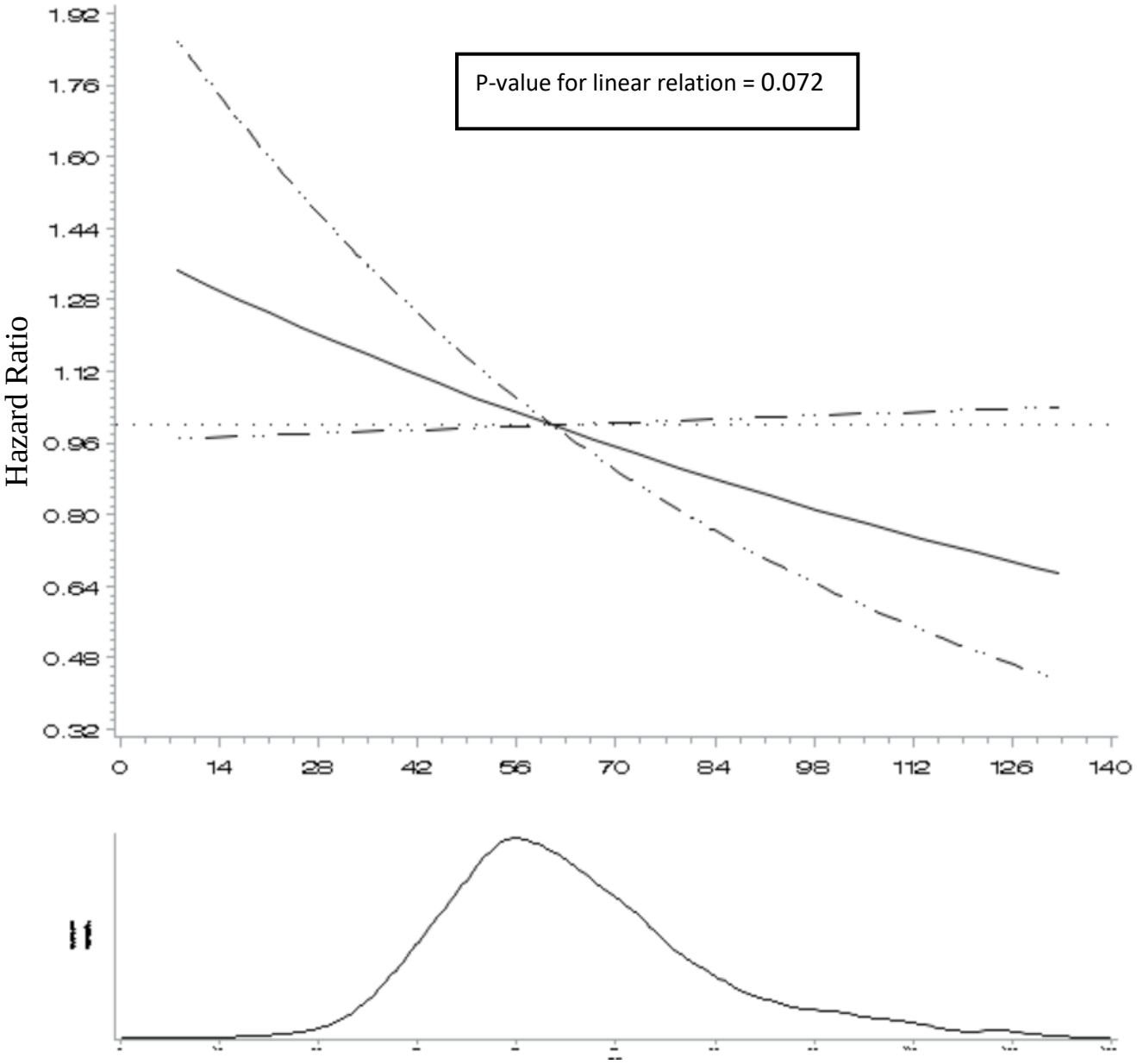
Supplementary Figure 1. Linear spline regression model for concentration of Se (µg/L) and breast cancer specific death. Reference: 60.98 µg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



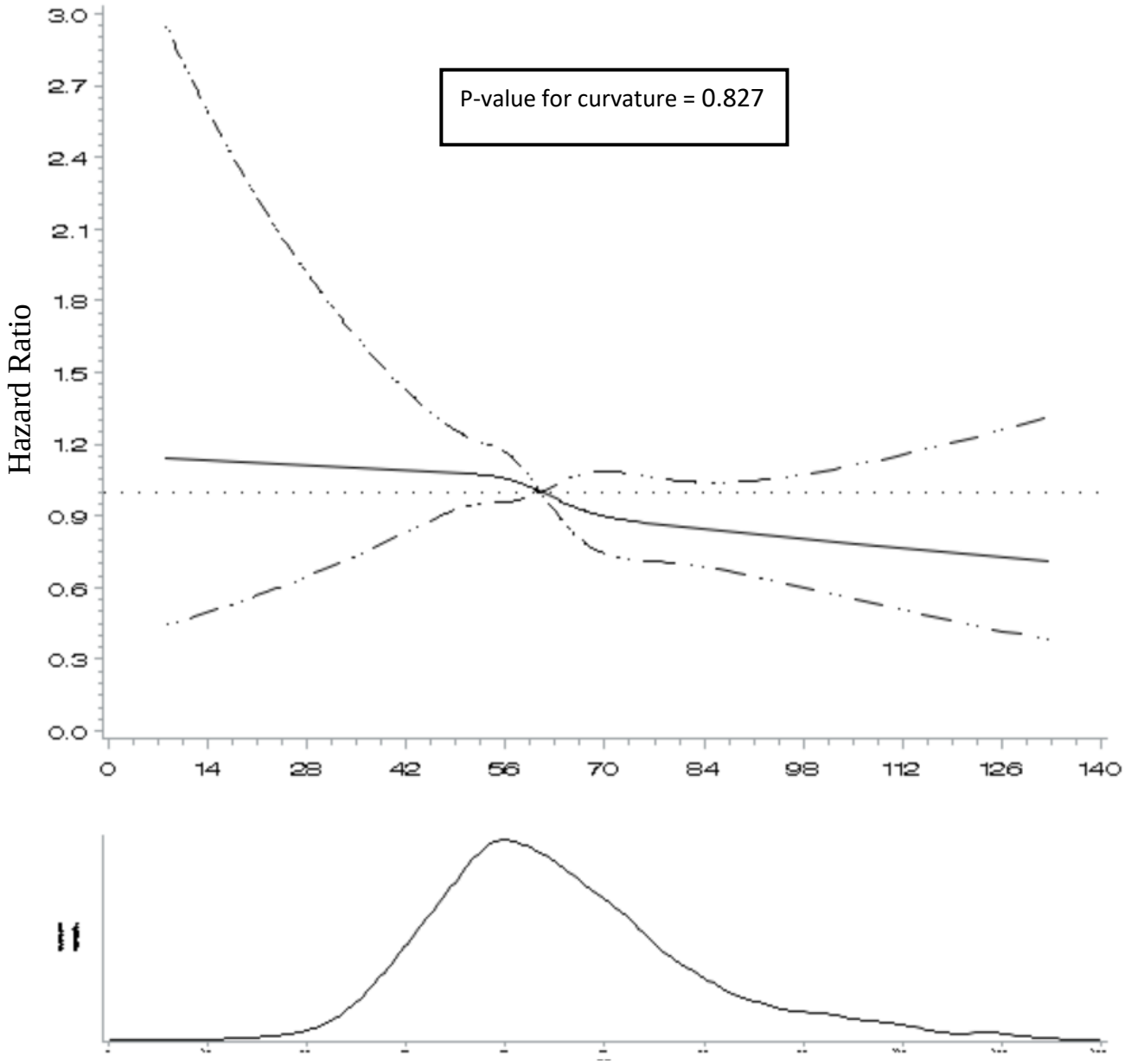
Supplementary Figure 2. Cubic spline regression model for concentration of Se (µg/L) and breast cancer specific death. Reference: 60.98 µg/L. Knots: 49.44, 57.44, 66.04, and 78.12 µg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



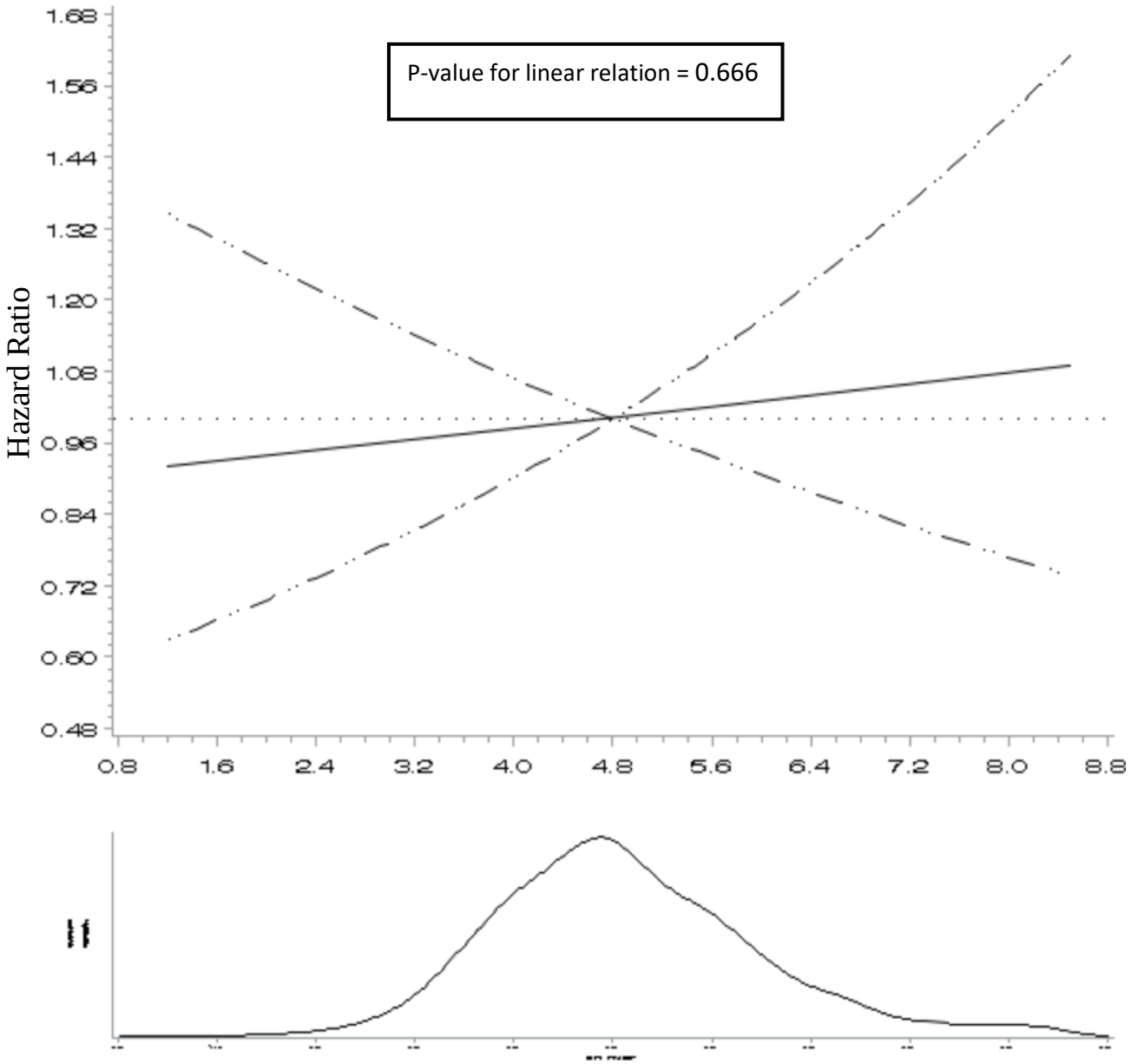
Supplementary Figure 3. Linear spline regression model for concentration of Se (µg/L) and all cause death. Reference: 60.98 µg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



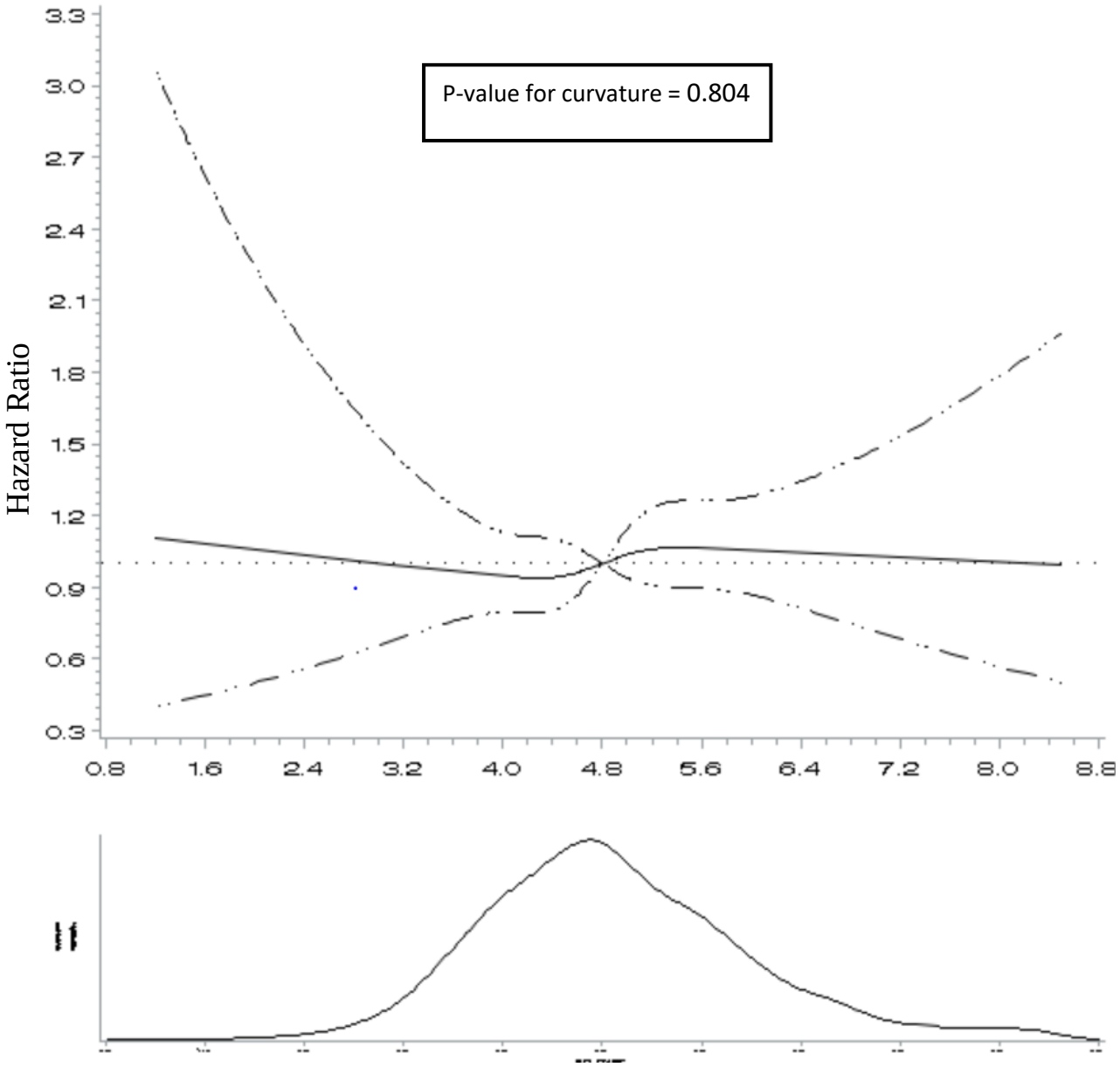
Supplementary Figure 4. Cubic spline regression model for concentration of Se (µg/L) and all cause death. Reference: 60.98 µg/L. Knots: 49.44, 57.44, 66.04, and 78.12 µg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



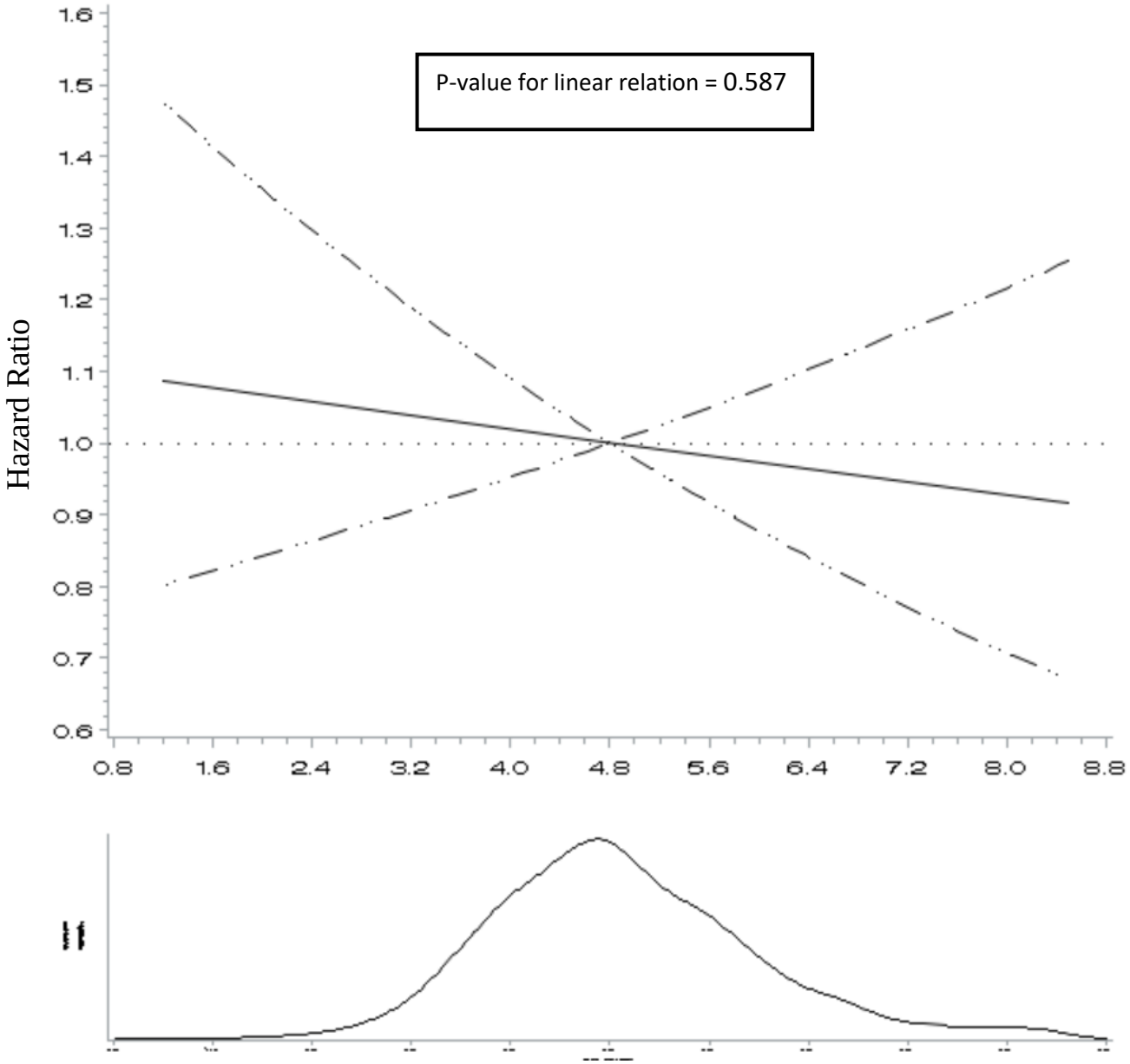
Supplementary Figure 5. Linear spline regression model for concentration of SePP (mg/L) and breast cancer specific death. Reference: 4.8 mg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



Supplementary Figure 6. Cubic spline regression model for concentration of SePP (mg/L) and breast cancer specific death. Reference: 4.8 mg/L. Knots: 4 4.5 5 5.7 mg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



Supplementary Figure 7. Linear spline regression model for concentration of SePP (mg/L) and all cause death. Reference: 4.8 mg/L. *Solid line*- HR, *dashed lines*- 95 % CI.



Supplementary Figure 8. Cubic spline regression model for concentration of SePP (mg/L) and all cause death. Reference: 4.8 mg/L. Knots: 4 4.5 5 5.7 mg/L. *Solid line*- HR, *dashed lines*- 95 % CI.