Women with lower levels of serum selenium (Se) may have a worse survival in breast cancer than women with higher levels, despite no difference in incidence of the disease. Our study was conducted to test whether Se is associated with the aggressiveness of breast tumors. Both the risk of having a tumor characteristic associated with worse prognosis, as well as the overall and breast cancer-specific mortality, were studied. We identified breast cancer cases and controls within the Malmö Diet and Cancer Study, a population-based cohort with 17 035 women recruited between 1991 and 1996. Inclusion criteria were incident breast cancer. Exclusion criteria were carcinoma in situ and bilateral breast cancer. Controls were selected among breast cancer-free women both from matching (n = 694) as well as randomization (n = 492). After exclusion, 1066 cases remained and were compared to controls regarding their prediagnostic serum Se levels and subsequent risk of having a certain tumor characteristic or intrinsic subtype. We also followed breast cancer patients regarding overall and breast cancer-specific mortality, comparing different Se quartiles. No association between serum Se quartile and any tumor characteristic or intrinsic subtype was found. Lower overall mortality was found among women in the highest Se quartile compared to the lowest using an adjusted Cox proportional hazards model, hazard ratio 0.63 (95% confidence interval: 0.44-0.89). Similar results were seen for breast cancer-specific mortality, 0.60 (0.37-0.98). The results of our study support that Se is associated with a lower mortality in breast cancer, not related to established prognostic factors.

KEYWORDS
breast cancer, prognostic factors, selenium, survival, tumor

1 | INTRODUCTION

Selenium (Se) has been studied regarding a protective effect against breast cancer risk and these studies have rendered mixed but mainly null results.1,2 Our results from a previous study, as well as a recent Cochrane systematic review, suggest that it is unlikely that Se is associated with the overall risk of breast cancer.2,3 However, two studies have reported
increased breast cancer mortality among women in the lowest quartile of serum Se as well as Se intake, and thus a potential protective effect of Se might be seen in clinical outcome rather than in incidence.4,5

Se is an essential mineral important in the anti-oxidation system and for thyroid hormone function, mediating its functions through selenoproteins, a group of proteins with incorporated Se.6 Several possible mechanisms for how Se impacts breast cancer development and progression have been suggested. The selenoprotein glutathione peroxidase-1 (GPx-1), which has a role in anti-oxidation and DNA stability, could be the mediator of a protective effect.7,8 Indeed, loss of heterozygosity in the GPx-1 gene is a common event in human breast cancer, and overexpression of GPx-1 has been shown to protect against chromosome damage in cell cultures.9,10 However, the Se pathways are complex and another major selenoprotein, thioredoxin reductase (TRx), has on the contrary been suggested to support the growth of cancer cells. Inhibition of this protein has recently rendered promising results in animal studies on the use of TRx inhibition as a cancer therapy.11 With these conflicting mechanisms in mind, the effect that Se might have on breast cancer prognosis is yet to be established.

Breast cancer should not be seen as a single entity. Tumor characteristics such as size and intrinsic subtype, together with clinical data such as lymph node positivity are essential factors to take into account when considering prognosis and treatment.12-14 Our study is, to the best of our knowledge, the first one using prediagnostic serum Se levels to study the risk of developing breast cancer in relation to specific tumor characteristics or intrinsic subtype, as well as the first to study prediagnostic serum Se and breast cancer mortality. We hypothesized that higher Se levels would be associated with lower breast cancer mortality as well as a reduced risk of developing prognostically unfavorable tumors.

2 | MATERIALS AND METHODS

2.1 | Design

Our study applied a prospective design to follow-up survival among women diagnosed with breast cancer, as well as a nested case-control design to study the risk of specific breast tumor characteristics relevant to prognosis. This approach was followed within the population-based cohort the Malmö Diet and Cancer Study (MDCS). The MDCS includes individuals born between 1923 and 1950 that were living in Malmö, Sweden, during the recruitment period, 1991 to 1996. A total of 17,035 women were included in the MDCS, representing 43% of eligible women.15 The baseline examination included a questionnaire, blood sample collection and measurements of height and weight.16

2.2 | Case and control selection

Information about breast cancer diagnosis was gained through linking MDCS participants to the Swedish Cancer Registry using their Swedish personal identity number. Excluding those with prevalent breast cancer at baseline, all women in the MDCS diagnosed with breast cancer for the first time from baseline up until December 31, 2013, were identified and eligible as cases in our study (n = 1,186). An equal number of controls (n = 1,186) were selected. The controls were selected using two different methods. One group of controls (n = 694) was selected based on a previous breast cancer study in the MDCS by Almquist et al that used incidence density matching, matching for age, menopausal status and time of inclusion.17 All unique controls from that study that still remained free from breast cancer up until December 31, 2013, were included as controls in the present study. The remaining controls (n = 492) were randomized from the female population of the cardiovascular (CV) subcohort in the MDCS. The CV-subcohort was created to be an extension of the MDCS with extra baseline examinations, including extra blood sampling and ultrasound of the carotid arteries, and 50% of those invited to the MDCS between 1991 to 1994 were also invited to the CV-subcohort.18,19 In total, 3,531 women accepted the invitation, however, among those, not everyone completed the MDCS baseline examination and was thus never included in the MDCS cohort. Our randomization was performed among those 3,363 women who were also among the 17,035 complete participants in the MDCS.18,19 The reason for selecting our control group using the described methods was that future studies will investigate thyroid hormones (analyzed in the study by Almquist et al) and genetic data (available in the CV subcohort).17 After including eligible cases and controls, a total of 120 cases were excluded due to bilateral breast cancer (n = 20) or carcinoma in situ (n = 100). Furthermore, 63 patients were excluded from risk analysis of tumor characteristics due to missing tumor material for pathology re-evaluation at the time of data collection. The inclusions and exclusions are visualized in Figure 1.

2.3 | Selenium analysis

Blood samples were taken at baseline and stored at −80°C. Serum Se was analyzed from these samples for all participants in the present study during October 2015 by ALS Scandinavia AB, Luleå, Sweden.
Single element standards traceable to the National Institute of Standards and Technology were used on an ICP-SFMS (Thermo Element 2). Every sample was diluted to 10 mL from 0.15 mL of serum by adding an alkali solution containing 0.1% NH₃ and 0.005% EDTA/Triton-X. Reference samples were included in all batches (Seronorm; Sero AS, Norway, Lot 0608414). The inter-batch coefficient of variation was 0.03, and the limit of detection was 4 ng/mL.

2.4 | Baseline and patient characteristics

Descriptive data such as age as well as life-style and reproductive factors were collected from the baseline questionnaire. BMI was calculated from measured height and weight. Menopausal status was defined based on information from the questionnaire as described previously. Data regarding lymph node status, distant metastasis, as well as oncological and surgical treatment were collected from medical records.

2.5 | Tumor characteristics

Information about tumor characteristics was collected in three different time periods. During all periods, tumor size, nodal status and information on metastasis were collected from medical records. Tumors from cases diagnosed from 1991 to December 31, 2004, were re-evaluated regarding histological grade, and a tissue micro-array (TMA) was constructed to assess proliferation (Ki67), HER2⁻ and hormone receptor status as described by Borgquist et al.²⁰ and Butt et al.²¹ For all cases diagnosed from 2005 to December 31, 2007, a re-evaluation of Ki67 as well as HER2⁻ and hormone receptor status were performed by using a TMA.²² However, when available, clinical data was used regarding PgR-status and ER-status from 2005 onwards, and all other data regarding tumor characteristics from 2005 onwards was collected from medical records. For the present study, the Ki67 variable was divided into subcategories (low, intermediate and high) depending on internal rank, with 1/3 of the cases in each category.
### TABLE 1  Descriptive data of cases, controls and excluded groups

|                                | Controls (n = 1186) | Invasive tumors with tumor material (n = 1003) | Invasive tumors with missing material (n = 63) | Bilateral cancers (n = 20) | Carcinoma in situ (n = 100) |
|--------------------------------|---------------------|-----------------------------------------------|-----------------------------------------------|---------------------------|-----------------------------|
| **Mean selenium ng/mL**        | 91.3                | 92.2                                          | 91.2                                          | 93.4                      | 90.5                        |
| **Mean age**                   | 57.1                | 56.3                                          | 56.9                                          | 60.4                      | 55.4                        |
| **Age**                        |                     |                                               |                                               |                           |                             |
| ≤49                            | 19.2                | 25.8                                          | 27.0                                          | 15.0                      | 27.0                        |
| 50-54                          | 23.5                | 23.4                                          | 14.3                                          | 5.0                       | 34.0                        |
| 55-59                          | 20.9                | 19.5                                          | 25.4                                          | 25.0                      | 11.0                        |
| ≥60                            | 36.3                | 31.2                                          | 33.3                                          | 55.0                      | 28.0                        |
| **Socioeconomic index**        |                     |                                               |                                               |                           |                             |
| Manual                         | 40.1                | 33.6                                          | 31.7                                          | 45.0                      | 24.0                        |
| Nonmanual                      | 52.4                | 59.6                                          | 63.5                                          | 55.0                      | 67.0                        |
| Employer                       | 6.8                 | 5.9                                           | 3.2                                           | 0.0                       | 6.0                         |
| **Education**                  |                     |                                               |                                               |                           |                             |
| ≤9 years                       | 71.2                | 68.0                                          | 61.9                                          | 70.0                      | 55.0                        |
| 10-12 years                    | 7.8                 | 6.9                                           | 9.5                                           | 5.0                       | 6.0                         |
| University                     | 20.9                | 24.8                                          | 28.6                                          | 25.0                      | 39.0                        |
| **Married or cohabiting**      |                     |                                               |                                               |                           |                             |
| No                             | 32.1                | 33.4                                          | 41.3                                          | 15.0                      | 26.0                        |
| Yes                            | 67.9                | 66.6                                          | 58.7                                          | 85.0                      | 74.0                        |
| **Parity**                     |                     |                                               |                                               |                           |                             |
| 0                              | 12.0                | 13.9                                          | 15.9                                          | 5.0                       | 16.0                        |
| 1                              | 20.8                | 19.0                                          | 19.0                                          | 20.0                      | 18.0                        |
| 2                              | 41.0                | 45.1                                          | 47.6                                          | 45.0                      | 46.0                        |
| 3                              | 16.9                | 15.7                                          | 9.5                                           | 10.0                      | 14.0                        |
| ≥4                             | 5.6                 | 4.5                                           | 4.8                                           | 20.0                      | 3.0                         |
| Missing                        | 3.6                 | 1.9                                           | 3.2                                           | 0.0                       | 3.0                         |
| **Age at first childbirth**    |                     |                                               |                                               |                           |                             |
| ≤20                            | 17.5                | 16.0                                          | 19.0                                          | 20.0                      | 10.0                        |
| 21-25                          | 34.7                | 35.0                                          | 31.7                                          | 30.0                      | 35.0                        |
| 26-30                          | 23.4                | 23.5                                          | 19.0                                          | 35.0                      | 26.0                        |
| ≥30                            | 8.9                 | 9.7                                           | 11.1                                          | 10.0                      | 10.0                        |
| Nullipara                      | 12.0                | 13.9                                          | 15.9                                          | 5.0                       | 16.0                        |
| Missing                        | 3.6                 | 2.0                                           | 3.2                                           | 0.0                       | 3.0                         |
| **Mean age at menarche**       | 13.7                | 13.5                                          | 13.3                                          | 13.9                      | 13.3                        |
| **Ever use of oral contraceptives** |                   |                                               |                                               |                           |                             |
| No                             | 52.2                | 45.4                                          | 52.4                                          | 60.0                      | 44.0                        |
| Yes                            | 47.8                | 54.6                                          | 46.0                                          | 40.0                      | 56.0                        |
| **Menopausal status**          |                     |                                               |                                               |                           |                             |
| Pre                            | 22.2                | 29.5                                          | 27.0                                          | 20.0                      | 40.0                        |
| Peri/post                      | 77.8                | 70.5                                          | 73.0                                          | 80.0                      | 60.0                        |
| **Oophorectomy bilateral**     |                     |                                               |                                               |                           |                             |
| No                             | 98.5                | 98.5                                          | 98.4                                          | 95.0                      | 98.0                        |
| Yes                            | 1.5                 | 1.5                                           | 1.6                                           | 5.0                       | 2.0                         |
| **HRT**                        |                     |                                               |                                               |                           |                             |
| No                             | 81.1                | 73.6                                          | 73.0                                          | 70.0                      | 73.0                        |
| Yes                            | 18.5                | 26.2                                          | 25.4                                          | 30.0                      | 27.0                        |

(Continues)
Due to the different data collection periods, the ranking was carried out separately for cases diagnosed in the periods 1991-2004, 2005-2007 and 2008-2013. Histological grade was based on the Nottingham classification as described by Elston and Ellis.\textsuperscript{23} ER\textsuperscript{−} and PgR receptor status was defined as positive if >10% of nuclei were positive.\textsuperscript{21} HER2 receptor status was regarded as positive if in situ hybridization (ISH) was amplified or if ISH data was missing and immunohistochemistry (IHC) was graded as 3+. HER2 was regarded as negative if ISH was not amplified or if ISH data was missing and IHC was 0 or 1+. In tumors with 2+ in IHC analysis and no data from ISH, the HER2 variable was classified as missing.\textsuperscript{22}

\subsection*{2.6 Surrogate intrinsic subtypes}

A surrogate intrinsic subtype variable was created. Tumors were divided into Luminal A-like, Luminal B-like, HER2-positive, and triple-negative breast cancer (TNBC) according to the following local criteria within the south Swedish health care system\textsuperscript{24}: Luminal A-like was defined as all ER-positive and HER2-negative tumors with a histological grade 1 or histological grade 2 and low Ki67 or histological grade 2, an intermediate Ki67 and a positive PgR. Luminal B-like was defined as all ER-positive and HER2-negative tumors with a histological grade 3 or histological grade 2 and a high Ki67 or histological grade 2, an intermediate Ki67 and a negative PgR. Luminal B-like was defined as all ER-positive and HER2-negative tumors with a histological grade 3 or histological grade 2 and a high Ki67 or histological grade 2, an intermediate Ki67 and a negative PgR. All HER2-positive tumors were put in the HER2-positive category. TNBC was defined as all tumors with a negative ER and PgR expression as well as a negative HER2-expression.

\subsection*{2.7 Endpoint follow-up}

Mortality data, both overall and breast cancer-specific, was collected from the Swedish Cause of Death Registry, including deaths up until
| Mortality status and prognostic factors among cases in main survival analysis |
|-------------------------------------------------|
| Alive (n = 714) | Breast cancer death (n = 179) | Other death (n = 170) | Unknown/emigrated (n = 3) | Total (n = 1066) |
| Mean selenium ng/mL | 91.8 | 90.0 | 90.6 | 92.9 | 91.3 |
| Mean age at baseline | 54.4 | 58.8 | 61.9 | 52.5 | 56.3 |
| Mean age at diagnosis | 65.6 | 67.7 | 70.7 | 62.2 | 66.7 |
| Distant metastasis at diagnosis | |
| No | 95.8 | 83.8 | 91.2 | 100.0 | 93.1 |
| Yes | 0.0 | 7.8 | 0.0 | 0.0 | 1.3 |
| Missing | 4.2 | 8.4 | 8.8 | 0.0 | 5.6 |
| Tumor size | |
| ≤10.00 mm | 29.1 | 3.4 | 22.4 | 33.3 | 23.7 |
| 10.01-20.00 mm | 46.1 | 35.2 | 44.7 | 33.3 | 44.0 |
| 20.01-50.00 mm | 20.2 | 41.3 | 24.7 | 33.3 | 24.5 |
| >50.01 mm | 1.8 | 8.4 | 4.1 | 0.0 | 3.3 |
| Missing | 2.8 | 11.7 | 4.1 | 0.0 | 4.5 |
| Lymph node status | |
| Negative | 68.9 | 34.6 | 59.4 | 0.0 | 61.4 |
| Positive | 23.0 | 53.1 | 25.3 | 66.7 | 28.5 |
| Missing | 8.1 | 12.3 | 15.3 | 33.3 | 10.0 |
| Intrinsic subtype | |
| Luminal A-like | 45.2 | 19.0 | 40.6 | 66.7 | 40.2 |
| Luminal B-like | 16.7 | 23.5 | 20.6 | 0.0 | 18.4 |
| HER2+ | 6.9 | 10.1 | 7.1 | 0.0 | 7.4 |
| Triple negative | 6.0 | 9.5 | 4.7 | 0.0 | 6.4 |
| Missing | 25.2 | 38.0 | 27.1 | 33.3 | 27.7 |
| Histological grade | |
| Grade 1 | 28.3 | 8.4 | 25.3 | 66.7 | 24.6 |
| Grade 2 | 46.2 | 35.2 | 41.2 | 33.3 | 43.5 |
| Grade 3 | 19.0 | 41.3 | 26.5 | 0.0 | 23.9 |
| Missing | 6.4 | 15.1 | 7.1 | 0.0 | 8.0 |
| KI-67 | |
| Low | 31.9 | 19.6 | 24.1 | 66.7 | 28.7 |
| Intermediate | 22.0 | 21.2 | 29.4 | 0.0 | 23.0 |
| High | 18.1 | 31.3 | 24.7 | 0.0 | 21.3 |
| Missing | 28.0 | 27.9 | 21.8 | 33.3 | 27.0 |
| ER | |
| ≤10 | 9.0 | 12.8 | 7.1 | 33.3 | 9.4 |
| >10 | 80.4 | 62.0 | 81.8 | 66.7 | 77.5 |
| Missing | 10.6 | 25.1 | 11.2 | 0.0 | 13.1 |
| PgR | |
| ≤10 | 31.0 | 39.7 | 34.7 | 33.3 | 33.0 |
| >10 | 55.6 | 32.4 | 48.8 | 66.7 | 50.7 |
| Missing | 13.4 | 27.9 | 16.5 | 0.0 | 16.3 |
| HER2+ | |
| Negative | 76.1 | 60.9 | 73.5 | 66.7 | 73.1 |
| Positive | 6.9 | 10.1 | 7.1 | 0.0 | 7.4 |
| Missing | 17.1 | 29.1 | 19.4 | 33.3 | 19.5 |

Note: All data is presented as column percentage except on rows stated as mean. Missing data is not presented if missing <1%. 
| Tumor characteristics | Quartile<sup>a</sup> | Case/controls | OR (95% CI) | OR<sup>b</sup> (95% CI) |
|-----------------------|----------------------|---------------|-------------|---------------------|
| Invasive breast cancer with tumor material | 1 | 224/264 | 1.00 | 1.00 |
| | 2 | 229/255 | 1.06 (0.82-1.36) | 1.06 (0.82-1.38) |
| | 3 | 233/265 | 1.04 (0.81-1.33) | 1.06 (0.81-1.37) |
| | 4 | 200/270 | 0.87 (0.68-1.13) | 0.95 (0.73-1.24) |
| | Missing | 117/132 | 1.05 (0.77-1.42) | 1.06 (0.77-1.46) |
| All invasive breast cancer | 1 | 235/264 | 1.00 | 1.00 |
| | 2 | 244/255 | 1.08 (0.84-1.38) | 1.08 (0.84-1.40) |
| | 3 | 245/265 | 1.04 (0.81-1.33) | 1.05 (0.81-1.35) |
| | 4 | 219/270 | 0.91 (0.71-1.17) | 1.00 (0.77-1.30) |
| | Missing | 123/132 | 1.05 (0.77-1.42) | 1.04 (0.76-1.44) |
| Tumor size ≤20 mm | 1 | 148/264 | 1.00 | 1.00 |
| | 2 | 157/255 | 1.10 (0.83-1.46) | 1.09 (0.81-1.47) |
| | 3 | 162/265 | 1.09 (0.82-1.44) | 1.11 (0.83-1.50) |
| | 4 | 135/270 | 0.89 (0.67-1.19) | 0.97 (0.72-1.32) |
| Tumor size >20 mm | 1 | 67/264 | 1.00 | 1.00 |
| | 2 | 66/255 | 1.02 (0.70-1.49) | 0.95 (0.64-1.40) |
| | 3 | 60/265 | 0.89 (0.61-1.32) | 0.88 (0.59-1.31) |
| | 4 | 61/270 | 0.89 (0.61-1.31) | 0.91 (0.61-1.36) |
| Lymph node positive | 1 | 64/264 | 1.00 | 1.00 |
| | 2 | 68/255 | 1.10 (0.75-1.61) | 1.06 (0.71-1.58) |
| | 3 | 67/265 | 1.04 (0.71-1.53) | 1.09 (0.73-1.63) |
| | 4 | 60/270 | 0.92 (0.62-1.36) | 0.99 (0.66-1.50) |
| Lymph node negative | 1 | 138/264 | 1.00 | 1.00 |
| | 2 | 142/255 | 1.07 (0.80-1.43) | 1.06 (0.78-1.44) |
| | 3 | 146/265 | 1.05 (0.79-1.41) | 1.08 (0.80-1.46) |
| | 4 | 125/270 | 0.89 (0.66-1.19) | 0.95 (0.70-1.30) |
| Luminal A-like | 1 | 84/264 | 1.00 | 1.00 |
| | 2 | 104/255 | 1.28 (0.92-1.79) | 1.23 (0.86-1.75) |
| | 3 | 95/265 | 1.13 (0.80-1.58) | 1.16 (0.81-1.66) |
| | 4 | 90/270 | 1.05 (0.74-1.48) | 1.17 (0.81-1.67) |
| Luminal B-like | 1 | 49/264 | 1.00 | 1.00 |
| | 2 | 46/255 | 0.97 (0.63-1.51) | 0.94 (0.60-1.47) |
| | 3 | 42/265 | 0.85 (0.55-1.33) | 0.84 (0.53-1.33) |
| | 4 | 36/270 | 0.72 (0.45-1.14) | 0.75 (0.47-1.22) |
| TNBC | 1 | 15/264 | 1.00 | 1.00 |
| | 2 | 16/255 | 1.10 (0.54-2.28) | 1.06 (0.51-2.22) |
| | 3 | 16/265 | 1.06 (0.52-2.19) | 1.07 (0.51-2.24) |
| | 4 | 16/270 | 1.04 (0.51-2.15) | 1.07 (0.51-2.25) |
| HER2 + | 1 | 20/264 | 1.00 | 1.00 |
| | 2 | 19/255 | 0.98 (0.51-1.89) | 0.94 (0.48-1.83) |
| | 3 | 19/265 | 0.95 (0.49-1.81) | 1.00 (0.51-1.96) |
| | 4 | 15/270 | 0.73 (0.37-1.46) | 0.78 (0.38-1.59) |
| HER2− | 1 | 175/264 | 1.00 | 1.00 |
| | 2 | 193/255 | 1.13 (0.86-1.48) | 1.11 (0.84-1.48) |
| | 3 | 193/265 | 1.06 (0.81-1.39) | 1.07 (0.80-1.42) |
| | 4 | 162/270 | 0.91 (0.69-1.20) | 1.01 (0.75-1.35) |

<sup>a</sup>Serum selenium quartiles: 1: ≤81 ng/mL, 2: 81.01-90.50 ng/mL, 3: 90.51-100.00 ng/mL, 4: ≥100.01 ng/mL.

<sup>b</sup>Adjusted for age, menopausal status, socio-economic index, use of oral contraceptives, use of HRT, year and season the sample was taken.
December 31, 2016, by record-linking to the MDCS using the Swedish personal identity number. Breast cancer-specific mortality was defined as breast cancer being the underlying or a contributing factor to death. Three women included as cases had emigrated and the end of follow-up was set to the date of emigration.

2.8 | Statistical analyses

All cases and controls were ranked according to serum Se values, ties were assigned the mean rank and the upper cut-points were included in the quartiles. Cut points were set to quartiles (Q): Q1: ≤81 ng/mL, Q2:81.01-90.50 ng/mL, Q3:90.51-100.00 ng/mL and Q4: ≥100.01 ng/mL. For sensitivity analyses we also created quartiles based only on controls, rendering following cut-points: Q1: ≤81 ng/mL, Q2:81.01-90.75 ng/mL, Q3:90.76-101.00 ng/mL and Q4: ≥101.01 ng/mL.

Odds ratios (OR) with 95% confidence intervals of having a certain tumor characteristic were calculated using logistic regression, comparing each characteristic with controls based on Se quartile. Crude as well as adjusted analyses were performed, and factors with at least a five percentage points difference between cases and controls in Table 1 were adjusted for: age, socioeconomic index, year and the season the sample was taken, use of hormone replacement therapy (HRT), use of oral contraceptives and menopausal status at baseline. P-trend over quartiles was analyzed with the quartiles as a continuous variable and excluding the missing category of the same analysis. The 63 individuals with missing tumor material were excluded from these analyses except when calculating the odds of having any invasive breast cancer as compared to being selected as a control. Women with missing Se values were included in all analyses but treated as a separate group to detect any systematic differences. Missing values in baseline and tumor characteristics were also treated accordingly. Missing values were only reported in descriptive tables if they constituted at least 1% of the variable data. Regarding the risk analyses, the OR for the category including women with missing Se values was only reported for the risk of invasive cancer not for the risk of specific tumor characteristics.

All cases were followed from the date of diagnosis until the end of follow-up, and were censored if death or emigration occurred. The mean follow-up time was 10.3 years and the total number of person-years included in the follow-up amounted to 10 949. Kaplan-Meier curves were used to visually estimate relative mortality, both breast cancer-specific and overall, between Se quartiles. Cox proportional hazards models were used to calculate the relative mortality, crude as well as adjusted. Adjustments were made for known prognostic factors in breast cancer, and factors influencing Se in our sample: age at diagnosis, lymph node status, tumor size, intrinsic subtype, BMI, age at baseline, year and the season the sample was taken. A sensitivity analysis was performed when excluding cases diagnosed within the first year of follow-up.

A sensitivity analysis concerning mortality was also performed when excluding the 63 patients with invasive tumors with missing material, as well as another 27 patients with missing information concerning all of the following: ER, PgR, HER2, histological grade and Ki67. Another sensitivity analysis was performed on only those with complete information on intrinsic subtype.

In an additional analysis, the same methods as above were used to compare mortality between Se quartiles among the controls. Adjustment of the Cox proportional hazards model was made for factors with a theoretical effect on survival or Se status in our cohort: age, BMI, smoking, year and the season the sample was taken.

Finally, to validate the intrinsic subtype variable, mortality was compared between different quartiles. Additional factors included in these analyses were age at baseline, age at diagnosis, BMI, lymph node status and tumor size.

3 | RESULTS

In Table 1, we present descriptive data among included and excluded women. The mean serum Se level was 91.3 ng/mL among the controls, 91.2 ng/mL among the breast cancer cases with invasive material, and 92.2 ng/mL among the rest of the cases. The women with breast cancer were younger than the controls at baseline, more likely to have used oral contraceptives or HRT, and

![Figure 2](Kaplan-Meier curves of survival comparing selenium quartiles)
more likely to be obese but less likely to be manual workers. In Table S1, we present descriptive data for the different Se quartiles. The women with the highest Se levels (Q4) were older compared to those having the lowest Se levels (Q1). They were also more likely to have had their blood sample collected during the winter and to be postmenopausal, and were less likely to be current smokers or manual workers. In total, 262 women had missing Se values and they were older, less educated, less likely to have used oral contraceptives and were more likely to be obese. In Table 2, we present tumor characteristics among women with invasive breast cancer. Out of the 1066 women with primary invasive breast cancer, 714 were still alive, three had emigrated, 179 had died from breast cancer and 170 had died from other causes. The women who had died from breast cancer were older at baseline and diagnosis compared to those who were still alive. They were more likely to have larger tumors, positive lymph node status, distant metastasis at diagnosis, to have Luminal B-like, HER2+ or TNBC breast tumors. In Table S2 we present what treatment patients received. Those with breast cancer who had died from breast cancer had undergone mastectomy and chemotherapy to a higher extent compared to those who were still alive. As presented in Tables 3 and S3, there were no associations between Se and overall risk of invasive breast cancer or for any specific tumor characteristic or intrinsic subtype. The adjusted OR for invasive breast cancer in Q4 vs Q1 was 1.00 (0.77-1.30). Similarly, for lymph node-positive status the adjusted OR for Q4 vs Q1 was 0.99 (0.78-1.25) and for HER2-positive tumors 0.79 (0.61-1.01). We found no evidence of a trend over the Se quartiles for any tumor characteristics, for luminal B-like tumors \( P \)-trend was .19 (results for other characteristics not presented). We found no evidence of missing Se status being associated with a higher risk of invasive breast cancer, adjusted OR for Missing vs Q1 was 1.04 (0.76-1.44) and similarly no evidence of differences was seen when comparing Missing to Q1 within the different tumor characteristics and intrinsic subtypes (results not presented). In Table 4, we present hazard ratios (HR) and mortality for breast cancer-specific mortality and overall mortality per selenium quartile.

| Selenium quartile | Women (n) | Dead (n) | Total person years | Mortality/1000 | HR (95% CI) | HR* (95% CI) | HR** (95% CI) |
|-------------------|-----------|---------|--------------------|---------------|-------------|--------------|--------------|
| Breast cancer specific | 1 | 235 | 45 | 2248 | 20.01 | 1.00 | 1.00 |
|                   | 2 | 244 | 41 | 2376 | 17.26 | 0.86 (0.57-1.32) | 0.93 (0.60-1.45) | 0.93 (0.60-1.45) |
|                   | 3 | 245 | 36 | 2592 | 13.89 | 0.70 (0.45-1.09) | 0.66 (0.42-1.04) | 0.66 (0.42-1.04) |
|                   | 4 | 219 | 30 | 2321 | 12.92 | 0.66 (0.41-1.04) | 0.60 (0.37-0.98) | 0.60 (0.37-0.98) |
| Missing           | 123 | 27 | 1413 | 19.11 | 0.98 (0.61-1.58) | 0.95 (0.56-1.60) | 0.95 (0.56-1.60) |
| Overall           | 1 | 235 | 79 | 2248 | 35.14 | 1.00 | 1.00 |
|                   | 2 | 244 | 35 | 2376 | 31.62 | 0.94 (0.69-1.28) | 0.92 (0.67-1.27) | 0.92 (0.67-1.27) |
|                   | 3 | 245 | 36 | 2592 | 28.94 | 0.82 (0.60-1.13) | 0.74 (0.53-1.02) | 0.74 (0.53-1.02) |
|                   | 4 | 219 | 30 | 2321 | 25.42 | 0.71 (0.51-1.00) | 0.63 (0.44-0.89) | 0.63 (0.44-0.89) |
| Missing           | 123 | 58 | 1413 | 41.05 | 1.14 (0.82-1.61) | 1.04 (0.72-1.49) | 1.04 (0.72-1.49) |

aAdjusted for age at diagnosis, lymph node status, tumor size, intrinsic subtype, BMI, age at baseline, year and season the sample was taken.
bAdjusted for same as a but with all factors by themselves instead of intrinsic subtype (ER, PgR, HER2, Grade, Ki67).
true for HER2-positive tumors; HR 3.10 (1.74-5.53) and TNBC, HR 2.93 (1.63-5.28; Table S6).

In sensitivity analyses when using the quartiles based only on controls, similar differences in risk and mortality were seen in all analyses as when using quartiles based on both cases and controls. The adjusted OR for invasive breast cancer in Q4 vs Q1 among cases and controls was 1.03 (0.79-1.35) and the HR for breast cancer-specific mortality among cases was 0.59 (0.36-0.98).

4 | DISCUSSION

In the present study, we found evidence of lower mortality in women who had the highest prediagnostic serum Se levels. This was seen for both breast cancer-specific and overall mortality. However, no correlation between Se status and specific breast cancer characteristics or intrinsic subtype was found, suggesting that Se might be an independent prognostic factor for mortality in breast cancer patients.

Our study is the largest to date examining serum Se and breast cancer prognosis, with 1066 included cases and 179 breast cancer-specific deaths. The only previous study on serum Se and breast cancer mortality is a case-control study by Lubinski et al,5 including 546 cases with 58 deaths from breast cancer and serum Se levels after diagnosis. They found, similarly to us, that women with lower Se had worse survival. In three studies regarding dietary Se and breast cancer mortality, one found a strong correlation between a higher Se intake and a lower breast cancer-specific mortality while the other two studies reported weak evidence of such an association.4,5,25,26

There are several potential biological mechanisms that may link Se to breast cancer survival. Se exerts its effect on human health mainly through being incorporated in selenoproteins. Selenoproteins have a range of different functions in the body and interestingly, Charalabopoulos et al27 found that serum Se levels were lower among breast cancer cases compared to age-matched controls, and Se levels were more than twofold higher in the neoplastic breast tissue compared to healthy breast tissue, indicating that Se exerts a local effect. The anti-oxidant activity is emphasized as having a central role in selenoproteins’ possible protective mechanisms in tumor development and progression. The selenoproteins GPx-1 and TRx have been highlighted as two of the most important intracellular anti-oxidative proteins.7 GPx-1 has also been shown to increase DNA stability, although the exact pathway is not known.10 Specific polymorphisms in the GPx-1 gene have been shown both to be more common in breast cancer tissue compared to normal breast tissue as well as to reduce GPx activity and the effect of Se supplementation.9,28,29 Also, Se is important for normal thyroid function as well as for thyroid hormone conversion through the thyroid deiodinases.30 Thyroid hormones as well as thyroid hormone receptors have been linked to the development and prognosis of breast cancer.31-34 Thus, Se may play an indirect part in that pathway to affect breast cancer. Additionally, selenium binding protein-1 is involved in cell-growth regulation and has been found to be correlated with poor survival in breast cancer.35

All the above-mentioned mechanisms offer potential explanations for why Se could have a protective effect against breast cancer mortality among women with higher Se values. However, several of these mechanisms are not specific to breast cancer, which may explain why we also found the highest overall mortality among the controls with the lowest Se values in our study. One biological example is the loss of heterozygosity at a specific locus in the GPx-1 gene that is found in both lung cancer and breast cancer.9,36 Indeed, previous epidemiologic data suggest a link between low Se and an increased mortality in other cancer types than breast cancer, for example, esophageal and gastric cancer.37 Bleys et al38 reported from the NHANES III study with 13 887 included individuals that the overall risk of mortality and cancer mortality, but not cardiovascular death, was lower in the highest tertile of Se compared to the lowest. A Swedish study including 668 individuals between the ages of 70 to 80 years also found an increased overall mortality among individuals with the lowest serum Se compared to those with the highest levels. However, in contrast to the NHANES III it reported increased cardiovascular death, but found no differences in cancer death.39 Thus, other diseases could represent part of the increased overall mortality.

With the St Gallen consensus of 2013, classification of intrinsic breast cancer subtypes is a well-established model for assessing prognosis and deciding treatment.12 However, given the present cohort with data from 1991 onwards, information regarding intrinsic subtypes would not be available for a large proportion of the data set. We therefore constructed a surrogate intrinsic subtype variable based on the south Swedish health care region guidelines, to better present information regarding prognosis than by individual prognostic factors.24 Using histological grade as the first watershed instead of Ki67 is not the international standard but is supported by research findings for example, by Maisonneuve et al and Ehinger et al, which suggest that histological grade might be a better predictor of outcome than Ki67.30,41 A survival analysis comparing the different intrinsic subtypes confirmed the internal validity of the method where Luminal A-like tumors predicted superior survival as compared to Luminal B-like, HER2-positive and TNBC in the MDCS data set (data presented in Table S6).

In this present study, the Se levels were only measured once from a single blood sample, taken prediagnostically. The reported values could therefore have been affected by short-term Se intake prior to blood sampling, although some evidence supports that this is an adequate way of measuring Se status and that long-term ranking between individuals is maintained.32,43 The fact that we used controls based on incidence density matching in another study, and added new by randomization, adds two considerations. First, the controls selected by incidence density matching are probably more similar to the cases regarding risk factors than anyone selected at random. Also, the randomization process was performed only on the CV subcohort, only including individuals recruited during 1991-1994 while the cases were selected from the whole recruitment period of the MDMS, 1991-1996. This could present a skewness in the data, for example, due to different sample storage times between cases and controls. However, all our analyses were adjusted for year of inclusion, correcting any potential difference. The finding that the
controls were older than the cases at baseline should be commented on. One possible reason is that inclusion during later years was extended to younger women, and most controls were recruited during the first part of the study as they were selected from the CV cohort.44 The controls were defined following the end of follow-up, and the higher age at baseline seen among the controls may be an effect of competing mortality since women who were older at baseline had a higher risk of dying from causes other than breast cancer. However, age was adjusted for in the analyses and we do not believe that our selection of controls led to any strong bias. Descriptive data on cases and controls are presented in Table 1. The validity and completeness of the Swedish cancer registry, which was used in the present study to identify women with breast cancer, has been reported to be high and the Swedish cause of death registry has been reported as nearly complete.45,46 Regarding the cohort, has been reported to be high and the Swedish cause of death was used in the present study to identify women with breast cancer. We believe that our selection of controls led to any strong bias. However, age was adjusted for in the analyses and we do not believe that our selection of controls led to any strong bias.

Descriptive data on cases and controls are presented in Table 1. Lower overall and breast cancer-specific mortality was found among women in the highest Se quartile (≥100.01 ng/mL) as compared to the lowest quartile (≤81.00 ng/mL) in prediagnostically measured serum Se levels. There were no associations between Se levels and tumor characteristics, including intrinsic subtypes. This suggests that Se is associated with breast cancer survival, not related to classic prognostic factors.

5 CONCLUSION

Lower overall and breast cancer-specific mortality was found among women in the highest Se quartile (≥100.01 ng/mL) as compared to the lowest quartile (≤81.00 ng/mL) in prediagnostically measured serum Se levels. There were no associations between Se levels and tumor characteristics, including intrinsic subtypes. This suggests that Se is associated with breast cancer survival, not related to classic prognostic factors.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA ACCESSIBILITY

The datasets used and analyzed during the current study are available from the corresponding author on request.

ETHICS STATEMENT

Ethics approval for our study was granted by the Regional Ethics Board in Lund; DNR 2015/283. An individual consent form was signed by each participant in the MDCS-cohort at inclusion and an ad was placed in a local newspaper in 2015 to inform participants in the MDCS about the current study and how to withdraw consent.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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