Anticoagulants and breast cancer survival, a nationwide cohort study

Running title: Anticoagulants and breast cancer survival

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ABSTRACT

Background: Various components of the coagulation cascade have been linked to breast cancer (BrCa) progression. *In vivo* results suggest that anticoagulants possess anticancer properties, but there are virtually no studies in human populations. Our nationwide study explored the association between anticoagulant use and BrCa survival.

Methods: All anticoagulants used from 1995–2015 in women (n=73,170) diagnosed with invasive BrCa in Finland between 1995–2013 were identified from the national prescription database; women were identified from the Finnish Cancer Registry. Cox regressions were performed to analyze BrCa survival as a function of pre- and post-diagnostic anticoagulant use; analyses were conducted for different anticoagulant subtypes and overall. Models were adjusted for age, mammography screening, tumor clinical characteristics, comorbidities, statin use, antidiabetic use, and antihypertensive use. To control for immortal time bias, post-diagnostic anticoagulant use was analyzed as a time-dependent variable.

Results: At a median of 5.8 years after BrCa diagnosis, 10,900 (15%) women had died from BrCa. In total, 25,622 (35%) women had used anticoagulants during the study period. Post-diagnostic anticoagulant use increased the risk of BrCa death (HR=1.41, 95% CI 1.33-1.49). The risk was especially high for low-molecular weight heparin, although the effect disappeared in long-term users.

Conclusion: Anticoagulant use provides no clinical benefit for BrCa survival; however, the association between thrombosis and cancer might mask potential survival benefits.

Impact: Future pharmacoepidemiological studies should adjust for anticoagulant use. Research should focus on the use of new oral anticoagulants because these are rarely studied and might be associated with improved BrCa survival.

INTRODUCTION

It is well known that venous thromboembolism (VTE) is more common in patients with advanced cancer [1], including breast cancer, and that VTE is associated with a worse prognosis [2]. In addition to their anti-clotting properties *in vivo*, anticoagulants exhibit direct antitumor properties in breast cancer cells *in vitro* [3-4].

In recent experimental studies, the expression of protease activated receptors (PARs) 1 and 2 has been associated with breast cancer histology and tumor size [5]. PAR1 signaling is driven by thrombin; which is not only a crucial element in the coagulation cascade, but it also promotes breast cancer cell growth and invasion [6-7]. The PAR2 (coagulation factor II [thrombin] receptor-like 1) pathway, which is dependent on tissue factor, is another important element in the coagulation cascade and has been crucial in evoking angiogenesis during *in vivo* breast cancer experiments [8-9], possibly involving insulin-like growth factor [10]. Thus, it is tempting to speculate that anticoagulants may limit breast cancer growth, with this effect being mediated through the PAR signaling pathways. And, indeed, direct thrombin inhibitors have been shown to reduce breast cancer metastasis [11-13]. In addition, studies suggest that warfarin exerts anti-adhesion properties in breast cancer cells; thus, warfarin has been further postulated to have direct anti-metastatic properties, especially when combined with cimetidine [14]. Furthermore, heparins have been reported to delay tumor growth in breast cancer independent of their anticoagulant properties [15]. In particular, low-molecular weight heparin (LMWH) administration is associated with improved survival in patients with T3, T4, and M1 breast cancer [16] as classified in the international TNM classification of malignant tumors [TNM-classification by Union of International Cancer Control (UICC). Available at: https://www.uicc.org/resources/tnm]; however, the benefit for M1 patients has been challenged [17].

Few observational studies examine the association between breast cancer mortality and anticoagulant use. To the authors' knowledge, only one previous study has been published on this topic, and it was limited to warfarin use [18]. That study found no risk association for pre-diagnostic warfarin use, but an increased risk of breast cancer death was associated with post-diagnostic warfarin use in subjects who had not previously received the drug (HR 1.36, 95% CI 1.12-1.64). Notably, however, although the cohort included 16,523 breast cancer patients, only 400 participants (2.4%) had used warfarin. Because experimental studies with a variety of different anticoagulant drugs

show promising results, and because observational studies are limited, we conducted a retrospective cohort study to explore the association between breast cancer survival and anticoagulant drug use in Finland between 1995–2015.

MATERIALS AND METHODS

Study cohort

All diagnoses of female breast cancer in Finland between 1995–2013 were obtained from the national comprehensive Finnish Cancer Registry (as described in previous publications) [19-20]. Information on each patient's primary therapy was also obtained from the Finnish Cancer Registry. After excluding 4,504 cases of carcinoma *in situ* with no later diagnosis of invasive carcinoma, a total of 73,170 women were left in the final cohort.

Death-related information was obtained from the national death certificate registry of Statistics Finland, which assigns official causes of death based on mandatory death certificates. The available information included the death date and the immediate, primary, and contributory causes of death. Only deaths with breast cancer (ICD-10: C50) listed as the primary cause were regarded as breast cancer deaths.

A mammography screening program was started in Finland in 1987 and became government-mandated in 1992. Between 1992–2009, the program had an average national coverage rate of 86.7% [20]; invitations to participate were sent at two-year intervals [21]. By linking our study cohort to the national Mass Screening Registry maintained by the Finnish Cancer Registry, we were able to obtain data on cohort members' participation in the mammography screening program and the number of screening rounds attended by each.

We obtained information on conditions that are major indications for anticoagulant use from the national Care Register for Health Care (HILMO), which is maintained by the National Institute for Health and Welfare; the relevant conditions included pulmonary embolism (ICD-10: I26.0, I26.9), venous thromboembolism (ICD-10: I82.0-82.9), and atrial fibrillation (ICD-10: I48). HILMO covers all public hospitals in Finland and records all diagnoses from in- and outpatient visits. We examined HILMO data for diagnoses made between 1995–2013. Diagnoses made during primary care visits are not reported in this database. Charlson comorbidity scores [22] were calculated based on diagnoses recorded in the HILMO database during the follow-up timeframe.

Information on anticoagulant usage

In order to obtain information on anticoagulant use, the study cohort was linked to the national medication reimbursement database maintained by the Social Insurance Institution (SII) of Finland. As a part of the national health insurance program that covers all Finnish citizens, SII provides reimbursement for physician-prescribed drugs [23]. In Finland, all citizens of every age are eligible for medication reimbursements. The Government-directed Pharmaceutical Pricing Board (HILA) decides which medications are eligible for reimbursement; most physician-prescribed drugs used for the treatment of disease are covered. During the study period, drug reimbursements ranged from 35% to 100%, depending on condition severity [Finnish Statistics on Medicines. Available at: https://www.kela.fi/documents/10180/1889281/SLT+2013_net.pdf/0758ba68-1886-4b69-bdb6-b7566e9daa2c]. The reimbursement system began in 1970 [History of Medication reimbursements in Finland. Available at: https://www.kela.fi/web/en/operations-history]. The study cohort was linked to the reimbursement database using the unique personal identity code assigned to all residents of Finland. All anticoagulant drugs are physician-prescribed in Finland; thus, all reimbursements in outpatient settings are recorded in the database However, drugs used during inpatient hospital visits are not recorded in the database.

All 14 anticoagulant drugs used in an outpatient setting during 1995–2015 were identified based on their Anatomical-Therapeutic-Chemical codes. The identified drugs included two vitamin K antagonists (warfarin [B01AA03] and phenindione [B01AA02]), three heparins (dalteparin [B01AB04], enoxaparin [B01AB06], and tinzaparin [B01AB10]), four platelet aggregation inhibitors (clopidogrel [B01AC04], dipyridamole [B01AC30], iloprost [B01AC11], and ticlopidine [B01AC05]), two direct thrombin inhibitors (dabigatran [B01AE07] and ximelagatran [B01AE05]), and three factor Xa inhibitors (rivaroxaban [B01AX06], apixaban [B01AF02], and fondaparinux [B01AX05]). We also obtained information on the use of cholesterol-lowering drugs, antihypertensive drugs, and antidiabetic drugs (Supplementary Table 1) that may influence breast cancer survival [24-26].

Statistical analysis

Cox proportional hazard regressions were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer death. The follow-up timeframe started at breast cancer diagnosis and continued until death, emigration, or January 1, 2016 (whichever came first).

During analysis, we used two different model adjustments. First, the Cox regression model was adjusted for age only. Then, the Cox regression model was adjusted for age, breast cancer spreading at diagnosis (localized, locally advanced, or metastatic; information available for 85.6% of cases), primary treatment (available for 99.6% of cases), Charlson comorbidity score, number of mammography screening rounds attended, diabetes diagnosis, antihypertensive drug use, statin use, hormonal treatment, tumor histology (ductal invasive, lobular invasive, or other invasive; available for 99.9% of cases), and indications for anticoagulant use.

We performed four separate analyses related to the pre- and post-diagnostic use of anticoagulants: 1) users of any anticoagulant drug compared to nonusers; 2) warfarin users compared to anticoagulant nonusers; 3) LMWH users compared to anticoagulant nonusers; 4) warfarin users compared to users of other anticoagulants.

We calculated each woman's total annual anticoagulant use; this amount was calculated for each calendar year and separately for the pre- and post-diagnostic periods. Doses between different anticoagulants were standardized by dividing the annual total milligram amount by the standard defined daily dose (DDD) listed by the World Health Organization [WHO ATC/DDD index 2016. Available at: http://www.whocc.no/atc_ddd_index/]. Each year with a registered anticoagulant purchase was considered a year of usage, regardless of the purchase amount. The intensity of a woman's anticoagulant use was estimated by dividing the sum of all her annual doses by the number of years she used anticoagulants.

Pre-diagnostic anticoagulant use was analyzed as a time-fixed variable. Cumulative use from 1995 onward was stratified by tertiles. Post-diagnostic use was analyzed as a time-dependent variable. Both user status and cumulative use were updated separately for each year of follow-up, beginning from the year of breast cancer diagnosis. Women were categorized as nonusers until their first recorded anticoagulant purchase; they maintained

their user status for each year with recorded anticoagulant purchases. Women who discontinued anticoagulant use were categorized as previous users. Women remained categorized as previous users unless anticoagulant use was restarted at another point during the follow-up timeframe; their status was then changed back to current user. User status was updated annually. Similarly, cumulative amount, use duration, and average annual dose were updated for each follow-up year. In the main analysis, we included user status and cumulative duration of use. Cumulative amounts and usage intensities were included in additional analyses.

In the analysis comparing warfarin users to anticoagulant nonusers, women were categorized as warfarin users each year with recorded purchases of warfarin, even if other anticoagulants were also used during that year. A similar methodology was used to compare LMWH users to anticoagulant nonusers. In the analysis comparing warfarin users to users of other anticoagulants, women were considered users of other anticoagulant drugs if they had recorded purchases of an anticoagulant other than warfarin.

The long-term impact of post-diagnostic anticoagulant use was assessed in a lag-time analysis. More specifically, anticoagulant exposure was lagged 1 to 3 years forward from the actual year of usage (i.e., its effect was ignored for the first 1 to 3 years following initial exposure). This approach is crucial because anticoagulants are used to treat breast cancer symptoms, and thus anticoagulant use may be associated with an elevated risk of breast cancer death in the short term. This phenomenon is known as protopathic bias [27].

We performed subgroup analyses for post-diagnostic anticoagulant use stratified by clinical and background characteristics. In the subgroup analyses, Charlson comorbidity scores were stratified into 3 groups (0 points, 1 point, and 2 or more points). We tested the statistical significance of each background variable's effect modification; this was done by adding an interaction term between anticoagulant use and the tested background variable to the Cox regression analysis and determining whether or not inclusion of the interaction term improved model fit. A *P* value <0.05 was considered statistically significant.

To evaluate the impact of advanced disease on breast cancer survival, we performed a sensitivity analysis in which patients with metastases or unknown spreading at diagnosis were excluded. The role that the timing of breast

cancer diagnosis plays in survival was determined by including diagnosis year in the Cox regression model during sensitivity analysis.

For 5,412 women in the Tampere University Hospital area, we also obtained information on hormone receptor status (estrogen receptor [ER] and progesterone receptor [PR]) and HER2 (human epidermal growth factor receptor 2) receptor status during a woman's first breast cancer diagnosis between 1995–2013. Adjusting for the same control variables included in the main analysis, we conducted multivariate analyses relating anticoagulant use, warfarin use, and LMWH use to breast cancer survival; this allowed us to evaluate the prognostic value of these factors.

All statistical analyses were carried out using IBM SPSS Statistics 22.0. All statistical tests were two-sided.

RESULTS

Population characteristics

At a median follow-up of 5.8 years after breast cancer diagnosis, a total of 22,520 (31%) women had died; for 10,900 (15%) of those women, breast cancer was the primary cause of death. A total of 25,622 (35%) women had used anticoagulants between 1995–2015. Of those women, 10,594 (41%) had used warfarin, and 14,224 (56%) had been prescribed LMWH. Compared to nonusers, women who used anticoagulants were older at breast cancer diagnosis (median age 66 and 59 years, respectively). The median age at breast cancer diagnosis among warfarin users was 72. The pattern of tumor spreading and tumor histology at diagnosis were similar in all groups. Curative-intent surgery was more common among anticoagulant nonusers than users (66.8% and 33.3%, respectively). The distribution of each background variable is presented in Table 1.

Breast cancer survival in relation to pre-diagnostic use of anticoagulants

When comparing pre-diagnostic users of any anticoagulant to nonusers, there was no significant risk association for breast cancer survival (multivariable HR=1.00, 95% CI 0.93-1.07) (Table 2). Short-term use (≤ 1 year) was associated with a statistically significant risk decrease in both the age- and multivariable-adjusted analyses. In the analysis

comparing pre-diagnostic warfarin users to anticoagulant nonusers, there was no statistically significant risk association (overall multivariable HR=0.97, 95% CI 0.89-1.06) (Table 2). When comparing pre-diagnostic LMWH users to anticoagulant nonusers, there was a statistically significant risk decrease (multivariable HR=0.71, 95% CI 0.59-0.85). This risk decrease was limited to short-term LMWH users. Table 2 shows results subdivided by the pre-diagnostic use of different anticoagulants. Results stratified by the amount and intensity of anticoagulant use are presented in Supplementary Table 2. The results were similar to those stratified by duration of use.

Breast cancer survival in relation to post-diagnostic use of anticoagulants

When comparing breast cancer survival in post-diagnostic anticoagulant users and nonusers, the risk was significantly increased for both current users (HR=1.41, 95% CI 1.33-1.49) and previous users (HR=1.30, 95% CI 1.22-1.39) (Table 3). With the exception of those using anticoagulants for 4 years or more (multivariable HR=1.06, 95% 0.96-1.19), the risk associations were similar in the analyses stratified by duration of anticoagulant use. In the lag-time analysis, the increased risk was attenuated but remained significant in all cases, except among short-term users and previous users.

When comparing warfarin users to anticoagulant nonusers, the risk of breast cancer death was elevated among previous users and current users (HR=1.58, 95%CI 1.45-1.72 and HR=1.10, 95% CI 1.02-1.19, respectively) (Table 3). However, the risk increase disappeared for previous users in the lag-time analyses. In contrast, the risk of breast cancer death remained practically unchanged for current warfarin users.

When compared to anticoagulant nonusers, current LMWH users were at a significantly higher risk of breast cancer death (HR=2.62, 95% CI 2.42-2.83); but previous LMWH use was not associated with any risk increase. The risk increase was limited to long-term users (≥ 2 years) (HR=3.39, 95% CI 3.00-3.82). When LMWH use was lagged for 3 years in the lag-time analysis, the observed risk increases were attenuated and disappeared. The results for post-diagnostic use of any anticoagulant stratified by use amount and intensity are presented in Supplementary Table 3.

Subgroup analysis

After a Bonferroni correction for multiple testing, age at diagnosis, number of mammography screens, tumor spreading at diagnosis, primary breast cancer treatment, antihypertensive use, and indications for anticoagulant use all significantly modified the breast cancer mortality associations for anticoagulants in general (Figure 1). The risk increase among anticoagulant users was only significant for participants diagnosed with breast cancer at age 62 or younger (p value for interaction term <0.001). Having metastatic disease at diagnosis, nonsurgical primary treatment (p<0.001 for each), and histology other than ductal or lobular carcinoma (p=0.002) removed the risk association. Simultaneous use of antihypertensive drugs and anticoagulants ameliorated the risk increase (p<0.001), except among LMWH users; in LMWH users, the risk increase was stronger when antihypertensive drugs had been used simultaneously (p=0.002). When patients had not received a mammography screening, there was no elevated risk associated with anticoagulant use (p<0.001). Furthermore, the risk of breast cancer death was higher among anticoagulant users with a recorded diagnosis of atrial fibrillation (p=0.002). When comparing women who had initiated anticoagulant use before breast cancer diagnosis to those who used anticoagulants after diagnosis, the risk of breast cancer death was significantly lower among the (p=0.034) (Figure 1). This effect modification was generally similar for all anticoagulant subtypes (not shown).

Sensitivity analysis

After excluding patients with metastatic disease or unknown spreading at diagnosis, the risk of breast cancer death increased slightly among anticoagulant users (multivariable HR=1.67, 95% CI 1.56-1.79) compared to those in the main analysis. A similar association was observed for other anticoagulant user categories, except for previous LMWH users. However, the effect was attenuated or missing for some categories in the lag-time analyses (Table 4). Including the timing of breast cancer diagnosis (year of diagnosis) in the sensitivity analysis of the Cox regression had virtually no effect on results (Supplementary Table 4).

Among 5,412 women with data on ER, PR, and HER2 status, the risk of breast cancer death was elevated among anticoagulant users compared to nonusers. The risk was also elevated in LMWH users compared to anticoagulant nonusers independent of ER or PR status; however, this elevation was not evident in patients receiving warfarin. On the other hand, among women with triple negative disease, anticoagulant use (both overall and for each subtype) was associated with a significantly elevated risk of breast cancer death (Supplementary Table 5).

We also compared the risk of breast cancer death between warfarin-only and LMWH-only users and found no risk association in general (HR=0.94, 95% CI 0.85-1.05). However, when women were stratified by treatment duration, those who used these medications for 2 years or less had a 1.3-fold risk of breast cancer death. In contrast, use of these medications for 3 years or more was associated with 0.6-fold risk of breast cancer death (not shown).

In an additional sensitivity analysis, we restricted the cohort to those who underwent curative-intent surgery. In this analysis, the risk of breast cancer death was elevated 1.7-fold in anticoagulant users compared to nonusers. The risk increase was attenuated in the lag-time analysis (not shown).

DISCUSSION

According to the literature, there are several specific pathways that may link the coagulation cascade to breast cancer growth and progression (e.g., via angiogenesis, which is vital for tumor cells) [5-10]. Several anticoagulant subtypes (i.e., warfarin, direct thrombin inhibitors, and LMWH) are postulated to improve breast cancer survival by affecting the growth and spread of tumors [11-16]. Therefore, there has been increasing interest in clarifying the association between anticoagulant use and breast cancer survival. In this epidemiological study, we did not observe a positive effect of anticoagulant use on survival; this was true when examining anticoagulants in general and by subtype.

To the authors' knowledge, only one previous observational study [17] has evaluated the association between breast cancer survival and anticoagulant use. That study was limited to warfarin users; moreover, though the study included a relatively large number of breast cancer cases, its number of warfarin users was low (400). That study reported an approximately 1.4-fold increase in breast cancer mortality among warfarin users. While that result is similar to those described here, that study did not examine other anticoagulant drugs. Furthermore, that study did not evaluate the impact that the timing of anticoagulant use has on the survival of breast cancer patients.

Our study revealed that, although post-diagnostic anticoagulant use was associated with an increased risk of breast cancer death, using anticoagulants for more than 4 years eliminated that risk. The risk association was particularly elevated among women who used LMWH for 2 years or more (3.4-fold risk of breast cancer death). However, the increase in risk appeared limited to active LMWH users; it did not persist when LMWH use was terminated. Using warfarin for 2 years or less after breast cancer diagnosis was also associated with an increased risk of breast cancer death. The risk increase was more significant for previous warfarin use than for current use. In a supplemental analysis, the risk of breast cancer death was significantly lower in warfarin users compared to women being treated with other anticoagulant drugs. However, this likely reflects the high risk associated with LMWH use. For LMWH, the risk increase was most likely attributable to reverse causation by terminal-phase breast cancer because LMWH is the drug of choice for all VTE treatment, including VTEs caused by advanced cancer. Because the risk association between anticoagulant use and breast cancer death was attenuated or eliminated in lag-time analyses, the relationship is not causal. In addition, no risk association was observed between pre-diagnostic anticoagulant use and breast cancer death. Furthermore, women initiating anticoagulant therapy after breast cancer diagnosis were at a higher risk of dying from breast cancer than those who became anticoagulant users prior to diagnosis.

In the analysis evaluating the risk associated with pre-diagnostic anticoagulant use, we found that low-dose, short-term use of LMWH was associated with a reduced risk of breast cancer death. This association is unlikely to be causal because no such effect was observed with long-term use. Therefore, our study does not support the theory postulated following previous *in vivo* and *in vitro* experiments that LMWH use improves survival [15-16].

In lag-time analyses evaluating the impact that the timing of anticoagulant use plays, the risk increase observed in the main analysis was attenuated but remained elevated. For LMWH users, the timing of anticoagulant use reduced the risk of breast cancer death; however, among women who had used LMWH for 2 years or more, the risk of death from breast cancer remained nearly double that of nonusers. Risk associations were greatly modified by having an indication for anticoagulant use. Unfortunately, however, this finding is less conclusive because data on indications for anticoagulant use in primary healthcare settings were not available. The increased risk of breast cancer death seems limited to short-term use because thromboembolic events are more common in patients with advanced

cancer [1] and are associated with a poorer prognosis [2]. However, thrombosis could be a long-term risk factor because the risk increase did not disappear in the lag-time analysis.

According to our subgroup analysis, the risk of breast cancer death was particularly high for those who had a local or locally advanced disease. An elevated risk of breast cancer death was also observed among those diagnosed with atrial fibrillation. Because the risk of cancer-induced thrombosis can be assumed to be lowest for those with atrial fibrillation, this finding supports the theory that thrombosis promotes cancer progression and worsens patient prognosis (rather than the reverse). On the other hand, compared to exclusively post-diagnostic anticoagulant users, those individuals who were both pre-diagnostic and post-diagnostic users had a lower risk of breast cancer death. In either case, the use of anticoagulants was not associated with an elevated risk of breast cancer death.

This study has several strengths. The sample includes all breast cancer cases occurring in a nationwide population over a period of 19 years (n=73,170), thereby minimizing selection bias. We believe that this is the largest and most comprehensive epidemiological study on breast cancer survival in anticoagulant users. Moreover, we had detailed information on tumor spread and histology at the time of diagnosis. We also had comprehensive data on anticoagulant use, including the timing of anticoagulant use. Furthermore, it was possible to stratify cohort members according to anticoagulant subtype, usage amount, duration of use, and intensity of use. The analyses were adjusted for multiple background variables to minimize bias. In a subpopulation of our cohort, we were able to evaluate the effect that important prognostic factors, such as ER, PR, and HER2 status, have on breast cancer survival.

This study also had some limitations. We had no information on smoking habits, but smoking has been associated with worse breast cancer prognoses [28]. We had no data on hormone replacement therapy or body mass index. Similarly, clinicians report only the most severe cases of obesity to health registries; as such, information related to obesity was limited. In addition, we did not have data on socioeconomic factors that may influence breast cancer survival [29]. We did not have data on anticoagulant use prior to 1995, which limits our ability to evaluate long-term risk associations. Moreover, we did not have information on indications for anticoagulant use identified during primary healthcare visits; without this data we could not fully evaluate the effect that indications for anticoagulant use have on breast cancer survival. Furthermore, our data did not include anticoagulants used during hospital

inpatient visits; therefore, some exposure misclassification is possible. However, we consider this to be a minor concern because indications for anticoagulant use other than thromboprophylaxis would continue after hospital discharge; thus, those indications would be captured in our anticoagulant use data. Finally, we did not have data on mammography screenings performed outside the national screening program, and this could be a source of healthy user bias.

For clinicians, the primary takeaway of this study is that anticoagulants provide no benefit for cancer control, even though they are useful in the treatment of thrombosis. Our study does not find any support for the prophylactic use of such potentially dangerous medications. Moreover, because our results suggest that anticoagulant use is correlated with cancer death, epidemiologists should adjust for anticoagulant use in future studies. Despite promising results from previous *in vitro* and *in vivo* studies, bioscience researchers should be aware that the positive effects of anticoagulant use on cancer survival are not evident at the population level.

Conclusion

General anticoagulant use, warfarin use, and LMWH use confer no clinical benefits against breast cancer. In fact, the risk of breast cancer death is increased for post-diagnostic anticoagulant users. Among pre-diagnostic users, the low-dose, short-term use of LMWH is associated with improved survival; however, other patterns of LMWH use predict a risk of death similar to that of nonusers. It is possible that the association between thrombosis and cancer masks some potential survival benefit. Future studies should focus on determining whether the administration of newer oral anticoagulants is associated with breast cancer survival; direct thrombin inhibitors, such as dabigatran, have been reported to reduce breast cancer metastasis [7-9].

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REFERENCES

1. Cronin-Fenton DP, Sondergaard F, Pedersen LA, et al (2010) Hospitalisation for venous thromboembolism in cancer patients and the general population: a population-based cohort study in Denmark, 1997-2006. Br J Cancer 103: 947-953.
2. Prandoni P, Lensing AW, Prins MR (1997) The natural history of deep-vein thrombosis. Semin Thromb Hemost 23: 185-188.
3. DeFeo K, Hayes C, Chernick M et al (2010) Use of dabigatran etexilate to reduce breast cancer progression. Cancer Biol Ther 10:1001-1008.
4. Schulze EB, Hedley BD, Goodale D et al (2008) The thrombin inhibitor Argatroban reduces breast cancer malignancy and metastasis via osteopontin-dependent and osteopontin-independent mechanisms. Breast Cancer Res Treat 112:243-254
5. Rydén L, Grabau D, Schaffner F et al (2010) Evidence for tissue factor phosphorylation and its correlation with protease-activated receptor expression and the prognosis of primary breast cancer. Int J Cancer 126:2330-2340. DOI:http://dx.doi.org/10.1002/ijc.24921
6. Ohshiro K, Bui-Nguyen TM, Divijendra Natha RS et al (2012) Thrombin stimulation of inflammatory breast cancer cells leads to aggressiveness via the EGFR-PAR1-Pak1 pathway. Int J Biol Markers 27:e305-13. DOI:http://dx.doi.org/10.5301/JBM.2012.10437
7. Arora P, Cuevas BD, Russo A et al (2008) Persistent transactivation of EGFR and ErbB2/HER2 by protease-activated receptor-1 promotes breast carcinoma cell invasion. Oncogene 27:4434-4445. DOI:http://dx.doi.org/10.1038/onc.2008.84
8. Versteeg HH, Schaffner F, Kerver M et al (2008) Protease-activated receptor (PAR) 2, but not PAR1, signaling promotes the development of mammary adenocarcinoma in polyoma middle T mice. Cancer Res 68:7219-7227. DOI:http://dx.doi.org/10.1158/0008-5472.CAN-08-0419
9. Schaffner F, Versteeg HH, Schillert A et al (2010) Cooperation of tissue factor cytoplasmic domain and PAR2 signaling in breast cancer development. Blood 116:6106-6113. DOI:http://dx.doi.org/10.1182/blood-2010-06-289314

10. Åberg M, Eriksson O, Mokhtari D et al (2014) Tissue factor/factor VIIa induces cell survival and gene transcription by transactivation of the insulin-like growth factor 1 receptor. Thromb Haemost 111:748-760

11. DeFeo K, Hayes C, Chernick M et al (2010) Use of dabigatran etexilate to reduce breast cancer progression. Cancer Biol Ther 10:1001-1008. DOI:http://dx.doi.org/10.4161/cbt.10.10.13236

12. Schulze EB, Hedley BD, Goodale D et al (2008) The thrombin inhibitor Argatroban reduces breast cancer malignancy and metastasis via osteopontin-dependent and osteopontin-independent mechanisms. Breast Cancer Res Treat 112:243-254

13. Asanuma K, Wakabayashi H, Okamoto T et al (2013) The thrombin inhibitor, argatroban, inhibits breast cancer metastasis to bone. Breast Cancer 20:241-246. DOI:http://dx.doi.org/10.1007/s12282-012-0334-5

14. Bobek V, Boubelik M, Kovarik J et al (2003) Inhibition of adhesion breast cancer cells by anticoagulant drugs and cimetidine. Neoplasma 50:148-151

15. Fluhr H, Seitz T, Zygmunt M (2013) Heparins modulate the IFN-gamma-induced production of chemokines in human breast cancer cells. Breast Cancer Res Treat 137:109-118. DOI:https://dx.doi.org/10.1007/s10549-012-2334-8

16. Nagy Z, Turcsik V, Blaskó G (2009) The effect of LMWH (Nadroparin) on tumor progression. Pathol Oncol Res 15:689-692. DOI:http://dx.doi.org/10.1007/s12253-009-9204-7

17. Haas SK, Freund M, Heigener D et al (2012) Low-molecular-weight heparin versus placebo for the prevention of venous thromboembolism in metastatic breast cancer or stage III/IV lung cancer. Clin Appl Thromb Hemost 18:159-165. DOI:http://dx.doi.org/10.1177/1076029611433769

18. O'Rorke MA, Murray LJ, Hughes CM et al (2015) The effect of warfarin therapy on breast, colorectal, lung, and prostate cancer survival: a population-based cohort study using the Clinical Practice Research Datalink. Cancer Causes Control 26:355-366. DOI:http://dx.doi.org/10.1007/s10552-014-0511-2

19. Murto MO, Artama M, Pukkala E et al (2018) Breast cancer extent and survival among diabetic women in a Finnish nationwide cohort study. International Journal of Cancer. DOI: https://dx.doi.org/10.1002/ijc.31250

20. Sarkeala T, Näveri T, Malila N et al (2013) Performance of population-based breast cancer screening in Finland in 1992-2009. Finnish Med J 68: 225–231.

21. Pukkala E, Engholm G, Højsgaard Schmidt LK et al (2018) Nordic Cancer Registries - an overview of their procedures and data comparability. Acta Oncol 57:440-455. DOI:https://dx.doi.org/10.1080/0284186X.2017.1407039

22. Charlson ME, Pompei P, Ales KL et al (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40:373-383

23. Martikainen J, Rajaniemi S. Drug reimbursement systems in EU Member States, Iceland and Norway**.** Helsinki: The Social Insurance Institution, Finland, Social security and health reports 54, 2002. Available at: https://helda.helsinki.fi/handle/10138/13

24. Murtola TJ, Visvanathan K, Artama M et al (2014) Statin use and breast cancer survival: a nationwide cohort study from Finland. PLoS ONE 9:e110231. DOI:http://dx.doi.org/10.1371/journal.pone.0110231

25. Raimondi S, Botteri E, Munzone E et al (2016) Use of beta-blockers, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers and breast cancer survival: Systematic review and meta-analysis. Int J Cancer 139:212-219. DOI:http://dx.doi.org/10.1002/ijc.30062

26. He X, Esteva FJ, Ensor J et al (2012) Metformin and thiazolidinediones are associated with improved breast cancer-specific survival of diabetic women with HER2+ breast cancer. Ann Oncol 23:1771-1780. DOI:http://dx.doi.org/10.1093/annonc/mdr534

27. Horwitz RI, Feinstein AR (1980): The problem of "protopathic bias" in case-control studies. Am J Med. 1980, 68 (2): 255-8.

28. Berube S, Lemieux J, Moore L et al (2014) Smoking at time of diagnosis and breast cancer-specific survival: new findings and systematic review with meta-analysis. Breast Cancer Res 16:R42. DOI:http://dx.doi.org/10.1186/bcr3646

29. Larsen SB, Kroman N, Ibfelt EH et al (2015) Influence of metabolic indicators, smoking, alcohol and socioeconomic position on mortality after breast cancer. Acta Oncol 54:780-788. DOI:http://dx.doi.org/10.3109/0284186X.2014.9987

Table 1 Population characteristis of the study population stratified by anticoagulant usage during 1995-2015.

		Anticoagulant usage		Type of anticoagulant	
		No	Yes	Warfarin users*	LMWH users*
n of women in the study population		47,548	25,622	10,594	14,224
Median year of breast cancer diagnosis		2005	2006	2004	2007
Breast cancer deaths		8,085 (17.0%)	2,815 (11.0%)	1,404 (13.3%)	1,312 (9.2%)
All deaths		14,418 (30.3%)	8,102 (31.6%)	4,531 (42.3%)	2,961 (20.8%)
Median age at diagnosis (years)		59	66	72	62
Median follow-up after breast cancer diagnosis (years)		5.7	6.0	6.3	5.5
Median follow-up from diagnosis to breast cancer death (years)		2.9	3.8	3.5	4.7
Age at diagnosis in 2 groups					
	≤62 years	28,546 (60.0%)	10,359 (40.4%)	2,628 (24.8%)	7,605 (53.5%)
	>62 years	19,002 (40.0%)	15,263 (59.6%)	7,966 (75.2%)	6,619 (46.5%)
Tumor spreading at diagnosis					
	Localized	23,782 (50.0%)	12,854 (50.2%)	5,326 (50.3%)	6,943 (48.8%)
	Locally advanced	15,569 (32.7%)	8,474 (33.1%)	3,407 (32.2%)	5,040 (35.4%)
	Metastatic	4,071 (8.6%)	2,054 (8.2%)	855 (8.1%)	1,182 (8.3%)
	Unknown	4,126 (8.7%)	2,240 (8.7%)	1,006 (9.5%)	1,059 (7.4%)
Tumor histology					
	Invasive ductal	35,930 (75.6%)	19,118 (74.6%)	7,800 (73.6%)	10,700 (75.2%)
	Invasive lobular	7,785 (16.4%)	4,461 (17.4%)	1,805 (17.0%)	2,581 (18.1%)
	Other invasive	3,801 (8.0%)	2,013 (7.9%)	980 (9.3%)	923 (6.5%)
	Unknown	32 (0.06%)	30 (0.1%)	9 (0.08%)	20 (0.1%)
Primary therapy					
	Curative-intent surgery	31,748 (66.8%)	8,539 (33.3%)	6,939 (65.5%)	9,630 (67.7%)

	Other/unknown	15,800 (33.2%)	17,083 (66.7%)	3,655 (34.5%)	4,594 (32.2%)
Charlson co-morbidity score (points)					
	0	37,173 (78.2%)	17,221 (67.2%)	6,917 (65.3%)	9,299 (65.4%)
	1	1,783 (3.7%)	1,756 (6.9%)	746 (7.0%)	948 (6.7%)
	2 or more	8,592 (18.1%)	6,645 (25.9%)	2,931 (27.7%)	3,977 (28.0%)
Mammography screening participation (n)					
	0	18,595 (39.1%)	12,185 (47.6%)	6,216 (58.7%)	5,357 (37.7%)
	1-3	14,225 (29.9%)	7,021 (27.4%)	2,551 (24.1%)	4,432 (31.2%)
	4 or more	14,728 (31.0%)	6,416 (25.0%)	1,827 (17.2%)	4,425 (31.1%)
Obesity**					
	Yes	193 (0.4%)	234 (0.9%)	102 (1.0%)	154 (1.1%)
	No	47,355 (99.6%)	25,388 (99.1%)	10,492 (99.0%)	14,070 (98.9%)
Diabetes					
	Yes	41,345 (87.0%)	20,149 (78.6%)	2,751 (26.0%)	2,566 (18.0%)
	No	6,203 (12.7%)	5,473 (21.4%)	7,843 (74.0%)	11,658 (82.0%)
Use of other medication					
	Anti-hypertensive drug users	29,533 (62.1%)	21,336 (83.3%)	9,981 (94.2%)	10,812 (76.0%)
	Statin users	13,460 (28.3%)	11,986 (46.8%)	5,269 (49.7%)	5,993 (42.1%)
	Hormonal therapy	18,682 (39.3%)	8,982 (35.1%)	3,157 (30.0%)	5,552 (39.0%)
Recorded diagnoses of:					
	Atrial fibrillation	207 (0.4%)	2,011 (7.8%)	1,886 (17.8%)	802 (5.6%)
	Pulmonary embolism	22 (0.05%)	274 (1.1%)	190 (1.8%)	204 (1.4%)
	Venous thromboembolism	13 (0.03%)	74 (0.3%)	52 (0.5%)	63 (0.4%)

* = Anticoagulant user status not mutually exclusive, LMWH = low-molecular weight heparin

**=Obesity is based on diagnosis obtained from HILMO as reported by clinicians, exact definition cannot be given.

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Table 2 Pre-diagnostic use of 1) anticoagulants overall, 2) warfarin compared to anticoagulant non-users, 3) low-molecular weight heparin (LMWH) compared to anticoagulant non-users and stratified by duration of usage. Age-adjusted and multivariable-adjusted hazard ratios (95% CI) related to all breast cancer deaths. Statistically significant results are bolded.

		n of deaths	Age-adjusted	Multivariable-adjusted
Anticoagulants compared to non-users				
	None	9,940	Ref	Ref
	Any	960	0.94 (0.87-1.00)	1.00 (0.93-1.07)
Duration of anticoagulant use				
	≤1 year	321	**0.76 (0.68-0.85)**	**0.85 (0.76-0.95)**
	2-3 years	269	1.04 (0.92-1.18)	1.11 (0.98-1.26)
	4 or more years	370	1.09 (0.98-1.21)	1.09 (0.98-1.21)
Warfarin compared to anticoagulant non-users**				
	Non-user	9,940	Ref	Ref
	Any	579	0.93 (0.85-1.01)	0.97 (0.89-1.06)
Duration of warfarin use				
	≤1 year	190	**0.84 (0.73-0.97)**	0.89 (0.77-1.03)
	2-4 years	211	0.98 (0.85-1.12)	1.01 (0.88-1.16)
	5 or more years	178	0.97 (0.84-1.13)	1.02 (0.88-1.19)
LMWH compared to anticoagulant non-users**				
	Non-user	9,940	Ref	Ref
	Any	127	**0.62 (0.52-0.74)**	**0.71 (0.59-0.85)**
Duration of LMWH use				
	≤1 year	105	**0.59 (0.48-0.71)**	**0.69 (0.57-0.83)**
	2 or more years	22	0.80 (0.53-1.22)	0.84 (0.55-1.27)

* Adjusted variables are: age, stage of disease at diagnosis, BrCa treatment, Charlson Score, obesity, participation in mammography screening, diabetes, use of antihypertensive drugs, use of statins, hormonal therapy, tumor histology, atrial fibrillation, venous thromboembolism, pulmonary embolism.

**=Use of warfarin/LMWH with or without use of other anticoagulants

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Table 3 Post-diagnostic use of 1) anticoagulants overall, 2) Warfarin compared to anticoagulant non-users, 3) low-molecular weight heparin (LMWH) compared to anticoagulant non-users. Stratified by duration of usage. Multivariable-adjusted and lag-time hazard ratios (95% CI) related to all breast cancer deaths. Statistically significant results are bolded.

		n of deaths	Multivariable-adjusted*	1-year lag-time	2-year lag-time	3-year lag-time
Anticoagulants compared to non-users						
	None	8,085	Ref	Ref	Ref	Ref
	current	1,323	**1.41 (1.33-1.49)**	**1.29 (1.21-1.36)**	**1.13 (1.06-1.20)**	**1.10 (1.03-1.18)**
	previous	1,492	**1.30 (1.22-1.39)**	1.05 (0.97-1.14)	0.97 (0.88-1.08)	0.97 (0.87-1.10)
Duration of anticoagulant use						
	≤1 year	1,217	**1.40 (1.32-1.49)**	**1.26 (1.17-1.35)**	1.03 (0.95-1.12)	1.00 (0.92-1.09)
	2-3 years	796	**1.54 (1.42-1.67)**	**1.27 (1.16-1.39)**	**1.27 (1.15-1.40)**	**1.25 (1.13-1.39)**
	4 or more years	802	1.06 (0.96-1.19)	**1.21 (1.10-1.34)**	**1.15 (1.04-1.28)**	**1.16 (1.04-1.28)**
Warfarin compared to anticoagulant non-users**						
	Non-user	8,085	Ref	Ref	Ref	Ref
	current	589	**1.10 (1.02-1.19)**	**1.22 (1.13-1.31)**	**1.12 (1.04-1.22)**	**1.13 (1.04-1.22)**
	previous	815	**1.58 (1.45-1.72)**	**1.15 (1.02-1.29)**	1.08 (0.94-1.24)	1.02 (0.87-1.21)
Duration of warfarin use						
	≤2 year	758	**1.32 (1.23-1.43)**	**1.17 (1.08-1.28)**	1.01 (0.92-1.11)	1.03 (0.92-1.14)
	3-6 years	383	1.01 (0.89-1.14)	**1.17 (1.04-1.32)**	1.13 (1.00-1.28)	1.11 (0.98-1.26)
	7 or more years	263	0.91 (0.72-1.15)	1.14 (0.96-1.37)	1.14 (0.97-1.35)	1.11 (0.94-1.30)
LMWH compared to anticoagulant non-users**						
	Non-user	8,085	Ref	Ref	Ref	Ref
	current	643	**2.62 (2.42-2.83)**	**1.74 (1.57-1.93)**	**1.30 (1.15-1.46)**	1.11 (0.96-1.27)
	previous	669	0.99 (0.90-1.09)	1.00 (0.89-1.11)	0.90 (0.78-1.03)	0.85 (0.71-1.01)
Duration of LMWH use						
	≤1 year	911	**1.40 (1.31-1.51)**	**1.16 (1.06-1.27)**	0.95 (0.85-1.06)	0.89 (0.79-1.01)
	2 or more years	401	**3.39 (3.00-3.82)**	**2.28 (1.92-2.70)**	**2.01 (1.63-2.49)**	**1.74 (1.35-2.24)**

* Adjusted variables are: age, stage of disease at diagnosis, BrCa treatment, Charlson Score, obesity, participation in mammography screening, diabetes, use of antihypertensive drugs, use of statins, hormonal therapy, tumor histology, atrial fibrillation, venous thromboembolism, pulmonary embolism.

**=Use of warfarin/LMWH with or without use of other anticoagulants

Table 4 Sensitivity analysis where metastatic and unknown disease at diagnosis have been excluded, leaving 60,679 women. Analyzed by post-diagnostic use of 1) anticoagulants overall, 2) Warfarin compared to anticoagulant non-users, 3) low-molecular weight heparin (LMWH) compared to anticaoagulant non-users and 4) warfarin compared to non-warfarin anticoagulant users. Age-adjusted, multivariable-adjusted and lag-time hazard ratios (95% CI) related to all breast cancer deaths. Statistically significant results are bolded.

		n of deaths	Age-adjusted	Multivariable-adjusted*	1-year lag-time	2-year lag-time	3-year lag-time
Anticoagulants compared to non-users							
	None	5,084	Ref	Ref	Ref	Ref	Ref
	current	860	**1.90 (1.78-2.03)**	**1.67 (1.56-1.79)**	**1.46 (1.35-1.57)**	**1.21 (1.11-1.31)**	**1.19 (1.09-1.29)**
	previous	960	**1.51 (1.39-1.64)**	**1.35 (1.24-1.47)**	1.08 (0.98-1.19)	1.02 (0.91-1.15)	1.01 (0.89-1.16)
Warfarin compared to anticoagulant non-users**							
	Non-user	5,084	Ref	Ref	Ref	Ref	Ref
	current	384	**1.16 (1.05-1.27)**	**1.22 (1.11-1.34)**	**1.30 (1.19-1.44)**	**1.13 (1.01-1.25)**	**1.16 (1.04-1.28)**
	previous	520	**1.65 (1.48-1.84)**	**1.65 (1.48-1.84)**	**1.20 (1.04-1.38)**	1.15 (0.99-1.35)	1.06 (0.87-1.28)
LMWH compared to anticoagulant non-users**							
	Non-user	5,084	Ref	Ref	Ref	Ref	Ref
	current	427	**3.32 (3.01-3.65)**	**3.16 (2.87-3.48)**	**2.11 (1.86-2.38)**	**1.44 (1.23-1.68)**	1.19 (1.00-1.42)
	previous	453	0.95 (0.84-1.06)	1.01 (0.90-1.13)	0.92 (0.81-1.06)	0.86 (0.73-1.01)	0.82 (0.67-1.00)
Warfarin compared to other anticoagulants							
	Other anticoagulants	906	Ref	Ref	Ref	Ref	Ref
	Warfarin users	914	0.95 (0.86-1.05)	**0.88 (0.80-0.98)**	**1.16 (1.04-1.30)**	1.11 (0.98-1.26)	**1.18 (1.04-1.35)**

* Adjusted variables are: age, stage of disease at diagnosis, BrCa treatment, Charlson Score, obesity, participation in mammography screening, diabetes, use of antihypertensive drugs, use of statins, hormonal therapy, tumor histology, atrial fibrillation, venous thromboembolism, pulmonary embolism.

**=Use of warfarin/LMWH with or without use of other anticoagulants

Figure Legends

Figure 1 - Subgroup analysis between anticoagulant users and non-users for breast cancer death in post-diagnostic setting. P for interaction is given under the variable if an effect modification was considered possible. Statistically significant P values are bolded.