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Antiplatelet drugs and breast cancer risk in a large nationwide Danish case-control study

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Abstract

Low-dose aspirin has been hypothesized to prevent cancer risk by inhibiting platelet aggregation. However, the anti-cancer effect of low-dose aspirin has recently been questioned and its effect on breast cancer development remains unclear. The impact of other antiplatelet drugs on breast cancer risk has rarely been evaluated. Thus, this study aimed to investigate the associations between breast cancer risk and antiplatelet drug use in a nationwide nested case-control study. From the Danish healthcare registries, we identified as cases all women with invasive breast cancer diagnosis between 2001 and 2018 (n = 68 852). The date of diagnosis corresponded to the index date. We matched cases to 10 population controls on age and calendar time, using risk set sampling. Controls were assigned the same index date as their matched case. We used the prescription registry to identify exposure to low-dose aspirin, clopidogrel and dipyridamole. We defined ever use of antiplatelet drugs as at least two prescriptions filled up to 1 year before the index date. We applied conditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals for breast cancer associated with the use of antiplatelet drugs, overall, by breast cancer subtype and by cumulative dose. Twelve percent of women had ever been exposed to low-dose aspirin, 2% to clopidogrel and 2% to dipyridamole. In multivariable models, breast cancer risk was not associated with ever use of low-dose aspirin (OR = 1.00 [0.97-1.03]), clopidogrel (OR = 0.93 [0.87-1.00]), and dipyridamole (OR = 1.02 [0.94-1.10]), compared with never use, and there was no evidence of a dose-response relation. However, we found an inverse association between dipyridamole use and breast cancer risk among women aged <55 years old, with suggestion of a dose-response relationship (OR per 1000 Defined Daily Doses = 0.72 [0.54-0.95]). Associations did not differ by breast cancer histological type, estrogen receptor status or clinical stage at diagnosis. Overall, the findings from this study do not support the use of antiplatelet drugs for breast cancer prevention.

KEYWORDS
antiplatelet drugs, breast cancer, low-dose aspirin, registries

Abbreviations: BMI, body mass index; CI, confidence interval; DDD, defined daily dose; ER, estrogen receptor; OR, odd ratio.

What's new?

Low-dose aspirin has been hypothesized to prevent cancer risk by inhibiting platelet aggregation while the impact of other antiplatelet drugs on breast cancer risk has rarely been evaluated. The findings from this large nationwide nested case-control study add to the growing evidence from randomized controls trials that low-dose aspirin does not appear to be a suitable pharmacological candidate for breast cancer prevention. Further, our results do not provide strong support to the use of other antiplatelet drugs for breast cancer prevention.

1 | INTRODUCTION

It has been suggested that low-dose aspirin may prevent several cancers, including breast cancer, by inhibiting platelet aggregation.[1] In a recent meta-analysis of 22 cohort and 16 case-control studies, aspirin use was associated with a 4% decreased risk of breast cancer in cohorts and a 17% decreased risk in case-control studies.[2] However, there was substantial heterogeneity in the results between studies, which may be attributable to differences in the doses or durations of aspirin use that were evaluated or in the populations studied. The potential preventive effect of low-dose aspirin on breast cancer incidence has not been confirmed in two randomized double-blind placebo-controlled trials.[3,4] The overall cancer preventive effect of low-dose aspirin has even been questioned with emerging evidence from one of these trials, published in 2020, which reported a positive association between low-dose aspirin and the risk of any stage 4 cancer among participants aged ≥70 years.[3] A meta-analysis of randomized controlled trials, published in 2018, found that aspirin's effects on cancer might differ by body size, age, dose and timing of aspirin use.[5] Among participants aged ≥70 years and weighing <70 kg, aspirin exposure was associated with an increased risk of any cancer in the first 3 years of follow-up, with a subsequent reduced risk after 5 years of follow-up.[5] A recent study among women from the French E3N cohort (median age at follow-up start: 63 years old), performed by our group, found the same pattern between low-dose aspirin use and breast cancer incidence, with a transient higher breast cancer risk a few years after starting low-dose aspirin use, followed by a lower risk after 4 years of use.[6]

If low-dose aspirin impacts breast cancer through its antiplatelet properties, other antiplatelet drugs are likely to elicit similar effects on breast cancer incidence. However, our analysis based on the E3N cohort was the only epidemiological study to consider the use of antiplatelet drugs other than aspirin in relation to breast cancer risk. We found some evidence that clopidogrel use was associated with a higher estrogen receptor (ER) negative breast cancer risk,[6] however this was based on a limited number of ER-negative breast cancer cases ($n_{ever\ exposed} = 23$).

Thus, we aimed to further evaluate this putative association using data from the nationwide Danish registries. In particular, we evaluated the associations between breast cancer incidence and antiplatelet drug use, overall and by breast cancer subtypes, types of antiplatelet drugs and cumulative dose (as a proxy for duration of use).

2 | MATERIALS AND METHODS

We performed a nested case-control analysis based on data from Danish nationwide registries. We compared the use of antiplatelet drugs among women diagnosed with invasive breast cancer (cases) with use among cancer-free women (controls), estimating odds ratios (ORs) for breast cancer associated with antiplatelet drug use.

2.1 | Nationwide registry sources

We used data from six nationwide registry sources: the Danish Cancer Registry,[7] the National Prescription Registry,[8] the National Patient Registry,[9] the Population Education Registry,[10] the Danish Pathology Register[11] and the Civil Registration System.[12,13] We described these registries in Data S1 (Additional File 1).

Almost all medical care in Denmark is funded by the Danish National Health Service, allowing population-based register linkage studies covering all residents of Denmark.[14] Data sources were linked by a unique personal identification number, assigned to all residents since 1968.[13] All linkages were performed by Statistics Denmark, a government institution that collects and processes information for a variety of statistical and scientific purposes.

2.2 | Selection of breast cancer cases and population controls

We described the selection of breast cancer cases in Figure 1 and codes for cancer diagnoses in Data S2 (Additional File 1). From the Danish Cancer Registry, we identified cases as all women with a primary diagnosis of invasive breast cancer between January 1, 2001 and December 31, 2018 (n = 82 031). First, we excluded cases that were diagnosed at death/by death certificate (n = 158). The date of diagnosis corresponded to the index date. We excluded cases aged ≤18 and ≥85 years at the index date (n = 4403) and cases that were not histologically verified (n = 467). We further excluded women with any cancer diagnosis (except non-melanoma skin cancer, n = 4422) or mastectomy before the index date (n = 220). Cases with any residency outside Denmark within 10 years prior to the index date were also excluded (n = 2051), thus ensuring at least 10 years of follow-up for all study subjects and a minimum of 5 years of drug prescription data (the prescription registry opened in 1995). We restricted the study sample to women with no prescription of antiplatelet drugs between January 1, 1995 and December 31, 1995 in

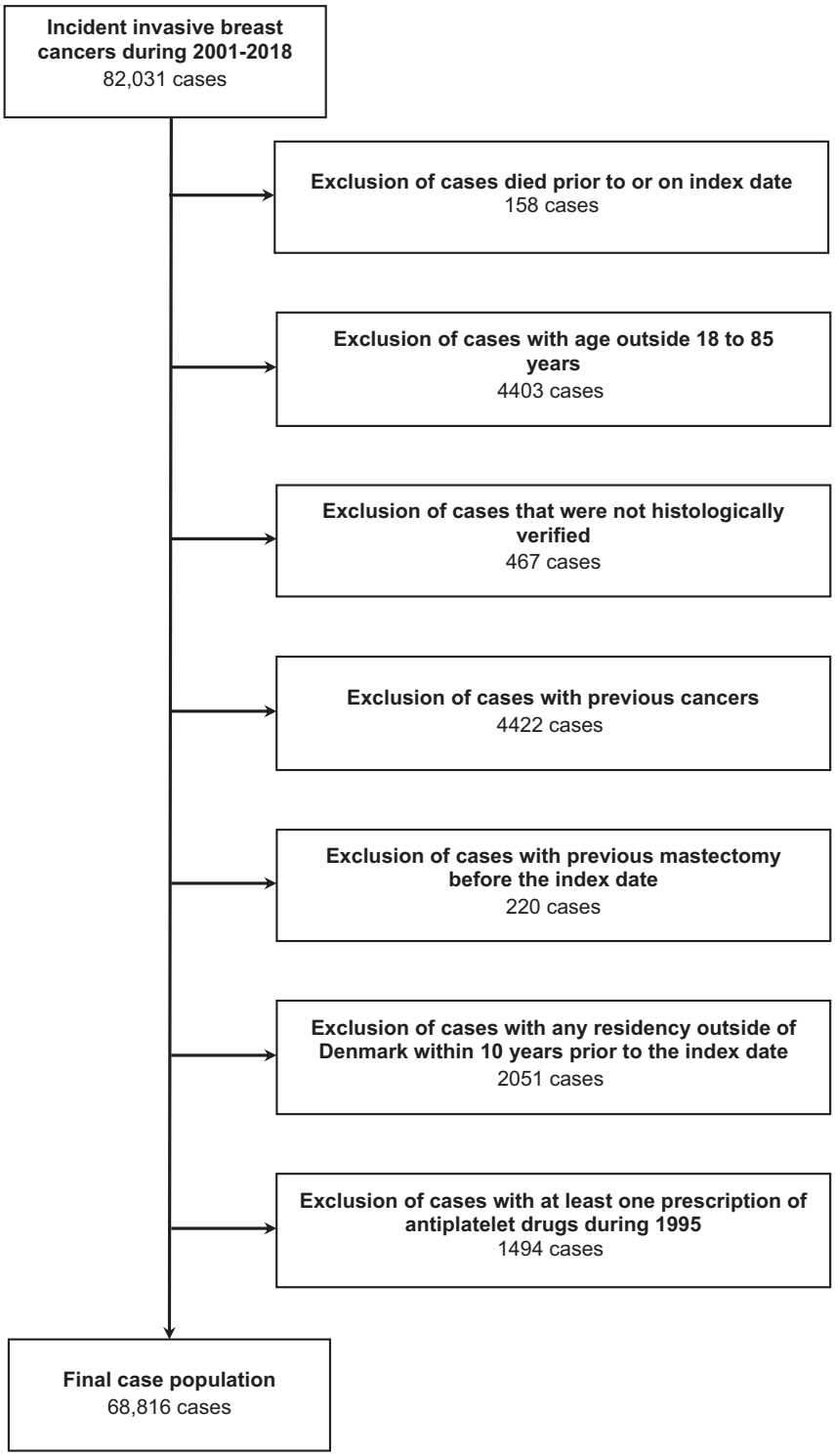

FIGURE 1 Flow-chart of the selection of cases

order to exclude women who had probably begun these drugs before the availability of prescription data. We ended-up with 68 816 histologically verified invasive breast cancer cases. For each case, we selected 10 controls among Danish women matched by exact birth year and calendar time, and the selection criteria listed above was applied to both cases and controls. Controls were selected using risk set sampling and were assigned the same index date as the case to whom they were matched. Subjects were eligible for sampling as controls before they became cases, thereby the calculated ORs provide unbiased estimates of the incidence rate ratios that would be estimated from a cohort study utilizing the source population.[15] The final study population included 68 816 cases matched with 688 160 controls.

2.3 | Exposure

Low-dose aspirin (≤150 mg), clopidogrel and dipyridamole are the most frequently prescribed antiplatelet drugs in Denmark. As they act through distinct pharmacological mechanisms,[16] they were analyzed separately. We described codes for drug exposure in Data S2 (additional file 1). We

TABLE 1 Characteristics of breast cancer cases and matched controls

	Cases, n = 68 816	Controls, n = 688 160
Age, median (IQR, years)	62 (53-70)	62 (53-70)
Use of low-dose aspirin, n (%)		
Never	60 268 (88%)	603 363 (88%)
Ever	8548 (12%)	84 797 (12%)
Long-term	5186 (7.5%)	50 867 (7.4%)
Cumulative DDDs, median (IQR)	1300 (500-2500)	1300 (500-2470)
Use of clopidogrel, n (%)		
Never	67 737 (98%)	676 397 (98%)
Ever	1079 (1.6%)	11 763 (1.7%)
Long-term	287 (0.4%)	2981 (0.4%)
Cumulative DDDs, median (IQR)	500 (340-1074)	448 (330-1000)
Use of dipyridamole, n (%)		
Never	67 792 (99%)	677 946 (99%)
Ever	1024 (1.5%)	10 214 (1.5%)
Long-term	527 (0.8%)	5205 (0.8%)
Cumulative DDDs, median (IQR)	1050 (360-2130)	1034 (360-2100)
Ever use of other drugs, n (%)		
Antidiabetics	3432 (5.0%)	33 756 (4.9%)
Statins	10 504 (15%)	106 227 (15%)
Spironolactone	1127 (1.6%)	10 182 (1.5%)
Loop diuretics	4701 (6.8%)	43 627 (6.3%)
Beta-blockers	2480 (3.6%)	22 602 (3.3%)
Vascular calcium-channel blockers	8238 (12%)	79 809 (12%)
Selective serotonin reuptake inhibitors	10 532 (15%)	100 842 (15%)
Raloxifene	126 (0.2%)	1986 (0.3%)
Recent use of oral contraceptives	3053 (4.4%)	24 678 (3.6%)
Former use of oral contraceptives	13 729 (20%)	130 851 (19%)
Recent use of hormone replacement therapy	8434 (12%)	48 379 (7.0%)
Former use of hormone replacement therapy	18 162 (26%)	146 237 (21%)
Comorbidities, n (%)		
Alcohol related diseases	2251 (3.3%)	20 032 (2.9%)
Chronic obstructive pulmonary disease	3354 (4.9%)	31 051 (4.5%)
Hypertension	30 618 (44%)	296 223 (43%)
Hypercholesterolemia	12 278 (18%)	125 082 (18%)
Diabetes	4127 (6.0%)	40 489 (5.9%)
Acute myocardial infarction	879 (1.3%)	9558 (1.4%)
Other ischemic heart disease	68 (0.1%)	789 (0.1%)
Angina pectoris	2536 (3.7%)	25 757 (3.7%)
Heart failure	931 (1.4%)	8507 (1.2%)
Stroke	1689 (2.5%)	16 982 (2.5%)
Other cerebrovascular diseases	992 (1.4%)	10 092 (1.5%)
Atrial fibrillation or atrial flutter	1948 (2.8%)	16 319 (2.4%)
Pulmonary embolism and infarction	342 (0.5%)	3010 (0.4%)
Phlebitis and thrombophlebitis	785 (1.1%)	7124 (1.0%)
Portal vein thrombosis	10 (0.0%)	89 (0.0%)
Other venous embolism and thrombosis	130 (0.2%)	1020 (0.1%)

TABLE 1 (Continued)

	Cases, n = 68 816	Controls, n = 688 160
Charlson Comorbidity Index, n (%)		
None (Score = 0)	54 398 (79%)	547 371 (80%)
Low (Score = 1)	8760 (13%)	88 909 (13%)
Medium (Score = 2)	3326 (4.8%)	30 579 (4.4%)
High (Score ≥ 3)	2332 (3.4%)	21 301 (3.1%)
Highest achieved education, n (%)		
Basic (7-10 years)	198 (0.3%)	3236 (0.5%)
Medium (11-12 years)	48 315 (70%)	495 434 (72%)
Higher (≥13 years)	18 845 (27%)	174 428 (25%)
Unknown	1458 (2.1%)	15 062 (2.2%)

Abbreviation: IQR, InterQuartile Range.

defined ever users of the drug of interest as women with at least two prescriptions between January 1, 1996 and 1 year prior to the index date. Women with 0 to 1 prescription were considered never users of the drug of interest (reference category). We also classified exposure according to cumulative number of defined daily doses (DDD). Long-term use of antiplatelet drugs was defined as filled prescriptions equivalent to ≥1000 DDDs of antiplatelet drugs, corresponding to approximately 3 years of cumulative use. For all analyses, prescriptions filled in the year prior to the index date were disregarded as to allow for a minimum latency period and to account for potential reverse causality.[17]

2.4 | Covariates

Potential confounders were selected a priori based on the literature and availability in the registries. From the Prescription Registry, we retrieved prescriptions of drugs suspected to modify breast cancer risk and likely to be associated with the use of antiplatelet drugs including antidiabetics, statins, spironolactone, loop diuretics, β-blockers, vascular calcium channel blockers and selective serotonin reuptake inhibitors. We defined ever users as women with at least two prescriptions of the drug of interest from 1995 to 1 year prior to the index date. For oral contraceptives or hormone replacement therapy, recent users were defined as women with at least two prescriptions in the penultimate year preceding the index date, and former users were defined as women with at least two prescriptions from 1995 to the penultimate year preceding the index date but who were not recent users.

From the Danish National Patient Registry, we retrieved information on diagnoses of chronic obstructive pulmonary disease and alcohol-related diseases as proxies for heavy smoking and heavy alcohol consumption. We also considered comorbidities including hypertension, hypercholesterolemia, type 1 or 2 diabetes, acute myocardial infarction, other ischemic heart disease, angina pectoris, heart failure, stroke, other cerebrovascular diseases, atrial fibrillation or atrial flutter, pulmonary embolism and infarction, phlebitis and thrombophlebitis, portal vein thrombosis, and other venous embolism and thrombosis. Comorbidities were defined as a primary or secondary discharge or outpatient diagnosis or by related medications. The Charlson comorbidity index score (0 [low], 1-2 [medium], or ≥ 3 [high]) was defined based on the prevalence of 19 chronic conditions.[18,19] Information within 1 year prior to the index date was also disregarded for comorbidities. From registries at Statistics Denmark and the Civil Registration System, we retrieved information on educational level as a crude measure of socioeconomic status (basic, medium, higher or unknown). Codes for all covariates are listed in Data S2 (Additional File 1).

2.5 | Statistical analyses

We computed the frequency and proportion of cases and controls within categories of exposure and covariates. We used conditional logistic regression to estimate ORs for the association of ever or long-term antiplatelet drug use with breast cancer incidence. Secondary analyses examined potential dose-response associations stratifying cumulative doses of antiplatelet drugs by predefined categories that is, <500, ≥500 to <1000, ≥1000 to <2000, ≥2000 to <3000 and ≥3000 DDDs. In all analyses, never use of antiplatelet drugs (defined as <2 prescriptions) served as the reference category. All models were adjusted for all potential confounders outlined previously and listed in the Data S3. In addition, analyses of low-dose aspirin, clopidogrel and dipyridamole were simultaneously adjusted for each other.

We performed several subgroup and sensitivity analyses. First, we examined the association between antiplatelet drug exposure and breast cancer risk by histological type (ductal adenocarcinoma, lobular adenocarcinoma, and others), estrogen receptor (ER) status (ER-positive, ER-negative, and unknown), and clinical stage at diagnosis (localized, non-localized and unknown). Then, we stratified our analyses by age at index date (<55, ≥55 to <70 and ≥70). It has been suggested that combination or concomitant use of low-dose aspirin with other antiplatelet drugs might be associated with an increased cancer risk.[6,20,21] Therefore, we also defined exposure as follows: (i) use of low-dose aspirin but never concomitantly with clopidogrel or dipyridamole, (ii) use of clopidogrel but never concomitantly with low-dose

TABLE 2 Associations between antiplatelet drug use and invasive breast cancer risk

	n cases	n controls	OR (95% CI)[a]	OR (95% CI)[b]
Low-dose aspirin				
Use categories				
Never use	60 268	603 363	1.00 (ref.)	1.00 (ref.)
Ever use	8548	84 797	1.01 (0.99-1.04)	1.00 (0.97-1.03)
Long-term use	5186	50 867	1.02 (0.99-1.06)	1.00 (0.97-1.04)
Cumulative DDDs				
Never use	60 268	603 363	1.00 (ref.)	1.00 (ref.)
<500	1822	17 895	1.02 (0.97-1.07)	1.00 (0.95-1.06)
≥500 to <1000	1540	16 035	0.96 (0.91-1.02)	0.95 (0.90-1.00)
≥1000 to <2000	2197	21 785	1.01 (0.97-1.06)	1.00 (0.95-1.05)
≥2000 to <3000	1437	14 046	1.03 (0.97-1.09)	1.02 (0.96-1.08)
≥3000	1552	15 036	1.04 (0.98-1.09)	1.03 (0.97-1.09)
OR per 1000 DDDs	8548	84 797	1.01 (1.00-1.02)	1.01 (0.99-1.02)
Clopidogrel				
Use categories				
Never use	67 737	676 397	1.00 (ref.)	1.00 (ref.)
Ever use	1079	11 763	0.91 (0.86-0.97)	0.93 (0.87–1.00)
Long-term use	287	2981	0.96 (0.85-1.08)	0.96 (0.85-1.09)
Cumulative DDDs				
Never use	67 737	676 397	1.00 (ref.)	1.00 (ref.)
<500	533	6226	0.85 (0.78-0.93)	0.87 (0.79–0.96)
≥500 to <1000	259	2556	1.01 (0.89-1.15)	1.02 (0.89-1.16)
≥1000 to <2000	196	1947	1.00 (0.87-1.16)	1.01 (0.87-1.17)
≥2000 to <3000	60	599	1.00 (0.76-1.30)	1.00 (0.77-1.31)
≥3000	31	435	0.71 (0.49-1.02)	0.71 (0.49-1.02)
OR per 1000 DDDs	1079	11 763	0.96 (0.91-1.01)	0.97 (0.91-1.03)
Dipyridamole				
Use categories				
Never use	67 792	677 946	1.00 (ref.)	1.00 (ref.)
Ever use	1024	10 214	1.00 (0.94-1.07)	1.02 (0.94–1.10)
Long-term use	527	5205	1.01 (0.92-1.11)	1.03 (0.93-1.14)
Cumulative DDDs				
Never use	67 792	677 946	1.00 (ref.)	1.00 (ref.)
<500	316	3204	0.99 (0.88-1.11)	0.99 (0.87-1.11)
≥500 to <1000	181	1805	1.00 (0.86-1.17)	1.02 (0.87-1.20)
≥1000 to <2000	250	2455	1.02 (0.89–1.16)	1.04 (0.91-1.19)
≥2000 to <3000	145	1531	0.95 (0.80-1.12)	0.96 (0.81-1.15)
≥3000	132	1219	1.08 (0.90-1.30)	1.12 (0.93-1.35)
OR per 1000 DDDs	1024	10 214	1.01 (0.98-1.05)	1.02 (0.98-1.06)

Abbreviations: CI, confidence interval; DDD, defined daily dose; OR, Odds ratio.
[a]Adjusted for age and calendar time (by risk-set matching and the conditional analysis).
[b]Adjusted for age and calendar time (by risk-set matching and the conditional analysis) and covariates listed in the Data S3.

aspirin, (iii) use of dipyridamole but never concomitantly with low-dose aspirin, (iv) ever concomitant use of low-dose aspirin and clopidogrel, and (v) ever concomitant use of low-dose aspirin and dipyridamole. Women were considered concomitant users of two different antiplatelet drugs when they had a prescription of another drug on the same day or until ≤30 days after the prescription of a first drug. If antiplatelet drugs impacted breast cancer risk through their antithrombotic properties, other antithrombotic drugs such as Vitamin

TABLE 3 Associations of antiplatelet drug use with risk of invasive breast cancer, stratified by age at index date

	Age < 55 years			Age ≥ 55 and <70 years			Age ≥ 70 years			
	n cases	n controls	OR (95% CI)[a]	n cases	n controls	OR (95% CI)[a]	n cases	n controls	OR (95% CI)[a]	$p_{heterogeneity}$
Low-dose aspirin										
Use categories										
Never use	19 788	197 360	1.00 (ref.)	27 448	274 983	1.00 (ref.)	13 032	131 020	1.00 (ref.)	
Ever use	401	4530	0.95 (0.85-1.07)	3228	31 777	0.98 (0.94-1.03)	4919	48 490	1.00 (0.96-1.05)	.64
Long-term use	130	1765	0.81 (0.66-0.99)	1816	17 714	0.99 (0.93-1.05)	3240	31 388	1.02 (0.97-1.07)	.05
Cumulative DDDs										
Never use	19 788	197 360	1.00 (ref.)	27 448	274 983	1.00 (ref.)	13 032	131 020	1.00 (ref.)	
<500	181	1816	1.04 (0.89-1.22)	765	7600	0.97 (0.90-1.05)	876	8479	1.02 (0.94-1.09)	
≥500 to <1000	90	949	1.02 (0.82-1.28)	647	6463	0.97 (0.89-1.05)	803	8623	0.92 (0.85-0.99)	
≥1000 to <2000	80	1029	0.84 (0.66-1.07)	849	8311	0.99 (0.92-1.07)	1268	12 445	1.01 (0.95-1.08)	
≥2000 to <3000	29	471	0.67 (0.46-0.99)	504	4979	0.98 (0.89-1.08)	904	8596	1.05 (0.97-1.13)	
≥3000	21	265	0.85 (0.54-1.33)	463	4424	1.01 (0.91-1.12)	1068	10 347	1.04 (0.97-1.12)	
OR per 1000 DDDs	401	4530	0.92 (0.84-1.00)	3228	31 777	1.00 (0.98-1.02)	4919	48 490	1.01 (0.99-1.03)	
Clopidogrel										
Use categories										
Never use	20 120	201 096	1.00 (ref.)	30 285	302 584	1.00 (ref.)	17 332	172 717	1.00 (ref.)	
Ever use	69	794	1.00 (0.75-1.32)	391	4176	0.95 (0.85–1.07)	619	6793	0.92 (0.83-1.01)	.80
Long-term use	18	166	1.28 (0.77-2.12)	107	982	1.07 (0.87-1.32)	162	1833	0.88 (0.75-1.05)	.24
Cumulative DDDs										
Never use	20 120	201 096	1.00 (ref.)	30 285	302 584	1.00 (ref.)	17 332	172 717	1.00 (ref.)	
<500	42	459	1.07 (0.75-1.51)	191	2321	0.85 (0.73-1.00)	300	3446	0.87 (0.77-0.99)	
≥500 to <1000	9	169	0.60 (0.30-1.18)	93	873	1.06 (0.85-1.32)	157	1514	1.04 (0.88-1.24)	
≥1000 to <2000	17	110	1.74 (1.03-2.96)	72	683	1.03 (0.80–1.32)	107	1154	0.94 (0.76-1.15)	
≥2000 to <3000	< 5	39	(–)	23	166	1.37 (0.88-2.13)	36	394	0.92 (0.65-1.31)	
≥3000	< 5	17	(–)	12	133	0.89 (0.49-1.61)	19	285	0.67 (0.42-1.06)	
OR per 1000 DDDs	69	794	0.89 (0.66-1.18)	391	4176	1.03 (0.94-1.13)	619	6793	0.95 (0.88-1.02)	
Dipyridamole										
Use categories										
Never use	20 141	201 316	1.00 (ref.)	30 287	303 094	1.00 (ref.)	17 364	173 536	1.00 (ref.)	
Ever use	48	574	0.86 (0.62-1.19)	389	3666	1.03 (0.91-1.17)	587	5974	1.02 (0.93-1.13)	.52
Long-term use	12	226	0.51 (0.28-0.94)	209	1891	1.08 (0.92-1.28)	306	3088	1.05 (0.92-1.19)	.005

(Continues)

TABLE 3 (Continued)

	Age < 55 years			Age ≥ 55 and <70 years			Age ≥ 70 years			$p_{heterogeneity}$
	n cases	n controls	OR (95% CI)[a]	n cases	n controls	OR (95% CI)[a]	n cases	n controls	OR (95% CI)[a]	
Cumulative DDDs										
Never use	20 141	201 316	1.00 (ref.)	30 287	303 094	1.00 (ref.)	17 364	173 536	1.00 (ref.)	
<500	26	222	1.20 (0.79-1.84)	115	1132	0.98 (0.80-1.19)	175	1850	0.97 (0.82-1.14)	
≥500 to <1000	10	126	0.82 (0.42-1.58)	65	643	1.00 (0.77–1.31)	106	1036	1.07 (0.87-1.31)	
≥1000 to <2000	9	125	0.72 (0.36-1.45)	105	897	1.15 (0.93-1.42)	136	1433	1.00 (0.83-1.20)	
≥2000 to <3000	< 5	73	(–)	55	566	0.94 (0.71-1.25)	87	892	1.03 (0.82-1.29)	
≥3000	< 5	28	(–)	49	428	1.13 (0.83-1.53)	83	763	1.17 (0.92-1.48)	
OR per 1000 DDDs	48	574	0.71 (0.53–0.95)	389	3666	1.03 (0.97-1.10)	587	5974	1.03 (0.98-1.08)	

Abbreviations: CI, confidence interval; DDD, defined daily dose; OR, Odds ratio.
[a]Adjusted for age, calendar time (by risk-set matching and the conditional analysis) and and covariates listed in the Data S3.

K antagonists would have similar effects on breast cancer incidence. We therefore examined the associations between Vitamin K antagonists (ATC code: B01AA) exposure and breast cancer risk (using the same primary exposure definition as previously outlined for antiplatelets). We repeated the main analyses varying the minimum latency period from 0 to 2 years. Finally, we restricted our analyses to women over the age of 55 years (ie, likely postmenopausal) who were never users of hormone replacement therapy and to those with a diagnosis of stroke or myocardial infarction. All statistical analyses were conducted using STATA version 17.0.

3 | RESULTS

Among the 68 816 invasive breast cancer cancers, 75% were ductal adenocarcinomas, 13% lobular adenocarcinomas and 12% others. Among cases, 49 845 had information on ER status, of which 81% were ER-positive and 19% ER-negative. Among cases with information on stage (n = 55 651), 57% were localized and 43% non-localized. The characteristics of the study population are presented in Table 1. The median age at index date was 62 years (interquartile range, 53-70). Differences in characteristics at index date between cases and controls were generally minor, except for a higher use of hormone replacement therapy among cases compared to controls. At the index date, 12% women had ever used low-dose aspirin, 2% clopidogrel and 2% dipyridamole, while 7% women were long-term users of low-dose aspirin, <1% of clopidogrel and <1% of dipyridamole.

Associations between antiplatelet drug use and breast cancer diagnosis are presented in Table 2. In age and calendar time-adjusted models, ever use of low-dose aspirin or dipyridamole, compared with never use, was not associated with breast cancer risk (low-dose aspirin: OR = 1.01 [95% CI, 0.99 to 1.04] and dipyridamole: OR = 1.00 [95% CI, 0.94 to 1.07]). Ever use of clopidogrel was associated with lower breast cancer risk (OR = 0.91 [95% CI, 0.86 to 0.97]). Adjustment for measured potential confounders had little influence on the magnitude of the estimates (low-dose aspirin: OR = 1.00 [95% CI, 0.97 to 1.03], clopidogrel: OR = 0.93 [95% CI, 0.86 to 1.00]; dipyridamole: OR = 1.02 [95% CI, 0.94 to 1.10]).

Compared to never use, long-term use (≥1000 DDDs) of each antiplatelet drug was not associated with breast cancer risk (low-dose aspirin: OR = 1.00 [95% CI, 0.97 to 1.04], clopidogrel: OR = 0.96 [95% CI, 0.85 to 1.09]; dipyridamole: OR = 1.03 [95% CI, 0.93 to 1.14]).

Analyses according to number of DDDs revealed an inverse association between short-term clopidogrel use (<500 DDDs) and breast cancer risk (OR = 0.87 [0.79-0.96]). However, this was not apparent for other dose categories and there was no evidence of a dose response relationship ($OR_{per1000DDDs}$ = 0.97 [95% CI, 0.91 to 1.03]). Similarly, there was no evidence of a dose response trend for other antiplatelet drugs (low-dose aspirin: $OR_{per1000DDDs}$ = 1.01 [95% CI, 0.99 to 1.02]; dipyridamole: $OR_{per1000DDDs}$ = 1.02 [95% CI, 0.98 to 1.06]).

The associations between long-term use of dipyridamole and breast cancer risk differed by age ($p_{homogeneity}$ = <.01, Table 3) suggesting an

inverse association among women aged <55 years (OR = 0.53 [95% CI, 0.30 to 0.95]) but not among women aged between 55 and 69 years old (OR = 1.08 [95% CI, 0.92 to 1.28]) or women aged ≥70 years old (OR = 1.05 [95% CI, 0.92 to 1.19]). Among women aged <55 years old, there was a dose-response relationship between dipyridamole and breast cancer risk ($OR_{per\ 1000\ DDDs}$ = 0.71 [0.53-0.95]). Associations between breast cancer risk and low-dose aspirin or clopidogrel did not differ by age ($p_{homogeneity} \geq .05$).

Overall, there was little evidence that associations between antiplatelet drugs and breast cancer differed by ER status ($p_{homogeneity} \geq .08$, Table S1). There was no evidence of associations with ever use of antiplatelets and the risk of breast cancer with unknown ER status (low-dose aspirin: OR = 0.99 [95% CI, 0.93 to 1.05], clopidogrel: OR = 1.00 [95% CI, 0.89 to 1.13], dipyridamole: OR = 1.13 [95% CI, 0.98 to 1.31]; data not shown). In addition, the associations between antiplatelet drugs and breast cancer risk did not differ by breast cancer histological type ($p_{homogeneity} \geq .10$, Table S2), and stage ($p_{homogeneity} \geq .06$, Table S3). Changing the minimum latency period to 0 or 2 years instead of 1 year (main analysis) only marginally altered the estimates (Table S4). Our findings remained unchanged after restricting the study sample to women with a diagnosis of stroke or myocardial infarction (Table S5) or to women over the age of 55 years who were never users of hormone replacement therapy (Table S6). Overall, analyses of antiplatelets alone or of clopidogrel or dipyridamole used concomitantly with low-dose aspirin revealed similar results (Table S7).

Weak positive associations were observed between ever and long-term use of Vitamin K antagonists and breast cancer risk with no evidence of a dose-response relation (Table S8).

4 | DISCUSSION

In this large registry-based case-control study, overall we did not observe strong evidence of association between antiplatelet drugs and breast cancer risk. While we did observe a lower breast cancer risk with short-term use of clopidogrel (<500 DDD, that is, approximately <1.5 year), there was no evidence of a dose response relationship. There was no association between long-term use of any antiplatelet drug and breast cancer risk. In sub-group analyses, we found that dipyridamole was associated with a lower breast cancer risk only among women aged <55 years old, with some evidence for a dose-response relationship.

In our study, we found no association between low-dose aspirin and breast cancer risk, overall and by subgroups. Our results on low-dose aspirin are consistent with two randomized controlled trials suggesting that its use (at a 100 mg daily dose) had no effect on breast cancer risk compared to placebo (observational follow-up of the Women's Health Study: HR = 1.02 95%CI 0.89-1.18, 385 exposed cases[4]; and the ASPREE trial: HR = 1.03 95%CI 0.80-1.32, 127 exposed cases[3]). In contrast to our results, two recent meta-analyses of observational studies suggested that a long duration of any aspirin use was associated with a lower breast cancer risk.[2,22] However, interpretation of these results was difficult because of high heterogeneity in terms of exposure definition and study design. The protective effect of aspirin on breast cancer was supported mostly in studies with selection and recall biases due to their retrospective designs.[2,22] Furthermore, these meta-analyses did not distinguish between low-dose and high-dose aspirin. Among those prospective studies which have examined low-dose aspirin,[23-30] four noted no association with breast cancer risk,[26-29] three noted a lower breast cancer risk with long-term exposure[24-26] and one reported an higher breast cancer risk.[30] A recent study performed by our group using data from the E3N cohort suggested that use of low-dose aspirin was associated with a transient higher risk of postmenopausal breast cancer few years after treatment start, followed by a lower risk after 4 years of treatment.[6] However, the E3N cohort includes women insured by a health insurance scheme that covers mainly teachers and results from this population cannot be directly extrapolated to other populations.

To our knowledge, the only previous epidemiological study evaluating the association between clopidogrel and breast cancer was performed by our group.[6] In the E3N study, clopidogrel use was associated with a higher ER− breast cancer risk, with no clear trend according to duration of use. This result was based on a limited number of ER− breast cancer cases (ever exposed n = 23) and, to the best of our knowledge, we are not aware of any biological mechanism that may explain this association. Our current study, which included 132 ER− breast cancer cases exposed to clopidogrel, found that women exposed to clopidogrel were not at higher risk for ER− breast cancer than nonexposed women. However, while there was some evidence that use of clopidogrel may be associated with small reductions in the odds of breast cancer, this was observed for very short-term use and no evidence of a dose-response was observed.

To our knowledge, this is the first study to evaluate the association between dipyridamole use and breast cancer risk. Overall results suggest null associations; however, subgroup analysis by age did suggest an inverse association only among women aged <55 years old, with evidence of a dose response. However, because our findings were based on relatively small numbers of exposed cases (n = 48) and since we performed a relatively large number of tests, these results may be due to chance and should be interpreted carefully before replication in other settings.

This was the largest study to evaluate the antiplatelet drugs-breast cancer associations to date. The principal strength of the present study is the use of nationwide registries of high validity,[7,31] with complete coverage of an entire nation, that allowed us to capture histologically verified breast cancer cases and risk-set sampling of controls with low risk of selection bias. In addition, the prospective design with the use of information from a drug prescription database to identify antiplatelet drug exposure for up to 23 years allowed us to minimize differential recall bias between cases and noncases and to consider precise information on exposure. We were also able to adjust our models for socioeconomic parameters, use of other drugs and comorbidities.

Despite these strengths, this study also had a number of limitations. Firstly, there is the potential for exposure misclassification for

those considered unexposed to aspirin due to a lack of information available on over-the-counter low-dose aspirin purchases. However, misclassification is likely to be minimal as, in Denmark, the prescribed proportion of low-dose aspirin varied between 60% and 87% from 1999 to 2007 and remained around 90% from 2006, with evidence suggesting the influence on study estimates to be negligible.[32] In addition, we assumed that the long-term use of low-dose aspirin is primarily managed through prescriptions due to the need for medical surveillance and possibility of financial reimbursement. Misclassification for clopidogrel or dipyridamole treatments are unlikely because these are only available upon prescription in Denmark. While we had no data regarding compliance and adherence to dispensed antiplatelets, it is likely that this may be less of a concern among long-term users of antiplatelets. Then, we were not able to consider medical follow-up in our analyses which might mask any decreased breast cancer risk associated with antiplatelet drug use. Further, we used chronic obstructive pulmonary disease and alcohol-related diseases as proxies for heavy smoking and alcohol consumption, but residual confounding may remain due to lack of information on these factors. We also had no data on other risk factors for breast cancer, including obesity, physical activity and parity. These factors might also be associated with use of antiplatelet drugs either positively or inversely, and uncontrolled confounding from these factors might bias our findings towards the null. Finally, the latest meta-analysis of randomized controlled trials on aspirin and cancer risk reported that aspirin's effects on cancer might differ by body size.[5] However, as data was not available, we were unable to stratify our analyses by that factor.

5 | CONCLUSION

The findings from this large nationwide nested case-control study add to the growing evidence from randomized controls trials that low-dose aspirin does not appear to be a suitable pharmacological candidate for breast cancer prevention. Further, our results do not provide strong support to the use of other antiplatelet drugs for breast cancer prevention.

AUTHOR CONTRIBUTIONS

Anton Pottegård and Morten Olesen analyzed the data. Manon Cairat and Blánaid Hicks were responsible for drafting the manuscript. Anton Pottegård, Agnès Fournier and Laure Dossus provided advice on the analysis and interpretation of the results. All authors read and approved the final manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

This study is based on anonymized registry data located on a secure platform at Statistics Denmark, which can be accessed given the relevant data permits. Further information is available from the corresponding author upon request.

DISCLAIMER

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

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SUPPORTING INFORMATION

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

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