Antiplatelet Drug Use and Breast Cancer Risk in a Prospective Cohort of Postmenopausal Women

Manon Cairat[1], Marie Al Rahmoun[2], Marc J. Gunter[1], Gianluca Severi[2,3], Laure Dossus[1], and Agnès Fournier[2]

ABSTRACT

Background: Epidemiologic evidence is insufficient to draw conclusions on the impact of low-dose aspirin use on breast cancer risk, and the potential impact of other antiplatelet drugs such as clopidogrel needs to be explored.

Methods: We investigated the association between breast cancer risk and low-dose aspirin or clopidogrel use in the E3N cohort, which includes 98,995 women, with information on breast cancer risk factors collected from biennial questionnaires matched with drug reimbursement data available from 2004. Women with at least two reimbursements of the drug of interest in any previous 3-month period were considered "ever" exposed. Exposure was considered as time-varying and multivariable Cox regression models were used to estimate HRs of breast cancer.

Results: Among 62,512 postmenopausal women followed during 9 years on average, 2,864 breast cancer cases were identified. Compared with never use, a transient higher breast cancer risk was observed during the third year of low-dose aspirin use [$HR_{2-\leq 3 \text{ years of use}} = 1.49$ (1.08–2.07)], followed by a lower risk [$HR_{4+ \text{ years of use}} = 0.72$ (0.52–0.99)]. Clopidogrel ever use was associated with a higher breast cancer risk [HR, 1.30 (1.02–1.68)], restricted to estrogen receptor negative (ER^-) tumors [$HR_{ER+} = 1.14$ (0.83–1.57), $HR_{ER-} = 3.07$ (1.64–5.76), $P_{homogeneity} = 0.01$].

Conclusions: Low-dose aspirin was associated with a lower breast cancer risk only after several years of use, while ever use of clopidogrel was associated with a higher ER^- breast cancer risk.

Impact: Antiplatelet drugs are not good pharmacologic candidates for breast cancer prevention.

Introduction

Although the role of platelets in hemostasis and thrombosis has been known for many decades, platelets have more recently emerged as contributors to inflammation, tumorigenesis, and metastasis (1, 2). Antiplatelet drugs, such as clopidogrel or low-dose aspirin, used in the prevention of cerebrovascular and cardiovascular diseases, are consequently considered as potential agents for cancer prevention (2). Of note, aspirin is only considered as an antiplatelet drug when it is used at low doses (≤325 mg/tablet). At higher doses (≥500 mg/tablet), aspirin is mainly used sporadically as an antipyretic, analgesic or anti-inflammatory drug. Low-dose aspirin has been consistently associated with a reduction in colorectal cancer incidence in epidemiologic studies (3–5). Evidence for other cancers such as breast cancer is less clear. Recent meta-analyses reported an approximate 10% decreased breast cancer risk with aspirin use (regardless of the dose). However, the beneficial effect of aspirin was more evident in case–control than in cohort studies (3, 6, 7). In addition, there was evidence of publication bias as well as significant heterogeneity of results and individual studies did not systematically report results on duration of aspirin use or breast cancer subtypes. More epidemiologic studies are thus needed with more accurate assessment of exposure to aspirin, which should in particular distinguish between low-dose and high-dose formulations, because low-dose and high-dose aspirin have different pharmacologic properties and might therefore influence breast cancer risk differently. We recently showed that the use of high-dose aspirin was not associated with breast cancer risk in the E3N cohort (8). The potential impact of other antiplatelet drugs such as clopidogrel on breast cancer risk has never been assessed in epidemiologic studies. Contrasting the results on clopidogrel and low-dose aspirin could help to elucidate whether use of aspirin might impact breast cancer risk through platelet inhibition or through other mechanisms (9–11).

We therefore evaluated the associations between breast cancer incidence and both the use of low-dose aspirin and the use of clopidogrel, overall and by breast cancer subtypes, risk factors and comorbidities in the prospective E3N (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Education Nationale) cohort.

Materials and Methods

E3N cohort

The E3N cohort includes 98,995 French women born between 1925 and 1950 and insured by the health insurance scheme that covers mainly teachers (12). In 1990, volunteers provided their written informed consent and completed the baseline questionnaire. Every 2 or 3 years thereafter, participants completed self-administered follow-up questionnaires regarding various characteristics and medical events. Furthermore, for each cohort member, the health insurance plan provided data on all outpatient reimbursements for health expenditure issued since January 1, 2004. The E3N cohort was conducted in accordance with the Declaration of Helsinki and received ethical approval from the French National Commission for Data Protection and Privacy.

[1]Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France. [2]Paris-Saclay University, UVSQ, Univ. Paris-Sud, Inserm, Gustave Roussy, "Exposome and Heredity" team, CESP, Villejuif, France. [3]Department of Statistics, Computer Science and Applications "G. Parenti" (DISIA), University of Florence, Florence, Italy.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

L. Dossus and A. Fournier contributed as co-last authors of this article.

Corresponding Author: Agnès Fournier, Université Paris-Saclay, UVSQ, Univ. Paris-Sud, Inserm, Gustave Roussy, "Exposome and Heredity" team, CESP, Villejuif 94805, France. Phone: 331-42 11-5163. E-mail: agnes.fournier@gustave.roussy.fr

Cancer Epidemiol Biomarkers Prev 2021;30:643–52

doi: 10.1158/1055-9965.EPI-20-1292

Study population and follow-up

Follow-up started on July 1, 2004 and ended at the date of diagnosis of any cancer (with the exception of basal cell carcinoma and *in situ* colorectal tumor), latest completed questionnaire, or November 17, 2014 (date at which the last considered questionnaire was sent to participants), whichever occurred first.

The study population included 62,512 postmenopausal women who were free of cancer on July 1, 2004 (**Fig. 1**).

Identification of breast cancer cases

Most breast cancer cases were identified using self-reports in the questionnaires and, to a lesser extent, spontaneous reports by participants' next of kin, or causes of death data. Pathology reports and other medical documents were then obtained from the participants and/or their physicians for 95% of the incident cases and were used to confirm the cases and to extract information on tumor characteristics such as hormonal receptor status, histologic type, grade, and stage at diagnosis. As the proportion of false-positive self-reports was low (<5%), we did not exclude from our analyses cases for which we could not obtain pathology reports.

Exposure to antiplatelet drugs

Only clopidogrel and low-dose aspirin were considered in our analyses because less than 1% of the women were exposed to other antiplatelet drugs (e.g., prasugrel or ticlopidine). Because they act through distinct pharmacologic mechanisms (13), clopidogrel and low-dose aspirin were analyzed separately. For each reimbursement of medicines containing clopidogrel or low-dose aspirin, we extracted data on date of purchase, number of pills per package, and dose per pill.

We defined as "ever" users, women with at least two reimbursements of the drug of interest during any previous 3-month period since January 1, 2004. Other women were considered as "never" users. We also classified exposure according to time since last and first use, age at first use, cumulative duration of use, and cumulative number of defined daily doses (DDDs). The DDD is the assumed average daily maintenance dose for a drug used for its main indication in adults (14). Here, we assumed that the DDD was equal to one tablet per day and the standard duration of a box was the number of pills it contained. For each box delivered, the duration of use was calculated as the shortest length of time between its standard duration and the time until the next antiplatelet drug delivery. The cumulative duration of use was

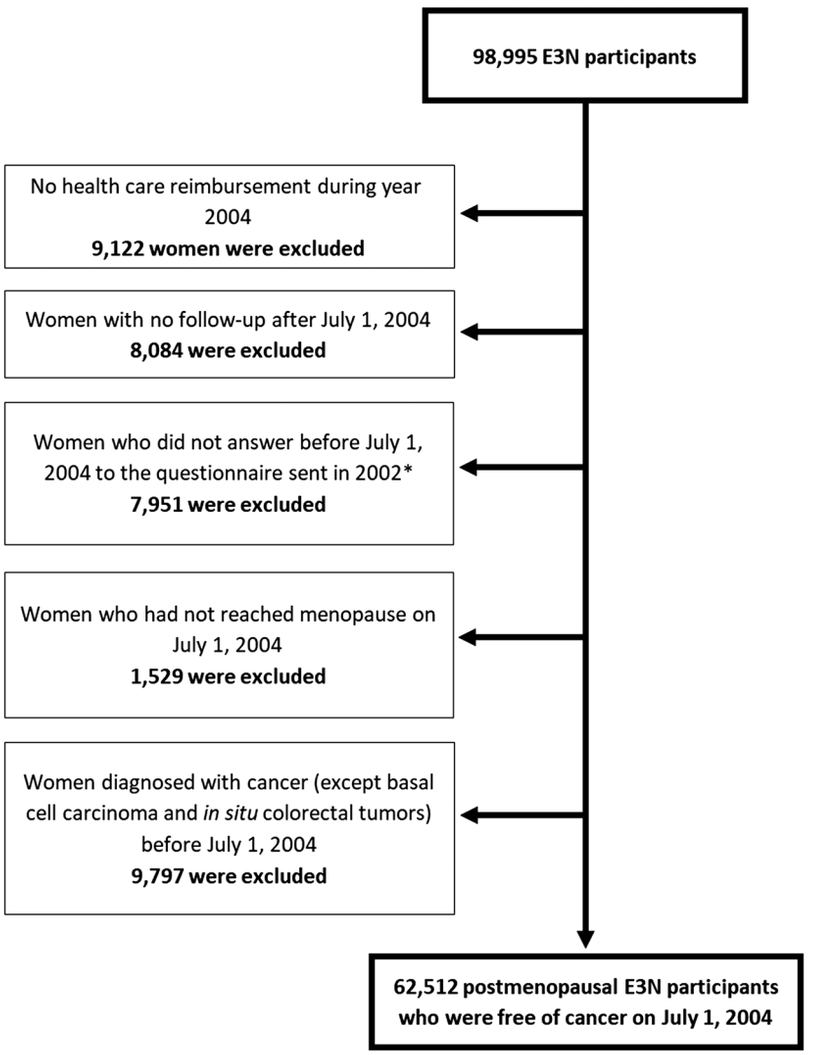

Figure 1.
Flow chart and study population.

*Last follow-up questionnaire sent before the beginning of follow-up for the current study (2004).

calculated as the sum of durations of use corresponding to each delivery since January 1, 2004. The date of last use was calculated as the date of last purchase + the standard duration of the last reimbursed box. Women with antiplatelet drug deliveries between January 1 and April 1, 2004 were likely to have begun use before the availability of reimbursement data. Therefore, cumulative duration and dose, age at first use and time since first use were in that case assigned to an "unknown" category, unless they could be assigned to the highest category of duration/dose/time since first use or the lowest category of age at first use. Results for time since last use were not shown because most users (>70%) were still taking the drugs at the end of follow-up.

Covariates

Parameters considered as potential confounders are listed in **Table 1**. Number of consultations with the doctor during the preceding 6 months and "ever" use (defined as at least two reimbursements of the drug of interest during any previous 3-month period since January 1, 2004) of other drugs likely to be used by antiplatelet drug users [antidiabetics (Anatomical Therapeutic Chemical codes: A10), statins (C10AA), antihypertensives (C02), diuretics (C03), β-blocking agents (C07), calcium channel blockers (C08), agents acting on the renin–angiotensin system (C09), other antithrombotic agents (B01A except B01AC) and proton pump inhibitors (A02BC)] were identified using the reimbursement database. Use of menopausal hormone therapy (MHT) was identified using both the drug reimbursement database and self-reported information from the questionnaires sent out before 2004 (15). Information on educational level, breastfeeding, age at menopause, age at menarche, parity and age at first full-term pregnancy, current level of physical activity, familial history of breast cancer and lifetime use of oral contraceptives originated from the biennial self-administered questionnaires sent before the start of follow-up. Information on current body mass index (BMI), smoking status, lifetime personal history of benign breast disease, alcohol intake, self-report of a mammogram performed during the previous follow-up cycle, lifetime histories of cardiovascular disease risk factors (hypertension, diabetes or hypercholesterolemia), coronary heart disease (angina pectoris or myocardial infarction), stroke, cardiac dysrhythmia, venous thromboembolism (pulmonary embolism or deep vein thrombosis), and arteritis originated from the biennial self-administered questionnaires sent before the start of follow-up, with subsequent updates in 2005, 2008, and 2011.

Statistical analysis

Multivariable Cox regression models, stratified by birth cohort (in 5-year categories) and with age as the time scale, were used to estimate HRs for the association of antiplatelet drug exposure with breast cancer incidence.

Exposure as well as other factors issued from the reimbursement database (including use of MHT, number of consultations, and use of other drugs) and covariates updated during follow-up were considered as time-varying parameters. For a given drug, participants contributed follow-up as nonexposed until purchasing the drug for the second time in a 3-month period. Cumulative duration/dose and time since last and first use were also updated during follow-up. Undiagnosed breast cancer may cause symptoms resulting in an increased use of drugs a few months prior to the diagnosis. Exposure as well as other variables coming from the reimbursement database were, therefore, lagged by 6 months to minimize any reverse causation bias due to early symptoms (16).

Educational level, recent mammogram, and established breast cancer risk factors (BMI, physical activity level, lifetime history of benign breast disease, family history of breast cancer, age at menarche, age at menopause, parity and age at first full-term pregnancy, lifetime use of oral contraceptives, lifetime use of MHT and alcohol consumption) were systematically included in the multivariable models. In addition, analyses of low-dose aspirin and clopidogrel were simultaneously adjusted for each other. Breastfeeding, number of consultations with the doctor during the preceding 6 months, smoking status, lifetime histories of comorbidities (cardiovascular disease risk factors, coronary heart disease, stroke, cardiac dysrhythmia, venous thromboembolism, and arteritis) and ever use of other drugs since January 1, 2004 (listed above) were tested as potential confounding factors. The categories used are displayed in **Table 1**. All drugs as well as number of consultations modified the HR of certain breast cancer subtypes associated with antiplatelet drug exposure by at least 0.05 point and were, therefore, included in the multivariable models. Alcohol consumption and breastfeeding had >5% missing values, which were accommodated by using a "missing" category in our models. All other covariates had <5% missing values, which were replaced either with the previous nonmissing questionnaire value, or with the mode or the median values observed among the subjects with complete data. A complete case analysis was also conducted (not shown because results were similar).

Effect modification by age, BMI, MHT use, comorbidities, and other drugs (all considered as time-varying parameters) was evaluated by including cross-product interaction terms in the Cox models.

When studying the risk of different breast cancers characterized by their invasiveness, molecular, histologic or stage/grade subtype, competing risk analysis was performed using the cause-specific hazards approach (17, 18). Cases with missing information on a given tumor characteristic were excluded from the corresponding analyses.

In sensitivity analyses, we changed the 6-month lag to a 2-year lag, and we restricted the study sample to women who self-reported having had a mammogram performed in the previous follow-up cycle. Finally, we defined exposure as low-dose aspirin only, clopidogrel only and combinations of low-dose aspirin and clopidogrel. A woman could successively take different types of antiplatelet drugs and therefore contribute to several categories (e.g., user of low-dose aspirin only and user of a combination of low-dose aspirin and clopidogrel).

All tests of statistical significance were two sided, and significance was set at the 0.05 level. We performed all analyses using SAS software, version 9.4 (SAS Institute Inc).

Data availability

The data and computing code required to replicate the results reported in this article are available upon duly motivated request by contacting A. Fournier.

Results

During a median follow-up time of 9 years, 2,864 breast cancer cases were diagnosed (335 *in situ* and 2,353 invasive) among the 62,512 participants. The characteristics of the participants are presented in **Table 1**. At the end of follow-up, 10,557 women (17%) had been exposed to low-dose aspirin and 2,130 (3%) to clopidogrel. The mean time elapsed between baseline and the date of purchasing the drug for the second time in a 3-month period was 3.8 years for low-dose aspirin and 4.0 years for clopidogrel. Compared with nonusers, antiplatelet

Table 1. Characteristics of participants, overall and according to antiplatelet drug use at the end of follow-up (E3N cohort; 2004–2014; $n = 62{,}512$).

Characteristics at the end of follow-up[a]	All women ($n = 62{,}512$)	Antiplatelet drug use at the end of follow-up: Low-dose aspirin ever users ($n = 10{,}557$)	Antiplatelet drug use at the end of follow-up: Clopidogrel ever users ($n = 2{,}130$)
Sociodemographic factors			
Age (years), mean (SD)	72.1 (6.4)	75.5 (6.5)	76.2 (6.4)
Educational level, *N* (%)			
<High school	6,516 (10)	1,295 (12)	278 (13)
From high school to 4 years higher education	45,138 (72)	7,569 (72)	1,528 (72)
At least 5 years higher education	10,858 (17)	1,693 (16)	324 (15)
Lifestyle and reproductive factors			
BMI (kg/m^2), *N* (%)			
<18.5	2,626 (4)	479 (5)	102 (5)
≥18.5–<23	25,409 (41)	3,668 (35)	742 (35)
≥23–<25	13,197 (21)	2,227 (21)	454 (21)
≥25–<30	16,139 (26)	3,034 (29)	597 (28)
≥30	5,141 (8)	1,149 (11)	235 (11)
Physical activity (MET-H/week), *N* (%)			
≤34.8	15,649 (25)	2,709 (26)	537 (25)
>34.8–≤57.6	15,680 (25)	2,512 (24)	507 (24)
>57.6–≤88.8	15,567 (25)	2,608 (25)	517 (24)
>88.8	15,616 (25)	2,728 (26)	569 (27)
Smoking status, *N* (%)			
Never smoker	33,281 (53)	5,899 (56)	1,169 (55)
Current smoker	4,741 (8)	738 (7)	192 (9)
Past smoker	24,490 (39)	3,920 (37)	769 (36)
Alcohol intake (grams/day), *N* (%)			
Abstainer	7,832 (13)	1,576 (15)	335 (16)
≤5	16,796 (27)	2,764 (26)	577 (27)
>5–≤10	9,357 (15)	1,482 (14)	302 (14)
>10–≤20	12,053 (19)	1,994 (19)	357 (17)
>20	12,522 (20)	1,994 (19)	392 (18)
Missing	3,952 (6)	747 (7)	167 (8)
Breastfeeding, *N* (%)			
Never	23,425 (37)	4,011 (38)	782 (37)
Ever	34,174 (55)	5,684 (54)	1,171 (55)
Missing	4,913 (8)	862 (8)	177 (8)
Age at menopause (years), mean (SD)	50.5 (3.7)	50.3 (4.0)	50.1 (4.3)
Age at menarche, years, *N* (%)			
<13	28,078 (45)	4,705 (45)	924 (43)
≥13	34,434 (55)	5,852 (55)	1,206 (57)
Parity and age at first full-term pregnancy, *N* (%)			
Nulliparous	7,282 (12)	1,253 (12)	224 (11)
First child before age 30 years, one or two children	31,393 (50)	4,922 (47)	1,006 (47)
First child before age 30 years, three or more children	17,373 (28)	3,340 (32)	691 (32)
First child after age 30 years	6,464 (10)	1,042 (10)	209 (10)
Lifetime oral contraceptive use, *N* (%)	38,570 (62)	5,304 (50)	1,069 (50)
Lifetime MHT use, *N* (%)	45,239 (72)	7,170 (68)	1,380 (65)
Self-report of a mammogram performed during the previous follow-up cycle, *N* (%)	51,097 (82)	7,673 (73)	1,468 (69)
Personal history of benign breast disease, *N* (%)	23,268 (37)	3,751 (36)	742 (35)
History of breast cancer in first-degree relatives, *N* (%)	7,139 (11)	1,200 (11)	231 (11)
Number of medical consultations/visits during the preceding 6 months, *N* (%)			
0	3,277 (5)	173 (2)	22 (1)
1–3	25,413 (41)	3,284 (31)	569 (27)
≥4	33,491 (54)	7,086 (67)	1,539 (72)
Missing	331 (1)	14 (0)	0 (0)
Lifetime medical history, *N* (%)			
Cardiac dysrhythmia	12,912 (21)	3,664 (35)	755 (35)
Cardiovascular disease risk factors[b]	41,355 (66)	8,606 (82)	1,804 (85)
Stroke	2,247 (4)	1,348 (13)	453 (21)
Coronary heart diseases[c]	1,913 (3)	1,300 (12)	619 (29)

(Continued on the following page)

Table 1. Characteristics of participants, overall and according to antiplatelet drug use at the end of follow-up (E3N cohort; 2004–2014; $n = 62{,}512$). (Cont'd)

Characteristics at the end of follow-up[a]	All women ($n = 62{,}512$)	Antiplatelet drug use at the end of follow-up: Low-dose aspirin ever users ($n = 10{,}557$)	Antiplatelet drug use at the end of follow-up: Clopidogrel ever users ($n = 2{,}130$)
Arteritis	716 (1)	358 (3)	242 (11)
Venous thromboembolism[d]	5,024 (8)	1,361 (13)	309 (15)
"Ever" use of other drugs[e], *N* (%)			
Proton pump inhibitors	30,063 (48)	6,514 (62)	1,527 (72)
Antihypertensives	2,662 (4)	911 (9)	213 (10)
Diuretics	8,859 (14)	2,774 (26)	681 (32)
Beta-blocking agents	14,271 (23)	4,793 (45)	1,228 (58)
Calcium channel blockers	8,561 (14)	3,132 (30)	844 (40)
Agents acting on the renin-angiotensin system	18,671 (30)	5,532 (53)	1,418 (67)
Statins	20,116 (32)	6,295 (60)	1,699 (80)
Antidiabetics	3,187 (5)	1,283 (12)	316 (15)
Other antithrombotic agents	8,163 (13)	2,340 (22)	539 (25)
Low-dose aspirin	10,557 (17)		1,391 (65)
Clopidogrel	2,130 (3)	1,374 (13)	

Abbreviations: MET-H, metabolic equivalent task-hour; SD, standard deviation.

[a]Except for years of schooling, physical activity level, age at menarche, parity and age at first birth, lifetime use of oral contraceptives, history of breast cancer in first-degree relatives and age at menopause, which were assessed before the start of follow-up.

[b]Cardiovascular disease risk factors include hypertension, diabetes or hypercholesterolemia.

[c]Coronary heart diseases include angina pectoris or myocardial infarction.

[d]Venous thromboembolisms include pulmonary embolism or deep vein thrombosis.

[e]"Ever" use was defined as at least two reimbursements of the drug of interest during any previous 3-month period since January 1, 2004.

drug users were older, more often overweight or obese, had more frequent medical follow-up, were less likely to have ever used oral contraceptives or MHT, and had more frequent histories of cardiovascular conditions as well as exposure to various drugs used in cardiovascular prevention.

The age-adjusted HR for breast cancer risk associated with having ever been exposed to low-dose aspirin, compared with having never been exposed, was 1.00 [95% confidence interval (CI), 0.89–1.14]. The multivariable HR was 0.99 (95% CI, 0.87–1.13; **Fig. 2**). No statistically significant heterogeneity across breast cancer subtypes was found ($P_{\text{homogeneity}} \geq 0.12$; **Fig. 2**). Analyses according to characteristics of use are presented in **Table 2**. Compared with never use, a higher breast cancer risk was found during the third year of cumulative use [$HR_{2-\leq 3 \text{ years of use}} = 1.49$ (1.08–2.07)] or when the cumulative number of DDDs was between 700 and 1,200 [$HR_{700-\leq 1{,}200 \text{ DDDs}} = 1.38$ (1.02–1.87)], while a lower breast cancer risk was found in the highest categories of cumulative number of DDDs [>1,700 DDDs: HR, 0.64 (0.45–0.92)], cumulative duration of use [>4 years: HR, 0.72 (0.52–0.99)] and time since first use [>4 years: 0.77 (0.62–0.96)]. The low-dose aspirin–breast cancer risk association differed according to history of venous thromboembolism ($P_{\text{homogeneity}} = 0.01$; Online Supplementary Table S1), suggesting a negative association among women with a history of venous thromboembolism [HR, 0.62 (0.41–0.93), n exposed cases = 29] but not among women without such a history [HR, 1.05 (0.92–1.21), *n* exposed cases = 270]. No effect modification was found by current age, BMI, ever use of MHT, other comorbidities and use of selected drugs ($P_{\text{interaction}} \geq 0.13$; **Table 3**; Online Supplementary Table S1).

The age-adjusted HR of breast cancer associated with having ever been exposed to clopidogrel, compared with having never been exposed, was 1.28 (95% CI, 1.00–1.64). The multivariable HR was 1.30 (95% CI, 1.02–1.68; **Fig. 3**). No statistically significant heterogeneity across breast cancer subtypes was found ($P_{\text{homogeneity}} \geq 0.17$; **Fig. 3**), except for estrogen receptor (ER) status. A statistically significant positive association was noted only for ER negative (ER^-) breast cancer [$HR_{ER^+} = 1.14$ (0.83–1.57); $HR_{ER^-} = 3.07$ (1.64–5.76); $P_{\text{homogeneity}} = 0.01$; **Fig. 3**]. Analyses according to characteristics of use yielded no statistically significant trend ($P_{\text{trend}} \geq 0.26$; **Table 2**). No effect modification was found by current age, BMI, comorbidities, and drugs ($P_{\text{interaction}} \geq 0.06$; **Table 3**; Online Supplementary Table S1). However, there was a statistically significant interaction by MHT use ($P_{\text{interaction}} = 0.01$; **Table 3**) suggesting a higher breast cancer risk with clopidogrel use only among MHT never users [HR, 1.94 (1.29–2.92)] but not among MHT ever users [HR, 1.07 (0.77–1.47)].

The results were not affected when we restricted the study sample to women with a recent mammogram (Online Supplementary Figs. S1 and S2), or applied a lag-time of 2 years (Online Supplementary Figs. S3 and S4). We still observed a higher breast cancer risk associated with 2–3 years cumulative duration of low-dose aspirin use when we restricted the analyses to women with a recent mammogram or to women without history of cardiovascular disease risk factors, coronary heart diseases, stroke, cardiac dysrhythmia, venous thromboembolism, and arteritis (Online Supplementary Table S2). When exposure was defined as low-dose aspirin only, clopidogrel only, combination of low-dose aspirin and clopidogrel, or none (reference), a statistically significant higher breast cancer risk was found with ever use of aspirin and clopidogrel combinations [HR, 1.55 (1.04–2.33), *n* exposed cases = 28], but not low-dose aspirin only [HR, 0.98 (0.86–1.12), *n* exposed cases = 288], or clopidogrel only [HR, 1.18 (0.88–1.57), *n* exposed cases = 55] ($P_{\text{homogeneity}} = 0.05$).

Discussion

In this cohort of postmenopausal women, a transient higher breast cancer risk was observed during the third year of low-dose aspirin use followed by a lower risk. Clopidogrel use was associated with higher

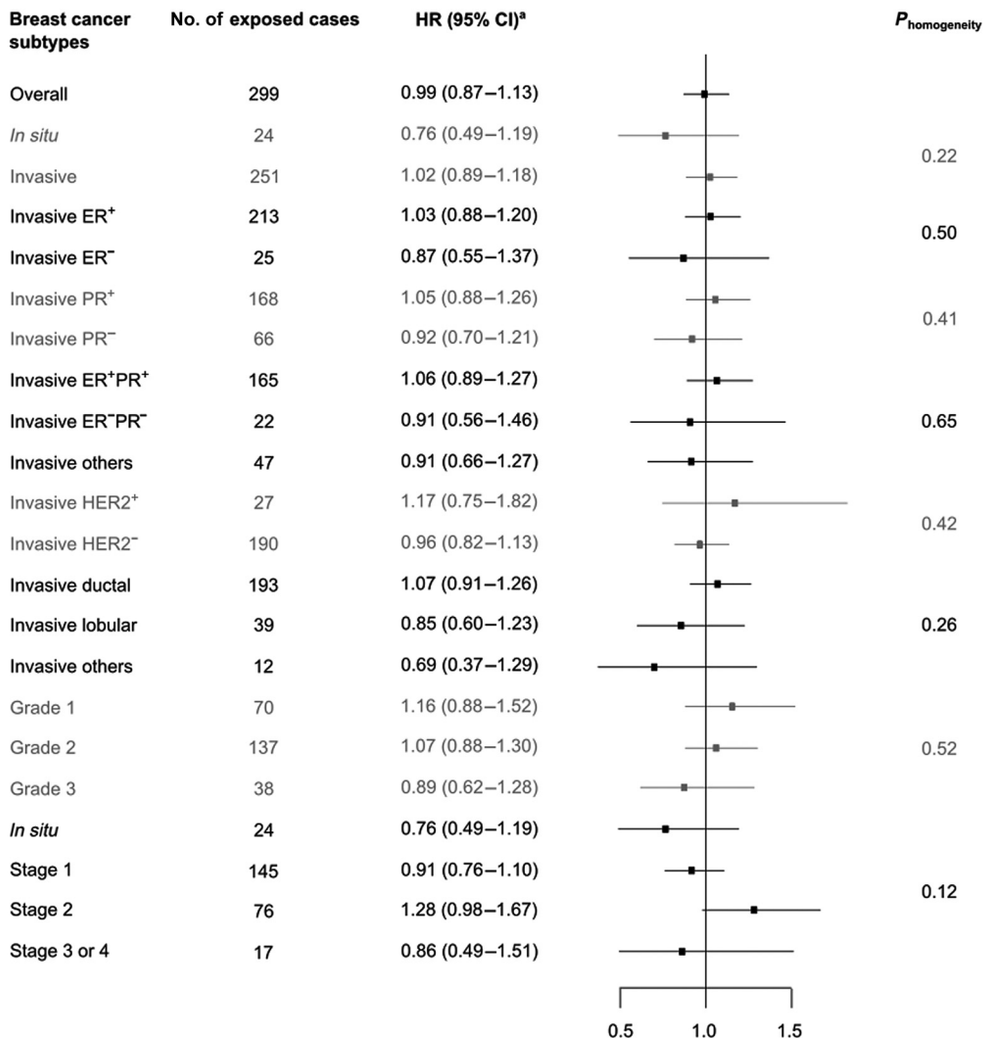

Figure 2.

Associations of low-dose aspirin ever use with breast cancer risk, compared with never use, overall and by breast cancer subtypes (E3N cohort; 2004–2014; n = 62,512). [a]Adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), BMI (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at menopause (baseline), history of breast cancer in first-degree relatives (baseline), personal history of benign breast disease (time-varying), lifetime use of menopausal hormone therapy (time-varying), self-report of a mammogram performed during the previous follow-up cycle (time-varying), number of medical consultations/visits during the preceding 6 months (time-varying), "ever" use of clopidogrel, statins, antihypertensives, diuretics, β-blocking agents, calcium channel blockers, agents acting on the renin–angiotensin system, antidiabetics, other antithrombotic agents, and proton pump inhibitors (time-varying). Categories used are those displayed in **Table 1**. HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

breast cancer risk that seemed restricted to ER^- tumors, with no clear trend according to duration of use.

Our results on low-dose aspirin are consistent with two recent meta-analyses which suggested that a long duration of any aspirin use was associated with a lower breast cancer risk (6, 7). However, these meta-analyses did not distinguish between low-dose and high-dose aspirin. We found few published prospective studies which have examined low-dose aspirin (19–26). Three studies noted a lower breast cancer risk with long-term exposure (19–21), while four suggested no statistically significant associations between low-dose aspirin and post-menopausal breast cancer risk (22–25), and one reported an increased breast cancer risk in women with at least 6 months of low-dose aspirin prescriptions (26). In a randomized controlled trial published in 2013 (Women's Health Study: participants ages ≥45 years old, median follow-up of 17.5 years), low-dose aspirin (100 mg) use every other day for an average of 10 years had no effect on breast cancer risk compared with placebo [HR, 1.02 (0.89–1.18), n exposed cases = 385] (4). This lack of effect could be due to the fact that low-dose aspirin was used less frequently than in usual prescriptions for cardiovascular prevention (i.e., daily), which could be less effective in terms of cancer prevention. In the latest randomized double-blind placebo-controlled trial (ASPREE: participants ages ≥ 65 years old, median follow-up of 4.7 years), daily use of low-dose aspirin (100 mg) had no effect on breast cancer incidence [HR, 1.03 (0.80–1.32), n exposed cases = 127] (27). This lack of effect could be due to the fact that follow-up as well as duration of aspirin use were not long

Table 2. Associations of low-dose aspirin and clopidogrel use with breast cancer risk, according to characteristics of use (E3N cohort; 2004–2014; $n = 62{,}512$).

	Low-dose aspirin exposure		Clopidogrel exposure	
	No. of cases	HR[a] (95% CI)	No. of cases	HR[a] (95% CI)
Cumulative number of defined daily doses				
Never exposed	2,565	1 (reference)	2,795	1 (reference)
≤200	58	1.00 (0.77–1.30)	12	1.20 (0.68–2.14)
>200–≤700	55	0.87 (0.66–1.15)	19	1.43 (0.90–2.27)
>700–≤1,200	46	1.38 (1.02–1.87)	8	1.23 (0.61–2.48)
>1,200–≤1,700	25	1.32 (0.88–1.97)	2	0.52 (0.13–2.09)
>1,700	32	0.64 (0.45–0.92)	14	1.53 (0.90–2.60)
Unknown	83	1.06 (0.84–1.33)	14	1.33 (0.78–2.25)
P_{trend}[b]		*0.71*		*0.27*
Cumulative duration of use (years)				
Never exposed	2,565	1 (reference)	2,795	1 (reference)
≤1	88	0.98 (0.79–1.22)	20	1.20 (0.77–1.88)
>1–≤2	34	0.88 (0.62–1.24)	14	1.69 (0.99–2.88)
>2–≤3	38	1.49 (1.08–2.07)	5	1.06 (0.44–2.57)
>3–≤4	20	1.24 (0.79–1.94)	2	0.64 (0.16–2.56)
>4	40	0.72 (0.52–0.99)	14	1.35 (0.73–2.30)
Unknown	79	1.05 (0.83–1.32)	14	1.39 (0.82–2.36)
P_{trend}[b]		*0.71*		*0.26*
Time since first use (years)				
Never exposed	2,565	1 (reference)	2,795	1 (reference)
≤1	43	1.12 (0.82–1.52)	8	0.97 (0.48–1.97)
>1–≤2	36	1.05 (0.75–1.47)	10	1.39 (0.74–2.62)
>2–≤3	36	1.21 (0.87–1.70)	10	1.64 (0.87–3.10)
>3–≤4	25	1.03 (0.69–1.54)	5	1.09 (0.45–2.66)
>4	98	0.77 (0.62–0.96)	26	1.41 (0.95–2.11)
Unknown	61	1.21 (0.93–1.57)	10	1.31 (0.70–2.45)
P_{trend}[b]		*0.77*		*0.90*
Age at first use (years)				
Never exposed	2,565		2,795	
≤65	86	0.86 (0.67–1.03)	23	1.75 (1.14–2.67)
>65–≤75	109	1.13 (0.93–1.39)	22	1.08 (0.70–1.67)
>75	32	0.95 (0.65–1.39)	11	1.22 (0.66–2.26)
Unknown	72	1.07 (0.84–1.37)	13	1.23 (0.71–2.13)
P_{trend}[b]		*0.77*		*0.90*

[a]Adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), body mass index (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at menopause (baseline), history of breast cancer in first-degree relatives (baseline), personal history of benign breast disease (time-varying), lifetime use of menopausal hormone therapy (time-varying), self-report of a mammogram performed during the previous follow-up cycle (time-varying), number of medical consultations/visits during the preceding 6 months (time-varying), "ever" use of statins, antihypertensives, diuretics, β-blocking agents, calcium channel blockers, agents acting on the renin–angiotensin system, antidiabetics, other antithrombotic agents and proton pump inhibitors (time-varying). Categories used are those displayed in **Table 1**. Low-dose aspirin and clopidogrel exposure were simultaneously adjusted for each other. HRs were obtained from separate models including one characteristic of exposure at a time.

[b]Tests for linear trends were performed among exposed women with known characteristics of exposure. The characteristics of use were considered as continuous variables.

enough to expect a benefit of aspirin. Indeed, in a meta-analysis of randomized controlled trials published in 2018, among participants aged ≥ 70 years and weighing less than 70 kg, an increased risk of any cancer was observed during the first 3 years of follow-up [HR, 1.31 (1.07–1.61)], followed by a reduced cancer incidence after 5 years of follow-up [HR, 0.62 (0.41–0.94)] (28). The increased cancer risk with aspirin use within 3 years of follow-up was stronger among women [HR, 1.44 (1.11–1.87)]. In our study, we found a transient higher breast cancer risk during the third year of use [HR, 1.49 (1.08–2.07)], followed by a lower breast cancer risk after 4 years of use [HR, 0.72 (0.52–0.99)]. After 5 years of aspirin use, we also found a lower breast cancer risk [HR, 0.66 (0.44–1.01)]; however, the association did not reach the significance probably due to the fact that the number of cases was limited (n exposed cases = 23). Our study thus shows close estimates compared with the ones found in the latest meta-analysis of randomized controlled trials on any cancer incidence and aspirin use. These results indicate that low-dose aspirin might have a tumor-promoting effect, that is, accelerate the growth of preexisting tumors that would have otherwise been diagnosed later. It seems thus important to be able to assess the low-dose aspirin–breast cancer risk associations according to duration of use.

To our knowledge, this is the first study to evaluate the clopidogrel–breast cancer association. In a randomized controlled trial, 30 months of simultaneous use of low-dose aspirin and clopidogrel compared with low-dose aspirin used alone was associated with a nonsignificant increase in risk of any solid cancer and a statistically significant increase in cancer-related death (29). Since then, three observational studies noted that clopidogrel use, alone or in combination with low-dose aspirin, might reduce the risk of several cancers (30–32) including colorectal, other gastrointestinal, nongastrointestinal, or hematologic

Table 3. Associations of low-dose aspirin and clopidogrel ever use with breast cancer risk, compared with never use of the considered drug, stratified by current age, BMI and MHT use (E3N cohort; 2004–2014; $n = 62{,}512$).

	Low-dose aspirin–breast cancer associations			Clopidogrel–breast cancer associations		
Strata	**No. of exposed cases**	**HR[a] (95% CI)**	**$P_{interaction}$**	**No. of exposed cases**	**HR[a] (95% CI)**	**$P_{interaction}$**
Current age (years)[b]						
≤71.2 (median age)	145	0.99 (0.82–1.19)	0.81	29	1.36 (0.93–2.00)	0.85
>71.2 (median age)	154	0.98 (0.81–1.18)		40	1.36 (0.97–1.91)	
BMI (kg/m^2)[b]						
<25	177	1.05 (0.88–1.24)	0.28	34	1.11 (0.78–1.58)	0.16
≥25	122	0.92 (0.75–1.13)		35	1.60 (1.12–2.29)	
MHT use[b]						
Never	77	0.82 (0.63–1.07)	0.17	28	1.94 (1.29–2.92)	0.01
Ever	222	1.06 (0.91–1.23)		41	1.07 (0.77–1.47)	

[a]Adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), body mass index (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at menopause (baseline), history of breast cancer in first-degree relatives (baseline), personal history of benign breast disease (time-varying), lifetime use of menopausal hormone therapy (time-varying), self-report of a mammogram performed during the previous follow-up cycle (time-varying), number of medical consultations/visits during the preceding 6 months (time-varying), "ever" use of statins, antihypertensives, diuretics, β-blocking agents, calcium channel blockers, agents acting on the renin–angiotensin system, antidiabetics, other antithrombotic agents and proton pump inhibitors (time-varying). Categories used are those displayed in **Table 1**. Low-dose aspirin and clopidogrel exposure were simultaneously adjusted for each other.

[b]All selected factors were considered as time-varying parameters.

cancers (32). In one of these studies, use of clopidogrel for less than 1 year was associated with an increased risk of colorectal cancer but the authors suggested that this association could be explained by detection bias (30).

Platelet inhibition could have different effects depending on the drug used, because low-dose aspirin and clopidogrel have specific targets. Although low-dose aspirin inhibits cyclooxygenase-1 enzyme synthesis in platelets and consequently suppresses the production of prostaglandins and thromboxanes (33), the active metabolite of clopidogrel selectively inhibits the binding of adenosine diphosphate to its platelet receptor, resulting in stabilization of platelet aggregation. Low-dose aspirin might interfere with additional antiplatelet-independent mechanisms, such as the inhibition of Wnt/β-catenin and NFκB signaling and the acetylation of extra-cyclooxygenase proteins, which could explain why low-dose aspirin would be chemopreventive and not clopidogrel (11). The mechanisms by which clopidogrel might increase breast cancer risk must be investigated. It has been suggested that the simultaneous use of low-dose aspirin and clopidogrel could lead to an excess of platelet inactivation and to the dissemination of cancer cells due to the unstable aggregates of platelet-tumor cells (34). In addition, a recent experimental study in mice reported that simultaneous injection of low-dose aspirin and clopidogrel could promote mammary tumor progression by inducting vascular mimicry (35). Our results support this hypothesis as the higher breast cancer risk was found with simultaneous use of low-dose aspirin and clopidogrel but not with use of low-dose aspirin or clopidogrel only, even though the power of this subanalysis was limited.

We found a statistically significant interaction between use of low-dose aspirin and history of venous thromboembolism and between clopidogrel and MHT use, regarding the risk of breast cancer. The clopidogrel–breast cancer association also differed according to ER status of breast tumors. To our knowledge, these heterogeneities have not been explored previously. However, because our findings were based on relatively small numbers of cases in subgroups and because 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.

The main strengths of this study included its prospective design and the use of information from a drug reimbursement database to identify antiplatelet drug exposure, which avoids differential recall bias between cases and noncases and allowed us to consider precise information on exposure (including duration, DDD, and timing of use). However, because most users were still taking the drugs at the end of follow-up, we did not consider time since last use of antiplatelet drugs. Because data on antiplatelet drug reimbursement were combined with self-reported data on lifestyle, reproductive and medical factors, we were able to take into consideration potential confounders and effect modifiers. Power to detect differences was limited because of the low number of cases in some categories (especially for clopidogrel), and we cannot exclude residual confounding, including confounding by indication for drug use. Because of the lack of information available on over-the-counter low-dose aspirin purchases, we could have misclassified over-the-counter users as nonexposed. However, we assumed that the long-term use of low-dose aspirin is primarily managed through prescriptions (36). Misclassification for clopidogrel treatment is unlikely because it is a prescription drug. We had no data regarding the compliance/adherence to the dispensed treatment, but we defined "ever" users as women with at least two reimbursements during a 3-month period, which would suggest that they took the drug. Another concern is the possibility of surveillance bias, but we adjusted our analyses for recent mammogram and number of consultations with a physician to overcome it. Finally, results on antiplatelet drugs other than low-dose aspirin and clopidogrel could help to clarify how antiplatelet drugs might influence breast cancer risk, but our power was too limited to investigate the associations between breast cancer risk and other antiplatelet drugs such as ticlopidine and prasugrel.

To conclude, in this large, prospective cohort of postmenopausal women, use of low-dose aspirin was associated with a transient higher breast cancer risk few years after treatment start, followed by a lower breast cancer risk, while ever use of clopidogrel was associated with a higher breast cancer risk that seemed restricted to ER$^-$ tumors. These results are novel and need to be replicated in other settings.

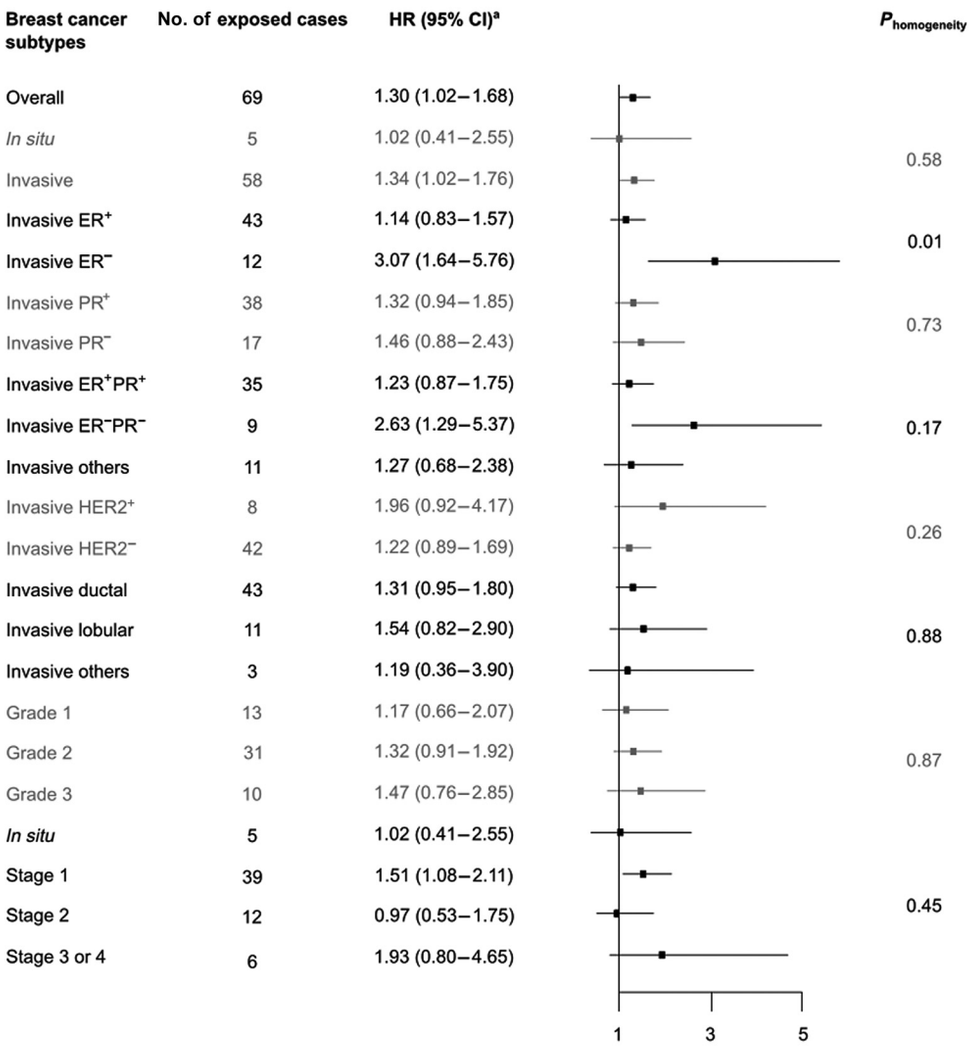

Figure 3.

Associations of clopidogrel ever use with breast cancer risk, compared with never use, overall and by breast cancer subtypes (E3N cohort; 2004–2014; $n = 62{,}512$). [a]Adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), body mass index (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at menopause (baseline), history of breast cancer in first-degree relatives (baseline), personal history of benign breast disease (time-varying), lifetime use of menopausal hormone therapy (time-varying), self-report of a mammogram performed during the previous follow-up cycle (time-varying), number of medical consultations/visits during the preceding 6 months (time-varying), "ever" use of low-dose aspirin, statins, antihypertensives, diuretics, β-blocking agents, calcium channel blockers, agents acting on the renin–angiotensin system, antidiabetics, other antithrombotic agents, and proton pump inhibitors (time-varying). Categories used are those displayed in **Table 1**. HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Authors' Disclosures

No disclosures were reported.

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.

Authors' Contributions

M. Cairat: Conceptualization, software, formal analysis, funding acquisition, writing–original draft. **M. Al Rahmoun:** Writing–review and editing. **M.J. Gunter:** Resources, Writing–review and editing. **G. Severi:** Resources, investigation, writing–review and editing. **L. Dossus:** Conceptualization, supervision, funding acquisition, writing–review and editing. **A. Fournier:** Conceptualization, software, supervision, writing–review and editing.

Acknowledgments

The research was carried out using data from the Inserm (French National Institute for Health and Medical Research) E3N cohort, which was established and maintained with the support of the Mutuelle Générale de l'Education Nationale (MGEN), Gustave Roussy, and the French League against Cancer (LNCC). E3N-E4N is also supported by the French National Research Agency (ANR) under the Investment for the future Program (PIA; ANR-10-COHO-0006) and by the French Ministry of Higher Education, Research and Innovation (subsidy for public service charges No. 2102 918823). This study is listed at clinicaltrials.gov as NCT03285230. We are grateful to the study participants for their continued participation and to medical practitioners for providing pathology reports. We also thank all members of

the E3N-EPIC study group, in particular, Rafika Chaït, Ghizlane Esselma, Marie Fangon, Pascale Gerbouin-Rérolle, Lyan Hoang, Roselyn Gomes and the data management team, and Amandine Gelot for data management and/or technical assistance. The work reported in this article was performed during A. Fournier's term as a Visiting Scientist at the International Agency for Research on Cancer.

M. Cairat was supported by a research scholarship from the Ligue Contre le Cancer. M. Al Rahmoun was supported by a research scholarship from the French National Cancer Institute (INCa_13539).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 1, 2020; revised December 3, 2020; accepted January 15, 2021; published first February 2, 2021.

References

1. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. Blood 2015;126:582–8.
2. Xu XR, Yousef GM, Ni H. Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents. Blood 2018;131:1777–89.
3. Qiao Y, Yang T, Gan Y, Li W, Wang C, Gong Y, et al. Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. BMC Cancer 2018;18:288.
4. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Ann Intern Med 2013;159:77–85.
5. Ye X, Fu J, Yang Y, Chen S. Dose-risk and duration-risk relationships between aspirin and colorectal cancer: a meta-analysis of published cohort studies. PLoS One 2013;8:e57578.
6. Lu L, Shi L, Zeng J, Wen Z. Aspirin as a potential modality for the chemoprevention of breast cancer: a dose-response meta-analysis of cohort studies from 857,831 participants. Oncotarget 2017;8:40389–401.
7. Cao Y, Tan A. Aspirin might reduce the incidence of breast cancer: an updated meta-analysis of 38 observational studies. Medicine 2020;99:e21917.
8. Cairat M, Al Rahmoun M, Gunter MJ, Severi G, Dossus L, Fournier A. Use of nonsteroidal anti-inflammatory drugs and breast cancer risk in a prospective cohort of postmenopausal women. Breast Cancer Res 2020;22:118.
9. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 1998;396:77–80.
10. Zhang X, Feng Y, Liu X, Ma J, Li Y, Wang T, et al. Beyond a chemopreventive reagent, aspirin is a master regulator of the hallmarks of cancer. J Cancer Res Clin Oncol 2019;145:1387–403.
11. Dovizio M, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. Recent Results Cancer Res 2013;191:39–65.
12. Clavel-Chapelon F. Cohort profile: the French E3N cohort study. Int J Epidemiol 2015;44:801–9.
13. Mega JL, Simon T. Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments. Lancet 2015;386:281–91.
14. WHO Collaborating Centre for Drug Statistics Methodology. DDD: Definition and general considerations. Available from: http://www.whocc.no/ddd/definition_and_general_considera/.
15. Fournier A, Fabre A, Mesrine S, Boutron-Ruault MC, Berrino F, Clavel-Chapelon F. Use of different postmenopausal hormone therapies and risk of histology- and hormone receptor-defined invasive breast cancer. J Clin Oncol 2008;26:1260–8.
16. Pottegard A, Hallas J. New use of prescription drugs prior to a cancer diagnosis. Pharmacoepidemiol Drug Saf 2017;26:223–7.
17. Pintilie M. Analysing and interpreting competing risk data. Stat Med 2007;26:1360–7.
18. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995;51:524–32.
19. Ready A, Velicer CM, McTiernan A, White E. NSAID use and breast cancer risk in the VITAL cohort. Breast Cancer Res Treat 2008;109:533–43.
20. Clarke CA, Canchola AJ, Moy LM, Neuhausen SL, Chung NT, Lacey JV Jr, et al. Regular and low-dose aspirin, other non-steroidal anti-inflammatory medications and prospective risk of HER2-defined breast cancer: the California Teachers Study. Breast Cancer Res 2017;19:52.
21. Yang YS, Kornelius E, Chiou JY, Lai YR, Lo SC, Peng CH, et al. Low-dose aspirin reduces breast cancer risk in women with diabetes: a nationwide retrospective cohort study in Taiwan. J Womens Health 2017;26:1278–84.
22. Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, et al. Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. Cancer Res 2003;63:6096–101.
23. Ajrouche A, De Rycke Y, Dalichampt M, Messika Zeitoun D, Hulot JS, Estellat C, et al. Reduced risk of cancer among low-dose aspirin users: data from French health care databases. Pharmacoepidemiol Drug Saf 2019;28:1258–66.
24. Kim S, Shore DL, Wilson LE, Sanniez EI, Kim JH, Taylor JA, et al. Lifetime use of nonsteroidal anti-inflammatory drugs and breast cancer risk: results from a prospective study of women with a sister with breast cancer. BMC Cancer 2015;15:960.
25. Hollestein LM, van Herk-Sukel MP, Ruiter R, de Vries E, Mathijssen RH, Wiemer EA, et al. Incident cancer risk after the start of aspirin use: results from a Dutch population-based cohort study of low dose aspirin users. Int J Cancer 2014;135:157–65.
26. Tsoi KKF, Ho JMW, Chan FCH, Sung JJY. Long-term use of low-dose aspirin for cancer prevention: a 10-year population cohort study in Hong Kong. Int J Cancer 2019;145:267–73.
27. McNeil JJ, Gibbs P, Orchard SG, Lockery JE, Bernstein WB, Cao Y, et al. Effect of aspirin on cancer incidence and mortality in older adults. J Natl Cancer Inst 2020 Aug 11 [Epub ahead of print].
28. Rothwell PM, Cook NR, Gaziano JM, Price JF, Belch JFF, Roncaglioni MC, et al. Effects of aspirin on risks of vascular events and cancer according to bodyweight and dose: analysis of individual patient data from randomised trials. Lancet 2018;392:387–99.
29. Serebruany VL, Cherepanov V, Golukhova EZ, Kim MH. The dual antiplatelet therapy trial after the FDA update: noncardiovascular deaths, cancer and optimal treatment duration. Cardiology 2015;132:74–80.
30. Rodriguez-Miguel A, Garcia-Rodriguez LA, Gil M, Montoya H, Rodriguez-Martin S, de Abajo FJ. Clopidogrel and low-dose aspirin, alone or together, reduce risk of colorectal cancer. Clin Gastroenterol Hepatol 2019;17:2024–33.e2.
31. Kuan YC, Huang KW, Lin CL, Luo JC, Kao CH. Effects of aspirin or clopidogrel on colorectal cancer chemoprevention in patients with type 2 diabetes mellitus. Cancers 2019;11:1468.
32. Leader A, Zelikson-Saporta R, Pereg D, Spectre G, Rozovski U, Raanani P, et al. The effect of combined aspirin and clopidogrel treatment on cancer incidence. Am J Med 2017;130:826–32.
33. Li H, Lee MH, Liu K, Wang T, Song M, Han Y, et al. Inhibiting breast cancer by targeting the thromboxane A2 pathway. NPJ Precis Oncol 2017;1:8.
34. Serebruany VL, Cherepanov V, Cabrera-Fuentes HA, Kim MH. Solid cancers after antiplatelet therapy: confirmations, controversies, and challenges. Thromb Haemost 2015;114:1104–12.
35. Smeda M, Kieronska A, Proniewski B, Jasztal A, Selmi A, Wandzel K, et al. Dual antiplatelet therapy with clopidogrel and aspirin increases mortality in 4T1 metastatic breast cancer-bearing mice by inducing vascular mimicry in primary tumour. Oncotarget 2018;9:17810–24.
36. Cea Soriano L, Soriano-Gabarró M, García Rodríguez LA. Validation of low-dose aspirin prescription data in The Health Improvement Network: how much misclassification due to over-the-counter use? Pharmacoepidemiol Drug Saf 2016;25:392–8.