



# Inflammation and breast cancer risk in the EPIC cohort

Manon Cairat

## ► To cite this version:

Manon Cairat. Inflammation and breast cancer risk in the EPIC cohort. Cancer. Université de Lyon, 2020. English. NNT : 2020LYSE1211 . tel-03656232

**HAL Id: tel-03656232**

**<https://theses.hal.science/tel-03656232>**

Submitted on 2 May 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



N° d'ordre NNT : 2020LYSE1211

**THESE de DOCTORAT DE L'UNIVERSITE DE LYON**  
opérée au sein de  
**l'Université Claude Bernard Lyon 1**

**Ecole Doctorale N° 205**  
**Ecole Doctorale Interdisciplinaire Science-Santé (EDISS)**

Spécialité de doctorat :  
**Epidémiologie, Santé Publique, Recherche sur les services de santé**  
Discipline :  
**Epidémiologie**

Soutenue publiquement le 02/11/2020, par :  
**Manon Cairat**

---

# **INFLAMMATION ET RISQUE DE CANCER DU SEIN DANS LA COHORTE EPIC**

---

Devant le jury composé de :

<b>Antoine PARIENTE</b> , MD, PhD	Inserm, UMR 1219, Bordeaux (France) Université de Bordeaux, Bordeaux (France)	Rapporteur
<b>Anton POTTEGÅRD</b> , PhD	University of Southern Denmark, Odense (Denmark)	Rapporteur
<b>Edoardo BOTTERI</b> , PhD	Oslo University Hospital, Oslo (Norway)	Rapporteur
<b>Béatrice FERVERS</b> , MD, PhD	Centre Léon Bérard, Lyon (France) Université Claude Bernard Lyon I, Lyon (France)	Examinatrice
<b>Florence MENEGAUX</b> , MD, PhD	Inserm, UMR 1018, Villejuif (France)	Examinatrice
<b>Mahmoud ZUREIK</b> , PhD	Agence nationale de sécurité du médicament et des produits de santé, Saint-Denis (France)	Examineur
<b>Talita DUARTE SALLES</b> , PhD	Institut Universitari d'Investigació en Atenció Primària Jordi Gol, Barcelone (Espagne)	Examinatrice
<b>Laure DOSSUS</b> , PhD	Centre international de recherche sur le cancer, Lyon (France)	Directrice de thèse



## **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.

## **THIS THESIS HAS BEEN PREPARED IN THE FOLLOWING INSTITUTE**

International Agency for Research on Cancer  
World Health Organization  
150, cours Albert Thomas 69372 Lyon Cedex 08, France

## **UNDER THE SUPERVISION OF**

Laure DOSSUS, PhD, Biomarker Group, Nutrition and Metabolism Section  
International Agency for Research on Cancer

## **AND SUPPORTED BY**

Ligue nationale contre le cancer  
Allocation doctorale de recherche 2017

**International Agency for Research on Cancer**



# **INFLAMMATION AND BREAST CANCER RISK IN THE EPIC COHORT**

## ABSTRACT

Chronic inflammation might promote breast cancer development. Therefore, the possibility of stemming tumorigenic inflammatory effects with pharmacological compounds has drawn significant interest.

The objective of my doctoral thesis was to better understand the role of inflammation on breast cancer development.

First, I evaluated the associations of eleven inflammatory biomarkers (cytokines and adipokines) with breast cancer risk in about 1,600 case-control pairs from the EPIC cohort. The results suggested that a higher leptin-to-adiponectin ratio decreased the risk of peri/premenopausal breast cancer, while high levels of TNF- $\alpha$  increased postmenopausal breast cancer risk.

Then, I evaluated the associations of several anti-inflammatory and antiplatelet drugs with breast cancer risk using self-reported (EPIC cohort) and drug reimbursement (E3N cohort) data. Nonsteroidal anti-inflammatory drugs were not associated with breast cancer risk. However, at antiplatelet dosage, aspirin use was associated with a transient higher breast cancer risk few years after treatment start, followed by a lower breast cancer risk. Another antiplatelet drug, clopidogrel, was associated with a higher breast cancer risk, regardless the duration of use. Glucocorticoid use was associated with a lower risk of invasive breast cancer, that seemed restricted to oestrogen-dependant and stage 1 or 2 tumours and was associated with a higher risk of *in situ* and stage 3/4 breast cancers.

The findings of this thesis suggest that inflammation plays a modest role in breast cancer development and that its impact on breast cancer could be limited to certain sub-populations or certain breast cancer subtypes.

### Keywords

Breast Cancer, Inflammation, Interleukins, Adipokines, Nonsteroidal Anti-Inflammatory Drugs, Glucocorticoids, Antiplatelet Drugs, Aspirin, Epidemiology.

## RESUME

Le cancer du sein pourrait être favorisé par l'inflammation chronique et par conséquent être prévenu par l'utilisation de médicaments agissant sur l'inflammation.

L'objectif de ma thèse était de mieux comprendre le rôle de l'inflammation sur le développement du cancer du sein.

J'ai d'abord évalué les associations entre onze biomarqueurs de l'inflammation (cytokines et adipokines) et le risque de cancer du sein chez environ 1600 paires cas-témoin de la cohorte EPIC. Le ratio leptine/adiponectine était associé à une diminution du risque de cancer du sein chez les femmes avant la ménopause alors que des niveaux élevés de TNF- $\alpha$  étaient associés à un risque accru de cancer du sein chez les femmes ménopausées.

Ensuite, j'ai évalué les associations entre plusieurs médicaments anti-inflammatoires et antiagrégants plaquettaires et le risque de cancer du sein en utilisant des données auto-rapportées (cohorte EPIC) ou de remboursement de médicaments (cohorte E3N). Globalement, l'utilisation d'anti-inflammatoires non stéroïdiens n'était pas associée au risque de cancer du sein alors, alors qu'une durée d'utilisation longue d'aspirine, en tant qu'antiagrégant plaquettaire et donc à faible dose ( $\leq 325$  mg), était associée à une diminution du risque de ce cancer. En revanche, un autre antiagrégant plaquettaire, le clopidogrel, était associé à une augmentation du risque de ce cancer, indépendamment de sa durée d'utilisation. L'utilisation de glucocorticoïdes était associée à une diminution du risque de cancer du sein infiltrants, dépendants aux œstrogènes et de stades 1 et 2 mais à une augmentation du risque de cancer *in situ* et de stades 3/4.

Les résultats de cette thèse suggèrent que l'inflammation joue un rôle mineur dans le développement du cancer du sein.

### Mots-clés

Cancer du sein, inflammation, interleukines, adipokines, anti-inflammatoires non stéroïdiens, antiagrégants plaquettaires, glucocorticoïdes, épidémiologie.

## RESUME SUBSTANTIEL

Au cours des dernières décennies, les résultats des études expérimentales et épidémiologiques ont suggéré que l'inflammation chronique pourrait favoriser le développement du cancer du sein. Ces observations ont conduit à l'hypothèse d'une possible prévention de ce cancer grâce à l'utilisation de médicaments agissant sur l'inflammation tels que les anti-inflammatoires non stéroïdiens (AINS), l'aspirine à faible dose et les glucocorticoïdes. Cependant, les études épidémiologiques sur les biomarqueurs d'inflammation ou sur les médicaments anti-inflammatoires et le risque de cancer du sein sont soit rares, soit hétérogènes.

L'objectif de ma thèse était de mieux comprendre le rôle de l'inflammation sur le développement du cancer du sein grâce à deux approches complémentaires :

- 1) En évaluant les associations entre 11 cytokines et adipokines et le risque de cancer du sein.
- 2) En évaluant les associations entre plusieurs médicaments anti-inflammatoires et antiagrégants plaquettaires et le risque de cancer du sein, par le biais de données auto-rapportées ou de remboursement de médicaments.

Différents projets ont été menés dans l'étude prospective européenne sur le cancer et la nutrition (cohorte EPIC), et sa composante française, l'étude épidémiologique auprès de femmes de la mutuelle générale de l'éducation nationale (cohorte E3N).

La cohorte européenne EPIC regroupe plus de 500 000 participants dans 10 pays européens, suivis depuis 1990. Les informations relatives au mode de vie et aux facteurs reproductifs ont été recueillis par auto-questionnaire à l'inclusion. Cinq pays (France, Allemagne, Pays-Bas, Royaume-Uni et Danemark) ont aussi collecté des données auto-rapportées sur l'utilisation régulière d'AINS. La plupart des participants ont également fourni un échantillon sanguin. L'identification des cas de cancers incidents pendant le suivi repose sur le croisement de registres de cancers, des dossiers d'assurance et hospitaliers, et d'un suivi actif par le contact des participants et de leurs proches.

L'étude E3N s'appuie sur une cohorte de 98 995 femmes volontaires françaises, adhérentes à la Mutuelle Générale de l'Education Nationale (MGEN), suivies depuis 1990. Les informations relatives au mode de vie, aux facteurs reproductifs et aux antécédents médicaux ont été recueillies tous les deux à trois ans par auto-questionnaires. Les cancers auto-déclarés

sont validés par obtention des comptes rendus anatomopathologiques. Depuis 2004, les données de remboursement de médicaments issues de la MGEN sont aussi disponibles

Dans une première partie, j'ai évalué les associations entre des niveaux circulants de biomarqueurs inflammatoires (cytokines et adipokines) et le risque de cancer du sein, globalement et selon le sous-type tumoral, le statut ménopausique, l'adiposité et l'utilisation d'hormones exogènes chez environ 1 600 paires cas-témoin sélectionnés dans la cohorte EPIC. Les résultats de cette étude ont montré que le ratio leptine/adiponectine étaient associés à une diminution du risque de cancer du sein chez les femmes avant la ménopause alors que des niveaux élevés de TNF- $\alpha$  étaient associés à un risque accru de cancer du sein chez les femmes ménopausées.

Dans une seconde partie, j'ai évalué les associations entre l'utilisation d'AINS et le risque de cancer, globalement et selon le sous-type tumoral, le statut ménopausique et les principaux facteurs de risque de cancer du sein. Durant un suivi moyen de 13 ans, 7 379 cas de cancer du sein incidents ont été diagnostiqués chez les 140 981 participantes incluses (âge moyen au début du suivi : 53 ans). L'utilisation régulière d'AINS n'était pas associée au risque de cancer du sein, globalement et par sous-type de cancer du sein. Cependant, une interaction a été observée entre l'utilisation régulière d'AINS et l'utilisation de traitement hormonaux de la ménopause (THM) chez les femmes ménopausées suggérant une diminution du risque de cancer du sein chez celles ayant déjà utilisées des THMs.

Ensuite, j'ai évalué les associations entre l'utilisation d'AINS, d'aspirine faible dose et d'un autre antiagrégant plaquettaire, le clopidogrel, et de glucocorticoïdes avec le risque de cancer du sein dans la cohorte E3N. Durant un suivi moyen de 9 ans, 2 887 cas de cancer du sein ont été identifiés chez les 62 512 femmes ménopausées suivies (âge moyen au début du suivi : 63 ans). Globalement, l'utilisation d'AINS n'était pas associée au risque de cancer du sein. Cependant, l'utilisation d'AINS était associée à une diminution du risque de cancer du sein chez les femmes ayant aussi utilisées des inhibiteurs de la pompe à protons.

Une longue durée d'utilisation d'aspirine à faible dose ( $\leq 325$  mg et donc en tant qu'antiagrégant plaquettaire) était associée à une diminution du risque de cancer du sein tandis qu'un autre antiagrégant plaquettaire, le clopidogrel, était associé à une augmentation du risque de ce cancer, plus particulièrement pour le cancer du sein non-dépendant aux œstrogènes, et ce quelle que soit sa durée d'utilisation. L'utilisation de glucocorticoïdes était

associée à une augmentation du risque de cancer du sein *in situ* et une diminution du risque de cancer infiltrants. Parmi les cancers du sein infiltrants, les glucocorticoïdes étaient associés à une diminution des cancers du sein de stades 1, 2 et dépendants aux œstrogènes, et associés à une augmentation du risque des cancers du sein de stades 3 et 4.

Les résultats de cette thèse suggèrent que :

- L'inflammation joue un rôle mineur dans le développement du cancer du sein. Cependant, elle pourrait être impliquée dans certains sous-groupes, et plus particulièrement chez les femmes ménopausées.
- Les effets des médicaments antiagrégants plaquettaires/anti-inflammatoires sur le risque de cancer du sein chez les femmes ménopausées semblent complexes et dépendre des sous-types et facteurs de risque de cancer du sein, de la durée d'utilisation ainsi que de l'utilisation d'autres médicaments.

Le rôle de l'inflammation ainsi que celui des médicaments anti-inflammatoires/anti-agrégants plaquettaires sur le développement du cancer du sein doit être confirmé dans des études supplémentaires. Les prochaines études épidémiologiques doivent prendre en compte les sous-types et facteurs de risque de cancer du sein, et pour les médicaments, la dose et la durée d'utilisation ainsi que l'utilisation d'autres médicaments.

## ACKNOWLEDGMENTS

I would like to express my grateful acknowledgments to all the people who make this adventure possible and support me throughout these 3 years

To Laure Dossus, my doctoral supervisor, who gave me the opportunity to discover the field of cancer research and pursue a doctoral thesis. I am very grateful for your incredible support and your valuable day-to-day guidance.

To Agnès Fournier, who co-supervised a large part of my doctoral thesis and make me benefit from her expertise. Thank you for your esteemed advice and numerous feedbacks.

To all the people who contributed directly or indirectly to the achievement of my PhD projects: Laure Dossus, Agnès Fournier, Marc J. Gunter, Sabina Rinaldi, Anne-Sophie Navionis, Isabelle Romieu, Carine Biessy, Bertrand Hemon, Elisabete Weiderpass, Gianluca Severi, Marie Al Rahmoun, Neil Murphy, Augustin Scalbert, and all the members of the EPIC breast cancer working group. Thank you for sharing your knowledge and expertise.

To Marc Gunter and Augustin Scalbert for hosting me during these 3 years of PhD in their group/section.

To all the people who contributed to this thesis manuscript: Laure Dossus, Agnès Fournier, Mathilde His, Michèle Matta, Carine Biessy, Bertrand Hemon, and Sally Moldan. Thank you for your time, precious advice, and correction!

To my financial supports: The Ligue National Contre le Cancer for funding this PhD, the International Agency for Research on Cancer for completing my doctoral grant, as well as the Institut national du cancer and World Cancer Research Fund for funding the study on inflammatory biomarkers and breast cancer I was fortunate to work on.

To Antoine Pariente, Anton Pottegård and Edoardo Botteri for accepting to be reviewers of my thesis manuscript, and to Béatrice Fervers, Mahmoud Zureik, Florence Menegaux and Talita Duarte Salles for taking part as jury members to my thesis defence. I am also grateful



to Béatrice Fervers and Florence Menegaux for actively participating to the thesis follow-up committee and giving me precious feedbacks.

To my closest friends, Aricia, Benji, Marina, Ben, Vincent (known as GG), David, Hélène, Candice, and Morgane. Because student life was much more fun at your side.

To all members of my family for contributing to my personal growth and for your constant support. I would like to particularly thank my parents, Pierre-Henri, my sisters, and Léo.

## LIST OF SCIENTIFIC WORKS

### Published/accepted papers

- Cairat M, Fournier A, Murphy N, Biessy C, Scalbert A, Rinaldi S, Tjønneland A, Olsen A, Overvad K, Arveux P, Boutron-Ruault MC, Cadeau C, Fortner RT, Kaaks R, Boeing H, Aleksandrova K, Peeters PHM, Van Gils CH, Wareham NJ, Khaw KT, Aune D, Riboli E, Gunter MJ, Dossus L. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a European prospective cohort study**. *Int J Cancer*. 2018 Apr 29. doi: 10.1002/ijc.31570. PMID: 29707771. Incorporated in the chapter 4.
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a prospective cohort study of postmenopausal women**. *Breast Cancer Res*. 2020 Oct 31. doi: 10.1186/s13058-020-01343-1. PMID: 33129324. Incorporated in the chapter 5.
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Antiplatelet drug use and breast cancer risk in a prospective cohort study of postmenopausal women**. Manuscript accepted to *Cancer Epidemiology, Biomarkers and Prevention*. Incorporated in the chapter 5.

### Submitted papers

- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Glucocorticoid use and breast cancer risk in a prospective cohort study of postmenopausal women**. Manuscript submitted to BMC Medicine. Incorporated in the chapter 5.
- Castro-Espin C, Agudo A, Bonet C, [EPIC co-authors], Cairat M, Weiderpass E, Riboli E, Dossus L, Jakszyn P. **Inflammatory potential of the diet and risk of breast cancer in the European Investigation into Cancer and Nutrition (EPIC) study**. Manuscript submitted to American Journal of Clinical Nutrition.
- Al Rahmoun M, Ghiasvand R, Cairat M, Mahamat-Saleh Y, Cervenka I, Severi G, Boutron-Ruault MC, Eid Robsahm T, Kulle Andreassen B, Kvaskoff M, Fournier A. **Statin Use and Skin Cancer Risk: A Prospective Cohort Study**. Manuscript submitted to British Journal of Dermatology.

### Ongoing paper

- Cairat M, Rinaldi S, Navionis AS, Romieu I, Biessy C, Weiderpass E, [EPIC co-authors], Gunter M, Dossus L. **Circulating inflammatory biomarkers and breast cancer risk: results from the EPIC study**. Manuscript in preparation. Incorporated in the chapter 3.

### Conference abstracts

- Cairat M, Fournier A, Murphy N, Biessy C, Gunter M, Dossus L. (2018). **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a prospective cohort study**. *Revue d'Épidémiologie et de Santé Publique*. 66. S247-S248. 10.1016/j.respe.2018.05.038.
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. (2019). **Utilisation d'aspirine faible dose et risque de cancer du sein dans une cohorte**

**prospective de femmes ménopausées.** Revue d'Épidémiologie et de Santé Publique. 67. S195. 10.1016/j.respe.2019.04.018.

- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. (2019). **Utilisation d'anti-inflammatoires non stéroïdiens et risque de cancer du sein dans une cohorte prospective de femmes ménopausées.** Revue d'Épidémiologie et de Santé Publique. 67. S188. 10.1016/j.respe.2019.04.046.

## CONFERENCES

### Oral presentations

- Cairat M, Fournier A, Murphy N, Biessy C, Scalbert A, Rinaldi S, Gunter MJ, Dossus L, on behalf of EPIC. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a European prospective cohort study.** European Congress of Epidemiology, 4<sup>th</sup> – 6<sup>th</sup> June 2018, Lyon (France).
- Cairat M, Rinaldi S, Navionis AS, Romieu I, Biessy C, Weiderpass E, Gunter M, Dossus L, on behalf of EPIC. **Circulating inflammatory biomarkers and breast cancer risk: results from the EPIC study.** EPIC 25th Anniversary Workshop (EPIC Breast Cancer Working Group), 17<sup>th</sup> September 2018, Lyon (France).
- Cairat M, Rinaldi S, Navionis AS, Romieu I, Biessy C, Weiderpass E, Gunter M, Dossus L, on behalf of EPIC. **Circulating inflammatory biomarkers and breast cancer risk: results from the EPIC study.** French Nutrition and Cancer Network (Journée Nacre Partenariat), 4<sup>th</sup> April 2019, Paris (France). Presented by Laure Dossus.
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Antiplatelet drug use and breast cancer risk in a prospective cohort study of postmenopausal women.** IARC Early Career Scientists Association Scientific and Career Day, 23<sup>rd</sup> May 2019, Lyon (France).

### Poster presentations

- Cairat M, Fournier A, Murphy N, Biessy C, Scalbert A, Rinaldi S, Gunter MJ, Dossus L, on behalf of EPIC. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a European prospective cohort study.** Forum du Cancéropôle Lyon Auvergne Rhône-Alpes, 3<sup>rd</sup> – 4<sup>th</sup> April 2018, Lyon (France). *Best poster award.*
- Cairat M, Fournier A, Murphy N, Biessy C, Scalbert A, Rinaldi S, Gunter MJ, Dossus L, on behalf of EPIC. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a European prospective cohort study.** IARC Early Career Scientists Association Scientific and Career Day, 24<sup>th</sup> April 2018, Lyon (France).
- Cairat M, Fournier A, Murphy N, Biessy C, Scalbert A, Rinaldi S, Gunter MJ, Dossus L, on behalf of EPIC. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a European prospective cohort study.** The Doctoral School Scientific Day, 11<sup>th</sup> October 2018, Lyon (France).
- Cairat M, Rinaldi S, Navionis AS, Romieu I, Biessy C, Weiderpass E, Gunter M, Dossus L, on behalf of EPIC. **Circulating inflammatory biomarkers and breast**

**cancer risk: results from the EPIC study.** Annual meeting of the NCI cohort consortium, 28<sup>th</sup> – 30<sup>th</sup> November 2018, Rockville (USA). Presented by Laure Dossus.

- Cairat M, Rinaldi S, Navionis AS, Romieu I, Biessy C, Weiderpass E, Gunter M, Dossus L, on behalf of EPIC. **Circulating inflammatory biomarkers and breast cancer risk: results from the EPIC study.** Forum du Cancéropôle Lyon Auvergne Rhône-Alpes, 4<sup>th</sup> – 5<sup>th</sup> April 2019, Lyon (France).
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Utilisation d'aspirine faible dose et risque de cancer du sein dans une cohorte prospective de femmes ménopausées.** Colloque AFCROs, 18<sup>th</sup> June 2019, Paris (France). *This poster was the subject of a despatch by APMnews (<https://www.apmnews.com>).*
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Utilisation d'anti-inflammatoires non stéroïdiens et risque de cancer du sein dans une cohorte prospective de femmes ménopausées.** Colloque AFCROs, 18<sup>th</sup> June 2019, Paris (France). *This poster was the subject of a despatch by APMnews (<https://www.apmnews.com>).*
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Nonsteroidal anti-inflammatory drug use and breast cancer risk in a prospective cohort study of postmenopausal women.** 35<sup>th</sup> International Conference on Pharmacoepidemiology & Therapeutic Risk Management, 24<sup>th</sup> – 28<sup>th</sup> August 2019, Philadelphia (USA).
- Cairat M, Rinaldi S, Navionis AS, Romieu I, Biessy C, Weiderpass E, Gunter M, Dossus L, on behalf of EPIC. **Circulating inflammatory biomarkers and breast cancer risk: results from the EPIC study.** IARC Scientific Council, 5<sup>th</sup> February 2020, Lyon (France). **Presented by Sabina Rinaldi.**
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Antiplatelet drug use and breast cancer risk in a prospective cohort study of postmenopausal women.** 36<sup>th</sup> International Conference on Pharmacoepidemiology & Therapeutic Risk Management, 16<sup>th</sup> – 17<sup>th</sup> September 2020, virtual event.
- Cairat M, Al Rahmoun M, Gunter M, Severi G, Dossus L, Fournier A. **Glucocorticoid use and breast cancer risk in a prospective cohort study of postmenopausal women.** 36<sup>th</sup> International Conference on Pharmacoepidemiology & Therapeutic Risk Management, 16<sup>th</sup> – 17<sup>th</sup> September 2020, virtual event.

## TABLE OF CONTENTS

<b>LIST OF FIGURES/TABLES .....</b>	<b>17</b>
<b>LIST OF ANNEXES .....</b>	<b>17</b>
<b>LIST OF ABBREVIATIONS .....</b>	<b>18</b>
<b>CHAPTER 1: INTRODUCTION .....</b>	<b>19</b>
1. Breast cancer .....	20
1.1. The breast: anatomy and functions .....	20
1.2. An overview of carcinogenesis .....	20
1.3. Tumour characteristics .....	21
1.4. Descriptive epidemiology .....	23
1.5. Aetiology .....	24
2. Inflammation .....	29
2.1. Principles and functions .....	29
2.2. Chronic inflammation .....	30
2.3. Inflammation and carcinogenesis .....	30
2.4. Inflammation and breast cancer risk: up-to-date epidemiological evidence .....	31
3. Nonsteroidal anti-inflammatory drugs .....	33
3.1. Definition and classification .....	33
3.2. Pharmacological properties and therapeutic use .....	34
3.3. Antitumor effects .....	36
3.4. Breast cancer prevention: up-to-date epidemiological evidence .....	37
4. Antiplatelet drugs .....	39
4.1. Definition and classification .....	39
4.2. Pharmacological properties and therapeutic use .....	39
4.3. Antitumor effects .....	41
4.4. Breast cancer prevention: up-to-date epidemiological evidence .....	43
5. Glucocorticoids .....	46
5.1. Definition and classification .....	46
5.2. Pharmacological properties and therapeutic use .....	46
5.3. Antitumor effects .....	48
5.4. Breast cancer prevention: up-to-date epidemiological evidence .....	48
6. Thesis objectives .....	50
<b>CHAPTER 2: DATABASES .....</b>	<b>51</b>
1. The EPIC study .....	51
1.1. Presentation of the cohort .....	51
1.2. Recruitment and baseline information .....	51
1.3. Data on cancer and vital status .....	52
1.4. Exposure to aspirin or nonsteroidal anti-inflammatory drugs .....	53

1.5. Co-variables.....	53
2. The E3N study.....	57
2.1. Presentation of the cohort.....	57
2.2. Recruitment and baseline information .....	57
2.3. Follow-up .....	57
2.4. Data on cancer and vital status.....	60
2.5. Exposure to anti-inflammatory and antiplatelet drugs .....	61
2.6. Co-variables.....	61
<b>CHAPTER 3: CIRCULATING INFLAMMATORY BIOMARKERS AND BREAST CANCER RISK IN THE EPIC STUDY.....</b>	<b>66</b>
1. Objective .....	66
2. Materials and methods.....	66
3. Main findings .....	67
4. Conclusions .....	68
5. Paper.....	68
<b>CHAPTER 4: NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND BREAST CANCER RISK IN THE EPIC STUDY.....</b>	<b>98</b>
1. Objective .....	98
2. Materials and methods.....	98
3. Main findings .....	99
4. Conclusions .....	100
5. Paper.....	100
<b>CHAPTER 5: ANTI-INFLAMMATORY DRUGS, ANTIPLATELET DRUGS AND BREAST CANCER RISK IN THE E3N STUDY .....</b>	<b>109</b>
1. Objectives.....	109
2. Materials and Methods .....	109
3. Nonsteroidal anti-inflammatory drugs and breast cancer risk.....	111
3.1. Main findings.....	111
3.2. Conclusions .....	111
3.3. Paper.....	111
4. Antiplatelet drugs and breast cancer risk .....	126
4.1. Main findings .....	126
4.2. Conclusions .....	126
4.3. Paper.....	127
5. Glucocorticoid use and breast cancer risk.....	154
5.1. Main findings .....	154
5.2. Conclusions .....	154
5.3. Paper.....	155
<b>CHAPTER 6: DISCUSSION.....</b>	<b>173</b>
1. Inflammatory biomarkers and breast cancer risk .....	173

2. Drugs with anti-inflammatory properties and breast cancer risk .....	174
3. Perspectives .....	178
<b>ANNEXES</b> .....	180
<b>REFERENCES</b> .....	187

## LIST OF FIGURES/TABLES

Figure 1: Age standardized incidence rates of breast cancer. .	23
Figure 2: Age standardized mortality rates of breast cancer.	24
Figure 3: An overview of eicosanoid synthesis pathways.	35
Figure 4: EPIC countries and centres.	51
Figure 5: Timeline of data collected between 1990 and 2014 (E3N Cohort).	59
Figure 6: Photo of one questionnaire including question on the use of anti-inflammatory drugs	61
Table 1: Data collected on NSAIDs in EPIC	99

## LIST OF ANNEXES

Annex 1: Different types of nonsteroidal anti-inflammatory drugs.	180
Annex 2: Different types of antiplatelet drugs.	182
Annex 3: Different types of systemic glucocorticoids.	183
Annex 4: Description of the SIDIAP database and study population.	184



## LIST OF ABBREVIATIONS

ADP: Adenosine Diphosphate  
AJCC: American Joint Committee on Cancer  
ATC: Anatomical Therapeutic Chemical  
BMI: Body Mass Index  
CI: Confidence Interval  
CIP: *Code Identifiant de Presentation*  
COX: Cyclooxygenase  
COXib: Selective Cox-2 Inhibitors  
CRP: C-Reactive Protein  
DDD: Defined Daily Dose  
E3N : *Etude Epidémiologique Auprès De Femmes De l'Education Nationale*  
EPIC: European Prospective Investigation into Cancer and Nutrition  
ER: Estrogen Receptor  
GR: Glucocorticoid Receptor  
HER2: Human Epidermal Growth Factor Receptor 2  
HR: Hazard Ratio  
IARC: International Agency for Research on Cancer  
IFN: Interferon  
ICD: International Classification of Diseases  
IL: Interleukin  
INCa : *Institut National du Cancer*  
LOX: Lypooxygenase  
MET: Metabolic Equivalent of Task  
MGEN : *Mutuelle Générale de l'Education Nationale*  
MHT: Menopausal Hormone Therapy  
NCI: National Cancer Institute  
OC: Oral Contraceptive  
OR: Odd Ratio  
OTC: Over the counter  
SCORE: Coronary Risk Evaluation  
UICC: *Union for International Cancer Control*  
UKPDS: United Kingdom Prospective Diabetes Study  
PCA: Principal Component Analysis  
PG: Prostaglandin  
PPI: Proton Pump Inhibitor  
PR: Progesterone Receptor  
RR: Relative Risk  
SBR: Scarff-Bloom Richardson grading  
SD: Standard Deviation  
TNF: Tumour Necrosis Factor  
TNM: Tumour, Node, Metastasis  
TX: Thromboxane  
WC: Waist Circumference  
WCRF: World Research Cancer Fund  
WHO: World Health Organization

## CHAPTER 1: INTRODUCTION

Although breast cancer has been intensively studied and many breast cancer risk factors have been identified, there are still more than two million breast cancer cases diagnosed each year worldwide (1). A better understanding of the disease aetiology as well as new prevention strategies are urgently needed.

In the past decades, experimental and observational studies have accumulated evidence that chronic inflammation could promote breast cancer development (2). The possibility of stemming tumorigenic inflammatory effects with pharmacological compounds has drawn significant interest. Consequently, nonsteroidal anti-inflammatory drugs (NSAIDs), antiplatelet drugs and glucocorticoids are seen as potential candidates of cancer preventive medications (3, 4).

In this first chapter, I will highlight the need for a better understanding of breast cancer development by presenting an overview of its aetiology and epidemiology. In addition, I will describe the potential role of inflammation in breast carcinogenesis and summarize results from epidemiological studies. I will then proceed to explain why NSAIDs, antiplatelet drugs and glucocorticoids are potential cancer preventive medications and highlight the necessity for more epidemiological studies.

## 1. Breast cancer

### 1.1. The breast: anatomy and functions

The breast is the tissue overlying the chest muscles. Each breast is composed of a mammary gland surrounded by connective, glandular, and adipose tissue. The mammary gland is organized into 15 to 20 sections, called lobes. Within each lobe are smaller structures, called lobules, where milk is produced. The lobes and lobules are linked by thin tubes called ducts. During lactation, each mammary gland drains milk towards the nipples via the ducts. Lymph nodes, located near the breast in the axilla (armpit), filter the lymphatic fluid (also called lymph) and store lymphocytes. Blood vessels and lymph vessels are present in each breast and respectively drain blood and lymph. The development of mammary glands is influenced from puberty by steroid hormones produced by the ovaries, such as oestrogen and progesterone (5).

### 1.2. An overview of carcinogenesis

Cancers are characterized by the uncontrollable growth of abnormal cells which go beyond their usual boundaries to invade adjoining parts of the body and/or spread to other organs. Carcinogenesis, the transformation of normal cells into cancerous cells, is a multistep process that develops through a latency period of several years before the clinical onset of the disease. It is the result of an accumulation of detrimental variations in the genome (mutations). These mutations might be inherited/transmitted during cell division, or acquired by random replication errors or exposure to carcinogens (e.g. smoking) (6). These successive alterations confer a selective advantage to the affected cells, leading to an intense proliferation. These cancerous cells have some biological properties in common which allow them to evolve, subsist, proliferate, and invade other tissue. These properties, also called the hallmarks of cancer, were proposed by Douglas Hanahan and Robert Weinberg (7, 8) and are as follow:

- sustaining proliferative signalling
- deregulating cellular energetics
- resisting cell death
- genome instability and mutation
- inducing angiogenesis
- evading growth suppressor
- enabling replicative immortality
- avoiding immune destruction

- tumour-promoting inflammation
- activating invasion and metastasis.

### 1.3. Tumour characteristics

Breast cancer comprises multiple entities that have different histological and biological features, clinical presentations, and behaviours.

#### 1.3.1. Histological classification

Breast cancers are distinguished according to their tissue origin. The most frequent types of breast cancer begin in the cells of the ducts (called ductal breast cancer and representing 40 to 75% of all breast cancers) or in the cells of the lobules (called lobular breast cancer and representing 5 to 15% of all breast cancers). Other breast cancers including tubular, medullary, papillary, and mucinous are less frequent (each representing less than 2% of all breast cancers) (9).

#### 1.3.2. Classification according to their invasiveness

When cancer cells stay within their emerging location (e.g. ducts or lobules), the cancer is called *in situ*. In contrast, when cancer cells infiltrate neighbouring tissue, this is called invasive breast cancer.

#### 1.3.3. Tumour stage

The stage of the cancer is defined by the Tumour, Node, Metastasis (TNM) system established by the American Joint Committee on Cancer (AJCC) and the *Union for International Cancer Control* (UICC) (10). The stage is generally determined at the time of diagnosis and describe the extent of the disease. The TNM system is based on the assessment of three components: the tumour size (T), the absence/presence and extent of regional lymph nodes metastasis (N), and the absence/presence of distant metastasis (M). Each of these components are characterized and combined to define the five stages of breast cancer ranging from stage 0 for carcinoma *in situ* to stage IV for metastatic cancers.

#### 1.3.4. Histological grade

Histological grade, as defined by the Scarff-Bloom-Richardson grading system (SBR), involves the evaluation of three morphological features: the percentage of tubule formations, the change in cell size, and the uniformity and frequency of cell mitosis/division. A score

between 1 and 3 is assigned to these three categories. The sum of the three scores defines the SBR grade ranging from 1 to 3.

This grading system represents how closely cancer cells look like healthy breast cells. The higher the grade is, the more the appearance and growth of cancer cells differs from healthy breast cells. Histological grades provide important prognostic information: the lower the grade is, the better the survival (11, 12).

#### 1.3.5. Molecular subtypes

Hormones play an important role in breast cancer progression because they influence the cell function and contribute to the proliferation of epithelial tumour cells (13). Breast cancer can be defined by the presence on cancer cells of receptors for oestrogen (ER) and progesterone (PR).

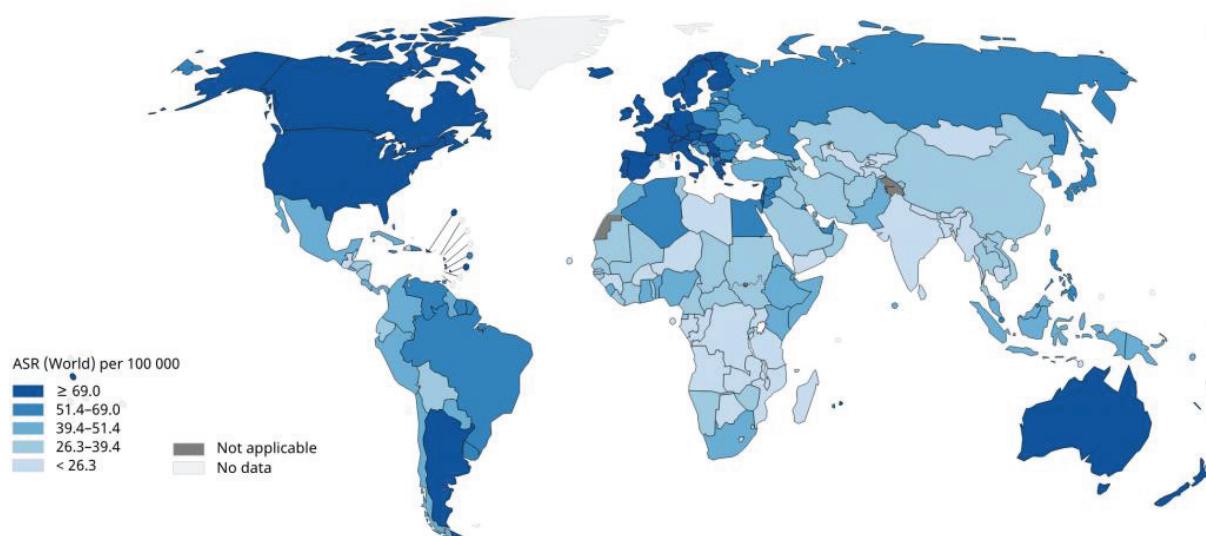
Breast cancers are classified as oestrogen-receptor positive (ER+) when the proportion of cells expressing ER is above 1%, and progesterone-positive (PR+) when the proportion of cells expressing PR is above 1%. On the contrary, breast cancers are classified as oestrogen-receptor negative (ER-) or progesterone-receptor negative (PR-) when the proportion of cells expressing ER or PR is less than 1% (14). The hormone receptor status on all invasive breast cancers is determined at diagnosis as it is an important indicator for the potential response to endocrine therapy (15). Breast cancer can also be classified by the overexpression of the human epidermal growth factor receptor 2 (HER2), an oncogene promoting cancer growth (16). The HER2 status is determined on all invasive breast cancers as targeted therapy (e.g. trastuzumab) is needed to treat HER2+ tumours (17). Women with ER+, PR+ or HER2+ breast cancers generally have a better survival rate than those with ER-, PR- or HER2- breast cancers (18, 19).

## 1.4. Descriptive epidemiology

### 1.4.1. Incidence

In both sexes combined, breast cancer is the second most diagnosed cancer, with approximately 2.09 million new cases diagnosed worldwide in 2018 (11.6% of the total cases). Among women, breast cancer is the most diagnosed cancer in the world (accounting for almost 1 in 4 cancer cases). In 2018, the highest incidence rates of breast cancer were observed in developed countries (North American, Australia and Western Europe) (Figure 1). Elevated incidence rates in developed countries are attributed to a higher prevalence of known lifestyle risk factors, which are described in the following part (1.5. Aetiology). In Europe, it is the most diagnosed cancer, with 552,513 new cases diagnosed (12.4% of all cases) in 2018. In France, 56,162 new cases (12.3% of all cases) were diagnosed in 2018 (1, 20).

Figure 1: Age standardized incidence rates of breast cancer.



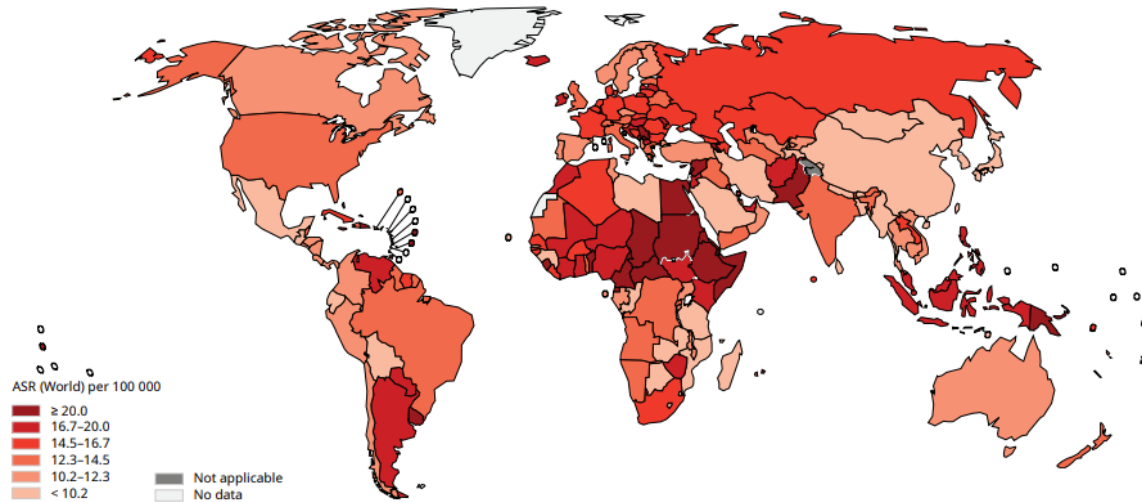
Source: Global Cancer Observatory: Cancer Today (20).

### 1.4.2. Mortality

In both sexes combined, breast cancer is the fifth leading cause of cancer death, with 626,679 deaths by breast cancer (6.6 % of the total cancer deaths) worldwide in 2018 (1). Among of the total cancer deaths, the highest mortality rates were observed in developing countries compared to developed countries (Figure 2). These variations in mortality rates across countries have been attributed to differences in access to early diagnosis and optimum treatment (21). In Europe, breast cancer is the third leading cause of cancer death, with

137,707 deaths by breast cancer (7.1% of the total cancer deaths) in 2018. In France, 13,353 women (7.3% of the total cancer deaths) died of breast cancer in 2018 (1, 20).

Figure 2: Age standardized mortality rates of breast cancer.



Source: Global Cancer Observatory: Cancer Today (20).

### 1.5. Aetiology

Breast cancer is a multifactorial disease with several well identified risk factors, including genetic, medical, hormonal/reproductive, anthropometric, lifestyle, and environmental factors. IARC and the French national institute on cancer (“Institut National du Cancer”, INCa) suggested that alcohol consumption, obesity, low levels of physical activity and, to a lesser extent, smoking and unhealthy diet, were responsible for around 40% of new breast cancer cases in France in 2015 (22). However, the aetiology of breast cancer remains in part unknown, and among all the identified risk factors, few of them might be modifiable. Below, I describe the different risk factors of breast cancer.

- **Age and sex**

**Sex** is the major risk factor for breast cancer. Breast cancer affects both men and women. However, the incidence is much higher in women and breast cancer in men represents less than 1% of all breast cancers worldwide (23). Breast cancer incidence rates increase with **age**, exponentially before menopause and slowly after. Around 50% of breast cancers are diagnosed between 50 and 69 years old and around 28% after 69 years old (24).

- **Genetic/familial factors**

About 5% to 10% of breast cancers are thought to be **hereditary** (25). A woman's risk for breast cancer is around 80% higher if she has a **first-degree relative** who had breast cancer compared to women without family history (26). The risk goes up if more family members are affected and varies according to their age at diagnosis. This increased risk from family history of breast cancer might be explained in part by **genetic variants** which have been previously identified such as mutations in genes BRCA1 and BRCA2 (27). However, these mutations would contribute to only 25% of familial breast cancers and other genes might be involved such as PTEN or TP53 (28). A large number of low-penetrance risk alleles identified in Genome-Wide Association Studies may also explain part of the breast cancer heritability (29).

- **Medical factors**

**Proliferative benign breast diseases**, with or without atypia, are associated with a significant increase in the risk of developing breast cancer. Compared to women without proliferative benign breast diseases, breast cancer risk increases by 58% for women with proliferative benign breast diseases without atypia, and is multiplied by four for women with proliferative benign breast diseases with atypia (30).

**Breast density**, which reflects the proportion of fibrous and glandular tissues in the breast, is a strong risk factor for breast cancer (31, 32). Women with a breast density  $\geq 75\%$  have their breast cancer risk multiplied by five compared to women with a breast density  $<5\%$  (31).

- **Hormonal and reproductive factors**

Breast cancer is a hormone-dependant cancer, which appears only after puberty among women. There is strong evidence that **sex steroid hormones**, in particular oestrogens, both endogenous and exogenous, are involved in breast cancer development (13). A variety of other hormonal and reproductive factors, which increase lifetime exposure to oestrogen and progesterone, are identified as breast cancer risk factors. Overall, these factors are supposed to have a stronger effect on ER+ or PR+ breast cancers compared to ER- or PR- breast cancers (33).



Menstrual factors including **early age at menarche and late menopause** expose women to a longer time of ovulatory menstrual cycles and consequently hormones, raising their risk of getting breast cancer. Breast cancer risk increases by 5% for every year younger at menarche, and independently by 3% for every year older at menopause (34).

Additionally, pregnancy-related factors, including **age at first full-term pregnancy, number of children and breastfeeding**, are also known to influence breast cancer risk. Just after delivery, parous women have an elevated breast cancer risk that peaks around five years after childbirth and persists for more than twenty years. After, this risk decreases until conferring a protective effect 34 years after childbirth (35). After this long period, breast cancer decreases by 3% for every year younger at age at first full term pregnancy and by 7% for each birth (35). Pregnancies that end as a spontaneous or induced abortion do not increase a woman's risk of developing breast cancer (36). Breastfeeding is an established protective factor of breast cancer among pre and postmenopausal women, with breast cancer risk decreasing by 4% for each year of breastfeeding, independently of the number of births (37).

In 1999, IARC concluded in a monograph that there was sufficient evidence in humans for the carcinogenicity of **oral contraceptives** containing oestrogen in combination with a progestogen (38). Compared to never users, breast cancer risk increased by 24% for current oral contraceptive users. However, the effect of oral contraceptives on the risk of breast cancer decreases gradually after cessation of use, returning to that of never-users within 10 years after cessation (39). Use of **menopausal hormone therapies** (MHT) has been convincingly shown as a risk factor of postmenopausal breast cancer (40). Every MHT type, except vaginal oestrogens, is associated with excess breast cancer risk, even though the increased risk is stronger for oestrogen-progestogen than oestrogen-only preparations. The risk increases substantially with duration of use. In addition, other exogenous hormones such as diethylstilboestrol, a synthetic oestrogen used by pregnant women to prevent miscarriage from 1940 to 1970, are known to increase breast cancer risk among users and their offspring (41).

- **Body fatness and physical activity**

The effect of body fatness on breast cancer development differs according to menopausal status. In 2017, the World Cancer Research Fund International (WCRF), which updated its

previous analysis of all published prospective studies on diet, nutrition and physical activity and the risk of developing breast cancer (42), concluded that there was strong evidence that:

- being **overweight or obese in young adulthood** (between the ages of about 18 and 30 years) or in adulthood before the menopause decreases the risk of premenopausal breast cancer
- being overweight or obese in young adulthood decreases the risk of postmenopausal breast cancer while being overweight/obese or **gaining weight** throughout adulthood increases the risk of postmenopausal breast cancer
- being **physically active** decreases the risk of premenopausal (only vigorous activity) and postmenopausal breast cancer (any activity including vigorous, occupational, recreational, walking and household activity)
- being **tall** increases the risk of premenopausal and postmenopausal breast cancer.

- **Alcohol consumption and tobacco**

The WCRF concluded that the **consumption of alcoholic drinks** is probably a cause of premenopausal breast cancer and a convincing cause of postmenopausal breast cancer (42). In their meta-analyses, the risk of premenopausal breast cancer increases by 5% per 10 grams of alcohol per day while the risk of postmenopausal breast cancer increases by 9% per 10 grams of alcohol per day.

Hundreds of epidemiological studies have been published on the relationship between breast cancer and active or passive smoking. However, there is still no consensus on whether tobacco affects breast cancer development. In a monograph of 2004, IARC concluded that there was plausible biological evidence on the detrimental effect of tobacco smoke on breast cancer development. However, there was no association between active/passive tobacco smoking and breast cancer incidence in epidemiological studies (43). Since then, new epidemiological studies have consistently observed a positive association between current smoking and breast cancer risk, with relative risk estimates ranging from 1.10 to 1.30 (44).

- **Diet**

Currently, no specific dietary habits are identified as risk factors of breast cancer. However, in 2017, the WCRF suggested that there was some evidence that:

- consuming foods containing carotenoids and diets high in calcium might decrease the risk of breast cancer,
- non-starchy vegetables might decrease the risk of ER- breast cancer,
- the consumption of dairy products might decrease the risk of breast cancer among premenopausal women.

The potential influence of a specific dietary pattern (e.g. Mediterranean diet) on breast cancer development is unclear (42).

- **Environmental and occupational factors**

Few environmental and occupational exposure are identified as breast cancer risk factors except for ionising radiation from medical treatments such as X-ray, which increases the risk of breast cancer particularly when the patient is exposed during puberty (45). In a monograph published in 2020 (46), IARC concluded that there was limited evidence in humans for the breast carcinogenicity of night shift work.

## **Take-home messages**

### **Breast cancer**

- is the most diagnosed cancer and the most common cause of cancer death among women in the world
- is a heterogeneous and multifactorial disease, for which many risk factors are identified, such as age, height, ionizing radiation, genetic factors, a wide list of reproductive/hormonal factors, obesity, or alcohol consumption. However, only a few of these risk factors are modifiable.

**A better understanding of the aetiology of breast cancer as well as new strategies to prevent breast cancer occurrence are urgently needed to strengthen primary prevention of this disease.**

## 2. Inflammation

### 2.1. Principles and functions

Inflammation is a component of a complex biological response by the immune system to stimulants (e.g. microbial infection or tissue injury) (47). Acute inflammation is well-known to restore cellular homeostasis after any detrimental disorder. The classical symptoms of acute inflammation include redness, pain, swelling and heat.

The major immune cells contributing to inflammation are antigen-presenting cells (macrophages and dendritic cells), leukocytes including granulocytes (neutrophils, eosinophils, and basophils), mast cells, and lymphocytes including natural killer (NK) cells. Non-immune cells such as platelets, epithelial cells, endothelial cells, and fibroblasts also participate to inflammatory processes. A wide range of inflammatory mediators are released by these cells such as leukotrienes, vasoactive amines, peptides, eicosanoids, pro-inflammatory cytokines, and acute-phase proteins.

The inflammatory response depends on the nature of stimulants and on the level of damage. For instance, a bacterial infection induces the production of inflammatory mediators such as inflammatory cytokines [e.g. tumour necrosis factor (TNF), interleukin-1 (IL-1) and IL-6] and chemokines. In most cases, this inflammatory response extends toward systemic effects through the secretion of acute phase proteins [e.g. c-reactive protein (CRP) and coagulation factors] by the liver cells (47). TNF- $\alpha$  and IL-1 $\beta$  also induce the translocation of the cellular transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) to the nucleus where it activates genes for immune and inflammatory response (48). NF- $\kappa$ B activation leads to cyclooxygenase-2 (COX-2) expression and inflammatory cytokine release (e.g. IL-6). COX-2 induces the biosynthesis of prostaglandins (PGs) and other eicosanoids (Figure 3) (49). On the other hand, a viral infection leads to a distinct signalling pathway through the production of another class of cytokines such as type-1 interferons (IFNs) and involves cytotoxic lymphocytes. A parasitic infection as well as allergens induce the production of IL-4, IL-5, IL-13, and histamine.

Once the potential danger is eradicated, the inflammatory response is immediately followed by the production of anti-inflammatory mediators such as IL-10, TGF- $\beta$ , or endogenous

cortisol and therefore inflammation is terminated. The role of each mediator on the resolution of inflammation is not completely known.

## 2.2. Chronic inflammation

If inflammation fails to end, the acute inflammation turns into a chronic stage. For instance, it is recognized that inflammation might persist in the case of chronic infection (e.g. *Helicobacter Pylori* or sexually transmitted infections), auto-immune diseases, or among overweight or obese people (50-55). However, chronic inflammation is not well understood, and other triggers remain to be identified. Contrary to acute inflammation, chronic inflammation might be deleterious and is involved in many diseases such as atherosclerosis, obesity, type 2 diabetes, asthma, in inflammatory bowel diseases, neurodegenerative diseases, rheumatoid arthritis and cancers (47, 56).

## 2.3. Inflammation and carcinogenesis

Since the discovery, by Rudolf Virchow in 1863, of the infiltration of leukocytes into tumour tissue, many experiments have confirmed the role of the immune system and inflammation in tumour proliferation, immunosuppression, angiogenesis, invasion, and metastasis (57). In 2011, tumour-promoting inflammation and avoiding immune destruction were identified by Hanahan and Weinberg as hallmarks of cancer (7). Indeed, the tumour microenvironment contains many different inflammatory mediators including pro-inflammatory cytokines, chemokines, and eicosanoids [such as prostaglandins (PGs), thromboxane (TX), and leukotrienes (LTs)] that might generate an immunosuppressive microenvironment (58). Inflammation has been hypothesized to recruit and change the function of various immune cells (58) and to supply bioactive molecules to the tumor microenvironment including growth factors (sustaining proliferative signaling), survival factors (regulating cell death), proangiogenic factors and extracellular matrix-modifying enzymes (accelerating angiogenesis, invasion, and metastasis), signals that initiate the epithelial-to-mesenchymal transition (7), and reactive oxygen/nitrogen species (promoting the evolution of transformed/initiated cells) (59).

Inflammation might be particularly relevant for breast cancer because cytokines that are elevated in the event of chronic inflammation, such as IL-6 and TNF- $\alpha$ , have been shown to upregulate angiogenesis and to stimulate aromatase activity within breast tissues (leading to oestrogen synthesis) (60-62). Experimental data suggest that TNF- $\alpha$  could be involved in

carcinogenesis by activating NF- $\kappa$ B and consequently inducing the expression of genes associated with cell proliferation, apoptosis, angiogenesis, and metastasis (63). TNF- $\alpha$  has also been shown to induce insulin resistance (64), which is potentially involved in breast cancer development (65). Also, leptin, an adipokine (a hormone produced mainly by adipocytes) could promote breast cancer by stimulating the conversion of androgen into oestradiol or inducing a metabolic dysregulation leading to high levels of insulin and IGF-1 (66), which are hypothesized to increase breast cancer risk development (65, 67). On the contrary, adiponectin, an adipokine with anti-inflammatory properties, might prevent cancer development by inhibiting angiogenesis and promoting apoptosis (68, 69). The adiponectin to leptin ratio has been shown to decrease proliferation of ER+ breast cancer cell lines and increase proliferation of ER- breast cancer cells lines (70).

#### 2.4. Inflammation and breast cancer risk: up-to-date epidemiological evidence

Prospective studies have shown that not only local inflammation but also systemic inflammation could be associated with carcinogenesis. CRP is the most studied inflammatory biomarker within the framework of breast cancer risk. In a meta-analysis of twelve prospective studies conducted mainly on postmenopausal women (published in 2015), a doubling of serum concentrations of CRP was associated with a significant 7% increase in breast cancer risk (71). Since then, five prospective studies reported the CRP-breast cancer associations mainly among postmenopausal women (72-75). Among them, two studies in a joint publication noted no association between CRP and breast cancer risk (72), while others confirmed the positive association between CRP and breast cancer risk (73), or suggested that the association was confined to non-MHT users (74) or overweight women (75). One prospective study reported no association between CRP and breast cancer risk among premenopausal women (73). The CRP-breast cancer associations warrant further investigation since most epidemiological studies were not able to adjust/stratify by breast cancer subtypes, menopausal status, or risk factors such as adiposity or use of exogenous hormones.

With respect to adipokines, a meta-analysis of six prospective and nine case-control studies on adiponectin and breast cancer risk (published in 2014) concluded that low levels of adiponectin were positively associated with risk of postmenopausal breast cancer (76). However, among the studies in which blood samples were taken before breast cancer diagnosis, none of them found a significant association between pre-diagnostic levels of

adiponectin and postmenopausal breast cancer risk (77-80). Elevated leptin levels have been observed in serum of breast cancer patients compared to control subjects (81). However, few prospective studies evaluated the associations between pre-diagnostic levels of leptin and breast cancer risk (73, 74, 82, 83). Among them, one study noted a significant lower premenopausal breast cancer risk with high levels of leptin (73), while other suggested no significant association between levels of leptin and breast cancer risk among premenopausal (83) or postmenopausal women (74, 82). The leptin to adiponectin ratio, which is a marker of insulin resistance (84), has been positively associated with postmenopausal breast cancer diagnosis (85, 86), but no study evaluated it prospectively. The associations between adipokines and breast cancer risk, overall and by breast cancer subtypes or risk factors need to be evaluate in prospective studies.

TNF- $\alpha$  levels were not associated with breast cancer risk in several prospective studies (73, 82, 87-90). One prospective study found a lower breast cancer risk with high levels of TNF- $\alpha$  (91).

To my knowledge, very few prospective studies have investigated the role of other pro-inflammatory cytokines on breast cancer development. It is also unknown whether chronic inflammation in general is relevant to breast cancer risk, or whether specific pathways are involved.

## **Take-home messages**

### **Inflammation**

- is an essential protective part of the immune system's response to infection or injuries. However, when it persists over time, it might become deleterious.
- is a recognized hallmark of cancer
- could be involved in breast cancer development. However, epidemiological findings on inflammatory biomarkers and breast cancer risk are limited.

**Further well-designed prospective studies should evaluate the associations between inflammatory biomarkers and breast cancer risk by menopausal status and breast cancer subtypes.**

### 3. Nonsteroidal anti-inflammatory drugs

#### 3.1. Definition and classification

Acetylsalicylic acid is the first NSAID manufactured following the extraction of its ancestor, salicin, from bark of willow trees. Acetylsalicylic acid was introduced into the market under the commercial name Aspirin® in 1899 (92). Indomethacin and ibuprofen were among the first non-aspirin NSAIDs to be commercialized, respectively in 1964 and 1965 (93). Since then, a large number of other NSAIDs, with antipyretic, analgesic and anti-inflammatory properties, have been developed (Annex 1). In France, most NSAIDs are only available through prescriptions, except for aspirin, ibuprofen, throat pastilles of flurbiprofen and dermal diclofenac, which are also available over the counter (OTC) (94). The society which maintains and develops the Anatomical, therapeutic, chemical (ATC) classification system, the WHO Collaborating Centre for Drug Statistics Methodology (<https://www.whocc.no/>), classifies preparations referring to aspirin (intended for analgesic or anti-inflammatory use) into “Salicylic Acid and Derivatives” (ATC code: N02BA) and preparations referring to other NSAIDs into “Anti-Inflammatory and Anti-Rheumatic Products, Non-Steroids” (M01A). Of note, aspirin at low dose ( $\leq 325$  mg per pill) is not a NSAID (4. Antiplatelet drugs).

NSAIDs can be further classified in the following therapeutic subgroups (Annex 1):

- Acetylsalicylic acid ( $>325$  mg per pill) (N02BA01)
- Butylpyrazolidines (M01AA)
- Acetic acid derivatives and related substances (M01AB)
- Oxicams (M01AC)
- Propionic acid derivatives (M01AE)
- Fenamates (M01AE)
- Coxibs (M01AH)
- Other anti-inflammatory and anti-rheumatic agents, non-steroids (M01AX).

Preparations containing combinations of NSAIDs with opioids, muscle relaxants, antibacterials, or other anti-inflammatory drugs are classified in N02AJ, M03B, J01, or M01B, respectively. The ATC codes for topical use of NSAIDs preparations starts by M02AA.



## 3.2. Pharmacological properties and therapeutic use

### 3.2.1. Pharmacological actions

The mechanism of action of NSAIDs was first described during the early 1970's by John Vane. He demonstrated that NSAIDs disrupt the biosynthesis of PGs, which are responsible for pain, inflammation and fever (Figure 3) (95, 96). It was initially believed that a single COX was responsible for the biosynthesis of PGs and TxA<sub>2</sub> (97). After the identification of COX-1 (in 1976) and COX-2 (in 1991), NSAIDs appeared to cause adverse gastro-intestinal toxicities and bleeding via COX-1 inhibition (98). Soon after, NSAIDs selectively inhibiting the COX-2 (known as selective COX-2 inhibitors or coxibs) were developed in the hope for a reduction of gastrointestinal toxicities and an enhancement of anti-inflammatory properties (99). However, emerging concerns about cardiovascular toxicities of these new NSAIDs resulted in the withdrawal of some of them in the early 2000s (100). It is now well understood that NSAIDs block the synthesis of PGs and TxA<sub>2</sub> by irreversible acetylation of a serine in both COXs, limiting the access of arachidonic acid to the catalytic active site of the enzymes (Figure 3). NSAIDs inhibit both COXs to a different extent leading to different benefits and harms (101). All NSAIDs reversibly inhibit the COXs, except aspirin which permanently inhibits them (102).

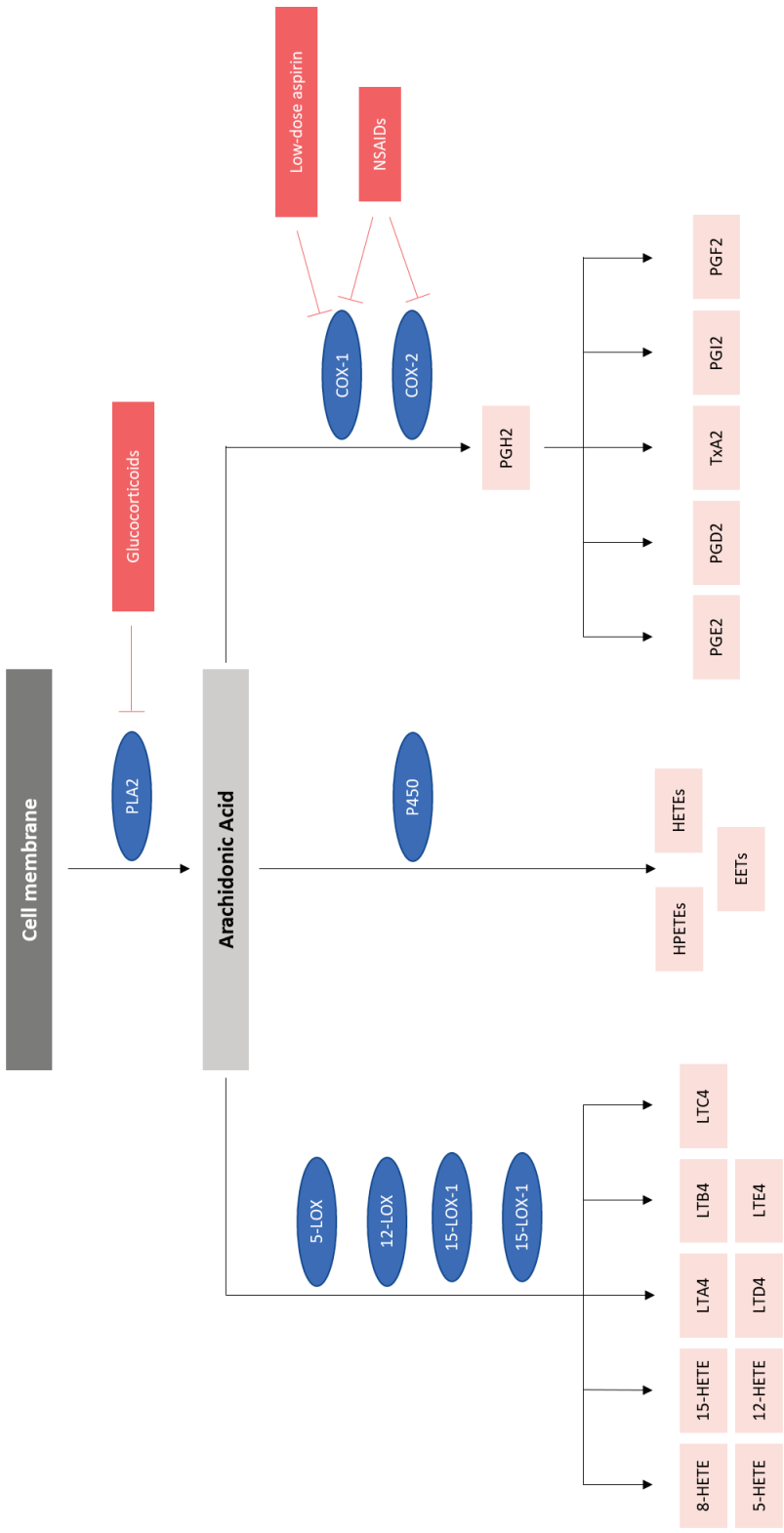
### 3.2.2. Indications

NSAIDs can be used for the temporary relief of minor aches and pains (for instance due to headache, toothache, backache, muscle pain, or menstrual cramps) and to reduce fever. Prescription-strength NSAID therapy is generally used to relieve chronic musculoskeletal pain and inflammation in conditions such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, or other rheumatologic conditions. Prescription-strength NSAIDs can also be used for the temporary relief of post-surgical pain or traumatic injury (103).

### 3.2.3. Main side effects

NSAIDs are associated with a wide well-recognized range of side effects including nervous system disorders (e.g. amnesia, seizure, or insomnia), gastrointestinal troubles (e.g. gastrointestinal bleeding, gastritis, or ulcers), liver and biliary disorders (e.g. hepatitis or liver failure), cardiovascular troubles (myocardial infarction, stroke, or hypertension), kidney problems (e.g. renal failure), respiratory problems (e.g. dyspnoea), blood disorders (e.g. haemorrhage), or allergies (e.g. pruritus or dermatitis) (103, 104). The side effects vary according to the molecule, dose, and duration of use.

Figure 3: An overview of eicosanoid synthesis pathways.



In response to a wide variety of stimuli, arachidonic acid, an unsaturated fatty acid embedded in cell membranes, is released into the cells by the phospholipase A2 (PLA2). This free arachidonic acid is subsequently converted via COX-1 or COX-2, lipoxygenases (LOXs) and P450 monooxygenase pathways into eicosanoids. COX-1 is constitutively expressed in many human organs (e.g. blood vessels, lung, kidneys, stomach, or colon) and tissues (e.g. smooth muscle cells, endothelial cells, or platelets). COX-1 induce the biosynthesis of prostaglandins (PGs) mainly involved in the maintenance of normal renal function in the kidneys (PGE2, PGI2), mucosal protection in the gastrointestinal tract (PGE2), vasodilatation (PGI2), vasoconstriction and induction of platelet aggregation (Thromboxane A2, Tx). COX-2 is constitutively found in kidneys, brain, endometrium, and ovary. However, when induced by pro-inflammatory cytokines or growth factors, COX-2 might be expressed in different cells including fibroblasts, endothelial cells, or inflammatory cells such as macrophages. COX-2 promote the biosynthesis of PGs, especially PGE2 which is particularly involved in inflammation. Different LOXs were identified including 5-LOX, 12-LOX, or 15-LOX. LOXs are expressed in leucocytes and are involved in the biosynthesis of leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs). 5-LOX is generally absent in normal epithelia but is induced by pro-inflammatory stimuli. P450 metabolizes arachidonic acid into epoxyeicosatrienoic acids (EETs), HETEs and hydroperoxyeicosatetraenoic acids (HPETEs). Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX-1 and COX2. Low-dose aspirin inhibits COX-1. Glucocorticoids inhibit PLA2.

#### 3.2.4. Main recommendations

Guidelines have been developed to reduce the risk of NSAID complications. In brief, NSAIDs should be used one at a time, at the minimal efficient dose, and during the shortest possible duration. NSAIDs should be used with caution among the elderly who run a far greater risk of side effects. NSAIDs are not recommended, or should be used with caution, among people with a history of peptic ulcer, stomach bleeding, any history of haemorrhage, any gastrointestinal problems, severe hepatocellular damage, severe renal impairment, severe heart failure, hyperlipidaemia, diabetes, hypertension, asthma, hypersensitivity to the medication or history of allergies. NSAIDs are not recommended during pregnancy, particularly during the third trimester (105). The previous guidelines vary according to the molecule.

In France, the concomitant use of proton pump inhibitors (PPI) is highly recommended to prevent or treat NSAID-induced gastrointestinal adverse events among people:

- Older than 65 years old,
- Or with a history of peptic ulcer disease,
- Or concomitantly using antiplatelet drugs, anticoagulants, or corticoids.

However, a French study published in 2020 reported that 80% of co-prescriptions of PPI with NSAIDs were not in line with these guidelines (106).

#### 3.3. Antitumor effects

Since Bennet and Del Tacca found in 1975 that PGE<sub>2</sub> were overexpressed in human colonic cancer tissues, compared to normal tissues, high PG and Tx levels have been also found in several human malignancies, such as colon, lung, breast, head and neck cancers (49, 107-109). These discoveries gave rise to the hypothesis that certain PGs and Tx were involved in the growth and spread of cancer cells and that NSAIDs, by inhibiting PG synthesis through COX-1 and COX-2 inhibition, could prevent or inhibit tumour growth (110, 111).

This hypothesis has been supported by the fact that abnormal levels of COX-1 and COX-2 were found in several human cancer tissues including breast tissues (112-119). Pro-inflammatory PGs such as PGE<sub>2</sub> might promote cancer cell growth, proliferation and angiogenesis, boost cell migration and invasion, and facilitate tumour cells to escape from immune system's attacks (49). PGE<sub>2</sub> has been shown to alter several pathways involved in cancer development (120) such as the activation the RAS-MAPK or epidermal growth factor

receptor pathways (121, 122), production of angiogenic factors (123), induction of antiapoptotic protein expression (124), or the activation of NF- $\kappa$ B transcriptional activity (125). Furthermore, PGE<sub>2</sub> might induce the massive infiltration of immune cells into the tumour microenvironment, which in turn results in the establishment of a chronic inflammatory microenvironment (120). There is also accumulating evidence that PGE<sub>2</sub> induces aromatase activity leading to oestrogen synthesis (111). Thus, NSAIDs might prevent breast cancer development (especially ER<sup>+</sup> breast cancers) by inhibiting COX-2 and consequently reducing the levels of PGE<sub>2</sub> and oestrogen. The inhibition of COX-1 could also partly explain the probable role of NSAIDs on the prevention of breast cancer. In brief, by inhibiting COX-1 in platelets, NSAIDs prevent the release of pro-inflammatory/growth mediators by platelets, which are supposed to be involved in breast cancer development [more details in the section on antiplatelet drugs (4.3. Antitumor effects)]. Furthermore, NSAIDs might alter other COX independent pathways that are involved in breast cancer development such as generation of reactive oxygen species, inhibition of NF- $\kappa$ B-mediated signals, decreasing insulin resistance, regulation of 15-LOX-1, or other apoptotic genes (103, 126-131).

### 3.4. Breast cancer prevention: up-to-date epidemiological evidence

In 1997, the IARC published its first handbook of cancer prevention focusing on NSAIDs (132). In this first volume, an international working group of experts evaluated the evidence of aspirin (regardless of the dose), sulindac, piroxicam and indomethacin as potential cancer preventive drugs. Although there was sufficient evidence for the cancer preventive activities of these compounds in experimental studies (126, 133-138), the experts concluded that epidemiological studies provided limited or inadequate evidence. At this time, epidemiological studies about the hypothetical benefits of NSAIDs were mainly based on colorectal, oesophageal, and gastric cancers. Only a few epidemiological studies on NSAIDs and breast cancer risk were published (139-143). Today, there is good evidence from epidemiological studies that NSAIDs reduce the risk of colorectal cancer (144, 145), but results for breast cancer are still not conclusive. A meta-analysis of 21 case-control and 12 cohort studies published in 2015 suggested that NSAIDs, especially aspirin (regardless of the dose) and selective COX-2 inhibitors, reduced breast cancer risk by about 10% (146). The authors also suggested that the protective effect of NSAIDs might be restricted to ER/PR<sup>+</sup> tumours. However, the benefits of aspirin or other NSAIDs was only statistically significant in case-control but not in cohort studies, where exposure was assessed prior to breast cancer

onset. The authors of this meta-analysis highlighted a high heterogeneity among the included studies. The major differences of the available epidemiological studies were in the accuracy of the exposure assessment (measured at baseline or updated during follow-up), in the exposure definition (ever use, regular use, long-term use, etc.), or in the types of NSAIDs considered (all NSAIDs, all NSAIDs but aspirin, aspirin, ibuprofen, COX-2 inhibitors, etc.). In addition, some studies reported heterogeneity of the NSAIDs-breast cancer risk associations according to BMI (147, 148), alcohol consumption (149), age at menarche (147), age at first full term pregnancy (148), menopausal status (147, 150, 151), or MHT use (151). While studies with self-reported data on NSAID use had limited data on exposure and were prone to exposure misclassification, studies using medico-administrative healthcare databases (149, 152-160) had very limited data on potential confounding factors and were not able to evaluate whether the associations differed by specific breast cancer subtypes or risk factors. Furthermore, there is a lack of epidemiological studies considering the use of other drugs as potential confounders/effect modifiers. For all these reasons, further studies are needed with well-controlled confounding factors, available data on breast cancer subtypes, and more accurate assessment of NSAID use, including types, dose, and duration of use.

## Take-home messages

### NSAIDs

- have anti-cancer properties
- have been intensively investigated as cancer preventive agents in epidemiological studies and consistently associated with a decreased risk of colorectal cancer
- are hypothesized to decrease breast cancer risk but epidemiological findings are still inconclusive.

**Further epidemiologic data, with accurate assessment of NSAID use, well-controlled confounders and long follow-up, are needed to confirm the role of NSAIDs and identify potential modalities of use (molecules, doses, duration, populations) that could be beneficial towards breast cancer prevention.**

## 4. Antiplatelet drugs

### 4.1. Definition and classification

Around 40 years after the commercialization of aspirin, an increased risk of bleeding was reported among patients treated with this molecule (92). Thereafter, aspirin had been suggested to have antithrombotic properties and to treat cardiovascular diseases (161). It is now recognized that aspirin decreases platelet aggregation and thrombus formation. At low dose ( $\leq 325$  mg per pill), aspirin is considered as an antiplatelet drug. Several other antiplatelet drugs are available such as ticlopidine and clopidogrel (Annex 2). The same molecule can exist in different dosages and administration routes (oral or parenteral). In France, antiplatelet drugs other than low-dose aspirin are only available on prescription (103). The WHO Collaborating Centre for Drug Statistics Methodology classifies antiplatelet drugs in the following group: “Platelet Aggregation Inhibitors Excl. Heparin” (ATC code: B01AC). Preparations containing combinations of low-dose aspirin with statins or angiotensin-converting-enzyme inhibitors are classified in C10BX. Preparations containing combinations of low-dose aspirin with beta blocking agents are classified in C07FX.

### 4.2. Pharmacological properties and therapeutic use

#### 4.2.1. Pharmacological actions

At low-dose, aspirin irreversibly inhibits COX-1 (particularly in platelets) and therefore the biosynthesis of  $\text{TxA}_2$  (Figure 3). The irreversible inactivation of COX-1 by low-dose aspirin leads to the virtually complete inhibition of  $\text{TxA}_2$  (162). Of note, NSAIDs which reversibly inhibit COX-isozymes do not have this feature.

Ticlopidine was the first agent of a new class of antiplatelet drugs, thienopyridines, which inhibit adenosine diphosphate (ADP). ADP, by binding the  $\text{P}_2\text{Y}_{12}$  receptor on platelets, plays a central role in platelet activation and aggregation. Because ticlopidine was associated with significant toxicities, it has been substituted by other thienopyridines including clopidogrel, ticagrelor, cangrelor and prasugrel. Other antiplatelet drugs, more recent and less often used, include glycoprotein platelet inhibitors (e.g., abciximab, eptifibatide, tirofiban), protease-activated receptor-1 antagonists (e.g., vorapaxar) or adenosine reuptake inhibitor (e.g., dipyridamole) (Annex 2).

#### 4.2.2. Indications

Antiplatelet drugs are widely used in primary or secondary cardio/cerebrovascular prevention. In 2010, the French National Authority for Health (Haute Autorité de Santé, HAS) and the French Medicine Agency (Agence Nationale de Sécurité du Médicament et des produits de santé, ANSM) recommended that the prescription of an antiplatelet treatment should be based on risk factors (assessed through a risk scale) and clinical characteristics such as risk of bleeding or history of peptic ulcer (163).

In primary prevention, the need for platelet inhibition is calculated according to a specific scale, called systematic coronary risk evaluation (SCORE), that was initiated by the European Society of Cardiology (<https://www.heartscore.org/>). The SCORE risk scale, which estimates the risk of fatal cardiovascular disease events over a ten-year period, is based on the following risk factors: gender, age, smoking, systolic blood pressure, and total cholesterol (164). Aspirin at a dose of 75-160 mg/day is recommended when cardiovascular risk is higher than 5%. Among people with diabetes, the cardiovascular risk is calculated according to the United Kingdom Prospective Diabetes Study (UKPDS) risk engine (<http://www.dtu.ox.ac.uk/riskengine/index.php>) (165).

In secondary prevention:

- administration of aspirin alone (at a dosage between 75 and 325 mg/day) is recommended after a stroke, transient ischemic attack or among people with a stable coronary heart disease (including after a coronary bypass) or peripheral arterial obstructive disease,
- co-administration of aspirin (75 to 160 mg/day) with clopidogrel (75 mg/day), prasugrel (10 mg/day) or ticagrelor (180 mg/day) is recommended during the year following a myocardial infarction (with or without stent). Then, low-dose aspirin will be taken alone,
- co-administration of aspirin (75 to 160 mg/day) with clopidogrel (75 mg/day) is recommended after a stent placement or balloon angioplasty among people without history of myocardial infarction,
- co-administration of aspirin (75 to 160 mg/day) with prasugrel (75 mg/day) or with ticagrelor (180 mg/day) is recommended after an acute coronary syndrome,
- administration of clopidogrel alone (75mg/day) is recommended when aspirin cannot be taken.



#### 4.2.3. Main side effects

A wide range of side effects associated with antiplatelet drugs is recorded (103). The side effects associated with low-dose aspirin or clopidogrel include asthenia, fever, dizziness, nervous system disorders (e.g. amnesia, somnolence), immune system disorders (e.g. allergies, oedema, or hives), skin disorders (e.g. eczema or pruritus), blood and lymphatic system disorders (e.g. haemorrhage or gastrointestinal bleeding), gastro-intestinal disorders (e.g. ulcers or gastritis), hepatic injuries (e.g. hepatitis or hepatic failure), renal and urinary troubles (renal failure or gynecomastia), musculoskeletal disorders (e.g. arthritis or myalgia), respiratory (e.g. asthma or dyspnoea), and cardio-vascular problems (e.g. vasculitis, pneumopathy).

#### 4.2.4. Main recommendations

Antiplatelet drugs should be used with caution among the elderly who run a far greater risk of side effects. Before starting antiplatelet agents, the patient should undergo assessment for bleeding risk. Temporary cessation of antiplatelet drugs increases the risk of cardiovascular events. Therefore, cessation must be professionally managed and based on a risk-benefit assessment. If the antiplatelet is an essential therapy, the medications should be resumed as quickly and safely as possible. Most common contraindications of antiplatelet agents include severe heart, hepatic or renal impairment, asthma, oesophagus or gastro-intestinal ulcers, stomach bleeding, any history of gastrointestinal problems, any haemorrhage, coagulation or haemostasis disorders, haemophilia, significant thrombocytopenia, recent surgical intervention, allergic or chronic rhinitis, and hypersensitivity to the medication. Antiplatelet drugs are not recommended during pregnancy (only during the third trimester for low-dose aspirin).

#### 4.3. Antitumor effects

Platelets, which are anucleate cell fragments generated by megakaryocytes in the bone marrow, are essentials in maintaining haemostasis and preventing bleeding (166). However, uncontrolled progression of platelets could lead to excessive thrombus formation, vascular occlusion, and subsequently vascular diseases (167). Emerging evidence suggested that platelet could contribute to inflammation and to the pathophysiology of cancer (107, 168, 169). Indeed, thrombocytosis, defined as a platelet count of  $>400 \text{ K}/\mu\text{L}$  of blood, was observed in cancer patients and associated with poor prognosis (170) in various cancers such as colorectal (171, 172), breast (173), lung (174, 175), renal (176), cervical (177), and



ovarian (178). In addition, platelet count has been positively correlated with serum levels of CRP and multiple cytokines including IL-1RA, IL-4, IL-6, IL-7, IL-8, IL-12, and IFN- $\gamma$  in colorectal cancer patients (179). Platelets might modulate inflammation and tumour progression by (107, 168, 169, 180, 181):

- interacting directly with leukocytes and affecting the immune system,
- secreting their granule components: cytokines, chemokines, platelet-derived growth factors, vascular endothelial growth factors, basic fibroblast growth factors and insulin growth factors,
- producing and releasing TxA<sub>2</sub> (which might accelerate tumour growth, invasion and promote angiogenesis),
- gathering around cancer cells, assisting tumour cells in evading immune destruction, and facilitating the circulation of cancer cells,
- inducing an invasive epithelial-mesenchymal transition phenotype of tumour cells,
- and influencing pathways that activate cell-bound protease-activated receptors, leading to the activation of inflammatory and angiogenic responses.

The possible roles of activated platelet in inflammation and cancer growth support the hypothesis that antiplatelet agents might reduce cancer development. Preclinical studies have demonstrated that aspirin inhibits the proliferation of breast tumour cells, promotes apoptosis and suppresses tumour growth and migration (182, 183). However, the mechanism of action of low-dose aspirin in the prevention of cancer has not been elucidated: both COX-dependent and COX-independent pathways could be involved. Aspirin has been hypothesized to decrease breast cancer by reducing eicosanoid biosynthesis/release through the inhibition of COX-1 (Figure 3). This hypothesis is supported by the fact that COX-1 and TXA<sub>2</sub> were found to be overexpressed in several cancers including breast cancers (108, 109, 117-119, 184). In addition, the inhibition of the TXA<sub>2</sub> biosynthesis was shown to suppress mammary tumorigenesis or breast cancer metastasis in animal models (107).

Aspirin could regulate other pathophysiological events in breast carcinogenesis, such as reprogramming the mesenchymal to epithelial transition (183, 185), inhibiting the Wnt/ $\beta$ -catenin and NF- $\kappa$ B signalling and the acetylation of extra-COX proteins (186).

There is also evidence that thienopyridines might have anti-cancer properties (187). Ticagrelor was found to inhibit platelet-tumour cell interactions and metastasis in human and murine breast cancer (188), and to inhibit lung, liver, and bone marrow cancer metastasis in mouse models (189). The inhibition by cangrelor (the in vitro equivalent of clopidogrel) of platelet secretion of vascular endothelial growth factor, thrombospondin, and transforming growth factor- $\beta$ 1 was stronger in breast cancer patients compared to healthy patients (190). In mice models, clopidogrel prevented the binding of cancer cell-derived microparticles to fibrinogen-platelets aggregates at the site of thrombosis, thereby suppressing the development of the tumour and reducing metastasis (191).

#### 4.4. Breast cancer prevention: up-to-date epidemiological evidence

Low-dose aspirin has been intensively evaluated as preventive cancer medication (192-196) and consistently associated with a lower colorectal cancer incidence in observational studies and randomized controlled trials (196, 197). Recent US guidelines even recommend low-dose aspirin for the prevention of colorectal cancer in a subset of patients (aged between 50 and 69 years old) with a favourable risk-benefit profile (198). However, findings for breast cancer are limited with few published results from randomized controlled trials. In a randomized controlled trial published in 2013 (Women's Health Study: participants aged  $\geq 45$  years old, median follow-up of 17.5 years), low-dose aspirin (100 mg) used every other day for an average of 10 years had no impact on breast cancer risk compared to placebo (Hazard Ratio [HR]=1.02, 95% confidence interval [CI]= 0.89 – 1.18, n exposed cases=385) (196). This could be because 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. The latest randomized double-blind placebo-controlled trial of daily low-dose aspirin (100 mg) (ASPREE: participants aged  $\geq 65$  years old, median follow-up of 4.7 years), showed no effect of low-dose aspirin on breast cancer incidence [HR=1.03 (0.80 – 1.32), n exposed cases=127] (199). This lack of effect could be due to the fact that follow-up as well as duration of aspirin use were not long enough to expect a benefit of aspirin. Indeed, in a meta-analysis of randomized controlled trials published in 2018, among participants aged  $\geq 70$  years, an increased risk of any cancer was observed during the first 3 years of follow-up [HR= 1.20 (1.03 – 1.47)], followed by a reduced incidence of any cancer after 5 years of follow up [HR= 0.62 (0.41 – 0.94)] (200). In the ASPREE trial, low-dose aspirin was associated with an increased risk of any solid cancer at stage 4. However, the authors did not present the associations between low-dose aspirin and stages of breast cancer (200). A meta-

analysis of 22 cohort studies and 16 case-control studies (published in 2019) suggested that aspirin use was associated with a 10% decreased risk of breast cancer, that seemed restricted to *in situ* or ER/PR+ tumours and postmenopausal women (201). However, the significant benefit for aspirin users was only found in case-control studies. In a meta-analysis of 13 cohort studies (published in 2017), a duration-response relationship between aspirin use and breast cancer risk was observed (202). The authors of these two meta-analyses detected substantial heterogeneity of results among studies as well as publication bias and highlighted the need for more epidemiological studies with well-controlled confounding and more accurate assessment of exposure to aspirin. Of note, the fact that most studies did not distinguish between low-dose and standard-dose formulations may have contributed to the reported heterogeneity of results.

Results on the associations between use of other antiplatelet drugs and breast cancer risk could help to further elucidate whether use of aspirin would impact breast cancer risk through platelet inhibition or through other mechanisms (9-11). A meta-analysis of 6 randomized controlled trials and 3 cohort studies published in 2017 concluded that there was no association between clopidogrel or prasugrel use and cancer risk (203). Since then, recent observational studies noted that clopidogrel use, alone or in combination with low-dose aspirin, might reduce the risk of several cancers (204-206) including colorectal, other gastro-intestinal, non-gastrointestinal, or haematological cancers (206). However, no epidemiological study evaluated the associations between antiplatelet drugs other than low-dose aspirin and breast cancer risk.

## **Take-home messages**

### **Low-dose aspirin**

- has been intensively investigated as a potential anti-cancer medication. However, epidemiological evidence is currently insufficient to draw conclusions on the impact of low-dose aspirin use on breast cancer risk.

### **Other antiplatelet drugs**

- have not been explored as potential breast cancer preventive medications in epidemiological studies.

**More epidemiological studies are needed and should distinguish between low-dose and standard-dose aspirin formulations. Results on other antiplatelet drugs could help to further elucidate whether aspirin would impact breast cancer risk through platelet inhibition rather than through its potential anti-inflammatory properties.**

## 5. Glucocorticoids

### 5.1. Definition and classification

Synthetic glucocorticoids emerged in the late 1940s with the chemical synthesis of cortisone (207). Nowadays, several glucocorticoids, with various anti-inflammatory, immunosuppressive, metabolic, and endocrine properties, are available on the market. All these drugs are structurally and pharmacologically similar to the endogenous hormone cortisol, mainly produced naturally by the adrenal glands. In France, all glucocorticoids are only available through prescriptions (103). The WHO Collaborating Centre for Drug Statistics Methodology classifies systemic glucocorticoids in the following group: “Glucocorticoids” (ATC code: H02AB). The different systemic glucocorticoids are presented in Annex 3. Other glucocorticoids are available including glucocorticoids for nasal use (R01AD), for inhalation (R03BA), for topical use (D07), for local treatment of acne (D10AA), for local intestinal treatment (A07E), for local oral treatment (A01AC), for eye treatment (S01), for ear treatment (S02), and for eye and ear treatment (S03).

### 5.2. Pharmacological properties and therapeutic use

#### 5.2.1. Pharmacological actions

Glucocorticoids are corticosteroids that bind to the glucocorticoid receptor (GR). GR is a member of the nuclear receptor superfamily of transcription factors expressed in various tissues. When glucocorticoids bind to the GR, the receptor becomes activated and the GR-glucocorticoid complex translocates into the cell nucleus (208). Once inside the nucleus, the complex can:

- Binds directly to DNA elements called glucocorticoid response elements to stimulate target gene expression and activate gene transcription. For instance, glucocorticoids induce the formation of lipocortin-1, an important inhibitor of the phospholipase A<sub>2</sub>, thereby blocking eicosanoid production including PGs and leukotrienes (Figure 3),
- repress transcription of genes containing a negative glucocorticoid response element,
- or bind to transcription factors (such as the NF- $\kappa$ B and activator protein-1), leading to the inhibition of various pro-inflammatory molecules including IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-18, COX-2, and TNF- $\alpha$ .

Likewise, high doses of glucocorticoids (depending on the molecules) bind to mineralocorticoid receptors influencing the electrolytes and water balance.

### 5.2.2. Indications

Synthetic glucocorticoids are used to treat a wide range of diseases (103). Systemic glucocorticoids are used as hormone replacement therapy in case of adrenal insufficiency (e.g., in Addison's disease [a rare condition which is primarily caused by autoimmune destruction of the adrenals, leading to deficiency of cortisol, aldosterone and adrenal androgens]), rheumatologic conditions (e.g. arthrosis, tendinitis, sprain, bursitis, arthritis, rheumatoid arthritis, or talalgia), autoimmune diseases (e.g. multiple sclerosis, inflammatory bowel disease, ulcerative colitis, psoriasis, autoimmune haemolytic anaemia, or systemic lupus erythematosus), nephrology disorders (e.g. nephrotic syndrome), allergies (e.g. allergic rhinitis), or respiratory diseases (e.g. chronic obstructive pulmonary disease). Systemic glucocorticoids can also be used at high doses to treat lymphomas, leukaemia, or multiple myeloma and to mitigate the side effects of anticancer drugs. Topical glucocorticoids are used to treat conditions like dermatoses, eye inflammations, or ear infections. Inhaled glucocorticoids are used to treat asthma (103).

### 5.2.3. Main side effects

Systemic glucocorticoids are known to increase blood sugar levels (which can lead to diabetes), suppress the body's ability to absorb calcium (which can lead to osteoporosis), increase cholesterol and triglyceride levels, suppress the immune system (by decreasing leukocytes and cytokines production), induce a loss of muscle tissue, and resorb sodium leading to fluid retention. Glucocorticoids could also lead to cardiovascular problems (e.g. arrhythmia, pulmonary embolism, heart failure, or hypertension), gastrointestinal troubles (e.g. gastrointestinal bleeding, or ulcers), kidney damages, metabolic troubles (e.g. weight gain or oedema), musculoskeletal disorders (arthritis or arthralgia), and skin disorders (acne, purpura, dermatitis or skin rash) (209, 210). The side effects of other routes of administration of glucocorticoids are mainly the same as those seen with systemic glucocorticoids (211). The most frequent adverse effects of dermal glucocorticoids include atrophy, striae, rosacea, perioral dermatitis, acne, and purpura (212).

### 5.2.4. Main recommendations

As many glucocorticoid-related adverse effects depend on dose and duration of treatment, it is recommended to use glucocorticoids during the shortest duration possible. Glucocorticoids must be mainly avoided in case of hypersensitivity, infection, psychiatric conditions, or surgical intervention. People with diabetes, history of peptic ulcer disease or ulcerative

colitis, hepatic or renal insufficiency, hypertension, severe myasthenia, hypokalaemia, sodium retention, lactase deficiency, or osteoporosis will need particular attention from medical services. A long glucocorticoid therapy could be complemented by a diet low in sodium, low in sugar, high in protein, and in some cases, using vitamin D supplements and PPI.

### 5.3. Antitumor effects

The action of glucocorticoids in tumor progression is controversial and might have diverse influences depending on the type of cancer (213). Regarding breast cancer, potential beneficial and harmful effects of glucocorticoids have been reported. On the one hand, glucocorticoids might prevent breast cancer development by affecting angiogenesis (214, 215), inhibiting growth and inflammatory factors (214), stimulating the expression of the sulfotransferase SULT1E1 (which plays a role in deactivating oestrogen) (213, 216), and suppressing cell migration/invasion (217, 218). In addition, glucocorticoids might exert some anti-tumour effects by reducing eicosanoid production through the inhibition of phospholipase A<sub>2</sub> (Figure 3). On the other hand, glucocorticoid might promote breast cancer growth by facilitating tumour cells to escape from immune surveillance and inducing insulin resistance (219, 220). Recent experiment using mice models suggested that glucocorticoids might promote breast cancer metastases (221). This hypothesis is not new: results from a study published in the 1960's indicated that, compared to non-use, the use of adrenal steroids (including glucocorticoids) increased the risk of metastasis among breast cancer patients (222).

Thus, whether glucocorticoids promote or inhibit breast cancer growth is still under investigation.

### 5.4. Breast cancer prevention: up-to-date epidemiological evidence

Previous epidemiological studies suggested that use of systemic glucocorticoids increases the risk of non-Hodgkin lymphoma (223), skin (223-225), bladder (226), and prostate (227, 228) cancers.

To my knowledge, only two epidemiological studies have been previously published, both based on data from nationwide medico-administrative databases from Northern Denmark (229, 230). The latest study, which was an extension of the previous one (229) suggested that

there was no association between at least 3 prescriptions of systemic or inhaled glucocorticoids and invasive breast cancer risk [inhaled glucocorticoids: odds ratio (OR)=1.00 (0.95 – 1.10), n exposed cases=593; systemic glucocorticoids: OR=1.00 (0.96 – 1.10), n exposed cases= 908)] (230). No associations were found when the authors categorized the glucocorticoid exposure into recent and former use, according to intensity or duration of use, or stratified the analysis by menopausal status (pre, peri, postmenopausal). As mentioned by the authors themselves, they were not able to stratify their analyses by breast cancer subtypes. However, glucocorticoids might have a different impact on breast cancer development according to breast cancer molecular status. Three other epidemiological studies suggested that “corticoid” or “steroid” use was associated with a decreased risk of breast cancer (160, 231, 232). However, there was no detailed information on exposure assessment and the number of exposed cases was small in two studies ( $n \leq 22$ ). There is thus a lack of epidemiological studies on the relation between glucocorticoid use and breast cancer risk. Further well-designed epidemiological studies should evaluate the glucocorticoids-breast cancer associations according to breast cancer subtypes or risk factors as well as dose, duration, and timing of glucocorticoid use.

## **Take-home messages**

### **Glucocorticoids**

- play a complex/dual role in breast cancer initiation and progression in experimental studies
- are not associated with breast cancer risk in the only epidemiological study with detailed data on glucocorticoid exposure

**Further well-designed epidemiological studies on the associations between glucocorticoid use and breast cancer risk, with data on potential confounders and breast cancer subtypes, are needed to address current knowledge gaps.**



## 6. Thesis objectives

My doctoral thesis aimed to better understand the role of inflammation on breast cancer development using two complementary approaches:

- 1) to evaluate the associations between breast cancer risk and a panel of 11 inflammatory biomarkers (TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, IL-10, IL-13, IL-17D, IL-1RA, CRP, leptin and adiponectin) in about 1600 case-control pairs in the European Prospective Investigation into Cancer and Nutrition (EPIC) study
- 2) to evaluate the associations between breast cancer risk and NSAIDs, antiplatelet drugs and glucocorticoids using self-reported and drug reimbursement data in the EPIC study and its French component, the E3N (*Étude Épidémiologique auprès de femmes de la Mutuelle Générale de l'Éducation Nationale*) cohort.

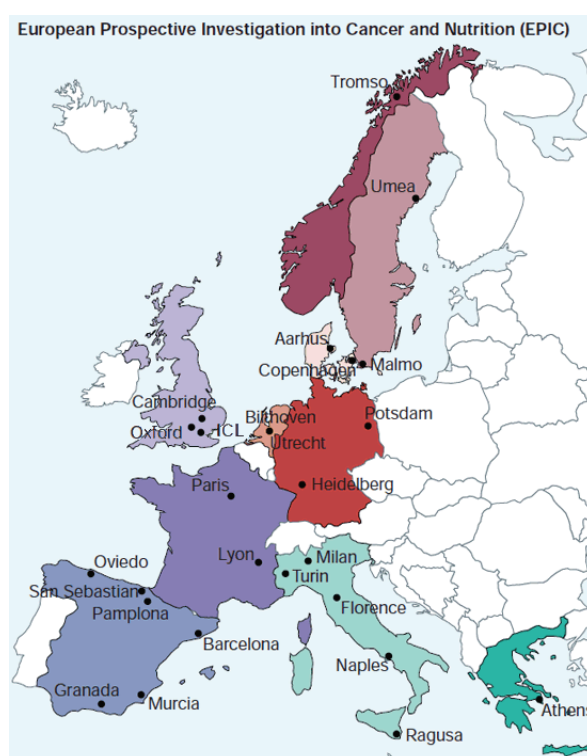
## CHAPTER 2: DATABASES

### 1. The EPIC study

#### 1.1. Presentation of the cohort

The European Prospective Investigation on Cancer and Nutrition (EPIC, <https://epic.iarc.fr/>) study is a multi-centre ongoing prospective cohort study, mainly designed to investigate the aetiology of cancers (233). This study enrolled more than 521,000 participants, aged between 25 and 70, recruited between 1992 and 2000 from 23 centres across 10 Western European countries including Spain, Italy, France, the United Kingdom, Greece, Germany, the Netherlands, Denmark, Sweden, and Norway (Figure 4). Approval for the study was obtained from the relevant ethical review boards of the participating institutions as well as from the IARC ethics committee.

Figure 4: EPIC countries and centres.



#### 1.2. Recruitment and baseline information

##### 1.2.1. Self-reported data

At recruitment, participants provided their informed consent and completed detailed questionnaires including questions on lifestyle habits (history of tobacco smoking, alcohol

consumption, physical activity, education level and diet), history of previous illnesses, reproductive factors, and anthropometric factors. Extensive details on the standardized recruitment procedures have been published previously (233). Below, I describe briefly the collected variables used in the present projects.

### 1.2.2. Biological data

At baseline, blood samples were collected from 387,889 individuals including 246,000 women from all countries. Blood was collected according to a standardized protocol in France, Germany, Greece, Italy, the Netherlands, Norway, Spain, and the United Kingdom (233). Serum (except in Norway), plasma, erythrocytes (except in Norway), and buffy coat were separated and aliquoted in 28 plastic straws of 0.5 ml each. These samples were then split into two mirror halves of 14 aliquots each and one was stored locally and the other half in a centralized biobank at the IARC in liquid nitrogen ( $-196^{\circ}\text{C}$ ). In Norway, blood fractions were collected in 20 plastic straws of 0.5 ml each (16 plasma and 4 buffy coat), half of which (8 plasma et 2 buffy coat) were shipped to IARC for storage in the central repository. In Denmark and Sweden, blood samples were stored in tubes (not in plastic straws) in local repositories (IARC is not suitable for storing tubes). In Sweden, the samples are kept in deep freezers at  $-70^{\circ}\text{C}$ , and in Denmark in nitrogen vapor ( $-150^{\circ}\text{C}$ ).

## 1.3. Data on cancer and vital status

### 1.3.1. Vital Status

Data on death were collected from mortality registries at the regional or national level. The follow-up period of this thesis project ended as follows: June 2008 in France, December 2009 in Heidelberg, Varese and Murcia, December 2010 in Florence, Ragusa, Turin, Asturias, Bilthoven, Naples and Utrecht, December 2011 in Granada, Navarra, San Sebastian and Cambridge, December 2012 in Greece, Oxford, Umeå, Norway and Denmark, and December 2013 in Malmö and Potsdam.

### 1.3.2. Cancer events

Cancer cases were identified during follow-up based on population cancer registries in Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom, and on a combination of methods, including health insurance records, contacts with cancer and pathology registries, and active follow-up of participants and their next of kin in France, Germany, and Greece. In the present work, first primary cases of breast cancer were

identified as primary incident tumours of the breast. Diagnosis of cancer cases in EPIC was based on the 2nd or 3rd revision of the International Classification of Diseases for Oncology (ICD-O-2 or ICD-O-3) (234). Around 20,000 incident breast cancers were diagnosed in EPIC in 2015.

#### 1.4. Exposure to aspirin or nonsteroidal anti-inflammatory drugs

Five countries have provided baseline self-reported data on NSAID use: the Netherlands (Utrecht centre), France (all centres), Denmark (all centres), Germany (all centres), and the United Kingdom (Cambridge centre). Heterogeneous data on NSAID use were collected at baseline. The following questions were asked to the participants:

- “Are you currently taking anti-inflammatory drugs at least three times per week?” in France.
- “Have you ever taken aspirin continuously for 3 months or more?” in the United Kingdom.
- “During the past 4 weeks, did you take any regular medications? If yes, please specify the medication.” in Germany.
- “Do you take aspirin daily?” in the Netherlands.
- “Have you used more than one painkilling tablet/month during the last year? If yes, how many pills containing aspirin and how many pills containing ibuprofen: none; 2–3/month; 1–2/week; 3–6/week; 1–3/day; 4–5/day; 6+/day?” in Denmark.

#### 1.5. Co-variables

##### 1.5.1. Education level

Educational level was collected at baseline for each centre and the replies were then categorized into a common classification using three categories (none or primary education, technical/professional/secondary school, and longer education).

##### 1.5.2. Familial history of breast cancer

Only in France, Spain, Utrecht, Norway and Bilthoven, participants were asked to report whether their mother or sister(s) were diagnosed with a breast cancer. The age at diagnosis was also asked.

### 1.5.3. Medical conditions

In all centres, participants were asked to report previous illnesses and age of onset of each of these events. Most centres included information on heart disease, diabetes, stroke, hypertension, hyperlipidaemia, gallstones, polyps of the large bowel, hysterectomy, oophorectomy, and breast surgery.

### 1.5.4. Hormonal and reproductive factors

#### **Age at menarche**

In all EPIC countries, women were asked to either report their age at menarche or to choose a predefined category of age at menarche. Age at menarche was then dichotomized ( $\leq 13$  years and  $>13$  years).

#### **Reproductive life**

In all EPIC countries, women were asked to report whether they had ever been pregnant and whether they had ever had any live born children or still births (miscarriage or abortion). They could also report the number of live births and their age at first delivery (second, third and last delivery). Women were considered to have had a full-term pregnancy if they reported at least one live birth or still birth. In Norway and Umeå, women were asked to directly give the number of full-term pregnancies. For the other countries, the number of full-term pregnancies was calculated from the number of live births and the number of still births. Age at first full term pregnancy was considered as the minimum age between the two types of full-term pregnancies (stillbirths or live births). This information was missing for Norway, Bilthoven, Malmö and Umeå. In all centres except Bilthoven and Umeå, women were asked whether they had ever breastfed one on their three first and last delivery. Women who reported to have breast fed at least one of her children was considered as having ever breastfed.

#### **Menopausal status**

A combination of different factors was used to determine the menopausal status at baseline. Women were considered premenopausal when they reported they were still menstruating or had at least nine menstrual periods over the previous 12 months. Women who had missing or incomplete questionnaire data or who had had a hysterectomy, were considered premenopausal when they were younger than 42 years (because among the female EPIC participants who had complete questionnaire data, almost all women who were younger than

42 years were premenopausal). Women were considered postmenopausal when they reported fewer than four menses in the past year, or when they reported a bilateral ovariectomy. Women who had missing or incomplete questionnaire data, were considered postmenopausal when they were older than 55 years (because among the female EPIC participants who had complete questionnaire data, almost all women who were older than 55 years were postmenopausal). Women between 42 and 55 years old who had had a hysterectomy and using exogenous hormones were considered perimenopausal women.

### **Phase of menstrual cycle among premenopausal women at the time of blood donation**

For all women classified as premenopausal at the time of blood donation, phases of menstrual cycle (early follicular, late follicular, peri-ovulatory, midluteal, and other luteal) were determined using two different methods: “forward” dating” (counted forward from the woman’s reported date of the start of her last menses) and “backward dating” (counted backward from the date of the start of her next menses after blood donation, which the woman reported on a prepaid postcard and sent back to the recruitment centre afterwards). Preference was given to backward dating because the length of the second half of the cycle (luteal phase) is generally more constant than the first half (follicular phase).

### **Exogenous hormone use**

All EPIC centres had information on oral contraceptive use including ever use, age at first use and duration of use. In all EPIC centres except Umeå, women were asked to report whether they were currently using oral contraceptives. Information on MHT use covered ever and current use, age at first use, duration of use and brand name of current MHT use. Umeå did not have information of current MHT use and Malmö did not have information on age at first MHT use. From the MHT brand name, the type of hormone and the route of administration could be deduced.

### **1.5.5. Anthropometric factors, physical activity, and energy intake**

#### **Anthropometry**

All EPIC centres except Umeå and Norway, have either self-reported (France and part of the UK) or measured information on weight, height, hip circumference, and waist circumference (WC). In Norway and Umeå, only self-reported information of height and weight were collected. In Oxford and in France anthropometric factors were measured only for a restricted number of participants, but self-reported weight and height were obtained from all

individuals. Sitting height was measured in France, Italy, Spain, Utrecht, Greece, Germany and Denmark (235). BMI was calculated for each participant and split into four categories: underweight ( $<18.5 \text{ kg/m}^2$ ), normoweight ( $\geq 18.5 - <25 \text{ kg/m}^2$ ), overweight ( $\geq 25 - <30$ ) and obese ( $\geq 30 \text{ kg/m}^2$ ).

### **Physical activity**

The baseline questionnaire assessed past-year physical activity including occupational, leisure/recreational and household activities. For recreational and household activities, participants reported the duration of activities during a typical week during the past summer and winter. Household activities included housework, home repair, gardening and stair climbing. Recreational activities included walking, cycling and sports activities. A metabolic equivalent of task (MET) value was assigned to each reported activity according to the Compendium of Physical Activities (236). The mean numbers of hours per week of summer and winter household and recreational activities were estimated and then multiplied by the appropriate MET values to obtain MET-hours per week of activity. A four-level physical activity index was derived by combining occupational physical activity together with time participating in cycling and other physical exercise (such as keep fit, aerobics, swimming, and jogging). Participants were categorized into inactive, moderately inactive, moderately active and active (237).

#### **1.5.6. Alcohol consumption and tobacco**

##### **Alcohol consumption**

Alcohol consumption at baseline was collected for the whole EPIC cohort. Participants reported the number of standard glasses of wine, beer, cider, sweet liquor, distilled spirits, or fortified wines they consumed daily or weekly during the 12 months before recruitment. These replies were used to estimate the quantity of ethanol consumption per day. Alcohol consumption was also assessed from 24-hour dietary recalls in a subset of the cohort, to standardise the dietary information received from all centres. Lifetime alcohol consumption was also collected for 76% of participants.

##### **Tobacco smoking**

The following information on cigarette smoking was collected through baseline questionnaires: smoking status (current, former, or never), age at which they started and quit smoking, number of cigarettes per day currently smoked, and number of cigarettes smoked

per day during different periods of life. Information on passive smoking was available in 6 countries (France, Italy, the Netherlands, Germany, Denmark, and Norway). All centres (except those in the Netherlands and Norway) also have information on current and past cigar and pipe smoking.

## 2. The E3N study



### 2.1. Presentation of the cohort

The French component of EPIC, E3N (*Etude Epidémiologique auprès de femmes de l'Education Nationale*, <https://www.e3n.fr/>), is an ongoing prospective cohort, which initially aimed to investigate the relationship between lifestyle, diet, hormones, environment and female cancers. Nowadays, other major chronic diseases such as diabetes and cardiovascular diseases are also being studied (238). The E3N study was approved by the French National Commission for Data Protection and Privacy (*Commission Nationale Informatique et Libertés*, CNIL).

### 2.2. Recruitment and baseline information

In 1990, 499,668 French women born between 1925 and 1950, living in metropolitan France and insured by a specific national health scheme covering mainly teachers (*Mutuelle Générale de l'Education Nationale*, MGEN), were invited to participate in the study. Twenty percent (98,995 women) agreed. Age and region of residence were not different between participants and non-participants (238). At baseline, participants signed an informed consent and replied to the first questionnaire (Q1), which was common to all EPIC centres, and included questions on socio-demographic factors (educational level and current employment status), anthropometric measures (current and at different times in life), menstrual and reproductive factors (age at menarche, parity, age at first pregnancy, breastfeeding, and menopausal status), lifetime medical and surgical history, family history of cancer, gynaecological follow-up (pap smear frequency and date of last mammogram), lifetime tobacco consumption, and current physical activity.

### 2.3. Follow-up

#### 2.3.1. Self-reported data

Every 2-3 years after recruitment, participants completed self-administered questionnaires to update previous information or to collect new information, as summarized in Figure 5. The



second questionnaire (Q2) asked detailed information on menstrual factors (age at menarche, menstrual cycle length, menopausal status, and age at menopause), reproductive history (treatments for infertility and for each pregnancy: age at pregnancy, duration and outcome and breastfeeding), lifetime history of use of oral contraceptives, MHT, or other hormonal treatments (for each episode of use: drug name, age at first use, and duration of use), lifetime personal history of benign gynaecological diseases (for each disease: type of disease, age at diagnosis, diagnostic procedures, and treatment), lifetime personal history of other diseases (diabetes, stroke, cancer, etc.), and medical follow-up (including information on recent mammogram). The third (Q3) and the eighth (Q8) questionnaires also included a diet history questionnaire, and the fourth questionnaire (Q4) focused on specific anthropometric measures. Q2 to Q9 updated information on menopausal status and use of hormonal treatments. Q2 to Q11 updated information on health status as well as data such as weight and tobacco consumption. Q3 to Q9 included questions on current use of drugs. Q10 included questions on individual's autonomy and well-being, oral health, visual, and hearing ability. Q11 also included information on quality of life and sleep patterns.

While Q3 and Q4 were only sent to women who responded to the previous questionnaire, Q2, Q5, Q6, Q7, Q8, Q9, Q10, and Q11 were sent to the whole cohort. The response rates were varied from 77% to 92%.

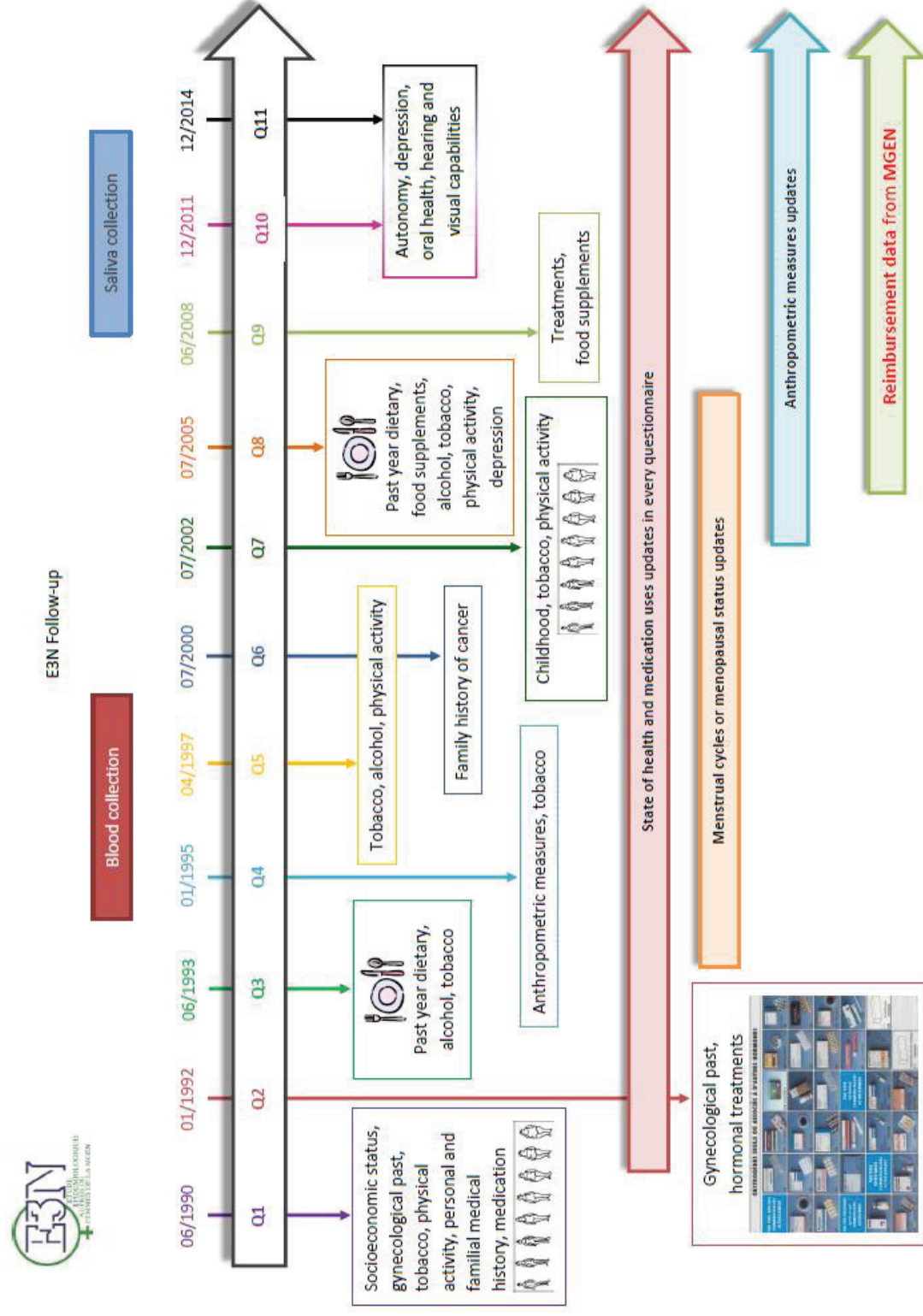
### 2.3.2. Data from the MGEN database

Every three months, the MGEN provides the E3N team with updated information on vital status and address of the participants that are still affiliated with this health scheme. Additionally, the MGEN provides, for each cohort participant, data on all outpatient reimbursements for health expenditure issued since January 1, 2004.

### 2.3.3. Biological data

Between 1994 and 1999, blood samples were collected among around 25,000 participants. Between 2009 and 2011, saliva samples were collected among around 45,000 women who did not previously provide blood samples.

Figure 5: Timeline of data collected between 1990 and 2014 (E3N Cohort).



## 2.4. Data on cancer and vital status

### 2.4.1 Vital status

As mentioned previously, the MGEN provides updated information on vital status of the participants that are still affiliated with this health scheme. Deaths can also be reported by family members or by the postal services (when the questionnaire is returned). When information on vital status is missing, the National Death Index is screened to check whether the participant is still alive. In the event of the death of a participant, cause of death is retrieved through the French National Causes of Death Registry (Cépi-DC Inserm).

### 2.4.2 Cancer events

As shown in Figure 5, each questionnaire included a specific section on health status including cancer occurrence. Participants were asked to report cancer site and date of diagnosis and to provide a copy of pathology reports. The contact details of the participant's physician were also requested in each follow-up questionnaire. When participants could not provide the pathology reports, physicians were contacted to confirm the reported cancers and were asked to send a copy of the pathology reports. The proportion of false-positive self-reports was low (<5%) for breast cancers. Death certificate information, mentioning the underlying cause of death, the chain of events that led to death, as well as all other diseases or conditions contributing to death, was also taken in consideration to identify any cancer cases which were not self-reported in the follow-up questionnaire. However, when a cancer event was identified through this means, it was impossible to investigate the event if the participant did not report the contact details of her physician(s) in any follow-up questionnaire. In addition, even with death certificates some cancer events might have been missed. When a cancer case was identified through death certificates and when no further information could be retrieved, including date of diagnosis, women were censored at the date of the last questionnaire returned and were considered as non-cases at that date.

Breast cancer characteristics were retrieved from the pathology reports. This includes exact localisation, morphology, grade and stage at diagnosis, and molecular status (ER, PR or HER2 status).

Breast cancer cases are coded by a staff member of the E3N team according to different international tables from the WHO and the UICC:

- the ICD-10 to define the exact localisation of the tumour (239).
- The ICD-0 2/3 to determine the tumour morphology and grade (234).
- The TNM system to describe the stage (10).

2,734 prevalent breast cancers were diagnosed in Q1 and 8,238 incident primary breast cancer were diagnosed between Q1 and Q11.

## 2.5. Exposure to anti-inflammatory and antiplatelet drugs

The MGEN provides, for each E3N participant, data on all drug reimbursements issued since January 1st, 2004. The available data for each reimbursement are date of drug purchase and national code number (*Code Identifiant de Presentation*, CIP). The national code number enabled us to retrieve the following information from Thériaque® (a French-drug database: [www.theriaque.org](http://www.theriaque.org)): ATC code, molecule, route of administration, number of pills/ampules per package, dose per pill/ampule, and DDD. In Q4, Q6, and Q7, women were asked to report whether they were currently using anti-inflammatory drugs at least three times per week (Figure 6).

Figure 6: Photo of one questionnaire including question on the use of anti-inflammatory drugs

**7 Autres médicaments : prenez-vous actuellement, au moins 3 fois par semaine ?**

Somnifères <input type="checkbox"/>	Anti-inflammatoires <input type="checkbox"/>
Psychotropes (tranquillisants, antidépresseurs...) <input type="checkbox"/>	Antihypertenseurs <input type="checkbox"/>
Vasodilatateurs (pour la circulation du sang) <input type="checkbox"/>	Antidiabétiques <input type="checkbox"/>
Supplémentation en :	Diurétiques <input type="checkbox"/>
Phytoestrogènes (Soja) <input type="checkbox"/>	Protecteurs gastriques <input type="checkbox"/>
Calcium <input type="checkbox"/>	(contre ulcères et aigreurs d'estomac)
Biphosphonates <input type="checkbox"/>	Laxatifs <input type="checkbox"/>
Autres Minéraux / Oligo-éléments <input type="checkbox"/>	(indiquer les noms en clair)
Vitamines :	.....
A <input type="checkbox"/>	.....
C <input type="checkbox"/>	.....
D <input type="checkbox"/>	.....
E <input type="checkbox"/>	.....
Groupe B <input type="checkbox"/>	.....
Acide folique <input type="checkbox"/>	.....
Béta-carotène <input type="checkbox"/>	.....
Autres vitamines <input type="checkbox"/>	.....

In Q5 women could also report (as free text) any other drug that they were currently using at least three times per week.

## 2.6. Co-variables

The co-variables of interest for the presented work are described below.

### 2.6.1. Educational level

Educational level was recorded in Q1. Participants could tick boxes corresponding to their educational level: 1) no schooling, 2) school certificates, 3) vocational education, 4) from high-school to two years of higher education, 5) from three to four years of higher education, 6) at least five years higher education.

### 2.6.2. Familial history of breast cancer

Familial history of cancer was collected in Q1 and Q6. In Q1, participants could tick boxes to declare whether their grand-parents, mother, father, sisters, brothers, uncles, aunts, or children had ever had breast, bowel, or other cancers. In Q6, participants could report whether a member of their family had ever had a cancer and specify the exact cancer site and the age at diagnosis.

### 2.6.3. Medical factors

In each questionnaire from Q3, participants could note in a free-text section any disease from which they were suffering. Additionally, participants were asked to specify if they had been diagnosed with specific diseases such as the following:

#### **History of benign breast diseases**

As mentioned above (2.3. Follow-up) information on lifetime personal history of benign breast diseases (such as adenoma and fibro-adenoma, mastitis, and fibrocystic diseases) was first collected in Q2. The age at diagnosis, diagnostic procedures and treatment was reported. This information was then updated in several questionnaires from Q3 to Q9.

#### **Other medical events**

Information on lifetime personal history of other medical events was first collected in Q1 (or Q2 for some of them) and then updated in several subsequent questionnaires. Indeed, women were asked to report, together with the corresponding age at / date of diagnosis (mm/yyyy), if they had had diabetes (all questionnaires), hypertension (all questionnaires except Q10), hypercholesterolemia (Q1, Q2, Q6, Q7, Q8, Q9, Q10 and Q11), angina pectoris (all questionnaires), myocardial infarction (all questionnaires), stroke, pulmonary embolism (all questionnaires), deep vein thrombosis (all questionnaires from Q2), arteritis of the lower limbs (all questionnaires from Q2), cardiac dysrhythmia (Q2-Q10),

polyarthritis, Horton's disease (Q9-Q11), rheumatism (Q1), spondylarthritis ankylosing (Q10-Q11), arthrosis (Q8, Q10), arthritis (Q8), migraine (all questionnaires from Q2 except Q8), asthma (all questionnaires except Q7), chronic bronchitis (all questionnaires), hay fever (Q2, Q3, Q7, and Q8), and chronic inflammatory bowel diseases (all questionnaires except Q11). We considered that a woman has a history of one of the previous diseases when she reported it for the first time in a questionnaire.

#### 2.6.4. Gynaecological follow-up

In Q1, women were asked to report if they had already undergone a mammogram together with the date of their last mammogram. In every other questionnaire, women reported whether they had undergone a mammogram since their answer to the previous questionnaire. In Q3, Q9, Q10, and Q11, the date of the last mammogram was asked.

#### 2.6.5. Hormonal and reproductive factors

##### **Age at menarche**

In the first two questionnaires, women were asked to report the age at which they had their first menstrual period.

##### **Reproductive life**

Number of pregnancies was recorded through Q1 and Q2. In Q2, other information was collected such as age at the beginning of each pregnancy (up to 12) and the duration of each pregnancy. For each birth, women were also asked to report if they breastfed their child and to specify the breastfeeding duration.

##### **Menopausal status and age at menopause**

Menopausal status was updated throughout the follow-up, based on self-reports of being postmenopausal (Q1, Q6, Q7, and Q8) as well as information on MHT use (Q2-Q8), oral contraceptives (Q2-Q8), menstrual cycles (Q2-Q8), date of last menses (Q2, Q3, Q4, Q6, and Q7), history of menopausal symptoms (Q2-Q7), history of hysterectomy and age at surgery (Q1, Q2, Q5, Q6, Q7, and Q8), history of simple or bilateral oophorectomy and age at surgery (Q2, Q5, and Q8), history of radiotherapy and / or chemotherapy (leading to definitive menses cessation; Q2). Women were considered postmenopausal if they reported 12 consecutive months of amenorrhea (unless due to hysterectomy), bilateral oophorectomy, ever use of MHT, or if they self-reported to be menopausal. Menopause was considered to be



artificial if prompted by bilateral oophorectomy, radiotherapy, or chemotherapy and to be natural otherwise. When menopausal status could not be determined, women were considered premenopausal if they had not reached the age of 51 years (the median age at natural menopause in the cohort) at end of follow-up and menopausal otherwise. After Q8, all E3N participants were at least 55 years old and therefore most of them had reached menopause.

Age at menopause was self-reported in Q1, Q6, and Q7. For menopausal women, age at menopause was defined as age at the latest menstruation (unless due to hysterectomy and if the last menstrual period occurred before MHT use) or as age at bilateral oophorectomy, if relevant. If this information was not available, other information was used to determine age at menopause, in decreasing order of priority: self-reported age at menopause, age at first MHT use, and age at start of menopausal symptoms. When age at menopause could not be determined, women were imputed a menopausal age of 51 years for natural menopause or unknown type of menopause and 47 years in case of artificial menopause, ages which correspond to the median ages for natural and artificial menopause in the cohort, respectively.

### **Exogenous hormone use**

Detailed information on lifetime use of oral contraceptives and MHT were collected through Q2. Participants were asked to report brand names (from which type and route of administration could be deduced for MHT), together with the start date and duration of each episode of hormone use. This information was then updated in each follow-up questionnaire until Q9. Since 1<sup>st</sup> January 2004, the MGEN provides, for each E3N participant, data on MHT reimbursements.

### **2.6.6. Anthropometric factors and physical activity**

Weight was available in each questionnaire. Height was available in Q1, Q4 and from Q6 to Q11. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist and hip circumferences were collected in Q4 and in all questionnaires from Q7.

In Q1, Q3, Q5, Q7, Q8, and Q11, women were asked to report their current physical activity habits including recreational and household activities and to specify the frequency and duration of these activities. For each physical activity, the MET-hours was estimated based on the Compendium of Physical Activities (236).

#### 2.6.7. Alcohol consumption and tobacco

In Q3, Q5, and Q8, women were asked to report their current consumption of alcohol. The type, frequency and quantity of alcohol consumption were collected. These data were used to estimate the quantity of ethanol consumption per day.

In all questionnaires except Q9, women were asked to report their smoking status. They were asked to report whether they were a current regular smoker (at least 1 cigarette per day), current occasional smoker, past regular smoker, past occasional smoker, or never smoker. Other information was collected in some questionnaires such as the type of tobacco, frequency, or duration of smoking.



## CHAPTER 3: CIRCULATING INFLAMMATORY BIOMARKERS AND BREAST CANCER RISK IN THE EPIC STUDY

### 1. Objective

The objective of this chapter was to conduct a case-control study nested within the EPIC cohort to evaluate the associations of pre-diagnostic circulating concentrations of 11 inflammatory biomarkers (TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, IL-10, IL-13, IL-17D, IL-1RA, CRP, leptin and adiponectin) with breast cancer risk, overall and by tumour receptor status, menopausal status, adiposity and exogenous hormone use.

### 2. Materials and methods

#### **Selection of cases and controls**

EPIC participants were eligible for inclusion in the current study if, at recruitment, they:

- donated their blood
- were cancer-free (other than non-melanoma skin cancer)
- were not pregnant.

Cases were selected among all EPIC eligible women with a first primary invasive breast cancer diagnosis at least two years after blood collection and before December 2012 and with known information on hormone receptor status. For each breast cancer case, one control was matched. Controls were selected randomly among all eligible EPIC participants who were alive and without cancer diagnosis (except non-melanoma skin cancer) at the time of cancer diagnosis of his matched case (incident density sampling). Controls were matched to cases on centre of recruitment, age, menopausal status, phase of the menstrual cycle for premenopausal women and fasting status at blood collection.

#### **Inflammatory biomarker assessment**

Pre-diagnostic plasma levels of cytokines and adipokines were measured using commercially available immunoassays provided by Meso Scale Discovery, a highly sensitive immunoassay platform, in the laboratories of the Biomarkers Group at IARC.

#### **Statistical analysis**

Geometric means were used to describe biomarker concentrations among cases and controls. Log-transformed biomarker concentrations were used in all following analyses. Partial

Pearson's correlations between biomarkers and with anthropometric factors, adjusted for age at blood collection and laboratory batch, were estimated among overall controls and by menopausal status. We used conditional logistic regression to estimate the ORs and 95% CI of breast cancer, overall and by menopausal status, per one standard deviation (SD) increase in biomarker concentration. All biomarkers, except IL-13, were considered as continuous variables and categorized into quartiles based on the distribution of the concentrations among controls. Most of the IL-13 measurements were below the LOQ and IL-13 was therefore dichotomized into value higher and lower than the LOQ.

All analyses were conditioned on the matching variables. Models were also adjusted either for BMI or waist circumference (WC).

Heterogeneity was investigated by use of exogenous hormones at blood collection, age at diagnosis, breast cancer subtypes, time between blood collection and diagnosis, WC, BMI, and country, by introducing interaction terms in the models. For WC and BMI unconditional logistic regressions adjusted for matching factors were used.

### 3. Main findings

Cases were diagnosed on average 8.4 years after blood collection at a median age of 61.4 years. Most tumours were ER-positive (80.4%), PR-positive (68.2%), and HER2-negative (78.6%). Characteristics and mean concentrations of the biomarkers were similar in cases and controls. All biomarkers were moderately correlated with each other, except for IL-17D which was not correlated with any other biomarkers and IL-13 which was only moderately correlated with IL-8 and IL-10. BMI and WC showed the highest correlations with leptin ( $r > 0.5$ ) and were also positively correlated with IL-6, IL-1RA and CRP ( $r > 0.3$ ) and negatively correlated with adiponectin ( $r < -0.25$ ). Similar correlations were observed among women before or after menopause.

Overall, no statistically significant association was observed between the inflammatory markers and breast cancer risk. Among pre/perimenopausal women (720 cases), the leptin-to-adiponectin ratio was inversely associated with breast cancer [WC-adjusted:  $OR_{1SD} = 0.87$  (0.76-0.99)]. Among postmenopausal women (838 cases), no statistically significant associations were found between biomarkers on a continuous scale and breast cancer risk.

However, in categorical models, higher TNF- $\alpha$  levels were positively associated with breast cancer risk [WC-adjusted: OR<sub>Q4 versus Q1</sub> = 1.44 (1.01 - 2.05)].

#### 4. Conclusions

These results suggest that the ratio of leptin-to-adiponectin might have relevance for peri/premenopausal breast cancer development, while higher levels of TNF- $\alpha$  could be involved in postmenopausal breast cancer development. Whether leptin-to-adiponectin ratio and TNF- $\alpha$  are associated with breast cancer risk independently of anthropometry deserves replication.

#### 5. Paper

Status: In preparation.

Contribution: First author, conducted statistical analyses and wrote the first draft of the manuscript.

**Circulating inflammatory biomarkers and breast cancer risk: results from the EPIC study**

Manon Cairat<sup>1</sup>, Sabina Rinaldi<sup>1</sup>, Anne-Sophie Navionis<sup>1</sup>, Isabelle Romieu<sup>2</sup>, Carine Biessy<sup>1</sup>, Vivian Viallon<sup>1</sup>, [EPIC co-authors], Elisabeth Weiderpass<sup>1</sup>, Marc J. Gunter<sup>1</sup>, Laure Dossus<sup>1</sup>

**Author details**

<sup>1</sup> International Agency for Research on Cancer, Lyon, France.

<sup>2</sup> National Institute of Public Health, Centre for Population Health Research, Cuernavaca, Morelos, Mexico City.

**Corresponding Author**

Laure Dossus, International Agency for Research on Cancer, 150 Cours Albert Thomas 69372 Lyon CEDEX 08; [Dossusl@iarc.fr](mailto:Dossusl@iarc.fr).

**Abstract**

Chronic inflammation has been hypothesized to play a role in the development and progression of breast cancer. However, few prospective studies have been conducted on the association between circulating inflammatory markers and breast cancer risk. The objective of this study was to examine whether pre-diagnostic circulating concentrations of 11 cytokines and adipokines (adiponectin, leptin, CRP, TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, IL-10, IL-1RA, IL-17D, IL-13) were associated with breast cancer risk, overall and by subtypes. Pre-diagnostic levels of cytokines and adipokines were measured in plasma from 1,558 matched breast cancer case-control pairs within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Conditional logistic regression models were used to estimate odds ratios (OR) for the biomarker-breast cancer associations with adjustment for body mass index (BMI) or waist circumference (WC), and other breast cancer risk factors. Overall, no statistically significant association was observed between the inflammatory markers and breast cancer risk. Among pre/perimenopausal women (720 cases), the leptin-to-adiponectin ratio was inversely associated with breast cancer [WC-adjusted: OR for 1 standard deviation increment = 0.87 (0.76-0.99)]. Among postmenopausal women (838 cases), no statistically significant associations were found between biomarkers on a continuous scale and breast cancer risk. However, in categorical models, higher TNF- $\alpha$  levels were positively associated with breast cancer risk [WC-adjusted: OR Q4 versus Q1 = 1.44 (1.01 - 2.05)]. These results suggest that inflammation represented by the ratio of leptin-to-adiponectin might have relevance for peri/premenopausal breast cancer development, while higher levels of TNF- $\alpha$  could be involved in postmenopausal breast cancer development. Whether leptin-to-adiponectin ratio and TNF- $\alpha$  are associated with breast cancer risk independently of anthropometry deserves replication.

**Key Words**

Breast Cancer, Immunity, Inflammation, Biomarkers, Menopausal Status, Anthropometry.

## Introduction

Since the discovery, by Rudolf Virchow in 1863, of the infiltration of leukocytes into tumour tissue, a large number of experimental studies have confirmed the role of the immune system in tumour proliferation, angiogenesis and metastasis (1). Inflammation is now recognized as a hallmark of cancer (2) and was demonstrated to promote lymphomas, melanomas and lung cancers (1). There is now emerging evidence that inflammation could be involved in the progression of several other cancers including breast cancer through the production of free radicals and the subsequent DNA damage as well as the promotion of survival of transformed cells (3).

Epidemiological data on the associations of inflammatory biomarkers with breast cancer risk are limited. The most studied inflammatory biomarkers within the framework of breast cancer risk is C-reactive protein (CRP), a marker of the acute-phase inflammatory response. In the most recent meta-analysis of twelve prospective studies on CRP-breast cancer associations, each doubling of CRP was associated with a 7% higher risk of breast cancer (4). These results warrant further investigation since most epidemiological studies were not able to adjust/stratify by breast cancer subtype, menopausal status, or risk factors such as adiposity or use of exogenous hormones. Yet, these last two were previously identified as potential modifiers/confounders of the inflammation-breast cancer association (5-9). In addition, it is also unknown whether chronic inflammation in general is relevant to breast cancer risk, or whether specific pathways are involved.

To address these gaps, we conducted a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort to evaluate the associations of biomarkers of immune function and low-grade inflammatory states [Tumour Necrosis Factor (TNF)- $\alpha$ , Interferon (IFN)- $\gamma$ , Interleukin (IL)-6, IL-8, IL-10, IL-13, IL-17D, IL-1RA, C-Reactive Protein (CRP)] and adipokines (leptin, adiponectin) with breast cancer risk, overall and by tumour receptor status, menopausal status, adiposity and exogenous hormone use.

## Material and methods

### EPIC presentation

The EPIC cohort comprises over 153,000 men and 368,000 women aged 35–75 years old and recruited between 1992 and 1998 in 10 Western European countries (10). At recruitment, dietary, lifestyle, reproductive, medical, and anthropometric data were collected through questionnaires (10). Around 246,000 women from all countries provided a baseline blood sample. Blood was collected according to a standardized protocol in France, Germany, Greece, Italy, the Netherlands, Norway, Spain, and the UK (10). Serum (except in Norway), plasma, erythrocytes, and buffy coat aliquots were stored in liquid nitrogen ( $-196^{\circ}\text{C}$ ) in a centralized biobank at the International Agency for Research on Cancer (IARC). In Denmark, blood fractions were stored locally



in the vapour phase of liquid nitrogen containers ( $-150^{\circ}\text{C}$ ), and in Sweden, they were stored locally at  $-80^{\circ}\text{C}$  in standard freezers.

Incident cases of cancer occurring after recruitment into the cohort are identified through local and national cancer registries in 7 of the 10 countries, and through health insurance records, cancer and pathology registries, and active follow-up in France, Germany, and Greece. Follow-up on vital status is achieved through record linkage with mortality registries.

All participants provided written informed consent to participate in the EPIC study, which was approved by the ethics committee of the International Agency for Research on Cancer (IARC) and local ethical committees in EPIC centres. Participants from Greece were not included in the current analyses.

### **Selection of cases and controls**

EPIC participants who had donated blood, were cancer-free (other than non-melanoma skin cancer), and not pregnant at recruitment were eligible for this study. Women with a first primary invasive breast cancer diagnosis at least 2 years after blood collection and before December 2012 were selected as cases for the current study if oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses of the tumours were available.

For each breast cancer case, one control was selected randomly among all female cohort members who were alive and without cancer diagnosis (except non-melanoma skin cancer) at the time of diagnosis of the index case (incident density sampling). Controls were matched to cases on centre of recruitment, age at blood collection ( $\pm 6$  months), menopausal status at blood collection (premenopausal, perimenopausal, postmenopausal, surgically postmenopausal)(11), phase of the menstrual cycle for premenopausal women (early follicular; late follicular; periovulatory; midluteal; other luteal), use of exogenous hormone at blood collection, time of the day ( $\pm 1$  h), and fasting status at blood collection [non-fasting ( $< 3$  h since last meal), in between (3–6 h), fasting ( $> 6$  h), unknown].

The final study sample included 1,558 breast cancer cases and 1,558 controls.

### **Inflammatory biomarker assessment**

All plasma sample measurements were performed in the laboratories of the Biomarkers Group at IARC. Cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, IL-10, IL-13, IL-17D, IL-1RA), adipokines, (leptin and adiponectin) and CRP were measured by Meso Scale Discovery, a commercially available, highly sensitive immunoassay platform. Samples from cases and matched controls were analysed together in the same analytical batch, and laboratory personnel were blinded as to the case or control status of samples. Leptin, CRP, IL-8, and IL-1-RA

had no measurement below the limit of quantification (LOQ). Measurements below the LOQ represented less than 5% for adiponectin, IFN- $\gamma$  and IL-17-D, less than 8% for TNF- $\alpha$ , around 25% for IL-6 and IL-10, and around 82% for IL-13 (Supplementary Table S1). When biomarker measurements were lower than the LOQ these values were imputed with half the LOQ. Since most of the IL-13 measurements were below the LOQ, IL-13 was dichotomized into value higher and lower than LOQ.

### Statistical analysis

Characteristics of cases and controls were described using median and standard deviation (SD) or frequency and percentages. Geometric means were used to describe biomarker concentrations among cases and controls. Log-transformed biomarker concentrations were used in all analyses. Partial Pearson's correlations between biomarkers and with anthropometric factors, adjusted for age at blood collection and laboratory batch, were estimated among overall controls and by menopausal status. Considering that all biomarkers studied are physiologically interrelated and are likely to reflect a more restricted number of underlying biological pathways, scores of inflammatory markers were constructed by principal component analyses (PCA) and then calculated for each subject.

We used conditional logistic regression to estimate the odds ratios (ORs) and 95% confidence intervals (CI) of breast cancer, overall and by menopausal status (at blood collection), per one standard deviation (SD) increase in biomarker concentration. All biomarkers and component scores were considered as continuous variables and categorized into quartiles based on the distribution of the concentrations among control, except IL-13 which was dichotomized into value higher and lower than LOQ.

To test linear trends across quartiles, participants were assigned consecutive scores (scores of 1, 2, 3, 4) in each category and the corresponding variable was modelled as a continuous term.

All analyses were conditioned on the matching variables. Known risk factors of breast cancer and other lifestyle covariates (assessed at recruitment) were tested as potential confounding factors by comparing models of the biomarkers of interest (continuous and quartiles) before and after adjustment for each potential confounder. The variables tested were: age at first menstrual period, number of full-term pregnancies, age at first full-term pregnancy, breastfeeding, ever use of oral contraceptive, ever use of menopausal hormone therapy (MHT), smoking status, level of physical activity, alcohol consumption, education level, energy intake, height, weight, body mass index, waist circumference (WC), hip circumference, waist-to-hip ratio, hypertension and diabetes (the categories used are displayed in Table 1). For these variables, missing values were assigned the median (continuous variables) or mode (categorical variables) if they represented less than 5% of the population or were otherwise classified in a "missing" category (breastfeeding and hypertension).

Only BMI (continuous) and WC (continuous) modified the OR by more than 0.05 points and were, therefore, included in the final models. Given the correlation between these variables (0.81), they were included separately in two different models.

Heterogeneity was investigated by use of exogenous hormones at blood collection, age at diagnosis (age 50 or older/younger than age 50), breast cancer subtypes (ER+PR+/-HER2+, ER+PR+/-HER2-, ER-PR-HER2+, ER-PR-HER2-), time between blood collection and age at diagnosis (quartiles: >2 – <5 years,  $\geq 5$  – <10 years and  $\geq 10$  years), WC ( $\leq 79$  cm and >79 cm), BMI ( $\leq 25$  kg/m<sup>2</sup> and >25 kg/m<sup>2</sup>) and country, by introducing interaction terms in the models. For WC and BMI unconditional logistic regressions adjusted for matching factors were used.

Sensitivity analyses were conducted excluding subjects who had a previous (self-reported) diagnosis of diabetes and hypertension and women who were non-fasting at blood collection.



## Results

Cases were diagnosed on average 8.4 years after blood collection at a median age of 61.4 years (Table 1). Most tumours were ER-positive (80.4%), PR-positive (68.2%), and HER2-negative (78.6%). Characteristics and mean concentrations of the biomarkers were similar in cases and controls (Table 1 and Supplementary Table S1). All biomarkers were moderately correlated with each other, except for IL-17D which was not correlated with any other biomarkers and IL-13 which was only moderately correlated with IL-8 and IL-10 (Table 2). The highest correlations were observed between IL-6 and CRP ( $r=0.40$ ), IFN- $\gamma$  and TNF- $\alpha$  ( $r=0.37$ ), CRP and leptin to adiponectin ratio ( $r=0.38$ ), CRP and leptin ( $r=0.34$ ), and IL-10 and TNF- $\alpha$  ( $r=0.33$ ). The leptin to adiponectin ratio was inversely correlation with adiponectin ( $r=-0.57$ ) and positively correlated with leptin (0.91). BMI and WC showed the highest correlations with the leptin-to-adiponectin ratio ( $r \geq 0.57$ ) or leptin ( $r \geq 0.53$ ) and were also positively correlated with IL-6, IL-1RA and CRP ( $r > 0.30$ ) and negatively correlated with adiponectin ( $r < -0.25$ ). Similar correlations were observed among women before or after menopause (Supplementary Table 2).

Overall, none of the 11 inflammatory biomarkers were significantly associated with the risk of breast cancer (Supplementary Figure S1).

Among pre/perimenopausal women (720 cases), none of the 11 inflammatory biomarkers was associated with breast cancer risk (Figure 1). Adjustment for BMI or WC did not change the results, although after adjustment for WC the leptin-to-adiponectin ratio was significantly inversely associated with breast cancer [OR for 1 SD increment = 0.87 (0.76 – 0.99)], and both leptin [OR for 1 SD increment = 0.88 (0.77 – 1.00)] and CRP [OR for 1 SD increment = 0.89 (0.80 – 1.00)] were borderline statistically significantly associated with breast cancer risk.

No significant association was found by quartiles of inflammatory biomarkers in BMI-adjusted models (Figure 2). In WC-adjusted models, a significant trend was observed with levels of CRP [OR<sub>Q4vsQ1</sub>=0.73 (0.52-1.02);  $P_{trend}=0.03$ ; Supplementary Figure S2] and with the leptin-to-adiponectin ratio [OR<sub>Q4 vs. Q1</sub>=0.67 (0.47 - 0.97);  $P_{trend}=0.04$ ; Supplementary Figure S2].

We found a statistically significant heterogeneity with age at diagnosis ( $P_{homogeneity}=0.04$ ; Supplementary Table S3) suggesting an increased breast cancer risk with IL-10 among pre/perimenopausal women younger than 50 years old [WC-adjusted: OR for 1 SD increment = 1.27 (1.00 – 1.61); n cases=180] but not among those older than 50 years old [BMI-adjusted or WC-adjusted: OR for 1 SD increment = 0.95 (0.83 – 1.08); n exposed cases=540]. The IL-10-breast cancer association also differed according to ER molecular status ( $P_{homogeneity}=0.02$ ) suggesting an increased ER- breast cancer risk [WC-adjusted: OR for 1 SD increment = 1.34 (1.03 – 1.75); n cases=148] and no association with ER+ breast cancer risk [WC-adjusted: OR for 1 SD increment = 0.95 (0.83 – 1.10); n cases=572]. Other inflammatory biomarkers-breast cancer associations were similar according to molecular status of breast cancer, time of follow-up, BMI, WC, or exogenous hormone use (Supplementary Table S3). A significant

heterogeneity by country was only observed for IL-17 ( $P_{\text{homogeneity}}=0.04$ ; data not shown). When analyses were restricted to premenopausal women, women without diabetes or hypertension, or women who were not fasting at the time of blood collection, results were virtually unchanged (data not shown).

Among postmenopausal women (838 cases), borderline statistically significant or significant associations were observed between levels of CRP [OR for 1 SD increment =1.09 (0.99 - 1.21)], leptin [OR for 1 SD increment =1.16 (1.05 - 1.29)], TNF- $\alpha$  [OR for 1 SD increment =1.14 (1.01 - 1.28)], leptin-to-adiponectin ratio [OR for 1 SD increment =1.11 (1.01 - 1.23)] and breast cancer risk. After adjustment for BMI or WC, the estimates were attenuated and became statistically non-significant (Figure 1).

In categorical analyses, compared to women in the lowest quartile of TNF- $\alpha$ , those in the highest quartile had statistically significant higher risk of breast cancer even after adjustment for BMI or WC (WC-adjusted: OR Q4 versus Q1= 1.44 (1.01 - 2.05); Figure 2 and Supplementary Figure S2).

None of the biomarker-postmenopausal breast cancer associations differed according to breast cancer molecular subtypes, exogenous hormone use at blood collection, BMI, and WC ( $P_{\text{homogeneity}} \geq 0.11$ ; Supplementary Table S3). A significant heterogeneity by time of follow-up was only found for IL-13 ( $P_{\text{homogeneity}}=0.01$ ; Supplementary Table S3) suggesting an increased breast cancer risk for cases diagnosed between two and five years after blood collection, a decreased breast cancer risk for cases diagnosed between five and ten years after blood collection and no significant association after ten years. A significant heterogeneity by country was only observed for IL-17 ( $P_{\text{homogeneity}}=0.01$ ; data not shown). Exclusion of women who had diabetes, hypertension or who were not fasting at the time of blood collection did not change the results (data not shown).

Principal component analysis identified four main components that explained 60% of the total variance of biomarkers (Supplementary Table S3). Loadings are presented in the Supplementary Table S3. Except for adiponectin and IL-17, all biomarkers had intermediate to high positive loadings (from 0.26 for IL-10 to 0.43 for TNF- $\alpha$ ) on Component 1. Component 1 was positively correlated with BMI and WC ( $r>0.40$ ; Supplementary Table S3). For Component 2, positive loadings were observed for adiponectin (0.46), TNF- $\alpha$ , IFN-gamma, IL-8, IL-10 and IL-17D (loadings  $> 0.24$ ) while negative loadings were observed for leptin, CRP, IL-6 and IL-1RA (loadings  $< -0.15$ ). Component 2 was negatively correlated with BMI and WC ( $r \leq -0.40$ ; Supplementary Table S3). Component 3 was characterized by high inverse loadings for IL-8 (-0.55) and IL-1-RA (-0.52) and moderate positive loadings for adiponectin, CRP, and IL-6 (~0.30). For Component 4, loadings were 0.59 for IL-17, -0.47 for IL-10 and -0.41 for IFN- $\gamma$ . Overall, none of the component scores was significantly associated with the risk of breast cancer (data not shown). Among pre/perimenopausal women, only the highest quartile of Component 2 was associated with an increased breast cancer risk [WC-adjusted: OR<sub>Q4 vs q1</sub>=1.58 (1.11 – 2.26); Table 3]. The OR was stronger when analyses were restricted to premenopausal



women [WC-adjusted:  $OR_{Q4 \text{ vs } Q1} = 1.78$  (1.12 – 2.81)] or to pre/perimenopausal women younger than 50 years old [WC-adjusted:  $OR_{Q4 \text{ vs. } Q1} = 2.28$  (1.14 – 4.59); data not shown]. Among postmenopausal women, no association was observed between component scores and breast cancer risk (Table 3).

## Discussion

In this prospective analysis of circulating inflammatory biomarkers and breast cancer risk, overall, no association was observed. Among pre/perimenopausal women, the leptin-to-adiponectin ratio was significantly associated with a decreased breast cancer risk. Among postmenopausal women, only high levels of TNF- $\alpha$  were associated with an increased risk of breast cancer. Results from the PCA suggested that a global score of inflammation inversely correlated to the leptin/adiponectin ratio was associated with an increased breast cancer risk among pre/perimenopausal women.

Leptin, a protein hormone produced mainly by adipocytes, could promote breast cancer by stimulating the conversion of aromatizable androgen into oestradiol (12). In our study, among pre/perimenopausal women, leptin was associated with decreased breast cancer risk, even though the association did not reach statistical significance. The leptin/adiponectin ratio, a suggested surrogate marker of insulin resistance (13), was significantly associated with decreased breast cancer risk. In addition, a PCA-derived component score characterized by low levels of leptin, CRP and TNF- $\alpha$  and high levels of adiponectin, IL-8 and IL-10 was associated with a decrease in breast cancer risk. We found no study on the associations between leptin-to-adiponectin ratio and premenopausal breast cancer risk but a few focusing on pre-diagnostic levels of leptin or adiponectin. Among them, one study noted a statistically significant decreased premenopausal breast cancer risk with elevated levels of leptin [ $OR_{Q3 \text{ vs } Q1} = 0.42$  (0.21 - 0.84)] (14) and the other one noted a non-significant decreased premenopausal breast cancer risk [ $OR_{Q4 \text{ vs } Q1} = 0.69$  (0.38 - 1.23)] (15). In premenopausal women, high levels of leptin have been hypothesized to reduce breast cancer risk through regulation of ovarian folliculogenesis (16) and the reduction of follicular oestradiol secretion (17). Similar to our study, two prospective studies suggested an increased premenopausal breast cancer risk with high pre-diagnostic levels of adiponectin even though the associations did not reach significance [ $OR_{Q4 \text{ vs } Q1} = 1.30$  (0.80 - 1.03); (18);  $OR_{Q3 \text{ vs } Q1} = 1.11$  (0.61 - 1.03); (14)]. On the contrary, one study found a 12% reduction in the risk of premenopausal breast cancer per unit increase of adiponectin (19). In our study, we observed a borderline inverse association with pre-diagnostic levels of CRP. Among the two prospective studies reporting results on CRP-breast cancer association among premenopausal women, one noted a non-significant inverse association (20) while the other reported a significant positive association (21).

Among postmenopausal women, only high levels of TNF- $\alpha$ , a macrophage-derived pro inflammatory cytokine, were associated with an increased postmenopausal breast cancer risk. Experimental data suggest

that TNF- $\alpha$  could be involved in carcinogenesis by activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and consequently inducing the expression of genes associated with cell proliferation, apoptosis, angiogenesis, and metastasis (22, 23). In addition, TNF- $\alpha$  could stimulate aromatase activity in breast tissue leading to oestrogen synthesis (24), important hormones involved in breast cancer growth in postmenopausal women. Our finding is not supported by previous prospective studies which reported a non-significant association between TNF- $\alpha$  levels and breast cancer risk (25, 26). However, because the number of cases included was smaller than in our study, most of the previous studies were not able to categorize TNF- $\alpha$  into quartile (14, 27-29). In contrast to our results, one case-control study on postmenopausal women nested in the Malmö Diet and Cancer Control found an inverse association between high levels of TNF- $\alpha$  and breast cancer risk [OR<sub>q3 vs q1</sub>=0.65 (0.43-0.99)] (30). In our study, CRP was not associated with an increased risk of postmenopausal breast cancer, although a borderline significant increase in risk was observed before BMI adjustment. In a recent meta-analysis of twelve prospective studies conducted mainly on postmenopausal women, a doubling of serum concentrations of CRP was associated with a significant 7% increase in breast cancer risk (4). Of note, 10 of the 12 studies noted a non-significant association between CRP levels and overall breast cancer risk, especially after BMI adjustment (5-8, 25, 27, 31-34). Some studies found a positive CRP-breast cancer association but this was strongly attenuated after adjustment for BMI suggesting that BMI was a confounder in the CRP-breast cancer association (6, 7). In our study, leptin was associated with an increased postmenopausal breast cancer risk in the crude model. After adjustment for anthropometric factors, the leptin-breast cancer association was strongly reduced and became non-statistically significant. A case-control study of postmenopausal women nested in the prospective study CLUE II and including 272 cases and matched controls, noted similar results to our study: high levels of leptin were associated with an increased breast cancer risk before adjustment for BMI and adjustment for BMI attenuated the association towards null (28). With respect to adipokines, a recent meta-analysis of published six prospective and nine case-control studies on adiponectin and breast cancer risk concluded that low levels of adiponectin were associated with elevated risk of postmenopausal breast cancer (35). However, among the studies in which blood samples were taken before breast cancer diagnosis, none of them found a significant association between pre-diagnostic levels of adiponectin and postmenopausal breast cancer risk (18, 33, 36, 37).

To our knowledge, no other prospective study has so far investigated the role of IFN- $\gamma$ , IL-13, IL-10, IL-17, IL-8, or IL-1RA on breast cancer development.

The main strength of our study is the use of a large European population-based prospective cohort which allowed us to select a large number of cases and controls and to prospectively examine associations between inflammatory biomarkers and breast cancer risk according to menopausal status and breast cancer subtypes. In addition, the long follow-up (median of 8.6 years among cases) allowed us to evaluate a potential bias in

reverse causality due to undiagnosed breast cancer by exploring whether the inflammation-breast cancer associations differ according to time of follow-up. Another advantage was that we had data on anthropometry, lifestyle and reproductive factors and were able to evaluate whether those factors were confounding or modifying the biomarker-breast cancer associations. However, some results from analysis subgroups were based on relatively small numbers of cases and, because of the number of comparisons evaluated, they might be due to chance. In our study, BMI and WC were strong confounders of the associations between leptin, CRP, TNF- $\alpha$  with postmenopausal breast cancer. It is very difficult to separate the independent effects of anthropometry and these markers due to their close correlations. As in many other studies on biomarker-cancer risk, the main limitation of our study is that inflammatory biomarkers were measured only once, even though most of the inflammatory markers have shown good reproducibility over time (38).

In summary, our results suggest that inflammation linked to the leptin-to-adiponectin ratio might play a role in peri/premenopausal breast cancer development, while high levels of TNF- $\alpha$  might be involved in postmenopausal breast cancer development. These results deserve replication in other settings.

## Tables

Table 1. Selected characteristics of the study population at blood collection (n cases/controls =1,558/1,558).

Variables	N	Cases Median (SD) or N (%)	Controls Median (SD) or N (%)
<b>Breast cancer cases characteristics</b>			
Time between blood collection and diagnosis in years	1,558	8.6 (2.8)	—
Age at diagnosis in years	1,558	61.4 (8.3)	—
<b>ER positive</b>			
Negative	305	305 (19.6)	—
Positive	1,253	1,253 (80.4)	—
<b>PR status</b>			
Negative	496	509 (31.8)	—
Positive	1,062	1,104 (68.2)	—
<b>HER2 status</b>			
Negative	1,224	1,224 (78.6)	—
Positive	334	334 (21.4)	—
<b>Molecular status</b>			
ER+PR+/-HER2+	210	210 (13.7)	—
ER+PR+/-HER2-	1,043	1,043 (68.3)	—
ER-PR-HER2-	160	160 (10.5)	—
ER-PR-HER2+	115	115 (7.5)	—
<b>Sociodemographic, lifestyle and reproductive factors</b>			
Age at blood collection in years	3,116	53.2 (7.9)	53.2 (7.9)
<b>Education level (N missing=63)</b>			
Primary/no schooling	1,128	557 (36.5)	571 (37.4)
Technical/professional/secondary	1,285	647 (42.3)	638 (41.8)
Longer education	640	324 (21.2)	316 (20.7)
<b>Time since last meal at blood collection in hours (N missing=49)</b>			
< 3 h	1,418	706 (46.1)	712 (46.4)
3–6 h	545	273 (17.8)	272 (17.7)
> 6 h	1,104	552 (36.1)	552 (35.9)
<b>Physical activity index (N missing=27)</b>			
Inactive	661	349 (22.5)	312 (20.3)
Moderately inactive	1,183	573 (37)	610 (39.6)
Moderately active	681	346 (22.3)	335 (21.8)
Active	564	282 (18.2)	282 (18.3)
<b>Smoking status (N missing=25)</b>			
Never	1,750	875 (56)	875 (56)
Former	685	351 (23)	334 (22)
Smoker	656	319 (21)	337 (22)
Energy intake in kcal (N missing=4)	3,112	1,972.6 (593.1)	1,949.8 (557.7)
Alcohol consumption at recruitment in g/day (N missing=4)	3,112	5.4 (13.6)	4.4 (12.4)
Age at first menstrual period in years (N missing=32)	3,084	13.0 (1.5)	13.0 (1.6)
<b>Number of full-term pregnancies* (N missing=61)</b>			
0	456	243 (34.8)	213 (45.7)



Variables	N	Cases	Controls
		Median (SD) or N (%)	Median (SD) or N (%)
1	546	299 (23.5)	247 (19)
2	1,254	607 (47.7)	647 (49.7)
≥ 3	774	366 (28.8)	408 (31.3)
Age at first full-term pregnancy in years* (N missing=5)	2,630	25.0 (4.4)	24.0 (4.4)
Ever breastfed*			
No	387	199 (15.3)	188 (14.1)
Yes	2,103	1,034 (79.5)	1,069 (80.1)
Missing	145	68 (5.2)	77 (5.8)
Use of exogenous hormones at blood collection	984	489 (31.4)	489 (31.4)
Ever use of oral contraceptive at baseline (N missing=11)	1,858	939 (50.5)	919 (49.5)
Ever use of menopausal hormone therapy at baseline** (N missing=5)	835	415 (49.7)	420 (50.3)
Menopausal status at blood collection (N missing=0)			
Premenopausal	826	413 (26.5)	413 (26.5)
Perimenopausal	614	307 (19.7)	307 (19.7)
Postmenopausal	1,676	838 (53.8)	838 (53.8)
<b>Anthropometric factors</b>			
Weight in kg			
Among peri/premenopausal women	1,440	65.9 (11.5)	65.7 (10.9)
Among postmenopausal women	1,676	69.1 (12.6)	66.8 (10.9)
Height in cm			
Among peri/premenopausal women	1,440	162.2 (6.3)	161.9 (6.7)
Among postmenopausal women	1,676	162.1 (6.6)	161.2 (6.5)
Body mass index in kg/m <sup>2</sup>			
Among peri/premenopausal women	1,440	24.3 (4.4)	24.3 (4.1)
Among postmenopausal women	1,676	25.5 (4.7)	25.4 (4.1)
Waist circumference in cm (N missing=36)			
Among peri/premenopausal women	1,404	77.4 (10.8)	77.3 (10.7)
Among postmenopausal women	1,634	81.0 (11.7)	81.0 (10.2)
Hip circumference in cm (N missing= 36)			
Among peri/premenopausal women	1,404	99.1 (8.7)	99.5 (8.4)
Among postmenopausal women	1,634	102.0 (9.5)	100.0 (8.0)
Waist to hip ratio (N missing= 36)			
Among peri/premenopausal women	1,404	0.79 (0.07)	0.78 (0.08)
Among postmenopausal women	1,634	0.80 (0.07)	0.80 (0.07)
<b>Comorbidities</b>			
History of diabetes (N missing=60)	62	40 (1)	22 (1)
History of hypertension (N missing=267)	677	354 (12)	323 (11)

Abbreviations: ER, oestrogen receptor; HER2, human epidermal growth factor 2; PR, progesterone receptor; SD, standard deviation.

\*among women with full term pregnancy

\*\* among postmenopausal women

Table 2: Spearman correlation coefficients between pre-diagnostic levels of inflammatory biomarkers and anthropometric factors among controls, adjusted for laboratory batch and age at blood donation.

Biomarkers	Adiponectin	Leptin	L/A	CRP	TNF- $\alpha$	IFN- $\gamma$	IL-6	IL-8	IL-10	IL-1-RA	IL-17-D	IL-13	BMI	WC
Adiponectin	1													
Leptin	-0.18	1												
L/A	-0.57	<b>0.91</b>	1											
CRP	-0.22	<b>0.34</b>	<b>0.38</b>	1										
TNF- $\alpha$	-0.05	0.08	0.09	<b>0.16</b>	1									
IFN- $\gamma$	-0.02	-0.01	0.01	<b>0.13</b>	<b>0.37</b>	1								
IL-6	-0.14	<b>0.25</b>	<b>0.27</b>	<b>0.40</b>	<b>0.28</b>	<b>0.20</b>	1							
IL-8	0.03	-0.03	-0.04	-0.01	<b>0.32</b>	<b>0.12</b>	<b>0.18</b>	1						
IL-10	-0.01	-0.08	-0.06	0.01	<b>0.33</b>	<b>0.27</b>	0.09	<b>0.16</b>	1					
IL-1-RA	-0.26	<b>0.24</b>	<b>0.31</b>	<b>0.27</b>	<b>0.29</b>	<b>0.18</b>	<b>0.24</b>	<b>0.23</b>	<b>0.11</b>	1				
IL-17-D	0.11	-0.05	-0.08	-0.07	0.07	-0.01	0.01	0.06	0.07	-0.02	1			
IL-13	-0.01	-0.03	-0.02	-0.04	0.08	0.09	0.06	<b>0.24</b>	<b>0.19</b>	0.09	0.07	1		
BMI	-0.25	<b>0.60</b>	<b>0.61</b>	<b>0.40</b>	0.14	0.03	<b>0.31</b>	0.02	-0.03	<b>0.34</b>	-0.07	-0.01	1	
WC	-0.29	<b>0.53</b>	<b>0.57</b>	<b>0.35</b>	0.15	0.05	<b>0.28</b>	0.01	-0.01	<b>0.33</b>	-0.06	-0.02	<b>0.81</b>	1

BMI, body mass index; CRP, c-reactive protein; IFN, interferon; IL, interleukin; L/A, leptin to adiponectin ratio; TNF, tumour necrosis factor; WC, waist circumference.

Spearman correlation coefficients with P-values <0.0001 are in bold.



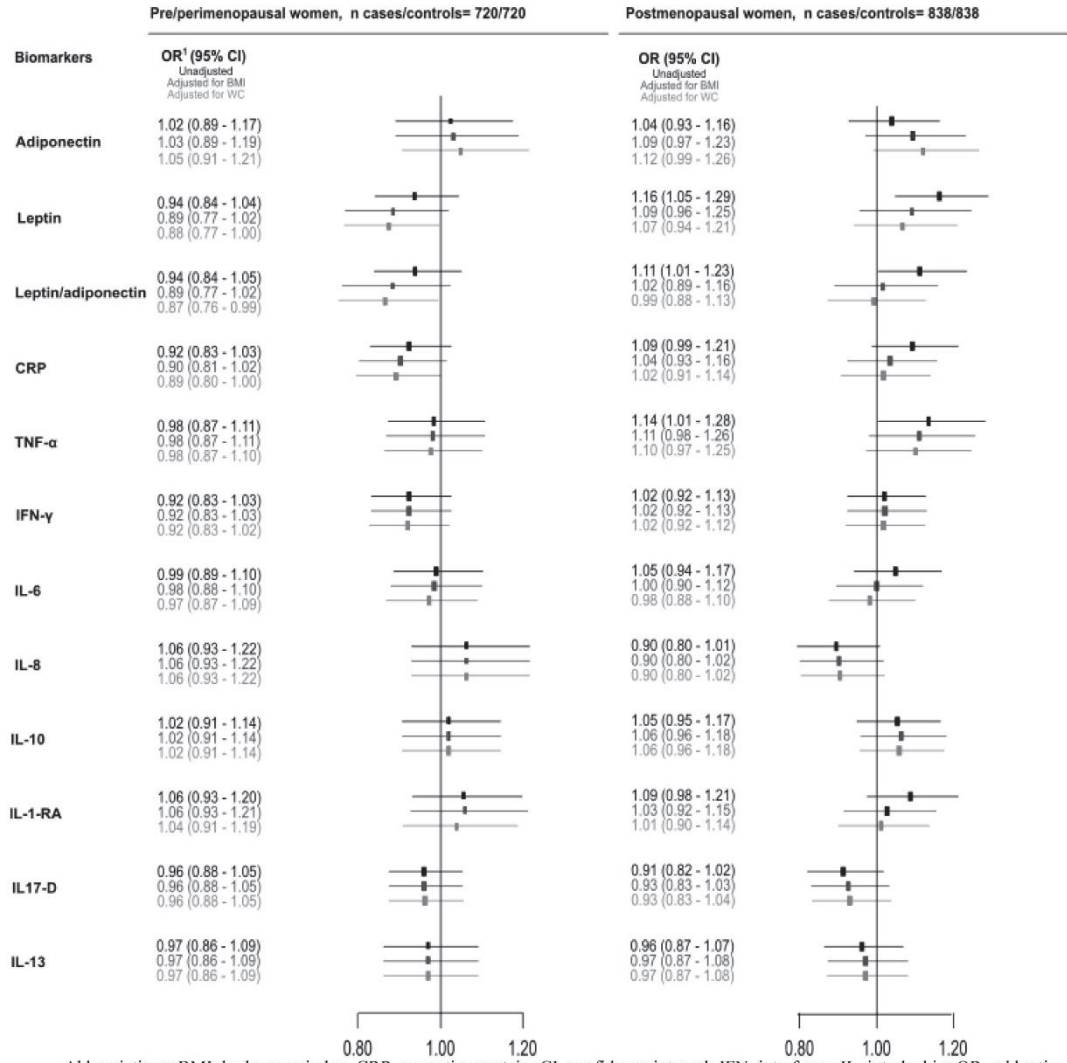
Table 3. Associations between inflammatory component scores and breast cancer risk according to menopausal status.

Pre/perimenopausal women (N cases/controls=720/720)				Postmenopausal women (N cases/controls=838/838)		
Components	N cases/controls	BMI-Adjusted OR (95% CI)	WC-Adjusted OR (95% CI)	N cases/controls	BMI-Adjusted OR (95% CI)	WC-Adjusted OR (95% CI)
<b>Component score 1</b>						
Continuous	718/718	0.96 (0.88 – 1.04)	0.94 (0.87 – 1.03)	838/838	1.04 (0.96 – 1.12)	1.02 (0.94 – 1.11)
<b>Quartiles</b>						
1	259/250	1.00 (reference)	1.00 (reference)	142/137	1.00 (reference)	1.00 (reference)
2	192/185	0.97 (0.73 – 1.28)	0.95 (0.72 – 1.26)	160/202	0.73 (0.53 – 1.00)	0.73 (0.53 – 1.00)
3	148/149	0.90 (0.65 – 1.24)	0.87 (0.63 – 1.20)	226/237	0.87 (0.63 – 1.19)	0.85 (0.61 – 1.16)
4	119/133	0.78 (0.54 – 1.14)	0.74 (0.51 – 1.08)	301/255	1.02 (0.72 – 1.45)	0.98 (0.70 – 1.40)
P trend		0.20	0.11		0.45	0.65
<b>Component score 2</b>						
Continuous	718/718	1.04 (0.94 – 1.15)	1.06 (0.96 – 1.17)	838/838	1.00 (0.90 – 1.10)	1.01 (0.92 – 1.12)
<b>Quartiles</b>						
1	191/208	1.00 (reference)	1.00 (reference)	203/179	1.00 (reference)	1.00 (reference)
2	184/185	1.13 (0.84 – 1.53)	1.16 (0.86 – 1.57)	204/203	0.97 (0.73 – 1.30)	1.00 (0.75 – 1.35)
3	173/185	1.11 (0.81 – 1.52)	1.15 (0.84 – 1.57)	201/201	0.98 (0.72 – 1.34)	1.02 (0.75 – 1.38)
4	170/140	1.50 (1.05 – 2.15)	1.58 (1.11 – 2.26)	221/248	0.93 (0.66 – 1.30)	0.99 (0.70 – 1.39)
P trend		0.05	0.02		0.70	0.94
<b>Component score 3</b>						
Continuous	718/718	0.88 (0.77 – 1.00)	0.88 (0.77 – 1.00)	838/838	1.10 (0.98 – 1.24)	1.11 (0.98 – 1.25)
<b>Quartiles</b>						
1	195/197	1.00 (reference)	1.00 (reference)	200/190	1.00 (reference)	1.00 (reference)
2	197/181	1.11 (0.81 – 1.52)	1.11 (0.81 – 1.52)	189/206	0.87 (0.64 – 1.18)	0.88 (0.65 – 1.20)
3	175/165	1.04 (0.75 – 1.44)	1.05 (0.76 – 1.45)	218/222	0.92 (0.67 – 1.25)	0.94 (0.69 – 1.28)
4	151/175	0.85 (0.60 – 1.20)	0.86 (0.61 – 1.21)	222/213	1.00 (0.72 – 1.39)	1.02 (0.73 – 1.42)
P trend		0.30	0.32		0.79	0.72
<b>Component score 4</b>						
Continuous	718/718	0.98 (0.88 – 1.10)	0.98 (0.87 – 1.09)	838/838	0.94 (0.84 – 1.05)	0.95 (0.85 – 1.06)
<b>Quartiles</b>						
1	247/238	1.00 (reference)	1.00 (reference)	157/149	1.00 (reference)	1.00 (reference)
2	181/191	0.89 (0.66 – 1.19)	0.89 (0.66 – 1.19)	195/197	0.92 (0.68 – 1.23)	0.93 (0.69 – 1.25)
3	158/162	0.92 (0.69 – 1.24)	0.92 (0.69 – 1.24)	228/225	0.92 (0.68 – 1.24)	0.93 (0.69 – 1.26)
4	132/127	0.98 (0.71 – 1.37)	0.98 (0.70 – 1.36)	249/260	0.82 (0.61 – 1.12)	0.86 (0.63 – 1.16)
P trend		0.89	0.86		0.53	0.34

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; WC, waist circumference.

## Figures

Figure 1. Associations between inflammatory biomarkers (continuous) and breast cancer risk according to menopausal status.

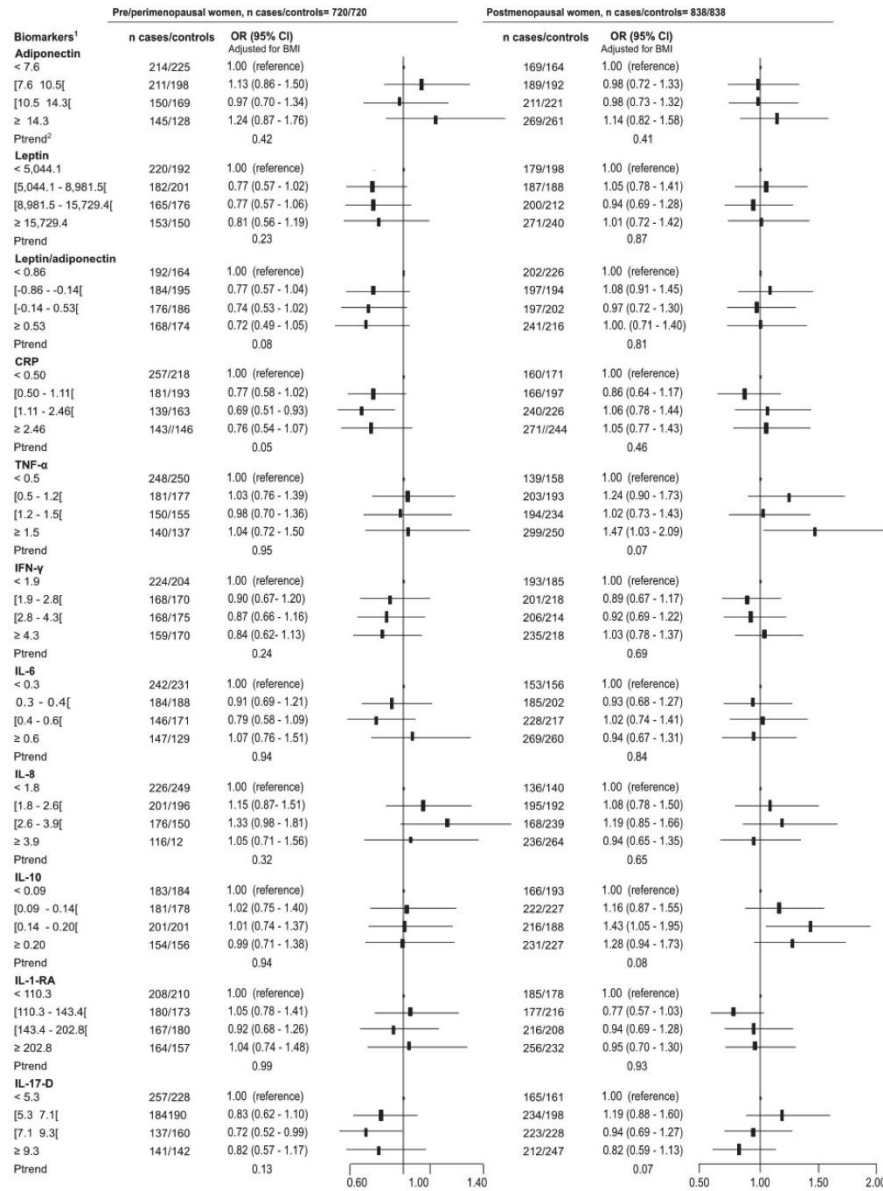


Abbreviations: BMI, body mass index; CRP, c-reactive protein; CI, confidence interval; IFN, interferon; IL, interleukin; OR, odd ratio; SD, standard deviation; TNF, tumour necrosis factor; WC, waist circumference.

P homogeneity was 0.03 for leptin, 0.10 for CRP and 0.07 for IL-8 and  $\geq 0.16$  for others inflammatory biomarkers.

<sup>1</sup> ORs were estimated per 1 SD increase in log-transformed biomarker concentrations, from logistic regression conditioned on matching variables. For IL-13, ORs were estimated according to the limit of quantification.

Figure 2. Associations between quartiles of inflammatory biomarkers and breast cancer risk after BMI adjustment and according to menopausal status.



Abbreviations: BMI, body mass index; CI, confidence interval; CRP, c-reactive protein; IFN, interferon; IL, interleukin; OR, odd ratio; SD, standard deviation; TNF, tumour necrosis factor.

<sup>1</sup> Quartile cut points were determined on control participants. Quartiles cut points for adiponectin and CRP were expressed in microgram per millilitre, those for other biomarkers were expressed in picogram per millilitre.

<sup>2</sup> For test of linear trends across quartiles, participants were assigned consecutive scores (scores of 1, 2, 3, 4) in each category and the corresponding variable was modelled as a continuous term.

**Supplementary tables.**

Table S1. Geometric mean values for inflammatory biomarkers in breast cancer cases and matched controls.

Markers	N Missing Values	N (%) Values $\leq$ LOQ	Cases	Controls
			Geometric Mean (95% CI)	Geometric Mean (95% CI)
Adiponectin ( $\mu$ g/mL)	0	7 (0.2)	10.50 (10.24 – 10.77)	10.39 (10.13 – 10.64)
Leptin (ng/mL)	2	0 (0)	9.05 (8.65 – 9.47)	8.70 (8.34 – 9.08)
CRP ( $\mu$ g/mL)	1	0 (0)	1.12 (1.05 – 1.19)	1.11 (1.05 – 1.18)
TNF- $\alpha$ (pg/mL)	8	795 (25.5)	1.06 (1.03– 1.09)	1.04 (1.01 – 1.07)
IFN- $\gamma$ (pg/mL)	8	2 (0.1)	2.97 (2.87 – 3.08)	3.02 (2.92 – 3.13)
IL-6 (pg/mL)	8	238 (7.6)	0.43 (0.42 – 0.45)	0.43 (0.41 – 0.44)
IL-8 (pg/mL)	8	0 (0)	2.71 (2.62 – 2.80)	2.75 (2.65 – 2.86)
IL-10 (pg/mL)	8	693 (22.2)	0.13 (0.13 – 0.14)	0.13 (0.13 – 0.14)
IL-1-RA (pg/mL)	9	0 (0)	159.11 (154.87 – 163.45)	154.51 (150.67 – 158.45)
IL-17-D (pg/mL)	9	76 (2.4)	6.67 (6.47 – 6.88)	6.89 (6.69 – 7.09)
IL-13 (pg/mL)	9	2,470 (79.3)	0.29 (0.28 – 0.29)	0.29 (0.29 – 0.30)

Abbreviations: CRP, c-reactive protein; IFN, interferon; IL, interleukin; LOQ, limit of quantification; mL, millilitre; pg, picogram; SD, standard deviation; TNF, tumour necrosis factor.

Table S2: Spearman correlation coefficients between pre-diagnostic levels of inflammation markers and anthropometric factors among controls, adjusted for laboratory batch and age at blood donation.

**a- among peri/premenopausal women**

Biomarkers	Adiponectin	Leptin	L/A	CRP	TNF- $\alpha$	IFN- $\gamma$	IL-6	IL-8	IL-10	IL-1-RA	IL-17-D	IL-13	BMI	WC
Adiponectin	1													
Leptin	-0.15	1												
L/A	<b>-0.55</b>	<b>0.91</b>	1											
CRP	-0.14	<b>0.33</b>	<b>0.34</b>	1										
TNF- $\alpha$	-0.02	0.07	0.07	<b>0.15</b>	1									
IFN- $\gamma$	-0.04	-0.04	-0.02	0.13	<b>0.37</b>	1								
IL-6	-0.11	<b>0.21</b>	<b>0.22</b>	<b>0.40</b>	<b>0.28</b>	<b>0.23</b>	1							
IL-8	0.02	-0.04	-0.05	0.02	<b>0.38</b>	0.15	0.14	1						
IL-10	0.01	-0.06	-0.06	0.01	<b>0.34</b>	<b>0.28</b>	0.09	0.11	1					
IL-1-RA	<b>-0.22</b>	<b>0.24</b>	<b>0.30</b>	<b>0.27</b>	<b>0.30</b>	<b>0.20</b>	<b>0.19</b>	<b>0.22</b>	0.12	1				
IL-17-D	0.08	-0.04	-0.07	-0.05	0.06	-0.02	0.02	0.06	0.08	-0.03	1			
IL-13	-0.03	<b>-0.05</b>	-0.03	-0.07	0.11	0.10	0.01	<b>0.21</b>	<b>0.16</b>	0.07	0.08	1		
BMI	<b>-0.23</b>	<b>0.60</b>	<b>0.60</b>	<b>0.41</b>	0.14	0.05	<b>0.30</b>	0.04	-0.01	<b>0.34</b>	-0.07	-0.02	1	
WC	<b>-0.26</b>	<b>0.52</b>	<b>0.56</b>	<b>0.34</b>	0.12	0.05	<b>0.28</b>	0.04	-0.01	<b>0.32</b>	-0.04	-0.02	<b>0.77</b>	1

Abbreviations: BMI, Body Mass Index; CRP, c-reactive protein; IFN, interferon; IL, Interleukin; L/A, Leptin to adiponectin ratio; TNF, tumour necrosis factor; WC, waist circumference. Spearman correlation coefficients with  $p$ -Values <0.0001 are in bold.

**b- among postmenopausal women**

Biomarkers	Adiponectin	Leptin	L/A	CRP	TNF- $\alpha$	IFN- $\gamma$	IL-6	IL-8	IL-10	IL-1-RA	IL-17-D	IL-13	BMI	WC
Adiponectin	1													
Leptin	<b>-0.18</b>	1												
L/A	<b>-0.57</b>	<b>0.91</b>	1											
CRP	<b>-0.28</b>	<b>0.35</b>	<b>0.41</b>	1										
TNF- $\alpha$	-0.08	0.09	0.11	<b>0.17</b>	1									
IFN- $\gamma$	-0.01	0.01	0.01	<b>0.13</b>	<b>0.37</b>	1								
IL-6	-0.16	<b>0.27</b>	<b>0.29</b>	<b>0.39</b>	<b>0.27</b>	<b>0.17</b>	1							
IL-8	0.01	-0.01	-0.01	-0.05	<b>0.26</b>	0.10	<b>0.23</b>	1						
IL-10	-0.02	-0.09	-0.07	0.03	<b>0.33</b>	<b>0.25</b>	0.10	<b>0.20</b>	1					
IL-1-RA	<b>-0.28</b>	<b>0.24</b>	<b>0.31</b>	<b>0.27</b>	<b>0.29</b>	<b>0.17</b>	<b>0.30</b>	<b>0.23</b>	0.10	1				
IL-17-D	0.13	-0.05	-0.09	-0.09	0.09	0.02	-0.01	0.06	0.05	-0.01	1			
IL-13	-0.01	<b>-0.02</b>	-0.01	-0.05	0.06	0.09	0.12	<b>0.26</b>	<b>0.22</b>	0.11	0.05	1		
BMI	<b>-0.26</b>	<b>0.59</b>	<b>0.60</b>	<b>0.38</b>	<b>0.15</b>	0.01	<b>0.30</b>	0.01	-0.05	<b>0.33</b>	-0.07	0.01	1	
WC	<b>-0.31</b>	<b>0.52</b>	<b>0.57</b>	<b>0.36</b>	<b>0.18</b>	0.05	<b>0.27</b>	0.01	-0.02	<b>0.34</b>	-0.06	-0.01	<b>0.84</b>	1

Abbreviations: BMI, Body Mass Index; CRP, c-reactive protein; IFN, interferon; IL, Interleukin; L/A, Leptin to adiponectin ratio; TNF, tumour necrosis factor; WC, waist circumference. Spearman correlation coefficients with  $p$ -Values <0.0001 are in bold.



Table S3. Associations between inflammatory biomarkers and breast cancer risk according to breast cancer subtypes and selected variables.

Biomarkers	Pre/perimenopausal women			Postmenopausal women		
	N cases/controls	OR <sup>1</sup> (95% CI)	P heterogeneity	N cases/controls	OR <sup>1</sup> (95% CI)	P heterogeneity
Adiponectin						
Breast cancer subtypes						
ER+PR+/-HER2+	210/210	0.96 (0.67 – 1.36)	0.88	210/210	1.25 (0.92 – 1.68)	0.73
ER+PR+/-HER2-	934/934	1.08 (0.91 – 1.29)		1,152/1,152	1.11 (0.95 – 1.27)	
ER-PR-HER2-	154/154	0.96 (0.56 – 1.63)		166/166	0.95 (0.64 – 1.41)	
ER-PR-HER2+	100/100	0.94 (0.56 – 1.58)		130/130	1.04 (0.64 – 1.68)	
Time between blood collection and diagnosis in years						
> 2 - <5	98/98	0.88 (0.60 – 1.28)	0.57	128/128	0.98 (0.70 – 1.39)	0.74
≥5 - <10	378/378	1.10 (0.90 – 1.34)		419/419	1.14 (0.96 – 1.34)	
≥10	244/244	1.00 (0.78 – 1.29)		291/291	1.08 (0.88 – 1.31)	
Age at diagnosis in years						
≤ 50	180/180	1.12 (0.87 – 1.46)	0.46			
> 50	540/540	1.00 (0.84 – 1.19)				
BMI in kg/m <sup>2</sup>						
<25	410/408	1.06 (0.83 – 1.36)	0.34	382/388	1.06 (0.94 – 1.19)	0.77
≥25 - <30	229/227	1.04 (0.75 – 1.44)		295/334	1.02 (0.89 – 1.17)	
≥30	81/85	0.73 (0.39 – 1.40)		161/116	0.99 (0.83 – 1.19)	
WC in cm (median)						
<79	399/403	1.08 (0.84 – 1.39)	0.55	349/379	1.05 (0.93 – 1.19)	0.63
≥79	303/299	0.94 (0.70 – 1.25)		468/438	1.04 (0.94 – 1.15)	
Use of hormones at blood collection						
No	576/576	1.05 (0.90 – 1.23)	0.55	493/493	1.11 (0.95 – 1.29)	0.71
Yes	144/144	0.93 (0.65 – 1.34)		345/345	1.06 (0.88 – 1.27)	
Leptin						
Breast cancer subtypes						
ER+PR+/-HER2+	210/210	0.71 (0.48 – 1.04)	0.57	210/210	0.98 (0.72 – 1.34)	0.80
ER+PR+/-HER2-	933/933	0.90 (0.75 – 1.07)		1,151/1,151	1.13 (0.96 – 1.34)	
ER-PR-HER2-	154/154	1.03 (0.66 – 1.62)		166/166	1.11 (0.73 – 1.68)	
ER-PR-HER2+	100/100	0.99 (0.59 – 1.67)		130/130	0.95 (0.59 – 1.52)	
Time between blood collection and diagnosis in years						
>2 - <5	98/98	0.63 (0.42 – 0.94)	0.15	128/128	0.98 (0.70 – 1.38)	0.67
≥5 - <10	378/378	0.97 (0.81 – 1.17)		419/419	1.07 (0.88 – 1.29)	
≥10	244/244	0.87 (0.66 – 1.13)		290/290	1.17 (0.94 – 1.45)	
Age at diagnosis in years						
≤ 50	180/180	0.82 (0.63 – 1.08)	0.51			
> 50	540/540	0.91 (0.78 – 1.08)				
BMI in kg/m <sup>2</sup>						
<25	410/408	0.88 (0.75 – 1.02)	0.19	381/388	1.01 (0.88 – 1.16)	0.17
≥25 - <30	229/226	1.12 (0.91 – 1.37)		295/334	0.98 (0.83 – 1.15)	
≥30	81/85	0.88 (0.59 – 1.32)		161/116	1.36 (1.06 – 1.76)	
WC in cm (median)						
<79	399/402	0.92 (0.79 – 1.08)	0.60	348/379	0.79 (0.60 – 1.05)	0.36
≥79	303/299	0.95 (0.75 – 1.06)		468/438	0.92 (0.78 – 1.11)	
Use of hormones at blood collection						
No	576/576	0.86 (0.74 – 1.00)	0.18	493/493	1.19 (1.00 – 1.41)	0.17
Yes	143/143	1.12 (0.78 – 1.61)		345/344	0.98 (0.80 – 1.21)	
Leptin/adiponectin						
Breast cancer subtypes						
ER+PR+/-HER2+	210/210	0.73 (0.49 – 1.11)	0.67	210/210	0.88 (0.64 – 1.19)	0.73
ER+PR+/-HER2-	933/933	0.87 (0.73 – 1.05)		1,151/1,151	1.04 (0.89 – 1.23)	
ER-PR-HER2-	154/154	1.05 (0.66 – 1.66)		166/166	1.12 (0.74 – 1.72)	
ER-PR-HER2+	100/100	1.03 (0.58 – 1.82)		130/130	0.95 (0.60 – 1.49)	
Time between blood collection and diagnosis in years						
>2 - <5	98/98	0.71 (0.47 – 1.08)	0.50	128/128	0.71 (0.47 – 1.08)	0.50
≥5 - <10	378/378	0.94 (0.77 – 1.13)		419/419	0.94 (0.77 – 1.13)	
≥10	244/244	0.87 (0.66 – 1.13)		290/290	0.87 (0.66 – 1.13)	
Age at diagnosis in years						
≤ 50	180/180	0.79 (0.60 – 1.05)	0.36			
> 50	540/540	0.92 (0.78 – 1.10)				
BMI in kg/m <sup>2</sup>						
<25	410/408	0.90 (0.79 – 1.02)	0.11	381/388	0.97 (0.84 – 1.11)	0.22
≥25 - <30	229/226	1.07 (0.91 – 1.27)		295/334	0.97 (0.82 – 1.14)	

Inflammatory biomarkers and breast cancer risk						
≥30	81/85	1.00 (0.71 – 1.41)		161/116	1.26 (0.99 – 1.59)	
<b>WC in cm (median)</b>						
<79	399/402	0.92 (0.81 – 1.06)	0.46	348/379	0.94 (0.81 – 1.10)	0.35
≥79	303/299	0.98 (0.84 – 1.15)		468/438	1.02 (0.90 – 1.16)	
<b>Use of hormones at blood collection</b>						
No	576/576	0.85 (0.72 – 0.99)	0.14	493/493	1.08 (0.91 – 1.28)	0.35
Yes	143/143	1.16 (0.79 – 1.70)		345/344	0.95 (0.77 – 1.18)	
<b>CRP</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	0.88 (0.67 – 1.17)	0.30	210/210	0.88 (0.66 – 1.18)	0.72
ER+PR+/-HER2-	934/934	0.90 (0.78 – 1.05)		1,151/1,151	1.05 (0.92 – 1.20)	
ER-PR-HER2-	154/154	0.70 (0.47 – 1.04)		166/166	1.11 (0.77 – 1.59)	
ER-PR-HER2+	100/100	1.29 (0.79 – 2.09)		130/130	1.01 (0.64 – 1.59)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	0.83 (0.61 – 1.13)	0.32	128/128	1.03 (0.78 – 1.35)	0.72
≥5 - <10	378/378	0.85 (0.73 – 1.00)		418/418	0.99 (0.84 – 1.17)	
≥10	244/244	1.03 (0.84 – 1.26)		291/291	1.10 (0.91 – 1.32)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	0.91 (0.72 – 1.14)	0.98			
> 50	540/540	0.91 (0.79 – 1.04)				
<b>BMI in kg/m²</b>						
<25	410/408	0.95 (0.86 – 1.04)	0.67	381/388	1.04 (0.94 – 1.16)	0.92
≥25 - <30	229/227	0.97 (0.85 – 1.10)		295/334	0.99 (0.86 – 1.14)	
≥30	81/85	0.94 (0.71 – 1.25)		161/116	1.10 (0.88 – 1.38)	
<b>WC in cm (median)</b>						
<79	399/403	0.95 (0.87 – 1.05)	0.65	348/379	1.05 (0.94 – 1.16)	0.62
≥79	303/299	0.96 (0.85 – 1.08)		468/438	1.00 (0.90 – 1.13)	
<b>Use of hormones at blood collection</b>						
No	576/576	0.92 (0.81 – 1.05)	0.59	490/490	0.97 (0.83 – 1.13)	0.11
Yes	144/144	0.85 (0.64 – 1.13)		345/345	1.16 (0.98 – 1.38)	
<b>TNF-α</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	0.87 (0.62 – 1.22)	0.45	210/210	1.20 (0.83 – 1.75)	0.32
ER+PR+/-HER2-	932/932	0.96 (0.83 – 1.11)		1,146/1,146	1.10 (0.95 – 1.28)	
ER-PR-HER2-	154/154	0.95 (0.62 – 1.45)		166/166	0.77 (0.49 – 1.21)	
ER-PR-HER2+	100/100	1.46 (0.85 – 2.52)		130/130	1.36 (0.89 – 2.08)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	1.07 (0.75 – 1.53)	0.57	128/128	1.26 (0.90 – 1.76)	0.67
≥5 - <10	378/378	0.92 (0.78 – 1.09)		417/417	1.11 (0.94 – 1.32)	
≥10	243/243	1.04 (0.86 – 1.28)		290/290	1.05 (0.84 – 1.30)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	1.02 (0.82 – 1.31)	0.69			
> 50	539/539	0.97 (0.84 – 1.11)				
<b>BMI in kg/m²</b>						
<25	409/407	1.01 (0.83 – 1.24)	0.23	381/386	1.00 (0.89 – 1.13)	0.70
≥25 - <30	229/227	1.03 (0.79 – 1.35)		293/333	1.10 (0.97 – 1.26)	
≥30	81/85	0.85 (0.53 – 1.35)		161/116	0.99 (0.82 – 1.20)	
<b>WC in cm (median)</b>						
<79	398/402	1.00 (0.82 – 1.23)	0.95	348/378	1.03 (0.91 – 1.17)	0.97
≥79	303/299	1.01 (0.81 – 1.28)		466/436	1.04 (0.94 – 1.15)	
<b>Use of hormones at blood collection</b>						
No	575/575	0.94 (0.83 – 1.08)	0.16	490/490	1.13 (0.95 – 1.34)	0.73
Yes	144/144	1.20 (0.88 – 1.63)		345/345	1.08 (0.90 – 1.30)	
<b>IFN-γ</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	0.76 (0.58 – 0.99)	0.38	210/210	1.21 (0.90 – 1.63)	0.44
ER+PR+/-HER2-	932/932	0.96 (0.84 – 1.09)		1,146/1,146	1.02 (0.91 – 1.15)	
ER-PR-HER2-	154/154	0.86 (0.62 – 1.20)		166/166	0.98 (0.72 – 1.33)	
ER-PR-HER2+	100/100	1.09 (0.70 – 1.69)		130/130	0.77 (0.48 – 1.23)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	0.96 (0.73 – 1.27)	0.81	128/128	0.93 (0.70 – 1.24)	0.56
≥5 - <10	378/378	0.89 (0.77 – 1.03)		417/417	1.08 (0.93 – 1.25)	
≥10	243/243	0.95 (0.80 – 1.13)		290/290	0.99 (0.84 – 1.16)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	1.04 (0.86 – 1.26)	0.13			
> 50	539/539	0.87 (0.77 – 0.99)				
<b>BMI in kg/m²</b>						
<25	409/407	0.99 (0.86 – 1.13)	0.21	381/386	0.99 (0.90 – 1.10)	0.61

Inflammatory biomarkers and breast cancer risk						
≥25 - <30	229/227	0.89 (0.73 – 1.09)		293/333	1.05 (0.94 – 1.18)	
≥30	81/85	0.76 (0.52 – 1.12)		161/116	0.94 (0.77 – 1.14)	
<b>WC in cm (median)</b>						
<79	398/402	0.93 (0.80 – 1.06)	0.63	348/378	1.00 (0.89 – 1.12)	0.95
≥79	303/299	0.98 (0.83 – 1.16)		466/436	1.01 (0.92 – 1.11)	
<b>Use of hormones at blood collection</b>						
No	575/575	0.90 (0.80 – 1.01)	0.23	490/490	1.08 (0.94 – 1.24)	0.28
Yes	144/144	1.06 (0.82 – 1.37)		345/345	0.97 (0.83 – 1.12)	
<b>IL-6</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	0.92 (0.66 – 1.27)		210/210	0.97 (0.71 – 1.32)	
ER+PR+/-HER2-	933/933	1.03 (0.90 – 1.18)	0.87	1,146/1,146	1.01 (0.89 – 1.16)	0.97
ER-PR-HER2-	154/154	0.78 (0.52 – 1.18)		166/166	0.95 (0.68 – 1.31)	
ER-PR-HER2+	100/100	0.94 (0.64 – 1.37)		130/130	0.95 (0.60 – 1.51)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	0.89 (0.67 – 1.20)		128/128	1.08 (0.81 – 1.45)	
≥5 - <10	378/378	0.98 (0.84 – 1.14)	0.73	417/417	1.04 (0.89 – 1.22)	0.48
≥10	243/243	1.03 (0.85 – 1.25)		290/290	0.91 (0.75 – 1.11)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	0.96 (0.76 – 1.22)	0.81			
> 50	539/539	1.00 (0.88 – 1.13)				
<b>BMI in kg/m<sup>2</sup></b>						
<25	409/407	1.01 (0.88 – 1.17)		381/386	1.06 (0.95 – 1.18)	0.84
≥25 - <30	229/227	0.91 (0.73 – 1.13)	0.89	293/333	0.92 (0.80 – 1.06)	
≥30	81/85	0.97 (0.63 – 1.50)		161/116	1.08 (0.88 – 1.32)	
<b>WC in cm (median)</b>						
<79	398/402	1.01 (0.87 – 1.17)	0.89	348/378	0.99 (0.88 – 1.11)	0.73
≥79	303/299	0.95 (0.78 – 1.16)		466/436	1.02 (0.91 – 1.14)	
<b>Use of hormones at blood collection</b>						
No	575/575	1.00 (0.89 – 1.13)	0.58	490/490	1.04 (0.90 – 1.22)	0.42
Yes	144/144	0.92 (0.68 – 1.23)		345/345	0.95 (0.81 – 1.13)	
<b>IL-8</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	1.17 (0.80 – 1.70)		210/210	0.91 (0.68 – 1.22)	
ER+PR+/-HER2-	932/932	1.06 (0.89 – 1.25)	0.93	1,146/1,146	0.95 (0.82 – 1.11)	0.49
ER-PR-HER2-	154/154	1.09 (0.72 – 1.64)		166/166	0.77 (0.53 – 1.12)	
ER-PR-HER2+	100/100	0.96 (0.60 – 1.51)		130/130	0.71 (0.45 – 1.10)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	0.97 (0.64 – 1.49)		128/128	0.92 (0.67 – 1.28)	
≥5 - <10	377/377	1.17 (0.97 – 1.40)	0.34	417/417	0.94 (0.81 – 1.09)	0.63
≥10	244/244	0.95 (0.75 – 1.19)		290/290	0.82 (0.64 – 1.04)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	1.21 (0.94 – 1.58)	0.23			
> 50	539/539	1.01 (0.86 – 1.18)				
<b>BMI in kg/m<sup>2</sup></b>						
<25	409/407	1.09 (0.94 – 1.27)		381/386	1.00 (0.88 – 1.14)	0.51
≥25 - <30	229/227	0.97 (0.75 – 1.25)	0.04	293/333	0.94 (0.80 – 1.10)	
≥30	81/85	0.91 (0.60 – 1.39)		161/116	0.91 (0.76 – 1.09)	
<b>WC in cm (median)</b>						
<79	398/402	1.13 (0.98 – 1.31)	0.12	348/379	0.94 (0.82 – 1.08)	0.94
≥79	303/299	0.93 (0.74 – 1.16)		468/438	0.96 (0.86 – 1.07)	
<b>Use of hormones at blood collection</b>						
No	575/575	1.08 (0.93 – 1.26)	0.61	490/490	0.94 (0.81 – 1.10)	0.35
Yes	144/144	0.99 (0.72 – 1.35)		345/345	0.84 (0.69 – 1.01)	
<b>IL-10</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	0.89 (0.63 – 1.27)		210/210	1.05 (0.78 – 1.40)	
ER+PR+/-HER2-	932/932	0.95 (0.83 – 1.10)	0.30	1,146/1,146	1.08 (0.96 – 1.23)	0.82
ER-PR-HER2-	154/154	1.05 (0.73 – 1.52)		166/166	0.91 (0.64 – 1.30)	
ER-PR-HER2+	100/100	1.47 (0.94 – 2.32)		130/130	1.00 (0.68 – 1.46)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	1.17 (0.84 – 1.64)		128/128	0.98 (0.75 – 1.27)	
≥5 - <10	377/377	0.96 (0.82 – 1.13)	0.52	417/417	1.02 (0.88 – 1.17)	0.31
≥10	244/244	1.06 (0.87 – 1.29)		290/290	1.20 (1.00 – 1.44)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	1.27 (1.00 – 1.61)	0.04			
> 50	539/539	0.95 (0.83 – 1.08)				
<b>BMI in kg/m<sup>2</sup></b>						
<25	409/407	0.99 (0.87 – 1.14)	0.99	381/386	1.04 (0.93 – 1.15)	0.75



Inflammatory biomarkers and breast cancer risk						
≥25 - <30	229/227	1.09 (0.90 – 1.31)		293/333	0.98 (0.87 – 1.11)	
≥30	81/85	1.04 (0.76 – 1.42)		161/116	1.10 (0.92 – 1.31)	
<b>WC in cm (median)</b>						
<79	398/402	0.99 (0.86 – 1.13)	0.55	348/379	1.02 (0.91 – 1.14)	0.99
≥79	303/299	1.08 (0.92 – 1.27)		468/438	1.02 (0.93 – 1.12)	
<b>Use of hormones at blood collection</b>						
No	575/575	1.01 (0.89 – 1.14)	0.64	490/490	1.04 (0.90 – 1.19)	0.49
Yes	144/144	1.08 (0.83 – 1.41)		345/345	1.12 (0.95 – 1.31)	
<b>IL-1-RA</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	0.98 (0.66 – 1.46)		210/210	1.14 (0.82 – 1.59)	
ER+PR+/-HER2-	933/933	1.05 (0.90 – 1.23)	0.95	1,146/1,146	1.07 (0.93 – 1.24)	0.40
ER-PR-HER2-	154/154	1.17 (0.74 – 1.83)		166/166	0.80 (0.57 – 1.13)	
ER-PR-HER2+	100/100	1.06 (0.63 – 1.77)		128/128	0.92 (0.64 – 1.34)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	1.20 (0.86 – 1.69)		127/127	1.24 (0.92 – 1.68)	
≥5 - <10	377/377	1.08 (0.89 – 1.30)	0.53	416/416	0.94 (0.80 – 1.11)	0.27
≥10	244/244	0.96 (0.75 – 1.22)		291/291	1.08 (0.89 – 1.32)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	0.96 (0.76 – 1.27)	0.45			
> 50	539/539	1.09 (0.93 – 1.27)				
<b>BMI in kg/m<sup>2</sup></b>						
<25	410/408	0.98 (0.78 – 1.24)		379/387	1.03 (0.90 – 1.17)	
≥25 - <30	228/227	1.17 (0.84 – 1.62)	0.72	294/331	0.96 (0.83 – 1.10)	0.43
≥30	81/85	1.12 (0.69 – 1.81)		161/116	1.12 (0.96 – 1.32)	
<b>WC in cm (median)</b>						
<79	398/403	1.10 (0.88 – 1.39)	0.60	348/379	1.02 (0.89 – 1.17)	0.99
≥79	303/299	0.99 (0.76 – 1.30)		468/438	1.00 (0.90 – 1.11)	
<b>Use of hormones at blood collection</b>						
No	575/575	1.04 (0.89 – 1.21)	0.53	490/490	1.07 (0.92 – 1.25)	0.49
Yes	144/144	1.16 (0.86 – 1.55)		345/345	0.99 (0.83 – 1.18)	
<b>IL-17-D</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	1.00 (0.79 – 1.27)		210/210	1.03 (0.78 – 1.37)	
ER+PR+/-HER2-	933/933	0.92 (0.82 – 1.04)	0.87	1,146/1,146	0.92 (0.80 – 1.05)	0.79
ER-PR-HER2-	154/154	1.00 (0.76 – 1.33)		166/166	1.09 (0.73 – 1.61)	
ER-PR-HER2+	100/100	1.01 (0.75 – 1.35)		128/128	0.93 (0.67 – 1.30)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	9898/	0.95 (0.76 – 1.20)		127/127	1.04 (0.81 – 1.34)	
≥5 - <10	377/377	0.99 (0.88 – 1.12)	0.69	416/416	0.85 (0.73 – 0.99)	0.27
≥10	244/244	0.90 (0.75 – 1.08)		291/291	1.00 (0.82 – 1.21)	
<b>Age at diagnosis in years</b>						
≤ 50	180/180	0.91 (0.74 – 1.11)	0.54			
> 50	539/539	0.97 (0.88 – 1.08)				
<b>BMI in kg/m<sup>2</sup></b>						
<25	410/410	0.91 (0.78 – 1.07)		379/387	0.94 (0.84 – 1.06)	
≥25 - <30	228/228	1.06 (0.86 – 1.32)	0.52	294/331	0.95 (0.85 – 1.05)	0.64
≥30	81/81	0.99 (0.73 – 1.35)		161/116	0.98 (0.81 – 1.18)	
<b>WC in cm (median)</b>						
<79	398/403	0.96 (0.82 – 1.12)	0.77	348/379	1.00 (0.88 – 1.13)	0.44
≥79	303/299	0.99 (0.83 – 1.20)		468/438	0.94 (0.86 – 1.03)	
<b>Use of hormones at blood collection</b>						
No	575/575	0.97 (0.87 – 1.07)	0.74	491/491	0.95 (0.82 – 1.09)	0.49
Yes	144/144	0.93 (0.76 – 1.14)		343/343	0.88 (0.74 – 1.03)	
<b>IL-13</b>						
<b>Breast cancer subtypes</b>						
ER+PR+/-HER2+	210/210	1.28 (0.93 – 1.76)		210/210	1.33 (0.97 – 1.83)	
ER+PR+/-HER2-	931/931	0.92 (0.79 – 1.06)	0.32	1,146/1,146	0.91 (0.80 – 1.03)	0.18
ER-PR-HER2-	154/154	1.01 (0.72 – 1.40)		166/166	1.00 (0.71 – 1.41)	
ER-PR-HER2+	100/100	0.91 (0.78 – 1.71)		128/128	1.00 (0.70 – 1.42)	
<b>Time between blood collection and diagnosis in years</b>						
>2 - <5	98/98	1.30 (0.95 – 1.78)		128/128	1.41 (1.03 – 1.92)	
≥5 - <10	378/378	0.91 (0.77 – 1.07)	0.14	417/417	0.84 (0.72 – 0.98)	0.01
≥10	242/242	0.94 (0.77 – 1.14)		290/290	1.02 (0.86 – 1.21)	
<b>Age at diagnosis in years</b>						
≤ 50	150/150	1.13 (0.88 – 1.45)	0.17			
> 50	538/538	0.93 (0.81 – 1.06)				
<b>BMI in kg/m<sup>2</sup></b>						
<25	408/407	1.00 (0.77 – 1.29)	0.84	381/386	1.02 (0.92 – 1.13)	0.50

Inflammatory biomarkers and breast cancer risk						
≥25 - <30	229/227	0.79 (0.54 – 1.17)		293/333	0.94 (0.83 – 1.07)	
≥30	81/85	1.34 (0.73 – 2.46)		161/116	0.96 (0.81 – 1.15)	
<b>WC in cm (median)</b>						
<79	398/402	1.01 (0.77 – 1.32)	0.63	348/378	0.99 (0.88 – 1.11)	0.79
≥79	303/299	0.91 (0.67 – 1.24)		466/436	0.96 (0.87 – 1.06)	
<b>Use of hormones at blood collection</b>						
No	575/575	1.04 (0.89 – 1.21)	0.53	490/490	1.02 (0.89 – 1.16)	0.27
Yes	143/143	1.16 (0.86 – 1.55)		345/345	0.90 (0.75 – 1.07)	

Abbreviations: CI, confidence interval; BMI, body mass index; CRP, c-reactive protein; ER, oestrogen receptor; HER2, human epidermal growth factor 2; IL, interleukin; IFN, interferon; OR, odd ratio; PR, progesterone receptor; SD, standard deviation; TNF, tumour Necrosis Factor; WC, waist circumference.

<sup>1</sup> ORs were estimated per 1 SD increase in log-transformed biomarkers concentrations, from logistic regression conditioned on matching variables and adjusted for BMI. For IL-13, ORs were estimated according to the limit of quantification.

Table S4: Loadings from component analysis of inflammatory biomarkers and correlations between components and selected factors.

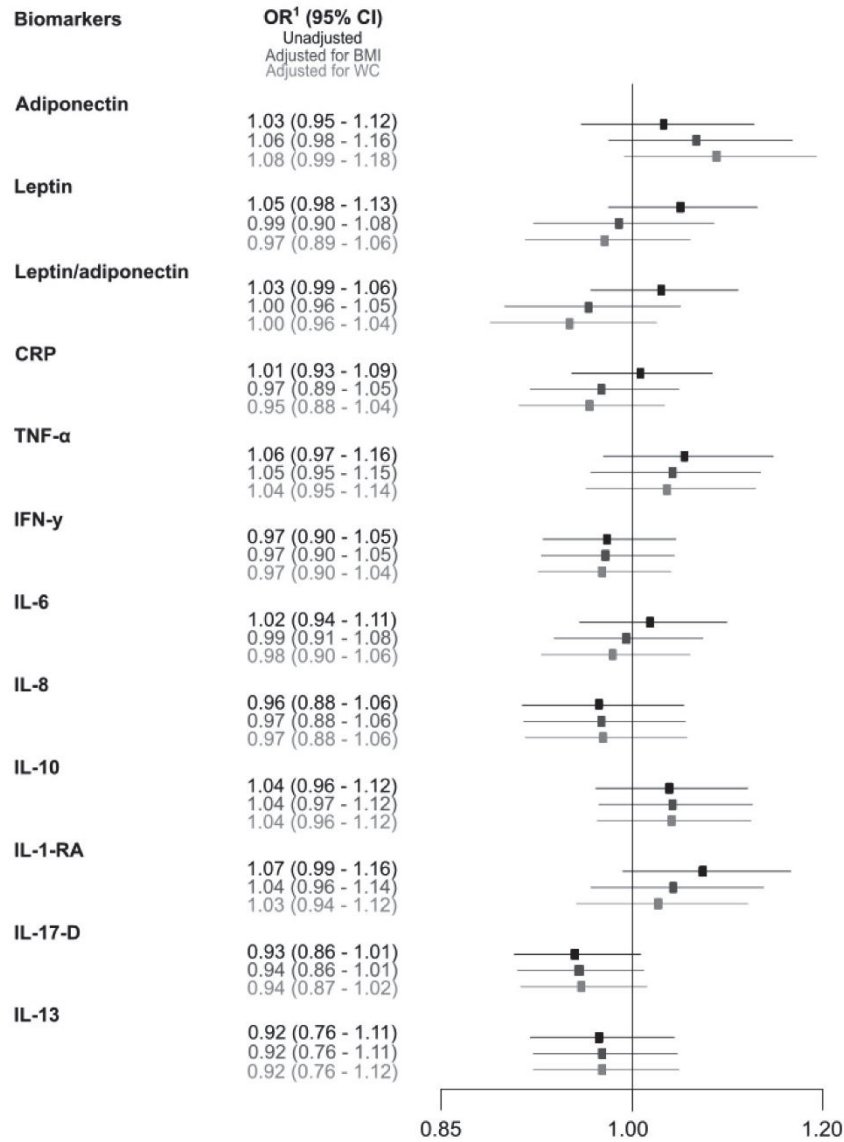
	Component 1	Component 2	Component 3	Component 4
<b>Loadings of inflammatory biomarkers</b>				
Adiponectin	-0.02	0.46	0.36	0.32
Leptin	0.30	-0.42	0.16	0.23
CRP	0.39	-0.34	0.27	0.14
TNF- $\alpha$	0.43	0.28	-0.10	-0.04
IFN- $\gamma$	0.35	0.24	0.17	-0.41
IL-6	0.40	-0.15	0.33	0.11
IL-8	0.27	0.30	-0.55	0.27
IL-10	0.26	0.32	0.18	-0.47
IL-1-RA	0.38	-0.19	-0.52	0.01
IL-17-D	0.08	0.33	0.13	0.59
Total variance %	25	16	11	10
<b>Correlations with selected factors</b>				
Age at blood collection	<b>0.29</b>	0.07	0.04	<b>0.18</b>
BMI	<b>0.46</b>	<b>-0.44</b>	0.04	<b>0.15</b>
Height	<b>-0.12</b>	0.05	-0.01	-0.07
Weight	<b>0.39</b>	<b>-0.42</b>	0.04	<b>0.12</b>
WC	<b>0.44</b>	<b>-0.40</b>	0.04	<b>0.11</b>
Hip circumference	<b>0.37</b>	<b>-0.38</b>	0.07	0.14
Waist/hip ratio	<b>0.31</b>	<b>-0.22</b>	-0.06	0.03
Leptin/adiponectin	<b>0.43</b>	<b>-0.70</b>	-0.01	<b>0.12</b>

Abbreviations: BMI, body mass index; CRP, c-reactive protein; IL, interleukin; IFN, interferon; TNF, tumour Necrosis Factor; WC, waist circumference.

Spearman correlation coefficients with  $p$ -Values <0.0001 are in bold.

**Supplementary Figures.**

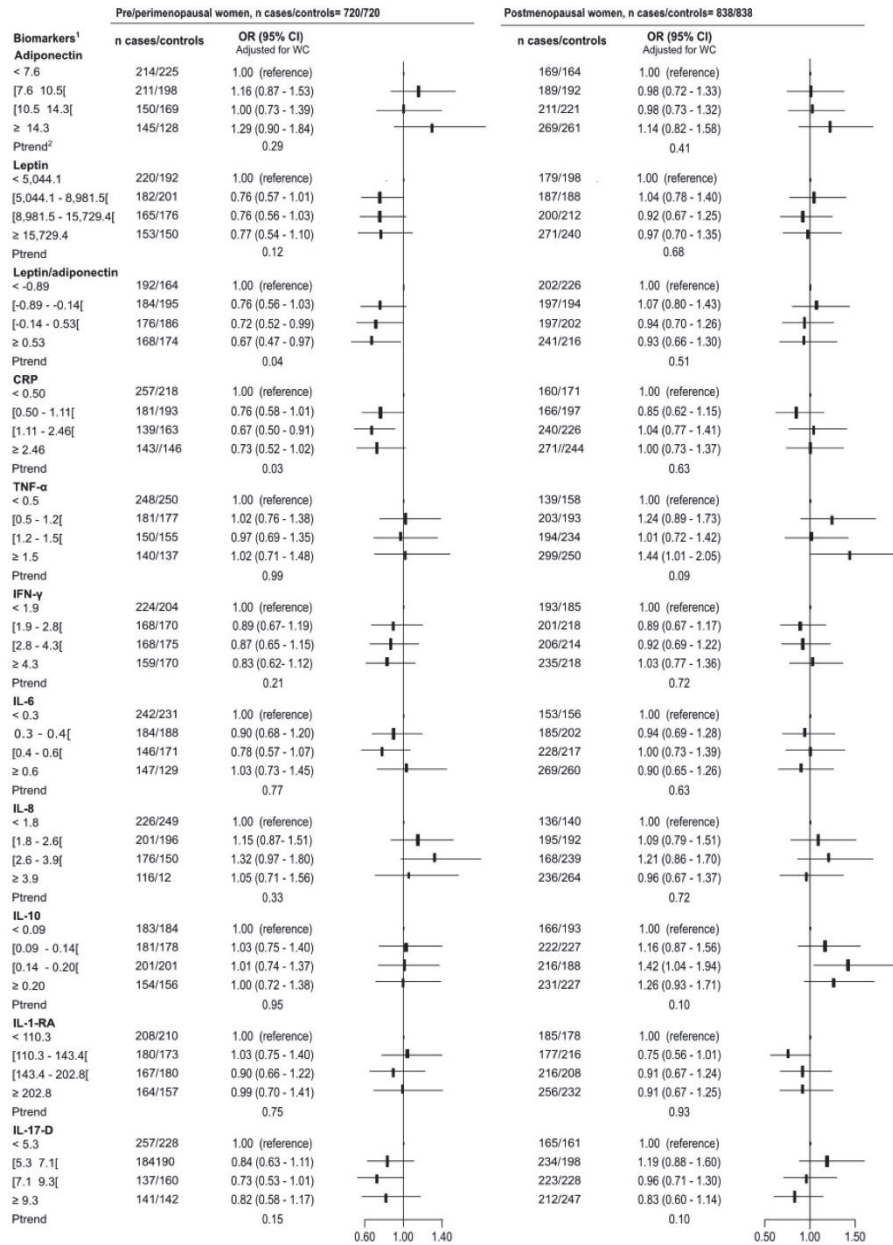
Figure S1. Associations between inflammatory biomarkers and breast cancer risk among all participants (n cases/controls = 1,558/1,558).



Abbreviations: CI, confidence interval; BMI, body mass index; CRP, c-reactive protein; ER, oestrogen receptor; HER2, human epidermal growth factor 2; IL, interleukin; IFN, interferon; OR, odd ratio; PR, progesterone receptor; SD, standard deviation; TNF, tumour Necrosis Factor; WC, waist circumference.

<sup>1</sup> ORs were estimated per 1 SD increase in log-transformed biomarkers concentrations, from logistic regression conditioned on matching variables. For IL-13, ORs were estimated according to the limit of quantification.

Figure S2. Associations between quartiles of inflammatory biomarkers and breast cancer risk after waist circumference adjustment and according to menopausal status.



Abbreviations: CI, confidence interval; BMI, body mass index; CRP, c-reactive protein; ER, oestrogen receptor; HER2, human epidermal growth factor 2; IL, interleukin; IFN, interferon; OR, odd ratio; PR, progesterone receptor; TNF, tumour necrosis factor; WC, waist circumference.



### Acknowledgements

The authors thank all participants in the EPIC cohort for their invaluable contribution to the study. The authors also thank Bertrand Hémon (IARC) for his precious help with the EPIC database.

### Funding

This work was funded by the French National Cancer Institute (grant number 2016-128) and the World Cancer Research Fund (grant number 2017/1614). Manon Cairat is supported by a PhD fellowship from la Ligue Nationale Contre le Cancer.

The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue National Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Éducation Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); European Research Council (ERC-2009-AdG 232997), and Nordforsk, Nordic Centre of Excellence Programme on Food, Nutrition and Health (Norway); Regional Governments of Andalucía, Asturias, Basque Country, Murcia (no. 6236), Navarra, and the CERCA Program (Generalitat de Catalunya) (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); and Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (United Kingdom). The funders were not involved in designing the study; collecting, analyzing or interpreting the data; or in writing or submitting the manuscript for publication.

### 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.

## Reference list

1. Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol*. 2012;30:677-706.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-74.
3. Jiang X, Shapiro DJ. The immune system and inflammation in breast cancer. *Molecular and cellular endocrinology*. 2014;382(1):673-82.
4. Chan DS, Bandera EV, Greenwood DC, Norat T. Circulating C-Reactive Protein and Breast Cancer Risk-Systematic Literature Review and Meta-analysis of Prospective Cohort Studies. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2015;24(10):1439-49.
5. Dossus L, Jimenez-Corona A, Romieu I, Boutron-Ruault MC, Bouitten A, Dupre T, et al. C-reactive protein and postmenopausal breast cancer risk: results from the E3N cohort study. *Cancer Causes Control*. 2014;25(4):533-9.
6. Zeleniuch-Jacquotte A, Gu Y, Bruning PF, Bonfrer JM, Koenig KL, Arslan AA, et al. Re: C-reactive protein and risk of breast cancer. *Journal of the National Cancer Institute*. 2008;100(6):443-4; author reply 4-5.
7. Ollberding NJ, Kim Y, Shvetsov YB, Wilkens LR, Franke AA, Cooney RV, et al. Prediagnostic leptin, adiponectin, C-reactive protein, and the risk of postmenopausal breast cancer. *Cancer Prev Res (Phila)*. 2013;6(3):188-95.
8. Zhang SM, Lin J, Cook NR, Lee IM, Manson JE, Buring JE, et al. C-reactive protein and risk of breast cancer. *Journal of the National Cancer Institute*. 2007;99(11):890-4.
9. Gunter MJ, Wang T, Cushman M, Xue X, Wassertheil-Smolter S, Strickler HD, et al. Circulating Adipokines and Inflammatory Markers and Postmenopausal Breast Cancer Risk. *Journal of the National Cancer Institute*. 2015;107(9).
10. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public health nutrition*. 2002;5(6b):1113-24.
11. Rinaldi S, Peeters PH, Berrino F, Dossus L, Biessy C, Olsen A, et al. IGF-I, IGFBP-3 and breast cancer risk in women: The European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocrine-related cancer*. 2006;13(2):593-605.
12. Jarde T, Perrier S, Vasson MP, Caldefie-Chezet F. Molecular mechanisms of leptin and adiponectin in breast cancer. *European journal of cancer (Oxford, England : 1990)*. 2011;47(1):33-43.
13. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, et al. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. *Diabetologia*. 2009;52(11):2345-9.
14. Agnoli C, Grioni S, Pala V, Allione A, Matullo G, Gaetano CD, et al. Biomarkers of inflammation and breast cancer risk: a case-control study nested in the EPIC-Varese cohort. *Scientific reports*. 2017;7(1):12708.
15. Harris HR, Tworoger SS, Hankinson SE, Rosner BA, Michels KB. Plasma leptin levels and risk of breast cancer in premenopausal women. *Cancer Prev Res (Phila)*. 2011;4(9):1449-56.
16. Brannan JD, Hansen KA. Leptin and ovarian folliculogenesis: implications for ovulation induction and ART outcomes. *Seminars in reproductive medicine*. 2002;20(2):103-12.
17. Falk RT, Brinton LA, Madigan MP, Potischman N, Sturgeon SR, Malone KE, et al. Interrelationships between serum leptin, IGF-1, IGFBP3, C-peptide and prolactin and breast cancer risk in young women. *Breast cancer research and treatment*. 2006;98(2):157-65.
18. Tworoger SS, Eliassen AH, Kesisidis T, Colditz GA, Willett WC, Mantzoros CS, et al. Plasma adiponectin concentrations and risk of incident breast cancer. *The Journal of clinical endocrinology and metabolism*. 2007;92(4):1510-6.
19. Macis D, Gandini S, Guerrieri-Gonzaga A, Johansson H, Magni P, Ruscica M, et al. Prognostic effect of circulating adiponectin in a randomized 2 x 2 trial of low-dose tamoxifen and fenretinide in premenopausal women at risk for breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012;30(2):151-7.
20. Zhang SM, Ridker PM. Response: Re: C-Reactive Protein and Risk of Breast Cancer. *JNCI: Journal of the National Cancer Institute*. 2008;100(6):444-5.
21. Wang G, Li N, Chang S, Bassig BA, Guo L, Ren J, et al. A prospective follow-up study of the relationship between C-reactive protein and human cancer risk in the Chinese Kailuan Female Cohort. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2015;24(2):459-65.
22. Piotrowski L, Kulcenty K, Suchorska W. Interplay between inflammation and cancer. *Reports of practical oncology and radiotherapy : journal of Great Poland Cancer Center in Poznan and Polish Society of Radiation Oncology*. 2020;25(3):422-7.
23. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nature reviews Immunology*. 2005;5(10):749-59.
24. Zhao Y, Nichols JE, Valdez R, Mendelson CR, Simpson ER. Tumor necrosis factor-alpha stimulates aromatase gene expression in human adipose stromal cells through use of an activating protein-1 binding site upstream of promoter 1.4. *Molecular endocrinology (Baltimore, Md)*. 1996;10(11):1350-7.
25. Heikkila K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, et al. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. *Cancer Causes Control*. 2009;20(1):15-26.
26. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer Epidemiol Biomarkers Prev*. 2005;14(10):2413-8.
27. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005;14(10):2413-8.
28. Gross AL, Newschaffer CJ, Hoffman-Bolton J, Rifai N, Visvanathan K. Adipocytokines, inflammation, and breast cancer risk in postmenopausal women: a prospective study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2013;22(7):1319-24.
29. Krajcik RA, Massardo S, Orentreich N. No association between serum levels of tumor necrosis factor-alpha (TNF-alpha) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2003;12(9):945-6.
30. Dias JA, Fredrikson GN, Ericson U, Gullberg B, Hedblad B, Engstrom G, et al. Low-Grade Inflammation, Oxidative Stress and Risk of Invasive Post-Menopausal Breast Cancer - A Nested Case-Control Study from the Malmo Diet and Cancer Cohort. *PLoS one*. 2016;11(7):e0158959.
31. Gaudet MM, Gapstur SM, Sun J, Diver WR, Hannan LM, Thun MJ. Active smoking and breast cancer risk: original cohort data and meta-analysis. *Journal of the National Cancer Institute*. 2013;105(8):515-25.

32. Wang J, Lee IM, Tworoger SS, Buring JE, Ridker PM, Rosner B, et al. Plasma C-reactive protein and risk of breast cancer in two prospective studies and a meta-analysis. *Cancer epidemiology, biomarkers & prevention* : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2015;24(8):1199-206.
33. Touvier M, Fezeu L, Ahluwalia N, Julia C, Charnaux N, Sutton A, et al. Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. *American journal of epidemiology*. 2013;177(1):3-13.
34. Allin KH, Bojesen SE, Nordestgaard BG. Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. *Journal of clinical oncology* : official journal of the American Society of Clinical Oncology. 2009;27(13):2217-24.
35. Chen X, Wang Y. Adiponectin and breast cancer. *Med Oncol*. 2011;28(4):1288-95.
36. Gaudet MM, Falk RT, Gierach GL, Lacey JV, Jr., Graubard BI, Dorgan JF, et al. Do adipokines underlie the association between known risk factors and breast cancer among a cohort of United States women? *Cancer epidemiology*. 2010;34(5):580-6.
37. Cust AE, Stocks T, Lukanova A, Lundin E, Hallmans G, Kaaks R, et al. The influence of overweight and insulin resistance on breast cancer risk and tumour stage at diagnosis: a prospective study. *Breast cancer research and treatment*. 2009;113(3):567-76.
38. Clendenen TV, Arslan AA, Lokshin AE, Idahl A, Hallmans G, Koenig KL, et al. Temporal reliability of cytokines and growth factors in EDTA plasma. *BMC research notes*. 2010;3:302.



## **CHAPTER 4: NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND BREAST CANCER RISK IN THE EPIC STUDY**

### **1. Objective**

The objective of this chapter was, using data from the EPIC study, to investigate the associations between “regular” NSAID use and breast cancer risk, overall and by breast cancer subtypes and according to various breast cancer risk factors.

### **2. Materials and methods**

#### **Study population**

Female participants in EPIC were eligible for inclusion in the current study if they had no history of cancer prior to recruitment, no missing date of cancer diagnosis or censoring and no missing data on exposure. A total of 140,981 women were therefore included in the current study.

#### **Follow-up**

Women were followed from study entry (around 1990) until the date of last known contact, cancer diagnosis, death, emigration, or the end of the follow-up period (between 2008 and 2014 depending on the centre), whichever came first.

#### **Exposure assessment**

The baseline questions asked to the participants are noted in Table 1. In EPIC, “regular” use of NSAIDs was defined by combining participants responding “yes” to the question in France, in the United Kingdom, and in the Netherlands, those who cited NSAID and/or aspirin as “regular” medications in Germany, and people responding at least “3 pills per week” for using aspirin or ibuprofen in Denmark.

Table 1: Data collected on NSAIDs in EPIC.

Definition of “regular” NSAID use					Questions asked to participants
Countries	Type	Frequency	Duration	Period	
The Netherlands	Aspirin	Daily		Current	“Do you take aspirin daily?”
United Kingdom	Aspirin		≥ 3 months	Current	“Have you ever taken aspirin continuously for 3 months or more?”
Denmark	Aspirin Ibuprofen	≥3 times/week		Last year	“Have you used more than one painkilling tablet/month during the last year? If yes, how many pills containing aspirin and how many pills containing ibuprofen: none; 2–3/month; 1–2/week; 3–6/week; 1–3/day; 4–5/day; 6+/day?”
France	Any anti-inflammatory drugs	≥3 times/week		Current	“Are you currently taking anti-inflammatory drugs at least three times per week?”
Germany	Any NSAIDs			Current	“During the past 4 weeks, did you take any “regular” medications? If yes, please specify the medication.”

### Statistical analysis

Multivariable Cox regression models with age as the time scale were used to estimate HRs for the association of “regular” NSAID exposure with BC incidence, overall and by BC subtypes. Models were adjusted for BMI, educational level, MHT use, age at first full term pregnancy, and alcohol intake. Effect modification by main breast cancer risk factors was evaluated by using likelihood ratio tests to compare models with and without cross-product interaction terms. Heterogeneity between breast cancer subtypes was evaluated by competing risk analyses, with women who developed the competing breast cancer subtypes censored at the time of occurrence and cases with missing information on the studied subtype excluded from the corresponding analysis (240).

### 3. Main findings

Of the 140,981 women included in the current analysis, 9,907 (7%) were “regular” NSAID users at baseline. During a median follow-up of 13 years, 7,379 incident breast cancer cases were diagnosed (816 *in situ*, and 6,563 invasive).

Compared to women who were not “regular” NSAID users, “regular” users were older, more often overweight, or obese, less educated, and had less frequent exposure to oral

contraceptive and more frequent exposure to MHT. “Regular” NSAID use was most frequent among women from Denmark and the United Kingdom than other EPIC countries.

In multivariable models, there was no statistically significant association between “regular” NSAID use and breast cancer risk overall. Compared with women who were not “regular” users at baseline, the HR for “regular” users of NSAIDs was 0.99 (95% CI= 0.90 to 1.08). There was a borderline significant heterogeneity between *in situ* and invasive breast cancers [ $HR_{in situ}=1.27$  (0.98 – 1.65);  $HR_{invasive}=0.96$  (0.87 – 1.05);  $P_{heterogeneity}=0.05$ ]. Risk estimates did not vary significantly by other breast cancer subtypes ( $P_{homogeneity} \geq 0.36$ ), breast cancer risk factors (all  $P_{interactions}$  between 0.10 and 0.96) or country ( $P_{heterogeneity}=0.95$ ). However, there was a borderline significant interaction by MHT use. “Regular” NSAID use was inversely associated with overall breast cancer risk among postmenopausal women who had ever used MHT [ $HR_{MHT}=0.87$  (0.76 – 0.99)] but not among never MHT users [ $HR_{noMHT}=1.09$  (0.95 – 1.26);  $P_{interaction}=0.06$ ]. The test for interaction was stronger for invasive breast cancer [ $HR_{MHT}=0.84$  (0.73 – 0.96);  $HR_{noMHT}=1.08$  (0.93 – 1.25);  $P_{interaction}=0.05$ ], but far from significance for *in situ* cases [ $HR_{MHT}=1.25$  (0.82 – 1.92);  $HR_{noMHT}=1.18$  (0.81 – 1.73);  $P_{interaction}=0.99$ ]. A similar pattern of associations by “regular” NSAID and ever MHT use was observed for all other invasive breast cancer subtypes examined.

#### 4. Conclusions

In this large prospective European cohort study, we found a decreased risk of invasive breast cancer with “regular” NSAID use only in postmenopausal women who ever used MHT. No association was found between NSAID use and breast cancer risk in non-MHT users. Additional investigation is needed to better understand interactions between MHT use, NSAIDs and breast cancer risk.

#### 5. Paper

Status: Published

Contribution: First author, conducted statistical analyses, wrote the first draft of the manuscript, submitted it to the journal and replied to reviewers’ comments.

## Short Report

# Nonsteroidal anti-inflammatory drug use and breast cancer risk in a European prospective cohort study

Manon Cairat<sup>1</sup>, Agnès Fournier<sup>2,3</sup>, Neil Murphy<sup>4</sup>, Carine Biessy<sup>1</sup>, Augustin Scalbert<sup>1</sup>, Sabina Rinaldi<sup>5</sup>, Anne Tjønneland<sup>4</sup>, Anja Olsen<sup>4</sup>, Kim Overvad<sup>5</sup>, Patrick Arveux<sup>2,3,6</sup>, Marie-Christine Boutron-Ruault<sup>2,3</sup>, Claire Cadeau<sup>2,3,7</sup>, Renée Turzanski Fortner<sup>8</sup>, Rudolf Kaaks<sup>8</sup>, Heiner Boeing<sup>9</sup>, Krasimira Aleksandrova<sup>9,10</sup>, Petra H.M. Peeters<sup>11</sup>, Carla H. Van Gils<sup>11</sup>, Nicholas J. Wareham<sup>12</sup>, Kay-Tee Khaw<sup>13</sup>, Dagfinn Aune<sup>14,15</sup>, Elio Riboli<sup>14</sup>, Marc J. Gunter<sup>1</sup> and Laure Dossus<sup>1</sup>

<sup>1</sup> Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France

<sup>2</sup> CESP "Health Across Generations", INSERM, Univ Paris-Sud, UVSQ, Univ Paris-Saclay, Villejuif, France

<sup>3</sup> Gustave Roussy, Villejuif, France

<sup>4</sup> Danish Cancer Society Research Center, Copenhagen, Denmark

<sup>5</sup> Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark

<sup>6</sup> Breast and Gynaecologic Cancer Registry of Côte d'Or, Georges-François Leclerc Comprehensive Cancer Care Centre, 1 rue du Professeur Marion, Dijon, France

<sup>7</sup> Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

<sup>8</sup> Division of Cancer Epidemiology, German Cancer Research Centre (DKFZ), Heidelberg, Germany

<sup>9</sup> Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

<sup>10</sup> Nutrition, Immunity and Metabolism Start-Up Lab, Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

<sup>11</sup> Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

<sup>12</sup> MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Institute of Metabolic Science, Cambridge Biomedical Campus, Cambridge, United Kingdom

<sup>13</sup> Cancer Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom

<sup>14</sup> Department of Epidemiology and Biostatistics, School of Public Health, Imperial College, London, United Kingdom

<sup>15</sup> Bjørknes University College, Oslo, Norway

Experimental studies have shown a protective effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on breast cancer development. However, results from epidemiological cohort studies are less consistent. Our objective was to assess the association between NSAID use and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). EPIC is a prospective cohort study initiated in 1992 in 10 European countries. Self-reported information on NSAID use at baseline has been collected in five EPIC countries. Multivariable Cox regression models were used to estimate hazard ratios for the association of NSAID use with breast cancer incidence with adjustment for potential confounders. We also assessed effect modification by breast cancer risk factors and examined the associations within specific breast cancer subtypes. Among the 140,981 women included in the analysis, 7% were regularly using NSAIDs at baseline. During a median follow-up time period

**Key words:** breast cancer, nonsteroidal anti-inflammatory agents, chemoprevention, cohort studies, postmenopausal hormone replacement therapy

**Grant sponsor:** The European Commission (DG-SANCO) and the International Agency for Research on Cancer; **Grant sponsor:** Danish Cancer Society (Denmark); **Grant sponsor:** Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Éducation Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); **Grant sponsor:** German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and Federal Ministry of Education and Research (Germany); **Grant sponsor:** Dutch Ministry of Public Health, Welfare and Sports (VWS), the Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); **Grant sponsor:** Cancer Research UK (14136 to EPIC-Norfolk), Medical Research Council (1000143 to EPIC-Norfolk) (United Kingdom); **Grant sponsor:** La Ligue contre le Cancer (LNCC) PhD Fellowship to Manon Cairat

DOI: 10.1002/ijc.31570

**History:** Received 14 Dec 2017; Accepted 7 Mar 2018; Online 29 Apr 2018

**Correspondence to:** Laure Dossus, Nutrition and Metabolism Section, International Agency for Research on Cancer, World Health Organization, 150 Cours Albert Thomas, 69372 Lyon CEDEX 08, France, Tel.: 33-472-738-330, E-mail: dossus@iarc.fr



of 13 years, 7,379 incident breast cancer cases were diagnosed (816 *in situ* and 6,563 invasive). There were no statistically significant associations between NSAID use and breast cancer risk, overall and by subtypes. However, a statistically significant interaction was observed for invasive cases between NSAID use and ever use of menopausal hormonal therapy (MHT) among postmenopausal women [MHT users:  $HR_{NSAID\ use} = 0.84$  (0.73–0.96); non MHT users:  $HR_{NSAID\ use} = 1.08$  (0.93–1.25);  $p_{interaction} = 0.05$ ]. Our results indicate potential effect modification of MHT use on the association between use of NSAIDs and breast cancer risk which deserves in-depth investigation in studies with accurate data on both NSAID and MHT use.

#### What's new?

Chronic inflammation is a suspected factor in breast cancer development. Hence, the possibility of stemming tumorigenic inflammatory effects with nonsteroidal anti-inflammatory drugs (NSAIDs) has drawn significant interest. Here, using data from the European Prospective Investigation into Cancer and Nutrition prospective cohort study, the authors reveal an association between NSAID use and decreased risk of invasive breast cancer specifically in postmenopausal women who ever used menopausal hormonal therapy (MHT). No association was found between NSAID use and breast cancer risk in non-MHT users. Additional investigation is needed to better understand interactions between MHT use, NSAIDs, and breast cancer risk.

#### Introduction

Breast cancer is the most frequently diagnosed cancer and the most common cause of cancer death among women worldwide.<sup>1</sup> In past decades, evidence from both experimental and observational studies has accumulated suggesting that chronic inflammation could promote breast cancer development.<sup>2</sup> Therefore, nonsteroidal anti-inflammatory drugs (NSAIDs), a class of drugs commonly used to treat pain, fever and inflammation, have been proposed as potential chemo-preventive agents for breast cancer.<sup>3</sup> Although some experimental studies have shown a protective effect of NSAIDs on breast cancer development,<sup>4</sup> epidemiological evidence is less consistent. Indeed, recent meta-analyses have found a statistically significant decreased risk of breast cancer among NSAID users in case-control studies but not in cohorts where NSAID use was assessed prior to breast cancer onset.<sup>5</sup> It also remains unclear whether the potential association between NSAID use and breast tumor would vary by breast cancer subtypes and by known breast cancer risk factors. We therefore examined the associations between regular NSAID use and breast cancer risk among women enrolled in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, overall and by breast cancer subtypes and according to various breast cancer risk factors.

#### Materials and Methods

##### EPIC cohort

EPIC is a multi-center prospective cohort study designed to investigate the relationships among diet, nutrition, metabolic factors and cancer and other chronic diseases. The study enrolled >521,000 participants, mostly aged 35–70 years, between 1992 and 2000, from 23 regional and national research centers located in 10 Western European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and The United Kingdom.

At baseline, participants completed questionnaires on diet and lifestyle including reproductive and medical history,

exogenous hormone use, occupation, education and physical activity. Participants were also invited to a study center for blood collection, anthropometric measurements and blood pressure measurement. Extensive details on the standardized recruitment procedures are provided elsewhere.<sup>6</sup>

##### Study population

Female participants in EPIC were eligible for inclusion in our study if they had no history of cancer prior to recruitment, and no missing date of cancer diagnosis or censoring. Only the five countries that have provided data on NSAID use were included in the current analysis: The Netherlands (Utrecht center), France (all centers), Denmark (all centers), Germany (all centers) and United Kingdom (Norfolk center). Women were further excluded if exposure was missing at baseline, and if they were diagnosed with non-epithelial breast cancer. A total of 140,981 women were therefore included in the analyses.

##### Follow-up

In the Netherlands, Denmark and the United Kingdom, incident breast cancer cases were identified using record linkage with cancer and pathologies registries. In France and Germany, cases were identified through regular direct contacts with participants and their next of kin or through national cause-of-death registries. All reported breast cancer cases were then systematically verified against clinical and pathological records. Vital status was collected from regional or national mortality registries. Cancer incidence data were classified according to the International Classification of Diseases, 10th Revision (ICD-10). Women were followed from study entry until the date of last known contact, cancer diagnosis, death, emigration or the end of the follow-up period (between 2008 and 2014 depending on the center), whichever came first.

##### Exposure assessment

Data on NSAID use were collected at baseline by questionnaire. The following questions were asked to the participants:

- “Are you currently taking anti-inflammatory drugs at least three times per week?” in France.
- “Have you ever taken aspirin continuously for 3 months or more?” in the United Kingdom.
- “During the past 4 weeks, did you take any regular medications? If yes, please specify the medication” in Germany.
- “Do you take aspirin daily?” in the Netherlands.
- “Have you used more than one painkilling tablet/month during the last year? If yes, how many pills containing aspirin and how many pills containing ibuprofen: none; 2–3/month; 1–2/week; 3–6/week; 1–3/day; 4–5/day; 6+/day?” in Denmark.

We defined “regular use” of NSAID by combining participants responding “yes” to the question in France, in the United Kingdom and in the Netherlands, those who cited NSAID and/or aspirin as regular medications in Germany, and people responding at least “3 pills per week” for using aspirin or ibuprofen in Denmark.

#### Statistical analysis

Comparison of participants’ characteristics according to NSAID use has been assessed by  $\chi^2$  test. Associations between regular NSAID use and breast cancer risk were evaluated using Cox proportional hazard models to estimate hazard ratios (HR) and 95% confidence intervals (CI), overall and by subtypes (invasive/*in situ*, invasive ductal/invasive lobular, invasive estrogen receptor (ER)-positive/invasive ER-negative, invasive progesterone receptor (PR)-positive/invasive PR-negative, invasive ER/PR-positive/invasive ER/PR-negative). Heterogeneity between breast cancer subtypes was evaluated by competing risk analyses, with women who developed the competing breast cancer subtypes censored at the time of occurrence and cases with missing information on the studied subtype excluded from the corresponding analysis.<sup>7</sup> All models were stratified by age in 1-year intervals and by study center to prevent violations of the proportional-hazards assumption. Of the breast cancer risk factors listed in Table 1, only those that modified the HR estimate between NSAID use and breast cancer risk by >10% were included in the models. The final adjusted models therefore included: body mass index (BMI) (continuous), educational level (primary/no schooling, technical/professional/secondary, longer education, missing), menopausal hormone therapy (MHT) use (never, former, current, missing), age at first full-term pregnancy (nulliparous, ≤24 years, >24 years, missing) and alcohol intake (non-drinker, >0 to 3 g/d, >3 to 12 g/d, >12 to 24 g/d, >24 to 60 g/d, >60 g/d, missing). A complete case analysis that excluded women with missing covariates was conducted and yielded similar results (data not shown). We also examined whether the NSAID use and breast cancer associations varied according to BMI (<25 kg/m<sup>2</sup>, ≥25 kg/m<sup>2</sup>), waist/hip ratio (<0.85, ≥0.85), level of physical activity (Cambridge index: inactive, moderately inactive, moderately

active, active), educational level (primary/no schooling, technical/professional/secondary, longer education), use of contraceptive pills (never, former, current and never, ever), full-term pregnancy (no, yes), age at first full-term pregnancy (<24 years, ≥24 years) and number of full-term pregnancies (1, ≥2) among parous women, breastfeeding among parous women (no, yes), menopausal status (premenopausal, postmenopausal), MHT use (never, former, current and never, ever) among postmenopausal women, type of MHT used (estrogen alone, estrogen-progestogen) and alcohol intake (non-drinker, >0 to 3 g/d, >3 to 12 g/d, >12 g/d). Effect modification was evaluated by using likelihood ratio tests to compare models with and without cross-product interaction terms. Two-sided *p*-values of <0.05 were considered to be statistically significant. All analyses were carried out using the SAS software system (SAS Institute, Inc., Cary, NC), version 9.4.

#### Results

Of the 140,981 women included in the current analysis, 9,907 (7%) were regular NSAID users at baseline (Table 1). Characteristics of participants according to NSAID use at baseline are shown in Table 1.

During a median follow-up time period of 13.1 (SD = 3.8) years, 7,379 incident breast cancer cases were diagnosed (816 *in situ* and 6,563 invasive). Among those, 5,360 invasive breast cancer cases had information on ER status (4,405 ER-positive, 955 ER-negative) and 4,290 on PR status (2,806 PR-positive, 1,484 PR-negative). In multivariable models, there was no statistically significant association between regular NSAID use and breast cancer risk overall (Table 2). Compared to women who were not regular users at baseline, the HR for regular users of NSAIDs was 0.99 (95% CI = 0.90 to 1.08). Risk estimates did not vary significantly by histological subtypes or by hormone receptor status of the tumor (all *p*<sub>heterogeneity</sub> ≥0.36, data not shown) but there was a borderline significant heterogeneity between *in situ* and invasive breast cancers [HR<sub>*in situ*</sub> = 1.27 (0.98–1.65); HR<sub>invasive</sub> = 0.96 (0.87–1.05); *p*<sub>heterogeneity</sub> = 0.05].

The association between regular NSAID use and breast cancer risk did not vary significantly by breast cancer risk factors (all *p*<sub>interactions</sub> between 0.10 and 0.96) or by country (*p*<sub>heterogeneity</sub> = 0.95; data not shown), however there was a statistically significant interaction by MHT use (Table 2 and Fig. 1). Regular NSAID use was inversely associated with overall breast cancer risk among postmenopausal women who had ever used MHT [HR<sub>MHT</sub> = 0.87 (0.76–0.99)] but not among never MHT users [HR<sub>noMHT</sub> = 1.09 (0.95–1.26); *p*<sub>interaction</sub> = 0.06]. The test for interaction reached statistical significance for invasive breast cancer cases [HR<sub>MHT</sub> = 0.84 (0.73–0.96); HR<sub>noMHT</sub> = 1.08 (0.93–1.25); *p*<sub>interaction</sub> = 0.05], but was not statistically significant for *in situ* cases [HR<sub>MHT</sub> = 1.25 (0.82–1.92); HR<sub>noMHT</sub> = 1.18 (0.81–1.73); *p*<sub>interaction</sub> = 0.99]. A similar pattern of associations by regular NSAID and ever MHT use was observed for all other



Table 1. Selected participant characteristics according to NSAID use at baseline

Characteristics	Total, N	Regular use of NSAIDs, n (%) <sup>1</sup>	
		No	Yes
Total	140,981	131,074 (93)	9,907 (7)
<i>Age at recruitment, missing N = 0</i>			
≤ Median (53 years)	66,923	64,027 (49)	2,896 (29)
> Median (53 years)	74,058	67,047 (51)	7,011 (71)
<i>Menopausal status, missing N = 0</i>			
Premenopausal	33,865	32,833 (25)	1,032 (10)
Postmenopausal	107,116	98,241 (75)	8,875 (90)
<i>Country, missing N = 0</i>			
France	61,388	57,710 (44)	3,678 (37)
United Kingdom	12,505	11,405 (9)	1,100 (11)
The Netherlands	10,812	10,413 (8)	399 (4)
Germany	27,908	26,500 (20)	1,408 (14)
Denmark	28,368	25,046 (19)	3,322 (34)
<i>Educational level, missing N = 4,037</i>			
Primary/no schooling	29,732	26,483 (21)	3,249 (34)
Technical/professional/secondary	72,287	67,506 (53)	4,781 (50)
Longer education	34,925	33,354 (26)	1,571 (16)
<i>Family history of breast cancer, missing N = 67,265</i>			
No	67,122	63,068 (91)	4,054 (92)
Yes	6,594	6,227 (9)	367 (8)
<i>BMI (WHO categories), missing N = 0</i>			
<18.5 (underweight)	3,378	3,192 (2)	186 (2)
18.5–24.9 (normal weight)	85,601	80,762 (62)	4,839 (49)
25–29.9 (overweight)	37,774	34,564 (26)	3,210 (32)
≥30 (obese)	14,228	12,556 (10)	1,672 (17)
<i>Physical activity, missing N = 967</i>			
Inactive	24,695	22,164 (17)	2,531 (26)
Moderately inactive	50,930	47,448 (36)	3,482 (35)
Moderately active	39,094	36,875 (28)	2,219 (23)
Active	25,295	23,697 (18)	1,598 (16)
<i>Alcohol intake, missing N = 2,131</i>			
Non-drinker	14,929	13,768 (11)	1,161 (12)
>0 to 3 g/d	36,189	33,433 (26)	2,756 (28)
>3 to 12 g/d	46,489	43,474 (33)	3,015 (31)
>12 to 24 g/d	23,552	22,035 (17)	1,517 (16)
>24 g/d	17,691	16,453 (13)	1,238 (13)
<i>Age at menarche, missing N = 2,959</i>			
≤13 years	83,712	78,157 (61)	5,555 (57)
>13 years	54,310	50,183 (39)	4,127 (43)
<i>Use of contraceptive pills, missing N = 1,449</i>			
Never	51,034	46,653 (36)	4,381 (45)
Former	82,965	77,730 (60)	5,235 (54)
Current	5,533	5,388 (4)	145 (1)

Table 1. Selected participant characteristics according to NSAID use at baseline (Continued)

Characteristics	Total, N	Regular use of NSAIDs, n (%) <sup>1</sup>	
		No	Yes
Use of MHT <sup>2</sup> , missing N = 3,028			
Never	57,390	53,012 (56)	4,378 (51)
Former	13,924	12,535 (13)	1,389 (16)
Current	32,774	29,903 (31)	2,871 (33)
Full-term pregnancy, missing N = 4,554			
No	15,819	14,727 (12)	1,092 (11)
Yes	120,608	112,147 (88)	8,461 (89)
Age at first full-term pregnancy <sup>3</sup> , missing N = 489			
≤24 years	66,084	60,958 (55)	5,126 (61)
>24 years	54,035	50,753 (45)	3,282 (39)
Number of full-term pregnancies <sup>3</sup> , missing N = 22			
1	23,776	22,134 (20)	1,642 (19)
≥2	96,810	89,992 (80)	6,818 (81)
Breastfeeding <sup>3</sup> , missing N = 2,489			
Never	23,247	21,769 (20)	1,478 (18)
Ever	94,872	88,138 (80)	6,734 (82)

<sup>1</sup>Variations between users and non-users of NSAIDs have been assessed by  $\chi^2$  test. All the *p*-values were <0.0001, except for family history of breast cancer (*p* = 0.12), full-term pregnancy (*p* = 0.60) and number of full-term pregnancies (*p* = 0.46).

<sup>2</sup>Among postmenopausal women.

<sup>3</sup>Among parous women.

invasive breast cancer subtypes examined, although the test for interaction only reached statistical significance among invasive ER- cases (data not shown). Figure 1 shows the results of the joint analysis of the effects of regular NSAID use and ever MHT use on invasive breast cancer risk among postmenopausal women. Ever MHT users had a significant increased risk of invasive breast cancer compared to non-MHT/non-NSAID users [ $HR_{NO\ NSAID-MHT} = 1.38$  (1.30–1.47)]. However, the risk estimate was significantly attenuated among women who also regularly used NSAIDs [ $HR_{NSAID-MHT} = 1.19$  (1.03–1.37)]. For ER- cases, no significant increased risk was observed among women who had used both MHT and NSAIDs [ $HR_{NSAID-MHT} = 1.07$  (0.73–1.58)]. The association between regular NSAID use and breast cancer risk (all subtypes) did not vary significantly between former and current MHT users and between different types and durations of MHT used at baseline (data not shown).

## Discussion

In this prospective cohort study there was no association between regular NSAID use and breast cancer risk (overall and by tumor subtypes). However, regular NSAID use was associated with a lower risk of invasive breast cancer diagnosis among MHT ever users.

Of the previous prospective studies that examined the NSAID-breast cancer association, only a few found a statistically significant inverse association between NSAID use with breast cancer.<sup>5</sup> These inconsistent findings could be due to

differences in the accuracy of the exposure assessment (measured at baseline or updated during follow-up), differences in the exposure period definition (ever use, regular use, long-term use, etc.), or in the exposure subtypes (all NSAIDs, all NSAIDs but aspirin, low-dose aspirin, aspirin, ibuprofen, COX-2 inhibitors, etc.). The associations between breast cancer and NSAID use could be limited to specific breast cancer subtypes. In fact, stronger inverse associations with NSAID use have been found for ER+ and/or PR+ breast cancer in some studies.<sup>5</sup> In our study, we did not observe differences according to breast cancer subtypes but the number of cases in some subtypes was small and our power to detect differences may be have limited. The NSAID-breast cancer association could also be limited to certain sub-population. In particular, our results suggest that the reported associations may depend on MHT use.

Estrogen-progestogen MHT use is a well-established risk factor for breast cancer<sup>8</sup> and any evidence of interaction between MHT and NSAID use in breast cancer risk would be of importance for public health. To our knowledge, only eight studies have examined the modifying effect of MHT use on the association between regular NSAID use and breast cancer risk. Seven of these studies did not observe any significant effect modification<sup>9–15</sup> and only one study reported an inverse association between NSAID use for at least 5 years and breast cancer risk among MHT users.<sup>14</sup> In the other six studies, risk estimates associated with NSAID use among MHT users were not reported. One other study showed a



Table 2. Associations between regular use of NSAIDs and the risk of breast cancer, overall, by breast cancer subtypes and stratified by MHT use

Breast cancer	Regular use of NSAIDs			
	All women (N = 140,981)	Postmenopausal women (N = 104,088)		<i>P</i> <sub>interaction</sub> <sup>3</sup>
	N exposed cases HR <sup>1</sup> (95% CI)	Never use of MHT N exposed cases HR <sup>2</sup> (95% CI)	Ever use of MHT N exposed cases HR <sup>2</sup> (95% CI)	
Overall	540	217	255	
	0.99 (0.90–1.08)	1.09 (0.95–1.26)	0.87 (0.76–0.99)	0.06
Invasive	477	194	225	
	0.96 (0.87–1.05)	1.08 (0.93–1.25)	0.84 (0.73–0.96)	0.05
<i>In situ</i>	63	23	30	
	1.27 (0.98–1.65)	1.25 (0.82–1.92)	1.18 (0.81–1.73)	0.99
Invasive ductal	351	141	168	
	0.97 (0.87–1.08)	1.09 (0.90–1.28)	0.87 (0.74–1.02)	0.16
Invasive lobular	74	24	38	
	0.98 (0.77–1.24)	0.96 (0.63–1.46)	0.86 (0.62–1.21)	0.92
Invasive ER+	307	118	150	
	0.93 (0.82–1.04)	1.02 (0.85–1.24)	0.82 (0.69–0.97)	0.19
Invasive ER–	72	34	29	
	1.05 (0.82–1.34)	1.39 (0.97–2.00)	0.77 (0.53–1.13)	0.04
Invasive PR+	181	73	83	
	0.94 (0.81–1.10)	1.11 (0.87–1.41)	0.78 (0.62–0.98)	0.09
Invasive PR–	92	33	45	
	0.91 (0.74–1.13)	0.94 (0.66–1.34)	0.80 (0.59–1.08)	0.69
Invasive ER+PR+	168	67	79	
	0.91 (0.78–1.07)	1.06 (0.82–1.36)	0.77 (0.61–0.97)	0.16
Invasive ER–PR–	44	19	19	
	0.91 (0.68–1.24)	1.14 (0.71–1.83)	0.70 (0.43–1.11)	0.23

<sup>1</sup>Hazard ratio stratified for recruitment center and age at baseline, and adjusted for BMI (continuous), educational level (primary/no schooling, technical/professional/secondary, longer education, missing), MHT use (never, former, current, missing), age at first full-term pregnancy (nulliparous, ≤24 years, >24 years, missing) and alcohol intake at recruitment (non-drinker, >0 to 3g/d, >3 to 12g/d, >12 to 24g/d, >24 to 60g/d, >60g/d, missing).

<sup>2</sup>Hazard ratio adjusted for all the above but MHT use.

<sup>3</sup>Likelihood ratio tests comparing models with and without cross-product interaction terms of NSAID × MHT use.

statistically significant positive association with regular/extra-strength aspirin use and breast cancer risk among MHT users for 10 years or more.<sup>16</sup> Nevertheless, this result should be considered cautiously because the number of exposed cases among MHT users for 10 years or more was very low (*n* = 25).

It has been suggested that NSAIDs could reduce breast cancer risk through the inhibition of the cyclooxygenase-2 (COX-2) enzymes.<sup>17</sup> Inactivating COX-2, that has been found to be overexpressed in breast cancer tissue when compared to normal tissue, may reduce the downstream synthesis of pro-inflammatory prostaglandins (PG), particularly PGE<sub>2</sub>, a potent mitogen. This reduction results in inhibition of angiogenesis, proliferation and in the promotion of apoptosis.<sup>18</sup> There is also accumulating evidence that the inhibition of

COX-2 and PGE<sub>2</sub> is associated with the suppression of estrogen synthesis *via* a decrease in aromatase activity.<sup>17</sup> Consistently, cross-sectional investigations among postmenopausal women, who were not recently exposed to exogenous hormones, suggest that current NSAID use may be associated with lower circulating estradiol levels.<sup>19</sup> However, results of a recent randomized controlled clinical trial have shown no effect of intake of 325 mg/day aspirin over 6 months on estradiol levels, among postmenopausal women who were not exposed to exogenous hormones.<sup>20</sup>

To date, the potential effect of the combination of NSAIDs and MHT on breast tissues has not been evaluated. However, MHT could modify exposure to various hepatically-derived proteins including many cytokines due to the hepatic first pass effect.<sup>21,22</sup> MHT may also reduce insulin

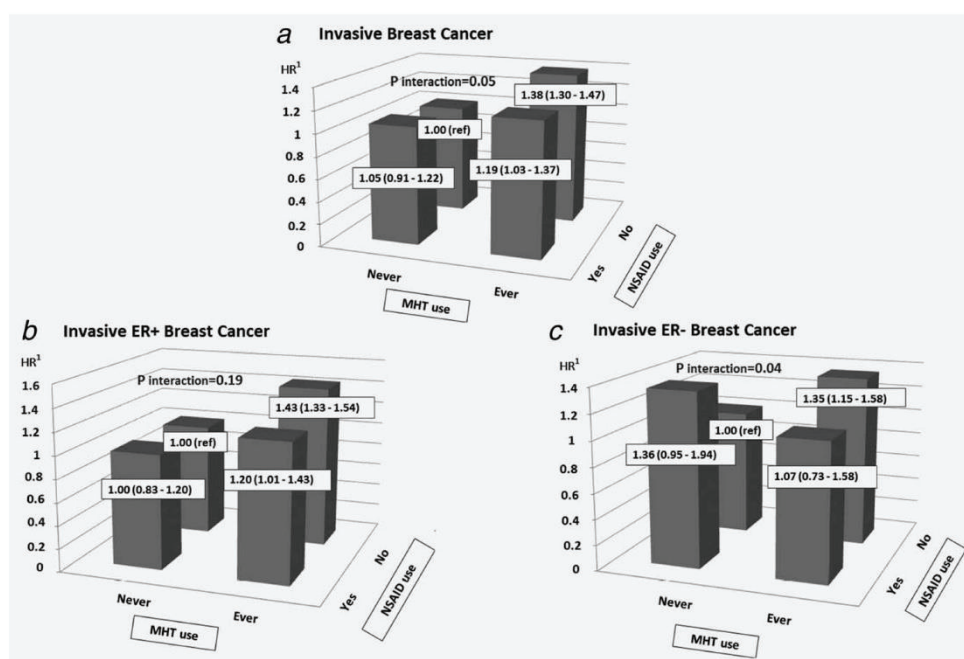


Figure 1. Associations between regular NSAID use, ever MHT use among postmenopausal women, and the risk of invasive (a), invasive ER+ (b) and invasive ER- (c) breast cancers. <sup>1</sup>Hazard ratio stratified for recruitment center and age at baseline, and adjusted for BMI (continuous), educational level (primary/no schooling, technical/professional/secondary, longer education, missing), age at first full-term pregnancy (nulliparous, <24 years, >24 years, missing) and alcohol intake at recruitment (non-drinker, >0 to 3g/d, >3 to 12g/d, >12 to 24g/d, >24 to 60g/d, >60g/d, missing).

sensitivity which would be expected to increase breast cancer risk.<sup>23</sup> NSAIDs can reduce insulin resistance<sup>24</sup> and it is therefore possible that NSAID use in MHT users reduces breast cancer risk by targeting some aspects of the insulin signaling pathways. An *in vitro* study has demonstrated that the combination of progestin and NSAIDs synergistically inhibits cell growth and induces apoptosis in the ovarian epithelium.<sup>25</sup> To our knowledge, there are no published studies examining the possible effect of the combination of exogenous estrogens and NSAIDs on the cancer development.

The main strength of our study is the use of a large European population-based prospective cohort, followed-up for a median period of 13 years. The prospective design minimizes recall bias and the large number of participants and incident breast cancer cases allowed us to examine interactions of NSAID use with breast cancer risk factors. Nevertheless, because of the number of comparisons evaluated, the effect modification by MHT may be a chance finding. The number of cases, although large overall,

remained nevertheless limited for some breast cancer subtypes. Thus, results for ER/PR status have to be considered with caution and replicated. As in many other cohorts, the main limitation of our study is the poor accuracy and heterogeneity in the assessment of NSAID use across countries. First, we used self-reported data, and NSAID exposure was assessed only once at baseline which could lead to a misclassification of longer term exposure. Second, we had no information on the type, frequency, duration and dosage of NSAID use. Thus, we were not able to characterize lifetime NSAID use to examine a dose-response relationship or to study the specific effect of each NSAID on breast cancer development. A further limitation of our study is the large number of missing data for family history of breast cancer, however, in previous studies it did not appear to modify the relationship between NSAID use and breast cancer risk.<sup>12</sup> Furthermore we performed a sensitivity analysis excluding women with family history of breast cancer in the three EPIC countries with data available and estimates were virtually unchanged.

## Conclusion

In this analysis of 140,981 women, NSAID use was associated with decreased invasive breast cancer risk in MHT ever users only. Our results require confirmation in other prospective cohorts that have collected accurate data on NSAID and MHT use, including type, dosage, frequency and duration of use.

## Acknowledgements

The authors thank all participants in the EPIC cohort for their invaluable contribution to the study. The authors also thank Bertrand Hémon (IARC) for his precious help with the EPIC database. The work reported in this article was undertaken during the stay of Agnès Fournier as Visiting Scientist at the International Agency for Research on Cancer.

## References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359–E86.
2. Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol* 2012;30:677–706.
3. Vainio H, Morgan G. Non-steroidal anti-inflammatory drugs and chemoprevention of cancer. *Ann Chir Gynaecol* 2000;89:173–6.
4. Howe LR, Subbaramaiah K, Brown AM, et al. Cyclooxygenase-2: a target for the prevention and treatment of breast cancer. *Endocr Relat Cancer* 2001;8:97–114.
5. Zhong S, Chen L, Zhang X, et al. Aspirin use and risk of breast cancer: systematic review and meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev* 2015;24:1645–55.
6. Riboli E, Hunt KJ, Slimani N, et al. European prospective investigation into cancer and nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5:1113–24.
7. Pintilie M. Analysing and interpreting competing risk data. *Stat Med* 2007;26:1360–7.
8. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans: combined estrogen-progestogen contraceptives and combined estrogen-progestogen menopause therapy. A review of human carcinogens. Lyon, France: International Agency for Research on Cancer, 2007.
9. Gierach GL, Lacey JV, Jr, Schatzkin A, et al. Nonsteroidal anti-inflammatory drugs and breast cancer risk in the National Institutes of Health-AARP Diet and Health Study. *Breast Cancer Res* 2008;10:1–12.
10. Gill JK, Maskarinec G, Wilkens LR, et al. Nonsteroidal antiinflammatory drugs and breast cancer risk: the multiethnic cohort. *Am J Epidemiol* 2007;166:1150–8.
11. Friis S, Thomassen L, Sorensen HT, et al. Nonsteroidal anti-inflammatory drug use and breast cancer risk: a Danish cohort study. *Eur J Cancer Prev* 2008;17:88–96.
12. Zhang X, Smith-Warner SA, Collins LC, et al. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and postmenopausal breast cancer incidence. *JCO* 2012;30:3468–77.
13. Egan KM, Stampfer MJ, Giovannucci E, et al. Prospective study of regular aspirin use and the risk of breast cancer. *J Natl Cancer Inst* 1996;88:988–93.
14. Harris RE, Chlebowski RT, Jackson RD, et al. Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. *Cancer Res* 2003;63:6096–101.
15. Harris RE, Kasbari S, Farrar WB. Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer. *Oncol Rep* 1999;6:71–3.
16. Ready A, Velicer CM, McTiernan A, et al. NSAID use and breast cancer risk in the VITAL cohort. *Breast Cancer Res Treat* 2008;109:533–43.
17. Diaz-Cruz ES, Shapiro CL, Brueggemeier RW. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *J Clin Endocrinol Metab* 2005;90:2563–70.
18. Yiannakopoulou E. Aspirin and NSAIDs for breast cancer chemoprevention. *Eur J Cancer Prev* 2015;24:416–21.
19. Gates MA, Tworoger SS, Eliassen AH, et al. Analgesic use and sex steroid hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2010;19:1033–41.
20. Duggan C, Wang CY, Xiao L, et al. Aspirin and serum estrogens in postmenopausal women: a randomized controlled clinical trial. *Cancer Prev Res (Phila)* 2014;7:906–12.
21. Tuck CH, Holleran S, Berglund L. Hormonal regulation of lipoprotein(a) levels: effects of estrogen replacement therapy on lipoprotein(a) and acute phase reactants in postmenopausal women. *Arterioscler Thromb Vasc Biol* 1997;17:1822–9.
22. Ahtiainen M, Pollanen E, Ronkainen PH, et al. Age and estrogen-based hormone therapy affect systemic and local IL-6 and IGF-1 pathways in women. *Age (Dordr)* 2012;34:1249–60.
23. Campagnoli C, Clavel-Chapelon F, Kaaks R, et al. Progestins and progesterone in hormone replacement therapy and the risk of breast cancer. *J Steroid Biochem Mol Biol* 2005;96:95–108.
24. Yuan M, Konstantopoulos N, Lee J, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 2001;293:1673–7.
25. Rodriguez GC, Turbov JM, Berchuck A, et al. Nonsteroidal antiinflammatory drugs and progestins synergistically enhance cell death in ovarian epithelial cells. *Am J Obstet Gynecol* 2012;206:253.e1–9.

## CHAPTER 5: ANTI-INFLAMMATORY DRUGS, ANTIPLATELET DRUGS AND BREAST CANCER RISK IN THE E3N STUDY

### 1. Objectives

The objectives of this chapter were, using data from the prospective E3N cohort, to evaluate the associations between the use of NSAIDs, antiplatelet drug and glucocorticoids with breast cancer incidence, overall and by breast cancer subtypes, risk factors, and use of other drugs.

### 2. Materials and Methods

#### Study Population and Follow-up

Postmenopausal E3N participants were eligible for inclusion if they:

- had no history of cancer (except basal cell carcinoma and *in situ* colorectal tumours) before study baseline (July 1<sup>st</sup>, 2004),
- had healthcare reimbursement during year 2004,
- answered before the beginning of follow-up for the current study to the last follow-up questionnaire sent in 2002,
- and no missing date of breast cancer diagnosis or censoring.

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

A total of 62,512 postmenopausal women, followed during a median time of 9 years, were included in the following projects (including 2,864 incident breast cancer cases diagnosed).

#### Exposure assessment

For each delivery of medicines containing the drugs of interest, we extracted data on date of purchase, molecule, number of pills per package, and dose per pill.

We defined as “recurrent” users of the drug of interest women with at least two reimbursements during any previous three-month period since January 1, 2004. For NSAIDs, drug reimbursement data were complemented by self-reports before follow-up start. Women who self-reported NSAID current use in the 2000 or 2002 questionnaires were considered



NSAID “recurrent” users from follow-up start. 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 (DDD). The DDD is the assumed average daily maintenance dose for a molecule used for its main indication in adults (241).

### **Statistical analysis**

Multivariable Cox regression models with age as the time scale and stratified by birth cohort (in 5-year categories) were used to estimate HRs for the association of exposure with breast cancer incidence, overall and by breast cancer subtypes. Exposure as well as other factors issued from the reimbursement database and covariates updated during follow-up were considered as time-varying parameters. Consequently, for a given drug, participants contributed follow-up as non-exposed until purchasing the drug for the second time in a three-month period. Cumulative duration/dose and time since last and first use were also updated during follow-up. Exposure, as well as other variables coming from the reimbursement database such as use of MHT, number of consultations and use of other drugs were, therefore, lagged by 6 months to minimize reverse causation bias due to early symptoms(242).

Educational level, recent mammogram, 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. Breastfeeding, number of consultations with the doctor during the preceding six months, smoking status, lifetime histories of comorbidities of interest and “recurrent” use of other drugs since January 1, 2004 were tested as potential confounding factors.

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, histological or stage/grade subtype, competing risk analysis was performed using the cause-specific hazards approach (240, 243). Cases with missing information on a given tumour characteristic were excluded from the corresponding analyses.

### 3. Nonsteroidal anti-inflammatory drugs and breast cancer risk

#### 3.1. Main findings

At the end of follow-up, 38,493 (62%) women had been recurrently exposed to NSAIDs. Compared to women who were occasionally or never exposed to NSAIDs, recurrent users were more often overweight or obese, had more frequent medical follow-up, exposure to other drugs (paracetamol, anti-arthritis, glucocorticoid, PPI and MHT), and comorbidities such as migraine and arthrosis. There were no other major differences.

Overall, recurrent NSAID use was not associated with breast cancer risk [HR= 1.00 (0.92 – 1.08)]. The NSAID-breast cancer associations did not differ by NSAID types, breast cancer subtypes, risk factors and comorbidities, nor by duration and dose of use. However, there was a statistically significant interaction by PPI use. Recurrent NSAID use was inversely associated with overall breast cancer risk among women who had ever recurrently used PPIs [HR =0.86 (0.74 – 0.99)] but not among never/occasional PPI users [HR = 1.07 (0.97 – 1.18);  $P_{\text{interaction}}=0.01$ ].

#### 3.2. Conclusions

In this large, prospective cohort of postmenopausal women with up to 10 years of follow-up, we observed a decreased risk of breast cancer with NSAID use only among women who previously used PPI. Our study is the first one to evaluate the modifying effect of PPI on the NSAIDs-breast cancer associations. Therefore, our finding of an interaction between NSAID and PPI use regarding the risk of breast cancer deserves replication in other settings.

#### 3.3. Paper

Status: Published.

Contribution: First author, conducted statistical analyses, wrote the first draft of the manuscript, submitted it to the journal, and replied to reviewers' comments.

RESEARCH ARTICLE

Open Access

# Use of nonsteroidal anti-inflammatory drugs and breast cancer risk in a prospective cohort of postmenopausal women



Manon Cairat<sup>1</sup>, Marie Al Rahmoun<sup>2,3</sup>, Marc J. Gunter<sup>1</sup>, Gianluca Severi<sup>2,3,4</sup>, Laure Dossus<sup>1†</sup> and Agnès Fournier<sup>2,3\*†</sup> 

## Abstract

**Background:** Although anti-inflammatory agents could theoretically have anticancer properties, results from cohort studies on nonsteroidal anti-inflammatory drugs (NSAIDs) and breast cancer (BC) risk are inconsistent.

**Methods:** We investigated the association between NSAID use and BC incidence in the French E3N prospective cohort, which includes 98,995 women born between 1925 and 1950 and insured by a health insurance plan that covers mostly teachers. Self-reported information on lifestyle and medical history has been collected biennially by questionnaires and matched with data from a drug reimbursement database covering the period 2004–2014. Women who self-reported current NSAID use in the 2000 or 2002 questionnaires or with at least two reimbursements in any previous 3-month period were defined as exposed to NSAIDs. Multivariable Cox regression models were used to estimate hazard ratios (HRs) for the association of NSAID use with BC risk.

**Results:** In the current analysis, 62,512 postmenopausal women were followed between 2004 and 2014 (9 years on average, starting at a mean age of 63 years; 2864 incident BC). In multivariable models, there was no statistically significant association between NSAID use and BC risk [HR = 1.00 (0.92–1.08), compared with non-exposed women]. The NSAID-BC associations did not differ by NSAID types, BC subtypes, risk factors, and comorbidities, nor by duration and dose of use. However, a statistically significant interaction was observed by proton pump inhibitor (PPI) drug use ( $P_{\text{interaction}} = 0.01$ ) whereby a decreased risk of BC with NSAID use was only observed among women who also used PPI before.

**Conclusion:** Only women who used NSAIDs after having used PPI had a lower risk of BC. This result is novel and requires replication in other studies.

**Keywords:** Nonsteroidal anti-inflammatory drugs, Breast cancer, Proton pump inhibitors, Postmenopausal women

\* Correspondence: Agnes.FOURNIER@gustaveroussy.fr

†Laure Dossus and Agnès Fournier are co-last authors.

<sup>2</sup>Centre de recherche en Épidémiologie et Santé des Populations (CESP),  
équipe "Exposome, Hérité, Cancer et Santé", Faculté de Médecine  
Université Paris-Saclay, UVSQ, Inserm U1018, Villejuif, France

<sup>3</sup>Gustave Roussy, Villejuif, France

Full list of author information is available at the end of the article.



© World Health Organization. 2020 The opinions expressed in this article are those of the authors and do not necessarily reflect the views of the World Health Organization, its Board of Directors, or the countries they represent. **Open Access** This article is licensed under the terms of the Creative Commons Attribution 3.0 IGO License, which permits use, sharing, and adaptation, distribution, and production in any medium or format, as long as appropriate credit is given to the World Health Organization, a link is provided to the Creative Commons license, and any changes made are indicated. The use of the World Health Organization's name, and the use of the World Health Organization's logo, shall be subject to a separate written licence agreement between the World Health Organization and the user and is not authorized as part of this CC-IGO licence. Note that the link provided below includes additional terms and conditions of the licence. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/3.0/igo/>.



## Introduction

Breast cancer is, by far, the most common cancer among women in the world. In France, an estimated 56,162 new cancer cases have been diagnosed in 2018 and 13,353 women died from the disease [1]. Inflammation is recognized as a hallmark of cancer [2], and hence, it has been postulated that nonsteroidal anti-inflammatory drugs (NSAIDs) could have anticancer properties [3]. NSAIDs, including high-dose aspirin (pills of 500 mg or more), ibuprofen, or diclofenac, are commonly used to reduce inflammation associated with conditions such as arthritis, tendonitis, and bursitis, or to alleviate pain from osteoarthritis, headaches, migraine, muscle aches, toothaches, back pain, and menstrual cramps. All NSAIDs inhibit the cyclooxygenase (COX) enzymes 1 and 2 to different extents, leading to a reduction in prostaglandin biosynthesis [4], which could hypothetically promote apoptosis and inhibit mutagenesis [5, 6]. The latest meta-analysis of case-control and cohort studies suggests a slightly protective effect of NSAIDs (especially aspirin and selective COX-2 inhibitors) against invasive breast cancer, which seems to be restricted to hormone receptor-positive tumors [7]. However, authors detected a high degree of heterogeneity among the results from the different studies (especially across cohort studies) and highlighted that studies had limited data on subtypes of breast cancer, types of NSAIDs, and NSAID dose or duration of use. The inconsistencies across studies could be explained by the heterogeneous definitions of exposure (ever use, long-term use), the NSAID subtypes studied (all NSAIDs, all NSAIDs but aspirin, aspirin, ibuprofen, COX-2 inhibitors, etc.), or the exposure assessment period (past, current). In addition, over half of the prospective studies relied on self-reported data on NSAID use, prone to exposure misclassification, while studies exploring the associations between NSAID use and breast cancer risk using medico-administrative healthcare databases [8–17] had very limited data on potential confounding factors and were not able to evaluate whether the associations differed by specific breast cancer subtypes. Furthermore, there is a lack of epidemiological studies considering the use of other drugs as potential confounders/effect modifiers even though NSAIDs are frequently used concomitantly with other drugs such as proton pump inhibitors (PPIs) [18, 19].

We therefore investigated the association between NSAID use and breast cancer incidence, overall and by breast cancer subtypes, risk factors, and use of other drugs, in the French prospective E3N (Etude Epidémiologique auprès de femmes de la Mutuelle Générale de l'Éducation Nationale) cohort in which there are both self-reported information on lifestyle, medical, and reproductive factors, and drug reimbursement data for the period 2004–2014.

## 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 all workers in the national education system, most of whom are teachers [20]. In 1990, these volunteers provided their informed consent and completed the first questionnaire on their lifestyle, as well as lifetime medical and reproductive history. Every 2–3 years thereafter, participants completed self-administered questionnaires to mostly update this information and address the occurrence of medical events. Furthermore, for each cohort member, the health insurance plan provided data on all outpatient reimbursements for health expenditure since January 1, 2004. The study was approved by the French National Commission for Data Protection and Privacy.

### Identification of breast cancer cases

Most breast cancer cases were self-reported in the questionnaires and, to a lesser extent, identified from the clinical records from the patients or their doctors or the national cause-of-death registry. Pathology reports were obtained for 95% of the incident cases identified in the entire cohort and were used to confirm the cases and to extract information on tumor characteristics such as stage, grade, hormonal receptor status, and histological type. 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.

### Study population and follow-up

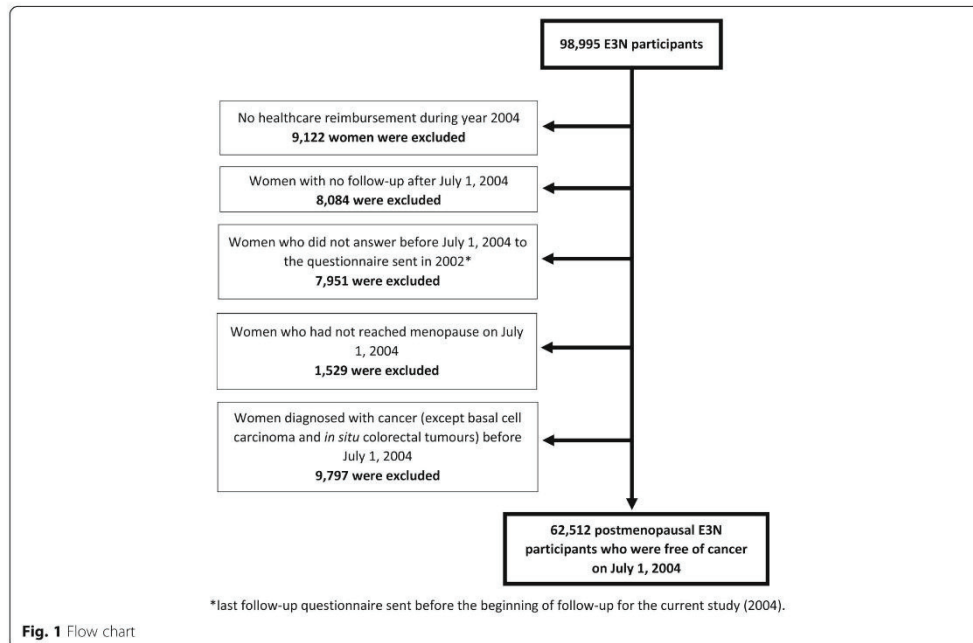
Since exposure was lagged by 6 months (see below), follow-up started on July 1, 2004. Participants contributed person-years of follow-up until the date of diagnosis of any cancer (with the exception of basal cell carcinoma and in situ colorectal tumor), the last completed questionnaire, or November 17, 2014 (date at which the last considered E3N 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).

### Exposure to NSAIDs

We considered all deliveries of NSAIDs since January 1, 2004 (as listed in Additional file 1, Table S1). Of note, aspirin used at low dose (pills of 325 mg or less) is not a NSAID but an antiplatelet drug.

The following data were extracted for each NSAID delivery: date of drug purchase, molecule, route of administration (oral, rectal, parenteral), number of pills/ampules per package, and dose per pill/ampule. E3N participants also reported whether they were currently



using anti-inflammatory drugs at least 3 three times per week in the 2000 and 2002 questionnaires.

We defined as “NSAID ever users” women with at least one NSAID reimbursement since January 1, 2004, or who self-reported NSAID current use in the 2000 or 2002 questionnaires. We defined as “NSAID recurrent users” women with at least two reimbursements during any previous 3-month period since January 1, 2004, or who self-reported NSAID current use in the 2000 or 2002 questionnaires. Ever users who were not recurrent users were considered “occasional NSAID users.”

The types of NSAIDs that were the most frequently used in the cohort were individually analyzed: high-dose aspirin ( $\geq 500$  mg), ibuprofen, ketoprofen, piroxicam, and diclofenac. Other NSAIDs were categorized according to their COX-selectivity [21]: selective COX-2 inhibitors (rofecoxib, etoricoxib, celecoxib), NSAIDs inhibiting preferentially COX-2 (meloxicam, etodolac, nimesulide), and other non-selective NSAIDs (mefenamic acid, indomethacin, tenoxicam, nabumetone, tiaprofenic acid, naproxen, fenoprofen, flurbiprofen, sulindac, phenylbutazone, aceclofenac, alminoprofen, niflumic acid, morniflumate, and floctafenine).

We also classified exposure among recurrent users according to characteristics of use: time since last use, time since first use, age at first use, cumulative duration of

use, average daily dose use, and cumulative number of defined daily doses (DDD). The DDD is the assumed average daily maintenance dose for a molecule used for its main indication in adults, available from the WHO Collaborating Centre for Drug Statistics Methodology [22]. The DDDs are based on the treatment of pain, osteoarthritis, and rheumatoid arthritis, respectively, for aspirin, selective COX-2 inhibitors, and other NSAIDs. The duration of use was calculated as the shortest length of time between the standard duration of treatment contained in the box delivered and the time until the next NSAID delivery. We assumed that the standard duration was the number of pills/ampules per package for piroxicam, tenoxicam, and meloxicam or parenteral drugs. For other NSAIDs which might be taken several times a day, we assumed that the standard duration was the number of pills per package divided by 2. The cumulative duration of use was 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 reimbursement + the standard duration of treatment contained in the last reimbursed box.

Women with NSAID deliveries between January 1, 2004, and April 1, 2004, were likely to have begun NSAID use before the availability of reimbursement data. Because the cumulative duration and dose, age at first use,



and time since first use are in that case likely to be left-truncated, they were assigned to an “unknown” category, unless they could be assigned to a “>  $x$ ” duration/dose/time since first/last use or a “<  $x$ ” age at first use category.

#### Covariates

Parameters considered as potential confounders are listed in Table 1. Some information on these parameters originated from the biennial self-administered questionnaires sent before year 2004 (educational level [1990], breastfeeding [1990 and 1992], age at menopause [up to 2004], age at menarche [1990], parity and age at first full-term pregnancies [1990 and 1992], current level of physical activity [2002], familial history of breast cancer [up to 2000], lifetime use of oral contraceptives [up to 2002]), with subsequent updates in 2005, 2008, and 2011 for most parameters that could change during follow-up (current body mass index [BMI], current smoking status, lifetime personal history of benign breast disease, current alcohol intake, self-report of a mammogram performed during the previous follow-up cycle, and lifetime histories of arthrosis, rheumatism, arthritis, polyarthritis, spondyloarthritis, or migraine). Number of consultations with the doctor during the preceding 6 months and drugs more likely to be used among NSAID users [paracetamol (Anatomical Therapeutic Chemical class (ATC, [http://www.whocc.no/atc\\_ddd\\_index/](http://www.whocc.no/atc_ddd_index/)) code: N02BE01), corticosteroids (H02AB), PPIs (A02BC), and symptomatic slow-acting drugs for osteoarthritis such as glucosamine (M01AX05), diacerein (M01AX21), oxaceprol (M01AX24), chondroitin sulfate (M01AX25), or avocado and soybean oil (M01AX26)] were identified using the drug reimbursement database which contains information starting from January 1, 2004. Lifetime use of menopausal hormone therapy (MHT) was identified using the drug reimbursement database as well as self-reported information from the questionnaires sent out before the year 2004 [23].

#### Statistical analysis

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

Exposure was considered as a time-varying parameter as well as other factors issued from the reimbursement database and covariates updated during follow-up. Consequently, participants classified as exposed to NSAIDs (either recurrent or occasional) or other drugs at the end of follow-up contributed follow-up as non-exposed until filling their first reimbursement (occasional) or their second reimbursement within a 3-month period

(recurrent). Our time-varying approach also allowed us to update cumulative duration/dose and time since last and first use during follow-up. Undiagnosed breast cancer may cause symptoms resulting in an increased use of drugs a few months prior the diagnosis. A Danish study published in 2017 suggested that a 6-month lag time would be sufficient to minimize any reverse causation bias for most drug-cancer associations, including breast cancer [24]. Thus, exposure and other variables coming from the reimbursement database such as MHT, number of consultations, and other drugs were lagged by 6 months. Changing the lag time to 0, 1 year, or 2 years did not make a difference in the estimates (data not shown).

Educational level, recent mammogram (self-report of a mammogram performed during the previous follow-up cycle), and known risk factors for breast cancer were systematically included in the multivariable models [BMI, physical activity level, lifetime history of breast benign disease, family history of breast cancer, age at menarche, age at menopause, parity, lifetime use of oral contraceptives, lifetime use of MHT, and alcohol consumption]. Other lifestyle factors [ever breastfed, number of consultations with the doctor during the preceding 6 months, and smoking status] as well as comorbidities [lifetime histories of arthritis, arthrosis, polyarthritis, rheumatism, spondyloarthritis, and migraine] and recurrent use of selected drugs since January 1, 2004 [anti-arthritis, systemic glucocorticoids, PPI, and paracetamol], were tested as potential confounding factors. The categories used are displayed in Table 1. None of the additional covariates modified the HR by more than 0.05 points, and therefore, none was included in the final multivariable models. Analyses of each type of NSAIDs were mutually adjusted for use of other NSAID types.

Alcohol consumption had > 5% missing values, which were accommodated by using a “missing category.” All other covariates had < 5% of missing values, which were replaced either with the previous non-missing questionnaire value where appropriate, 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 unchanged).

Effect modification by age, BMI, MHT use, comorbidities, and other drugs that might be associated with NSAID use (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, receptor status, and histological or stage/grade subtype, competing risk analysis was performed using the cause-specific hazards approach [25, 26]. Cases with missing information on a

**Table 1** Characteristics of participants, overall and according to NSAID exposure at the end of follow-up (E3N cohort, 2004–2014)

Characteristics at the end of follow-up <sup>1</sup>	All women (n = 62,512)	NSAID exposure at the end of follow-up	
		Never/occasional users (n = 24,019)	Recurrent users (n = 38,493)
Sociodemographic factors			
Age (years), mean (SD)	72.1 (6.7)	71.8 (6.7)	72.2 (6.2)
Educational level, N (%)			
< High school	6516 (10)	2160 (9)	4356 (11)
From high school to 4 years higher education	45,138 (72)	17,450 (73)	27,688 (72)
At least 5 years higher education	10,858 (18)	4409 (18)	6449 (17)
Lifestyle factors, N (%)			
BMI (kg/m <sup>2</sup> )			
< 18.5	2626 (4)	1284 (5)	1342 (3)
[18.5–23[	25,409 (41)	10,913 (45)	14,496 (38)
[23–25[	13,197 (21)	5020 (21)	8177 (21)
[25–30[	16,139 (26)	5359 (22)	10,780 (28)
≥ 30	5141 (8)	1443 (6)	3698 (10)
Physical activity (Met-h/week)			
≤ 34.8	15,649 (25)	5863 (24)	9786 (25)
]34.8–57.6]	15,680 (25)	5986 (25)	9694 (25)
]57.6–88.8]	15,567 (25)	6085 (25)	9482 (25)
> 88.8	15,616 (25)	6085 (25)	9531 (25)
Smoking status			
Never smoker	33,281 (53)	13,258 (55)	20,023 (52)
Current smoker	4741 (8)	1681 (7)	3060 (8)
Past smoker	24,490 (39)	9080 (38)	15,410 (40)
Alcohol intake (g/day)			
Abstainer	7832 (13)	3283 (14)	4549 (12)
≤ 5	16,796 (27)	6611 (28)	10,185 (26)
]5–10]	9357 (15)	3752 (16)	5605 (15)
]10–20]	12,053 (19)	4546 (19)	7507 (20)
> 20	12,522 (20)	4483 (19)	8039 (21)
Missing	3952 (6)	1344 (6)	2608 (7)
Reproductive factors			
Breastfeeding, N (%)			
Never	23,425 (37)	9088 (38)	14,337 (37)
Ever	34,174 (55)	13,248 (55)	20,926 (54)
Missing	4913 (8)	1683 (7)	3230 (9)
Age at menopause (years), mean (SD)	50.52 (3.73)	50.68 (3.68)	50.41 (3.76)
Age at menarche (years), N (%)			
< 13	28,078 (45)	10,413 (43)	17,665 (46)
≥ 13	34,434 (55)	13,606 (57)	20,828 (54)
Parity and age at first full-term pregnancy, N (%)			
Nulliparous	7282 (12)	3010 (13)	4272 (11)
First child before age 30 years, one or two children	31,393 (50)	11,739 (49)	19,654 (51)
First child before age 30 years, three or more children	17,373 (28)	6602 (27)	10,771 (28)

**Table 1** Characteristics of participants, overall and according to NSAID exposure at the end of follow-up (E3N cohort, 2004–2014) (Continued)

Characteristics at the end of follow-up <sup>1</sup>	All women (n = 62,512)	NSAID exposure at the end of follow-up	
		Never/occasional users (n = 24,019)	Recurrent users (n = 38,493)
First child after age 30 years	6464 (10)	2668 (11)	3796 (10)
Ever use of oral contraceptives, N (%)	38,570 (62)	14,191 (59)	24,379 (63)
Lifetime MHT use, N (%)			
Never	17,273 (28)	8076 (34)	9197 (24)
Recent	5300 (8)	1866 (8)	3434 (9)
Past	39,939 (64)	14,077 (59)	25,862 (67)
<b>Medical events and medical follow-up, N (%)</b>			
<b>Number of medical consultations/visits during the preceding 6 months</b>			
0	3277 (5)	1928 (8)	1349 (4)
[1–4[	25,413 (41)	11,387 (47)	14,026 (36)
≥ 4	33,491 (54)	10,434 (43)	23,057 (60)
Missing	331 (1)	270 (1)	61 (0)
Self-report of a mammogram performed during the previous follow-up cycle	51,097 (82)	19,194 (80)	31,903 (83)
Personal history of benign breast disease	23,268 (37)	8483 (35)	14,785 (38)
History of breast cancer in first-degree relatives	7139 (11)	2735 (11)	4404 (11)
<b>Recurrent use<sup>2</sup> of other drugs, N (%)</b>			
Systemic glucocorticoids	17,391 (28)	3551 (15)	13,840 (36)
Paracetamol	38,392 (61)	10,527 (44)	27,865 (72)
Proton pump inhibitors	30,063 (48)	6308 (26)	23,755 (62)
Anti-arthritis	26,400 (42)	6471 (27)	19,929 (52)
<b>Comorbidities, N (%)</b>			
History of arthrosis	21,475 (34)	5568 (23)	15,907 (41)
History of rheumatism	6111 (10)	2400 (10)	3711 (10)
History of arthritis	888 (1)	248 (1)	640 (2)
History of polyarthritis	2375 (4)	451 (2)	1924 (5)
History of spondyloarthritis	236 (1)	29 (1)	207 (1)
History of migraine	21,591 (35)	7073 (29)	14,518 (38)

Abbreviations: BMI body mass index, MET-h metabolic equivalent task-hour, MHT menopausal hormone therapy, NSAID nonsteroidal anti-inflammatory drug, SD standard deviation

<sup>1</sup>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

<sup>2</sup>At least two reimbursements during any previous 3-month period since January 1, 2004

considered characteristic were excluded from the corresponding analyses.

We also performed several sensitivity analyses. First, we restricted the study sample to women with incident use by excluding women who were using NSAIDs before the beginning of the follow-up. Second, we defined “recurrent users” as women with at least two reimbursements during any previous 6-month period since January 1, 2004, or who self-reported NSAID current use in the 2000 or 2002 questionnaires. Third, we did not consider self-reported information about current NSAID use in the 2000 or 2002 questionnaires to define

ever users or recurrent NSAID users. Fourth, we restricted the study sample to women who self-reported having had a mammogram performed in the previous follow-up cycle.

All tests of statistical significance were two-sided, and significance was set at the .05 level. We performed all analyses using SAS software, version 9.4 (SAS Institute Inc., Cary, NC).

## Results

The characteristics of the study population ( $n = 62,512$ ) are presented in Table 1 and Table S2 (Additional file 1).



At the end of follow-up, 56,031 women (90%) had been ever exposed to NSAIDs and 38,493 (62%) had been recurrently exposed. Among the recurrent NSAID users, 7% had been recurrently exposed to aspirin, 21% to ibuprofen, 20% to diclofenac, 17% to ketoprofen, 16% to piroxicam, 13% to selective COX-2 inhibitors, 9% to preferentially COX-2 inhibitors, and 27% to other non-selective NSAIDs during follow-up. A total of 12,897 women (21%) had been recurrently exposed to at least 2 categories of NSAIDs.

During a median follow-up time of 9 years, 2864 incident breast cancer cases were diagnosed (335 in situ and 2353 invasive). Compared to women who were occasionally or never exposed to NSAIDs, recurrent users were more often overweight or obese and had more frequent medical follow-up, exposure to other drugs (paracetamol, anti-arthritis, glucocorticoids, PPI, and MHT), and comorbidities such as migraine and arthrosis. There were no other major differences (Table 1).

The age-adjusted HR of breast cancer associated with having ever been exposed to NSAIDs, compared with having never been exposed, was 1.07 (95% CI, 0.98 to 1.18; Table 2). The multivariable HR was 1.02 (95% CI, 0.93 to 1.12). The multivariable HR of breast cancer associated with having been recurrently exposed to NSAIDs, compared with having been never or occasionally exposed, was 1.00 (95% CI, 0.92 to 1.08). No statistically significant heterogeneity across different types of NSAIDs ( $P$  homogeneity = 0.93; Table 2) and across breast cancer subtypes ( $P$  homogeneity between 0.14 and 0.98; Fig. 2) was found. Analyses according to characteristics of use yielded no statistically significant trend according to the number of DDDs, duration of use, average daily dose, age at first use, or time since first or last use ( $P_{\text{trend}}$  between 0.18 and 0.92; Table 3).

No effect modification by attained age, BMI, ever use of MHT, comorbidities, recurrent use of anti-arthritis, corticosteroids, and paracetamol was found ( $P_{\text{interaction}} > 0.14$ ) (Fig. 3). However, there was a statistically significant interaction by PPI use. Recurrent NSAID use was inversely associated with overall breast cancer risk among women who had ever recurrently used PPIs [HR = 0.86 (0.74–0.99)] but not among never/occasional PPI users [HR = 1.07 (0.97–1.18);  $P_{\text{interaction}} = 0.01$ ]. Associations between recurrent NSAID use and the risk of different breast cancer subtypes in strata of PPI use are shown in Table S3 (Additional file 1). Compared with women never exposed to NSAIDs nor PPIs, concomitant use of NSAIDs and PPIs was not associated with breast cancer risk [HR = 1.00 (0.87–1.14);  $n$  exposed cases = 559], NSAID use after PPI use was inversely associated with breast cancer risk [HR = 0.81 (0.69–0.95);  $n$  exposed cases = 365], and PPI use after NSAID use was positively associated with breast cancer risk [HR = 1.13 (1.00–

1.29);  $n$  exposed cases = 607]. Exclusive use of NSAID was not associated with breast cancer risk [HR = 1.05 (0.89–1.09);  $n$  exposed cases = 956], and exclusive use of PPI was not associated with breast cancer risk [HR = 0.99 (0.97–1.14);  $n$  exposed cases = 773] ( $P$  homogeneity = 0.03).

The results remained unchanged after restricting the study sample to women with incident use by excluding women who were using NSAIDs before the beginning of the follow-up, changing the definition of recurrent NSAID use to “at least 2 reimbursements during a 6-month period,” and when we did not consider questionnaire data for NSAID use (data not shown). Results were also the same when the study sample was restricted to women with a recent mammogram (HR = 0.97 (0.90–1.05);  $n$  exposed cases = 1264, Additional file 1, Table S4).

## Discussion

Overall, recurrent NSAID use was not associated with breast cancer risk in this large, prospective cohort of 62,512 postmenopausal women with up to 10 years of follow-up. We found no duration- or dose-response relationship or differential effect across types of NSAIDs or breast cancer subtypes. However, we found a statistically significant decreased risk of breast cancer with NSAID use among women who had been previously exposed to PPIs.

Our results are consistent with the majority of results from prospective studies, suggesting no reduced risk of breast cancer with NSAID use whether the exposure considered was any type of NSAIDs [12, 27–29], high-dose aspirin [17, 27, 30], ibuprofen [27, 31, 32], COX-2 inhibitors [27, 30], or non-aspirin NSAIDs [17, 33–38]. However, some other studies have reported a reduced risk of breast cancer with NSAIDs (any type) [16, 32, 35, 39], high-dose aspirin [32], ibuprofen [16], COX-2 inhibitors [11, 14, 16, 39], or non-aspirin NSAIDs [29, 30, 40, 41] while a few other studies indicated an increased risk of breast cancer with NSAIDs (any type) [10, 31], high-dose aspirin [10], ibuprofen [10], or COX-2 inhibitors [13, 15].

Among a dozen prospective studies with data on duration or dose of use, a few observed a dose/duration response [16, 28, 32, 40]. In our study, the NSAID-breast cancer association did not differ according to duration or dose of use.

In some previous studies, the NSAID-breast cancer associations were limited to ER+ and/or PR+ breast cancer [33, 42]. In our study, we did not observe any significant heterogeneity according to breast cancer subtypes. The indication for using NSAIDs and the concomitant use of other drugs could also be confounding factors that few studies were able to take into account. Even though we did not know the clinical indications for NSAID use, we were able to adjust or stratify the NSAID-breast cancer

**Table 2** Associations of NSAID exposure with breast cancer risk (E3N cohort, 2004–2014)

Characteristics of exposure	No. of cases	HR <sup>1</sup> (95% CI)	HR <sup>2</sup> (95% CI)
<b>Any NSAID</b>			
Never	596	1 (reference)	1 (reference)
Ever	2268	1.07 (0.98–1.18)	1.02 (0.93–1.12)
Never	596	1 (reference)	1 (reference)
Occasional	845	1.06 (0.95–1.18)	1.03 (0.92–1.15)
Recurrent	1423	1.09 (0.98–1.20)	1.01 (0.92–1.14)
Never/occasional	1441	1 (reference)	1 (reference)
Recurrent	1423	1.05 (0.97–1.13)	1.00 (0.92–1.08)
<b>Types of NSAID</b>			
<b>High-dose aspirin</b>			
Never/occasional use	2763	1 (reference)	1 (reference)
Recurrent use	101	1.06 (0.87–1.29)	1.02 (0.83–1.24)
<b>Ibuprofen</b>			
Never/occasional use	2659	1 (reference)	1 (reference)
Recurrent use	205	0.93 (0.81–1.08)	0.91 (0.79–1.05)
<b>Diclofenac</b>			
Never/occasional use	2650	1 (reference)	1 (reference)
Recurrent use	214	1.01 (0.88–1.17)	0.98 (0.85–1.14)
<b>Piroxicam</b>			
Never/occasional use	2651	1 (reference)	1 (reference)
Recurrent use	213	1.06 (0.92–1.22)	1.03 (0.90–1.19)
<b>Ketoprofen</b>			
Never/occasional use	2679	1 (reference)	1 (reference)
Recurrent use	185	1.06 (0.91–1.23)	1.02 (0.88–1.19)
<b>Selective COX-2 inhibitors</b>			
Never/occasional use	2694	1 (reference)	1 (reference)
Recurrent use	170	1.02 (0.87–1.20)	0.98 (0.84–1.15)
<b>Other NSAIDs inhibiting preferentially COX-2</b>			
Never/occasional use	2740	1 (reference)	1 (reference)
Recurrent use	124	1.04 (0.87–1.25)	1.03 (0.86–1.24)
<b>Other NSAIDs</b>			
Never/occasional use	2547	1 (reference)	1 (reference)
Recurrent use	317	1.07 (0.95–1.21)	1.04 (0.92–1.17)

Abbreviations: CI confidence interval, COX cyclooxygenase, HR hazard ratio, NSAID nonsteroidal anti-inflammatory drug

<sup>1</sup>Adjusted only for age (time scale)

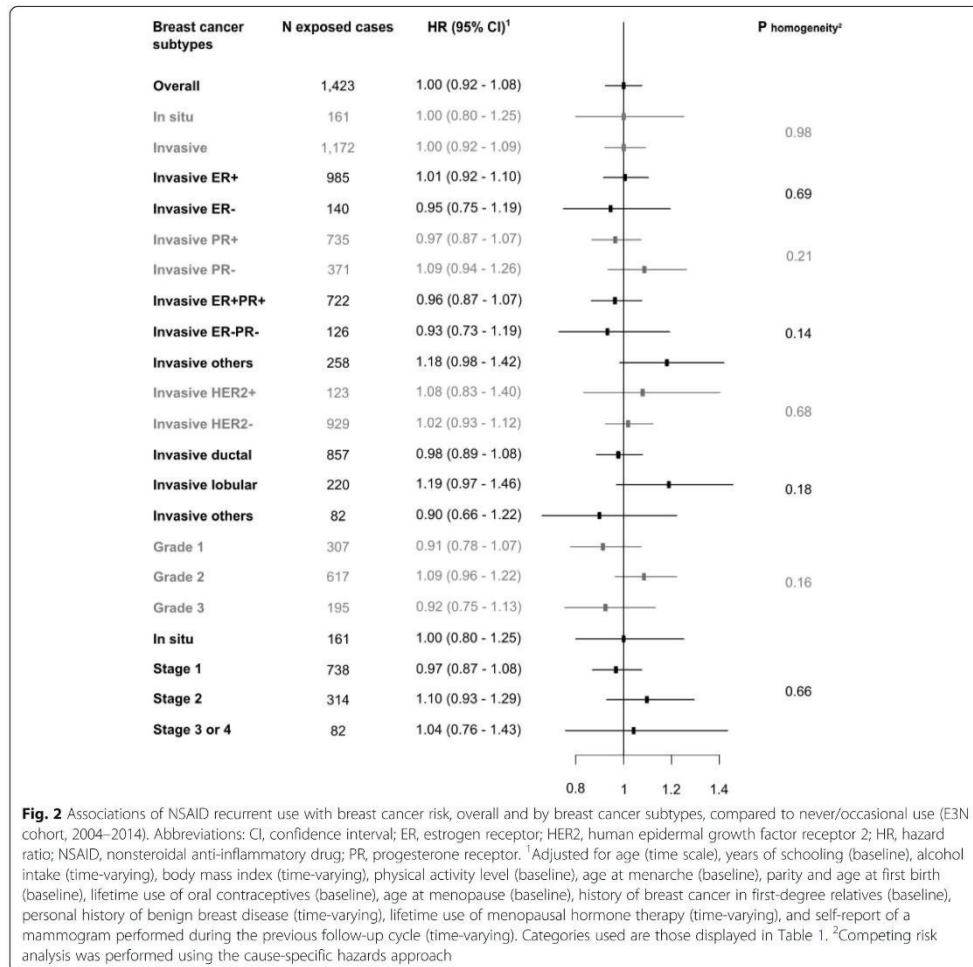
<sup>2</sup>Adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), body mass index (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at menopause (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), and other types of NSAIDs (except for "Any NSAID") (time-varying). Categories used are those displayed in Table 1

associations on several comorbidities that are indications for NSAID use (arthritis, arthrosis, polyarthritis, rheumatism, spondyloarthritis, and migraine), and on use of other drugs (PPI, glucocorticoids, paracetamol, and anti-arthritis). Women exposed to NSAIDs had more comorbidities and were more exposed to other drugs, but we found no confounding or modifying effect

of these parameters apart from the statistically significant interaction with recurrent PPI use. A lower risk of breast cancer was found when NSAID use followed PPI dispensation but not when NSAIDs were started at or before PPI dispensation.

Our study was the first one to evaluate the NSAID-breast cancer associations according to PPI use. PPIs,





inhibitors of gastric acid secretion, are approved for the treatment or prevention of a broad range of related gastric conditions such as gastroesophageal reflux, gastric and duodenal ulcers, or NSAID-induced gastrointestinal complications [18, 43]. PPIs alter (increase or decrease depending on the molecule) the absorption of other drugs by increasing the intragastric pH and interfering with the metabolism of other drugs [44, 45]. Surprisingly, several reviews concluded that few studies were published about potential drug interactions between PPIs and NSAIDs [45, 46]. These studies focused on the concomitant use of these two drugs and showed no influence of PPIs on the pharmacokinetics of NSAIDs [45, 46]. However, PPI use

might increase the risk of an imbalance in gut microbiota composition, even 8 weeks after PPI treatment [47, 48]. This change in microbiota composition has been shown to exacerbate NSAID side effects [49].

The main strengths of this study included its prospective design and the use of information from a drug reimbursement database to identify NSAID exposure, which avoids differential recall bias between cases and non-cases and minimizes exposure misclassification. Indeed, a French survey showed that among 250 French pregnant women, around 70% thought that high-dose aspirin and ibuprofen were not NSAIDs [50], and prescription analyses noted that among 184 prescriptions which

**Table 3** Associations of NSAID recurrent use with breast cancer, according to characteristics of use (E3N cohort, 2004–2014)

Characteristics of exposure	No. of cases	HR <sup>1</sup> (95% CI)	P <sub>trend</sub> <sup>2</sup>
<b>Cumulative number of DDDs</b>			<b>0.67</b>
Never/occasional use of NSAIDs	1441	1 (reference)	
≤ 200	763	1.02 (0.93–1.12)	
> 200 to ≤ 400	89	0.93 (0.75–1.16)	
> 400 to ≤ 600	22	1.28 (0.84–1.95)	
> 600	43	1.02 (0.75–1.39)	
Unknown	506	0.96 (0.91–1.13)	
<b>Cumulative duration of use, months</b>			<b>0.92</b>
Never/occasional use of NSAIDs	1441	1 (reference)	
≤ 3	516	1.00 (0.91–1.11)	
> 3 to ≤ 6	256	1.05 (0.91–1.20)	
> 6 to ≤ 12	89	1.02 (0.82–1.27)	
> 12	73	1.03 (0.81–1.31)	
Unknown	489	0.96 (0.87–1.07)	
<b>Time since first use, years</b>			<b>0.85</b>
Never/occasional use of NSAIDs	1441	1 (reference)	
≤ 4	491	1.00 (0.90–1.11)	
> 4 to ≤ 6	201	1.07 (0.92–1.25)	
> 6 to ≤ 8	138	1.04 (0.86–1.25)	
> 8	249	0.88 (0.76–1.02)	
Unknown	344	1.01 (0.90–1.14)	
<b>Age at first use, years</b>			<b>0.85</b>
Never/occasional use of NSAIDs	1441	1 (reference)	
≤ 60	461	0.97 (0.86–1.09)	
> 60 to ≤ 65	292	1.06 (0.93–1.22)	
> 65 to ≤ 70	215	1.03 (0.88–1.20)	
> 70	154	1.03 (0.85–1.24)	
Unknown	301	0.95 (0.83–1.09)	
<b>Time since last use, years</b>			<b>0.79</b>
Never/occasional use of NSAIDs	1441	1 (reference)	
Current use	845	1.01 (0.93–1.11)	
> Current use to ≤ 2	330	1.02 (0.91–1.16)	
> 2 to ≤ 5	103	0.99 (0.81–1.22)	
> 5	57	0.85 (0.65–1.11)	
Unknown	88	0.89 (0.72–1.11)	
<b>Average daily dose</b>			<b>0.18</b>
Never/occasional use of NSAIDs	1441	1 (reference)	
≤ 1 DDD	445	1.03 (0.92–1.14)	
> 1 to ≤ 1.5 DDD	587	1.02 (0.92–1.13)	

**Table 3** Associations of NSAID recurrent use with breast cancer, according to characteristics of use (E3N cohort, 2004–2014)

(Continued)

Characteristics of exposure	No. of cases	HR <sup>1</sup> (95% CI)	P <sub>trend</sub> <sup>2</sup>
> 1.5 to ≤ 2 DDDs	200	0.96 (0.83–1.12)	
> 2 DDDs	55	0.95 (0.73–1.25)	
Unknown	136	0.90 (0.76–1.08)	

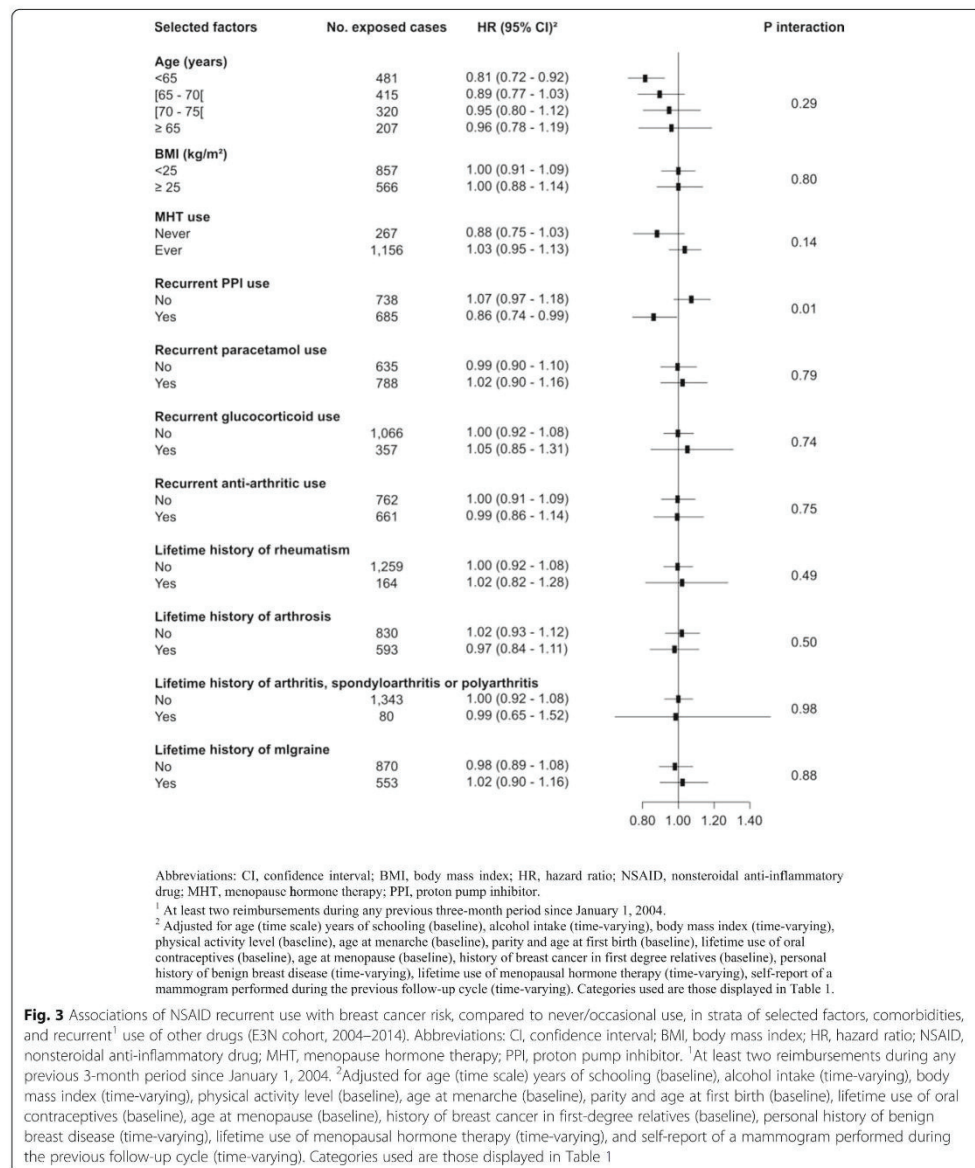
Abbreviations: CI confidence interval, DDD defined daily dose, HR hazard ratio, NSAID nonsteroidal anti-inflammatory drug

<sup>1</sup>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 use (time-varying), and self-report of a mammogram performed during the previous follow-up cycle (time-varying). Categories used are those displayed in Table 1. HRs were obtained from separate models including one characteristic of exposure at a time

<sup>2</sup>Tests for linear trends were performed among exposed women with known characteristics of exposure. The characteristics of use were considered as continuous variables

contained at least one NSAID, 98% were obtained for acute symptoms and consequently for a short duration, contributing to recall bias [51]. In addition, the detailed NSAID reimbursement data allowed us to consider precise information on NSAID exposure (including molecules, duration, dose, time since last and time since first use). In our cohort, we found that exposure to NSAIDs was associated with a decreased risk of colorectal cancer (HR = 0.67 (0.54–0.82) for ever versus never exposed women; Additional file 1, Table S5), in close agreement with estimates from meta-analyses [52, 53], which provides support for the accuracy of our assessment of NSAID exposure. However, because NSAID is mostly used sporadically (as noted in a study from the French National Health Insurance Claims [54]), our statistical power remained limited to evaluate the NSAID-breast cancer associations for a long duration of use. In addition, the period of follow-up was inadequate to examine the NSAID-breast cancer associations in different windows of susceptibility such as postpartum or perimenopause [55, 56]. Since data on NSAID reimbursements were combined with self-reported data on lifestyle, reproductive, and medical factors, we were able to take into consideration potential confounders and to examine interactions of NSAID use with breast cancer risk factors, comorbidities, and other drugs. However, although we adjusted for many potential confounders, we cannot exclude residual confounding. For example, we could not take into account the severity of rheumatoid polyarthritis or other comorbidities. In addition, a high number of comparisons were evaluated and the significant interaction with recurrent PPI use may be a chance finding.

Drug reimbursement data combined with information on lifestyle, reproductive factors, and medical events are



**Fig. 3** Associations of NSAID recurrent use with breast cancer risk, compared to never/occasional use, in strata of selected factors, comorbidities, and recurrent<sup>1</sup> use of other drugs (E3N cohort, 2004–2014). Abbreviations: CI, confidence interval; BMI, body mass index; HR, hazard ratio; NSAID, nonsteroidal anti-inflammatory drug; MHT, menopause hormone therapy; PPI, proton pump inhibitor. <sup>1</sup>At least two reimbursements during any previous 3-month period since January 1, 2004. <sup>2</sup>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), and self-report of a mammogram performed during the previous follow-up cycle (time-varying). Categories used are those displayed in Table 1

a great asset, but these data have some limitations. Exposure assessment based on reimbursements rather than intake leads to an obvious misclassification of NSAID use since, in France, NSAIDs can also be obtained over-the-counter. Indeed, several surveys in France noted that

between 70 and 80% interviewed persons had already used NSAIDs by self-medication [57, 58]. Furthermore, we had no data regarding the compliance/adherence to the purchased treatment. However, we assumed that women who need NSAIDs chronically will go to the



doctor to get reimbursed. We defined recurrent users as women with at least 2 reimbursements during any 3-month period which would suggest that they took the drug. Self-reported data on NSAID use, which therefore included NSAIDs purchased over-the-counter and captured only taken medications, were only available before year 2004 and consequently were used to assess prevalent use of NSAIDs.

## Conclusion

To conclude, in this large, prospective cohort of postmenopausal women with up to 10 years of follow-up, we observed a decreased risk of breast cancer with NSAID use only among women who previously used PPI. Our study is the first one to evaluate the modifying effect of PPI on the NSAID-breast cancer associations. Therefore, our finding of an interaction between NSAID and PPI use regarding the risk of breast cancer deserves replication in other settings.

## Supplementary information

**Supplementary information** accompanies this paper at <https://doi.org/10.1186/s13058-020-01343-1>.

**Additional file 1: Table S1.** ATC codes and COX-2 selectivity of NSAIDs. **Table S2.** Characteristics of participants, overall and according to NSAID exposure at the end of follow-up (E3N cohort, 2004–2014). **Table S3.** Associations of NSAID recurrent use with the risk of different breast cancer subtypes, compared with NSAID never/occasional use, in strata of PPI use (E3N cohort, 2004–2014). **Table S4.** Associations of NSAID recurrent use with breast cancer risk among women with a recent mammogram (E3N cohort, 2004–2014). **Table S5.** Associations of NSAID exposure with colorectal cancer risk (E3N cohort, 2004–2014).

## Abbreviations

BMI: Body mass index; CI: Confidence interval; COX: Cyclooxygenase; DDD: Defined daily dose; HR: Hazard ratio; MHT: Menopausal hormone therapy; NSAID: Nonsteroidal anti-inflammatory drug; PPI: Proton pump inhibitor

## Acknowledgements

The research was carried out using data from the Inserm (French National Institutes for Health and Medical Research) E3N cohort, which was established and maintained with the support of the Mutuelle Générale de l'Éducation Nationale (MGÉN), Gustave Roussy, and the French League against Cancer (LNCC). 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 Chait, Ghizlane Esselma, Marie Fangon, Pascale Gerboun-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 paper was performed during Agnès Fournier's term as a Visiting Scientist at the International Agency for Research on Cancer.

## 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

MC analyzed the data. MC, LD, and AF were responsible for drafting the manuscript. MA, MJG, and GS provided advice on the analysis and interpretation of the results. All authors read and approved the final manuscript.

## Funding

Ligue Contre le Cancer (support for the E3N cohort and PhD fellowship to Manon Cairat).

## Availability of data and materials

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

## Ethics approval and consent to participate

The study was approved by the French National Commission for Data Protection and Privacy, and all participants gave written informed consent for data collection and storage, as well as individual follow-up.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Author details

<sup>1</sup>Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France. <sup>2</sup>Centre de recherche en Épidémiologie et Santé des Populations (CESP), équipe "Exposome, Hérité, Cancer et Santé", Faculté de Médecine Université Paris-Saclay, UVSQ, Inserm U1018, Villejuif, France. <sup>3</sup>Gustave Roussy, Villejuif, France. <sup>4</sup>Department of Statistics, Computer Science and Applications "G. Parenti" (DISIA), University of Florence, Florence, Italy.

Received: 11 June 2020 Accepted: 16 September 2020

Published online: 31 October 2020

## References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–42.
- Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009;30(7):1073–81.
- Harris RE, Beebe-Donk J, Doss H, Burr DD. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review). *Oncol Rep*. 2005;13(4):559–83.
- Steinmeyer J. Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. *Arthritis Res*. 2000;2(5):379–85.
- Yiannakopoulou E. Aspirin and NSAIDs for breast cancer chemoprevention. *Eur J Cancer Prev*. 2015;24(5):416–21.
- Ornelas A, Zacharias-Millward N, Menter DG, Davis JS, Lichtenberger L, Hawke D, et al. Beyond COX-1: the effects of aspirin on platelet biology and potential mechanisms of chemoprevention. *Cancer Metastasis Rev*. 2017;36(2):289–303.
- de Pedro M, Baeza S, Escudero MT, Dierssen-Sotos T, Gomez-Acebo I, Pollán M, et al. Effect of COX-2 inhibitors and other non-steroidal inflammatory drugs on breast cancer risk: a meta-analysis. *Breast Cancer Res Treat*. 2015;149(2):525–36.
- Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA. Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage. *Br J Cancer*. 2000;83(1):112–20.
- Sorensen HT, Friis S, Norgaard B, Møller-Jensen L, Blot WJ, McLaughlin JK, et al. Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study. *Br J Cancer*. 2003;88(11):1687–92.
- Friis S, Thomassen L, Sorensen HT, Tjønneland A, Overvad K, Cronin-Fenton DP, et al. Nonsteroidal anti-inflammatory drug use and breast cancer risk: a Danish cohort study. *Eur J Cancer Prev*. 2008;17(2):88–96.

11. Rahme E, Ghosh J, Dasgupta K, Rajan R, Hudson M. Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer. *BMC Cancer*. 2005;5:159.
12. Meier CR, Schmitz S, Jick H. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. *Pharmacotherapy*. 2002;22(3):303–9.
13. Cronin-Fenton DP, Pedersen L, Lash TL, Friis S, Baron JA, Sørensen HT. Prescriptions for selective cyclooxygenase-2 inhibitors, non-selective non-steroidal anti-inflammatory drugs, and risk of breast cancer in a population-based case-control study. *Breast Cancer Res*. 2010;12(2):R15.
14. Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD. Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. *Breast (Edinburgh, Scotland)*. 2011; 20(1):66–70.
15. Vinogradova Y, Coupland C, Hippisley-Cox J. Exposure to cyclooxygenase-2 inhibitors and risk of cancer: nested case-control studies. *Br J Cancer*. 2011; 105(3):452–9.
16. Hung CH, Lin YC, Chang YH, Lin YC, Huang HY, Yeh WJ, et al. The effect of NSAIDs exposure on breast cancer risk in female patients with autoimmune diseases. *Eur J Cancer Prev*. 2019;28(5):428–34.
17. Garcia Rodriguez LA, Gonzalez-Perez A. Risk of breast cancer among users of aspirin and other anti-inflammatory drugs. *Br J Cancer*. 2004;91(3):525–9.
18. Lassalle M, Le Tri T, Bardou M, Biour M, Kirchgessner J, Rouby F, et al. Use of proton pump inhibitors in adults in France: a nationwide drug utilization study. *Eur J Clin Pharmacol*. 2020;76(3):449–57.
19. Lugardon S, Roussel H, Bourrel R, Sciotino V, Montastruc JL, Lapeyre-Mestre M. Patterns of non-steroidal anti-inflammatory drug use in ambulatory care. *Therapie*. 2006;61(2):109–14.
20. Clavel-Chapelon F. Cohort profile: the French E3N cohort study. *Int J Epidemiol*. 2015;44(3):801–9.
21. Fitzgerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med*. 2001;345(6):433–42.
22. WHO Collaborating Centre for Drug Statistics Methodology: DDD: definition and general considerations. [Available from: [http://www.whocc.no/ddd/definition\\_and\\_general\\_considera/](http://www.whocc.no/ddd/definition_and_general_considera/)].
23. 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(8):1260–8.
24. Pottegard A, Hallas J. New use of prescription drugs prior to a cancer diagnosis. *Pharmacoepidemiol Drug Saf*. 2017;26(2):223–7.
25. Pintilie M. Analysing and interpreting competing risk data. *Stat Med*. 2007; 26(6):1360–7.
26. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995;51(2):524–32.
27. 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(1):52.
28. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, et al. Nonsteroidal anti-inflammatory drug use and breast cancer risk by stage and hormone receptor status. *J Natl Cancer Inst*. 2005; 97(11):805–12.
29. Gill JK, Maskarinec C, Wilkens LR, Pike MC, Henderson BE, Kolonel LN. Nonsteroidal antiinflammatory drugs and breast cancer risk: the multiethnic cohort. *Am J Epidemiol*. 2007;166(10):1150–8.
30. 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.
31. Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, et al. Aspirin and other nonsteroidal anti-inflammatory drugs and breast cancer incidence in a large U.S. cohort. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(1):261–4.
32. 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(18):6096–101.
33. Gierach GL, Lacey JV Jr, Schatzkin A, Leitzmann MF, Richesson D, Hollenbeck AR, et al. Nonsteroidal anti-inflammatory drugs and breast cancer risk in the National Institutes of Health-AARP Diet and Health Study. *Breast Cancer Res*. 2008;10(2):R38.
34. Bardia A, Keenan TE, Ebbert JO, Lazovich D, Wang AH, Vierkant RA, et al. Personalizing aspirin use for targeted breast cancer chemoprevention in postmenopausal women. *Mayo Clin Proc*. 2016;91(1):71–80.
35. Egan KM, Stampfer MJ, Giovannucci E, Rosner BA, Colditz GA. Prospective study of regular aspirin use and the risk of breast cancer. *J Natl Cancer Inst*. 1996;88(14):988–93.
36. Johnson TW, Anderson KE, Lazovich D, Folsom AR. Association of aspirin and nonsteroidal anti-inflammatory drug use with breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2002;11(12):1586–91.
37. Eliassen AH, Chen WY, Spiegelman D, Willett WC, Hunter DJ, Hankinson SE. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. *Arch Intern Med*. 2009;169(2):115–21 discussion 21.
38. Brasky TM, Liu J, White E, Peters U, Potter JD, Walter RB, et al. Non-steroidal anti-inflammatory drugs and cancer risk in women: results from the Women's Health Initiative. *Int J Cancer*. 2014;135(8):1869–83.
39. Harris RE, Kasbari S, Farrar WB. Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer. *Oncol Rep*. 1999;6(1):71–3.
40. Bosco JL, Palmer JR, Boggs DA, Hatch EE, Rosenberg L. Regular aspirin use and breast cancer risk in US Black women. *Cancer Causes Control*. 2011; 22(11):1553–61.
41. Gallicchio L, Visvanathan K, Burke A, Hoffman SC, Helzlsouer KJ. Nonsteroidal anti-inflammatory drugs and the risk of developing breast cancer in a population-based prospective cohort study in Washington County, MD. *Int J Cancer*. 2007;121(1):211–5.
42. Bardia A, Olson JE, Vachon CM, Lazovich D, Vierkant RA, Wang AH, et al. Effect of aspirin and other NSAIDs on postmenopausal breast cancer incidence by hormone receptor status: results from a prospective cohort study. *Breast Cancer Res Treat*. 2011;126(1):149–55.
43. Savarino V, Marabotto E, Zentilin P, Furnari M, Bodini G, De Maria C, et al. The appropriate use of proton-pump inhibitors. *Minerva Med*. 2018;109(5): 386–99.
44. Euseibi LH, Rabitti S, Artesiani ML, Gelli D, Montagnani M, Zagari RM, et al. Proton pump inhibitors: risks of long-term use. *J Gastroenterol Hepatol*. 2017;32(7):1295–302.
45. Wedemeyer R-S, Blume H. Pharmacokinetic drug interaction profiles of proton pump inhibitors: an update. *Drug Saf*. 2014;37(4):201–11.
46. Lattuca B, Khoulery Z, Maldas G, Davy JM, Leclercq F. Drug interactions between non-steroidal anti-inflammatory drugs and cardiovascular treatments (except anti-agregant therapy). *Antiinflamm Antiallergy Agents Med Chem*. 2013;12(1):36–46.
47. Hojo M, Asahara T, Nagahara A, Takeda T, Matsumoto K, Ueyama H, et al. Gut microbiota composition before and after use of proton pump inhibitors. *Dig Dis Sci*. 2018;63(11):2940–9.
48. Bruno G, Zaccari P, Rocco G, Scalese G, Panetta C, Porowska B, et al. Proton pump inhibitors and dysbiosis: current knowledge and aspects to be clarified. *World J Gastroenterol*. 2019;25(22):2706–19.
49. Syer SD, Blackler RW, Martin R, de Palma G, Rossi L, Verdu E, et al. NSAID enteropathy and bacteria: a complicated relationship. *J Gastroenterol*. 2015; 50(4):387–93.
50. Damase-Michel C, Christaud J, Berrebi A, Lacroix I, Montastruc JL. What do pregnant women know about non-steroidal anti-inflammatory drugs? *Pharmacoepidemiol Drug Saf*. 2009;18(11):1034–8.
51. Grandin M. Les anti-inflammatoires non stéroïdiens, utilisation et conseils dans la pratique officinale quotidienne. Document étayé par une analyse d'ordonnances d'une pharmacie rurale. In: Angers. Tplddéddepu, editor.
52. Tomić T, Dominguez-López S, Barrios-Rodríguez R. Non-aspirin non-steroidal anti-inflammatory drugs in prevention of colorectal cancer in people aged 40 or older: a systematic review and meta-analysis. *Cancer Epidemiol*. 2019; 58:52–62.
53. Veettil SK, Jinatongthai P, Nathisuwan S, Teerawattanapong N, Ching SM, Lim KG, et al. Efficacy and safety of chemopreventive agents on colorectal cancer incidence and mortality: systematic review and network meta-analysis. *Clin Epidemiol*. 2018;10:1433–45.
54. Moore N, Diris H, Martin K, Viale R, Fourrier A, Moride Y, et al. NSAID use profiles derived from reimbursement data in France. *Therapie*. 2004;59(5): 541–6.
55. Martinson HA, Lyons TR, Giles ED, Borges VF, Schedin P. Developmental windows of breast cancer risk provide opportunities for targeted chemoprevention. *Exp Cell Res*. 2013;319(11):1671–8.



56. O'Brien J, Hansen K, Barkan D, Green J, Schedin P, O'Brien J, et al. Non-steroidal anti-inflammatory drugs target the pro-tumorigenic extracellular matrix of the postpartum mammary gland. *Int J Dev Biol*. 2011;55(7–9):745–55.
57. Sivry P. Anti-Inflammatoires non stéroïdiens consommés en automédication : évaluation du niveau de connaissance de 334 patients de cabinets de médecine générale des Alpes-Maritimes. *Médecine humaine et pathologie*. 2014. dumas-01219849.
58. Ghali M. Représentation des patients à propos des AINS oraux en vente libre. Thèse de Doctorat de Médecine. 2017.

#### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)



## 4. Antiplatelet drugs and breast cancer risk

### 4.1. Main findings

At the end of follow-up, 10,557 women (17%) had been exposed to low-dose aspirin and 2,130 (3%) to clopidogrel. Compared to non-users, antiplatelet 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.

Overall, compared with never/occasional use, recurrent use of low-dose aspirin was not associated with breast cancer risk [HR= 0.99 (0.87 – 1.13)]. However, a transient increase in breast cancer risk was observed during the 3<sup>rd</sup> year of use [HR<sub>2-<3 years of use</sub>= [1.49 (1.08 – 2.07)], followed by a decrease in risk [HR<sub>4+ years of use</sub>=0.72 (0.52 – 0.99)]. The low-dose aspirin-breast cancer association differed according to history of venous thromboembolism ( $P_{\text{homogeneity}}=0.01$ ) suggesting a decreased breast cancer risk among women with a history of venous thromboembolism [HR= 0.62 (0.41 – 0.93)] but not among women without such a history [HR = 1.05 (0.92 – 1.21)]. The low dose aspirin-breast cancer associations did not differ by breast cancer subtypes, risk factors, other comorbidities or use of selected drugs.

Compared with never/occasional use, recurrent use of clopidogrel was associated with an increased breast cancer risk [HR=1.30 (1.02 – 1.68)], restricted to ER- breast cancer [HR<sub>ER+</sub>=1.14 (0.83 – 1.57), HR<sub>ER-</sub>= 3.07 (1.64 – 5.76),  $P_{\text{homogeneity}}=0.01$ ]. A statistically significant interaction by MHT use was found suggesting an increased 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 clopidogrel-breast cancer associations did not differ by breast cancer subtypes, risk factors, other comorbidities or use of selected drugs.

### 4.2. Conclusions

In this cohort of French postmenopausal women, a transient increase in breast cancer risk was observed during the 3<sup>rd</sup> year of low-dose aspirin use followed by a decrease in risk. Clopidogrel use was associated with increased breast cancer risk that seemed restricted to ER- tumours, with no clear trend according to duration of use.

### 4.3. Paper

**Status:** Accepted.

**Contribution:** First author, conducted statistical analyses, wrote the first draft of the manuscript, submitted it to the journal, and replied to reviewers' comments.

Antiplatelet drugs and breast cancer risk

#### **Antiplatelet drug use and breast cancer risk in a prospective cohort of postmenopausal women.**

Manon Cairat<sup>1</sup>, Marie Al Rahmoun<sup>2,3</sup>, Marc J. Gunter, PhD<sup>1</sup>, Gianluca Severi, PhD<sup>3,4</sup>, Laure Dossus, PhD<sup>1,\*</sup>, Agnès Fournier, PhD<sup>2,3,\*</sup>

##### **Author details**

<sup>1</sup>Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France.

<sup>2</sup>Centre de recherche en Épidémiologie et Santé des Populations (CESP), équipe "Exposome, Hérité, Cancer et Santé", Faculté de Médecine Université Paris-Saclay, UVSQ, Inserm U1018, Villejuif, France.

<sup>3</sup>Gustave Roussy, Villejuif, France.

<sup>4</sup>Department of Statistics, Computer Science and Applications "G. Parenti" (DISIA), University of Florence, Florence, Italy.

\*The two authors are co-last authors.

##### **Corresponding Author**

Agnès Fournier, CESP-Inserm U1018, équipe "Exposome and Heredity", Gustave Roussy, 114 rue Edouard Vaillant, 94805 Villejuif Cedex; [Agnes.FOURNIER@gustaveroussy.fr](mailto:Agnes.FOURNIER@gustaveroussy.fr); +33 1 42 11 51 63.

##### **Abstract**

Epidemiological 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.

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 three-month period were considered "ever" exposed. Exposure was considered as time-varying and multivariable Cox regression models were used to estimate hazard ratios (HRs) of breast cancer.

Among 62,512 postmenopausal women followed during nine years on average, 2,864 breast cancer cases were identified. Compared with never use, a transient higher breast cancer risk was observed during the 3<sup>rd</sup> year of low-dose aspirin use [ $HR_{2-3 \text{ years of use}} = 1.49 (1.08 - 2.07)$ ], 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 [ $HR_{ER+} = 1.14 (0.83 - 1.57)$ ,  $HR_{ER-} = 3.07 (1.64 - 5.76)$ ,  $P_{\text{homogeneity}} = 0.01$ ].

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.

Antiplatelet drugs are not good pharmacological candidates for breast cancer prevention.

## Introduction

While the role of platelets in haemostasis and thrombosis has been known for many decades, platelets have more recently emerged as contributors to inflammation, tumorigenesis and metastasis <sup>1,2</sup>. 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 <sup>2</sup>. Of note, aspirin is only considered as an antiplatelet drug when it is used at low doses ( $\leq 325$  mg per tablet). At higher doses ( $\geq 500$  mg per 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 epidemiological studies <sup>3-5</sup>. 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 <sup>3,6,7</sup>. In addition, there was publication bias as well as significant heterogeneity of results and individual studies did not systematically report results on duration of aspirin use and breast cancer subtypes. More epidemiological studies are thus needed with more accurate assessment of exposure to aspirin, that should in particular distinguish between low-dose and high-dose formulations, because low-dose and high-dose aspirin have different pharmacological properties and might therefore influence differently breast cancer risk. We recently showed that the use of high-dose aspirin was not associated with breast cancer risk in the E3N cohort <sup>8</sup>. The potential impact of other antiplatelet drugs such as clopidogrel on breast cancer risk has never been assessed in epidemiological 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 <sup>9-11</sup>.

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 <sup>12</sup>. In 1990, volunteers provided their informed consent and completed the baseline questionnaire. Every two or three 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 since January 1, 2004. The study was approved by the French National Commission for Data Protection and Privacy.

### ***Study population and Follow-up***

Follow-up started on July 1<sup>st</sup>, 2004 and ended at the date of diagnosis of any cancer (with the exception of basal cell carcinoma and *in situ* colorectal tumour), latest completed questionnaire, or November 17<sup>th</sup>, 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<sup>st</sup>, 2004 (Figure 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 tumour characteristics such as hormonal receptor status, histological 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 pharmacological mechanisms<sup>13</sup>, clopidogrel and low-dose aspirin were analysed separately. For each delivery 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 three-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 (DDD). The DDD is the assumed average daily maintenance dose for a drug used for its main indication in adults<sup>14</sup>. Here, we assumed that the DDD was equal to 1 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 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 six months and “ever” use (defined as at least two reimbursements of the drug of interest during any previous three-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), beta-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<sup>15</sup>. 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 diseases (angina pectoris or myocardial infarction), stroke, cardiac dysrhythmia, venous thromboembolisms (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 five-year categories) and with age as the time scale, were used to estimate hazard ratios (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 non-exposed until purchasing

4

the drug for the second time in a three-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 <sup>16</sup>.

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 six months, smoking status, lifetime histories of comorbidities (cardiovascular disease risk factors, coronary heart diseases, 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 points 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 non missing 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, histological or stage/grade subtype, competing risk analysis was performed using the cause-specific hazards approach <sup>17,18</sup>. Cases with missing information on a given tumour characteristic were excluded from the corresponding analyses.

In sensitivity analyses, we changed the six-month lag to a two-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 .05 level. We performed all analyses using SAS software, version 9.4 (SAS Institute Inc, Cary, NC).

## Results

During a median follow-up time of nine 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 three-month period was 3.8 years for low-dose aspirin and 4.0 years for clopidogrel. Compared to non-users, antiplatelet 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% CI 0.89 – 1.14). The multivariable HR was 0.99 (95% CI 0.87 – 1.13) (Figure 2). No statistically significant heterogeneity across breast cancer subtypes was found ( $P_{\text{homogeneity}} \geq 0.12$ , Figure 2). Analyses according to characteristics of use are presented in Table 2. Compared to never use, a higher breast cancer risk was found during the third year of cumulative use [ $\text{HR}_{2-3 \text{ years of use}} = 1.49$  (1.08 – 2.07)] or when the cumulative number of DDDs was between 700 and 1,200 [ $\text{HR}_{700-1200 \text{ DDDs}} = 1.38$  (1.02 – 1.87)], while a lower breast cancer risk was found in the highest categories of cumulative number of DDDs [ $>1,700$  DDDs:  $\text{HR} = 0.64$  (0.45 – 0.92)], cumulative duration of use [ $>4$  years:  $\text{HR} = 0.72$  (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 [ $\text{HR} = 0.62$  (0.41 – 0.93),  $n$  exposed cases= 29] but not among women without such a history [ $\text{HR} = 1.05$  (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 and 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) (Figure 3). No statistically significant heterogeneity across breast cancer subtypes was found

6



( $P_{\text{homogeneity}} \geq 0.17$ ; Figure 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);  $P_{\text{homogeneity}} = 0.01$ ; Figure 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 and 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 Figures S1 and S2), or applied a lag-time of two years (Online Supplementary Figure S3 and Figure 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 3<sup>rd</sup> year of low-dose aspirin use followed by a lower risk. Clopidogrel use was associated with higher breast cancer risk that seemed restricted to ER- tumours, 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 <sup>6,7</sup>. 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 <sup>19-26</sup>. Three of them noted a lower breast cancer risk with long-term exposure <sup>19-21</sup> while four suggested no statistically significant associations between low-dose aspirin and postmenopausal breast cancer risk <sup>22-25</sup>, and one reported an increased breast cancer risk in women with at least 6 months of low-dose aspirin prescriptions <sup>26</sup>. In a randomized controlled trial published in 2013 (Women's Health Study: participants aged  $\geq 45$  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 to placebo [HR= 1.02 (0.89 – 1.18), n exposed cases= 385] <sup>4</sup>. 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 aged  $\geq 65$  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] <sup>27</sup>. This lack of effect could be due to the fact that follow-up as well as duration of aspirin use were not long enough to expect a benefit of aspirin. Indeed, in a meta-analysis of randomized controlled trials published in 2018, among participants aged  $\geq 70$  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)] <sup>28</sup>. 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 3<sup>rd</sup> year of use [HR= 1.49 (1.08 – 2.07)], followed by a lower breast cancer risk after four years of use [HR= 0.72 (0.52 – 0.99)]. After five 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=23). Our study thus shows close estimates compared to 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, i.e. accelerate the growth of pre-existing 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 to low-dose aspirin used alone was associated with a non-significant increase in risk of any solid cancer and a statistically significant increase in cancer-related death <sup>29</sup>. Since then, three observational studies noted that clopidogrel use, alone or in combination with low-dose aspirin, might reduce the risk of several cancers <sup>30-32</sup> including colorectal, other gastro-intestinal, non-gastrointestinal, or haematological cancers <sup>32</sup>. 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 <sup>30</sup>.

Platelet inhibition could have different effects depending on the drug used, because low-dose aspirin and clopidogrel have specific targets. While low-dose aspirin inhibits cyclooxygenase-1 enzyme synthesis in platelets and consequently suppresses the production of prostaglandins and thromboxanes <sup>33</sup>, 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/b-catenin and NF- $\kappa$ B signalling and the acetylation of extra-COX proteins, which could explain why low-dose aspirin would be chemo-preventive and not clopidogrel<sup>11</sup>. 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-tumour cells<sup>34</sup>. In addition, a recent experimental study in mice reported that simultaneous injection of low-dose aspirin and clopidogrel could promote mammary tumour progression by inducing vascular mimicry<sup>35</sup>. 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 sub-analysis 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 tumours. 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 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.

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 non-cases 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. Since 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 non-exposed. However, we assumed that the long-term use of low-dose aspirin is primarily managed through prescriptions<sup>36</sup>. 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 2 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. Lastly, 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- tumours. These results are novel and need to be replicated in other settings.

## Tables

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

	Antiplatelet drug use at the end of follow-up		
Characteristics at the end of follow-up <sup>a</sup>	All women (n=62,512)	Low-dose aspirin ever users (n=10,557)	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 <sup>2</sup> ), 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 (g/d), 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)

# Antiplatelet drugs and breast cancer risk

Ever	34,174 (55)	5,684 (54)	1,171 (55)
Missing	4,913 (8)	862 (8)	177 (8)
<b>Age at menopause (years), mean (SD)</b>	50.5 (3.7)	50.3 (4.0)	50.1 (4.3)
<b>Age at menarche, years, N (%)</b>			
< 13	28,078 (45)	4,705 (45)	924 (43)
≥ 13	34,434 (55)	5,852 (55)	1,206 (57)
<b>Parity and age at first full-term pregnancy, N (%)</b>			
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)
<b>Lifetime oral contraceptive use, N (%)</b>	38,570 (62)	5,304 (50)	1,069 (50)
<b>Lifetime MHT use, N (%)</b>	45,239 (72)	7,170 (68)	1,380 (65)
<b>Self-report of a mammogram performed during the previous follow-up cycle, N (%)</b>	51,097 (82)	7,673 (73)	1,468 (69)
<b>Personal history of benign breast disease, N (%)</b>	23,268 (37)	3,751 (36)	742 (35)
<b>History of breast cancer in first-degree relatives, N (%)</b>	7,139 (11)	1,200 (11)	231 (11)
<b>Number of medical consultations/visits during the preceding 6 months, N (%)</b>			
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)
<b>Lifetime medical history, N (%)</b>			
Cardiac dysrhythmia	12,912 (21)	3,664 (35)	755 (35)
Cardiovascular disease risk factors <sup>b</sup>	41,355 (66)	8,606 (82)	1,804 (85)
Stroke	2,247 (4)	1,348 (13)	453 (21)
Coronary heart diseases <sup>c</sup>	1,913 (3)	1,300 (12)	619 (29)
Arteritis	716 (1)	358 (3)	242 (11)
Venous thromboembolism <sup>d</sup>	5,024 (8)	1,361 (13)	309 (15)
<b>“Ever” use of other drugs<sup>e</sup>, N (%)</b>			
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)

Antiplatelet drugs and breast cancer risk			
<b>Antidiabetics</b>	3,187 (5)	1,283 (12)	316 (15)
<b>Other antithrombotic agents</b>	8,163 (13)	2,340 (22)	539 (25)
<b>Low-dose aspirin</b>	10,557 (17)		1,391 (65)
<b>Clopidogrel</b>	2,130 (3)	1,374 (13)	

<sup>a</sup> 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.

<sup>b</sup> Cardiovascular disease risk factors include hypertension, diabetes or hypercholesterolemia.

<sup>c</sup> Coronary heart diseases include angina pectoris or myocardial infarction.

<sup>d</sup> Venous thromboembolisms include pulmonary embolism or deep vein thrombosis.

<sup>e</sup> "Ever" use was defined as at least two reimbursements of the drug of interest during any previous three-month period since January 1, 2004. Abbreviations: MET-H, metabolic equivalent task-hour; SD, standard deviation.



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

	Low-dose aspirin exposure		Clopidogrel exposure	
	No. cases	HR <sup>a</sup> (95% CI)	No. cases	HR <sup>a</sup> (95% CI)
<b>Cumulative number of DDDs</b>				
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)
<i>P<sub>trend</sub></i> <sup>b</sup>		0.71		0.27
<b>Cumulative duration of use (years)</b>				
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)
<i>P<sub>trend</sub></i> <sup>b</sup>		0.71		0.26
<b>Time since first use (years)</b>				
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)
<i>P<sub>trend</sub></i> <sup>b</sup>		0.77		0.90
<b>Age at first use (years)</b>				
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)
<i>P<sub>trend</sub></i> <sup>b</sup>		0.77		0.90

<sup>a</sup> 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, beta 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.

<sup>b</sup> Tests for linear trends were performed among exposed women with known characteristics of exposure. The characteristics of use were considered as continuous variables.

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

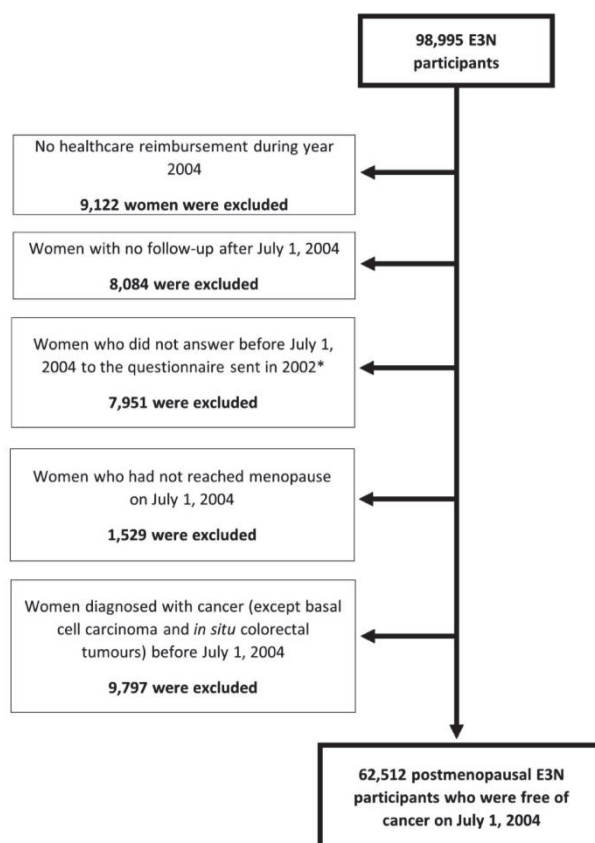
Strata	Low-dose aspirin-breast cancer associations			Clopidogrel-breast cancer associations		
	No. exposed cases	HR <sup>a</sup> (95% CI)	P interaction	No. exposed cases	HR <sup>a</sup> (95% CI)	P interaction
<b>Current age (years)<sup>b</sup></b>						
≤ 71.2 (median age)	145	0.99 (0.82 – 1.19)	0.81	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)	
<b>BMI (kg/m<sup>2</sup>)<sup>b</sup></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)	
<b>MHT use<sup>b</sup></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)	

<sup>a</sup> 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, beta 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.

<sup>b</sup> All selected factors were considered as time-varying parameters.

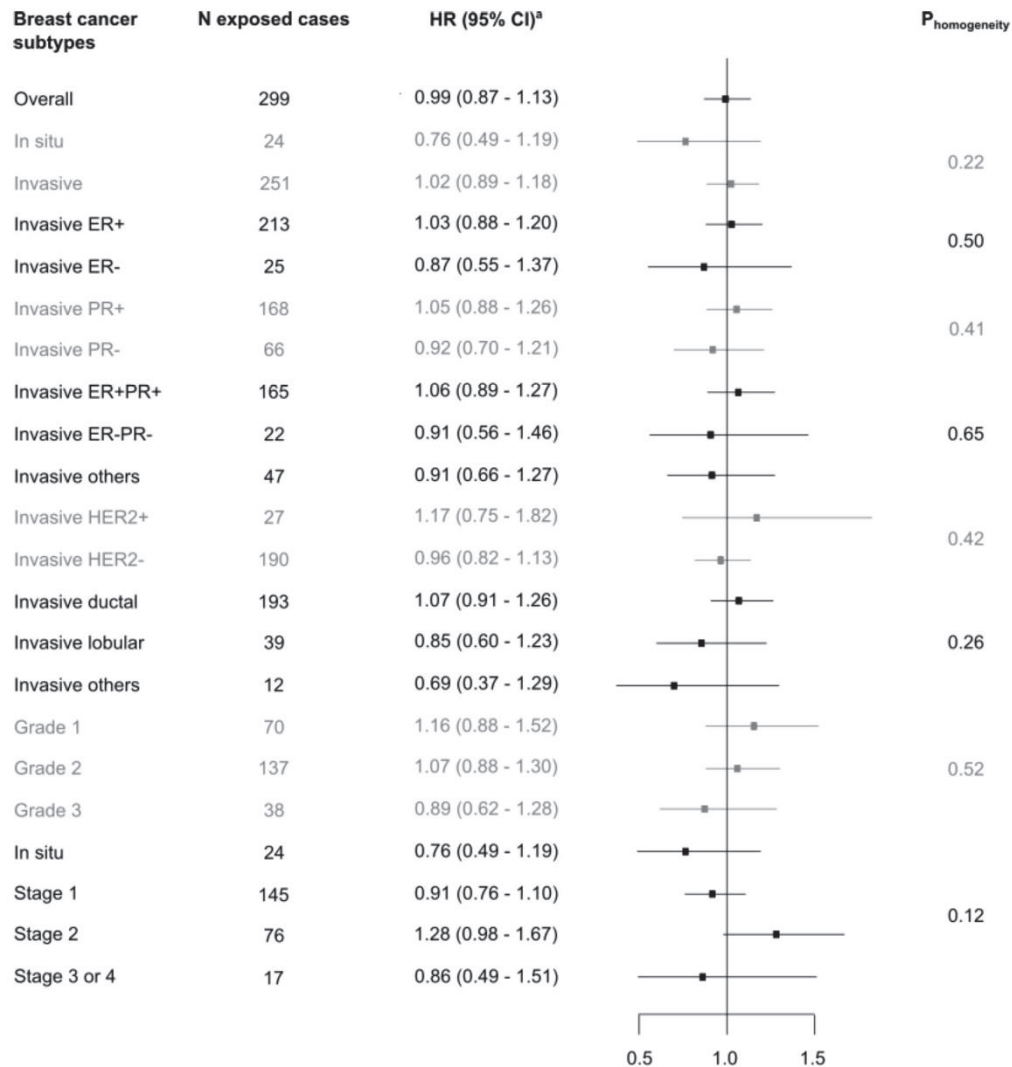
## Figures

Figure 1. Flow Chart.



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

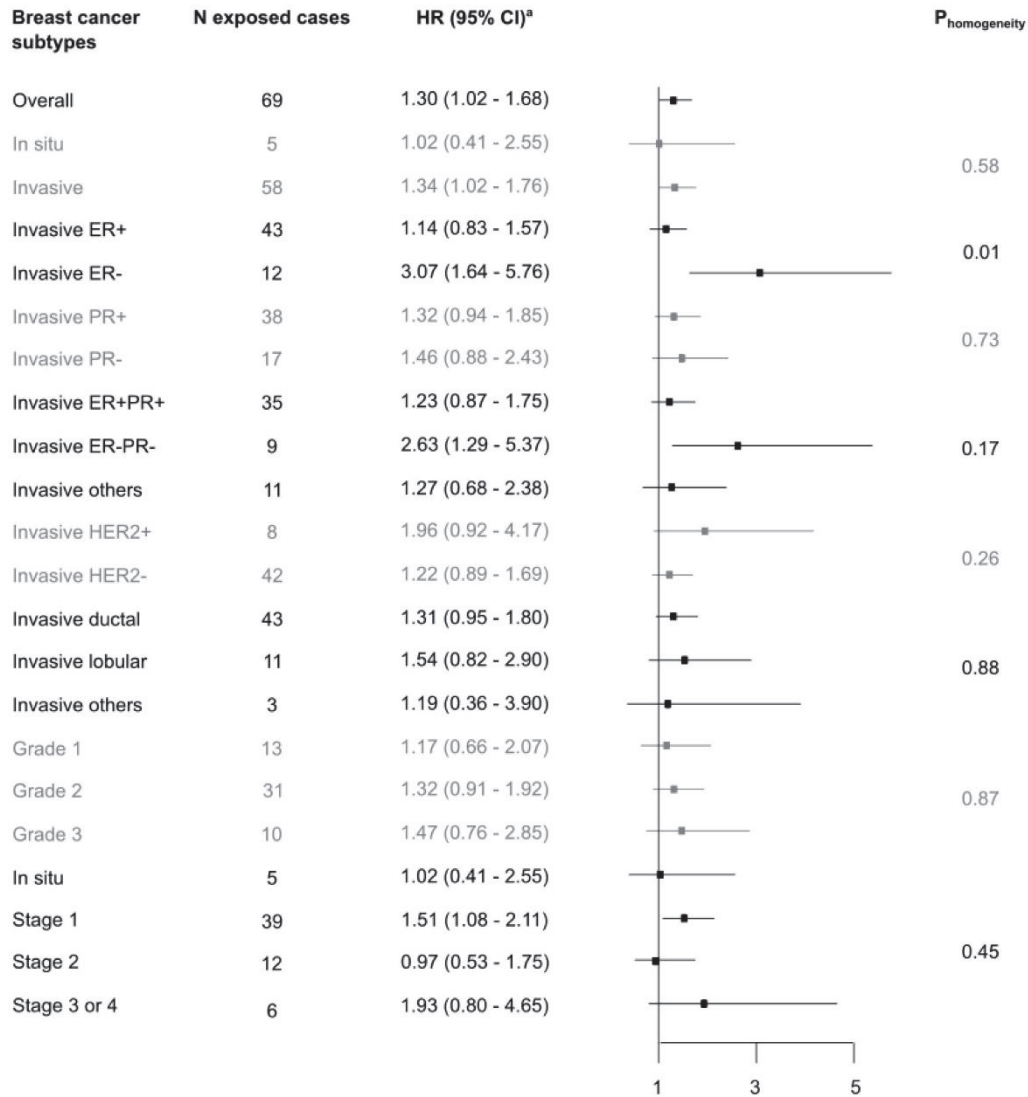
Figure 2. Associations of low-dose aspirin ever use with breast cancer risk, compared to never use, overall and by breast cancer subtypes (E3N Cohort; 2004 to 2014; n=62,512).



<sup>a</sup> 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 clopidogrel, statins, antihypertensives, diuretics, beta 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. Abbreviations: HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.



Figure 3. Associations of clopidogrel ever use with breast cancer risk, compared to never use, overall and by breast cancer subtypes (E3N Cohort; 2004 to 2014; n=62,512).



<sup>a</sup> 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, beta 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.

Abbreviations: HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

**Supplementary Tables.**

Supplementary Table S1. Associations of low-dose aspirin and clopidogrel ever use with breast cancer risk, compared to never use, according to ever use of other drugs and comorbidities (E3N Cohort; 2004 to 2014; n=62,512).

Strata	Associations between low-dose aspirin ever use and breast cancer risk			Associations between clopidogrel ever use and breast cancer risk		
	No. exposed cases	HR <sup>a</sup> (95% CI)	P <sub>interaction</sub>	No. exposed cases	HR <sup>a</sup> (95% CI)	P <sub>interaction</sub>
Ever use of other drugs <sup>b</sup>						
Proton pump inhibitors						
Never	158	1.05 (0.88 – 1.25)	0.22	31	1.51 (1.05 – 2.18)	0.23
Ever	141	0.94 (0.77 – 1.15)		38	1.19 (0.84 – 1.68)	
Antihypertensives						
Never	275	0.97 (0.85 - 1.12)	0.13	65	1.35 (1.04 – 1.74)	0.63
Ever	24	1.41 (0.81 - 2.46)		4	0.81 (0.28 – 2.36)	
Diuretics						
Never	218	0.95 (0.82 – 1.10)	0.15	42	1.13 (0.83 – 1.55)	0.08
Ever	81	1.16 (0.88 – 1.53)		27	1.77 (1.16 – 2.72)	
Beta blocking agents						
Never	178	1.03 (0.88 – 1.21)	0.61	31	1.25 (0.87 – 1.80)	0.81
Ever	121	0.94 (0.75 – 1.17)		38	1.41 (0.99 – 2.01)	
Calcium channel blockers						
Never	232	1.04 (0.90 – 1.20)	0.29	44	1.23 (0.90 – 1.68)	0.62
Ever	67	0.84 (0.62 – 1.14)		25	1.48 (0.95 – 2.30)	
Agents acting on the renin-angiotensin system						
Never	166	1.01 (0.86 – 1.20)	0.61	28	1.28 (0.88 – 1.88)	0.97
Ever	133	0.97 (0.78 – 1.19)		41	1.36 (0.97 – 1.91)	
Statins						
Never	139	1.00 (0.84 – 1.20)	0.71	16	1.17 (0.71 – 1.92)	0.70
Ever	160	1.00 (0.82 – 1.21)		53	1.39 (1.03 – 1.88)	
Antidiabetics						
Never	272	1.01 (0.88 – 1.15)	0.69	60	1.30 (1.00 – 1.71)	0.93
Ever	27	0.83 (0.51 – 1.35)		9	1.25 (0.59 – 2.63)	
Other antithrombotic agents						
Never	258	1.03 (0.90 – 1.19)	0.27	53	1.23 (0.93 – 1.63)	0.34
Ever	41	0.76 (0.52 – 1.11)		16	1.70 (0.99 – 2.93)	
Low-dose aspirin						
Never				27	1.14 (0.78 – 1.68)	0.41
Ever				42	1.55 (1.10 – 2.20)	
Clopidogrel						
Never	257	0.98 (0.85 – 1.12)	0.41			
Ever	42	1.07 (0.63 – 1.81)				
Lifetime history of comorbidities						
Cardiac dysrhythmia						
No	192	1.00 (0.86 – 1.18)	0.98	38	1.09 (0.78 – 1.53)	0.06
Yes	107	0.97 (0.77 – 1.23)		31	1.76 (1.20 – 2.59)	

# Antiplatelet drugs and breast cancer risk

Stroke						
No	261	0.97 (0.84 – 1.11)	0.25	54	1.31 (0.99 – 1.73)	0.93
Yes	38	1.37 (0.81 – 2.33)		15	1.68 (0.89 – 3.17)	
Arteritis						
No	287	0.99 (0.86 – 1.13)	0.85	60	1.27 (0.97 – 1.65)	0.83
Yes	12	1.02 (0.42 – 2.47)		9	1.54 (0.58 – 4.14)	
Cardiovascular disease risk factors <sup>c,f</sup>						
No	54	0.92 (0.69 – 1.23)	0.57	7	0.73 (0.34 – 1.57)	0.16
Yes	245	1.01 (0.87 – 1.17)		62	1.40 (1.07 – 1.83)	
Coronary heart diseases <sup>d,f</sup>						
No	261	1.00 (0.87 – 1.15)	0.57	47	1.21 (0.90 – 1.63)	0.26
Yes	38	0.91 (0.53 – 1.57)		22	1.72 (0.94 – 3.14)	
Venous thromboembolism <sup>e,f</sup>						
No	270	1.05 (0.92 – 1.21)	0.01	59	1.27 (0.97 – 1.66)	0.66
Yes	29	0.62 (0.41 – 0.93)		10	1.47 (0.75 – 2.86)	

<sup>a</sup> 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 use (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, beta blocking agents, calcium channel blockers, agents acting on the renin-angiotensin system, antidiabetics, other antithrombotic agents and proton pump inhibitors (time-varying). Low-dose aspirin and clopidogrel exposure were simultaneously adjusted for each other. Categories used are those displayed in Table 1.

<sup>b</sup> Ever use of other drugs was defined as at least two reimbursements of the drug of interest during any previous three-month period since January 1, 2004.

<sup>c</sup> Cardiovascular disease risk factors include hypertension, diabetes, or hypercholesterolemia.

<sup>d</sup> Coronary heart diseases include angina pectoris or myocardial infarction.

<sup>e</sup> Venous thromboembolisms include pulmonary embolism or deep vein thrombosis.

<sup>f</sup> Associations were similar when comorbidities were ungrouped.

Abbreviations: CI, confidence interval; HR, hazard ratio.

Table S2. Associations of  $>2\leq 3$  years of low-dose aspirin use, compared to never exposed, with breast cancer risk (E3N Cohort; 2004 to 2014).

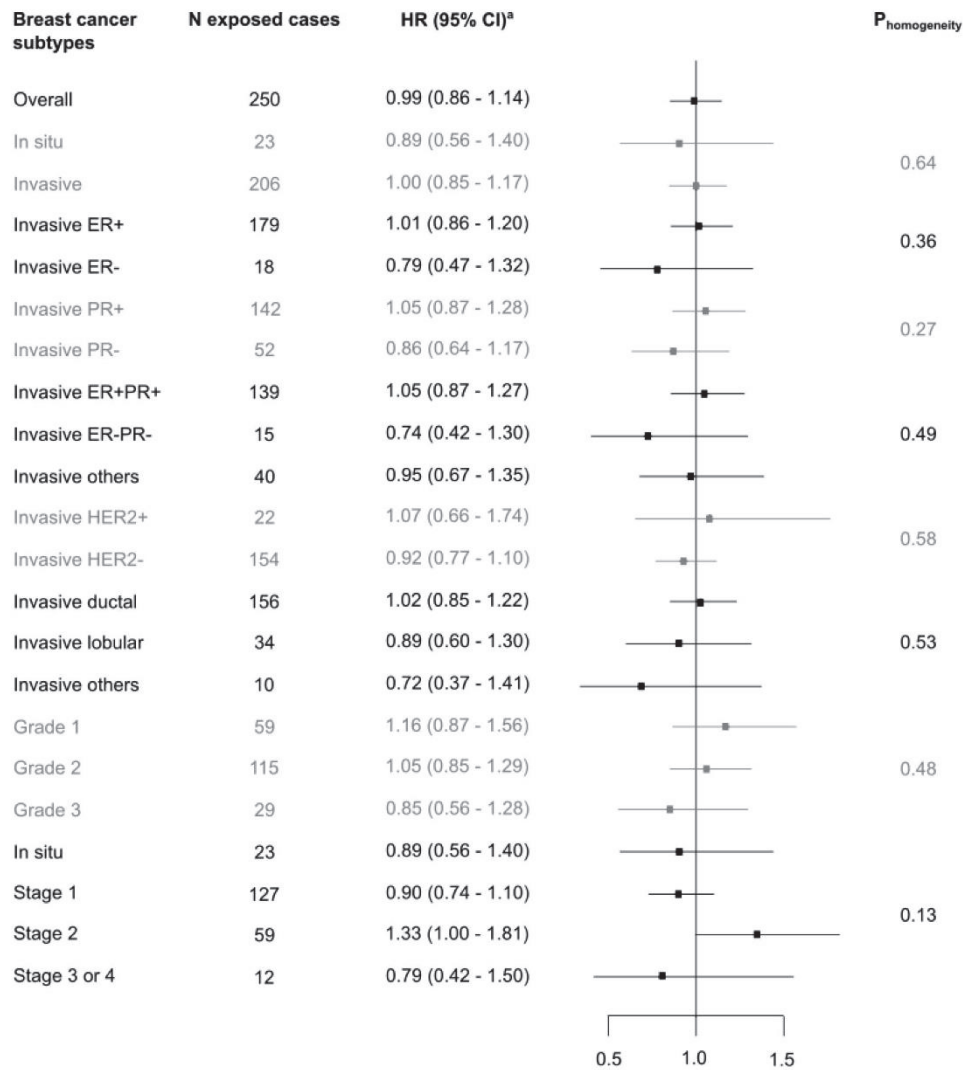
	No. cases	HR <sup>a</sup> (95% CI)
Among all women	38	1.49 (1.08 – 2.07)
Among women who had had a recent mammogram	33	1.56 (1.11 – 2.20)
Among women without history of:		
- arteritis	34	1.39 (0.98 – 1.96)
- pulmonary embolism	33	1.35 (0.95 – 1.92)
- deep vein thrombosis	34	1.53 (1.08 – 2.17)
- hypertension	21	1.92 (1.24 – 2.98)
- hypercholesterolemia	19	1.81 (1.14 – 2.88)
- diabetes	33	1.46 (1.03 – 2.08)
- stroke	34	1.53 (1.08 – 2.16)
- myocardial infarction	33	1.37 (0.96 – 1.94)
- cardiac dysrhythmia	20	1.30 (0.83 – 2.03)

<sup>a</sup> 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 use (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, beta 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.

Abbreviations: CI, confidence interval; HR, hazard ratio.

**Supplementary Figures.**

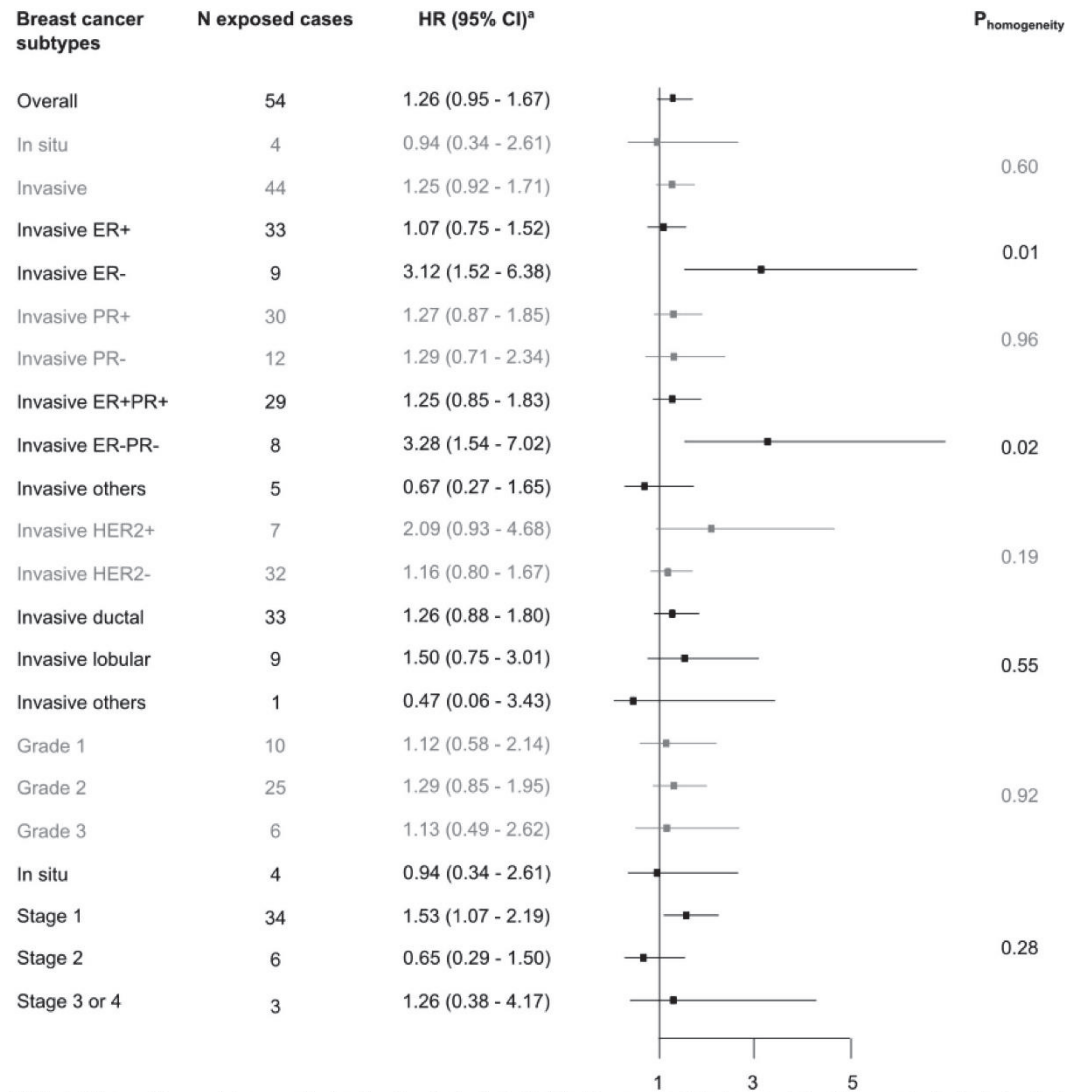
Supplementary Figure S1. Associations of low-dose aspirin ever use with breast cancer risk, compared to never use, among women with a recent mammography (E3N Cohort; 2004 to 2014; n=51,097).



<sup>a</sup> 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 clopidogrel, statins, antihypertensives, diuretics, beta 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. Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; PR, progesterone receptor.

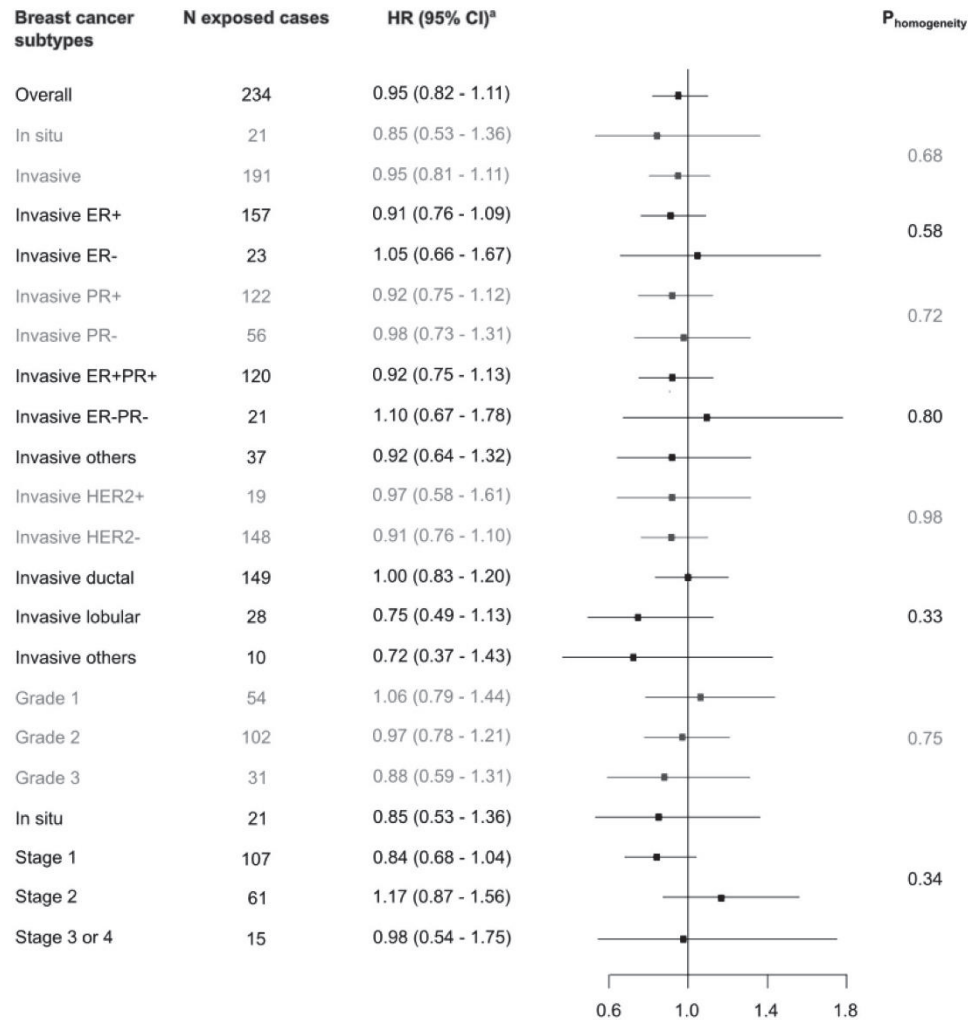


Supplementary Figure S2. Associations of clopidogrel ever use with breast cancer risk, compared to never use, among women with a recent mammogram (E3N Cohort; 2004 to 2014; n=51,097).



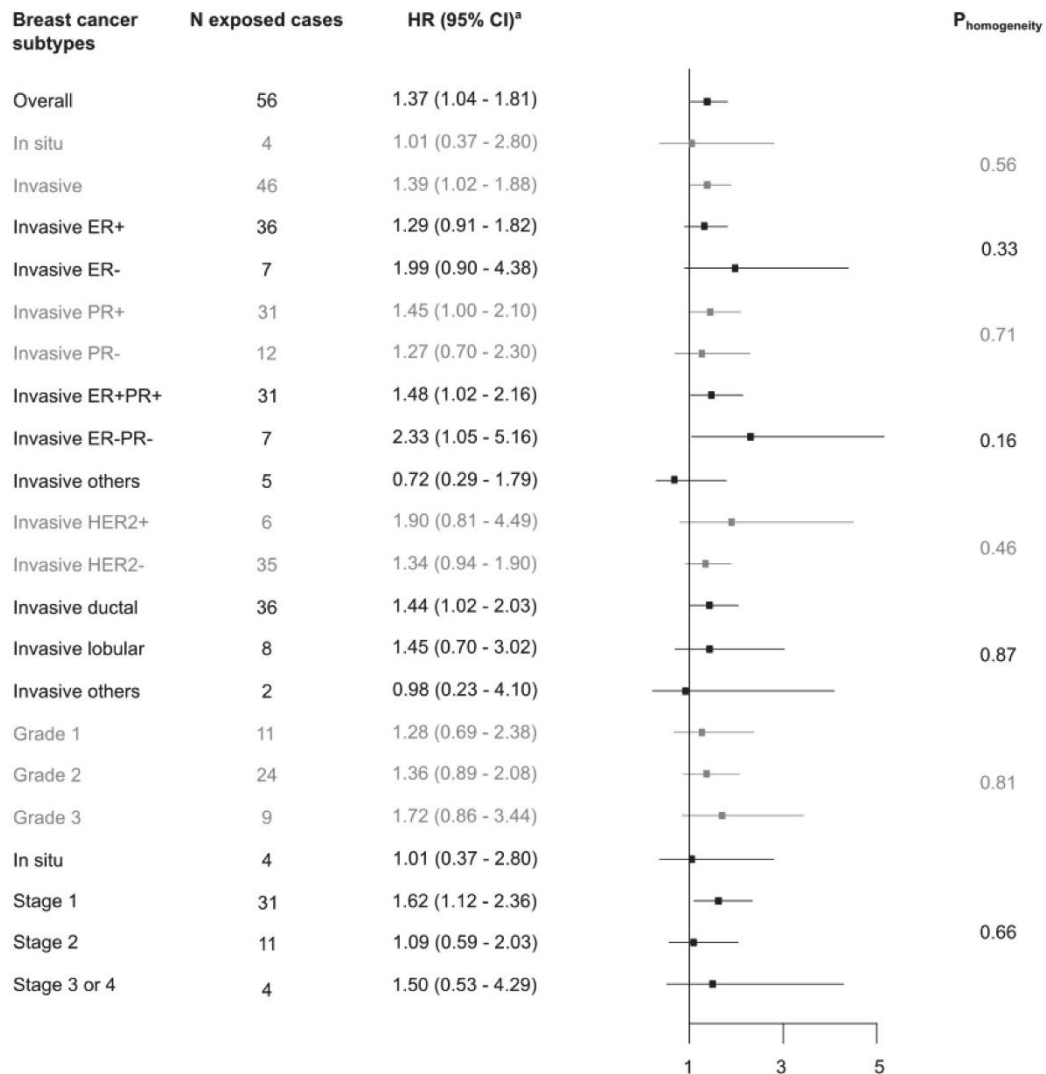
<sup>a</sup> Adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), body mass index (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at menopause (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, beta 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. Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, Hazard Ratio; PR, progesterone receptor.

Supplementary Figure S3. Associations of low-dose aspirin ever use with breast cancer risk, compared to never use, with exposure and other co-variables coming from the reimbursement database lagged by 2 years (E3N Cohort; 2004 to 2014; n=62,512).



<sup>a</sup> 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 clopidogrel, statins, antihypertensives, diuretics, beta 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. Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, Hazard Ratio; PR, progesterone receptor.

Supplementary Figure S4. Associations of clopidogrel ever use with breast cancer risk, compared to never use, with exposure and other co-variables coming from the reimbursement database lagged by 2 years (E3N Cohort; 2004 to 2014; n=62,512).



<sup>a</sup> 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, beta 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. Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, Hazard Ratio; PR, progesterone receptor.



## Reference list

1. Franco AT, Corken A, Ware J: Platelets at the interface of thrombosis, inflammation, and cancer. *Blood* 126:582-8, 2015
2. Xu XR, Yousef GM, Ni H: Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents. *Blood* 131:1777-1789, 2018
3. Qiao Y, Yang T, Gan Y, et al: Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. *BMC Cancer* 18:288, 2018
4. Cook NR, Lee IM, Zhang SM, et al: Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. *Ann Intern Med* 159:77-85, 2013
5. Ye X, Fu J, Yang Y, et al: Dose-risk and duration-risk relationships between aspirin and colorectal cancer: a meta-analysis of published cohort studies. *PLoS One* 8:e57578, 2013
6. Lu L, Shi L, Zeng J, et al: 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
7. Cao Y, Tan A: Aspirin might reduce the incidence of breast cancer: An updated meta-analysis of 38 observational studies. *Medicine (Baltimore)* 99:e21917, 2020
8. Cairat M, Al Rahmoun M, Gunter MJ, et al: Use of nonsteroidal anti-inflammatory drugs and breast cancer risk in a prospective cohort of postmenopausal women. *Breast Cancer Res* 22:118, 2020
9. Yin MJ, Yamamoto Y, Gaynor RB: The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* 396:77-80, 1998
10. Zhang X, Feng Y, Liu X, et al: Beyond a chemopreventive reagent, aspirin is a master regulator of the hallmarks of cancer. *J Cancer Res Clin Oncol* 145:1387-1403, 2019
11. Dovizio M, Bruno A, Tacconelli S, et al: Mode of action of aspirin as a chemopreventive agent. *Recent Results Cancer Res* 191:39-65, 2013
12. Clavel-Chapelon F: Cohort Profile: The French E3N Cohort Study. *Int J Epidemiol* 44:801-9, 2015
13. Mega JL, Simon T: Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments. *Lancet* 386:281-91, 2015
14. WHO Collaborating Centre for Drug Statistics Methodology: DDD: Definition and general considerations. [http://www.whocc.no/ddd/definition\\_and\\_general\\_considera/](http://www.whocc.no/ddd/definition_and_general_considera/).
15. Fournier A, Fabre A, Mesrine S, et al: Use of different postmenopausal hormone therapies and risk of histology- and hormone receptor-defined invasive breast cancer. *J Clin Oncol* 26:1260-8, 2008
16. Pottegard A, Hallas J: New use of prescription drugs prior to a cancer diagnosis. *Pharmacoepidemiol Drug Saf* 26:223-227, 2017
17. Pintilie M: Analysing and interpreting competing risk data. *Stat Med* 26:1360-7, 2007
18. Lunn M, McNeil D: Applying Cox regression to competing risks. *Biometrics* 51:524-32, 1995
19. Ready A, Velicer CM, McTiernan A, et al: NSAID use and breast cancer risk in the VITAL cohort. *Breast Cancer Res Treat* 109:533-43, 2008
20. Clarke CA, Canchola AJ, Moy LM, 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* 19:52, 2017
21. Yang YS, Kornelius E, Chiou JY, et al: Low-Dose Aspirin Reduces Breast Cancer Risk in Women with Diabetes: A Nationwide Retrospective Cohort Study in Taiwan. *J Womens Health (Larchmt)*, 2017
22. Harris RE, Chlebowski RT, Jackson RD, et al: Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. *Cancer Res* 63:6096-101, 2003
23. Ajrouche A, De Rycke Y, Dalichampt M, et al: Reduced risk of cancer among low-dose aspirin users: Data from French health care databases. *Pharmacoepidemiol Drug Saf* 28:1258-1266, 2019
24. Kim S, Shore DL, Wilson LE, 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* 15:960, 2015
25. Hollestein LM, van Herk-Sukel MP, Ruiter R, 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* 135:157-65, 2014
26. Tsoi KKF, Ho JMW, Chan FCH, et al: Long-term use of low-dose aspirin for cancer prevention: A 10-year population cohort study in Hong Kong. *Int J Cancer* 145:267-273, 2019
27. McNeil JJ, Gibbs P, Orchard SG, et al: Effect of aspirin on cancer incidence and mortality in older adults. *J Natl Cancer Inst*, 2020
28. Rothwell PM, Cook NR, Gaziano JM, 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* 392:387-399, 2018
29. Serebruany VL, Cherepanov V, Golukhova EZ, et al: The Dual Antiplatelet Therapy Trial after the FDA Update: Noncardiovascular Deaths, Cancer and Optimal Treatment Duration. *Cardiology* 132:74-80, 2015
30. Rodriguez-Miguel A, Garcia-Rodriguez LA, Gil M, et al: Clopidogrel and Low-Dose Aspirin, Alone or Together, Reduce Risk of Colorectal Cancer. *Clin Gastroenterol Hepatol* 17:2024-2033.e2, 2019
31. Kuan YC, Huang KW, Lin CL, et al: Effects of Aspirin or Clopidogrel on Colorectal Cancer Chemoprevention in Patients with Type 2 Diabetes Mellitus. *Cancers (Basel)* 11, 2019
32. Leader A, Zelikson-Saporta R, Pereg D, et al: The Effect of Combined Aspirin and Clopidogrel Treatment on Cancer Incidence. *Am J Med* 130:826-832, 2017
33. Li H, Lee MH, Liu K, et al: Inhibiting breast cancer by targeting the thromboxane A2 pathway. *NPJ Precis Oncol* 1:8, 2017
34. Serebruany VL, Cherepanov V, Cabrera-Fuentes HA, et al: Solid cancers after antiplatelet therapy: Confirmations, controversies, and challenges. *Thromb Haemost* 114:1104-12, 2015
35. Smeda M, Kieronska A, Proniewski B, 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* 9:17810-17824, 2018
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* 25:392-8, 2016

## 5. Glucocorticoid use and breast cancer risk

### 5.1. Main findings

At the end of follow-up, 17,374 women (28%) had been recurrently exposed to systemic glucocorticoids. Glucocorticoids that were the most frequently used in the cohort were betamethasone (5%), prednisolone (11%), prednisone (5%) and cortivazol (5%). Less than 1% of women had been recurrently exposed to dexamethasone, methylprednisolone, hydrocortisone, or triamcinolone. Compared to glucocorticoid never/occasional users, recurrent glucocorticoid users were older, had a higher BMI, had more frequent medical follow-up, were more likely to have ever used MHT and had more frequent histories of arthrosis as well as recurrent exposure to NSAIDs, paracetamol, PPI, and anti-arthritics.

Overall, compared with never/occasional use, recurrent use of glucocorticoids was not associated with breast cancer risk [HR= 0.94 (0.85 – 1.05)]. However, we found a statistically significant heterogeneity according to the invasiveness status ( $P_{\text{homogeneity}}=0.01$ ). Recurrent use of glucocorticoids was associated with an increased risk of *in situ* breast cancer [HR= 1.34 (1.01 – 1.78)] and a decreased risk of invasive breast cancer [HR=0.86 (0.79 – 0.97)]. Regarding the risk of *in situ* breast cancer, no statistically significant trend according to molecules, routes of administration, time since first/last use or age at first use were found. Among invasive cases, recurrent glucocorticoid use was associated with a decreased risk only for ER+ breast cancer [HR<sub>ER+</sub>= 0.82 (0.72 – 0.94); HR<sub>ER-</sub>= 1.21 (0.88 – 1.66);  $P_{\text{homogeneity}}=0.03$ ], stage 1 and 2 breast cancer [HR<sub>stage1</sub>= 0.87 (0.75 – 1.01); HR<sub>stage2</sub>= 0.67 (0.52 – 0.86); HR<sub>stage 3 or 4</sub>= 1.49 (1.02 – 2.20),  $P_{\text{homogeneity}}=0.01$ ], or with the highest categories of cumulative number of reimbursments [ $>15$  DDDs: HR=0.53 (0.34 – 0.81);  $P_{\text{trend}}=0.02$ ]. The glucocorticoids-invasive breast cancer associations did not differ across other breast cancer subtypes, other characteristic of use (molecules, routes of administration, time since first/last use or age at first use, or according to current age, BMI, ever use of MHT, comorbidities and use of selected drug.

### 5.2. Conclusions

This study suggests that breast cancer subtypes should be considered when evaluating the associations between glucocorticoid use and breast cancer risk.



### 5.3. Paper

**Status:** Submitted.

**Contribution:** First author, conducted statistical analyses, wrote the first draft of the manuscript and submitted it to the journal.

Glucocorticoids and breast cancer risk

#### Use of glucocorticoids and risk of breast cancer in a prospective cohort of postmenopausal women.

Manon Cairat<sup>1</sup>, Marie Al Rahmoun<sup>2,3</sup>, Marc J. Gunter<sup>1</sup>, Gianluca Severi<sup>2,3,4</sup>, Laure Dossus<sup>1,\*</sup>, Agnès Fournier<sup>2,3\*</sup>.

##### Author details

<sup>1</sup>Nutrition and Metabolism Section, International Agency for Research on Cancer, Lyon, France.

<sup>2</sup>Centre de recherche en Épidémiologie et Santé des Populations (CESP), équipe "Exposome, Hérité, Cancer et Santé", Faculté de Médecine Université Paris-Saclay, UVSQ, Inserm U1018, Villejuif, France

<sup>3</sup>Gustave Roussy, Villejuif, France

<sup>4</sup>Department of Statistics, Computer Science and Applications "G. Parenti" (DISIA), University of Florence, Florence, Italy.

\*The two authors are co-last authors.

##### Corresponding Author

Agnès Fournier, CESP-Inserm U1018, équipe "exposome et hérité", Gustave Roussy, 114 rue Edouard Vaillant, 94805 Villejuif Cedex; [Agnes.FOURNIER@gustaveroussy.fr](mailto:Agnes.FOURNIER@gustaveroussy.fr); +33 1 42 11 51 63.

##### Abstract

Experiments have suggested that glucocorticoids might play a role in breast cancer development. However, epidemiological evidence is limited regarding the associations between glucocorticoid use and breast cancer risk. We investigated the association between the use of systemic glucocorticoids and breast cancer incidence in the E3N cohort, which includes 98,995 women with information on various characteristics collected from repeated questionnaires complemented with drug reimbursement data available from 2004. Women with at least two reimbursements of systemic glucocorticoids in any previous three-month period were defined as ever exposed. Exposure was considered as a time-varying parameter and multivariable Cox regression models were used to estimate hazard ratios (HRs) of breast cancer. Among 62,512 postmenopausal women (median follow-up of 9 years, median age at inclusion of 63 years old), 28% had been exposed to glucocorticoids by the end of the follow-up. Ever use of glucocorticoids was not associated with overall breast cancer risk [HR=0.94 (0.85 – 1.05)]. Heterogeneity was found according to invasiveness status ( $P_{\text{homogeneity}}=0.01$ ) suggesting a higher risk of *in situ* breast cancer [HR=1.34 (1.01 – 1.78)] and a lower risk of invasive breast cancer [HR=0.86 (0.76 – 0.97)] with glucocorticoid exposure. The associations between glucocorticoids and invasive breast cancer risk differed by tumor stages ( $P_{\text{homogeneity}}=0.03$ ). Glucocorticoids ever use was associated with lower risk of stage 1 [HR=0.87 (0.75 – 1.01)] and stage 2 [HR=0.67 (0.52 – 0.86)] breast cancer and higher risk of stage 3/4 breast cancer [HR=1.49 (1.02 – 2.20)]. The lower risk of invasive breast cancer was only found for estrogen receptor positive (ER+) breast cancer [HR<sub>ER+</sub>=0.82 (0.72 – 0.94); HR<sub>ER-</sub>=1.21 (0.88 – 1.66);  $P_{\text{homogeneity}}=0.01$ ], or with the highest categories of cumulative number of glucocorticoid reimbursements [ $>15$ : HR=0.53 (0.34 – 0.81);  $P_{\text{trend}}=0.02$ ]. This study suggests that the association between systemic glucocorticoid use and breast cancer risk may differ by tumour subtypes and stages.

##### Keywords

Glucocorticoid, Breast Cancer, Molecular Status, Stages, Postmenopausal Women.

## Introduction

Inflammation has been hypothesized to be involved in the pathophysiology of breast cancer <sup>1</sup>. Hence, the possibility of stemming tumorigenic inflammatory effects with anti-inflammatory drugs has drawn significant interest. Synthetic glucocorticoids, drugs that are structurally and pharmacologically similar to the endogenous hormone cortisol, are used to treat a wide range of diseases, most often chronic diseases, such as rheumatologic disorders, autoimmune diseases, allergies, or respiratory diseases <sup>2</sup>. Glucocorticoids possess various anti-inflammatory, immunosuppressive, metabolic, and endocrine properties, and have been hypothesized to play a dual role in breast cancer development <sup>3,4</sup>. Some experimental studies reported that glucocorticoids decreased breast cancer growth <sup>4</sup>, while other experiments suggested that they might promote breast cancer progression and metastasis <sup>3-5</sup>. On one hand, glucocorticoids decrease the levels of various mediators potentially involved in breast cancer development such as pro-inflammatory cytokines, estrogens and eicosanoids <sup>4,6</sup>. On the other hand, they might facilitate tumour cells to escape from immune surveillance or induce insulin resistance <sup>7,8</sup>, which is also a suspected risk factor for breast cancer development <sup>9</sup>. To date, there is a lack of epidemiological studies addressing the relationship between synthetic glucocorticoid use and breast cancer risk. To our knowledge, only two epidemiological studies have been published previously, both based on data from nationwide medico-administrative databases from Northern Denmark <sup>10,11</sup>. The most recent study, which was an extension of the previous one <sup>10</sup>, reported no association between at least three prescriptions of systemic or inhaled glucocorticoids and invasive breast cancer risk <sup>11</sup>. There was also no association when the authors categorized glucocorticoid exposure into recent and former use, according to intensity or duration of use, or stratified the analyses by menopausal status. However, they were not able to stratify their analyses by breast cancer subtypes.

To advance knowledge on the role of glucocorticoids on breast cancer development, we evaluated the associations between systemic glucocorticoid use and breast cancer incidence, overall and by breast cancer subtypes, in the 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 a specific national health scheme covering mainly teachers <sup>12</sup>. In 1990, participants signed an informed consent and responded to a first questionnaire, which included questions on various characteristics and medical events such as socio-demographic factors, current anthropometric measurements, menstrual and reproductive factors, lifetime medical history, family history of cancer, gynaecological follow-up, and current physical activity. Every two or three years thereafter, participants completed self-administered follow-up questionnaires to

update previous information or to collect new ones. For each cohort member, the health insurance plan provided data on all outpatient reimbursements for health expenditure since January 1, 2004. The study was approved by the French National Commission for Data Protection and Privacy.

### ***Study Population and follow-up***

Follow-up started on July 1<sup>st</sup>, 2004 and ended at the date of diagnosis of any cancer (except for basal cell carcinoma and in situ colorectal tumour), latest completed questionnaire, or November 17<sup>th</sup>, 2014 (date at which the last considered E3N questionnaire was sent to participants), whichever occurred first. The selection of the study population has been already described previously <sup>13</sup>. In brief, it included 62,512 E3N postmenopausal women who were free of cancer at the study baseline (July 1<sup>st</sup>, 2004).

### ***Identification of breast cancer cases***

Most breast cancer cases were self-reported in the questionnaires or, to a lesser extent, spontaneously reported by participants' next-of-kin, or identified from cause of death data. Pathology reports were obtained for 95% of the incident cases identified in the entire cohort and were used to confirm the cases and to extract information on tumour characteristics such as stage, grade, hormonal receptor status and histological type. The proportion of false-positive self-reports was less than 5% and therefore cases for which we could not obtain pathology reports were not excluded from our analyses.

### ***Exposure to systemic glucocorticoids***

We considered all deliveries of systemic glucocorticoids (Anatomical Therapeutic Chemical [ATC] code: H02AB) since January 1<sup>st</sup>, 2004. The following data were extracted for each glucocorticoid delivery: date of purchase, active ingredient, number of pills/phials per package, and route of administration.

We defined as "recurrent" users, women with at least two reimbursements of the drug of interest during any previous three-month period since January 1, 2004. Other women were considered as never/occasional users and served as the reference category. We classified exposure among recurrent users according to the following characteristics: active ingredient, route of administration, time since first or last use, age at first use, and cumulative number of reimbursements. The date of last use was calculated as the date of last purchase + the number of pills/phials contained in the last reimbursed box. Women with glucocorticoid reimbursements between January 1, 2004 and April 1, 2004 were likely to have begun their treatment before the availability of reimbursement data and, in that case, the cumulative number of reimbursements, age at first use and time since first use might be left-truncated. Thus, we assigned these women to an "unknown" category, unless they could be assigned to the highest category of reimbursement/time since first use or the lowest category of age at first use.



### *Covariates*

Variables considered as potential confounders are listed in Table 1. Number of consultations with any doctor during the preceding six months and “recurrent” use (defined as at least two reimbursements of the drug of interest during any previous three-month period since January 1, 2004) of other drugs likely to be also used by glucocorticoid users [Paracetamol (ATC code: N02BE01), nonsteroidal-anti-inflammatory drugs (M01A), proton-pump inhibitors (A02BC), immunosuppressants (L04) and symptomatic slow acting drugs for osteoarthritis (glucosamine: M01AX05, diacerein: M01AX21, oxaceprol: M01AX24, chondroitin sulfate: M01AX25 or avocado and soybean oil: M01AX26)] were identified using the drug reimbursement database. Lifetime use of menopausal hormone therapy (MHT) was identified using both self-reported information from the questionnaires sent out before 2004 and the drug reimbursement database since 1<sup>st</sup> January 2004. Education 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 were generated from the biennial self-administered questionnaires sent before the study baseline. 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 comorbidities that might be treated with glucocorticoids (rheumatism, arthrosis, and arthritis, spondyloarthritis, polyarthritis, asthma, chronic bronchitis, hay fever, or chronic inflammatory bowel diseases) originated from the biennial self-administered questionnaire sent before the study baseline with subsequent updates until 2011.

### *Statistical analysis*

We used multivariable Cox regression models with age as the time scale and stratified by birth cohort (in five-year categories) to estimate hazard ratios (HRs) and 95% confidence interval (CI) for the association of recurrent glucocorticoid use with breast cancer risk, overall and by breast cancer subtypes. We considered exposure, other factors issued from the reimbursement database and covariates updated during follow-up as time-varying parameters. Thus, for a given drug, participants contributed follow-up as unexposed until purchasing the drug for the second time in a three-month period. The cumulative number of reimbursements, age at first use, and time since first/last use were also updated during follow-up.

We systematically included in the multivariable models the following covariates: educational level, recent mammogram, and established risk factors for breast cancer (BMI, physical activity level, lifetime personal history of benign breast disease, family history of breast cancer, age at menarche, age at menopause, parity and age first full-term pregnancy, lifetime use of oral contraceptives, lifetime use of MHT and alcohol consumption). Number of consultations with the doctor during the preceding six months and recurrent use of proton pump inhibitors modified the HR of certain breast cancer subtypes associated with exposure to

4

glucocorticoids by at least 0.05 point and were therefore included in the final multivariable models. None of the other factors tested as potential confounding factors (breastfeeding, smoking status, lifetime histories of comorbidities that might be treated with glucocorticoids, or drugs likely to be used by glucocorticoid users) were included in the final models. Categories used are displayed in Table 1. For these covariates, when missing values represented < 5%, they were replaced either with the previous non missing questionnaire value where appropriate, or with the mode or the median values observed among the subjects with complete data. Only alcohol consumption and breastfeeding had  $\geq 5\%$  of missing values, which was accommodated by using a “missing” category in our models. A complete case analysis was also conducted (not shown because results were similar).

We lagged by six months all variables coming from the reimbursement database (exposure, use of MHT, use of other drugs, and number of consultations) to allow a period of latency and to minimize reverse causation bias due to any early breast cancer symptoms<sup>14</sup>. The results were similar when exposure and other variables were lagged or not (data not shown).

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

We performed competing risk analysis using the cause-specific hazards approach to study the glucocorticoids-breast cancer associations according to the invasiveness, molecular, histological or stage/grade breast cancer subtypes<sup>15,16</sup>. We excluded cases with missing information on a given tumour characteristic from the corresponding analyses.

As sensitivity analyses, we defined users as women with at least three reimbursements. We also repeated the analyses i) using a 2-year exposure lag instead of the 6-month lag, and ii) only among women who self-reported having had a mammogram performed in the previous follow-up cycle to minimize surveillance bias.

All tests of statistical significance were two-sided, and significance was set at .05. We performed all analyses using SAS software, version 9.4 (SAS Institute Inc, Cary, NC).

## Results

During a median follow-up time of nine years, 2,864 breast cancer cases were diagnosed (335 *in situ* and 2,353 invasive) among the 62,512 participants. At the end of follow-up, 17,374 women (28%) had been recurrently exposed to systemic glucocorticoids. Glucocorticoids that were the most frequently used in the cohort were



betamethasone (5%), prednisolone (11%), prednisone (5%) and cortivazol (5%). Less than 1% of women had been recurrently exposed to dexamethasone, methylprednisolone, hydrocortisone, or triamcinolone.

Compared to glucocorticoid never/occasional users, recurrent glucocorticoid users were older, had a higher BMI, more frequent medical follow-up, were more likely to have ever used MHT and had more frequent histories of arthrosis, asthma, chronic bronchitis, or hay fever as well as recurrent exposure to nonsteroidal anti-inflammatory drugs, paracetamol, proton pump inhibitors, and anti-arthritis (Table 1).

The age-adjusted HR of breast cancer associated with having recurrently been exposed to glucocorticoids, compared with having never/occasionally been exposed, was 0.98 (95% CI 0.89 – 1.09). The multivariable HR was 0.94 (95% CI 0.85 – 1.05) (Figure 1). Significant heterogeneity was found according to the invasiveness status ( $P_{\text{homogeneity}}=0.01$ ), with a positive association of glucocorticoid use with the risk of *in situ* breast cancer [HR= 1.34 (1.01 – 1.78)] and an inverse association with the risk of invasive breast cancer [HR= 0.86 (0.76 – 0.97)].

Regarding the risk of *in situ* breast cancer, no statistically significant heterogeneity/trend according to active ingredients, routes of administration, time since first/last use, age at first use, or cumulative number of reimbursements was found ( $P_{\text{trend/homogeneity}} \geq 0.11$ , Supplementary Table S1).

Regarding the risk of invasive breast cancer, statistically significant heterogeneities by tumor stages ( $P_{\text{homogeneity}}=0.01$ ) and estrogen receptor (ER) status ( $P_{\text{homogeneity}}=0.03$ ) were found (Figure 1). Recurrent use of glucocorticoids was associated with lower risk of stage 1 and stage 2 breast cancer [HR<sub>stage1</sub>= 0.87 (0.75 – 1.01); HR<sub>stage2</sub>= 0.67 (0.52 – 0.86)] and higher risk of stage 3/4 breast cancer [HR<sub>stage 3 or 4</sub>= 1.49 (1.02 – 2.20)]. The lower risk of invasive breast cancer was only found for ER+ breast cancer [HR<sub>ER+</sub>= 0.82 (0.72 – 0.94); HR<sub>ER-</sub>= 1.21 (0.88 – 1.66)]. The breast cancer-glucocorticoid association did not differ by other breast cancer subtypes. No statistically significant heterogeneity/trend of invasive breast cancer risk according to active ingredients, routes of administration, time since first/last use, or age at first were found ( $P_{\text{trend/homogeneity}} \geq 0.29$ , Table 2). However, analyses according to cumulative number of reimbursements yielded statistically significant trend suggesting a decreased risk of invasive breast cancer in the highest categories of reimbursements [ $>15$  reimbursements: HR=0.53 (0.34 – 0.81);  $P_{\text{trend}}=0.02$ ]. No effect modification was found by current age, BMI, ever use of MHT, selected comorbidities and use of selected drugs ( $P_{\text{interaction}} > 0.24$ , Supplementary Figure S1).

When we changed the definition of exposure to at least three reimbursements (data not shown), applied a lag-time of two years (Supplementary Figure S2) or restricted the study sample to women with a recent mammogram (Supplementary Figure S3), the results remained virtually unchanged.

## Discussion

In this large cohort of postmenopausal women, glucocorticoid exposure was associated with lower risk of invasive breast cancer that was restricted to ER+ and to stage 1 or 2 tumours. There was also a statistically significant trend according to cumulative number of reimbursements with a lower risk of invasive breast cancer in the highest categories of cumulative number of reimbursements. Conversely, glucocorticoid use was positively associated with risk of *in situ* and stage 3/4 breast cancers.

The only epidemiological study published to date to specifically evaluate glucocorticoid-breast cancer associations reported a null association between glucocorticoid use and invasive breast cancer risk [systemic glucocorticoids: OR= 1.00 (0.96 – 1.10), n exposed cases= 908]<sup>11</sup>. The associations did not differ by intensity, or duration of use. However, the authors were not able to stratify their analyses by breast cancer subtypes. Our results, suggesting a lower risk of invasive breast cancer especially for ER+ tumours, are supported by experimental models showing that the expression of the glucocorticoid receptor was correlated with improved breast cancer prognosis especially for ER+ breast cancer tumours and that activation of the glucocorticoid receptor may reduce estrogen-induced cell proliferation in ER+ breast cancer<sup>17,18</sup>. Indeed, glucocorticoids were shown to have preventive breast cancer effects by stimulating the expression of sulfotransferase SULT1E1 (which plays a role in deactivating oestrogen)<sup>6</sup>, affecting angiogenesis<sup>19</sup>, or inhibiting inflammatory and growth factors<sup>20,21</sup>. Our finding supporting an increased risk of *in situ* breast cancer with glucocorticoid exposure could be explained by the fact that glucocorticoids might slow down tumour growth (e.g. through the inhibition of the NF-κB activity and growth/proliferation signalling pathways<sup>22,23</sup>) and therefore keep the cancer cells located *in situ*. The higher risk of stage 3/4 breast cancers with glucocorticoid exposure is also in line with experiments suggesting that glucocorticoids promote breast cancer metastasis<sup>5</sup>, or that the glucocorticoid receptor was over-expressed in metastatic breast cancer<sup>24</sup> and might be a strong inducer of epithelial-to-mesenchymal transition<sup>24</sup>. In addition, glucocorticoids have been hypothesized to promote metastasis through the activation of the TEA domain transcription factor 4<sup>25</sup>. Studies conducted in the 1960s among breast cancer patients reported that the use of adrenal steroids (including glucocorticoids), compared to non-use, increased the risk of metastasis<sup>26</sup>. However, in our study, the number of advanced stage cases was too small to separate stage 3 and stage 4 breast cancers.

The main strengths of this study included its prospective design and the use of information from a drug reimbursement database to identify glucocorticoid exposure, which avoids differential recall bias between

cases and non-cases. In addition, the exhaustive data on glucocorticoid reimbursement allowed us to consider precise information on exposure (including number of reimbursements and timing of use). However, we lacked data regarding the compliance/adherence to the dispensed treatment but defining users as women with at least two reimbursements during a three-month period makes it likely that they took the drug. Since data on glucocorticoid reimbursements were combined with self-reported data on lifestyle, reproductive, and medical factors, we were able to consider potential confounders and to examine interactions of glucocorticoid use with breast cancer risk factors, comorbidities and other drugs. However, the power to detect differences was limited because of the low number of cases in some categories and we cannot exclude residual confounding. In addition, while some autoimmune diseases were associated with breast cancer risk <sup>27</sup>, we were not able to adjust or stratify on several comorbidities of interest such as adrenal insufficiency or some autoimmune diseases (e.g. systemic lupus erythematosus, pernicious anaemia, or psoriasis). Nevertheless, the prevalence of these comorbidities is low and because they are not established breast cancer risk factors, we do not expect that they would confound the glucocorticoid-breast cancer associations. We minimized any surveillance bias by adjusting for recent mammogram and number of consultations with medical doctors. Lastly, we were able to stratify the analyses according to breast cancer subtypes, but some subgroups were limited, and results must be interpreted carefully.

To conclude, this study suggests that breast cancer subtypes are important to consider when evaluating the associations between glucocorticoid use and breast cancer risk. Our results require confirmation in other prospective studies with data on breast cancer subtypes.



## Tables

Table 1. Characteristics of participants, overall and according to glucocorticoid exposure at the end of follow-up (E3N Cohort; 2004 to 2014; n = 62,512).

Characteristics at the end of follow-up <sup>1</sup>	All women (n=62,512)	Exposure at the end of follow-up Never/occasional users (n=45,138)	Recurrent users (n=17,374)
<b>Sociodemographic factors</b>			
Age (years), mean (SD)	72.1 (6.7)	71.8 (6.4)	72.8 (6.3)
<b>Educational level, N (%)</b>			
< High-school	6,516 (11)	4,536 (10)	1,980 (11)
From high-school to 4 years higher education	45,138 (72)	32,771 (73)	12,367 (71)
At least 5 years higher education	10,858 (17)	7,831 (17)	3,027 (18)
<b>Lifestyle and reproductive factors</b>			
<b>BMI (kg/m<sup>2</sup>), N (%)</b>			
< 18.5	2,626 (4)	1,978 (4)	648 (4)
≥ 18.5 to < 23	25,409 (41)	19,037 (42)	6,372 (37)
≥ 23 to < 25	13,197 (21)	9,499 (21)	3,698 (21)
≥ 25 to < 30	16,139 (26)	11,207 (25)	4,932 (28)
≥ 30	5,141 (8)	3,417 (8)	1,724 (10)
<b>Physical activity (Met-h/week), N (%)</b>			
≤ 34.8	15,649 (25)	11,115 (25)	4,534 (26)
> 34.8 to ≤ 57.6	15,680 (25)	11,329 (25)	4,351 (25)
> 57.6 to ≤ 88.8	15,567 (25)	11,306 (25)	4,261 (25)
> 88.8	15,616 (25)	11,388 (25)	4,228 (24)
<b>Smoking status, N (%)</b>			
Never smoker	33,281 (53)	24,229 (54)	9,052 (52)
Current smoker	4,741 (8)	3,307 (7)	1,434 (8)
Past smoker	24,490 (39)	17,602 (39)	6,888 (40)
<b>Alcohol intake (g/d), N (%)</b>			
Abstainer	7,832 (13)	5,694 (13)	2,138 (13)
≤ 5	16,796 (27)	12,198 (27)	4,598 (26)
> 5 to ≤ 10	9,357 (15)	6,863 (15)	2,494 (14)
> 10 to ≤ 20	12,053 (19)	8,700 (19)	3,353 (19)
> 20	12,522 (20)	8,957 (20)	3,565 (21)
Missing	3,952 (6)	2,726 (6)	1,226 (7)
<b>Breastfeeding, N (%)</b>			
Never	23,425 (37)	16,829 (37)	6,596 (38)
Ever	34,174 (55)	24,941 (55)	9,233 (53)
Missing	4,913 (8)	3,368 (8)	1,545 (9)
<b>Age at menopause (years), mean (SD)</b>	50.5 (3.7)	50.6 (3.7)	50.3 (3.9)
<b>Age at menarche, years, N (%)</b>			
< 13	28,078 (45)	20,054 (44)	8,024 (46)
≥ 13	34,434 (55)	25,084 (56)	9,350 (54)
<b>Parity and age at first full-term pregnancy, N (%)</b>			
Nulliparous	7,282 (12)	5,346 (12)	1,936 (11)
First child before age 30 years, one or two children	31,393 (50)	22,434 (50)	8,959 (51)
First child before age 30 years, three or more children	17,373 (28)	12,592 (28)	4,781 (28)
First child after age 30 years	6,464 (10)	4,766 (11)	1,698 (10)
<b>Lifetime oral contraceptive use, N (%)</b>	38,570 (62)	27,719 (61)	10,851 (62)
<b>Lifetime MHT use, N (%)</b>	45,239 (72)	31,843 (71)	13,396 (77)
<b>Number of medical consultations/visits during the preceding 6 months, N (%)</b>			
0	3,277 (5)	2,865 (6)	412 (2)
1 to 3	25,413 (41)	20,289 (45)	5,124 (30)

Glucocorticoids and breast cancer risk

≥ 4	33,491 (54)	21,667 (48)	11,824 (68)
Missing	331 (1)	317 (1)	14 (0)
<b>Self-report of a mammogram performed during the previous follow-up cycle, N (%)</b>	51,097 (82)	36,800 (82)	14,297 (82)
<b>Lifetime personal history of benign breast disease, N (%)</b>	23,268 (37)	16,435 (36)	6,833 (39)
<b>History of breast cancer in first-degree relatives, N (%)</b>	7,139 (11)	5,196 (12)	1,943 (11)
<b>Comorbidities, N (%)</b>			
<b>Lifetime history of arthrosis</b>	21,475 (34)	14,152 (31)	7,323 (42)
<b>Lifetime history of rheumatism</b>	6,111 (10)	4,504 (10)	1,607 (9)
<b>Lifetime history of arthritis</b>	888 (1)	591 (1)	297 (2)
<b>Lifetime history of polyarthritis</b>	2,375 (4)	1,180 (3)	1,195 (7)
<b>Lifetime history of spondyloarthritis</b>	236 (1)	123 (0)	113 (1)
<b>Lifetime history of asthma</b>	6,043 (10)	3,484 (8)	2,559 (15)
<b>Lifetime history of chronic bronchitis</b>	7,102 (11)	4,278 (9)	2,824 (16)
<b>Lifetime history of hay fever</b>	12,633 (20)	8,629 (19)	4,004 (23)
<b>Lifetime history of chronic inflammatory bowel diseases</b>	2,284 (4)	1,576 (3)	708 (4)
<b>Recurrent use of other drugs<sup>1</sup>, N (%)</b>			
<b>NSAIDs</b>	38,493 (62)	14,238 (47)	24,255 (75)
<b>Immunosuppressants</b>	895 (1)	171 (0)	724 (4)
<b>Paracetamol</b>	38,392 (61)	24,725 (55)	13,667 (79)
<b>PPIs</b>	30,063 (48)	18,011 (40)	12,052 (69)
<b>Anti-arthritis</b>	26,400 (42)	17,095 (38)	9,305 (54)

Abbreviations: BMI, body mass index; MHT, menopausal hormone therapy; MET-H, metabolic equivalent task-hour; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor; SD, standard deviation.

<sup>1</sup> 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.

<sup>2</sup> Recurrent use was defined as at least two reimbursements of the drug of interest during any previous three-month period since January 1, 2004.



Table 2. Associations of glucocorticoid recurrent use with invasive breast cancer risk, according to characteristics of use (E3N Cohort; 2004 to 2014; n= 62,512).

Characteristics of exposure	N cases	HR <sup>1</sup> (95% CI)
<b>Route of administration<sup>2</sup></b>		
Oral	198	0.82 (0.70 – 0.95)
Parenteral	130	0.90 (0.75 – 1.08)
<i>P<sub>homogeneity</sub></i>		0.40
<b>Active ingredient<sup>3</sup></b>		
Betamethasone	56	0.88 (0.66 – 1.14)
Prednisolone	128	0.90 (0.75 – 1.08)
Prednisone	47	0.80 (0.59 – 1.07)
Cortivazol	57	0.94 (0.72 – 1.23)
Others <sup>3</sup>	10	0.47 (0.25 – 0.87)
<i>P<sub>homogeneity</sub></i>		0.98
<b>Cumulative number of reimbursements</b>		
Occasional/never use	2,001	1.00 (ref)
≤ 5	179	0.88 (0.75 – 1.03)
> 5 to ≤ 10	84	0.84 (0.67 – 1.05)
> 10 to ≤ 15	18	0.68 (0.42 – 1.08)
> 15	21	0.53 (0.34 – 0.81)
Unknown	50	1.16 (0.87 – 1.53)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.02
<b>Time since first use (years)</b>		
Occasional/never use	2,001	1.00 (ref)
≤ 2	112	0.88 (0.73 – 1.07)
> 2 to ≤ 4	76	0.77 (0.61 – 0.98)
> 4 to ≤ 6	61	0.86 (0.66 – 1.11)
> 6	69	0.92 (0.72 – 1.18)
Unknown	34	0.86 (0.61 – 1.21)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.82
<b>Time since last use (years)</b>		
Occasional/never use	2,001	1.00 (ref)
≤ 1	255	0.84 (0.74 – 0.97)
> 1 to ≤ 2	44	0.96 (0.71 – 1.29)
> 2 to ≤ 3	25	0.94 (0.63 – 1.40)
> 3 to ≤ 4	12	0.76 (0.43 – 1.35)
> 4	16	0.78 (0.48 – 1.28)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.81
<b>Age at first use (years)</b>		
Occasional/never use	2,001	1.00 (ref)
≤ 60	79	0.96 (0.76 – 1.22)
> 60 to ≤ 70	161	0.81 (0.69 – 0.96)
> 70	67	0.73 (0.57 – 0.95)
Unknown	45	1.09 (0.81 – 1.47)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.29

<sup>1</sup> HR 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), and recurrent use of proton pump inhibitors (time-varying). Categories used are those displayed in Table 1. HRs were obtained from separate models including one characteristic of exposure at a time.

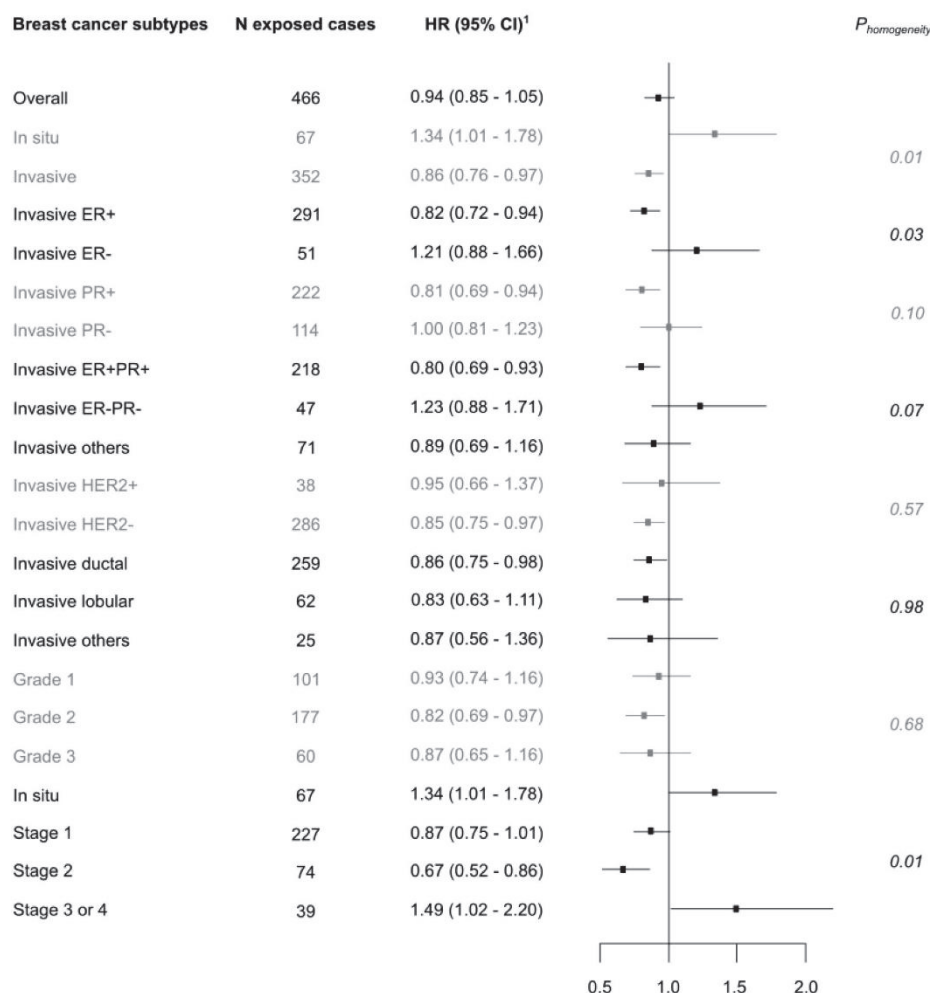
<sup>2</sup> The variables corresponding to recurrent use (versus never/occasional use) of each route of administration/active ingredients displayed in the table were introduced simultaneously in the model.

<sup>3</sup> Other molecules include dexamethasone, methylprednisolone, triamcinolone, and hydrocortisone.

<sup>4</sup> Tests for linear trends were performed among recurrently exposed women with known characteristics of exposure using an ordinal variable across categories. The corresponding variables were introduced in the models as continuous variables.

**Figure**

Figure 1. Associations of glucocorticoid recurrent use with breast cancer risk, compared to never/occasional use, overall and by breast cancer subtypes (E3N Cohort; 2004 to 2014; n=62,512).



Abbreviations: CI, confidence interval; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

<sup>1</sup>HR 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), and recurrent use of proton pump inhibitors (time-varying). Categories used are those displayed in Table 1. introduced in the models as continuous variables.

## Supplementary Figures.

Table S1. Associations of glucocorticoid recurrent use with *in situ* breast cancer risk, according to characteristics of use (E3N Cohort; 2004 to 2014; n= 62,512).

Characteristics of exposure	N cases	HR <sup>1</sup> (95% CI)
<b>Route of administration<sup>2</sup></b>		
Oral	46	1.50 (1.09 – 2.08)
Parenteral	19	0.93 (0.58 – 1.50)
<i>P<sub>homogeneity</sub></i>		0.86
<b>Active ingredient<sup>2</sup></b>		
Betamethasone	6	0.61 (0.27 – 1.37)
Prednisolone	25	1.22 (0.80 – 1.86)
Prednisone	14	1.63 (0.94 – 2.85)
Cortivazol	9	1.07 (0.55 – 2.11)
Others <sup>3</sup>	8	2.48 (1.20 – 5.11)
<i>P<sub>homogeneity</sub></i>		0.11
<b>Cumulative number of reimbursements</b>		
Occasional/never use	268	1.00 (ref)
≤ 5	31	1.24 (0.85 – 1.92)
> 5 to ≤ 10	17	1.43 (0.86 – 2.37)
> 10	12	1.46 (0.80 – 2.65)
Unknown	7	1.44 (0.68 – 3.06)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.45
<b>Time since first use (years)</b>		
Occasional/never use	268	1.00 (ref)
< 2	20	1.29 (0.82 – 2.06)
> 2 to ≤ 4	17	1.45 (0.88 – 2.40)
> 4 to ≤ 6	13	1.51 (0.85 – 2.68)
> 6	11	1.19 (0.63 – 2.23)
Unknown	6	1.20 (0.53 – 2.72)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.83
<b>Time since last use (years)</b>		
Occasional/never use	268	1.00 (ref)
< 1	47	1.28 (0.92 – 1.77)
> 1 to ≤ 2	13	2.29 (1.30 – 4.03)
> 2	7	0.91 (0.43 – 1.94)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.79
<b>Age at first use (years)</b>		
Occasional/never use	268	1.00 (ref)
≤ 60	12	0.90 (0.50 – 1.65)
> 60 to ≤ 70	34	1.37 (0.93 – 2.00)
≥ 70	11	1.48 (0.76 – 2.90)
Unknown	10	2.25 (1.18 – 4.29)
<i>P<sub>trend</sub></i> <sup>4</sup>		0.66

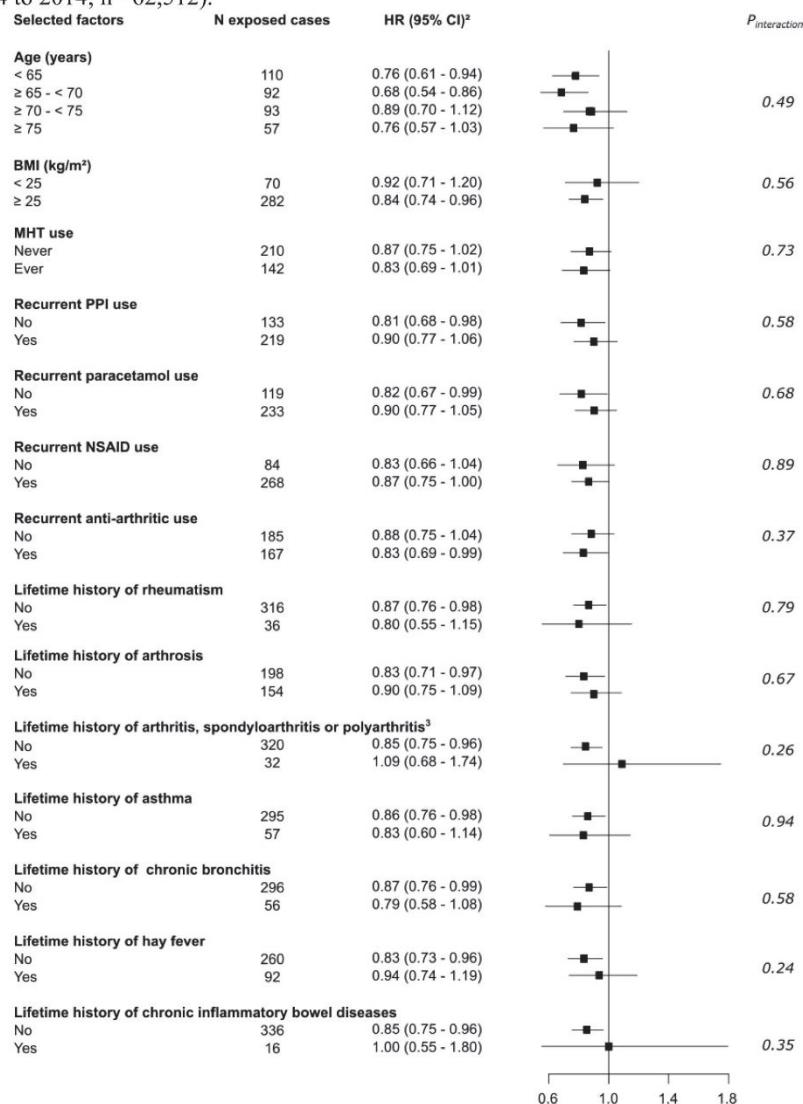
<sup>1</sup> HR adjusted for age (time scale), years of schooling (baseline), alcohol intake (time-varying), body mass index (time-varying), physical activity level (baseline), age at menarche (baseline), parity and age at first birth (baseline), lifetime use of oral contraceptives (baseline), age at 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), and recurrent use of proton pump inhibitors (time-varying). Categories used are those displayed in Table 1. HRs were obtained from separate models including one characteristic of exposure at a time.

<sup>2</sup> The variables corresponding to recurrent use (versus never/occasional use) of each route of administration/active ingredient displayed in the table were introduced simultaneously in the model.

<sup>3</sup> Other molecules include dexamethasone, methylprednisolone, triamcinolone, and hydrocortisone.

<sup>4</sup> Tests for linear trends were performed among exposed women with known characteristics of exposure using an ordinal variable across categories. The corresponding variables were introduced in the models as continuous variables.

Figure S1. Associations of glucocorticoid recurrent use with invasive breast cancer risk, compared to never/occasional use, in strata of selected factors, comorbidities and recurrent use<sup>1</sup> of other drugs (E3N Cohort; 2004 to 2014; n= 62,512).



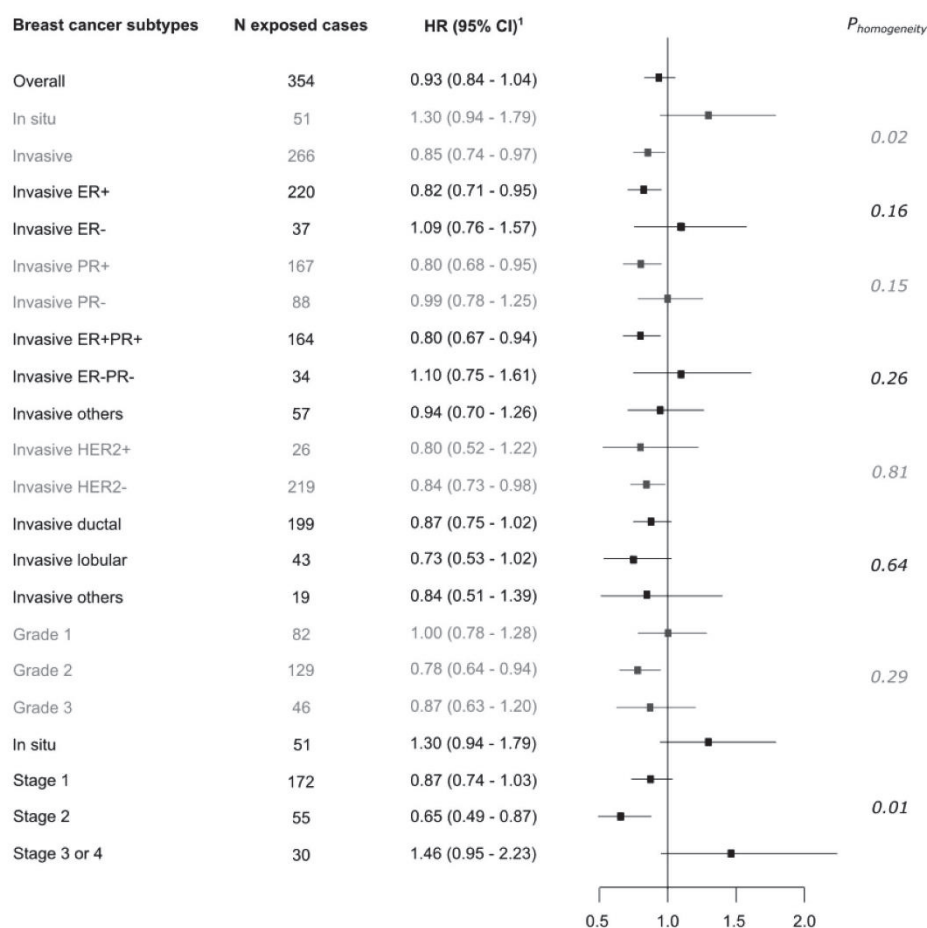
Abbreviations: BMI; body mass index, CI, confidence interval; HR, hazard ratio; MHT, menopausal hormone therapy; NSAID, nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor.

<sup>1</sup> At least two reimbursements during any previous three-month period since January 1, 2004.

<sup>2</sup> HR 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), and recurrent use of proton pump inhibitors (time-varying). Categories used are those displayed in Table 1.

<sup>3</sup> Associations were similar when comorbidities were ungrouped.

Figure S2. Associations of glucocorticoid recurrent use with breast cancer risk, compared to never/occasional use, overall and by breast cancer subtypes, with exposure and other co-variables coming from the reimbursement database lagged by 2 years (E3N Cohort; 2004 to 2014; n=62,512).

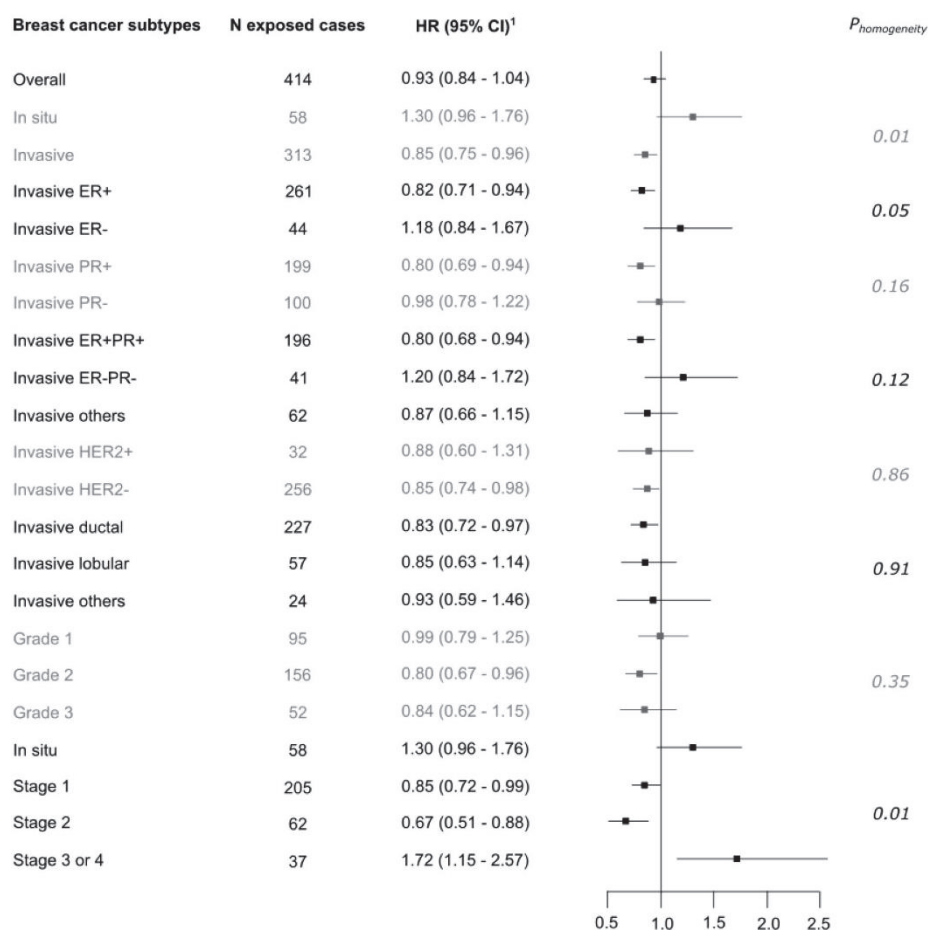


Abbreviations: CI, confidence interval; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

<sup>1</sup>HR 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), and recurrent use of proton pump inhibitors (time-varying). Categories used are those displayed in Table 1.



Figure S3. Associations of glucocorticoid recurrent use with breast cancer risk, compared to never/occasional use, overall and by breast cancer subtypes, among women with a recent mammogram (E3N Cohort; 2004 to 2014; n=51,097).



Abbreviations: CI, confidence interval; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

<sup>1</sup>HR 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), number of medical consultations/visits during the preceding 6 months (time-varying), and recurrent use of proton pump inhibitors (time-varying). Categories used are those displayed in Table 1.

**Financial support:** The Ligue Contre le Cancer supports the E3N cohort and PhD fellowship to Manon Cairat.

**Corresponding Author:** Agnès Fournier, CESP-Inserm U1018, équipe “Exposome and Heredity”, Gustave Roussy, 114 rue Edouard Vaillant, 94805 Villejuif Cedex; [Agnes.FOURNIER@gustaveroussy.fr](mailto:Agnes.FOURNIER@gustaveroussy.fr); +33 1 42 11 51 63.

**Author disclosures:** The authors declare no potential conflicts of interest.

**Data availability:** The data and computing code required to replicate the results reported in this paper are available upon duly motivated request by contacting Dr Agnès Fournier.

**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.

**Acknowledgements:** The research was carried out using data from the Inserm (French National Institutes 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). 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 paper was performed during Agnès Fournier’s term as a Visiting Scientist at the International Agency for Research on Cancer.

## Reference list

1. Jiang X, Shapiro DJ: The immune system and inflammation in breast cancer. *Mol Cell Endocrinol* 382:673-82, 2014
2. Six M, Morin C, Fardet L: [Association between prescription of long-term systemic glucocorticoid therapy associated measures and prescriber's medical speciality]. *Rev Med Interne* 40:427-432, 2019
3. McNamara KM, Kannai A, Sasano H: Possible roles for glucocorticoid signalling in breast cancer. *Mol Cell Endocrinol* 466:38-50, 2018
4. Lin KT, Wang LH: New dimension of glucocorticoids in cancer treatment. *Steroids* 111:84-88, 2016
5. Obradović MMS, Hamelin B, Manevski N, et al: Glucocorticoids promote breast cancer metastasis. *Nature* 567:540-544, 2019
6. Gong H, Jarzynka MJ, Cole TJ, et al: Glucocorticoids antagonize estrogens by glucocorticoid receptor-mediated activation of estrogen sulfotransferase. *Cancer Res* 68:7386-93, 2008
7. Zarkovic M, Beleslin B, Ciric J, et al: Glucocorticoid effect on insulin sensitivity: a time frame. *J Endocrinol Invest* 31:238-42, 2008
8. Geer EB, Islam J, Buettner C: Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism. *Endocrinol Metab Clin North Am* 43:75-102, 2014
9. Hernandez AV, Guarnizo M, Miranda Y, et al: Association between insulin resistance and breast carcinoma: a systematic review and meta-analysis. *PLoS One* 9:e99317, 2014
10. Sørensen HT, Møllekjær L, Skriver MV, et al: NO excess risk of breast cancer among female users of systemic glucocorticoids. *Cancer Epidemiol Biomarkers Prev* 14:1022-3, 2005
11. Sørensen GV, Cronin-Fenton DP, Sørensen HT, et al: Use of glucocorticoids and risk of breast cancer: a Danish population-based case-control study. *Breast Cancer Res* 14:R21, 2012
12. Clavel-Chapelon F: Cohort Profile: The French E3N Cohort Study. *Int J Epidemiol* 44:801-9, 2015
13. Cairat M, Al Rahmoun M, Gunter MJ, et al: Use of nonsteroidal anti-inflammatory drugs and breast cancer risk in a prospective cohort of postmenopausal women. *Breast Cancer Res* 22:118, 2020
14. Pottgard A, Hallas J: New use of prescription drugs prior to a cancer diagnosis. *Pharmacoepidemiol Drug Saf* 26:223-227, 2017
15. Pintilie M: Analysing and interpreting competing risk data. *Stat Med* 26:1360-7, 2007
16. Lunn M, McNeil D: Applying Cox regression to competing risks. *Biometrics* 51:524-32, 1995
17. Karmakar S, Jin Y, Nagaich AK: Interaction of glucocorticoid receptor (GR) with estrogen receptor (ER)  $\alpha$  and activator protein 1 (AP1) in dexamethasone-mediated interference of ER $\alpha$  activity. *J Biol Chem* 288:24020-34, 2013
18. Abduljabbar R, Negm OH, Lai CF, et al: Clinical and biological significance of glucocorticoid receptor (GR) expression in breast cancer. *Breast Cancer Res Treat* 150:335-46, 2015
19. Martens B, Drebert Z: Glucocorticoid-mediated effects on angiogenesis in solid tumors. *J Steroid Biochem Mol Biol* 188:147-155, 2019
20. Mitre-Aguilar IB, Cabrera-Quintero AJ, Zentella-Dehesa A: Genomic and non-genomic effects of glucocorticoids: implications for breast cancer. *Int J Clin Exp Pathol* 8:1-10, 2015
21. Meng G, Tang X, Yang Z, et al: Dexamethasone decreases the autotaxin-lysophosphatidate-inflammatory axis in adipose tissue: implications for the metabolic syndrome and breast cancer. *Faseb j* 33:1899-1910, 2019
22. Pufall MA: Glucocorticoids and Cancer. *Advances in experimental medicine and biology* 872:315-333, 2015
23. Yemelyanov A, Czornog J, Chebotaev D, et al: Tumor suppressor activity of glucocorticoid receptor in the prostate. *Oncogene* 26:1885-96, 2007
24. Shi W, Wang D, Yuan X, et al: Glucocorticoid receptor-IRS-1 axis controls EMT and the metastasis of breast cancers. *J Mol Cell Biol* 11:1042-1055, 2019
25. He L, Yuan L, Sun Y, et al: Glucocorticoid Receptor Signaling Activates TEAD4 to Promote Breast Cancer Progression. *Cancer Res* 79:4399-4411, 2019
26. Sherlock P, Hartmann WH: Adrenal steroids and the pattern of metastases of breast cancer. *Jama* 181:313-7, 1962
27. Schairer C, Pfeiffer RM, Gadalla SM: Autoimmune diseases and breast cancer risk by tumor hormone-receptor status among elderly women. *Int J Cancer* 142:1202-1208, 2018

## CHAPTER 6: DISCUSSION

My doctoral thesis aimed to provide additional epidemiological evidence on the potential role of inflammation on breast cancer development by i) identifying inflammatory biomarkers associated with breast cancer risk and ii) investigating whether drugs with anti-inflammatory properties might prevent breast cancer occurrence. Overall, results from this thesis suggested that inflammation plays a modest role in the development of breast cancer and that its impact on breast cancer risk could be limited to certain sub-populations or certain breast cancer subtypes.

### 1. Inflammatory biomarkers and breast cancer risk

In the EPIC cohort, we found no overall association between pre-diagnostic blood levels of eleven inflammatory markers and breast cancer risk. However, an inverse association was observed with the leptin to adiponectin ratio among pre/perimenopausal women and a positive association with TNF- $\alpha$  levels among postmenopausal women.

No previous epidemiological study evaluated the leptin to adiponectin ratio regarding breast cancer risk. However, a few prospective epidemiological studies reported that high levels of leptin and low levels of adiponectin were associated with a decreased risk of breast cancer among premenopausal women (73, 77, 244), even though the associations did not reach statistical significance in some of them (73, 77). The plausibility of the associations between leptin to adiponectin ratio and premenopausal breast cancer risk needs to be further addressed in experimental studies. In premenopausal women, high levels of leptin have been hypothesized to reduce breast cancer risk through regulation of ovarian folliculogenesis (245) and reduction of follicular oestradiol secretion (246). Because the leptin to adiponectin ratio is a marker of insulin resistance (84), the inverse association found between this ratio and breast cancer risk might not be due to inflammation.

Our finding on TNF- $\alpha$  is not in line with previous prospective epidemiological studies. Most of them reported no significant associations between TNF- $\alpha$  levels and breast cancer risk (73, 82, 87-90). However, because the number of cases included was smaller than in our study, most of the previous studies were not able to categorize TNF- $\alpha$  into quartiles (73, 82, 89, 90). In contrast to our results, one prospective study reported an inverse association between high levels of TNF- $\alpha$  and breast cancer risk (91). In EPIC, results from a previous study suggested

also an increased risk of endometrial cancer in the highest quartile of TNF- $\alpha$ , compared to the lowest (247). The positive association between TNF- $\alpha$  and breast cancer is highly plausible since experimental data suggested that TNF- $\alpha$  could be involved in carcinogenesis by activating NF- $\kappa$ B and consequently inducing the expression of genes associated with cell proliferation, apoptosis, metastasis and angiogenesis (63), or by stimulating aromatase activity in breast tissue leading to oestrogen synthesis (62).

Our study, which was the largest population-based prospective cohort examining the inflammatory biomarkers-breast cancer associations, supports that menopausal status needs to be considered when assessing these associations. In our study, we did not find any heterogeneity according to molecular subtypes of breast cancer. However, some results from subgroup analyses were based on relatively small numbers of cases. Since breast cancer is a heterogeneous disease, there is a need to robustly evaluate the role of inflammation according to histologic subtypes and provide data on less common but more aggressive tumour types e.g. ER-PR- or triple negative breast cancers. Results from our study suggested that anthropometry factors were strong confounders of the associations between some inflammatory biomarkers with postmenopausal breast cancer. Indeed, obesity is an established risk factor of postmenopausal breast cancer and contribute to chronic inflammation (51-55). It is important that further studies evaluate whether anthropometry confounds or modifies the inflammation-breast cancer associations. It would also be interesting to evaluate to what extent inflammation may mediate the obesity-breast cancer association. Lastly, the inflammation pathways are correlated to the oestrogen and insulin pathways, and there is also a need to disentangle the effect on breast cancer development of inflammation pathways from the two others.

## 2. Drugs with anti-inflammatory properties and breast cancer risk

Results from our pharmacoepidemiologic studies conducted in the EPIC or E3N cohorts suggested that NSAIDs, low-dose aspirin and glucocorticoids might have different effects on breast cancer development and that these effects may differ according to the type and duration of drug use but also according to the breast cancer subtypes considered. These associations may also vary depending on subgroups of the population or on the use of other drugs. Indeed, in the EPIC and E3N cohorts, no overall association was observed between NSAID use and breast cancer risk. However, in the EPIC cohort (where NSAID use was self-reported), there was a borderline significant interaction by MHT use suggesting an inverse



association between NSAID use and breast cancer risk only among postmenopausal women who had ever used MHT. In the E3N cohort, using drug reimbursement data updated during follow-up, there was a significant interaction by PPI use suggesting an inverse association between NSAID use and breast cancer risk only among postmenopausal women who previously used PPI.

In the E3N cohort, use of aspirin at antiplatelet dosage was associated with a higher breast cancer risk few year after treatment star, followed by a lower breast cancer risk. In the study on NSAIDs, overall, aspirin at anti-inflammatory dosage was not associated with breast cancer risk. However, because it is mainly used sporadically, it was not possible to capture a long duration of use. Therefore, comparison of results across different dosage of aspirin was difficult. We also evaluated the association between clopidogrel, another antiplatelet drug, and breast cancer risk to test whether low-dose aspirin and clopidogrel showed similar associations with breast cancer risk, which was not the case, suggesting that the decreased risk of breast cancer found with a long duration of low-dose aspirin might be due to other properties than its antiplatelet properties. Indeed, clopidogrel use was associated with increased breast cancer risk that seemed restricted to ER- tumours, with no clear trend according to duration of use.

In the E3N cohort, glucocorticoid use was associated with an increased risk of *in situ* breast cancer and a decreased risk of invasive breast cancer. Among invasive cases, glucocorticoid use was associated with a decreased risk only for ER+ breast cancer, stage 1 and 2 breast cancer, or with the highest categories of cumulative number of reimbursements, and was associated with an increased risk of stage 3/4 breast cancers.

Our results on NSAIDs are consistent with the majority of results from prospective studies, suggesting no reduced risk of breast cancer with NSAID use (142, 147, 148, 150, 155, 160, 248-255). To our knowledge, most of the studies evaluating whether the NSAID-breast cancer associations differed by MHT use did not observe any significant effect modification (142, 148, 149, 250, 252, 256, 257) and only one study reported an inverse association between NSAID use for at least five years and breast cancer risk among MHT users (148). Another study showed a statistically significant positive association between standard dose aspirin use and breast cancer risk among MHT users for ten years or more (151). Nevertheless, this result should be considered cautiously because the number of exposed cases in that category was very low (n=25). In all the other studies, risk estimates associated with NSAID use among MHT users were not reported. In addition, the interaction

with MHT use was not confirmed in the E3N cohort, which has more accurate data on both MHT and NSAID use than the EPIC cohort. The modifying effect of PPI on the NSAIDs-breast cancer associations we observed within the E3N cohort was not examined in previous epidemiological studies.

Our results on low-dose aspirin are consistent with two meta-analyses which suggested a duration-response relationship between aspirin use and breast cancer risk (194, 202). In the latest randomized double-blind placebo-controlled trial (ASPREE: participants aged  $\geq 65$  years old, median follow-up of 4.7 years), daily use of low-dose aspirin (100 mg) had no effect on breast cancer incidence (199). This lack of effect could be due to the fact that follow-up as well as duration of aspirin use were not long enough to expect a benefit of aspirin (34)(35) (200)(201). However, our study shows close estimates compared to the ones found in the latest meta-analysis of randomized controlled trials on any cancer incidence and aspirin use, with a transient increase in risk in the three first years of use, followed by a decrease in risk (200). It therefore seems important to be able to assess the low-dose aspirin-breast cancer risk associations according to duration of use. In our analyses, the number of cases was too limited to evaluate the low-dose aspirin-breast cancer associations for a duration longer than five years of use. No previous epidemiological studies evaluated the clopidogrel-breast cancer association.

Our results on glucocorticoids are not in line with the only epidemiological study specifically designed to evaluate the glucocorticoid-breast cancer associations, which reported a null association between glucocorticoid use and invasive breast cancer risk (230). However, the authors were not able to stratify their analyses by breast cancer subtypes. Our results suggesting a decreased risk of invasive breast cancer especially for ER+ tumour are supported by experiments showing that glucocorticoids might stimulate the expression of the sulfotransferase SULT1E1 (which plays a role in deactivating oestrogen) (213, 216), affect angiogenesis (215), or inhibit inflammatory and growth factors (214, 258). Our findings supporting an increased risk of *in situ* breast cancer could be explained by the fact that glucocorticoids might slow down tumour growth (259, 260). An increased risk of stage 3/4 breast cancers with glucocorticoid exposure is also in line with experiments suggesting that glucocorticoids promote breast cancer metastasis (221), induce the epithelial-to-mesenchymal transition (261), or activate the TEA domain transcription factor 4 (262). In addition, in the

1960's, a study among breast cancer patients reported that the use of adrenal steroids (including glucocorticoids), compared to non-use, increased the risk of metastasis (222).

The prospective design of our studies minimizes recall bias and the large number of participants and incident breast cancer cases allowed us to examine interactions of drug use with breast cancer risk factors and to estimate associations with different breast cancer subtypes. While many medico-administrative databases do not have data on breast cancer risk factors, our studies showed that breast cancer risk factors are not major confounders in the associations between the studied drugs and breast cancer risk. Our results suggested that future pharmacoepidemiologic studies should consider breast cancer subtypes, risk factors, and use of other drugs because, in our analyses, they were found to modify the associations between anti-inflammatory/antiplatelet drug use and breast cancer risk. However, some of our subgroup analyses were based on low numbers of cases and, because of the large number of comparisons evaluated, they need to be interpreted cautiously before confirmation in other prospective studies. In the EPIC cohort, NSAID exposure was self-reported in a baseline questionnaire and there was a lack of data on characteristics of NSAID use, such as duration, dose, or frequency of use. Thus, we were not able to examine a potential dose- or duration-response relationship. In the E3N cohort, drug exposure was identified through a drug reimbursement database, which avoids differential recall bias between cases and non-cases and allowed us to consider precise information on exposure (including duration, dose, and timing of use). However, our statistical power remained limited to evaluate breast cancer risk associated with a long duration of use and future studies should be able to do so. We might be able to do so in the E3N cohort when the follow-up will be longer. In the EPIC cohort the NSAIDs-breast cancer associations did not differ according to menopausal status. In the E3N cohort, we only evaluated the effect of drugs on breast cancer development among postmenopausal women. The associations between NSAIDs, antiplatelet drugs, glucocorticoids and breast cancer risk among premenopausal is undervalued and further studies should consider menopausal status. In conclusion, there is a real need to perform large, well-powered analyses where less common tumour subtypes or subgroups can be addressed, and a long duration of use can be captured.

### 3. Perspectives

Additional evidence on the role of inflammation/anti-inflammatory drugs on breast cancer development are needed. Therefore, we planned several additional analyses (listed below).

First, in the EPIC cohort, we will use mediation analysis to evaluate and quantify:

- the role of leptin to adiponectin ratio as a mediator in the association between obesity and pre/perimenopausal breast cancer,
- and the role of TNF- $\alpha$  as a mediator in the association between obesity and postmenopausal breast cancer.

Then, we will use data from the Information System for the Development of Research on Primary Care (SIDIAP) database, in which data on drug prescription are available from 2006 and 2018, to evaluate the associations between NSAIDs use and breast cancer risk, overall and by menopausal status. During my thesis, I spent 1 month at the Institut Universitari d'Investigació en Atenció Primària (IDIAP) Jordi Gol (in Barcelona), after being awarded a French Mobility Scholarship from the Cancéropôle Lyon Auvergne Rhône-Alpes, to investigate the feasibility of this study. The data source and the selection of the study population are presented in the Annex 4.

Lastly, to provide more definitive evidence on the association of inflammation with breast cancer development, data from prospective cohort studies collaborating within the National Cancer Institute (NCI) Cohort Consortium will be pooled (project approved by the NCI cohort consortium steering committee and pending funding). Data from 22 prospective cohorts (including more than 44,000 breast cancer cases, among over 1.4 million study participants) will be pooled to evaluate the association between aspirin/NSAIDs and breast cancer risk, according to breast cancer subtypes and risk factors. Data from 14 case-control studies nested within prospective cohorts (including more than 7,200 breast cancer cases and 15,000 matched controls) will be pooled to evaluate the associations between CRP, leptin, and adiponectin with breast cancer risk, according to breast cancer subtypes and risk factors.

## Take-home messages

**The results of this thesis suggest that:**

- Inflammation plays a modest role in breast cancer development and its role might be limited to postmenopausal women.
- The relationships between the drugs with anti-inflammatory properties and breast cancer risk are complexes. In addition, because the studied drugs have several pharmacological properties, we do not know whether the drugs might impact breast cancer development through their anti-inflammatory properties or through other ones.

**Since breast cancer is a heterogeneous disease, there is a need to robustly evaluate the role of inflammation or anti-inflammatory drugs according to breast cancer subtypes within large and well-powered analyses where less common tumour subtypes or subgroups can be addressed, and, for medications, a long duration of use can be captured.**



## ANNEXES

### Annex 1: Different types of nonsteroidal anti-inflammatory drugs<sup>∞</sup>.

ATC codes <sup>‡</sup>	Molecules
<b>SALICYLIC ACID AND DERIVATIVES (N02BA) §</b>	
N02BA01	Acetylsalicylic acid
<b>BUTYLPYRAZOLIDINES (M01AA)</b>	
M01AA01	Phenylbutazone
M01AA02	Mofebutazone
M01AA03	Oxyphenbutazone
M01AA05	Clofezone
M01AA06	Kebuzone
<b>ACETIC ACID DERIVATIVES AND RELATED SUBSTANCES (M01AB)</b>	
M01AB01	Indometacin
M01AB02	Sulindac
M01AB03	Tolmetin
M01AB04	Zomepirac
M01AB05	Diclofenac
M01AB06	Alclofenac
M01AB07	Bumadizone
M01AB08	Etodolac
M01AB09	Ionazolac
M01AB10	Fentiazac
M01AB11	Acemetacin
M01AB12	Difenpiramide
M01AB13	Oxametacin
M01AB14	Proglumetacin
M01AB15	Ketorolac
M01AB16	Aceclofenac
M01AB17	Bufexamac
M01AB51	Combinations of indometacin
M01AB55	Combinations of diclofenac
<b>OXICAMS (M01AC)</b>	
M01AC01	Piroxicam
M01AC02	Tenoxicam
M01AC04	Droxicam
M01AC05	Iornoxicam
M01AC06	Meloxicam
M01AC56	Combinations of Meloxicam
<b>PROPIONIC ACID DERIVATIVES (M01AE)</b>	
M01AE01	Ibuprofen
M01AE02	Naproxen
M01AE03	Ketoprofen
M01AE04	Fenoprofen
M01AE05	Fenbufen
M01AE06	Benoxaprofen
M01AE07	Suprofen
M01AE08	Pirprofen
M01AE09	Flurbiprofen
M01AE10	Indoprofen
M01AE11	Tiaprofenic acid
M01AE12	Oxaprozin
M01AE13	Ibuproxam
M01AE14	Dexibuprofen
M01AE15	Flunocaprofen
M01AE16	Alminoprofen
M01AE17	Dexketoprofen
M01AE18	Naproxcinod
M01AE51	Combinations of ibuprofen
M01AE52	Naproxen and esomeprazole
M01AE53	Combinations of Ketoprofen
M01AE56	Naproxen and misoprostol
<b>FENAMATES (M01AG)</b>	
M01AG01	Mefenamic acid

M01AG02	Tolfenamic acid
M01AG03	Fufenamic acid
M01AG04	Meclofenamic acid
<b>COXIBS (M01AH)</b>	
M01AH01	Celecoxib
M01AH02	Rofecoxib
M01AH03	Valdecoxib
M01AH04	Parecoxib
M01AH05	Etoricoxib
M01AH06	Lumiracoxib
M01AH07	Polmacoxib
<b>OTHER ANTI-INFLAMMATORY AND ANTI-RHEUMATIC AGENTS, NON-STERIODS (M01AX)</b>	
M01AX01	Nabumetone
M01AX02	Niflumic acid
M01AX04	Azapropazone
M01AX07	Benzydamine
M01AX13	Proquazone
M01AX14	Orgotein
M01AX17	Nimesulide
M01AX18	Feprazone
M01AX22	Morniflumate
M01AX23	Tenidap
M01AX68	Combinations of Feprazone

≠ “In the ATC classification system, the active substances are classified in a hierarchy with five different levels. The system has fourteen main anatomical/pharmacological groups or 1st levels. Each ATC main group is divided into 2nd levels which could be either pharmacological or therapeutic groups. The 3rd and 4th levels are chemical, pharmacological or therapeutic subgroups and the 5th level is the chemical substance”.

§ I reported only preparations containing acetylsalicylic acid.

∞ Excluding topical preparations.

## Annex 2: Different types of antiplatelet drugs.

ATC codes <sup>‡</sup>	Molecules
<b>Platelet aggregation inhibitors excl. heparin (B01AC)</b>	
B01AC01	Ditazole
B01AC02	Cloricromen
B01AC03	Picotamide
B01AC04	Clopidogrel
B01AC05	Ticlopidine
B01AC06	Acetylsalicylic acid
B01AC07	Dipyridamole
B01AC08	Carbasalate calcium
B01AC09	Epoprostenol
B01AC10	Indobufen
B01AC11	Iloprost
B01AC13	Abciximab
B01AC15	Aloxiaprin
B01AC16	Eptifibatide
B01AC17	Tirofiban
B01AC18	Triflusal
B01AC19	Beraprost
B01AC21	Treprostinil
B01AC22	Prasugrel
B01AC23	Cilostazol
B01AC24	Ticagrelor
B01AC25	Cangrelor
B01AC26	Vorapaxar
B01AC27	Selexipag
B01AC30	Combinations of acetylsalicylic acid
B01AC56	Acetylsalicylic acid and proton pump inhibitors

‡ “In the ATC classification system, the active substances are classified in a hierarchy with five different levels. The system has fourteen main anatomical/pharmacological groups or 1st levels. Each ATC main group is divided into 2nd levels which could be either pharmacological or therapeutic groups. The 3rd and 4th levels are chemical, pharmacological or therapeutic subgroups and the 5th level is the chemical substance”.

### Annex 3: Different types of systemic glucocorticoids.

ATC codes <sup>‡</sup>	Molecules
<b>Glucocorticoids for systemic use (H02AB)</b>	
H02AB01	Betamethasone
H02AB02	Dexamethasone
H02AB03	Fluocortolone
H02AB04	Methylprednisolone
H02AB05	Paramethasone
H02AB06	Prednisolone
H02AB07	Prednisone
H02AB08	Triamcinolone
H02AB09	Hydrocortisone
H02AB10	Cortisone
H02AB11	Prednylidene
H02AB12	Rimexolone
H02AB13	Deflazacort
H02AB14	Cloprednol
H02AB15	Meprednisone
H02AB17	Cortivazol

<sup>‡</sup> “In the ATC classification system, the active substances are classified in a hierarchy with five different levels. The system has fourteen main anatomical/pharmacological groups or 1st levels. Each ATC main group is divided into 2nd levels which could be either pharmacological or therapeutic groups. The 3rd and 4th levels are chemical, pharmacological or therapeutic subgroups and the 5th level is the chemical substance”.

#### Annex 4: Description of the SIDIAP database and study population.

##### **The Information System for the Development of Research in Primary Care database**

SIDIAP (<http://www.sidiap.org/index.php/en>) is a large prospective population-based database in Catalonia. In Spain, general practitioners (GP)s and nurses play an essential role in the public health care system as they are responsible for primary health care, long-term prescriptions, and specialist/hospital referrals. The Spanish public healthcare system covers more than 98% of the population, and more than two thirds of the Catalan population see their GP at least once a year (263). The computerisation of the primary care patient records of the Catalan Health Institute (CHI) was completed in 2005. SIDIAP was designed to provide a valid and reliable database of selected information from clinical records of patients registered in primary care centres for use in biomedical research. SIDIAP contains data of anonymized patients' healthcare records for nearly six million people (approximately 80% of the Catalan population) registered in 287 primary care practices throughout Catalonia since 2005 (264, 265). It was previously shown that SIDIAP population is highly representative of the entire Catalan region in terms of geographic, age, and sex distributions (265).

SIDIAP includes data collected by health professionals during routine visits in primary care, including anthropometric measurements, clinical diagnoses (International Classification of Diseases 10<sup>th</sup> revision [ICD-10]), laboratory tests, treatments, hospital referrals, demographic and lifestyle information. It provides an excellent source of population-based data and reliably reproduces the actual conditions of clinical practice. The high quality of these data has been previously documented (264, 265), and SIDIAP has been successfully applied to epidemiological studies of key exposures and outcomes including cancers (266). In addition, data on cancer cases recorded in primary care were validated through a comparison of SIDIAP and two Catalonia population-based cancer registries (267). High sensitivity was observed for most cancer types, with the highest sensitivity for breast (89%, 95% CI: 88–90%). Lifestyle variables and diagnoses of health problems are recorded by GPs following the Catalan Clinical Practice Guidelines. Quality checks to identify duplicate patient IDs are performed centrally at each SIDIAP database update, which is done annually. Checks for logical values and data harmonisation are performed. For biochemistry data, consistency for measurements taken in different laboratories is assessed, and unit conversion is undertaken when needed.



### Case-control selection

In the following, we have defined SIDIAP0 as the date of entry into the SIDIAP database. In a first step, we extracted data from women with at least 1.5 years of medical history in the SIDIAP database (i.e. not deceased, not lost to-follow-up, not reaching the end of data availability into SIDIAP within 1.5 years after SIDIAP0). Women with any cancer (except basal cell carcinoma) within 1.5 years after SIDIAP0 are excluded.

Within the cohort defined above, we will perform a nested case-control study using risk set sampling (cases are eligible for sampling as controls before their diagnosis date).

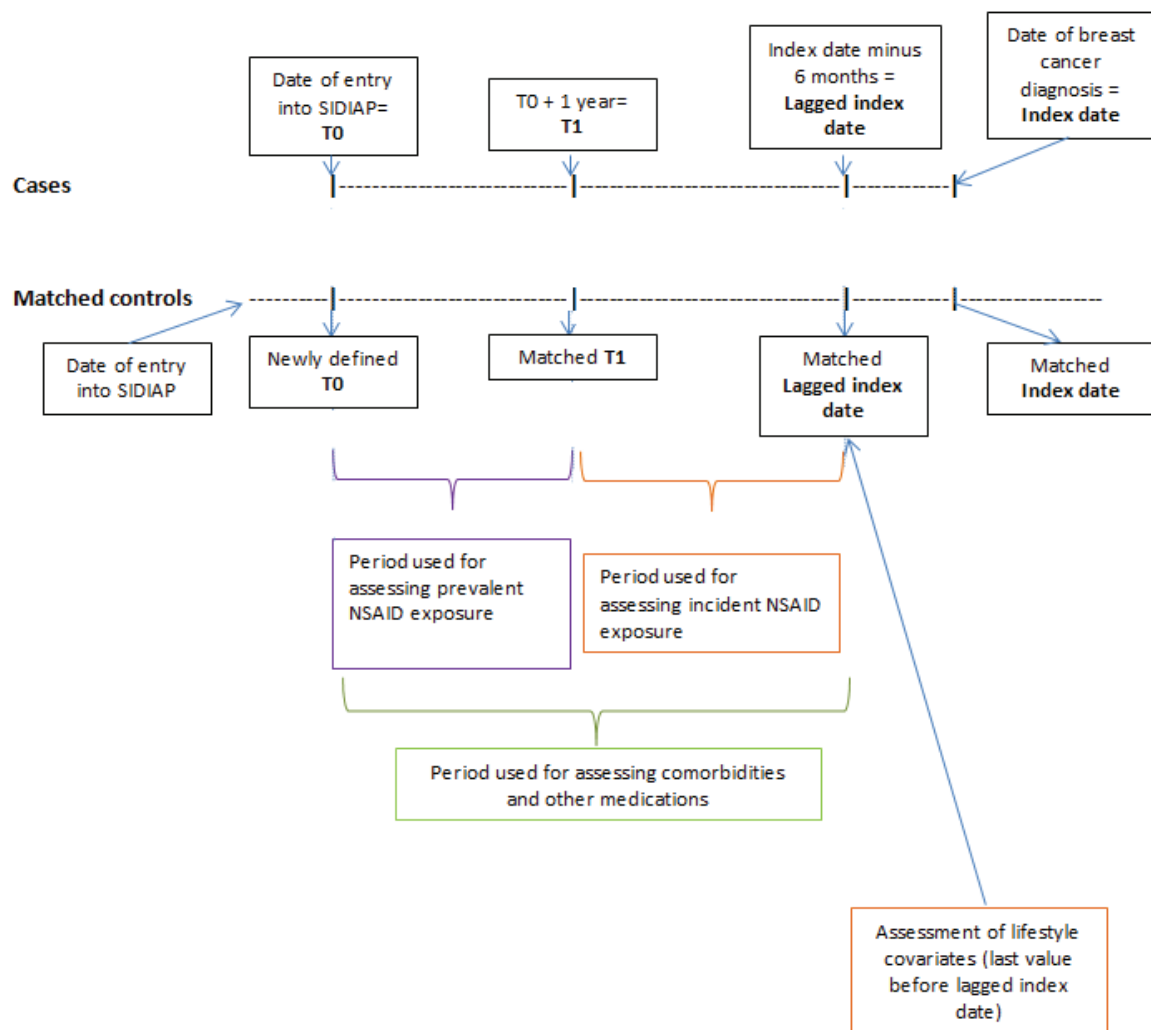
**Cases** are women with breast cancer diagnosed after SIDIAP0+1.5 years. **For cases**, the date of breast cancer diagnosis defines the index date, and T0 is equal to SIDIAP0. We further define T1 as T0 + one year.

**Controls** (up to 10 per case) are women with no cancer (any cancer except basal cell carcinoma) diagnosed between SIDIAP0 and the time of the case's breast cancer diagnosis, alive and still registered into SIDIAP at that date. They are matched on year of birth ( $\pm 1$  year), primary care centre, menopausal status, and must have a medical history duration (time between SIDIAP0 and the time of the matched case's breast cancer diagnosis)  $\geq$  the medical history duration of the matched case. **Controls** are assigned the same index date, the same T0, and the same T1 as those of the matched case (since medical history of the control is longer than the one of the case, a new T0 is defined, equal to the T0 of the case). For latency considerations and in order to minimize reverse causality, we define a lagged index date which is the index date minus 6 months (242). Any exposure or adjustment data recorded after the lagged index date (likely not causal) is disregarded.

***Index date = date of each case's breast cancer diagnosis***

***Lagged index date = Index date minus 6 months***

The T0-T1 period is used to identify prevalent NSAID exposure. It is a period with no cancer diagnoses (see Figure below). Finally, 24,697 cases will be included and 117,916 matched controls. The median age of the study population at T0 is 53 years old. In the study population 67% of women had at least 1 prescription of NSAID or aspirin and 34% had at least 2 prescriptions in any three-month period.



## REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2018.
2. Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol*. 2012;30:677-706.
3. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. *Nature reviews Clinical oncology*. 2015;12(10):584-96.
4. Vainio H, Morgan G. Non-steroidal anti-inflammatory drugs and chemoprevention of cancer. *Ann Chir Gynaecol*. 2000;89(3):173-6.
5. Hassiotou F, Geddes D. Anatomy of the human mammary gland: Current status of knowledge. *Clinical anatomy (New York, NY)*. 2013;26(1):29-48.
6. Pitot HC. The molecular biology of carcinogenesis. *Cancer*. 1993;72(3 Suppl):962-70.
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-74.
8. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57-70.
9. Lakhani SR, Ellis IO, Schnitt S, Tan PH, van de Vijver MJ. *WHO Classification of Tumours of the Breast*. IARC: Lyon, France. 2012.
10. Sobin LH, Gospodarowicz MK, Wittekind C. *International Union Against Cancer (UICC). TNM classification of malignant tumours*. 7th Edition. Wiley, Chichester. 2009.
11. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*. 1991;19(5):403-10.
12. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*. 2002;41(3a):151-2, discussion 2-3.
13. Key TJ, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *European journal of cancer & clinical oncology*. 1988;24(1):29-43.
14. Yip CH, Rhodes A. Estrogen and progesterone receptors in breast cancer. *Future oncology (London, England)*. 2014;10(14):2293-301.
15. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010;28(16):2784-95.
16. Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. *Archives of pathology & laboratory medicine*. 2011;135(1):55-62.
17. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013;31(31):3997-4013.
18. Arciero CA, Guo Y, Jiang R, Behera M, O'Regan R, Peng L, et al. ER(+)/HER2(+) Breast Cancer Has Different Metastatic Patterns and Better Survival Than ER(-)/HER2(+) Breast Cancer. *Clin Breast Cancer*. 2019;19(4):236-45.
19. Hwang KT, Kim J, Jung J, Chang JH, Chai YJ, Oh SW, et al. Impact of Breast Cancer Subtypes on Prognosis of Women with Operable Invasive Breast Cancer: A Population-based Study Using SEER Database. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2019;25(6):1970-9.
20. Ferlay J EM, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2018). *Global Cancer Observatory: Cancer Today*. Lyon, France: International Agency for Research on Cancer. Available from: <https://gco.iarc.fr/today>, accessed on 22 July 2020.

21. Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, et al. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet* (London, England). 2015;385(9972):977-1010.
22. Soerjomataram I, Shield K, Marant-Micallef C, Vignat J, Hill C, Rogel A, et al. Cancers related to lifestyle and environmental factors in France in 2015. *European journal of cancer* (Oxford, England : 1990). 2018;105:103-13.
23. Korde LA, Zujewski JA, Kamin L, Giordano S, Domchek S, Anderson WF, et al. Multidisciplinary meeting on male breast cancer: summary and research recommendations. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010;28(12):2114-22.
24. Fidler MM, Gupta S, Soerjomataram I, Ferlay J, Steliarova-Foucher E, Bray F. Cancer incidence and mortality among young adults aged 20-39 years worldwide in 2012: a population-based study. *The Lancet Oncology*. 2017;18(12):1579-89.
25. Kwong A, Shin VY, Ho JCW, Kang E, Nakamura S, Teo S-H, et al. Comprehensive spectrum of BRCA1 and BRCA2 deleterious mutations in breast cancer in Asian countries. *J Med Genet*. 2016;53(1):15-23.
26. Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. *Lancet* (London, England). 2001;358(9291):1389-99.
27. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *American journal of human genetics*. 2003;72(5):1117-30.
28. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. *Molecular oncology*. 2010;4(3):174-91.
29. Skol AD, Sasaki MM, Onel K. The genetics of breast cancer risk in the post-genome era: thoughts on study design to move past BRCA and towards clinical relevance. *Breast cancer research : BCR*. 2016;18(1):99.
30. Salamat FM, Niakan BM, Keshtkar AP, Rafiei EM, Zendehdel MM. Subtypes of Benign Breast Disease as a Risk Factor of Breast Cancer: A Systematic Review and Meta Analyses. *Iranian journal of medical sciences*. 2018;43(4):355-64.
31. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2006;15(6):1159-69.
32. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. *The New England journal of medicine*. 2007;356(3):227-36.
33. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2004;13(10):1558-68.
34. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *The Lancet Oncology*. 2012;13(11):1141-51.
35. Nichols HB, Schoemaker MJ, Cai J, Xu J, Wright LB, Brook MN, et al. Breast Cancer Risk After Recent Childbirth: A Pooled Analysis of 15 Prospective Studies. *Annals of internal medicine*. 2019;170(1):22-30.
36. Beral V, Bull D, Doll R, Peto R, Reeves G. Breast cancer and abortion: collaborative reanalysis of data from 53 epidemiological studies, including 83,000 women with breast cancer from 16 countries. *Lancet* (London, England). 2004;363(9414):1007-16.

37. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet* (London, England). 2002;360(9328):187-95.
38. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans :combined estrogen-progestogen contraceptives and combined estrogen-progestogen menopausal therapy. A review of human carcinogens. Lyon, France: International Agency for Research on Cancer; 2007.
39. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet* (London, England). 1996;347(9017):1713-27.
40. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. *Lancet* (London, England). 2019;394(10204):1159-68.
41. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. A review of human carcinogens. Part A : Pharmaceuticals. Lyon: 2012.
42. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Report: Diet, Nutrition, Physical activity an Breast Cancer. 2017. Available at: [wcrf.org/breast.cancer-2017](http://wcrf.org/breast.cancer-2017).
43. Tobacco smoke and involuntary smoking. IARC monographs on the evaluation of carcinogenic risks to humans. 2004;83:1-1438.
44. A review of human carcinogens. Part E: Personal habits and indoor combustions / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2009: Lyon, France).
45. Ronckers CM, Erdmann CA, Land CE. Radiation and breast cancer: a review of current evidence. *Breast cancer research : BCR*. 2005;7(1):21-32.
46. IARC (2020). Night shift work. *IARC Monogr Identif Carcinog Hazards Hum*, 124:1–371.
47. Ahmed A. An overview of inflammation: Mechanism and consequences. *Frontiers in Biology*. 2011;6.
48. Tergaonkar V. NFkappaB pathway: a good signaling paradigm and therapeutic target. *The international journal of biochemistry & cell biology*. 2006;38(10):1647-53.
49. Gomes RN, Felipe da Costa S, Colquhoun A. Eicosanoids and cancer. *Clinics*. 2018;73(suppl 1):e530s.
50. Engin AB. Adipocyte-Macrophage Cross-Talk in Obesity. *Adv Exp Med Biol*. 2017;960:327-43.
51. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm*. 2006;74:443-77.
52. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259(5091):87-91.
53. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89(6):2548-56.
54. Schaffler A, Muller-Ladner U, Scholmerich J, Buchler C. Role of adipose tissue as an inflammatory organ in human diseases. *Endocr Rev*. 2006;27(5):449-67.
55. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Molecular and cellular endocrinology*. 2010;316(2):129-39.
56. Medzhitov R. Origin and physiological roles of inflammation. *Nature*. 2008;454(7203):428-35.
57. Piotrowski I, Kulcenty K, Suchorska W. Interplay between inflammation and cancer. Reports of practical oncology and radiotherapy : journal of Greatpoland Cancer Center in Poznan and Polish Society of Radiation Oncology. 2020;25(3):422-7.



58. Wang D, DuBois RN. Immunosuppression associated with chronic inflammation in the tumor microenvironment. *Carcinogenesis*. 2015;36(10):1085-93.
59. Trinchieri G. Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol*. 2012;30:677-706.
60. Hong DS, Angelo LS, Kurzrock R. Interleukin-6 and its receptor in cancer: implications for Translational Therapeutics. *Cancer*. 2007;110(9):1911-28.
61. Knupfer H, Preiss R. Significance of interleukin-6 (IL-6) in breast cancer (review). *Breast Cancer Res Treat*. 2007;102(2):129-35.
62. Zhao Y, Nichols JE, Valdez R, Mendelson CR, Simpson ER. Tumor necrosis factor- $\alpha$  stimulates aromatase gene expression in human adipose stromal cells through use of an activating protein-1 binding site upstream of promoter 1.4. *Molecular endocrinology* (Baltimore, Md). 1996;10(11):1350-7.
63. Karin M. NF-kappaB and cancer: mechanisms and targets. *Molecular carcinogenesis*. 2006;45(6):355-61.
64. Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. *The Journal of endocrinology*. 2014;222(3):R113-27.
65. Hernandez AV, Guarnizo M, Miranda Y, Pasupuleti V, Deshpande A, Paico S, et al. Association between insulin resistance and breast carcinoma: a systematic review and meta-analysis. *PloS one*. 2014;9(6):e99317.
66. Jarde T, Perrier S, Vasson MP, Caldefie-Chezet F. Molecular mechanisms of leptin and adiponectin in breast cancer. *European journal of cancer* (Oxford, England : 1990). 2011;47(1):33-43.
67. Key TJ, Appleby PN, Reeves GK, Roddam AW. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. *The Lancet Oncology*. 2010;11(6):530-42.
68. Sugiyama M, Takahashi H, Hosono K, Endo H, Kato S, Yoneda K, et al. Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. *International journal of oncology*. 2009;34(2):339-44.
69. Bråkenhielm E, Veitonmäki N, Cao R, Kihara S, Matsuzawa Y, Zhiotovskiy B, et al. Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(8):2476-81.
70. Nkhata KJ, Ray A, Schuster TF, Grossmann ME, Cleary MP. Effects of adiponectin and leptin co-treatment on human breast cancer cell growth. *Oncology reports*. 2009;21(6):1611-9.
71. Chan DS, Bandera EV, Greenwood DC, Norat T. Circulating C-Reactive Protein and Breast Cancer Risk-Systematic Literature Review and Meta-analysis of Prospective Cohort Studies. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2015;24(10):1439-49.
72. Wang J, Lee IM, Tworoger SS, Buring JE, Ridker PM, Rosner B, et al. Plasma C-Reactive Protein and Risk of Breast Cancer in Two Prospective Studies and a Meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2015.
73. Agnoli C, Grioni S, Pala V, Allione A, Matullo G, Gaetano CD, et al. Biomarkers of inflammation and breast cancer risk: a case-control study nested in the EPIC-Varese cohort. *Scientific reports*. 2017;7(1):12708.
74. Gunter MJ, Wang T, Cushman M, Xue X, Wassertheil-Smoller S, Strickler HD, et al. Circulating Adipokines and Inflammatory Markers and Postmenopausal Breast Cancer Risk. *J Natl Cancer Inst*. 2015;107(9).
75. Dossus L, Jimenez-Corona A, Romieu I, Boutron-Ruault MC, Boutten A, Dupre T, et al. C-reactive protein and postmenopausal breast cancer risk: results from the E3N cohort study. *Cancer Causes Control*. 2014;25(4):533-9.
76. Macis D, Guerrieri-Gonzaga A, Gandini S. Circulating adiponectin and breast cancer risk: a systematic review and meta-analysis. *International journal of epidemiology*. 2014;43(4):1226-36.

77. Tworoger SS, Eliassen AH, Kelesidis T, Colditz GA, Willett WC, Mantzoros CS, et al. Plasma adiponectin concentrations and risk of incident breast cancer. *The Journal of clinical endocrinology and metabolism*. 2007;92(4):1510-6.
78. Gaudet MM, Falk RT, Gierach GL, Lacey JV, Jr., Graubard BI, Dorgan JF, et al. Do adipokines underlie the association between known risk factors and breast cancer among a cohort of United States women? *Cancer epidemiology*. 2010;34(5):580-6.
79. Touvier M, Fezeu L, Ahluwalia N, Julia C, Charnaux N, Sutton A, et al. Association between prediagnostic biomarkers of inflammation and endothelial function and cancer risk: a nested case-control study. *American journal of epidemiology*. 2013;177(1):3-13.
80. Cust AE, Stocks T, Lukanova A, Lundin E, Hallmans G, Kaaks R, et al. The influence of overweight and insulin resistance on breast cancer risk and tumour stage at diagnosis: a prospective study. *Breast cancer research and treatment*. 2009;113(3):567-76.
81. Pan H, Deng LL, Cui JQ, Shi L, Yang YC, Luo JH, et al. Association between serum leptin levels and breast cancer risk: An updated systematic review and meta-analysis. *Medicine*. 2018;97(27):e11345.
82. Gross AL, Newschaffer CJ, Hoffman-Bolton J, Rifai N, Visvanathan K. Adipocytokines, inflammation, and breast cancer risk in postmenopausal women: a prospective study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2013;22(7):1319-24.
83. Harris HR, Tworoger SS, Hankinson SE, Rosner BA, Michels KB. Plasma leptin levels and risk of breast cancer in premenopausal women. *Cancer prevention research*. 2011;4(9):1449-56.
84. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, et al. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. *Diabetologia*. 2009;52(11):2345-9.
85. Korner A, Pazaitou-Panayiotou K, Kelesidis T, Kelesidis I, Williams CJ, Kaprara A, et al. Total and high-molecular-weight adiponectin in breast cancer: in vitro and in vivo studies. *J Clin Endocrinol Metab*. 2007;92(3):1041-8.
86. Chen DC, Chung YF, Yeh YT, Chaung HC, Kuo FC, Fu OY, et al. Serum adiponectin and leptin levels in Taiwanese breast cancer patients. *Cancer Lett*. 2006;237(1):109-14.
87. Heikkila K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, et al. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. *Cancer Causes Control*. 2009;20(1):15-26.
88. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer Epidemiol Biomarkers Prev*. 2005;14(10):2413-8.
89. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005;14(10):2413-8.
90. Krajcik RA, Massardo S, Orentreich N. No association between serum levels of tumor necrosis factor-alpha (TNF-alpha) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2003;12(9):945-6.
91. Dias JA, Fredrikson GN, Ericson U, Gullberg B, Hedblad B, Engstrom G, et al. Low-Grade Inflammation, Oxidative Stress and Risk of Invasive Post-Menopausal Breast Cancer - A Nested Case-Control Study from the Malmo Diet and Cancer Cohort. *PloS one*. 2016;11(7):e0158959.
92. Montinari MR, Minelli S, De Caterina R. The first 3500 years of aspirin history from its roots - A concise summary. *Vascular pharmacology*. 2019;113:1-8.

93. Conaghan PG. A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity. *Rheumatol Int.* 2012;32(6):1491-502.
94. Ameli, le site de l'Assurance Maladie en ligne. Bien utiliser les anti-inflammatoires non stéroïdiens (AINS). Available from: <https://www.ameli.fr/assure/sante/utiliser-medicaments/utiliser-anti-inflammatoires>, accessed on 31 August 2020. [
95. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature: New biology.* 1971;231(25):232-5.
96. Flower RJ, Vane JR. Inhibition of prostaglandin biosynthesis. *Biochemical pharmacology.* 1974;23(10):1439-50.
97. Hemler M, Lands WE. Purification of the cyclooxygenase that forms prostaglandins. Demonstration of two forms of iron in the holoenzyme. *The Journal of biological chemistry.* 1976;251(18):5575-9.
98. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annual review of pharmacology and toxicology.* 1998;38:97-120.
99. Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. *The American journal of medicine.* 1998;104(3a):2S-8S; discussion 21S-2S.
100. Pitt B, Pepine C, Willerson JT. Cyclooxygenase-2 inhibition and cardiovascular events. *Circulation.* 2002;106(2):167-9.
101. Rao P, Knaus EE. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques.* 2008;11(2):81s-110s.
102. Steinmeyer J. Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. *Arthritis research.* 2000;2(5):379-85.
103. Banque de donnée sur les médicaments Thériaque. Available from: <https://www.theriaque.org>, accessed 31 August 2020.
104. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *The New England journal of medicine.* 2001;345(6):433-42.
105. Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM). Rappel des règles de bon usage des anti-inflammatoires non stéroïdiens (AINS). Juillet 2013. Available from: [https://ansm.sante.fr/var/ansm\\_site/storage/original/application/53960970b52f1b0c30da77518e8c86d7.pdf](https://ansm.sante.fr/var/ansm_site/storage/original/application/53960970b52f1b0c30da77518e8c86d7.pdf), accessed on 31 August 2020.
106. Lassalle M, Le Tri T, Bardou M, Biour M, Kirchgesner J, Rouby F, et al. Use of proton pump inhibitors in adults in France: a nationwide drug utilization study. *European journal of clinical pharmacology.* 2020;76(3):449-57.
107. Li H, Lee MH, Liu K, Wang T, Song M, Han Y, et al. Inhibiting breast cancer by targeting the thromboxane A2 pathway. *NPJ precision oncology.* 2017;1(1):8.
108. Karmali RA, Welt S, Thaler HT, Lefevre F. Prostaglandins in breast cancer: relationship to disease stage and hormone status. *British journal of cancer.* 1983;48(5):689-96.
109. Bennett A, Del Tacca M. Proceedings: Prostaglandins in human colonic carcinoma. *Gut.* 1975;16(5):409.
110. Robertson FM, Parrett ML, Joarder FS, Ross M, Abou-Issa HM, Alshafie G, et al. Ibuprofen-induced inhibition of cyclooxygenase isoform gene expression and regression of rat mammary carcinomas. *Cancer letters.* 1998;122(1-2):165-75.
111. Diaz-Cruz ES, Shapiro CL, Brueggemeier RW. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *The Journal of clinical endocrinology and metabolism.* 2005;90(5):2563-70.
112. Jiang XH, Wong BC. Cyclooxygenase-2 inhibition and gastric cancer. *Current pharmaceutical design.* 2003;9(27):2281-8.
113. Mann JR, DuBois RN. Cyclooxygenase-2 and gastrointestinal cancer. *Cancer journal (Sudbury, Mass.).* 2004;10(3):145-52.

114. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: A review. *Journal of cellular physiology*. 2019;234(5):5683-99.
115. Hugo HJ, Saunders C, Ramsay RG, Thompson EW. New Insights on COX-2 in Chronic Inflammation Driving Breast Cancer Growth and Metastasis. *Journal of mammary gland biology and neoplasia*. 2015;20(3-4):109-19.
116. Howe LR. Inflammation and breast cancer. *Cyclooxygenase/prostaglandin signaling and breast cancer*. *Breast cancer research : BCR*. 2007;9(4):210.
117. Fahlén M, Zhang H, Löfgren L, Masironi B, von Schoultz E, von Schoultz B, et al. Expression of cyclooxygenase-1 and cyclooxygenase-2, syndecan-1 and connective tissue growth factor in benign and malignant breast tissue from premenopausal women. *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology*. 2017;33(5):353-8.
118. Erovic BM, Woegerbauer M, Pammer J, Selzer E, Grasl M, Thurnher D. Strong evidence for up-regulation of cyclooxygenase-1 in head and neck cancer. *European journal of clinical investigation*. 2008;38(1):61-6.
119. Gupta RA, Tejada LV, Tong BJ, Das SK, Morrow JD, Dey SK, et al. Cyclooxygenase-1 is overexpressed and promotes angiogenic growth factor production in ovarian cancer. *Cancer research*. 2003;63(5):906-11.
120. Wang D, Dubois RN. Prostaglandins and cancer. *Gut*. 2006;55(1):115-22.
121. Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. *The Journal of biological chemistry*. 2003;278(37):35451-7.
122. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nature medicine*. 2002;8(3):289-93.
123. Lala PK, Nandi P, Majumder M. Roles of prostaglandins in tumor-associated lymphangiogenesis with special reference to breast cancer. *Cancer Metastasis Rev*. 2018;37(2-3):369-84.
124. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer research*. 1998;58(2):362-6.
125. Wang D, Buchanan FG, Wang H, Dey SK, DuBois RN. Prostaglandin E2 enhances intestinal adenoma growth via activation of the Ras-mitogen-activated protein kinase cascade. *Cancer research*. 2005;65(5):1822-9.
126. Thompson HJ, Briggs S, Paranka NS, Piazza GA, Brendel K, Gross PH, et al. Inhibition of mammary carcinogenesis in rats by sulfone metabolite of sulindac. *Journal of the National Cancer Institute*. 1995;87(16):1259-60.
127. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science (New York, NY)*. 2001;293(5535):1673-7.
128. Yiannakopoulou E. Aspirin and NSAIDs for breast cancer chemoprevention. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)*. 2015;24(5):416-21.
129. Zhang Z, DuBois RN. Par-4, a proapoptotic gene, is regulated by NSAIDs in human colon carcinoma cells. *Gastroenterology*. 2000;118(6):1012-7.
130. Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of BAX in the apoptotic response to anticancer agents. *Science (New York, NY)*. 2000;290(5493):989-92.
131. Yamamoto Y, Yin MJ, Lin KM, Gaynor RB. Sulindac inhibits activation of the NF-kappaB pathway. *The Journal of biological chemistry*. 1999;274(38):27307-14.
132. International Agency for Research on Cancer (1997). *IARC Handbooks of Cancer Prevention: Nonsteroidal Anti-inflammatory Drugs*. Volume 1. Lyon, France: International Agency for Research on Cancer. Available on <http://publications.iarc.fr/Book-And-Report-Series/Iarc-Handbooks-Of-CancerPrevention/Non-Steroidal-Anti-Inflammatory-Drugs-1997>.
133. Kudo T, Narisawa T, Abo S. Antitumor activity of indomethacin on methylazoxymethanol-induced large bowel tumors in rats. *Gan*. 1980;71(2):260-4.



134. Narisawa T, Satoh M, Sano M, Takahashi T. Inhibition of initiation and promotion by N-methylnitrosourea-induced colon carcinogenesis in rats by non-steroid anti-inflammatory agent indomethacin. *Carcinogenesis*. 1983;4(10):1225-7.
135. Pollard M, Luckert PH. Prolonged antitumor effect of indomethacin on autochthonous intestinal tumors in rats. *Journal of the National Cancer Institute*. 1983;70(6):1103-5.
136. Jalbert G, Castonguay A. Effects of NSAIDs on NNK-induced pulmonary and gastric tumorigenesis in A/J mice. *Cancer letters*. 1992;66(1):21-8.
137. Thompson HJ, Jiang C, Lu J, Mehta RG, Piazza GA, Paranka NS, et al. Sulfone metabolite of sulindac inhibits mammary carcinogenesis. *Cancer research*. 1997;57(2):267-71.
138. McCormick DL, Madigan MJ, Moon RC. Modulation of rat mammary carcinogenesis by indomethacin. *Cancer research*. 1985;45(4):1803-8.
139. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology (Cambridge, Mass)*. 1994;5(2):138-46.
140. Harris RE, Namboodiri KK, Farrar WB. Nonsteroidal antiinflammatory drugs and breast cancer. *Epidemiology (Cambridge, Mass)*. 1996;7(2):203-5.
141. Paganini-Hill A, Chao A, Ross RK, Henderson BE. Aspirin use and chronic diseases: a cohort study of the elderly. *BMJ (Clinical research ed)*. 1989;299(6710):1247-50.
142. Egan KM, Stampfer MJ, Giovannucci E, Rosner BA, Colditz GA. Prospective study of regular aspirin use and the risk of breast cancer. *Journal of the National Cancer Institute*. 1996;88(14):988-93.
143. Rosenberg L. Nonsteroidal anti-inflammatory drugs and cancer. *Preventive medicine*. 1995;24(2):107-9.
144. Veettil SK, Jinatongthai P, Nathisuwan S, Teerawattanapong N, Ching SM, Lim KG, et al. Efficacy and safety of chemopreventive agents on colorectal cancer incidence and mortality: systematic review and network meta-analysis. *Clinical epidemiology*. 2018;10:1433-45.
145. Tomic T, Dominguez-Lopez S, Barrios-Rodriguez R. Non-aspirin non-steroidal anti-inflammatory drugs in prevention of colorectal cancer in people aged 40 or older: A systematic review and meta-analysis. *Cancer epidemiology*. 2019;58:52-62.
146. de Pedro M, Baeza S, Escudero MT, Dierssen-Sotos T, Gomez-Acebo I, Pollan M, et al. Effect of COX-2 inhibitors and other non-steroidal inflammatory drugs on breast cancer risk: a meta-analysis. *Breast cancer research and treatment*. 2015;149(2):525-36.
147. Bardia A, Keenan TE, Ebbert JO, Lazovich D, Wang AH, Vierkant RA, et al. Personalizing Aspirin Use for Targeted Breast Cancer Chemoprevention in Postmenopausal Women. *Mayo Clin Proc*. 2016;91(1):71-80.
148. 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 research*. 2003;63(18):6096-101.
149. Friis S, Thomassen L, Sorensen HT, Tjonneland A, Overvad K, Cronin-Fenton DP, et al. Nonsteroidal anti-inflammatory drug use and breast cancer risk: a Danish cohort study. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)*. 2008;17(2):88-96.
150. 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.
151. Ready A, Velicer CM, McTiernan A, White E. NSAID use and breast cancer risk in the VITAL cohort. *Breast cancer research and treatment*. 2008;109(3):533-43.
152. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA. Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage. *British journal of cancer*. 2000;83(1):112-20.
153. Sorensen HT, Friis S, Norgard B, Mellemkjaer L, Blot WJ, McLaughlin JK, et al. Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study. *British journal of cancer*. 2003;88(11):1687-92.



154. Rahme E, Ghosn J, Dasgupta K, Rajan R, Hudson M. Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer. *BMC cancer*. 2005;5:159.
155. Meier CR, Schmitz S, Jick H. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. *Pharmacotherapy*. 2002;22(3):303-9.
156. Cronin-Fenton DP, Pedersen L, Lash TL, Friis S, Baron JA, Sørensen HT. Prescriptions for selective cyclooxygenase-2 inhibitors, non-selective non-steroidal anti-inflammatory drugs, and risk of breast cancer in a population-based case-control study. *Breast cancer research : BCR*. 2010;12(2):R15.
157. Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD. Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. *Breast (Edinburgh, Scotland)*. 2011;20(1):66-70.
158. Vinogradova Y, Coupland C, Hippisley-Cox J. Exposure to cyclooxygenase-2 inhibitors and risk of cancer: nested case-control studies. *British journal of cancer*. 2011;105(3):452-9.
159. Hung CH, Lin YC, Chang YH, Lin YC, Huang HY, Yeh WJ, et al. The effect of NSAIDs exposure on breast cancer risk in female patients with autoimmune diseases. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)*. 2018.
160. Garcia Rodriguez LA, Gonzalez-Perez A. Risk of breast cancer among users of aspirin and other anti-inflammatory drugs. *British journal of cancer*. 2004;91(3):525-9.
161. Gibson PC. Aspirin in the treatment of vascular diseases. *Lancet (London, England)*. 1949;2(6591):1172-4.
162. Bruno A, Dovizio M, Tacconelli S, Contursi A, Ballerini P, Patrignani P. Antithrombotic Agents and Cancer. *Cancers*. 2018;10(8):253.
163. Haute Autorité de Santé and Agence Nationale de Sécurité du Médicament et des produits de santé. Bon usage des agents antiplaquettaires. Juin 2012. Available from: <https://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/Recommandations-ANSM-HAS-Bon-usage-des-agents-antiplaquettaires-Point-d-information>, accessed on 30 August 2020.
164. Conroy RM, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *European heart journal*. 2003;24(11):987-1003.
165. Stevens RJ, Kothari V, Adler AI, Stratton IM. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clinical science (London, England : 1979)*. 2001;101(6):671-9.
166. van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. *Nature reviews Cardiology*. 2019;16(3):166-79.
167. Eikelboom JW, Hirsh J, Spencer FA, Baglin TP, Weitz JI. Antiplatelet drugs: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2 Suppl):e89S-e119S.
168. Franco AT, Corken A, Ware J. Platelets at the interface of thrombosis, inflammation, and cancer. *Blood*. 2015;126(5):582-8.
169. Olsson AK, Cedervall J. The pro-inflammatory role of platelets in cancer. *Platelets*. 2018;29(6):569-73.
170. Sylman JL, Boyce HB, Mitrugno A, Tormoen GW, Thomas IC, Wagner TH, et al. A Temporal Examination of Platelet Counts as a Predictor of Prognosis in Lung, Prostate, and Colon Cancer Patients. *Scientific reports*. 2018;8(1):6564.
171. Wan S, Lai Y, Myers RE, Li B, Hyslop T, London J, et al. Preoperative platelet count associates with survival and distant metastasis in surgically resected colorectal cancer patients. *Journal of gastrointestinal cancer*. 2013;44(3):293-304.
172. Lee YS, Suh KW, Oh SY. Preoperative thrombocytosis predicts prognosis in stage II colorectal cancer patients. *Annals of surgical treatment and research*. 2016;90(6):322-7.

173. Taucher S, Salat A, Gnant M, Kwasny W, Mlineritsch B, Menzel RC, et al. Impact of pretreatment thrombocytosis on survival in primary breast cancer. *Thrombosis and haemostasis*. 2003;89(6):1098-106.
174. Yu D, Liu B, Zhang L, Du K. Platelet count predicts prognosis in operable non-small cell lung cancer. *Experimental and therapeutic medicine*. 2013;5(5):1351-4.
175. Aoe K, Hiraki A, Ueoka H, Kiura K, Tabata M, Tanaka M, et al. Thrombocytosis as a useful prognostic indicator in patients with lung cancer. *Respiration; international review of thoracic diseases*. 2004;71(2):170-3.
176. Suppiah R, Shaheen PE, Elson P, Misbah SA, Wood L, Motzer RJ, et al. Thrombocytosis as a prognostic factor for survival in patients with metastatic renal cell carcinoma. *Cancer*. 2006;107(8):1793-800.
177. Lopes A, Daras V, Cross PA, Robertson G, Beynon G, Monaghan JM. Thrombocytosis as a prognostic factor in women with cervical cancer. *Cancer*. 1994;74(1):90-2.
178. Eggemann H, Ehrlicke J, Ignatov T, Fettke F, Semczuk A, Costa SD, et al. Platelet Count After Chemotherapy is a Predictor for Outcome for Ovarian Cancer Patients. *Cancer investigation*. 2015;33(5):193-6.
179. Väyrynen JP, Väyrynen SA, Sirniö P, Minkkinen I, Klintrup K, Karhu T, et al. Platelet count, aspirin use, and characteristics of host inflammatory responses in colorectal cancer. *Journal of translational medicine*. 2019;17(1):199.
180. Schaffner F, Ruf W. Tissue factor and PAR2 signaling in the tumor microenvironment. *Arteriosclerosis, thrombosis, and vascular biology*. 2009;29(12):1999-2004.
181. Pradono P, Tazawa R, Maemondo M, Tanaka M, Usui K, Saijo Y, et al. Gene transfer of thromboxane A(2) synthase and prostaglandin I(2) synthase antithetically altered tumor angiogenesis and tumor growth. *Cancer research*. 2002;62(1):63-6.
182. Alfonso L, Ai G, Spitale RC, Bhat GJ. Molecular targets of aspirin and cancer prevention. *British journal of cancer*. 2014;111(1):61-7.
183. Maity G, De A, Das A, Banerjee S, Sarkar S, Banerjee SK. Aspirin blocks growth of breast tumor cells and tumor-initiating cells and induces reprogramming factors of mesenchymal to epithelial transition. *Laboratory investigation; a journal of technical methods and pathology*. 2015;95(7):702-17.
184. Li H, Lee MH, Liu K, Wang T, Song M, Han Y, et al. Inhibiting breast cancer by targeting the thromboxane A(2) pathway. *NPJ precision oncology*. 2017;1(1):8.
185. Guillem-Llobat P, Dovizio M, Bruno A, Ricciotti E, Cufino V, Sacco A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. *Oncotarget*. 2016;7(22):32462-77.
186. Dovizio M, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. Recent results in cancer research *Fortschritte der Krebsforschung Progres dans les recherches sur le cancer*. 2013;191:39-65.
187. Wojtukiewicz MZ, Hempel D, Sierko E, Tucker SC, Honn KV. Antiplatelet agents for cancer treatment: a real perspective or just an echo from the past? *Cancer Metastasis Rev*. 2017;36(2):305-29.
188. Gareau AJ, Brien C, Gebremeskel S, Liwski RS, Johnston B, Bezuhly M. Ticagrelor inhibits platelet-tumor cell interactions and metastasis in human and murine breast cancer. *Clinical & experimental metastasis*. 2018;35(1-2):25-35.
189. Gebremeskel S, LeVatte T, Liwski RS, Johnston B, Bezuhly M. The reversible P2Y12 inhibitor ticagrelor inhibits metastasis and improves survival in mouse models of cancer. *International journal of cancer*. 2015;136(1):234-40.
190. Holmes CE, Levis JE, Schneider DJ, Bambace NM, Sharma D, Lal I, et al. Platelet phenotype changes associated with breast cancer and its treatment. *Platelets*. 2016;27(7):703-11.
191. Mezouar S, Darbousset R, Dignat-George F, Panicot-Dubois L, Dubois C. Inhibition of platelet activation prevents the P-selectin and integrin-dependent accumulation of cancer

cell microparticles and reduces tumor growth and metastasis in vivo. *International journal of cancer*. 2015;136(2):462-75.

192. Xu XR, Yousef GM, Ni H. Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents. *Blood*. 2018;131(16):1777-89.

193. Rohwer N, Kuhl AA, Ostermann AI, Hartung NM, Schebb NH, Zopf D, et al. Effects of chronic low-dose aspirin treatment on tumor prevention in three mouse models of intestinal tumorigenesis. *Cancer medicine*. 2020.

194. Zhong S, Chen L, Zhang X, Yu D, Tang J, Zhao J. Aspirin use and risk of breast cancer: systematic review and meta-analysis of observational studies. *Cancer Epidemiol Biomarkers Prev*. 2015;24(11):1645-55.

195. 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(1):288.

196. 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. *Annals of internal medicine*. 2013;159(2):77-85.

197. 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(2):e57578.

198. Bibbins-Domingo K. Aspirin Use for the Primary Prevention of Cardiovascular Disease and Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Annals of internal medicine*. 2016;164(12):836-45.

199. 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. *Journal of the National Cancer Institute*. 2020.

200. 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 (London, England)*. 2018;392(10145):387-99.

201. Cao Y, Tan A. Aspirin might reduce the incidence of breast cancer: An updated meta-analysis of 38 observational studies. *Medicine*. 2020;99(38):e21917.

202. 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.

203. Kotronias RA, Kwok CS, Wong CW, Kinnaird T, Zaman A, Mamas MA. Cancer Event Rate and Mortality with Thienopyridines: A Systematic Review and Meta-Analysis. *Drug Saf*. 2017;40(3):229-40.

204. 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. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*. 2019;17(10):2024-33.e2.

205. 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(10).

206. 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. *The American journal of medicine*. 2017;130(7):826-32.

207. Chast F. [History of corticotherapy]. *La Revue de medecine interne*. 2013;34(5):258-63.

208. Saklatvala J. Glucocorticoids: do we know how they work? *Arthritis research*. 2002;4(3):146-50.

209. Schäcke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacology & therapeutics*. 2002;96(1):23-43.

210. Ramamoorthy S, Cidlowski JA. Corticosteroids: Mechanisms of Action in Health and Disease. *Rheumatic diseases clinics of North America*. 2016;42(1):15-31, vii.

211. Daley-Yates PT. Inhaled corticosteroids: potency, dose equivalence and therapeutic index. *Br J Clin Pharmacol*. 2015;80(3):372-80.
212. Hengge UR, Ruzicka T, Schwartz RA, Cork MJ. Adverse effects of topical glucocorticosteroids. *Journal of the American Academy of Dermatology*. 2006;54(1):1-15; quiz 6-8.
213. Lin KT, Wang LH. New dimension of glucocorticoids in cancer treatment. *Steroids*. 2016;111:84-8.
214. Mitre-Aguilar IB, Cabrera-Quintero AJ, Zentella-Dehesa A. Genomic and non-genomic effects of glucocorticoids: implications for breast cancer. *International journal of clinical and experimental pathology*. 2015;8(1):1-10.
215. Martens B, Drebert Z. Glucocorticoid-mediated effects on angiogenesis in solid tumors. *The Journal of steroid biochemistry and molecular biology*. 2019;188:147-55.
216. Gong H, Jarzynka MJ, Cole TJ, Lee JH, Wada T, Zhang B, et al. Glucocorticoids antagonize estrogens by glucocorticoid receptor-mediated activation of estrogen sulfotransferase. *Cancer research*. 2008;68(18):7386-93.
217. Rubenstein NM, Guan Y, Woo PL, Firestone GL. Glucocorticoid down-regulation of RhoA is required for the steroid-induced organization of the junctional complex and tight junction formation in rat mammary epithelial tumor cells. *The Journal of biological chemistry*. 2003;278(12):10353-60.
218. Law ME, Corsino PE, Jahn SC, Davis BJ, Chen S, Patel B, et al. Glucocorticoids and histone deacetylase inhibitors cooperate to block the invasiveness of basal-like breast cancer cells through novel mechanisms. *Oncogene*. 2013;32(10):1316-29.
219. Zarkovic M, Beleslin B, Ciric J, Penezic Z, Stojkovic M, Trbojevic B, et al. Glucocorticoid effect on insulin sensitivity: a time frame. *Journal of endocrinological investigation*. 2008;31(3):238-42.
220. Geer EB, Islam J, Buettner C. Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism. *Endocrinology and metabolism clinics of North America*. 2014;43(1):75-102.
221. Obradović MMS, Hamelin B, Manevski N, Couto JP, Sethi A, Coissieux MM, et al. Glucocorticoids promote breast cancer metastasis. *Nature*. 2019;567(7749):540-4.
222. Sherlock P, Hartmann WH. Adrenal steroids and the pattern of metastases of breast cancer. *Jama*. 1962;181:313-7.
223. Sørensen HT, Møller M, Nielsen GL, Baron JA, Olsen JH, Karagas MR. Skin cancers and non-hodgkin lymphoma among users of systemic glucocorticoids: a population-based cohort study. *Journal of the National Cancer Institute*. 2004;96(9):709-11.
224. Karagas MR, Cushing GL, Jr., Greenberg ER, Mott LA, Spencer SK, Nierenberg DW. Non-melanoma skin cancers and glucocorticoid therapy. *British journal of cancer*. 2001;85(5):683-6.
225. Jensen A, Thomsen HF, Engebjerg MC, Olesen AB, Friis S, Karagas MR, et al. Use of oral glucocorticoids and risk of skin cancer and non-Hodgkin's lymphoma: a population-based case-control study. *British journal of cancer*. 2009;100(1):200-5.
226. Dietrich K, Schned A, Fortuny J, Heaney J, Marsit C, Kelsey KT, et al. Glucocorticoid therapy and risk of bladder cancer. *British journal of cancer*. 2009;101(8):1316-20.
227. Beckmann K, Russell B, Josephs D, Garmo H, Haggstrom C, Holmberg L, et al. Chronic inflammatory diseases, anti-inflammatory medications and risk of prostate cancer: a population-based case-control study. *BMC cancer*. 2019;19(1):612.
228. Severi G, Baglietto L, Muller DC, English DR, Jenkins MA, Abramson MJ, et al. Asthma, asthma medications, and prostate cancer risk. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2010;19(9):2318-24.
229. Sørensen HT, Møller M, Skriver MV, Lash TL, Olsen JH, Baron JA. NO excess risk of breast cancer among female users of systemic glucocorticoids. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005;14(4):1022-3.



230. Sorensen GV, Cronin-Fenton DP, Sorensen HT, Ulrichsen SP, Pedersen L, Lash TL. Use of glucocorticoids and risk of breast cancer: a Danish population-based case-control study. *Breast cancer research : BCR*. 2012;14(1):R21.
231. Davis S, Mirick DK. Medication use and the risk of breast cancer. *European journal of epidemiology*. 2007;22(5):319-25.
232. Mackenzie IS, Macdonald TM, Thompson A, Morant S, Wei L. Spironolactone and risk of incident breast cancer in women older than 55 years: retrospective, matched cohort study. *BMJ (Clinical research ed)*. 2012;345:e4447.
233. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public health nutrition*. 2002;5(6b):1113-24.
234. Ewertz M MS, Boice JD, Jr., Jensen OM. Endometrial cancer following treatment, 687-92. *fbcac-csiDBJC*.
235. Haftenberger M, Lahmann PH, Panico S, Gonzalez CA, Seidell JC, Boeing H, et al. Overweight, obesity and fat distribution in 50- to 64-year-old participants in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public health nutrition*. 2002;5(6b):1147-62.
236. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and science in sports and exercise*. 2000;32(9 Suppl):S498-504.
237. Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public health nutrition*. 2003;6(4):407-13.
238. Clavel-Chapelon F. Cohort Profile: The French E3N Cohort Study. *International journal of epidemiology*. 2015;44(3):801-9.
239. World Health Organization. International Statistical Classification of Diseases and Related Health Problems. 10th Revision 2010.
240. Pintilie M. Analysing and interpreting competing risk data. *Stat Med*. 2007;26(6):1360-7.
241. WHO Collaborating Centre for Drug Statistics Methodology: DDD: Definition and general considerations. [http://www.whocc.no/ddd/definition\\_and\\_general\\_considera/](http://www.whocc.no/ddd/definition_and_general_considera/).
242. Pottegard A, Hallas J. New use of prescription drugs prior to a cancer diagnosis. *Pharmacoepidemiology and drug safety*. 2017;26(2):223-7.
243. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995;51(2):524-32.
244. Macis D, Gandini S, Guerrieri-Gonzaga A, Johansson H, Magni P, Ruscica M, et al. Prognostic effect of circulating adiponectin in a randomized 2 x 2 trial of low-dose tamoxifen and fenretinide in premenopausal women at risk for breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012;30(2):151-7.
245. Brannian JD, Hansen KA. Leptin and ovarian folliculogenesis: implications for ovulation induction and ART outcomes. *Seminars in reproductive medicine*. 2002;20(2):103-12.
246. Falk RT, Brinton LA, Madigan MP, Potischman N, Sturgeon SR, Malone KE, et al. Interrelationships between serum leptin, IGF-1, IGFBP3, C-peptide and prolactin and breast cancer risk in young women. *Breast cancer research and treatment*. 2006;98(2):157-65.
247. Dossus L, Becker S, Rinaldi S, Lukanova A, Tjønneland A, Olsen A, et al. Tumor necrosis factor (TNF)- $\alpha$ , soluble TNF receptors and endometrial cancer risk: the EPIC study. *International journal of cancer*. 2011;129(8):2032-7.
248. 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 research : BCR*. 2017;19(1):52.



249. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, et al. Nonsteroidal anti-inflammatory drug use and breast cancer risk by stage and hormone receptor status. *Journal of the National Cancer Institute*. 2005;97(11):805-12.
250. Gill JK, Maskarinec G, Wilkens LR, Pike MC, Henderson BE, Kolonel LN. Nonsteroidal antiinflammatory drugs and breast cancer risk: the multiethnic cohort. *American journal of epidemiology*. 2007;166(10):1150-8.
251. Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, et al. Aspirin and other nonsteroidal anti-inflammatory drugs and breast cancer incidence in a large U.S. cohort. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2005;14(1):261-4.
252. Gierach GL, Lacey JV, Jr., Schatzkin A, Leitzmann MF, Richesson D, Hollenbeck AR, et al. Nonsteroidal anti-inflammatory drugs and breast cancer risk in the National Institutes of Health-AARP Diet and Health Study. *Breast cancer research : BCR*. 2008;10(2):R38.
253. Johnson TW, Anderson KE, Lazovich D, Folsom AR. Association of aspirin and nonsteroidal anti-inflammatory drug use with breast cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. 2002;11(12):1586-91.
254. Eliassen AH, Chen WY, Spiegelman D, Willett WC, Hunter DJ, Hankinson SE. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. *Arch Intern Med*. 2009;169(2):115-21; discussion 21.
255. Brasky TM, Liu J, White E, Peters U, Potter JD, Walter RB, et al. Non-steroidal anti-inflammatory drugs and cancer risk in women: results from the Women's Health Initiative. *International journal of cancer*. 2014;135(8):1869-83.
256. Zhang X, Smith-Warner SA, Collins LC, Rosner B, Willett WC, Hankinson SE. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and postmenopausal breast cancer incidence. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2012;30(28):3468-77.
257. Harris RE, Kasbari S, Farrar WB. Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer. *Oncology reports*. 1999;6(1):71-3.
258. Meng G, Tang X, Yang Z, Zhao Y, Curtis JM, McMullen TPW, et al. Dexamethasone decreases the autotaxin-lysophosphatidate-inflammatory axis in adipose tissue: implications for the metabolic syndrome and breast cancer. *FASEB J*. 2019;33(2):1899-910.
259. Pufall MA. Glucocorticoids and Cancer. *Adv Exp Med Biol*. 2015;872:315-33.
260. Yemelyanov A, Czornog J, Chebotaev D, Karseladze A, Kulevitch E, Yang X, et al. Tumor suppressor activity of glucocorticoid receptor in the prostate. *Oncogene*. 2007;26(13):1885-96.
261. Shi W, Wang D, Yuan X, Liu Y, Guo X, Li J, et al. Glucocorticoid receptor-IRS-1 axis controls EMT and the metastasis of breast cancers. *Journal of molecular cell biology*. 2019;11(12):1042-55.
262. He L, Yuan L, Sun Y, Wang P, Zhang H, Feng X, et al. Glucocorticoid Receptor Signaling Activates TEAD4 to Promote Breast Cancer Progression. *Cancer research*. 2019;79(17):4399-411.
263. Catalunya & Departament de Salut. Pla de salut de Catalunya a l'horitzó 2010: informe de salut a Catalunya. (Generalitat de Catalunya, Departament de Salut, 2009).
264. Bolibar B, Fina Aviles F, Morros R, Garcia-Gil Mdel M, Hermosilla E, Ramos R, et al. [SIDIAP database: electronic clinical records in primary care as a source of information for epidemiologic research]. *Medicina clinica*. 2012;138(14):617-21.
265. Garcia-Gil Mdel M, Hermosilla E, Prieto-Alhambra D, Fina F, Rosell M, Ramos R, et al. Construction and validation of a scoring system for the selection of high-quality data in a Spanish population primary care database (SIDIAP). *Informatics in primary care*. 2011;19(3):135-45.

266. Garcia-Gil M, Elorza JM, Banque M, Comas-Cufí M, Blanch J, Ramos R, et al. Linking of primary care records to census data to study the association between socioeconomic status and cancer incidence in Southern Europe: a nation-wide ecological study. *PloS one*. 2014;9(10):e109706.
267. Recalde M, Manzano-Salgado CB, Díaz Y, Puente D, Garcia-Gil MDM, Marcos-Gragera R, et al. Validation Of Cancer Diagnoses In Electronic Health Records: Results From The Information System For Research In Primary Care (SIDIAP) In Northeast Spain. *Clinical epidemiology*. 2019;11:1015-24.