UNIVERSITÉ DE LILLE

FACULTÉ DE MÉDECINE HENRI WAREMBOURG

Année : 2022

THÈSE POUR LE DIPLÔME D'ÉTAT

DE DOCTEUR EN MÉDECINE

Etude de l'association entre l'exposition à la pollution atmosphérique et différents biomarqueurs inflammatoires dans une population issue de l'étude transversale ELISABET réalisée dans le Nord de la France entre 2011 et 2013.

Présentée et soutenue publiquement le 18 mai à 18h
au Pôle Formation
par Marion HOSTENS

_____

JURY
Président :
Monsieur le Professeur Philippe AMOUYEL
Assesseurs :
Monsieur le Docteur Jean DALLONGEVILLE
Monsieur le Docteur Jean-Marc Lo Guidice
Directeur de thèse :
Monsieur le Docteur Luc DAUCHET

Travail du Laboratoire de INSERM U1167 – Université de Lille – CHU de Lille – Institut Pasteur de Lille

_____

Avertissement

La faculté n'entend donner aucune approbation aux opinions émises dans les thèses : celles-ci sont propres à leur auteur

Liens d’intérêts

L’auteur et son directeur de thèse ne déclarent aucun lien d’intérêt en rapport avec le sujet traité au cours des trois années précédant la présentation de cette thèse

Tables des matières

Avant-propos

Cette thèse d'exercice a été réalisée au sein du laboratoire INSERM U1167 du Professeur Philippe Amouyel à l'Institut Pasteur de Lille. Ce travail s'inscrit dans le cadre de l'*Enquête Littoral Souffle Air Biolologie Environnement* (ELISABET). Elle a été réalisée sous la direction du Docteur Luc Dauchet.

Les résultats obtenus ont été suivis de la publication de l'article : Darras-Hostens M, Achour D, Muntaner M, Grare C, Zarcone G, Garçon G, et al. Short-term and residential exposure to air pollution: Associations with inflammatory biomarker levels in adults living in northern France. Sci Total Environ. 2022 Apr 7;833:154985 (1).

Résumé

Exposition à court terme et résidentielle à la pollution atmosphérique: associations avec les taux de biomarqueurs inflammatoires chez des adultes du Nord de la France

Contexte: La pollution atmosphérique a un impact sur la santé, et l'inflammation de bas grade pourrait être l'un des mécanismes sous-jacents. L'objectif de la présente étude portant sur des adultes du Nord de la France était d'évaluer les associations entre l'exposition à court terme et résidentielle à la pollution atmosphérique et les taux de divers biomarqueurs inflammatoires.

Méthodes: L'étude transversale *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) a été menée de 2011 à 2013 dans les aires urbaines de Lille et de Dunkerque, dans le nord de la France. Ici, nous avons évalué les associations entre l'exposition aux PM_{10}, NO_2 et O_3 (le jour et la veille du prélèvement de l'échantillon de sang, et les niveaux annuels moyens résidentiels) et les taux de biomarqueurs inflammatoires que sont la protéine C-réactive ultra-sensible (CRPus), les interleukines (IL)-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, et le facteur de nécrose tumorale (TNF) α.

Résultats: Nous avons évalué 3074 participants pour l'association avec la CRPus et un sous-échantillon de 982 non-fumeurs de Lille pour l'association avec les taux de cytokines plasmatiques. Une augmentation de 10 $\mu g/m^3$ des niveaux de PM_{10} et de NO_2 le jour et la veille du prélèvement de l'échantillon de sang était associée à une concentration plus élevée de CRPus (3.43% [0.68; 6.25] et 1.75% [-1.96; 5.61], respectivement, alors qu'une augmentation de 10 $\mu g/m^3$ de O_3 était associée à une concentration plus basse de CRPus (-1.2% [-3.95; 1.64]). Les associations entre l'exposition annuelle moyenne et le taux de CRPus n'étaient pas significative. De même, les associations entre l'exposition et les niveaux de cytokines plasmatiques n'étaient pas statistiquement significatives.

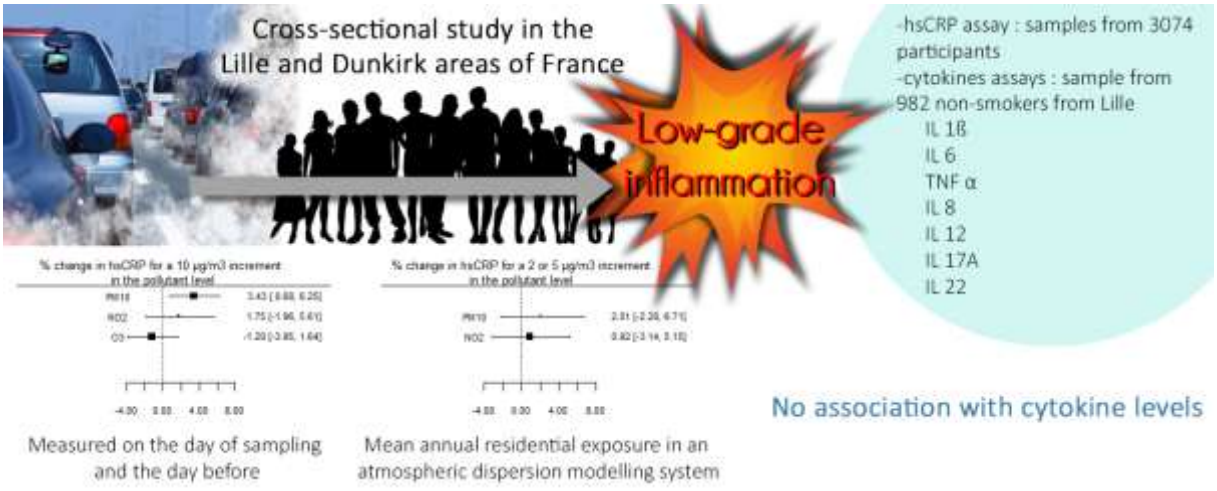
Conclusion: L'exposition à la pollution atmosphérique à court terme était associée à des taux de CRPus sériques plus élevés chez des adultes vivants dans deux zones urbaines du Nord de la France. Nos résultats suggèrent que, parmi d'autres facteurs, l'inflammation de bas grade pourrait expliquer les effets néfastes de la pollution atmosphérique sur la santé.

Abstract

Short-term and residential exposure to air pollution: associations with inflammatory biomarker levels in adults living in northern France

Background: Air pollution has an impact on health, and low-grade inflammation might be one of the underlying mechanisms. The objective of the present study of adults from northern France was to assess the associations between short-term and residential exposure to air pollution and levels of various inflammatory biomarkers.
Methods: The cross-sectional *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) study was conducted from 2011 to 2013 in the Lille and Dunkirk urban areas of northern France. Here, we evaluated the associations between PM_{10}, NO_2 and O_3 exposure (on the day of the blood sample collection and on the day before, and the mean annual residential level) and levels of the inflammatory biomarkers high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and tumor necrosis factor α.
Results: We assessed 3074 participants for the association with hsCRP and a subsample of 982 non-smokers from Lille for the association with plasma cytokine levels. A 10 µg/m^3 increment in PM_{10} and NO_2 levels on the day of sample collection and on the day before was associated with a higher hsCRP concentration (3.43% [0.68; 6.25] and 1.75% [-1.96; 5.61], respectively, whereas a 10 µg/m^3 increment in O_3 was associated with lower hsCRP concentration (-1.2% [-3.95; 1.64]). The associations between mean annual exposure and the hsCRP level were not significant. Likewise, the associations between exposure and plasma cytokine levels were not statistically significant.
Conclusion: Short-term exposure to air pollution was associated with higher serum hsCRP levels in adult residents of two urban areas in northern France. Our results suggest that along with other factors, low-grade inflammation might explain the harmful effects of air pollution on health.

Graphical abstract



Keywords: Atmospheric pollution ; Low-grade inflammation ; Population-based study; Human health ; Epidemiology

Liste des abréviations

BMI	Body Mass Index
CI	Confident Interval
CRP	C-Reactive protein
HDL	High-Density Lipoprotein
hsCRP	high-sensitivity C-reactive protein
IL	Interleukin
INSEE	Institut National de la Statistique et des Etudes Economique
IQR	Interquartile range
IRIS	Ilots Regroupés pour l'Information Statistique
NO_2	Nitrogen dioxide
O_3	Ozone
PM_{10}	Particulate matter with an aerodynamic diameter below 10µm
WHO	World Health Organization

1 Contexte

1.1. Pollution atmosphérique

1.1.1. Pollution atmosphérique et santé en général

La pollution atmosphérique a un impact majeur sur la santé. En effet, selon l'Organisation Mondiale de la Santé, elle provoque la mort de 7 millions de personnes par an (2).

Les principaux polluants de l'air mesurés par les stations de mesures sont :

- les particules fines ou particules de matières (PM), de taille microscopique. Elles sont classées en fonction de leur taille et les particules les plus souvent mesurées sont les particules de matières de diamètre inférieur à 10 µm (PM_{10}) ainsi que les particules de matières de diamètre inférieur à 2,5 µm ($PM_{2.5}$).
- les oxydes d'azote (NOx) avec en particulier le dioxyde d'azote (NO_2)
- l'ozone (O_3)

Les valeurs à ne pas dépasser d'après les recommandations de l'OMS pour la qualité de l'air sont (3):

	$PM_{2.5}$	**PM_{10}**	**NO_2**	**O_3**
Valeur moyenne annuelle	5 µg/m^3	15 µg/m^3	10 µg/m^3	
Valeur moyenne sur 24 heures	15 µg/m^3	45 µg/m^3	25 µg/m^3	
Valeur maximale journalière sur 8 heures				100 µg/m^3

Les PM_{10} sont fortement corrélées au NO_2. L'O_3 est inversement corrélé au NO_2 car il existe un processus chimique de transformation du NO_2 en O_3 lors de l'ensoleillement. L'O_3 est inversement corrélé aux PM_{10} par l'intermédiaire du NO_2.

En France, les particules de matière sont responsables de plus 40 000 morts par an (pour les $PM_{2.5}$), ce qui représente 9% de la mortalité totale. Cela entraîne également 950 000 années de vie perdues par an. Plus de la moitié de ces décès et années de vie perdues par an

surviennent dans des zones urbaines de plus de 100 000 habitants (4,5).

Les effets délétères de la pollution atmosphérique sur la santé concernent de nombreux organes du corps humain. Ainsi, la pollution atmosphérique favorise des maladies pulmonaires (4,6–9), cardiovasculaires (4,10), cérébrales et psychiques (4,11), des cancers (12–14) et entraînent des troubles métaboliques (15–19) et de la reproduction (4).

1.1.2. Effets de la pollution atmosphérique sur les différents organes

a) Appareil respiratoire

La pollution atmosphérique entraîne de nombreuses pathologies respiratoires. En effet, les polluants atmosphériques augmentent l'apparition et la fréquence de symptômes respiratoires et diminuent la fonction respiratoire. Ainsi, le nombre d'entrées aux urgences pour infections des voies respiratoires hautes est augmenté lors de plus fortes pollutions (6). En outre, le fait d'être exposé à la pollution atmosphérique entraine des exacerbations de l'asthme se manifestant par l'aggravation des symptômes asthmatiques et par une augmentation du nombre de visites aux urgences et d'hospitalisations. De plus une exposition sur le long terme à des polluants atmosphériques pourrait provoquer le développement d'un asthme (7,8).

La pollution participe également aux exacerbations de broncho-pneumopathies chroniques obstructives (BPCO) et accélère le développement de cette pathologie (4). Cela se traduit, par exemple, par une augmentation du nombre de passages aux urgences en lien avec la BPCO lorsque les taux de polluants atmosphériques sont plus élevés (9).

b) Cœur et appareil circulatoire

La pollution atmosphérique est associée à une altération de la fonction cardiaque, un risque d'ischémie myocardique ou encore d'arythmie (4). Par exemple, lorsque la concentration journalière de pollution atmosphérique augmente, le nombre de passages aux urgences

pour fibrillation atriale augmente la journée suivante (10).

Les polluants ont aussi un effet sur le système circulatoire en particulier sur l'hypertension artérielle (HTA), la coagulation qui s'accroît, le développement, l'accélération et la déstabilisation des plaques d'athérosclérose pouvant conduire ainsi à une ischémie (4).

c) Système nerveux

Les risques pour le cerveau sont une augmentation de l'ischémie cérébrale avec risque d'accidents vasculaires cérébraux (AVC), des troubles cognitifs et des maladies neurodégénératives (4). La pollution est en, outre, associée à la dépression, l'anxiété et au suicide (11).

d) Métabolisme

Il existe une association entre la pollution atmosphérique et les troubles métaboliques. Ainsi, l'incidence du diabète de type 2 augmente avec une exposition à la pollution (15).

Par ailleurs, une association a été montrée entre l'exposition à des taux modérés de pollution et l'hémoglobine glyquée (16). L'hémoglobine glyquée est le reflet de la glycémie moyenne sur les trois derniers mois et témoigne de l'équilibre (ou du déséquilibre) du diabète.

La pollution semble également être associée à des dyslipidémies (17) avec une augmentation des triglycérides et du cholestérol total (18) et une diminution du cholestérol HDL (19).

e) Cancer

La pollution atmosphérique est associée au développement de cancers. Par exemple une exposition à long terme à la pollution atmosphérique est associée à un risque plus élevé de cancers du poumon (12), du sein (13) ou du foie (14).

f) Appareil reproducteur

Enfin, la pollution atmosphérique provoque des troubles de la reproduction et du développement. En effet, une exposition aux polluants pourra entrainer infertilité, fausses-couches, retard de croissance fœtale, naissance prématurée ou encore faible poids de naissance (4).

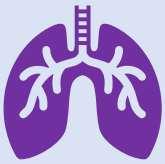
g) Schéma récapitulatif

Effets de la pollution atmosphérique sur les organes

SYSTEME NERVEUX

- ↗ ischémie cérébrale (AVC)
- ↗ troubles cognitifs
- ↗ maladies neurodégénératives
- ↗ troubles psychiatriques (dépression, anxiété, suicide)

POUMONS



- ↗ infections respiratoires
- ↗ exacerbations asthme
- ↗ exacerbations BPCO
- ↘ fonction respiratoire

CŒUR ET APPAREIL CIRCULATOIRE

- ↗ HTA
- ↗ coagulation
- ↗ ischémie myocardique
- ↗ arythmie

APPAREIL REPRODUCTEUR

- infertilité, fausse-couche
- retard de croissance fœtale, naissance prématurée, faible poids de naissance

METABOLISME

- ↗ incidence diabète
- ↗ dyslipidémie

CANCER

- ↗ risque de cancer

1.2. Inflammation

1.2.1. Rôle de l'inflammation

L'inflammation est un processus de réponse du corps humain à la suite d'une agression. La réaction inflammatoire est une composante de la réponse immune et est le plus souvent protectrice. On distingue l'inflammation aiguë de l'inflammation chronique. La première survient brutalement à la suite d'une souffrance telle qu'une brûlure, une pancréatite aiguë ou encore un choc septique. Elle dure quelques minutes, quelques jours ou quelques semaines. Parfois l'inflammation persiste et s'aggrave pendant plusieurs mois voire plusieurs années, on parle alors d'inflammation chronique. Cette dernière se voit dans de nombreuses pathologies comme le cancer, les maladies auto-immunes, le diabète ou encore les maladies cardiovasculaires.

Dans l'inflammation (aiguë ou chronique) causée par une pathologie, la valeur de la protéine C-réactive (CRP) est augmentée sur la prise de sang (généralement supérieure à 10 mg/L).

1.2.2. Inflammation de bas grade

L'inflammation de bas grade, quant à elle, est une inflammation systémique qui n'est pas due à une pathologie aiguë ou chronique. Ainsi, la valeur de la CRP est normale c'est-à-dire inférieure à 10 mg/L. Cette inflammation de bas grade est le reflet de l'inflammation de base que possède chaque personne. Néanmoins celle-ci peut évoluer à bas bruit et favoriser l'apparition et/ou la progression de certaines maladies chroniques, notamment les maladies cardiovasculaires (20), le diabète (21), le cancer (22) ou encore la dépression (23).

Ainsi, une inflammation pulmonaire et systémique de bas grade peut accroître le risque de coagulation, de thromboses et, en fin de compte, d'événements cardiovasculaires et ischémiques (24).

1.2.3. Marqueurs de l'inflammation de bas grade : CRPus et cytokines

Les marqueurs biologiques de l'inflammation de bas grade comprennent la protéine C-réactive ultra-sensible (CRPus) et les cytokines inflammatoires. La protéine C-réactive ultra-sensible (CRPus) est la même protéine que la protéine C-réactive (CRP) mais mesurée avec des techniques plus fines, permettant ainsi de doser des valeurs inférieures à 10 mg/L. La CRPus est un marqueur de l'inflammation de bas grade qui est mesurée assez fréquemment dans les études épidémiologiques; en revanche, les cytokines sont moins fréquemment dosées. La CRP est l'un des réactifs inflammatoires de phase aiguë les plus distinctifs. Les taux plasmatiques de CRP augmentent rapidement et de manière significative en réponse à une inflammation, une lésion cellulaire ou une lésion tissulaire. La CRP est un marqueur de l'inflammation utilisé couramment en pratique clinique. Les tests de CRP ultra-sensible peuvent mesurer avec précision de petites augmentations dans la plage normale de la CRP et peuvent ainsi identifier des niveaux d'inflammation faibles mais persistants. Étant donné que l'athérosclérose a une composante inflammatoire, le dosage de la CRPus fournit des informations sur le risque cardiovasculaire (25). Dans les études sur les mécanismes moléculaires et cellulaires sous-jacents de l'inflammation, les niveaux de cytokines sont généralement mesurés dans des échantillons de sérum ou de plasma provenant de patients ou d'individus sains. En effet, les cytokines sont des protéines solubles qui agissent comme des messagers chimiques dans la réponse immunitaire et jouent un rôle important dans la communication intercellulaire, la croissance et la différenciation cellulaires. Par ailleurs, la sécrétion anormale de cytokines spécifiques a été associée à la pathogenèse ou à la progression de maladies inflammatoires chroniques et à l'exposition à des contaminants chimiques ou biologiques dans l'environnement. Dans les grandes études épidémiologiques comme dans la pratique clinique courante, la détection et la quantification des cytokines

peuvent utilement compléter les données du dosage de la CRP. Dans la présente étude, nous avons analysé sept cytokines (interleukines (IL)-1ß, -6, -8, -10, -17A et -22, et facteur de nécrose tumorale (TNFα)) connues pour être impliquées dans les maladies inflammatoires ou la réponse à l'exposition aux polluants atmosphériques (26–29). Toutes ces cytokines sont pro-inflammatoires (elles favorisent l'inflammation) sauf l'interleukine 10 qui est une protéine anti-inflammatoire (elle diminue l'inflammation).

1.2.4. Rôle de pollution atmosphérique sur inflammation

Les particules de matière (PM) et certains de leurs constituants ont des effets pro-inflammatoires sur l'organisme. Les PM pourraient augmenter l'inflammation de bas grade et donc les problèmes de santé susmentionnés. Plus précisément, les changements biologiques induits par l'exposition aux PM peuvent se produire par plusieurs voies (30,31): (i) la libération de médiateurs pro-inflammatoires (tels que les cytokines) et d'espèces réactives de l'oxygène par les cellules pulmonaires; (ii), la perturbation du système nerveux autonome par des interactions entre les particules inhalées et les nerfs pulmonaires; et (iii) la translocation directe de particules ultrafines ou de constituants de particules dans la circulation systémique.

1.3. Association entre pollution atmosphérique et biomarqueurs de l'inflammation dans la littérature

1.3.1. Association entre pollution atmosphérique et CRPus dans la littérature

L'association entre la pollution atmosphérique et l'inflammation a été étudiée dans la population présentant un état de santé ou une exposition spécifiques dans des échantillons limités (de 15 à 175 sujets) et ces études hétérogènes ont donné des résultats incohérents (31–51). L'association entre la CRPus et l'inflammation a également été étudiée chez des femmes enceintes, montrant qu'une CRPus plus élevée était significativement associée à

une exposition moyenne annuelle aux PM_{10} mais pas aux NO_2 (52). Chez des nouveau-nés à terme, une association a été observée entre PM_{10} et IL1β (53). Chez des enfants asthmatiques comparés à des enfants non asthmatiques, une association a été observée entre le NO_2 et Il6 et IL10 (54). Les données de la littérature sont néanmoins contradictoires: certains chercheurs ont même observé des niveaux plus faibles de CRP (40,47,48) et de cytokines pro-inflammatoires (39,42,49,50,53) ou des taux plus élevés d'une cytokine anti-inflammatoire (41,54), associés à une exposition élevée.

Seules quelques études ont évalué l'association entre les marqueurs inflammatoires et la pollution atmosphérique chez les adultes non enceintes, dans le cadre d'études de population; laissant l'association entre la pollution atmosphérique et l'inflammation dans la population générale insuffisamment quantifiée. La plupart de ces études ne mesuraient que les taux de CRPus, et les résultats étaient contradictoires. Une association significative entre PM, NO_2 et CRPus a été observée chez des adultes âgés chinois. Cependant, le niveau de la pollution de l'air était très élevé à 91 $\mu g/m^3$ (55).

Quelques études, conduites dans des zones d'Europe ou d'Amérique avec des niveaux de pollution très modérés, n'ont pas observé d'association significative. Par exemple, une étude de 2252 personnes en Allemagne n'a pas trouvé d'association significative entre PM, NOx, O_3 d'une part et CRPus d'autre part (47). Dans une étude de 408 résidents de Boston (Etats-Unis), les PM n'étaient pas significativement associées à la CPRus (56). Dans un échantillon de 17000 personnes au Royaume-Uni, une association entre les PM_{10} et la CRPus n'a pas été observée (48). Une étude portant sur 3860 membres de la population générale suisse (57) n'a trouvé aucune association significative entre PM_{10} et CRP. A l'inverse, d'autres études ont trouvé des associations significatives. Ainsi, une association positive et statistiquement significative entre une exposition d'une année à la pollution ($PM_{2.5}$ et PM_{10}) et le taux sérique

de CRPus a été observée dans un échantillon de population générale allemande, avec des échantillons collectés pendant deux périodes de suivi (58). Par ailleurs, une association positive et significative entre une exposition d'un an aux $PM_{2.5}$ et de six mois à l'O_3 et le taux sérique de CRPus (avec échantillonnage répété) a été trouvée dans une étude portant sur 2086 femmes aux Etats-Unis (24). Enfin, dans une analyse transversale de l'étude *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) menée dans le Nord de la France, nous avons précédemment observé une association entre le NO_2 le jour et la veille du prélèvement de l'échantillon sanguin et le taux sérique de CRPus dans un sous-groupe spécifique, composé de 1506 non-fumeurs sains (c'est-à-dire des personnes ne présentant pas de maladie respiratoire chronique ou aiguë, de prise de médicaments pour les maladies pulmonaires, d'obstruction des voies respiratoires, d'exposition au tabagisme actif ou passif, ou de syndrome inflammatoire (défini par un taux sérique de CRPus > 10 mg/L)) (59). Le tableau suivant résume les recherches bibliographiques sur l'association entre la pollution atmosphérique et la CRPus (Tableau A).

Tableau A: Recherches bibliographiques sur l'association entre la pollution atmosphérique et la CRPus

	Auteur	Population	Pollution
Petit échantillon/ Population spécifique	Young et al 2020	54 femmes au Nicaragua	Pollution intérieure
	Pope et al 2004	88 résidents âgés de l'Utah (Etats-Unis)	$PM_{2.5}$
	Westberg et al 2016	67 travailleurs d'une usine de pâte à papier en Suède	Exposition professionnelle (poussières)
	Shakya et al 2019	53 agents de la police de la circulation volontaires au Népal (100% hommes)	Exposition professionnelle (pollution atmosphérique)
	Riaz et al 2020	87 conducteurs automobiles au Pakistan	Exposition professionnelle (pollution atmosphérique)
	Huang W-H et al 2014	175 patients dialysés à Taïwan	$PM_{2.5}$, PM_{10}, NO_2, SO_2, CO, O_3
Sujets exposés comparés à des sujets non exposés	Brucker et al 2013	39 chauffeurs de taxis comparés à 21 personnes non exposées professionnellement, au Brésil (100% hommes).	Exposition professionnelle (pollution atmosphérique)
	Jabbar et al 2020	72 travailleurs exposés professionnellement au pétrole (travail dans une station service) comparés à 75 sujets non-exposés, en Irak (étudiants et membres d'une faculté) (100% hommes)	Exposition professionnelle (pétrole)
	Bassig et al 2017	54 travailleurs hommes exposés aux gaz d'échappement des moteurs diesel et 55 hommes non exposés aux gaz d'échappement des moteurs diesel, en Chine	Exposition professionnelle (gaz d'échappement de moteurs diesel)
	Dai et al 2016	137 travailleurs hommes exposés au diesel comparés à 108 travailleurs hommes non exposés. En Chine	Exposition professionnelle (gaz d'échappement de moteurs diesel)
	Taj et al 2021	78 soudeurs d'acier doux et 96 témoins (non exposés) en Suède (100% hommes)	Exposition professionnelle (poussières)
Enfants et femmes enceintes	Van den Hooven et al 2012	6508 femmes enceintes aux Pays-Bas	PM_{10} et NO2
Etude en population générale	Elbarbary et al 2021	7915 adultes âgés en Chine	PM_1, $PM_{2.5}$, PM_{10} et NO_2
	Rioux et al 2010	1017 adultes d'origine portoricaine à Boston (Etats-Unis)	Trafic routier
	Tsai D-H et al 2019	3860 suisses	PM_{10}
	Lane et al 2016	408 individus à Boston	Particules ultrafines (< 100 nm)
	Frauke Hennig et al 2014	8204 allemands	$PM_{2.5}$ and PM10
	Pilz et al 2018	2252 allemands	$PM_{2.5}$, PM_{10}, NOx et O_3
	Green et al 2016	2086 femmes (âgées de 42 à 52 ans), aux Etats-Unis	$PM_{2.5}$ et O_3
	Forbes et al 2009	17000 individus en Angleterre	PM_{10}, NO_2 et O_3

Abréviations: PM_1 = particules de matière de diamètre inférieur à 1 µm; $PM_{2.5}$ = particules de matière de diamètre inférieur à 2,5 µm; PM_{10} = particules de matière de diamètre inférieur à 10 µm; NOx = oxydes d'azote; NO_2 = dioxyde d'azote; O_3 = ozone; SO_2 = dioxyde de soufre; CO = monoxyde de carbone

1 µm = 10^{-6} m; 1 nm = 10^{-9} m

1.3.2. Associations entre pollution atmosphérique et cytokines dans la littérature

En ce qui concerne les cytokines, moins de données ont été publiées. Dans une étude menée chez 3860 individus issus de la population générale suisse (une étude qui a aussi inclus deux périodes de suivi), il y avait des associations positives significatives entre une exposition de un jour, une semaine, un mois, trois mois et six mois aux PM_{10} et des cytokines inflammatoires (IL-6, IL-1β, and TNFα) (57). Cependant, la CRPus n'était pas associée aux PM_{10} (57). Ces associations significatives avec les taux de cytokines n'ont pas encore été confirmées dans d'autres études. Comme mentionné ci-dessus, une étude portant sur 408 résidents de Boston (56) n'a pas trouvé d'association significative. De plus, ces études en population chez les adultes (56,57) ont mesuré un petit nombre de cytokines (IL-1β, IL-6, and TNFα). Le tableau suivant résume les recherches bibliographiques sur l'association entre la pollution atmosphérique et les cytokines (Tableau B).

Tableau B: Recherches bibliographiques sur l'association entre la pollution atmosphérique et les cytokines

	Auteur	Cytokines étudiées	Population	Pollution
Petit échantillon/ Population spécifique	Negherbon et al 2017	IIβ; IL6; TNFα; IL8; IL10; IL2; IL4; IL13; INFγ	45 enfants: 30 asthmatiques et 15 témoins à Lima, Pérou	$PM_{2.5}$
	Buxton et al 2019	IL6,TFNα (au niveau cervicovaginal et dans le sérum)	104 femmes enceintes à Mexico	PM_{10} et CO
	Klumper et al 2015	IL6; IL8; IL10; TNFα; INFγ	27 enfants asthmatiques et 59 enfants non asthmatiques, en Allemagne	$PM_{2.5}$, PM_{10}, NO_2, NOx
Sujets exposés comparés à des sujets non exposés	Brucker et al 2013	IL1β; IL6; IL10;TNFα; INFγ	39 chauffeurs de taxis comparés à 21 non exposés, au Brésil (100% hommes)	Exposition professionnelle
	Matsuda et al 2015	IL2; IL5; IL10; INFγ (dans les sécrétions lacrymales)	19 exposés (chauffeurs de taxis et professionnels de la circulation) comparés à 11 non exposés au Brésil. (100% hommes)	Exposition professionnelle (très élevée)
	Jabbar et al 2020	IL6	72 travailleurs exposés professionnellement au pétrole (travail dans une station service) comparés à 75 sujets non exposés, en Irak (étudiants et membres d'une faculté) 100% hommes	Exposition professionnelle (pétrole)
	Riaz et al 2020	IL6; TNFα; TNFβ	87 conducteurs automobiles au Pakistan	$PM_{2.5}$, PM_{10} et NOx
	Bassig et al 2017	IL21; IL10, IL16	54 travailleurs hommes exposés aux gaz d'échappement des moteurs diesel et 55 hommes non exposés aux gaz d'échappement des moteurs diesel, en Chine	Exposition professionnelle aux gaz d'échappement de moteurs diesel
	Dai et al 2016	IL1β; IL6; IL8; TNFα	137 travailleurs hommes exposés au diesel comparés à 108 travailleurs hommes non exposés. En Chine	Exposition professionnelle aux gaz d'échappement de moteurs diesel
	Dai et al 2018	IL1; IL6; IL8; TNFα	41 travailleurs hommes responsables des tests des moteurs diesel dans une usine de fabrication de moteurs diesel et 46 hommes non exposés (travaillant dans un département d'embouteillage d'une brasserie, une usine de traitement de l'eau, une usine de conditionnement de la viande et une installation administrative)	Exposition professionnelle aux gaz d'échappement de moteurs diesel
Enfants et femmes enceintes	Latzin et al 2011	IL1β; IL6;IL10;TNFα (dans le sérum du sang de cordon)	265 nouveau-nés en bonne santé en Suisse	PM_{10} et pollution intérieure, exposition durant le dernier trimestre de grossesse
	Gruzieva et al 2017	IL1β ; IL2 ; IL4 ; IL6 ; IL8 ; IL10 ; IL13;TNFα	670 enfants asthmatiques à Stockholm (Suède)	Pollution atmosphérique liée à la circulation
Etude en population générale	Tsai D-H et al 2019	IL1β; IL6;TNFα	3860 suisses	PM_{10}
	Lane et al 2016	IL6	408 individus à Boston	Particules ultrafines (< 100 nm)

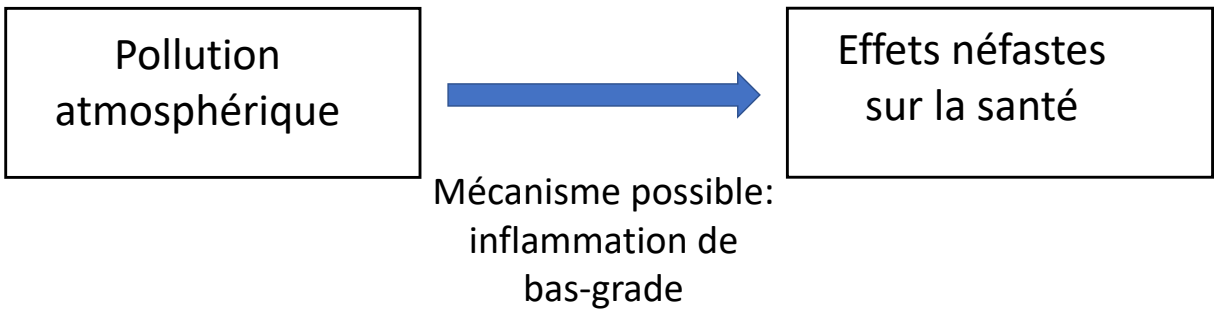
Abréviations: $PM_{2.5}$ = particules de matière de diamètre inférieur à 2,5 µm; PM_{10} = particules de matière de diamètre inférieur à 10 µm; NOx = oxydes d'azote; NO_2 = dioxyde d'azote; CO = monoxyde de carbone; IL = interleukine; TNF = facteur de nécrose tumorale; INF = interféron

1 µm = 10^{-6} m; 1 nm = 10^{-9} m

1.4. Objectif

L'objectif de ce travail est d'explorer les associations entre les niveaux de polluants atmosphériques et les biomarqueurs de l'inflammation de bas grade (CRPus, IL1β, IL6, IL8, IL10, IL17A, IL22, and TNFα). Nous avons étudié les associations avec la CRPus en utilisant 3074 participants de l'étude *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) menée dans le Nord de la France (Lille et Dunkerque) entre 2011 et 2013. Pour étudier les associations avec les cytokines inflammatoires, nous avons utilisé un sous-échantillon de l'étude ELISABET composé de 982 non-fumeurs de l'agglomération lilloise.

Nous avons étudié les effets de la pollution à court terme et à long terme (à l'adresse résidentielle). Nous avons défini la pollution à court terme comme le niveau moyen de pollution dans chaque zone d'étude (Lille ou Dunkerque) à chaque jour de prélèvement sanguin ainsi que la veille. Cette variable fournit des informations sur les effets réversibles à court terme des périodes de forte pollution. L'exposition à long terme était définie comme l'exposition annuelle moyenne à l'adresse résidentielle. Cette variable fournit des informations sur les différences entre les personnes vivant dans des zones fortement polluées et celles vivant dans des zones moins polluées, c'est-à-dire les effets à long terme de la pollution atmosphérique.



2 Article

Short-term and residential exposure to air pollution: associations with inflammatory biomarker levels in adults living in northern France

Authors

Marion Darras-Hostens[a], Djamal Achour[b], Manon Muntaner[a], Céline Grare[b], Gianni Zarcone[b], Guillaume Garçon[b], Philippe Amouyel[a], Farid Zerimech[2], Régis Matran[b], Jean-Marc Lo Guidice[b], Luc Dauchet[a]

[a] Univ. Lille, INSERM, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, F-59000 Lille, France

[b] Univ. Lille, CHU Lille, Institut Pasteur de Lille, ULR 4483 – IMPECS – IMPact de l'Environnement Chimique sur la Santé, F-59000 Lille, France

2.1. Introduction

Air pollution has a major impact on health: according to the World Health Organization, it causes the death of 7 million people per year (2). The effects of air pollution on the respiratory and cardiovascular systems are particularly harmful (30). Low-grade, systemic inflammation is not caused by a specific chronic or acute disease but promotes the onset and/or progression of certain chronic diseases, including cardiovascular disease(20), diabetes (21), cancer (22), and even depression (23). Thus, low-grade pulmonary and systemic inflammation might increase the potential for coagulation, thrombosis and, ultimately, cardiovascular and ischemic events (24).

Furthermore, particulate matter (PM) and some of its constituents have pro-inflammatory

effects on the body. PM might increase low-grade inflammation and thus the above-mentioned health problems. More specifically, the biological changes induced by PM exposure may occur through several mechanistic pathways (30,31): (i) the release of pro-inflammatory mediators (such as cytokines) and reactive oxygen species from lung cells; (ii), disruption of the autonomic nervous system via interactions between inhaled particles and pulmonary nerves; and (iii) direct translocation of ultrafine particles or particle constituents into the systemic circulation.

The biological markers of low-grade inflammation include high-sensitivity C-reactive protein (hsCRP) and inflammatory cytokines. High-sensitivity C-reactive protein (hsCRP) is a marker of low-grade inflammation and is measured quite frequently in epidemiological studies; in contrast, other cytokines are less frequently assayed. CRP is one of the most distinctive acute phase reactants. Plasma levels of CRP increase rapidly and significantly in response to inflammation, cell damage, or tissue injury. Hence, CRP has long been considered as a prime marker of inflammation. High-sensitivity CRP assays can accurately measure small increases within the normal CRP range and can thus identify low but persistent levels of inflammation. Given that atherosclerosis has an inflammatory component, an hsCRP assay provides information on the cardiovascular risk (25). In studies of the underlaying molecular and cellular mechanisms of inflammation, cytokine levels are generally measured in serum or plasma samples from patients or healthy individuals. Indeed, cytokines are soluble proteins that act as chemical messengers in the immune response and have an important role in cell-to-cell communication, cell growth, and cell differentiation. Moreover, abnormal secretion of specific cytokines has been associated with the pathogenesis or progression of chronic inflammatory diseases and with exposure to chemical or biological contaminants in the environment. In both large epidemiological studies and routine clinical practice, cytokine

detection and quantification can usefully complement CRP assay data. In the present study, we assayed seven cytokines (interleukins (IL)-1ß, -6, -8, -10, -17A and -22, and tumor necrosis factor alpha (TNFα)) known to be involved in inflammatory diseases or the response to air pollutant exposure (26–29).

Association between air pollution and inflammation has been studied in population with a specific health condition or exposure in limited samples (from 15 to 175 subjects) and these heterogeneous studies had inconsistent results (31–51). Association between hsCRP and inflammation has also been studied in pregnant women showing higher hsCRP was significantly associated with mean annual exposure to particulate matter with a diameter < 10 µm (PM_{10}) but not nitrogen dioxide (NO_2) (52). In term-born neonates, an association was observed between PM_{10} and IL1β (53) . In asthmatic and non-asthmatic children, an association was observed between NO_2 and Il6 and IL10 (54). The literature data are nevertheless inconsistent: some researchers even observed lower levels of CRP (40,47,48) and pro-inflammatory cytokines (39,42,49,50,53) or higher levels of an anti-inflammatory cytokine (41,54) associated with higher exposure.

Only a few studies evaluated the association between inflammatory markers and air pollution in non-pregnant adults, in population-based studies; leaving the association between atmospheric pollution and inflammation in the general population insufficiently quantified . Most of these studies measured only hsCRP levels, and the results were inconsistent. A significant association between PM, NO_2 and hsCRP was observed in older Chinese adults. However, the level of air pollution was very high at 91 µg/m^3 (55).

Some studies conducted in European or American areas with very moderate pollution levels

did not observe a significant association. For example, a study of 2252 people in Germany did not find significant associations between PM, nitrogen oxide (NOx), ozone (O_3) on one hand and hsCRP on the other (47). In a study of 408 residents in Boston (USA), PM was not significantly associated with hsCRP (56). In a sample of 17000 people in the United Kingdom, an association between PM_{10} and hsCRP was not observed (48). A study of 3860 members of the Swiss general population (57) did not find any significant association between PM_{10} and CRP. In contrast, other studies found significant associations. Conversely, a statistically significant, positive association between 1-year pollution exposure to $PM_{2.5}$ and PM_{10} and the serum hsCRP level was observed in a German general population sample, with samples collected during two follow-up periods (58). Furthermore, a significant positive association between 1-year $PM_{2.5}$ exposure and 6-month O_3 exposure and the serum hsCRP level (with repeat sampling) was found in a study of 2086 women in the USA (24). Lastly, in a cross-sectional analysis of the *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) study conducted in northern France, we previously observed an association between NO_2 on the day of blood sample collection and on the day before and the serum hsCRP level in a specific subgroup of 1506 healthy non-smokers (i.e. people without chronic or acute respiratory diseases, pulmonary medication use, airway obstruction, exposure to active or passive smoking, or inflammatory syndrome (defined as a serum hsCRP level > 10 mg/L)) (59)

With regard to cytokines, fewer data have been published. In a study of 3860 individuals from the Swiss general population (a study that also included two follow-up periods), there were significant positive associations between 1-day, 1-week, 1-month, 3-month and 6-month exposure to particulate matter with an aerodynamic diameter <10 µm (PM_{10}) and inflammatory cytokines (IL-6, IL-1β, and TNFα) (57). However, hsCRP was not associated with

PM_{10} (57). These significant associations with cytokine levels have not yet been confirmed in other studies. As mentioned above, a study of 408 Boston residents (56) did not find any significant association. Furthermore, these population-based studies in adults (56,57) measured a small set of cytokines (IL-1β, IL-6, and TNFα).

The objective of the present study was to explore (i) the associations between air pollutant levels and serum hsCRP levels in the ELISABET study population as a whole and (ii) the associations between air pollutant levels and plasma levels of a large set of cytokines in a subsample of non-smokers residing in the Lille urban area. We examined both short-term exposure (i.e. on the day of sample collection and on the day before) and long-term exposure (i.e. mean annual exposure at the residential address) and assayed the blood samples for biomarkers.

2.2. Methods

2.2.1. Population

The study population comprised male and female participants (aged 40-65) from the cross-sectional 2011-2013 ELISABET survey performed in northern France. All participants had lived in the same city (either Lille or Dunkirk) or its surrounding urban area for at least five years, and were recruited between January 2011 and November 2013 (60). The participants were selected from electoral rolls by random sampling, with stratification for sex, age and centre (Lille or Dunkirk), and were contacted in random order. Data were collected at home or (occasionally) during a consultation in a healthcare establishment. In all cases, a trained, registered nurse administered a detailed questionnaire. A blood sample was collected during the same visit (59).

We excluded ELISABET survey participants with a known inflammatory syndrome (defined as a serum hsCRP level > 10 mg/L) and participants with missing data for the hsCRP level, pollutant levels, or covariates.

Next, we studied other inflammatory biomarkers (IL1β, IL6, IL8, IL10, IL17A, IL22, and TNFα) in a subsample of 1125 non-smokers, in order to avoid the bias due to smoking-induced inflammation. We included participants from the Lille area only because the standard deviation of the residential air PM_{10} level was higher in Lille than in Dunkirk (1.936 $\mu g/m^3$ versus 0.999 $\mu g/m^3$, respectively), which therefore increased the statistical power of an analysis of a smaller number of participants. To enable subsequent genetic studies, we included participants of Caucasian origin with data on genetic variables. Lastly, we excluded patients with a known inflammatory syndrome, non-acceptable spirometry data, non-fasting data, or missing data.

The study was registered at ClinicalTrials.gov (NCT02490553) and the protocol was approved by the local investigational review (*CPP Nord Ouest IV*, Lille, France; reference: 2010-A00065-34), in compliance with the French legislation on biomedical research. All participants provided their written, informed consent to participation in the study (59).

2.2.2. Study areas

We studied participants living in two urban areas in northern France (Lille and Dunkirk, including the main city and the immediate suburbs). A map showing the cities' situation is shown in Supplemental Figure 1. Firstly, we checked that the characteristics of the two urban areas met the APHEKOM criteria, i.e. homogeneous exposure to a given air pollutant on a given day throughout the study area (61).

The Lille area is a dense urban area, with only a few non-urban zones between the three main cities (Lille, Roubaix and Tourcoing) and two smaller cities (Armentieres and Comines).

The road/motorway network in this area is very dense. The Lille urban area is crossed by five motorways; in 2012, the mean number of vehicles per day ranged from 36,000 to 176,000 (62,63).

The Dunkirk area is a coastal urban area. The sea is to the north of the city, and a harbour and an industrial area are located north-west of the city. The prevailing winds are south-westerly; as a consequence, air pollution from the industrial area is mostly pushed out to sea. The road and motorway network in this area is also dense (59). The Dunkirk urban area is crossed by two motorways; in 2012, the mean number of vehicles per day ranged from 27,000 to 61,000 (62,63)

2.2.3. Air pollution and meteorological measurements

The estimation of air pollution exposure has been described previously (16,59,64). We examined both short-term and residential exposure, using two different data sources. Short-term exposure was defined as the mean level of pollution in each study area (Lille or Dunkirk) on each measurement day and on the preceding day. This variable provides information on the short-term, reversible effects of high-pollution periods. Residential exposure was defined as the mean annual exposure at the residential address. This variable provides information on differences between people living in highly polluted areas vs. those living in less polluted areas, i.e. the long-term effects of air pollution. In brief, short-term exposure was assessed via daily measurements of PM_{10} and nitrogen dioxide (NO_2) and hourly measurements of ozone (O_3) at air pollution monitoring stations operated by the ATMO Hauts-de-France air quality monitoring association (Lille, France). The data from the urban and suburban stations met the APHEKOM homogeneity criteria and were included. The daily pollution levels for each city area were defined as the mean daily air concentration

for PM_{10} and NO_2 and the maximal 8-hour moving average for the O_3 air concentration, as measured at monitoring stations across the urban and suburban areas. We checked that the daily measurements from monitoring stations in the same city area were correlated (59,61). Meteorological data (including daily rainfall, air temperature, relative humidity, and atmospheric pressure at sea level) were obtained from the French National Meteorological Service.

For residential exposure, the annual mean concentrations of PM_{10} and NO_2 between 2010 and 2013 in Lille and between 2012 and 2013 in Dunkirk were taken from estimates produced by ATMO – Nord Pas de Calais, using the Atmospheric Dispersion Modelling System (ADMS, version 3.4, for urban areas) developed by Cambridge Environmental Research Consultants (65,66). This model could not be used to estimate residential exposure to 0_3.The latter incorporated meteorological, topographic and land-use data, pollutant emissions from natural sources and those related to human activity, and ambient air pollution data from monitoring stations. Concentration maps for PM_{10} and NO_2 in Lille and Dunkirk in 2015 are shown on the ATMO website (ATMO Nord-Pas-de-Calais, 2016a, 2016b). Maps of the spatial distribution of air pollutants for 2015 in the Lille and Dunkirk urban areas are available on the ATMO Hauts-de-France website (www.atmo-hdf.fr) (67,68). We applied a spatial resolution of 25 × 25 m. Each participant's place of residence was located within a 25 m grid. We assessed the annual exposure levels at the place of residence as the mean value of the four closest points in the grid, weighted by the inverse square distance to each point (16,69).

2.2.4. Biomarkers

The serum concentration of hsCRP was measured in a nephelometric assay (BN ProSpec

System, Siemens) with a detection range of 0.17 to 10 mg/L. Values below the limit of detection (LOD) were computed as 0.12 (LOD/√2) mg/L (59).

For cytokine measurements, we used the MesoScale Discovery® electrochemiluminescent multiplex immunoassay (Meso Scale Diagnostics LLC, Rockville, MA, USA). Multiplex assays (for IL-1β, IL-6, IL-8, IL-10, IL-17A, and TNFα) and the individual assay (for IL22) were provided on 10-spot plates (U-PLEX Plate K15067L-2, Meso Scale Diagnostics LLC). The assays were performed according to the manufacturer's instructions, except that we modified the range of the calibration curves. The manufacturer recommends eight-point calibration curves; however, in order to improve the assay's sensitivity, we added two points within the lower part of the curve and removed two points from the upper part of the curve.

Briefly, for a single plate, 200 µL of antibodies were first incubated with 300 µL of linkers for 30 minutes. Next, 200 µL of a stop solution were added, and the mixture was incubated for 30 minutes, loaded into QuickPlex 96-Well microplates (Meso Scale Diagnostics LLC), and incubated for 1 hour at room temperature. Thereafter, 25 µl of diluent and 25 µl of samples, standards or controls were added in duplicate and incubated overnight at 4°C. Each well was washed, incubated for 1 hour with a detection antibody, washed again, and supplemented with Read Buffer (Meso Scale Diagnostics LLC). Electrochemiluminescence signals were detected with the QuickPlex SQ 120 instrument and analyzed using Discovery Workbench 4.0 software (both from Meso Scale Diagnostics LLC).

Values below the lower limit of quantification (the lowest concentration on the standard curve which gave a percentage coefficient of variation of less than 20%) were counted as real concentrations.

The biomarker assay's stability over time was checked by running control samples on a regular basis during the study period. To this end, we pooled 40 residual plasma samples

collected during routine clinical care from adult patients admitted to Lille University Hospital (Lille, France). At least one control sample was included in each run.

2.2.5. Statistical analysis

We looked at both short-term and residential pollution. We calculated Pearson's coefficient for the correlations with residential exposure in each year and each city. Data for residential exposure in 2011 in Dunkirk area were not available (16). We imputed the 2011 residential exposure data for this area using the 2012 residential exposure data and mean of daily measure from pollution station using the following formula. "Imputed residential exposure in 2011" = "residential exposure in 2012" – "annual mean of daily air pollution station measurement in 2012" + "annual mean of daily air pollution station measurement in 2011". In order to evaluate the accuracy of this imputation, we imputed residential exposure in 2012 with 2013 data and calculated the agreement between the imputed values and the available values using the intra-class correlation (ICC) in the whole sample of the ELISABET study.

Associations between exposure to each air pollutant and the explanatory variables (the levels of inflammatory markers) were estimated using multiple linear regression models. In order to normalize the data distribution, all explanatory variables were log-transformed. Hence, the regression coefficients were expressed as the percentage change [95% confidence interval (CI)] per 10 $\mu g/m^3$ increment in each pollutant for short-term exposure and per 2 $\mu g/m^3$ of PM_{10} or 5 $\mu g/m^3$ of NO_2 increment for residential exposure. These increments for residential exposure were chosen because there were of the same order of magnitude as the interquartile range of the measurements in Lille and Dunkirk. The values were back-transformed and converted into a percentage change by multiplying by 100 and

subtracting 100 percentage points.

We tested for interactions between pollutant levels on one hand and the urban area, smoking status, and BMI on the other.

For short-term exposure, the models were adjusted for the urban area (Lille or Dunkirk), year of inclusion, age, sex, BMI, smoking status (non-smokers/current smokers), educational level (5 or more years of higher education, 2 to 4 years of higher education, no higher education, or no secondary education), population density (calculated from the IRIS submunicipal area databases obtained from the French National Institute for Statistics and Economic Studies: www.insee.fr), meteorological measurements (humidity, atmospheric pressure, air temperature, and rainfall), season (winter, spring, summer, or autumn), day of the week, the number of days since the beginning of the study, and school vacation periods.

For residential exposure, the models were adjusted for the urban area (Lille or Dunkirk), year of inclusion, age, sex, BMI, smoking status (non-smokers/current smokers) and educational level (5 or more years of higher education, 2 to 4 years of higher education, no higher education, or no secondary education).

Our primary analysis involved tests for associations between the explanatory variables and short-term exposure to PM_{10}, NO_2 and O_3, and associations between the explanatory variables and residential exposure to PM_{10} and NO_2. We then performed two sensitivity analyses; the first with the inclusion of PM_{10}, NO_2, and O_3 in a multipollutant model, and the second with the inclusion of wind direction and speed and cumulative hours of sunshine during the day (to determine whether the wind might bring industrial pollution into the Dunkirk area). Wind and sunshine data were obtained from the regional meteorological office (*Météo France,* Lille, France). Sunshine was included in the model because sunlight is involved in the formation of ground-level O_3 via the photodissociation of NO_2 (70)

The wind direction (averaged over an hour) was measured at a height of 10 meters.
We compared days with a northerly wind (between 270 and 89 degrees on the compass) with days with a southerly wind (between 90 and 269 degrees on the compass). The mean wind speed (in m/s) was calculated per hour and then per day. We excluded hourly wind speeds below 2 m/s, and calculated the hours per day with a northerly wind and the hours per day with a southerly wind. We only considered full days with a northerly wind or southerly wind for at least 70% of the day. Sunshine was considered as the cumulative duration per day. The latter variable was not measured in Dunkirk, and so we used data from the nearest station (Calais-Marck, about 30 km away).
All statistical analyses were performed using R software (version 1.3.1093, R Core Team, R Foundation for Statistical Computing, http:// www.r-project.org). The threshold for statistical significance was set to $p<0.05$.

2.3. Results

2.3.1. Population

A total of 3275 participants were included in the ELISABET study. For the hsCRP analysis, we excluded 198 participants (119 had missing hsCRP data and 79 an inflammatory syndrome), leaving 3077. The short-term pollution analyses included 3011 participants after the exclusion of 26 participants with missing data for at least one covariate and 40 participants with missing data for pollutants (PM_{10}, NO_2 or O_3). The residential pollution analyses included 3074 participants after the exclusion of 2 participants with missing data for at least one covariate and 1 participant with missing data for pollutants (PM_{10} or NO_2).

Of the 3275 participants, 1125 were included in the cytokine analysis. In the Lille Area, there were 1668 participants, of whom 1335 were non-smokers (either never-smokers or people having stopped smoking at least 12 months previously). Genetic data were available for 1125 of these 1335 participants. We then excluded 72 participants with non-acceptable spirometry data and 26 participants who had not fasted before blood sampling. 23 samples could not be assayed. After the exclusion of 22 participants with an inflammatory syndrome, the final sample comprised 982 participants.

The participants' characteristics are summarized in Table 1. The Lille and Dunkirk populations did not differ with regard to the main characteristics, except that the BMI was higher in the Dunkirk population and the educational level was higher in the Lille population. The participants' characteristics are described as a function of the BMI class (normal weight, overweight, or obesity) in Supplemental Table 1.

2.3.2. Pollution

The pollutant concentrations are summarized in Table 1. For short-term exposure, PM_{10} and NO_2 concentrations were higher in Lille than in Dunkirk, whereas O_3 concentrations were similar in the two urban areas. For residential exposure, PM_{10} and NO_2 concentrations were significantly higher in Lille than in Dunkirk.

We looked at the correlation between the various pollutants. For short-term exposure, PM_{10} and NO_2 were positively correlated with each other (r = 0.749 in Dunkirk and 0.635 in Lille), while O_3 was negatively correlated with NO_2 (r = -0.539 in Dunkirk and -0.660 in Lille) and PM_{10} (r = -0.261 in Dunkirk and -0.265 in Lille). For residential exposure, the coefficient for the correlation between PM_{10} and NO_2 was 0.762 in Dunkirk and 0.840 in Lille.

For residential exposure, the Pearson coefficients for the correlation with exposure in 2012 and 2013 in Dunkirk were 0.94 for PM_{10} and 0.97 for NO_2. For the 2012 imputed data using

the 2013 pollution data, the measurement of the agreement by ICC was 0.94 for PM_{10} and 0.89 for NO_2. The mean level of exposure in Dunkirk was 25.02 µg/m^3 for PM_{10} and 22.43 µg/m^3 for NO_2 (in 2012); and 25.93 µg/m^3 for PM_{10} and 23.08 µg/m^3 for NO_2 (in 2013). The correlation matrix for Lille is given in Supplemental Table 2; the correlation coefficients were very high.

2.3.3. Associations between pollution and inflammatory biomarkers

2.3.3.1. Association between pollution and hsCRP

For short-term exposure (Table 2), a 10 µg/m^3 increment in PM_{10} levels was associated with a significant increase [95%CI] in hsCRP (3.43% [0.68; 6.25] in the total study population (p=0.014). A 10 µg/m^3 increment in NO_2 levels was associated with a significant increase in hsCRP in Lille residents (7.85% [2.04; 13.98]; p=0.0076) but not in Dunkirk residents (-2.61% [-7.55; 2.59] p=0.32; p interaction = 0.030). Furthermore, the association was significant in current smokers (+12.37% [1.99; 23.8]; p=0.019) but not in non-smokers (-0.43% [-4.35; 3.64]; p=0.83), and the interaction was statistically significant (p=0.018). For O_3, the association was statistically significant in Lille residents (with a 5.32% [-8.98; -1.50] decrease in hsCRP; p=0.0068) but not in Dunkirk residents (+3.59% [-0.72; 8.09]; p=0.10), and the interaction was significant (p=0.025).

Moreover, we observed a significant interaction between BMI and PM_{10} (p< 0.01) and between BMI and NO_2 (p=0.043). The hsCRP level increased with PM_{10} exposure in participants with a normal BMI and in overweight participants, although the association was only statistically significant in the latter BMI class (p=0.0032). No significant associations were observed in the obese class. For NO_2 exposure, the changes in hsCRP levels were

similar but the associations were not significant in the normal weight and overweight BMI classes; however, a significant decrease was observed in the obese BMI class (p=0.029). After the exclusion of obese participants from the analysis, the associations were significant: +6.17% [2.72; 9.73] (p <0.001) for PM_{10} (p interaction <0.01) and +5.83% [1.06; 10.82] (p=0.016) for NO_2 (p interaction for obese vs non-obese = 0.016). Besides, the interaction with the urban area was no more significant after adjustment for the interaction between pollution and BMI.

For residential exposure (Table 2), the associations between air pollutants and the hsCRP level were not statistically significant. Likewise, the interactions between residential exposure to air pollutants and BMI were not statistically significant.

2.3.3.2. Association between pollution and cytokines

None of the associations between IL6, IL8, IL10, IL22, TNF, IL1, and IL17A and daily (short-term) exposure to PM_{10} and NO_2 were significant (Table 3). For short-term exposure to O_3 only, the association with TNFα was significant (+2.69% [0.44; 4.99]; p=0.019). None of the associations with mean annual residential exposure to PM_{10} or NO_2 were significant.

2.3.4. Sensitivity analyses

We performed linear regressions on multipollutant models. After adjustment for O_3, the results for short-term PM_{10} exposure (Supplemental Table 3) followed the same trend as the primary results. After adjustment for NO_2, the PM_{10} association was no longer significant for active smokers (+6.48% [-2.81; 16.66]; p=0.18) but became significant for non-smokers

(+5.03% [0.77; 9.47]; p=0.020), with a non-significant interaction (p=0.088). For NO_2 exposure, the associations were non-significant after adjustment for PM_{10} but the association with hsCRP was still significant in active smokers after adjustment for O_3 (+15.37% [2.97; 29.26] p=0.014), with a significant interaction (p=0.017). With regard to O_3 exposure, the associations with hsCRP levels were not significant after adjustment for PM_{10} or NO_2. The interaction with the urban area remained significant.

For residential exposure, multipollutant models did not show any statistically significant associations with hsCRP (Supplemental Table 4).

In multipollutant models, the associations between air pollution and cytokines were not statistically significant.

We also performed a linear regression by adding meteorological adjustment covariates (wind direction, wind speed, and sunshine) to the model for short-term pollution and hsCRP levels. For PM_{10} exposure, the associations with hsCRP were statistically significant in the total population, Lille residents, and non-smokers. The associations between NO_2 or O_3 and hsCRP were not significant. The interactions between pollutants and the urban area and between pollutants and smoking status were no longer significant. Lastly, the interactions between pollutants and the wind direction were not significant in either Lille or Dunkirk.

2.4. Discussion

Short-term exposure to air pollution was associated with serum hsCRP levels, and the association was influenced by smoking status, BMI, and urban area. Residential exposure was not significantly associated with serum hsCRP or plasma cytokine levels. The associations between short-term pollution and cytokine levels were not significant, with the exception of the association between O_3 and TNFα.

2.4.1. hsCRP

In a large survey of adults exposed to moderate levels of air pollution in two urban areas in northern France, we found that exposure on the day of the blood sample collection and the day before was significantly associated with higher hsCRP levels. The association was stronger in Lille residents, current smokers, and non-obese individuals.

Previous studies have found associations in specific, smaller populations (i.e. fewer than 100 people). For example, the blood CRP level was associated with occupational exposure among workers in a pulp and paper mill in Sweden (33) and among traffic police officers in Nepal (34). Furthermore, the CRP level in dried blood spots was associated with use of a traditional wood-burning cookstove in Nicaragua (51), and the serum CRP level was associated with exposure to metal fumes in Germany (46). A significant, positive association between daily atmospheric $PM_{2.5}$ exposure and CRP (repeated measurements) was observed in a sample of 88 elderly residents in the US state of Utah. (32)

Data for the general population are scarce. Two studies of general population samples did not find any significant associations. Green et al., (24) included 2086 women aged between 42 and 52 from six urban areas in the USA. The associations between air pollutant levels ($PM_{2.5}$ and O_3) and the serum hsCRP level were not significant for 1-day and 30-day exposures. Likewise, a study of 3860 members of the Swiss CoLaus cohort (57) did not find significant associations between 1-day, 1-week or 1-month PM_{10} exposures and serum hsCRP (with two blood samples per participant). Lastly, Hennig et al.'s study (58) of 8204 people from three large adjacent cities in the Ruhr area of Germany found an association between short-term (7-day) $PM_{2.5}$ or PM_{10} traffic-related exposure and serum hsCRP (two blood samples per participant). However, the associations with industry-generated pollution and total pollution were not significant.

It is well established that airborne pollutants can cause lung inflammation directly through the activation of alveolar macrophages and the secretion of inflammatory cytokines, such as TNFs and ILs (71–73). The overexpression of these cytokines (predominantly IL-6) might induce an acute hepatic response, with subsequent increases in the blood levels of various proteins associated with systemic inflammation - including CRP. The blood CRP concentration is usually low under normal conditions but rises quickly and markedly in response to pro-inflammatory stimuli (74–76). The plasma half-life of CRP is less than 20 hours and does not vary according to the pathophysiological conditions. Hence, the sole determinant of the circulating CRP concentration is the rate of synthesis, which directly reflects the intensity of the stimulus. The results of several *in vitro* and *in vivo* studies have suggested that CRP levels rise in response to PM exposure (77–84).

The effects of air pollution might differ according to the duration of exposure. Indeed, short-term exposure might trigger the phagocytosis of air pollutants by alveolar macrophages, which in turn might suddenly increase inflammatory biomarker levels (71). Long-term exposure might perpetuate the inflammatory process, with consequences for chronic disease (57).

Several of the studied interactions with pollutants were statistically significant. Firstly, we observed a significant interaction between smoking status and short-term exposure to NO_2. This interaction persisted in multipollutant models. Our results suggest that the effect of pollution on hsCRP levels is greater in smokers than in non-smokers.

Pilz et al., (47) found a significant interaction between pollution and smoking. However, it is hard to draw firm conclusions because the association was stronger in former smokers than in non-smokers and current smokers. The stronger association in smokers might be due to

synergistic effects of air pollution and tobacco. One hypothesis is that smokers are more susceptible to the effects of air pollution because their airways and lungs are already damaged. Indeed, smoking leads to disruption of the tight junctions and thus weakens the airways' epithelial barrier function (85). Furthermore, greater epithelial permeability in smokers might facilitate the penetration of pollutants into the body (86,87). Moreover, a decrease in pulmonary clearance in smokers might explain the greater effect of pollutants (88). Indeed, a study found that people exposed to cigarette smoke or smoking (89,90) had significantly lower plasma antioxidant levels, which might make these individuals more sensitive to the oxidative stress generated by air pollution (91). Consistently, Sun et al., (92) reported that the risk of mortality related to air pollution was greater in current or former smokers than in non-smokers.

However, a synergistic effect is not consistently observed. Panasevich et al., (93) found a significant association between air pollution and hsCRP in non-smokers but not in smokers. One hypothesis is that smokers already have a high level of low-grade inflammation due to tobacco particles, and so the additional effect of air pollution may be more difficult to detect in smokers than in non-smokers. Hence, the data on the interaction between tobacco and air pollution are sparse and inconclusive. This interaction warrants further study.

Secondly, we observed a significant interaction between pollutants (PM_{10} and NO_2) and BMI. Thus, PM_{10} was positively associated with hsCRP in non-obese people but not in obese people. These results are consistent with Pilz et al.'s (47) report of the association between elevated hsCRP levels and PM_{10} in non-obese individuals but not in obese individuals. In our study (and as expected, in view of Gentile et al.'s results (94)), the hsCRP level was much higher in obese people than in people of normal weight. This was probably because obesity is associated with chronic inflammation, the activation of pro-inflammatory pathways, and

higher levels of inflammatory biomarkers (95). Thus, a moderate effect of air pollution might be harder to detect in a population with a very high baseline level of low-grade inflammation.

In contrast, it has been suggested that effect of air pollution on health (not including low-grade inflammation) may be greater in obese people. For example, the association between $PM_{2.5}$ and diabetes found in the Danish Nurse cohort (15) was stronger in obese people. Surprisingly, we found NO_2 exposure was associated with significantly lower hsCRP levels in obese people. We do not have any physiological hypotheses for this result, which might be due to the negative correlations between NO_2 and O_3 and the harmful effects of O_3. Nevertheless, the association between O_3 and hsCRP was not significant and did not confirm this hypothesis. This finding might also be due to chance.

Thirdly, we found interactions between short-term NO_2 and O_3 exposure and the urban area. The association between NO_2 and O_3 exposure and hsCRP was statistically significant in Lille but not in Dunkirk, and the interaction was significant. The association between O_3 and hsCRP was in the opposite direction to that between NO_2 and hsCRP. Indeed, O_3 levels and NO_2 levels are negatively correlated. There is an industrial zone in the northwest part of the Dunkirk urban area; hence, the interaction between O_3 and NO_2 levels and the urban area might be explained by a difference in the nature of the pollutants between Lille and Dunkirk. Accordingly, the association in Hennig et al.'s study (58) (where industrial and traffic-related pollutants were modelled separately) was significant only for traffic-related pollution. A northerly wind would tend to bring industrial pollution to Dunkirk, whereas a southerly wind would bring traffic-related pollution to the city and thus resemble the situation in Lille. With a southerly wind, the associations between pollution and inflammation should be similar in Lille and Dunkirk. With a northerly wind, the nature of the pollutants would differ more.

Hence, one could reasonably hypothesize the presence of a pollution/wind interaction in Dunkirk. However, we did not find a significant interaction in this respect. The nature of the pollutants in Lille and Dunkirk and the differences between the two remain to be explored further. Lastly, the interaction between NO_2 and the urban area was not significant after the interaction with BMI had been taken into account – suggesting that the weaker association observed in Dunkirk (relative to Lille) was partly due to the first city's higher mean BMI.

We did not find a significant association between residential exposure and the hsCRP level. This might be because most of the effects of air pollution are short-term effects. Alternatively, the difference between the highest level of PM_{10} exposure and lowest level in our moderately exposed population might be too small to be produce a significant difference. Hence, the effect of air pollution on inflammation (if present) might not differ greatly from one area of a city to another. Lastly, environmental policies tend to focus on decreasing overall pollution levels and not solely reducing the number of highly exposed subjects.

In the literature, positive associations between occupational exposure to pollution and CRP were found among 87 Pakistani automobile vehicle drivers (35), 39 taxi drivers in Brazil (38), 72 workers in petrol station in Iraq (37), 137 people exposed to diesel engine exhaust in China (39), and 78 welders in Sweden (45). Conversely, a significant negative association between occupational exposure and CRP was found in a Chinese study of 54 workers exposed to diesel engine exhaust, relative to 55 non-exposed participants (40).
Some studies have found an association between air pollution and the CRP level in particular populations, such as 175 dialysis patients in Taiwan (36), 6508 pregnant women in Netherlands (52), and 7915 older adults in China (55). Furthermore, an association was

found between traffic density and the CRP level in 1017 participants of Puerto Rican origin living in Boston, USA (31).

Several studies of the general population (e.g. 3860 participants in Switzerland (57), 408 participants in Boston (USA) (56), 2252 participants in Germany (47) and about 17000 participants in the United Kingdom (48)) have not found a significant association between long-term exposure to air pollution and CRP. However, Green et al.'s (24) study of 2086 women in the USA found a significant association between hsCRP and 1-year $PM_{2.5}$ exposure and 6-month O_3 exposure. Lastly, a significant association between hsCRP and 1-year exposure to $PM_{2.5}$ or PM_{10} was found in 8204 German participants (58). The mean residential 365-day concentration of PM_{10} was 19.68 $\mu g/m^3$ for the overall pollution, including a mean of 0.81 $\mu g/m^3$ from traffic-related pollution and 2.47 $\mu g/m^3$ from industrial pollution. It is noteworthy that both of these studies featured repeated measurements of the hsCRP level.

2.4.2. Cytokines

The associations between plasma cytokine levels and short-term and residential exposures were not significant, with the exception of the association between short-term O_3 exposure and TNFα.

Previous studies have demonstrated associations between air pollution and cytokine in highly exposed people or in specific population. For example, studies of people occupationally exposed to pollution have found positive associations (35,37,38,40) or negative associations (39,49,50) between exposure and proinflammatory cytokine levels. Studies of children with asthma (41,44,54) have found positive associations between pollution exposure and blood levels of pro-inflammatory cytokines and (surprisingly, in two reports) the anti-inflammatory cytokine IL-10 (41,54). PM_{10} exposure was positively

correlated with IL-1β but negatively correlated with IL-6 in a cohort of newborns (53) and was positively correlated with IL6 but negatively with TNFα in pregnant women (42). In interventional studies, exposure to metal fumes was associated with higher cytokine levels (46), and the use of an air purifier was associated with lower IL-6 levels (43).

Conversely, population-based studies have published few data on moderate levels of exposure. Lane et al., (56) studied 408 Boston residents but did not find a significant association between mean annual air pollution exposure and cytokine levels. Lastly, Tsai et al., (57) studied a cohort of 3860 subjects aged from 35 to 75 years in Switzerland. Biomarker measurements were repeated twice, and PM_{10} exposure was assigned according to the participants' home address. Short-term exposure was defined variously as exposure one day, one week and one month before the blood sampling. On average, PM_{10} concentrations were 22.2 $\mu g/m^3$ one day before blood sampling, 24.5 $\mu g/m^3$ one week before blood sampling and 24.8 $\mu g/m^3$ one month before blood sampling. Long-term exposure was defined as exposure 3 months and 6 months before blood sampling. The concentrations of the pollutants in Tsai et al.'s study were similar to those in our study. Tsai et al., found a statistically significant association between short- and long-term exposure to PM_{10} and levels of three cytokines (IL1β, IL6, and TNFα); this is still the only study to have found an association with cytokine levels in the general population.

It is plausible that air pollution increases inflammation and cytokine secretion, since *in vitro* studies have evidenced the production of TNFα by alveolar macrophages exposed to particulate air pollutants (71,72). Furthermore, exposure to air pollution can lead to an increase in oxidative stress (96), a failure to eliminate apoptotic cells, and a state of chronic inflammation (97,98).

Not significant result concerning IL1β and IL-6 seems to be inconsistent with results of Tsai et

al.

With regard to the size of the effect observed by Tsai et al., (57) the power of our study for IL1β was 99%. Furthermore, when comparing the associations for IL-6 in Tsai et al.'s study and our study, the CIs did not overlap -suggesting that there was a true difference between the two sets of results. Indeed, the value was around 0.049 [0.029; 0.067] unit change per 1 $\mu g/m^3$ increment in PM_{10} for the Swiss cohort vs. -0.0036 [-0.025;0.018] for our study.

In contrast to Tsai et al., we observed a positive association between O_3 and TNFα. Tsai et al., observed a positive association between TNFα and PM_{10}. In most studies, PM_{10} and NO_2 levels are negatively correlated with O_3 levels; hence, we expected to see a positive association between TNFα and both PM_{10} and NO_2, and a negative correlation with O_3. However, this finding should be interpreted with caution because we performed multiple statistical tests on the cytokine data.

Lastly, our mainly non-significant results for cytokines are not consistent with those of Tsai et al., despite an apparently sufficient level of statistical power. Nevertheless ,the fact that we only considered a single blood sample might also explain the discrepancy between the two sets of results. Hence, further studies of the putative association between air pollution and cytokine levels are necessary.

2.4.3. Strengths and weaknesses

The present study had several strengths. Firstly, we studied a large, population-based sample. Secondly, our biomarker assays were performed with strict quality control procedures. In particular, a high-throughput analysis of plasma cytokine levels was performed on a multiplex assay platform equipped with a high-performance

electrochemiluminescence detection system (QuickPlex SQ 120, Meso Scale Diagnostics LLC). As previously described (99,100), this QuickPlex instrument was superior to conventional multiplex ELISA systems in terms of sensitivity, reproducibility, throughput, and the ability to test closely related antigens simultaneously without signal reduction due to antigen competition.

Our study also had some limitations. Firstly, we did not have data on residential exposure in 2011. However, Pearson's coefficient for the correlation between residential exposure levels in 2012 and 2013 in Dunkirk was 0.94 for PM_{10} and 0.97 for NO_2; and the ICC for the 2012 imputed data using 2013 pollution data was 0.94 for PM_{10} and 0.89 for NO_2– suggesting that the geographical distribution of mean annual air pollution levels is stable over time and that the imputation of residential exposure data in Dunkirk may only induce a limited classification bias. In addition, adjustment of the analysis for the year of inclusion might have reduce this bias. Furthermore, as defined in the present study, residential exposure depended mainly on the household's geographical location and less on the measurement timepoint. Therefore, the bias due to the use of data on geographical disparities from 2012 (rather than 2011) might be limited. Secondly, the difference in residential exposure between the most and least exposed participants was small. For example, the interquartile range was 25.84-27.19 $\mu g/m^3$ in Lille; this might have limited the study's power for the analysis of residential exposure. Thirdly, a lack of precision in the measurement of pollutant concentrations might have induced non-differential bias and reduces the study's power. Fourthly, our study's cross-sectional design (with a single blood sample) prevented us from controlling for the intra-individual variability in cytokine levels. In comparison, studies with repeated biomarker measurements have shown significant associations between pollution exposure and cytokine (57) or hsCRP (58) levels. Nevertheless, our statistically significant

results were in line with known associations (101–103), such as the associations with BMI, IL-6 ($p<10^{-17}$), TNF ($p<10^{-3}$) and the association between pulmonary obstruction and IL-6 ($p<0.05$) (data not shown). This confirmed our ability to evidence a strong association, even when cytokine levels varied significantly. Lastly, our population sample size might not have been large enough for some analyses (e.g. the cytokine analysis in particular). For example, the association between pollution and hsCRP in the subsample of 982 non-smokers from Lille was not significant (data not shown) - suggesting the need for a larger sample.

2.5. Conclusion

We observed a significant association between short-term exposure to air pollution and the serum hsCRP level; this association is consistent with the hypothesis whereby the health effects of air pollution are mediated by low-grade inflammation. This finding is consistent with some (but not all) previous observations. We found that BMI and smoking interacted with the association between short-term exposure to air pollution and the serum hsCRP level. This interaction might explain discrepancies in the literature data and might need to be taken in account in studies of hsCRP and pollution. We studied a large set of cytokines; all but one of the associations between air pollution exposure and cytokine levels were non-significant and so were not in agreement with Tsai et al.'s previous report (57). Hence, further research on this topic must include precise air pollutant measurements and must take account of intra-individual variability in cytokine levels.

Table 1. Characteristics of the study participants

	hsCRP population N = 3074		Cytokine population N = 982
	Dunkirk	Lille	
n (%)	1508 (49.06 %)	1566 (50.94 %)	982 (100 %)
Age (years), mean (SD)	53.26 (7.26)	53.16 (7.20)	53.84 (7.17)
Sex (H), n (%)	735 (48.74 %)	742 (47.38 %)	451 (45.93 %)
Non-smokers, n (%)	1229 (81.50 %)	1279 (81.67 %)	1004 (100 %)
BMI (kg/m²), median (SD)	27.38 (5.08)	26.54 (4.96)	26.72 (5.03)
Educational level, n (%)			
5 or more years of higher education	193 (12.80 %)	376 (24.01 %)	263 (26.78 %)
2 to 4 years of higher education	222 (14.72 %)	340 (21.71 %)	222 (22.61 %)
no higher education	902 (59.81 %)	716 (45.72 %)	422 (42.97 %)
no secondary education	191 (12.67 %)	134 (8.56 %)	75 (7.64 %)
hsCRP (mg/L), median [IQR]	1.14 [0.58-2.47]	0.99 [0.50-2.04]	0.94 [0.49-1.89]
Biomarkers (pg/mL), median [IQR]			
IL1β			0.13 [0.13-0.13]
IL6			0.44 [0.30-0.67]
IL8			2.72 [2.09-3.76]
IL10			0.19 [0.13-0.28]
IL17a			1.39 [1.39-1.39]
TNFα			2.35 [1.92-2.90]
IL22			0.23 [0.13-0.42]
Pollutants			
Short-term PM_{10} (µg/m^3), median [IQR]	21.50 [17.23-33.29]	22.50 [16.5-35.82]	22.56 [16.5-35.82]
Short-term NO_2 (µg/m^3), median [IQR]	20.25 [13.75-29.5]	24.00 [18-32.72]	24.00 [18.12-32.44]
Short-term O_3 (µg/m^3), median [IQR]	44.96 [32.84-55.06]	44.50 [30.97-56.51]	44.90 [31.16-56.43]
Long-term PM_{10} (µg/m^3), median [IQR]	26.54 [25.61-27.53]	27.03 [25.7-28.28]	26.87 [25.44-28.13]
Long-term NO_2 (µg/m^3), median [IQR]	22.12 [19.68-26.10]	26.01 [22.62-28.63]	25.53 [22.15-28.28]

Abbreviations: SD = standard deviation; BMI = body mass index; hsCRP = high sensitivity C-reactive protein; PM_{10} = particulate matter with an aerodynamic diameter below 10 µm; NO_2 = nitrogen dioxide; O_3 = ozone

Table 2. Associations between exposure to atmospheric pollutants and serum hsCRP (mg/L)

	PM_{10}				NO_2			O_3		
					short-term exposure					
Population	Number	Percentage change for 10 µg/m3 [95%CI]	p trend	p interaction	Percentage change for 10 µg/m3 [95%CI]	p trend	p interaction	Percentage change for 10 µg/m3 [95%CI]	p trend	p interaction
Total	3011	3.43 [0.68 ; 6.25]	0.014		1.75 [-1.96 ; 5.61]	0.36		-1.20 [-3.95 ; 1.64]	0.40	
City				0.074			0.030			0.025
Lille	1554	5.83 [2.26 ; 9.54]	0.0013		7.85 [2.04 ; 13.98]	0.0076		-5.32 [-8.98 ; -1.50]	0.0068	
Dunkirk	1457	0.16 [-4.21 ; 4.73]	0.94		-2.61 [-7.55 ; 2.59]	0.32		3.59 [-0.72 ; 8.09]	0.10	
Tobacco				0.082			0.018			0.84
Non-smokers	2461	2.22 [-0.71 ; 5.24]	0.14		-0.43 [-4.35 ; 3.64]	0.83		-1.01 [-3.99 ; 2.05]	0.51	
Current smokers	550	9.41 [2.16 ; 17.17]	0.010		12.37 [1.99 ; 23.80]	0.019		-1.82 [-8.78 ; 5.68]	0.63	
BMI				0.0074			0.043			0.57
BMI < 25	1163	3.15 [-1.74 ; 8.29]	0.21		3.34 [-3.38 ; 10.52]	0.34		-2.74 [-7.57 ; 2.34]	0.28	
25 ≤ BMI < 30	1167	6.69 [2.20 ; 11.37]	0.0032		4.26 [-1.86 ; 10.76]	0.18		-0.62 [-5.05 ; 4.01]	0.79	
BMI ≥ 30	681	-4.18 [-9.02 ; 0.92]	0.11		-7.47 [-13.66 ; -0.83]	0.029		2.79 [-2.59 ; 8.47]	0.32	
					residential exposure					
Population	Number	Percentage change for 2 µg/m3 [95%CI]	p trend	p interaction	Percentage change for 5 µg/m3 [95%CI]	p trend	p interaction			
Total	3074	1.98 [-2.35 ; 6.51]	0.38		0.88 [-3.19 ; 5.13]	0.68				
City				0.27			0.12			
Lille	1566	0.13 [-5.00 ; 5.55]	0.96		-0.91 [-5.27 ; 3.66]	0.69				
Dunkirk	1508	7.27 [-3.17 ; 18.83]	0.18		9.50 [-1.66 ; 21.92]	0.098				
Tobacco				0.18			0.082			
Non-smokers	2508	3.24 [-1.42 ; 8.13]	0.18		2.84 [-1.56 ; 7.43]	0.21				
Current smokers	566	-3.44 [-14.46 ; 9.00]	0.57		-8.92 [-18.98 ; 2.38]	0.12				
BMI				0.28			0.18			
BMI <25	1187	-0.23 [-7.31 ; 7.40]	0.95		-1.25 [-7.69 ; 5.65]	0.72				
25 ≤ BMI < 30	1190	2.93 [-3.83 ; 10.17]	0.40		3.76 [-3.03 ; 11.03]	0.29				
BMI ≥ 30	697	5.42 [-3.14 ; 14.72]	0.22		2.38 [-5.40 ; 10.8]	0.56				

Abbreviations: PM_{10} = particulate matter with an aerodynamic diameter below 10 µm; NO_2 = nitrogen dioxide; O_3 = ozone; BMI = body mass index (kg/m²)

Table 3. Associations between air pollution exposure and plasma biomarker levels (pg/mL)

	PM_{10}		NO_2		O_3	
			short-term exposure			
Biomarker	Percentage change for 10 µg/m3 [95%CI]	p trend	Percentage change for 10 µg/m3 [95%CI]	p trend	Percentage change for 10 µg/m3 [95%CI]	p trend
IL6	1.56 [-1.44 ; 4.65]	0.31	0.67 [-4.00 ; 5.57]	0.78	1.04 [-2.31 ; 4.52]	0.55
IL8	-1.39 [-3.64 ; 0.91]	0.23	-1.84 [-5.37 ; 1.83]	0.32	1.05 [-1.56 ; 3.72]	0.43
IL10	1.87 [-1.32 ; 5.16]	0.25	0.27 [-4.67 ; 5.47]	0.92	0.16 [-3.39 ; 3.83]	0.93
IL22	1.43 [-4.06 ; 7.24]	0.62	1.76 [-6.85 ; 11.16]	0.70	2.61 [-3.65 ; 9.27]	0.42
TNF	-0.72 [-2.65 ; 1.25]	0.47	-2.25 [-5.25 ; 0.83]	0.15	2.69 [0.44 ; 4.99]	0.019
IL1	-1.81 [-6.68 ; 3.32]	0.48	-1.25 [-8.96 ; 7.10]	0.76	1.07 [-4.63 ; 7.11]	0.72
IL17a	-0.23 [-3.95 ; 3.63]	0.91	-4.20 [-9.76 ; 1.72]	0.16	3.19 [-1.17 ; 7.75]	0.15
			residential exposure			
	Percentage change for 2 µg/m3 [95%CI]	p trend	Percentage change for 5 µg/m3 [95%CI]	p trend		
IL6	-0.39 [-4.68 ; 4.09]	0.86	-0.71 [-4.42 ; 3.16]	0.72		
IL8	-2.15 [-5.40 ; 1.21]	0.21	-2.03 [-4.86 ; 0.88]	0.17		
IL10	0.23 [-4.36 ; 5.05]	0.92	-0.60 [-4.58 ; 3.54]	0.77		
IL22	-2.13 [-9.82 ; 6.22]	0.61	-1.06 [-7.84 ; 6.22]	0.77		
TNF	-0.44 [-3.24 ; 2.45]	0.76	-0.53 [-2.96 ; 1.97]	0.68		
IL1	-3.77 [-10.83 ; 3.85]	0.32	-3.85 [-10.18 ; 2.93]	0.26		
IL17a	-0.067 [-5.48 ; 5.66]	0.98	-1,59 [-6.25 ; 3.30]	0.52		

Abbreviations: PM_{10} = particulate matter with an aerodynamic diameter below 10 µm; NO_2 = nitrogen dioxide; O_3 = ozone

Acknowledgements

We thank Florent Occelli for the map of northern France. We thank the ATMO Hauts-de-France air quality monitoring association for air pollution measurements and modelling in the Lille and Dunkirk urban areas. We also thank Lille University Hospital (especially the Centre de Biologie et de Pathologie), the University of Lille, the Institut Pasteur of Lille (especially the Departments of Médecine Préventive, Biologie Spécialisée and Médecine du Travail, and the Laboratoire d'Analyses Génomiques) and the Centre Hospitalier Général de Dunkerque (especially the Departments of Biology and Pneumology). We particularly thank the nurses, physicians and secretarial staff at the University of Lille and the Institut Pasteur of Lille. Lastly, we thank the French Ministère de l'Enseignement Supérieur et de la Recherche, the Hauts de France Region and the European Regional Development Fund for financial support.

Funding

This work was funded by Lille University Hospital (CHU de Lille, Lille, France), the Nord Pas-de-Calais Regional Council, and the European Regional Development Fund (ERDF-FEDER Presage N°36034) as part of the CPER Institut de Recherche en ENvironnement Industriel (IRENI) program. The work was also supported by the French government through the Programme Investissement d'Avenir (I-SITE ULNE / ANR-16-IDEX-0004 ULNE), managed by the Agence Nationale de la Recherche as part of the project "Santé Environnement: du risque territorial au risque individuel" (also funded by Lille European Metropole). Lastly, this work is part of the CPER's CLIMIBIO research project.

Competing interest

LD, RM and JMLG have contributed to an expert report (commissioned by Lille European Metropole) entitled "Rapport d'expertise à propos de la localisation de la piscine du projet

d’aménagement de la gare Saint Sauveur à Lille” [Expert report on the location of the swimming pool in the Saint Sauveur station development project in Lille] but did not receive any personal fees.

Other authors declare that they have no conflicts of interest.

3 Discussion (français)

L'exposition à court terme à la pollution atmosphérique était associée aux taux sériques de CRPus, et cette association était influencée par le tabagisme, l'IMC et la zone urbaine. L'exposition résidentielle n'était pas significativement associée aux taux sériques de CRPus ou de cytokines plasmatiques. Les associations entre la pollution à court terme et les niveaux de cytokines n'étaient pas significatives, à l'exception de l'association entre O_3 et TNFα.

La présente étude possède plusieurs points forts. Tout d'abord, nous avons étudié un grand échantillon de population. Ensuite, nos dosages de biomarqueurs ont été effectués selon des procédures strictes de contrôle de la qualité. En particulier, une analyse à haut débit des taux de cytokines plasmatiques a été réalisée sur une plateforme de dosage multiplex équipée d'un système de détection par électro-chimioluminescence haute performance (QuickPlex SQ 120, Meso Scale Diagnostics LLC). Comme décrit précédemment (99,100), cet instrument QuickPlex était supérieur aux systèmes ELISA multiplex classiques en termes de sensibilité, de reproductibilité, de débit et de capacité à tester simultanément des antigènes étroitement liés sans réduction du signal due à la compétition entre antigène.

Notre étude présente également certaines limites. Premièrement, nous ne disposions pas de données sur l'exposition résidentielle en 2011. Cependant, le coefficient de Pearson pour la corrélation entre les niveaux d'exposition résidentielle en 2012 et 2013 à Dunkerque était de 0,94 pour PM_{10} et de 0,97 pour NO_2; et le coefficient de corrélation intra-classe pour les données imputées de 2012 en utilisant les données de pollution de 2013 était de 0,94 pour PM_{10} et de 0.89 pour NO_2. Cela suggère ainsi que la distribution géographique des niveaux moyens annuels de pollution atmosphérique est stable dans le temps et que l'imputation des données d'exposition résidentielle à Dunkerque ne peut induire qu'un biais de

classification limité. De plus, l'ajustement de l'analyse en fonction de l'année d'inclusion a pu réduire ce biais. En outre, comme nous l'avons défini précédemment dans la présente étude, l'exposition résidentielle dépendait principalement de la localisation géographique du ménage et moins du moment de la mesure. Par conséquent, le biais dû à l'utilisation des données sur les disparités géographiques de 2012 (plutôt que de 2011) pourrait être limité. Deuxièmement, la différence d'exposition résidentielle entre les participants les plus et les moins exposés était faible. Par exemple, l'écart interquartile était de 25,84-27,19 $\mu g/m^3$ à Lille; cela pourrait avoir limité la puissance de l'étude pour l'analyse de l'exposition résidentielle. Troisièmement, un manque de précision dans la mesure des concentrations des polluants peut avoir induit un biais non différentiel et réduit la puissance de l'étude. Quatrièmement, la conception transversale de notre étude (avec un seul échantillon de sang) nous a empêchés de contrôler la variabilité intra-individuelle des niveaux de cytokines. En comparaison, des études avec des mesures répétées des biomarqueurs ont montré des associations significatives entre l'exposition à la pollution et les taux de cytokines (57) ou de CRPus (58). Néanmoins, nos résultats statistiquement significatifs étaient conformes aux associations connues (101–103), telles que les associations avec l'IMC et l'IL-6 ($p<10^{-17}$), le TNF ($p<10^{-3}$), et l'association entre l'obstruction pulmonaire et l'IL-6 ($p<0.05$) (données non présentées). Cela a confirmé notre capacité à mettre en évidence une forte association, même lorsque les niveaux de cytokines variaient de manière significative. Enfin, la taille de notre échantillon de population n'était peut-être pas suffisante pour certaines analyses (notamment l'analyse des cytokines). Par exemple, l'association entre pollution et CRPus dans le sous-échantillon de 982 non-fumeurs de Lille n'était pas significatif (données non présentées), suggérant la nécessité d'un échantillon plus large.

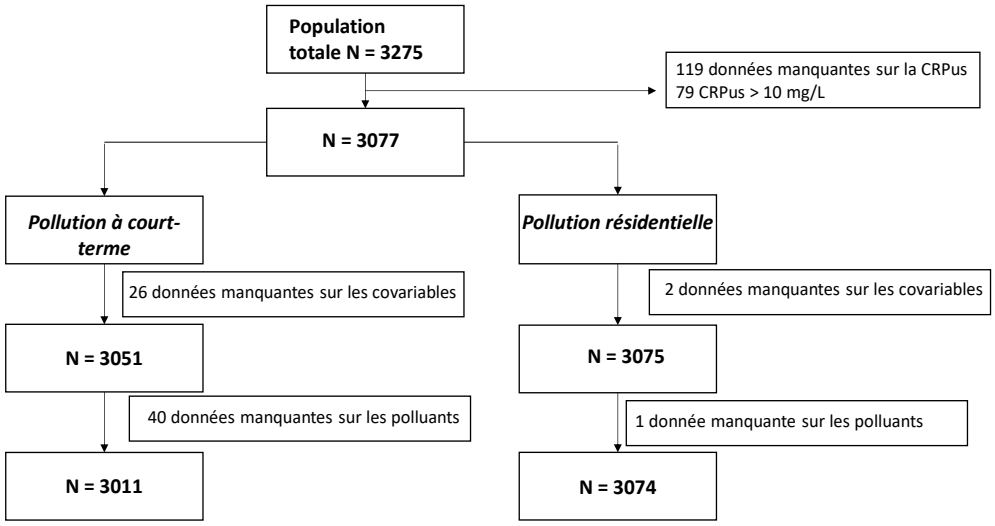
4 Conclusion (français)

Nous avons observé une association significative entre l'exposition à court terme à la pollution atmosphérique et le taux sérique de CRPus; cette association est cohérente avec l'hypothèse selon laquelle les effets de la pollution atmosphérique sur la santé sont médiés par une inflammation de bas grade. Ce résultat est conforme à certaines observations antérieures (mais pas toutes). Nous avons constaté que l'IMC et le tabagisme interagissaient avec l'association entre l'exposition à court terme à la pollution atmosphérique et le taux de CRPus sérique. Cette interaction pourrait expliquer les divergences entre les données de la littérature et devrait être prise en compte dans les études sur la CRPus et la pollution. Nous avons étudié un grand nombre de cytokines; toutes les associations entre l'exposition à la pollution atmosphérique et les niveaux de cytokines, sauf une, étaient non significatives et ne concordaient donc pas avec le rapport précédent de Tsai et al., (57). Les recherches futures sur ce sujet doivent donc inclure des mesures précises des polluants atmosphériques et tenir compte de la variabilité intra-individuelle des niveaux de cytokines.

5 Annexes

Annexe 1 : Population étudiée : diagrammes de flux (flowchart)

CRPus



Cytokines

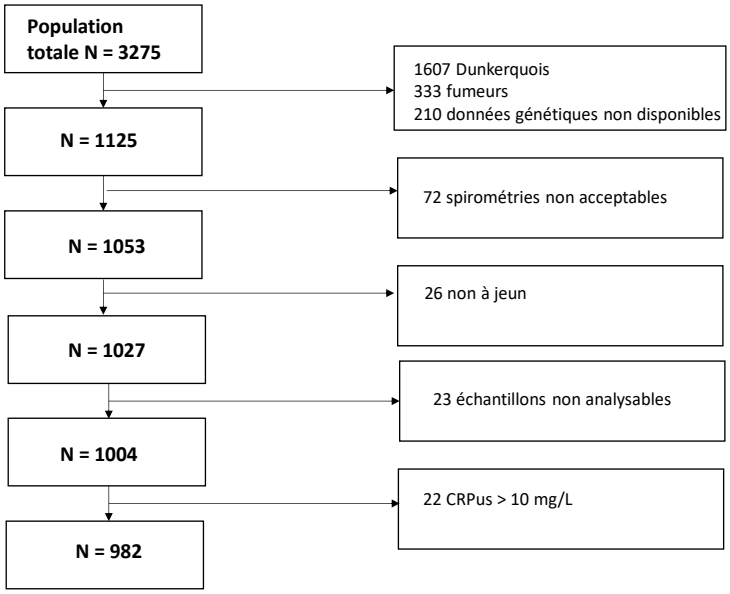
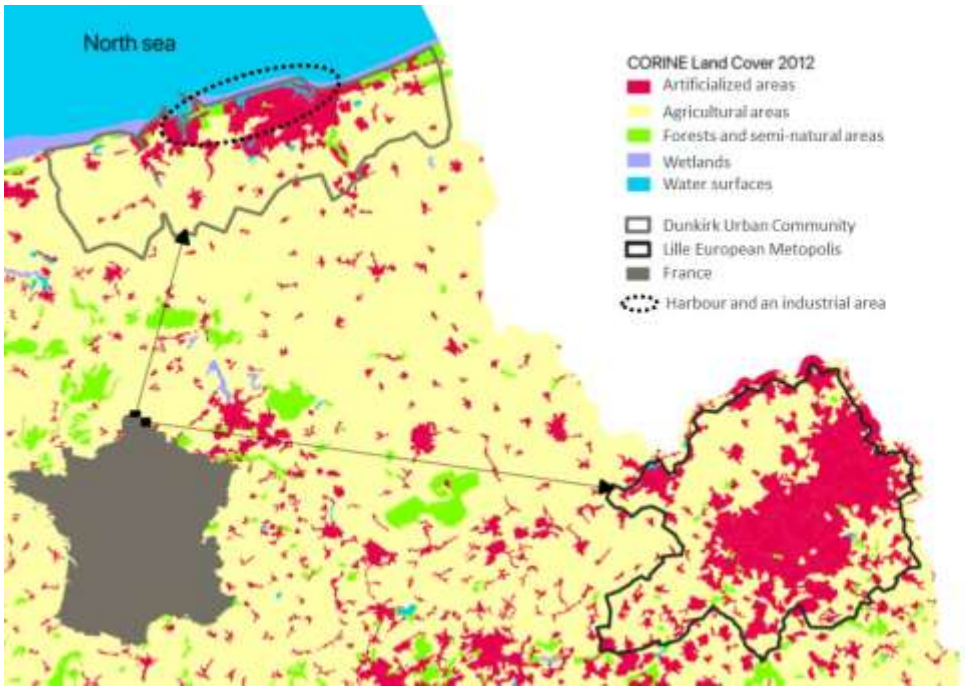


Figure supplémentaire 1. Carte du Nord de la France. (Source https://www.statistiques.developpement-durable.gouv.fr/corine-land-cover-0)


North sea
CORINE Land Cover 2012
Artificialized areas
Agricultural areas
Forests and semi-natural areas
Wetlands
Water surfaces
Dunkirk Urban Community
Lille European Metopolis
France
Harbour and an industrial area

Supplemental table 1. Characteristics of the participants, by BMI class (kg/m²) (N = 3074)

	BMI < 25	25 ≤ BMI <30	BMI ≥ 30
Number (%)	1187 (38.61%)	1190 (38.71%)	697 (22.67%)
Age (years), mean (SD)	51.43 (7.18)	54.04 (7.13)	54.83 (6.86)
Sex (H), n (%)	412 (34.71 %)	59.66 (59.66 %)	355 (50.93 %)
Non-smokers, n (%)	940 (79.19 %)	973 (81.76 %)	595 (85.37 %)
hsCRP (mg/L), median [IQR]	0.63 [0.34-1.33]	1.10 [0.63-2.12]	2.10 [1.15-3.99]

Abbreviations: BMI = body mass index; hsCRP = high sensitivity C-reactive protein; SD = standard deviation; IQR= Interquartile range

Supplemental Table 2. Pearson's coefficients for the correlations between residential air pollutant levels (µg/m^3) in different years in Lille

	Correlations between PM_{10} levels			
	2010	2011	2012	2013
2010	1.000	0.991	0.997	1.000
2011		1.000	0.997	0.992
2012			1.000	0.998
2013				1.000

	Correlations between NO_2 levels			
	2010	2011	2012	2013
2010	1.000	0.992	0.994	0.999
2011		1.000	0.996	0.991
2012			1.000	0.996
2013				1.000

Abbreviations: PM_{10} = particulate matter with an aerodynamic diameter below 10 µm; NO_2 = nitrogen dioxide

Supplemental table 3. Association between short-term exposure to atmospheric pollutants and serum hsCRP (mg/L) - multipollutant models

		PM_{10} adjusted for NO_2			**PM_{10} adjusted for O_3**		
Population	Number	Percentage change for 10 µg/m^3 [95%CI]	p trend	p interaction	Percentage change for 10 µg/m^3 [95%CI]	p trend	p interaction
Total	3011	5.03 [1.13 ; 9.08]	0.011		3.48 [0.52 ; 6.52]	0.021	
City				0.010			0.073
Lille	1554	4.92 [-0.24 ; 10.34]	0.062		4.52 [0.58 ; 8.62]	0.024	
Dunkirk	1457	3.40 [-2.81 ; 10.00]	0.29		1.37 [-3.25 ; 6.21]	0.57	
Tobacco				0.088			0.082
Non-smokers	2461	5.03 [0.77 ; 9.47]	0.020		2.17 [-1.01 ; 5.45]	0.18	
Current smokers	550	6.48 [-2.81 ; 16.66]	0.18		9.72 [2.13 ; 17.87]	0.012	
		NO_2 adjusted for PM_{10}			**NO_2 adjusted for O_3**		
Population	Number	Percentage change for 10 µg/m^3 [95%CI]	p trend	p interaction	Percentage change for 10 µg/m^3 [95%CI]	p trend	p interaction
Total	3011	-2.98 [-7.91 ; 2.22]	0.26		1.24 [-3.22 ; 5.92]	0.59	
City				0.067			0.032
Lille	1554	1.93 [-6.01 ; 10.54]	0.64		4.60 [-2.66 ; 12.4]	0.22	
Dunkirk	1457	-5.21 [-11.82 ; 1.89]	0.15		-0.54 [-6.43 ; 5.72]	0.86	
Tobacco				0.016			0.017
Non-smokers	2461	-5.11 [-10.37 ; 0.46]	0.071		-1.78 [-6.48 ; 3.15]	0.47	
Current smokers	550	5.97 [-6.85 ; 20.56]	0.38		15.37 [2.97 ; 29.26]	0.014	
		O_3 adjusted for PM_{10}			**O_3 adjusted for NO_2**		
Population	Number	Percentage change for 10 µg/m^3 [95%CI]	p trend	p interaction	Percentage change for 10 µg/m^3 [95%CI]	p trend	p interaction
Total	3011	0.13 [-2.87 ; 3.23]	0.93		-0.67 [-4.02 ; 2.80]	0.70	
City				0.039			0.027
Lille	1554	-3.14 [-7.32 ; 1.22]	0.16		-3.36 [-8.19 ; 1.74]	0.19	
Dunkirk	1457	3.98 [-0.54 ; 8.71]	0.085		3.35 [-1.67 ; 8.63]	0.19	
Tobacco				0.78			0.83
Non-smokers	2461	-0.14 [-3.39 ; 3.22]	0.93		-1.79 [-5.38 ; 1.94]	0.34	
Current smokers	550	1.04 [-6.39 ; 9.07]	0.79		3.88 [-4.67 ; 13.19]	0.39	

Abbreviations: PM_{10} = particulate matter with an aerodynamic diameter below 10 µm; NO_2 = nitrogen dioxide; O_3 = ozone

Supplemental table 4. Association between residential exposure to atmospheric pollutants and serum hsCRP (mg/L) - multipollutant models

		PM_{10} adjusted for NO_2			NO_2 adjusted for PM_{10}		
Population	Number	Percentage change for 2 µg/m^3 [95%CI]	p trend	p interaction	Percentage change for 5 µg/m^3 [95%CI]	p trend	p interaction
Total	3074	4.20 [-3.91 ; 12.99]	0.32		-2.39 [-9.61 ; 5.41]	0.54	
City				0.32			0.13
Lille	1566	5.56 [-6.15 ; 18.73]	0.37		-4.93 [-14.04 ; 5.15]	0.33	
Dunkirk	1508	-4.48 [-25.07 ; 21.75]	0.71		14.39 [-11.35 ; 47.61]	0.30	
Tobacco				0.16			0.080
Non-smokers	2508	2.47 [-6.23 ; 11.99]	0.59		0.83 [-7.28 ; 9.65]	0.85	
Current smokers	566	11.70 [-8.33 ; 36.1]	0.27		-16.31 [-30.86 ; 1.31]	0.068	

Abbreviations: PM_{10} = particulate matter with an aerodynamic diameter below 10 µm; NO_2 = nitrogen dioxide

6 Références bibliographiques

1. Darras-Hostens M, Achour D, Muntaner M, Grare C, Zarcone G, Garçon G, et al. Short-term and residential exposure to air pollution: Associations with inflammatory biomarker levels in adults living in northern France. Sci Total Environ. 2022 Apr 7;833:154985.

2. Air pollution [Internet]. [cited 2021 May 19]. Available from: https://www.who.int/news-room/air-pollution

3. Qualité de l'air ambiant et santé [Internet]. [cited 2022 Apr 28]. Available from: https://www.who.int/fr/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health

4. Pascal M, de Crouy Chanel P, Wagner V, Corso M, Tillier C, Bentayeb M, et al. The mortality impacts of fine particles in France. Sci Total Environ. 2016 Nov 15;571:416–25.

5. Adélaïde L. IMPACT DE LA POLLUTION DE L'AIR AMBIANT SUR LA MORTALITÉ EN FRANCE MÉTROPOLITAINE : RÉDUCTION EN LIEN AVEC LE CONFINEMENT DU PRINTEMPS 2020 ET IMPACT À LONG TERME POUR LA PÉRIODE 2016-2019 / IMPACT OF AIR POLLUTION ON MORTALITY IN METROPOLITAN FRANCE: REDUCTION RELATED TO THE SPRING 2020 LOCKDOWN AND LONG-TERM IMPACT FOR 2016-2019. :11.

6. Bono R, Romanazzi V, Bellisario V, Tassinari R, Trucco G, Urbino A, et al. Air pollution, aeroallergens and admissions to pediatric emergency room for respiratory reasons in Turin, northwestern Italy. BMC Public Health. 2016 Aug 5;16:722.

7. Madaniyazi L, Xerxes S. Outdoor air pollution and the onset and exacerbation of asthma. Chronic Dis Transl Med. 2021 Jun;7(2):100–6.

8. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet. 2014 May 3;383(9928):1581–92.

9. DeVries R, Kriebel D, Sama S. Outdoor Air Pollution and COPD-Related Emergency Department Visits, Hospital Admissions, and Mortality: A Meta-Analysis. COPD. 2017 Feb;14(1):113–21.

10. Solimini AG, Renzi M. Association between Air Pollution and Emergency Room Visits for Atrial Fibrillation. Int J Environ Res Public Health. 2017 Jun 20;14(6):E661.

11. Braithwaite I, Zhang S, Kirkbride JB, Osborn DPJ, Hayes JF. Air Pollution (Particulate Matter) Exposure and Associations with Depression, Anxiety, Bipolar, Psychosis and Suicide Risk: A Systematic Review and Meta-Analysis. Environ Health Perspect. 2019 Dec;127(12):126002.

12. Huang Y, Zhu M, Ji M, Fan J, Xie J, Wei X, et al. Air Pollution, Genetic Factors, and the Risk of Lung Cancer: A Prospective Study in the UK Biobank. Am J Respir Crit Care Med. 2021

Oct 1;204(7):817–25.

13. Li YC, Chiou JY, Lin CL, Wei JCC, Yeh MH. The association between air pollution level and breast cancer risk in Taiwan. Medicine (Baltimore). 2021 May 14;100(19):e25637.

14. So R, Chen J, Mehta AJ, Liu S, Strak M, Wolf K, et al. Long-term exposure to air pollution and liver cancer incidence in six European cohorts. Int J Cancer. 2021 Dec 1;149(11):1887–97.

15. Hansen AB, Ravnskjær L, Loft S, Andersen KK, Bräuner EV, Baastrup R, et al. Long-term exposure to fine particulate matter and incidence of diabetes in the Danish Nurse Cohort. Environ Int. 2016 May;91:243–50.

16. Riant M, Meirhaeghe A, Giovannelli J, Occelli F, Havet A, Cuny D, et al. Associations between long-term exposure to air pollution, glycosylated hemoglobin, fasting blood glucose and diabetes mellitus in northern France. Environ Int. 2018 Nov;120:121–9.

17. Yang BY, Bloom MS, Markevych I, Qian ZM, Vaughn MG, Cummings-Vaughn LA, et al. Exposure to ambient air pollution and blood lipids in adults: The 33 Communities Chinese Health Study. Environ Int. 2018 Oct;119:485–92.

18. Shanley RP, Hayes RB, Cromar KR, Ito K, Gordon T, Ahn J. Particulate Air Pollution and Clinical Cardiovascular Disease Risk Factors. Epidemiology. 2016 Mar;27(2):291–8.

19. Bell G, Mora S, Greenland P, Tsai M, Gill E, Kaufman JD. Association of Air Pollution Exposures With High-Density Lipoprotein Cholesterol and Particle Number: The Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2017 May;37(5):976–82.

20. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003 Jan 28;107(3):499–511.

21. León-Pedroza JI, González-Tapia LA, del Olmo-Gil E, Castellanos-Rodríguez D, Escobedo G, González-Chávez A. Inflamación sistémica de grado bajo y su relación con el desarrollo de enfermedades metabólicas: de la evidencia molecular a la aplicación clínica. Cirugía y Cirujanos. 2015 Nov 1;83(6):543–51.

22. Lee HM, Cha JM, Lee JL, Jeon JW, Shin HP, Joo KR, et al. High C-reactive protein level is associated with high-risk adenoma. Intest Res. 2017 Oct;15(4):511–7.

23. Leonard BE. Inflammation and depression: a causal or coincidental link to the pathophysiology? Acta Neuropsychiatr. 2018 Feb;30(1):1–16.

24. Green R, Broadwin R, Malig B, Basu R, Gold EB, Qi L, et al. Long- and Short-term Exposure to Air Pollution and Inflammatory/Hemostatic Markers in Midlife Women. Epidemiology. 2016 Mar;27(2):211–20.

25. Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of

coronary heart disease. Clin Chem. 2001 Mar;47(3):403–11.

26. Barnes PJ. Cellular and molecular mechanisms of asthma and COPD. Clin Sci (Lond). 2017 Jul 1;131(13):1541–58.

27. Akiki Z, Rava M, Diaz Gil O, Pin I, le Moual N, Siroux V, et al. Serum cytokine profiles as predictors of asthma control in adults from the EGEA study. Respir Med. 2017 Apr;125:57–64.

28. Shamsollahi HR, Jahanbin B, Rafieian S, Yunesian M. Particulates induced lung inflammation and its consequences in the development of restrictive and obstructive lung diseases: a systematic review. Environ Sci Pollut Res Int. 2021 May;28(20):25035–50.

29. Plé C, Fan Y, Ait Yahia S, Vorng H, Everaere L, Chenivesse C, et al. Polycyclic Aromatic Hydrocarbons Reciprocally Regulate IL-22 and IL-17 Cytokines in Peripheral Blood Mononuclear Cells from Both Healthy and Asthmatic Subjects. PLoS One. 2015 Apr 10;10(4):e0122372.

30. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation. 2010 Jun 1;121(21):2331–78.

31. Rioux CL, Tucker KL, Mwamburi M, Gute DM, Cohen SA, Brugge D. Residential traffic exposure, pulse pressure, and C-reactive protein: consistency and contrast among exposure characterization methods. Environ Health Perspect. 2010 Jun;118(6):803–11.

32. Pope CA, Hansen ML, Long RW, Nielsen KR, Eatough NL, Wilson WE, et al. Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. Environ Health Perspect. 2004 Mar;112(3):339–45.

33. Westberg H, Elihn K, Andersson E, Persson B, Andersson L, Bryngelsson IL, et al. Inflammatory markers and exposure to airborne particles among workers in a Swedish pulp and paper mill. Int Arch Occup Environ Health. 2016 Jul;89(5):813–22.

34. Shakya KM, Peltier RE, Zhang Y, Pandey BD. Roadside Exposure and Inflammation Biomarkers among a Cohort of Traffic Police in Kathmandu, Nepal. Int J Environ Res Public Health. 2019 Jan 29;16(3).

35. Riaz H, Syed BM, Laghari Z, Pirzada S. Analysis of inflammatory markers in apparently healthy automobile vehicle drivers in response to exposure to traffic pollution fumes. Pak J Med Sci. 2020 Jun;36(4):657–62.

36. Huang WH, Yen TH, Chan MJ, Su YJ. Environmental carbon monoxide level is associated with the level of high-sensitivity C-reactive protein in peritoneal dialysis patients. Medicine (Baltimore). 2014 Nov;93(26):e181.

37. Sajid Jabbar A, Ali ET. Impact of Petroleum Exposure on Some Hematological Indices,

Interleukin-6, and Inflammatory Markers of Workers at Petroleum Stations in Basra City. J Environ Public Health. 2020;2020:7693891.

38. Brucker N, Moro AM, Charão MF, Durgante J, Freitas F, Baierle M, et al. Biomarkers of occupational exposure to air pollution, inflammation and oxidative damage in taxi drivers. Sci Total Environ. 2013 Oct 1;463–464:884–93.

39. Dai Y, Zhang X, Zhang R, Zhao X, Duan H, Niu Y, et al. Long-term exposure to diesel engine exhaust affects cytokine expression among occupational population. Toxicol Res (Camb). 2016 Mar 1;5(2):674–81.

40. Bassig BA, Dai Y, Vermeulen R, Ren D, Hu W, Duan H, et al. Occupational exposure to diesel engine exhaust and alterations in immune/inflammatory markers: a cross-sectional molecular epidemiology study in China. Carcinogenesis. 2017 Oct 26;38(11):1104–11.

41. Negherbon JP, Romero K, Williams DL, Guerrero-Preston RE, Hartung T, Scott AL, et al. Whole Blood Cytokine Response to Local Traffic-Related Particulate Matter in Peruvian Children With and Without Asthma. Front Pharmacol. 2017;8:157.

42. Buxton MA, Meraz-Cruz N, Sanchez BN, Gronlund CJ, Foxman B, Vadillo-Ortega F, et al. Air pollution and inflammation: Findings from concurrent repeated measures of systemic and reproductive tract cytokines during term pregnancy in Mexico City. Sci Total Environ. 2019 Sep 1;681:235–41.

43. Chen R, Li H, Cai J, Wang C, Lin Z, Liu C, et al. Fine Particulate Air Pollution and the Expression of microRNAs and Circulating Cytokines Relevant to Inflammation, Coagulation, and Vasoconstriction. Environ Health Perspect. 2018 Jan 17;126(1):017007.

44. Klümper C, Krämer U, Lehmann I, von Berg A, Berdel D, Herberth G, et al. Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children. Environ Res. 2015 Apr;138:381–90.

45. Taj T, Gliga AR, Hedmer M, Wahlberg K, Assarsson E, Lundh T, et al. Effect of welding fumes on the cardiovascular system: a six-year longitudinal study. Scand J Work Environ Health. 2021 Jan 1;47(1):52–61.

46. Baumann R, Joraslafsky S, Markert A, Rack I, Davatgarbenam S, Kossack V, et al. IL-6, a central acute-phase mediator, as an early biomarker for exposure to zinc-based metal fumes. Toxicology. 2016 Dec 12;373:63–73.

47. Pilz V, Wolf K, Breitner S, Rückerl R, Koenig W, Rathmann W, et al. C-reactive protein (CRP) and long-term air pollution with a focus on ultrafine particles. Int J Hyg Environ Health. 2018 Apr;221(3):510–8.

48. Forbes LJL, Patel MD, Rudnicka AR, Cook DG, Bush T, Stedman JR, et al. Chronic exposure to outdoor air pollution and markers of systemic inflammation. Epidemiology. 2009 Mar;20(2):245–53.

49. Matsuda M, Bonatti R, Marquezini MV, Garcia MLB, Santos UP, Braga ALF, et al. Lacrimal Cytokines Assessment in Subjects Exposed to Different Levels of Ambient Air Pollution in a Large Metropolitan Area. PLoS One. 2015;10(11):e0143131.

50. Dai Y, Ren D, Bassig BA, Vermeulen R, Hu W, Niu Y, et al. Occupational exposure to diesel engine exhaust and serum cytokine levels. Environ Mol Mutagen. 2018 Mar;59(2):144–50.

51. Young BN, Peel JL, Nelson TL, Bachand AM, Heiderscheidt JM, Luna B, et al. C-reactive protein from dried blood spots: Application to household air pollution field studies. Indoor Air. 2020 Jan;30(1):24–30.

52. van den Hooven EH, de Kluizenaar Y, Pierik FH, Hofman A, van Ratingen SW, Zandveld PYJ, et al. Chronic air pollution exposure during pregnancy and maternal and fetal C-reactive protein levels: the Generation R Study. Environ Health Perspect. 2012 May;120(5):746–51.

53. Latzin P, Frey U, Armann J, Kieninger E, Fuchs O, Röösli M, et al. Exposure to moderate air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. PLoS One. 2011;6(8):e23130.

54. Gruzieva O, Merid SK, Gref A, Gajulapuri A, Lemonnier N, Ballereau S, et al. Exposure to Traffic-Related Air Pollution and Serum Inflammatory Cytokines in Children. Environ Health Perspect. 2017 Jun 16;125(6):067007.

55. Elbarbary M, Oganesyan A, Honda T, Morgan G, Guo Y, Guo Y, et al. Systemic Inflammation (C-Reactive Protein) in Older Chinese Adults Is Associated with Long-Term Exposure to Ambient Air Pollution. Int J Environ Res Public Health. 2021 Mar 22;18(6):3258.

56. Lane KJ, Levy JI, Scammell MK, Peters JL, Patton AP, Reisner E, et al. Association of modeled long-term personal exposure to ultrafine particles with inflammatory and coagulation biomarkers. Environ Int. 2016 Aug;92–93:173–82.

57. Tsai DH, Riediker M, Berchet A, Paccaud F, Waeber G, Vollenweider P, et al. Effects of short- and long-term exposures to particulate matter on inflammatory marker levels in the general population. Environ Sci Pollut Res Int. 2019 Jul;26(19):19697–704.

58. Hennig F, Fuks K, Moebus S, Weinmayr G, Memmesheimer M, Jakobs H, et al. Association between source-specific particulate matter air pollution and hs-CRP: local traffic and industrial emissions. Environ Health Perspect. 2014 Jul;122(7):703–10.

59. Dauchet L, Hulo S, Cherot-Kornobis N, Matran R, Amouyel P, Edmé JL, et al. Short-term exposure to air pollution: Associations with lung function and inflammatory markers in non-smoking, healthy adults. Environment International. 2018 Dec;121:610–9.

60. Quach A, Giovannelli J, Chérot-Kornobis N, Ciuchete A, Clément G, Matran R, et al. Prevalence and underdiagnosis of airway obstruction among middle-aged adults in northern France: The ELISABET study 2011-2013. Respir Med. 2015 Dec;109(12):1553–

61.

61. Pascal M, Corso M, Ung A, Declercq C, Medina S. Guidelines for assessing the health impacts of air pollution in European cities Work Package 5 Deliverable D5. :40.

62. Cartes de trafics annuels - DREAL HAUTS-DE-FRANCE [Internet]. [cited 2021 Dec 28]. Available from: https://www.hauts-de-france.developpement-durable.gouv.fr/?Cartes-de-trafics-annuels

63. https://www.hauts-de-france.developpement-durable.gouv.fr/IMG/pdf/recensement_circulation_region_nord_pas_de_calais_2012_trafic_tous_vehicules.pdf.

64. Havet A, Hulo S, Cuny D, Riant M, Occelli F, Cherot-Kornobis N, et al. Residential exposure to outdoor air pollution and adult lung function, with focus on small airway obstruction. Environ Res. 2020 Apr;183:109161.

65. CERC > Environmental software > ADMS-Urban model [Internet]. [cited 2021 Dec 28]. Available from: http://www.cerc.co.uk/environmental-software/ADMS-Urban-model.html

66. NUMTECH - Logiciels gamme ADMS - Conseils, vente exclusive, formation et assistance [Internet]. [cited 2021 Dec 28]. Available from: http://www.numtech.fr/log.php?rub=urban

67. ATMO Nord-Pas-de-Calais. Bilan territorial 2015 - Métropole Européenne de Lille [Internet]. 2016 [cited 2018 Jul 11]. Available from: http://www.atmo-hdf.fr/joomlatools-files/docman-files/Bilans-territoriaux/BT2015_MEL_VF.pdf.

68. ATMO Nord-Pas-de-Calais. Bilan territorial 2015 - Communauté Urbaine de Dunkerque [Internet]. 2016 [cited 2018 Jul 11]. Available from: http://www.atmo-hdf.fr/joomlatools-files/docman-files/BT2015_CUD_VF.compressed.pdf.

69. Babak O, Deutsch CV. Statistical approach to inverse distance interpolation. Stoch Environ Res Risk Assess. 2009 Jul 1;23(5):543–53.

70. Xiong Y, Du K. Source-resolved attribution of ground-level ozone formation potential from VOC emissions in Metropolitan Vancouver, BC. Science of The Total Environment. 2020 Jun 15;721:137698.

71. van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T, et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am J Respir Crit Care Med. 2001 Sep 1;164(5):826–30.

72. Becker S, Mundandhara S, Devlin RB, Madden M. Regulation of cytokine production in human alveolar macrophages and airway epithelial cells in response to ambient air pollution particles: further mechanistic studies. Toxicol Appl Pharmacol. 2005 Sep 1;207(2 Suppl):269–75.

73. Glencross DA, Ho TR, Camiña N, Hawrylowicz CM, Pfeffer PE. Air pollution and its effects

on the immune system. Free Radical Biology and Medicine. 2020 May;151:56–68.

74. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999 Feb 11;340(6):448–54.

75. Szalai AJ, Agrawal A, Greenhough TJ, Volanakis JE. C-reactive protein: structural biology and host defense function. Clin Chem Lab Med. 1999 Mar;37(3):265–70.

76. Sheriff A, Kayser S, Brunner P, Vogt B. C-Reactive Protein Triggers Cell Death in Ischemic Cells. Front Immunol. 2021 Feb 10;12:630430.

77. Watterson TL, Hamilton B, Martin R, Coulombe RA. Urban particulate matter causes ER stress and the unfolded protein response in human lung cells. Toxicol Sci. 2009 Nov;112(1):111–22.

78. Ramage L, Guy K. Expression of C-reactive protein and heat-shock protein-70 in the lung epithelial cell line A549, in response to PM10 exposure. Inhal Toxicol. 2004 Jun;16(6–7):447–52.

79. Ramage L, Proudfoot L, Guy K. Expression of C-reactive protein in human lung epithelial cells and upregulation by cytokines and carbon particles. Inhal Toxicol. 2004 Aug;16(9):607–13.

80. Vogel CFA, Sciullo E, Wong P, Kuzmicky P, Kado N, Matsumura F. Induction of proinflammatory cytokines and C-reactive protein in human macrophage cell line U937 exposed to air pollution particulates. Environ Health Perspect. 2005 Nov;113(11):1536–41.

81. Niwa Y, Hiura Y, Sawamura H, Iwai N. Inhalation exposure to carbon black induces inflammatory response in rats. Circ J. 2008 Jan;72(1):144–9.

82. Rohr AC, Wagner JG, Morishita M, Kamal A, Keeler GJ, Harkema JR. Cardiopulmonary responses in spontaneously hypertensive and Wistar-Kyoto rats exposed to concentrated ambient particles from Detroit, Michigan. Inhal Toxicol. 2010 May;22(6):522–33.

83. Upadhyay S, Ganguly K, Stoeger T, Semmler-Bhenke M, Takenaka S, Kreyling WG, et al. Cardiovascular and inflammatory effects of intratracheally instilled ambient dust from Augsburg, Germany, in spontaneously hypertensive rats (SHRs). Part Fibre Toxicol. 2010 Sep 29;7:27.

84. Lei YC, Hwang JS, Chan CC, Lee CT, Cheng TJ. Enhanced oxidative stress and endothelial dysfunction in streptozotocin-diabetic rats exposed to fine particles. Environ Res. 2005 Nov;99(3):335–43.

85. Heijink IH, Brandenburg SM, Postma DS, van Oosterhout AJM. Cigarette smoke impairs airway epithelial barrier function and cell-cell contact recovery. Eur Respir J. 2012 Feb;39(2):419–28.

86. Jones JG, Minty BD, Lawler P, Hulands G, Crawley JC, Veall N. Increased alveolar

epithelial permeability in cigarette smokers. Lancet. 1980 Jan 12;1(8159):66–8.

87. Danov O, Wolff M, Bartel S, Böhlen S, Obernolte H, Wronski S, et al. Cigarette Smoke Affects Dendritic Cell Populations, Epithelial Barrier Function, and the Immune Response to Viral Infection With H1N1. Front Med (Lausanne). 2020;7:571003.

88. Mehta H, Nazzal K, Sadikot RT. Cigarette smoking and innate immunity. Inflamm Res. 2008 Nov;57(11):497–503.

89. Dietrich M, Block G, Norkus EP, Hudes M, Traber MG, Cross CE, et al. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. Am J Clin Nutr. 2003 Jan;77(1):160–6.

90. Hemilä H. The effect of β-carotene on the mortality of male smokers is modified by smoking and by vitamins C and E: evidence against a uniform effect of nutrient. J Nutr Sci. 2020 Mar 11;9:e11.

91. Münzel T, Sørensen M, Gori T, Schmidt FP, Rao X, Brook FR, et al. Environmental stressors and cardio-metabolic disease: part II-mechanistic insights. Eur Heart J. 2017 Feb 21;38(8):557–64.

92. Sun S, Cao W, Chan KP, Ran J, Ge Y, Zhang Y, et al. Cigarette smoking increases deaths associated with air pollution in Hong Kong. Atmospheric Environment. 2020 Feb 15;223:117266.

93. Panasevich S, Leander K, Rosenlund M, Ljungman P, Bellander T, de Faire U, et al. Associations of long- and short-term air pollution exposure with markers of inflammation and coagulation in a population sample. Occup Environ Med. 2009 Nov;66(11):747–53.

94. Gentile M, Panico S, Rubba F, Mattiello A, Chiodini P, Jossa F, et al. Obesity, overweight, and weight gain over adult life are main determinants of elevated hs-CRP in a cohort of Mediterranean women. Eur J Clin Nutr. 2010 Aug;64(8):873–8.

95. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006 Mar;17(1):4–12.

96. Imrich A, Ning Y, Lawrence J, Coull B, Gitin E, Knutson M, et al. Alveolar macrophage cytokine response to air pollution particles: oxidant mechanisms. Toxicol Appl Pharmacol. 2007 Feb 1;218(3):256–64.

97. Grabiec AM, Hussell T. The role of airway macrophages in apoptotic cell clearance following acute and chronic lung inflammation. Semin Immunopathol. 2016 Jul;38(4):409–23.

98. Robb CT, Regan KH, Dorward DA, Rossi AG. Key mechanisms governing resolution of lung inflammation. Semin Immunopathol. 2016 Jul;38(4):425–48.

99. Bolton J, Chaudhury S, MacGill RS, Early AM, King CR, Locke E, et al. Multiplex serological assay for establishing serological profiles of polymorphic, closely related peptide antigens. MethodsX. 2021;8:101345.

100. Bolton JS, Chaudhury S, Dutta S, Gregory S, Locke E, Pierson T, et al. Comparison of ELISA with electro-chemiluminescence technology for the qualitative and quantitative assessment of serological responses to vaccination. Malaria Journal. 2020 Apr 17;19(1):159.

101. Celli BR, Locantore N, Yates J, Tal-Singer R, Miller BE, Bakke P, et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012 May 15;185(10):1065–72.

102. Huang H, Huang X, Zeng K, Deng F, Lin C, Huang W. Interleukin-6 is a Strong Predictor of the Frequency of COPD Exacerbation Within 1 Year. Int J Chron Obstruct Pulmon Dis. 2021;16:2945–51.

103. De Lorenzo A, Del Gobbo V, Premrov MG, Bigioni M, Galvano F, Di Renzo L. Normal-weight obese syndrome: early inflammation? Am J Clin Nutr. 2007 Jan;85(1):40–5.

AUTEUR(E) : Nom : HOSTENS **Prénom : MARION**

Date de soutenance : 18 mai 2022

Titre de la thèse : Etude de l'association entre l'exposition à la pollution atmosphérique et différents biomarqueurs inflammatoires dans une population issue de l'étude transversale ELISABET réalisée dans le Nord de la France entre 2011 et 2013.

Thèse - Médecine - Lille 2022

Cadre de classement : *Santé publique*
DES + FST/option : *Santé publique*
Mots-clés : Pollution atmosphérique, inflammation de bas grade, Etude de population, Santé humaine, Epidémiologie

Résumé :

Contexte: La pollution atmosphérique a un impact sur la santé, et l'inflammation de bas grade pourrait être l'un des mécanismes sous-jacents. L'objectif de la présente étude portant sur des adultes du Nord de la France était d'évaluer les associations entre l'exposition à court terme et résidentielle à la pollution atmosphérique et les taux de divers biomarqueurs inflammatoires.

Méthodes: L'étude transversale *Enquête Littoral Souffle Air Biologie Environnement* (ELISABET) a été menée de 2011 à 2013 dans les aires urbaines de Lille et de Dunkerque, dans le nord de la France. Ici, nous avons évalué les associations entre l'exposition aux PM_{10}, NO_2 et O_3 (le jour et la veille du prélèvement de l'échantillon de sang, et les niveaux annuels moyens résidentiels) et les taux de biomarqueurs inflammatoires que sont la protéine C-réactive ultra-sensible (CRPus), les interleukines (IL)-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, et le facteur de nécrose tumorale (TNF) α.

Résultats: Nous avons évalué 3074 participants pour l'association avec la CRPus et un sous-échantillon de 982 non-fumeurs de Lille pour l'association avec les taux de cytokines plasmatiques. Une augmentation de 10 µg/m^3 des niveaux de PM_{10} et de NO_2 le jour et la veille du prélèvement de l'échantillon de sang était associée à une concentration plus élevée de CRPus (3.43% [0.68; 6.25] et 1.75% [-1.96; 5.61], respectivement, alors qu'une augmentation de 10 µg/m^3 de O_3 était associée à une concentration plus basse de CRPus (-1.2% [-3.95; 1.64]). Les associations entre l'exposition annuelle moyenne et le taux de CRPus n'étaient pas significative. De même, les associations entre l'exposition et les niveaux de cytokines plasmatiques n'étaient pas statistiquement significatives.

Conclusion: L'exposition à la pollution atmosphérique à court terme était associée à des taux de CRPus sériques plus élevés chez des adultes vivants dans deux zones urbaines du Nord de la France. Nos résultats suggèrent que, parmi d'autres facteurs, l'inflammation de bas grade pourrait expliquer les effets néfastes de la pollution atmosphérique sur la santé.

Composition du Jury :

Président : Pr. Philippe Amouyel

Assesseurs : Dr. Jean Dallongeville
Dr. Jean-Marc Lo-Guidice
Directeur de thèse :Dr. Luc Dauchet