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# Broncho-pneumopathie chronique obstructive secondaire à l'exposition aux poussières organiques : caractérisation pulmonaire et systémique.

Thibaud Soumagne

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**UNIVERSITÉ BOURGOGNE-FRANCHE COMTÉ**

**École Doctorale Environnements – Santé**

**THÈSE**

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**Docteur de l'Université de Bourgogne Franche-Comté**

Sciences de la Vie et de la Santé

Présentée et soutenue publiquement le 13 Septembre 2019 par

**Thibaud SOUMAGNE**

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**Broncho-pneumopathie chronique obstructive secondaire à  
l'exposition aux poussières organiques:  
caractérisation pulmonaire et systémique**

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## **LISTE DES ABREVIATIONS**

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A1AT:  $\alpha$ -1-antitrypsine  
ALD: affection de longue durée  
ATS: American thoracic society  
AVC: accident vasculaire cérébral  
BDI: baseline dyspnea index  
BPCO: broncho-pneumopathie chronique obstructive  
CAT: COPD Assessment Test  
CDV: courbe débit-volume  
CI: capacité inspiratoire  
CPT: capacité pulmonaire totale  
CRF: capacité résiduelle fonctionnelle  
CVF: capacité vitale forcée  
CVL: capacité vitale forcée  
EFX: épreuve fonctionnelle d'exercice  
ERS: European respiratory society  
FeMaSaC: fédération des maisons de santé comtoises  
GLI: Global Lung function Initiative  
GOLD: Global initiative for Obstructive Lung Disease  
HAD: hospitalization anxiety depression  
IDM: infarctus du myocarde  
IL: interleukine  
IMC: indice de masse corporelle  
Ig: immunoglobulines  
LIN: Limite Inférieure à la Normale  
mMRC: modified Medical Research Council  
MSA: mutualité sociale agricole  
NO<sub>2</sub>: dioxyde d'azote  
OMS: Organisation Mondiale de la Santé  
PA: paquets-années  
PaO<sub>2</sub>: pression partielle en O<sub>2</sub> dans le sang artériel  
PI max: pression inspiratoire maximale  
PM: particules fines  
SGRQ: Saint-George's respiratory questionnaire  
SNIP: pression sniff nasale  
SPLF: société de pneumologie de langue française  
SpO<sub>2</sub>: la saturation pulsée en O<sub>2</sub>  
TLCO: transfert pulmonaire du monoxyde de carbone  
TM6: test de marche de 6 minutes  
TVO: trouble ventilatoire obstructif  
VCO<sub>2</sub>: débit de dioxyde de carbone rejeté  
VE: débit ventilatoire  
VEMS: volume expiré maximal lors de la première seconde de l'expiration  
V'E/V'O<sub>2</sub>: équivalent respiratoire en oxygène  
V'E/V'CO<sub>2</sub>: équivalent respiratoire en dioxyde de carbone  
VFC: variabilité de la fréquence cardiaque  
VO<sub>2</sub>: débit d'oxygène consommé  
VOP: vitesse d'onde de pouls  
VGDF: vapeur, gases, dust and fumes  
VR: volume résiduel

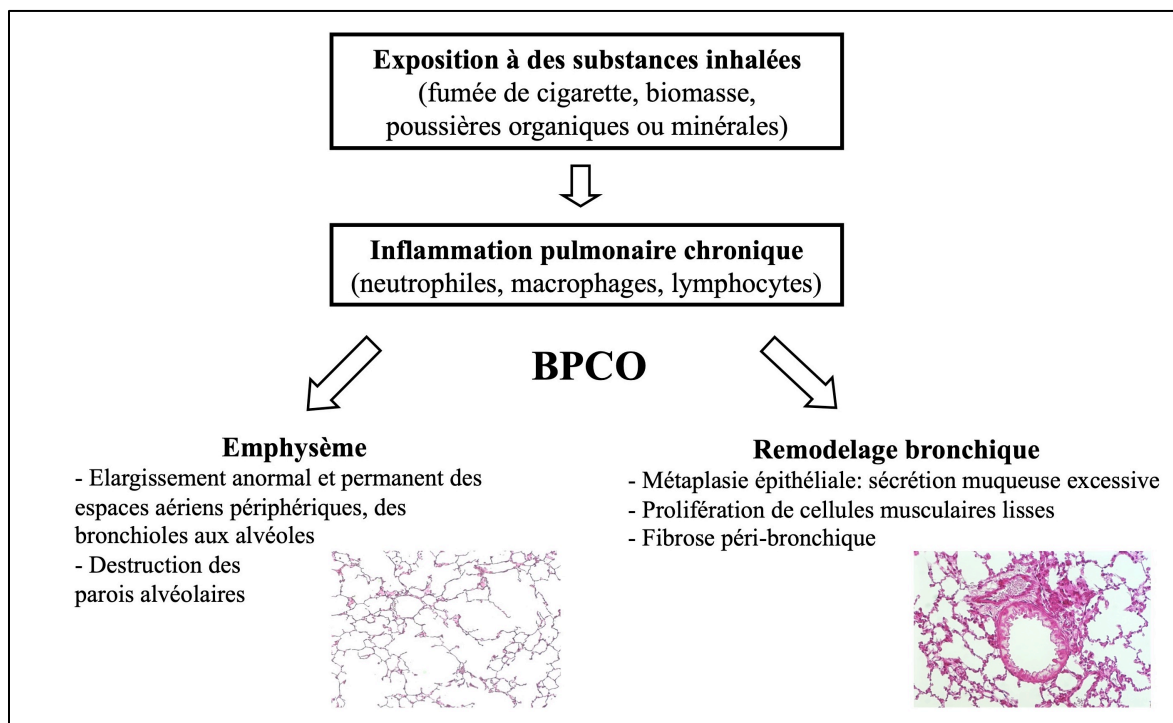


# I. INTRODUCTION

## A. La broncho-pneumopathie chronique obstructive

### 1. Définition et généralités

La broncho-pneumopathie chronique obstructive (BPCO) est une maladie respiratoire chronique qui atteint principalement les adultes de plus de 40 ans et qui est définie par la présence de symptômes respiratoire et d'une obstruction progressive et permanente des voies aériennes (1, 2). Cette obstruction est causée par l'association, variable selon les patients, d'une diminution du calibre des bronchioles du fait de modifications anatomiques (remodelage) et d'une destruction pulmonaire en aval des bronchioles terminales (emphysème) (figure 1) (1).



**Figure 1.** Physiopathologie de la BPCO

La BPCO a longtemps été confondue, y compris dans sa nosologie, avec la bronchite chronique qui pour sa part est définie par une toux productive quotidienne durant au moins 3 mois par an et au cours d'au moins 2 années consécutives. Cependant, la BPCO demeure longtemps peu symptomatique : la dyspnée, signe d'alerte pour le malade comme pour le médecin, apparaît lorsque la fonction respiratoire est déjà très altérée (3). La toux et l'expectoration font partie des symptômes rencontrés dans la BPCO, mais leur présence n'est ni sensible ni spécifique. La reconnaissance de la maladie sur des critères exclusivement cliniques s'est révélée désuète lorsque la BPCO, sous l'influence du GOLD (Global initiative

for Obstructive Lung Disease), s'est trouvée définie exclusivement sur un critère spirométrique (4). Le diagnostic était en effet porté sur les résultats d'une courbe débit-volume (CDV) grâce à laquelle sont mesurés la capacité vitale forcée (CVF) et le volume expiré maximal lors de la première seconde de l'expiration (VEMS). La nouvelle définition GOLD inclut à présent l'existence concomitante d'un trouble ventilatoire obstructif et de symptômes compatibles (1).

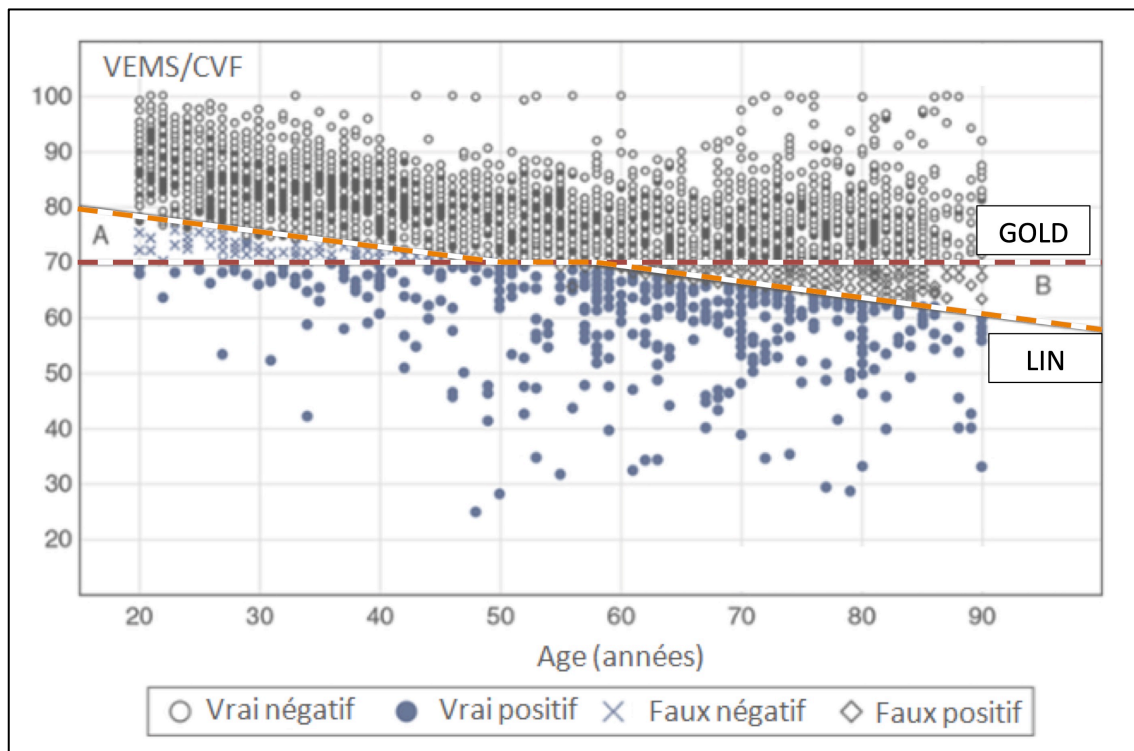
Le développement d'une BPCO est le plus souvent secondaire à l'exposition à des substances toxiques inhalées. C'est une affection d'installation lentement progressive, non réversible à l'arrêt de l'exposition. Sa gravité, chez les sujets sensibles à une ou plusieurs de ces substances inhalées, est proportionnelle à la durée et à l'intensité de l'exposition (5). La fumée de tabac est de loin le principal facteur de risque identifié (responsable de 80 % des BPCO actuellement). Certains milieux professionnels tels que l'extraction (mines, travaux publics, puits...), la cimenterie, la production laitière, l'élevage de porcs et de volailles ou le travail du bois sont associés à un risque accru de BPCO (5, 6). Dans certaines régions du monde, la fumée domestique issue de la combustion de biomasse destinée à se chauffer ou à faire la cuisine expose les femmes et les enfants à un risque important de BPCO (7). Enfin, certains sujets développent une BPCO sans qu'aucun facteur d'exposition ne soit identifié (8).

## **2. Confirmation de la BPCO**

La stratégie retenue en France consiste à adresser à un pneumologue les sujets suspects de BPCO, avec comme objectifs de confirmer la BPCO, d'éliminer une pathologie pulmonaire autre pouvant s'accompagner d'une obstruction bronchique, de rechercher des maladies associées, d'évaluer la sévérité et de proposer un « parcours de soins » (2).

Selon les critères du GOLD, le diagnostic de BPCO repose sur la mise en évidence d'un trouble ventilatoire obstructif (TVO) non réversible et l'existence de symptômes respiratoires compatibles; le critère fonctionnel en vigueur est un rapport VEMS/CVF < 70 % après administration d'un bronchodilatateur (1). Chez certains patients, la capacité vitale lente (CVL) est supérieure à la CVF (9). Les recommandations ATS/ ERS recommandent d'utiliser la CV (meilleur résultat des mesures de CVF et de CVL) pour calculer le rapport VEMS/CV. La CVL peut être mesurée à l'aide de spiromètres conventionnels, mais cette mesure (ainsi que celle de la capacité inspiratoire [CI]) est le plus souvent réalisée lors de la pléthysmographie, permettant de déterminer le rapport VEMS/CVL, généralement plus sensible que le rapport VEMS/CVF pourtant retenu par GOLD (10). Du fait d'une diminution du rapport VEMS/CVF avec l'âge, même chez les non-fumeurs ou les non exposés,

l'utilisation d'un seuil fixe à 70 % peut sembler discutable car il entraîne un diagnostic par excès chez les personnes âgées (figure 2) (11).

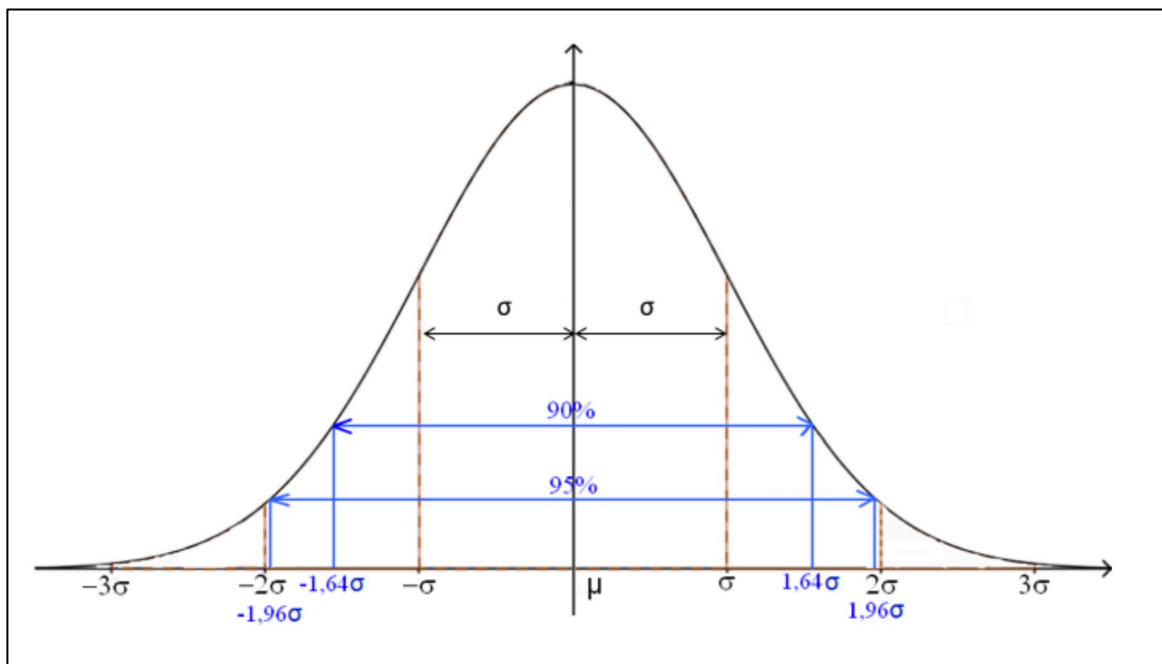


**Figure 2.** Rapport VEMS/CVF chez des sujets sains.

Pour pallier aux limites du critère GOLD, plusieurs groupes se sont donnés pour objectif de créer une équation prenant en compte différentes caractéristiques de l'individu testé de manière à fixer une valeur seuil différente pour chaque individu. Cependant, les équations développées jusqu'en 2010 souffraient de plusieurs écueils :

- Les populations de référence à partir desquelles elles ont été développées étaient de moyen ou de faible effectif induisant un manque de précision (11-13).
- Ces populations incluait un très faible effectif d'enfants de moins de 8 ans et de personnes âgées de plus de 80 ans rendant les équations d'autant plus imprécises pour ces catégories d'âge
- Le modèle statistique utilisé pour développer les équations était le plus souvent un modèle de régression linéaire : une régression linéaire croissante pour la période de l'enfance et l'adolescence et une régression linéaire décroissante pour la période adulte. Ce choix de deux équations différentes induisait une importante discontinuité de la valeur seuil lors de la transition entre la fin de l'adolescence et le début de l'âge adulte. Récemment, le groupe Global Lung function Initiative (GLI) a utilisé un outil statistique robuste pour améliorer les équations développées jusqu'alors et corriger les erreurs de diagnostic liées au critère GOLD.

Grâce à cette nouvelle équation, la Limite Inférieure à la Normale (LIN) permet d'obtenir une valeur seuil du rapport VEMS/CVF qui tient compte de l'âge, du sexe, de la taille et de l'ethnie de chaque individu soumis à une spirométrie (14). La valeur du rapport VEMS/CVF du sujet testé (dont on veut évaluer s'il présente un TVO ou non) est comparée à une LIN obtenues grâce aux valeurs de VEMS/CVF mesurées dans une « population de référence ». La population de référence est une population saine et non fumeuse de même âge, sexe, taille et ethnie que le sujet testé. En médecine, il est d'usage de considérer qu'on peut se tromper dans 5% des cas en affirmant à tort qu'un sujet de la population de référence a une valeur anormale (ce seuil de 5% est aussi appelé risque de première espèce, ou risque alpha). Même si un rapport VEMS/CVF très élevé peut être le témoin d'authentiques situations pathologiques (fibrose pulmonaire par exemple), on considère que le rapport VEMS/CVF ne sert qu'à rechercher un TVO, et seule une valeur du rapport VEMS/CVF anormalement basse sera considérée comme pathologique. Ainsi, le rapport VEMS/CVF d'un individu sera considéré comme anormal par la LIN s'il est inférieur au 5<sup>ème</sup> percentile de la distribution du rapport VEMS/CVF dans sa population de référence (Figure 3).



**Figure 3.** Représentation d'une distribution suivant une loi normale

En pratique, pour pouvoir comparer une valeur à une distribution de référence, il faut que cette dernière suive une loi normale. Or, ceci n'est pas le cas pour la plupart des paramètres spirométriques et notamment pour le rapport VEMS/CVF. Ainsi, la valeur étudiée

et celles de la population de référence subissent une transformation de Box-Cox-Cole-Green (15) afin de pouvoir être comparées. Cette transformation est de la forme suivante :

$$z = \frac{\left(\frac{Y}{\mu}\right)^\lambda - 1}{\lambda \times \sigma}$$

où Y est la valeur mesurée chez le sujet testé (ici le rapport VEMS/CVF),  $\mu$  est la moyenne des valeurs observées dans la population de référence de ce sujet ( $\mu$  est donc ce qui est souvent appelé « valeur de référence » ou « valeur théorique » de ce sujet),  $\sigma$  est le coefficient de variation des valeurs observées dans la population de référence de ce sujet, et  $\lambda$  l'indice d'asymétrie des valeurs observées dans la population de référence de ce sujet. L'objectif de la transformation de l'équation précédente est d'obtenir un z-score (z) ayant une distribution proche d'une distribution gaussienne centrée réduite (moyenne nulle et écart-type égal à 1) (15). En d'autres termes, cette transformation permet d'obtenir une distribution symétrique (toute asymétrie étant corrigée par le choix approprié du coefficient lambda).

La définition LIN (rapport VEMS/CVF < limite inférieure de la normale) semble plus pertinente, notamment pour le dépistage, puisqu'elle est spécifique à l'âge et non fixe. En effet, la présence de symptômes fait partie de la définition de la BPCO mais des patients présentant une obstruction bronchique définie par un rapport VEMS/CVF < LIN, qui ne rapportent pas de symptômes, peuvent révéler des anomalies physiologiques à l'exercice (16, 17). La présence de symptômes n'est donc pas indispensable pour que soit faite une spirométrie de dépistage de BPCO, si tant est que la définition ATS/ERS (VEMS/CVF < LIN) soit appliquée pour définir l'obstruction bronchique persistante.

En résumé, le diagnostic de BPCO se porte sur deux examens : un examen clinique pour l'évaluation des symptômes et une CDV pour la mesure du TVO. Ce dernier est défini par un rapport VEMS/CVF, mesuré après l'inhalation de broncho-dilatateurs, inférieur à 70% (critère GOLD) ou inférieur à une valeur déterminée selon l'âge, le sexe, la taille et l'ethnie du patient (critère LIN).

### **3. Exploration fonctionnelle complémentaires et évaluation de la sévérité**

La CDV est en général complétée par une pléthysmographie qui permet principalement de rechercher une distension thoracique (10). La mesure des volumes pulmonaires (volume résiduel [VR], capacité résiduelle fonctionnelle [CRF] et capacité pulmonaire totale [CPT]) n'a pas de valeur diagnostique dans la BPCO, mais la distension pulmonaire peut précéder une obstruction bronchique caractérisée sur le rapport VEMS/CVF.



La mesure du transfert pulmonaire du CO (TLCO) n'est pas indispensable au diagnostic de BPCO, mais cette mesure peut être utile lorsque le diagnostic différentiel avec un asthme se discute car l'asthme n'entraîne pas, dans la plupart des cas, de diminution du TLCO (10).

La seule mesure du VEMS est insuffisante pour évaluer fonctionnellement la sévérité de la BPCO et a fortiori son retentissement clinique (10, 18). Conformément aux nouveaux critères du GOLD, la sévérité devrait désormais être jugée à l'aune de la fonction et de la clinique (1). Le critère fonctionnel retenu pour définir la sévérité reste le VEMS ; celui-ci permet de déterminer 4 degrés de sévérité (stades 1 à 4) (figure 4). Les symptômes sont appréciés grâce au questionnaire mMRC (modified Medical Research Council) (19) ou au score CAT (COPD Assessment Test) (figure 5 A et B) (20). Les patients sont répartis en 4 catégories (A à D) (figure 3). Cette nouvelle classification n'a pour le moment pas été retenue par la société de pneumologie de langue française (SPLF) (21).

Groupe GOLD	Caractéristiques	Classification spirométrique <sup>1</sup>	Exacerbations par an	mMRC	CAT
A	Risque faible Peu de symptômes	GOLD 1-2	< 1	0-1	< 10
B	Risque faible Symptômes importants	GOLD 1-2	< 1	> 2	> 10
C	Haut risque Peu de symptômes	GOLD 3-4	> 2	0-1	< 10
D	Haut risque Symptômes importants	GOLD 3-4	> 2	> 2	> 10

**Figure 4.** Classification de la gravité de la BPCO proposée par le GOLD

<sup>1</sup>GOLD 1 : BPCO légère (VEMS > à 80 % du VEMS théorique) ; GOLD 2 : BPCO modérée (50 % < VEMS < 80 %) ; GOLD 3 : BPCO sévère (30 % < VEMS < 50 %) ; GOLD 4 : BPCO très sévère (VEMS < 30 %).

A. Echelle mMRC					B. COPD Assessment test (CAT)																											
<b>Stade 0</b> • Je ne suis essoufflé que pour un effort important <b>Stade 1</b> • Je suis essoufflé(e) lorsque je me presse en terrain plat ou lorsque je monte une petite côte <b>Stade 2</b> • Je marche plus lentement que les personnes de mon âge, en terrain plat, à cause de mon essoufflement, ou • Je dois m'arrêter pour respirer lorsque je marche à mon propre pas en terrain plat <b>Stade 3</b> • Je m'arrête pour respirer après avoir marché environ 100 m, ou • Je m'arrête pour respirer après avoir marché quelques minutes en terrain plat <b>Stade 4</b> • Je suis trop essoufflé(e) pour sortir de chez moi, ou • Je suis essoufflé(e) quand je m'habille ou me déshabille					<b>Je ne tousse jamais</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>Je tousse tout le temps</b> <b>Je n'ai pas du tout de glaires (mucus) dans les poumons</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>J'ai les poumons entièrement encombrés de glaires</b> <b>Je n'ai pas du tout la poitrine oppressée</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>J'ai la poitrine très oppressée</b> <b>Quand je monte une côte ou une volée de marches, je ne suis pas essoufflé(e)</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>Quand je monte une côte ou une volée de marches, je suis très essoufflé(e)</b> <b>Je ne suis pas limité(e) dans mes activités chez moi</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>Je suis très limité(e) dans mes activités chez moi</b> <b>Je ne suis pas inquiet quand je quitte la maison, en dépit de mes problèmes pulmonaires</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>Je suis très inquiet quand je quitte la maison, en raison de mes problèmes pulmonaires</b> <b>Je dors bien</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>Je dors mal à cause de mes problèmes pulmonaires</b> <b>Je suis plein d'énergie</b> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <b>Je n'ai pas d'énergie du tout</b>																											
<b>C. Index BODE</b> <table border="1"> <thead> <tr> <th>Critères</th> <th>0</th> <th>1</th> <th>2</th> <th>3</th> </tr> </thead> <tbody> <tr> <td>[B] IMC (kg/m<sup>2</sup>)</td> <td>&gt; 21</td> <td>≤ 21</td> <td></td> <td></td> </tr> <tr> <td>[O] VEMS (% de la valeur théorique)</td> <td>≥ 65</td> <td>50-64</td> <td>36-49</td> <td>≤ 35</td> </tr> <tr> <td>[D] mMRC (0-4)</td> <td>0-1</td> <td>2</td> <td>3</td> <td>4</td> </tr> <tr> <td>[E] Distance parcourue au TM6 (m)</td> <td>≥ 350</td> <td>250-349</td> <td>150-249</td> <td>≤ 149</td> </tr> </tbody> </table>					Critères	0	1	2	3	[B] IMC (kg/m <sup>2</sup> )	> 21	≤ 21			[O] VEMS (% de la valeur théorique)	≥ 65	50-64	36-49	≤ 35	[D] mMRC (0-4)	0-1	2	3	4	[E] Distance parcourue au TM6 (m)	≥ 350	250-349	150-249	≤ 149	Chaque réponse est cotée de 0 à 5, en fonction de la sévérité, et un score total est établi.		
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[E] Distance parcourue au TM6 (m)	≥ 350	250-349	150-249	≤ 149																												

**Figure 5.** Echelle mMRC (A), COPD Assessment test (B) et index BODE (C)

Un autre index composite qui intègre des données de fonction (VEMS et distance parcourue au test de marche de 6 minutes [TM6]) et des données cliniques (dyspnée et indice de masse corporelle [IMC]) est l'index BODE (figure 5C). Il est supérieur au VEMS pour prédire la mortalité globale, la mortalité d'origine respiratoire, la qualité de vie et le risque d'exacerbation (22-24).

La mesure de la distension peut donner des informations sur la sévérité de la BPCO. Sans revenir sur les discussions portant sur les définitions de la distension, on peut retenir que le rapport CI/CPT est probablement à la fois un bon reflet de la distension, de la sévérité et du pronostic de la BPCO (25). La mesure de la réversibilité de la distension – quand elle existe – pourrait apporter des arguments prédictifs de l'efficacité d'un traitement bronchodilatateur au long cours. En effet, une amélioration de la composante dynamique de la distension est expliquée par une amélioration des débits expiratoires sous l'effet des bronchodilatateurs, sans que le VEMS ni la CVF ne s'améliorent forcément de façon significative; les patients qui diminuent leur distension sous bronchodilatateurs améliorent leurs capacités d'exercice (26).

Comme rappelé dans les récentes recommandations françaises, la mesure du TLCO est un élément d'appréciation utile de la sévérité de l'emphysème, conjointement aux données morphologiques. La mesure de TLCO est également recommandée lorsque les symptômes paraissent disproportionnés par rapport aux anomalies des volumes et débits forcés (10). L'évaluation de la force des muscles respiratoires est indiquée en cas de facteur de risque de dysfonction (dénutrition, par exemple) ou en cas de dyspnée mal expliquée par le reste des examens de la fonction respiratoire (10). Compte tenu des discordances possibles, il est préférable de mesurer conjointement la pression inspiratoire maximale (PI max) et la pression sniff nasale (SNIP) si la première est basse (27). Si une réhabilitation est envisagée, il est utile de mesurer la force des muscles inspiratoires afin de savoir si un ré-entraînement spécifique des muscles inspiratoires est utile : en effet, une PI max < 60 cmH<sub>2</sub>O est prédictive d'un effet bénéfique de ce réentraînement (28).

La pression partielle en O<sub>2</sub> dans le sang artériel (PaO<sub>2</sub>) témoigne de façon fidèle de la gravité de la maladie. La recherche d'une hypoxémie (qui suppose la mesure de la PaO<sub>2</sub> par gazométrie artérielle) se justifie si une oxygénothérapie est envisagée. En pratique, une gazométrie artérielle est indiquée si le VEMS est inférieur à 50 % de la valeur théorique et/ou si la saturation pulsée en O<sub>2</sub> (SpO<sub>2</sub>) est < 92 % et/ou en cas de discordance entre la dyspnée et les valeurs spirométriques (10). La gazométrie peut être complétée par une oxymétrie nocturne chez les patients dont la PaO<sub>2</sub> est entre 56 et 59 mmHg, à la recherche de désaturation significatives justifiant une oxygénothérapie de longue durée (SpO<sub>2</sub> <90% plus

de 30% du temps). Dans la BPCO, les capacités d'exercice sont le plus souvent appréciées « en routine » par le TM6. La procédure standardisée consiste à mesurer la SpO<sub>2</sub>, évaluer la dyspnée et la fatigue musculaire à l'aide d'échelles validées (échelle de Borg modifiée ou échelle visuelle analogique). Le TM6 est utile pour évaluer la limitation à l'exercice et la désaturation à l'exercice. Une distance parcourue inférieure à 350 mètres est un facteur pronostique de mortalité. Chez les patients en stade 3 ou 4 du GOLD, la distance parcourue en 6 minutes est fortement corrélée au V'O<sub>2</sub> et au niveau d'activité physique quotidienne. Chez les patients les moins sévères dont la distance parcourue dépasse 450 m, le TM6 est moins discriminant (effet « plafond ») (29).

L'épreuve fonctionnelle d'exercice (EFX) sur cyclo-ergomètre connaît deux indications principales dans la BPCO. La première consiste à s'assurer d'une absence de contre-indication cardiaque à l'exercice physique et à aider à fixer les modalités d'un réentraînement à l'exercice dans le cadre plus vaste d'une réhabilitation. La seconde consiste à préciser les facteurs qui déterminent la dyspnée, comme par exemple la distension dynamique (16), lorsque cette dernière semble disproportionnée par rapport à la gravité de l'atteinte pulmonaire parenchymateuse.

#### **4. Epidémiologie et impact socio-économique**

La BPCO constitue un problème de santé publique majeur en raison de sa morbi-mortalité et des dépenses de santé qu'elle induit (30). En France, on estime qu'elle atteint 5 à 10 % de la population des plus de 45 ans (soit 2,5 à 3,5 millions de personnes) et est responsable d'environ 16000 décès par an (31). Parmi les porteurs de BPCO, on estime que 30000 d'entre eux sont au stade d'insuffisance respiratoire chronique et nécessitent un traitement par oxygénothérapie ou ventilation assistée. Dans le monde, la maladie touche 210 millions de personnes soit une prévalence chez les plus de 40 ans estimée à 10,1% (11,8% chez les hommes et 8,5% chez les femmes) mais cette prévalence est très variable d'une zone géographique à l'autre. L'étude BOLD, qui estimait la prévalence de BPCO dans 12 pays, a en effet démontré que les pays ayant la plus haute prévalence étaient l'Autriche et l'Afrique du Sud tandis que la Chine et l'Allemagne avaient la plus faible (32). La prévalence de la BPCO est en augmentation constante depuis 20 ans. Son incidence tend à se stabiliser chez les hommes alors qu'elle augmente chez les femmes, suivant la courbe du tabagisme. L'Organisation Mondiale de la Santé (OMS) estime que, d'ici quelques années, la BPCO pourrait devenir la quatrième cause de mortalité par maladie et la cinquième cause de handicap dans le monde. Le coût direct de la maladie est estimé à 3,5 milliards d'euros par

an, dont 60% sont liés aux hospitalisations pour exacerbations et 40% au suivi au long cours de la pathologie. Le coût moyen de la prise en charge d'une BPCO est estimé à 4000 euros par malade et par an, mais cette somme s'élève à 8000 euros pour les patients en mono affectation de longue durée (ALD) et à presque 18000 euros pour les patients en poly ALD (33).

La BPCO est la seule pathologie dont le taux de mortalité a augmenté durant les 50 dernières années (+163% depuis 1965, contre -59% pour les pathologies coronariennes, -64% pour les accidents vasculaires cérébraux ou -7% pour toutes les pathologies sans distinction entraînant un décès) (1). Du fait de sa prévalence croissante, le nombre de décès lié à la BPCO devrait doubler entre 1990 et 2020, ce qui en fera la 3<sup>ème</sup> cause de mortalité (34) et donc un enjeu sanitaire mais aussi socio-économique majeur.

## **5. Facteurs de risque de BPCO**

Bien que le tabagisme soit le facteur de risque de BPCO le plus étudié, ce n'est pas le seul facteur de risque et de nombreuses études épidémiologiques montrent que les non-fumeurs peuvent également développer une obstruction bronchique (8)

### ***a. Tabagisme actif et passif***

L'exposition active au tabac est le principal facteur de risque de la pathologie. En effet, près de 85% des cas recensés de BPCO sont le résultat d'une exposition tabagique. Il faut noter que l'inhalation de la fumée de cigarette contribue à augmenter l'incidence de nombreuses autres pathologies respiratoires telles que l'asthme ou la rhinite.

Néanmoins, tous les fumeurs ne sont pas égaux face à la BPCO. Dans une étude menée au Danemark, 8045 sujets âgés de 30 à 60 ans et présentant une fonction respiratoire normale au moment de l'inclusion ont été suivis sur une période de 25 ans. Les résultats montrent que 30 à 40% des fumeurs n'ayant pas stoppé leur tabagisme durant le suivi avaient développé une BPCO (dont 25% une BPCO de stade II ou plus), contre 8% seulement chez les non-fumeurs (35). Une précédente étude montrait que l'incidence de la BPCO chez les fumeurs, évaluée sur une période de 10 ans, était de 13,5% (36) : la durée du tabagisme a donc un impact direct sur le risque de BPCO (37).

Le tabagisme passif est également un facteur de risque important dans le développement d'une BPCO (5).

### ***b. Facteurs génétiques***

La BPCO est une pathologie multifactorielle. Seuls 20 à 25% des fumeurs actifs vont développer une BPCO. Par ailleurs, des études récentes montrent que ce pourcentage augmente avec l'âge (38). Néanmoins, il n'y a pas de relation linéaire entre consommation tabagique et effet dans la survenue de la pathologie (39). Ces éléments montrent l'implication d'une susceptibilité génétique dans la BPCO.

#### *Déficit en alpha-1-antitrypsine*

Le principal facteur génétique associé à la survenue d'un emphysème pulmonaire est un déficit de l' $\alpha$ -1-antitrypsine (A1AT), un inhibiteur des sérines protéases (élastase du neutrophile par exemple) de la famille des superserpines, synthétisé dans les hépatocytes (40). Dans la plupart des cas, ce déficit se traduit par une mutation du glutamate en position 342 en lysine, appelé aussi mutant A1AT (PiZZ). Cette mutation est associée à une polymérisation spontanée de l'A1AT, aboutissant à un défaut d'arrangement de la structure tertiaire de la molécule, ce qui la maintient dans les hépatocytes et diminue par conséquent sa concentration plasmatique (41). Cette séquestration aboutit à une baisse majeure de l'activité anti-élastasique du sérum, ainsi que dans le poumon. Cette pathologie représente environ 3% des cas de BPCO (42).

#### *Polymorphismes associés au développement de la BPCO*

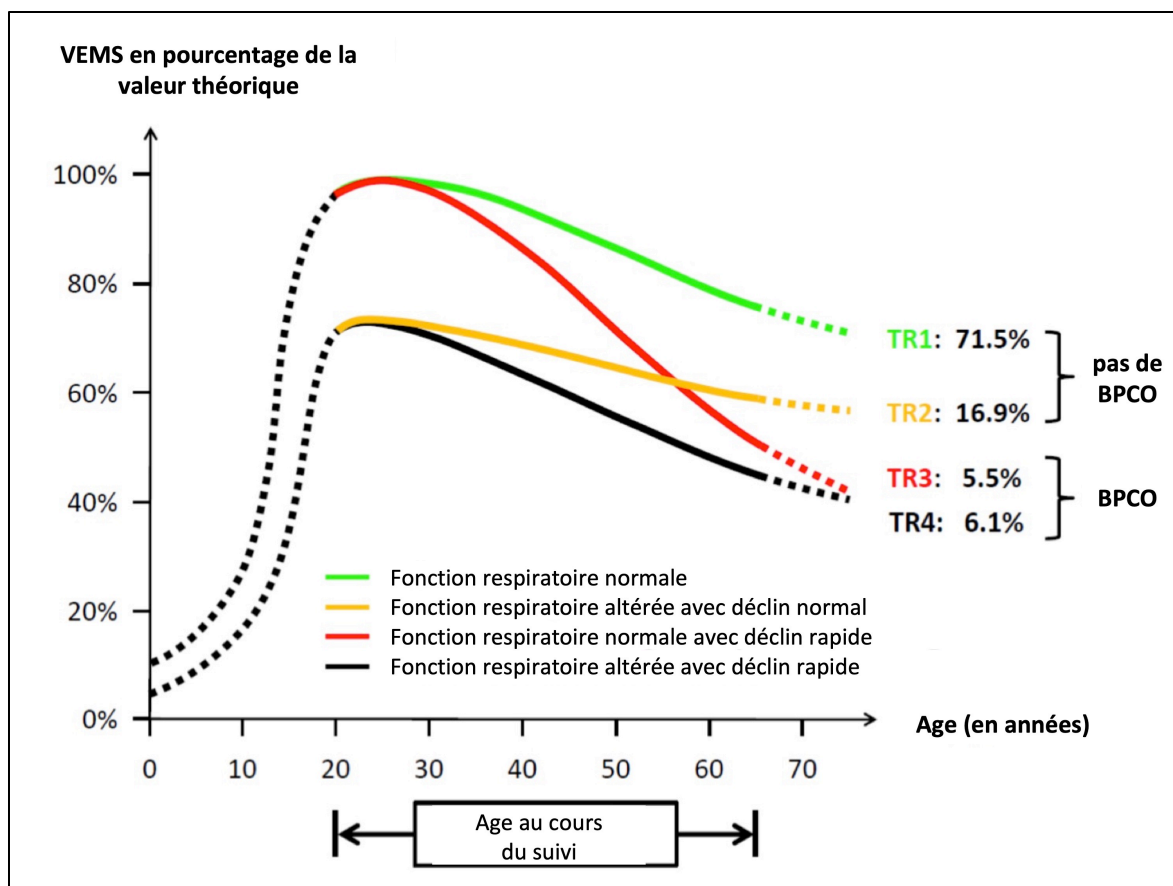
De nombreux polymorphismes ont été associés chez les sujets fumeurs au développement d'une BPCO. Ils vont inclure des gènes impliqués dans la réponse inflammatoire, dans la régulation de la balance protéases / antiprotéases, dans la régulation du système antioxydant ou encore des gènes impliqués dans le contrôle du cycle cellulaire (43 , 44).

### ***c. Développement pulmonaire***

Les évènements survenant pendant la grossesse (tabagisme maternel *in utero*), la naissance (prématurité) et les expositions au cours de l'enfance (infections respiratoires) et de l'adolescence ont une incidence sur la croissance pulmonaire (45, 46). Il a été en évidence que les sujets ayant une réduction de leur fonction pulmonaire maximale atteinte au début de l'âge adulte (mesurée par spirométrie) présentait un risque accru de développement de BPCO (47, 48). Tous les facteurs qui affectent la croissance pulmonaire pendant la grossesse et l'enfance peuvent potentiellement augmenter le risque de développer une BPCO. Plusieurs études ont mis en évidence une association positive entre le poids à la naissance ou l'existence

d'infections pulmonaires dans la petite enfance et le VEMS à l'âge adulte (49). Les événements anténataux (tabac pendant la grossesse, prématurité) et ceux survenant au cours des premières années de vie (tabagisme passif, infections graves des voies aériennes inférieures, asthme, pollution) semblent être aussi importants que le tabagisme pour prédire la fonction pulmonaire à l'âge adulte.

Plusieurs « trajectoires » de fonction respiratoire ont d'ailleurs été mises en évidence (figure 6) (50). Parmi les sujets présentant une BPCO, la moitié suivrait la trajectoire classique, c'est-à-dire partant d'un VEMS optimal à l'âge de 20 ans, et amorcerait ensuite un déclin accéléré de leur capacité respiratoire (1). L'autre moitié des sujets présentant une BPCO aurait déjà, à 20 ans, une fonction respiratoire altérée. Certains n'ont pas de déclin accéléré par la suite (50% d'entre eux) alors que les autres voient leur fonction respiratoire chuter anormalement, à l'origine d'une BPCO.



**Figure 6.** Trajectoires de fonction respiratoire Adapté de Lange et al. (50)

#### ***d. Expositions professionnelles***

La responsabilité des facteurs professionnels dans la genèse ou l'aggravation de la BPCO est restée longtemps méconnue, occultée en particulier par le poids prépondérant du tabagisme. Néanmoins, la prévalence de la BPCO chez des patients sans aucune exposition tabagique est estimée entre 2,5% à 6,5% selon de récentes études (51-53); 25 à 45% des sujets ayant une BPCO n'auraient jamais fumé. (54). Il existe désormais un corpus de données expérimentales et surtout épidémiologiques qui attestent du rôle important des expositions professionnelles en tant que facteur étiologique des BPCO.

En effet, certains milieux professionnels où les sujets sont exposés à des vapeurs, des gaz, des poussières et/ou des fumées (VGDF [vapeur, gases, dust and fumes]) sont associés à un risque accru d'aggravation et/ou de survenue de BPCO. Ceci concerne l'industrie du papier, le nettoyage, les industries de transformation des aliments et bois, l'extraction minière (charbon, or, potasse), les travaux publics et du bâtiments (asphaltage et ouvriers tunneliers), les secteurs des fonderies et de la sidérurgie, la cimenterie, la production laitière, l'élevage de porcs et de volailles, le travail du bois, l'usinage des métaux et l'activité de soudage (tableau 1) (5, 55). Dans une revue générale récente, Blanc et al ont estimé que la fraction des BPCO attribuable à l'exposition professionnelle (qui correspond au pourcentage de BPCO qui ne serait pas survenue s'il n'y avait pas eu d'exposition professionnelle) était d'environ 15%, chiffre corroborée par un récent consensus de l'American Thoracic Society (5, 56). Par rapport à des sujets non exposés, on estime que la probabilité de développer une BPCO est 1,7 à 3,8 fois plus élevé chez les travailleurs exposés à des matières inorganiques (fumée de soudure, ciment, charbon, ...) et 1,2 à 8,9 fois plus élevé chez les travailleurs exposés à des matières organiques (coton, laine, grain, poussières de fermes, ...) (57).

#### ***e. Pollution atmosphérique***

La pollution atmosphérique en milieu urbain est nocive pour les individus atteints de maladies cardiaque ou pulmonaire. L'importance de la pollution de l'air en tant que facteur de risque de BPCO n'est pas clair, mais semble être relativement plus faible que celui du tabagisme (5). Une récente étude transversale réalisée en Chine a montré une association entre le taux de particules fines (PM 2.5 et PM 10) et la prévalence de la BPCO (58). Par ailleurs, il est prouvé que la pollution atmosphérique a un impact significatif sur la croissance et le développement pulmonaire. Dans une étude menée en Californie, il a été montré que les enfants exposés à des niveaux élevés de dioxyde d'azote (NO<sub>2</sub>) et de particules fines (PM2.5) avaient un risque presque cinq fois plus élevé d'avoir une fonction respiratoire réduite

(définie par un VEMS <80% de la valeur prédite) par rapport aux enfants exposés à des taux plus faibles (59). A l'inverse, la réduction des concentrations ambiantes de NO<sub>2</sub> et de PM2.5 atténuerait considérablement le risque de ralentissement de la croissance pulmonaire (60). Cependant, les effets liés à des expositions à court terme de forte intensité (pics de pollution) et des expositions de faible intensité à long terme restent à déterminer.

#### ***f. Combustion de biomasse***

Le bois, les déjections animales, les résidus de récolte et le charbon, généralement brûlés dans des foyers ouverts ou des fourneaux en mauvais état, peuvent entraîner une très forte pollution de l'air intérieur (61). Il est de plus en plus évident que l'exposition à la biomasse intérieure aux combustibles modernes et traditionnels utilisés pendant la cuisson peut prédisposer au développement de la BPCO dans de nombreux pays en développement (62-65). Près de trois milliards de personnes dans le monde utilisent la biomasse et le charbon comme principale source d'énergie pour la cuisson, le chauffage et les autres besoins du ménage; la population exposée au risque est donc très importante (66).

### **6. Dépistage**

Le dépistage systématique de la BPCO dans la population générale se heurte à un rapport coût/efficacité qui, à certains égards, peut être jugé mauvais. En effet, même si la sensibilité et la spécificité de la CDV sont excellentes pour le diagnostic de BPCO, la prévalence de la maladie dans la population générale est telle que plus de 90 % des CDV réalisées sont normales.

La dernière version des recommandations du GOLD stipule que le diagnostic de BPCO doit être évoqué (et une CDV réalisée) chez des sujets qui rapportent à la fois des symptômes et une exposition à des facteurs de risque (1). Les symptômes considérés comme pertinents sont la dyspnée, la toux chronique et/ou l'expectoration chronique. Ces critères ont l'avantage d'être simples, mais méritent néanmoins d'être mis en perspective avec d'autres données de la littérature médicale.

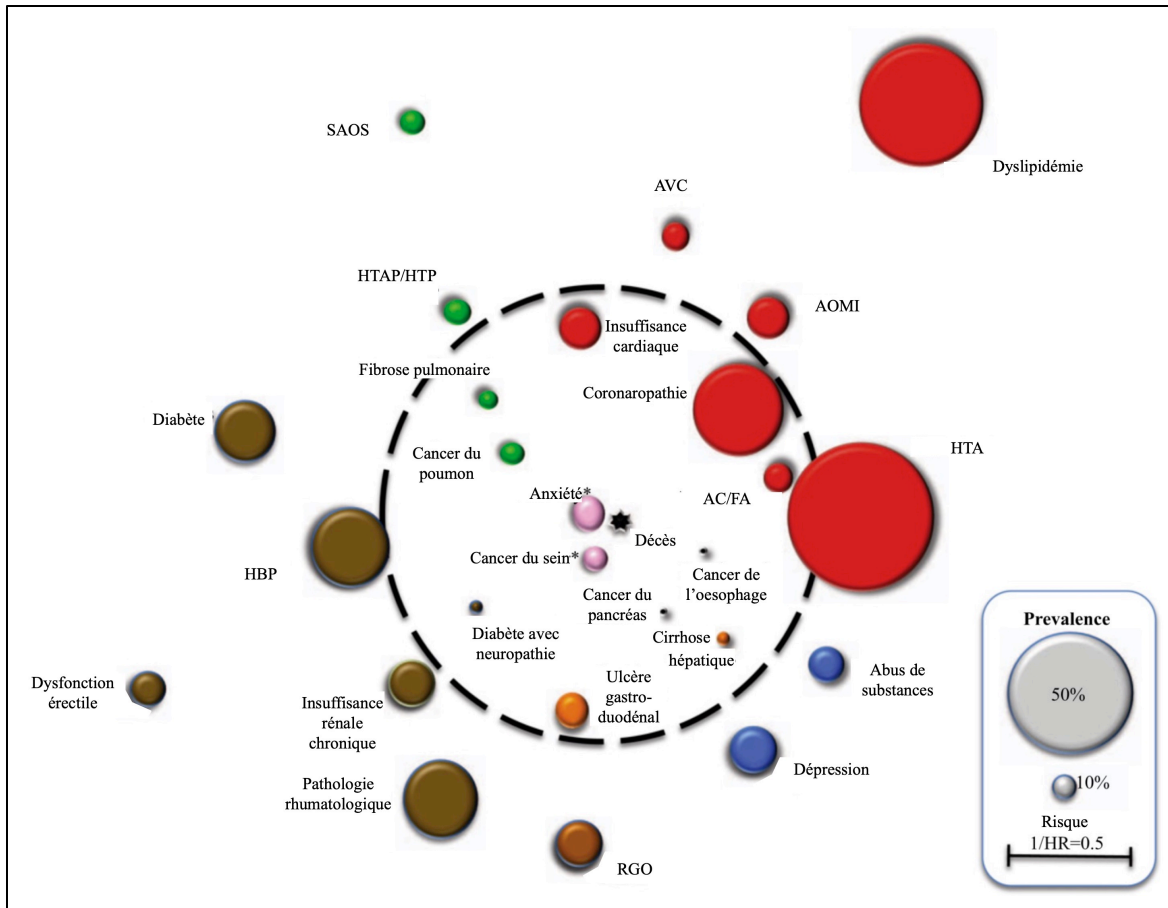
Le tabagisme est un facteur de risque de BPCO unanimement reconnu. Par ailleurs, on estime que près de 20% des BPCO sont d'origine professionnelles. Néanmoins, l'évaluation des pratiques montre que la démarche vis-à-vis du diagnostic de BPCO chez les patients tabagiques ou exerçant des professions à risque mérite d'être plus rigoureuse. À titre



d'exemple, la réalisation systématique d'une CDV chez 1024 sujets fumeurs a permis d'identifier une BPCO jusque-là méconnue chez 9 % d'entre eux et, à l'inverse, a montré que chez 11 % d'entre eux, un diagnostic erroné de BPCO avait été porté (67). Dans un autre travail portant sur la détection de la BPCO chez 146 patients âgés de 40 à 70 ans, ayant un tabagisme cumulé à plus de 15 paquets-années (PA), la prévalence de la BPCO était de 47 % alors que 17 % seulement étaient déjà connus pour avoir une BPCO (68). Ces deux études plaident en faveur d'une démarche de détection via la réalisation systématique d'une CDV chez les sujets ayant un tabagisme significatif, probablement supérieur à 15 PA. On estime que seulement un tiers des malades atteints de BPCO sont effectivement diagnostiqués comme tel. Ceci est en partie dû au fait que la BPCO est une maladie longtemps peu symptomatique (3). La dyspnée apparaît lorsque la fonction respiratoire est déjà très dégradée. Si on estime que la BPCO est due à une accélération de la dégradation de la fonction respiratoire par rapport à un vieillissement normal et que cette vitesse de dégradation reste élevée en l'absence d'intervention (aide au sevrage tabagique, éviction d'un toxique dans un contexte professionnel), il est probablement important de faire le diagnostic BPCO à un stade précoce afin de réaliser une prévention secondaire à même de limiter la dégradation de la fonction respiratoire et donc de réduire les complications (69).

## **B. Conséquences systémiques de la BPCO**

La BPCO est définie en partie comme une maladie inflammatoire du poumon associée à une inflammation systémique, avec de nombreuses comorbidités (figure 7) (70-72). Indépendamment de la sévérité de l'obstruction bronchique, les taux plasmatiques de marqueurs de l'inflammation comme le  $TNF\alpha$ , le fibrinogène, l'IL6 et la CRP sont plus élevés que chez les sujets fumeurs sans BPCO. L'inflammation systémique à l'état stable n'est pas constante, elle est observée chez environ 60 % des patients (73). Son mécanisme est multifactoriel et n'est pas totalement élucidée : l'hypothèse évoquée est celle d'un « débordement » (spill-over) de l'inflammation pulmonaire avec passage des médiateurs de l'inflammation dans la circulation sanguine ; d'autres mécanismes seraient en cause dont un effet direct de la fumée de tabac sur l'activation des polynucléaires neutrophiles ou un passage dans la circulation des gaz ou particules toxiques inhalés. Le stress oxydatif joue un rôle important dans la pathogénie de l'athérosclérose.



**Figure 7. Fréquence et mortalité attribuable des comorbidités dans la BPCO.**

Abréviations : AC/FA : arythmie cardiaque par fibrillation auriculaire ; AOMI : artérite oblitérante des membres inférieurs ; AVC : accident vasculaire cérébral ; HBP : hypertrophie bénigne de prostate ; HTA : hypertension artérielle ; HTAP/HTP : hypertension artérielle pulmonaire, RGO : reflux gastro-oesophagien ; SAOS : syndrome d'apnées obstructives du sommeil.

Adapté de Divo et al. (72)

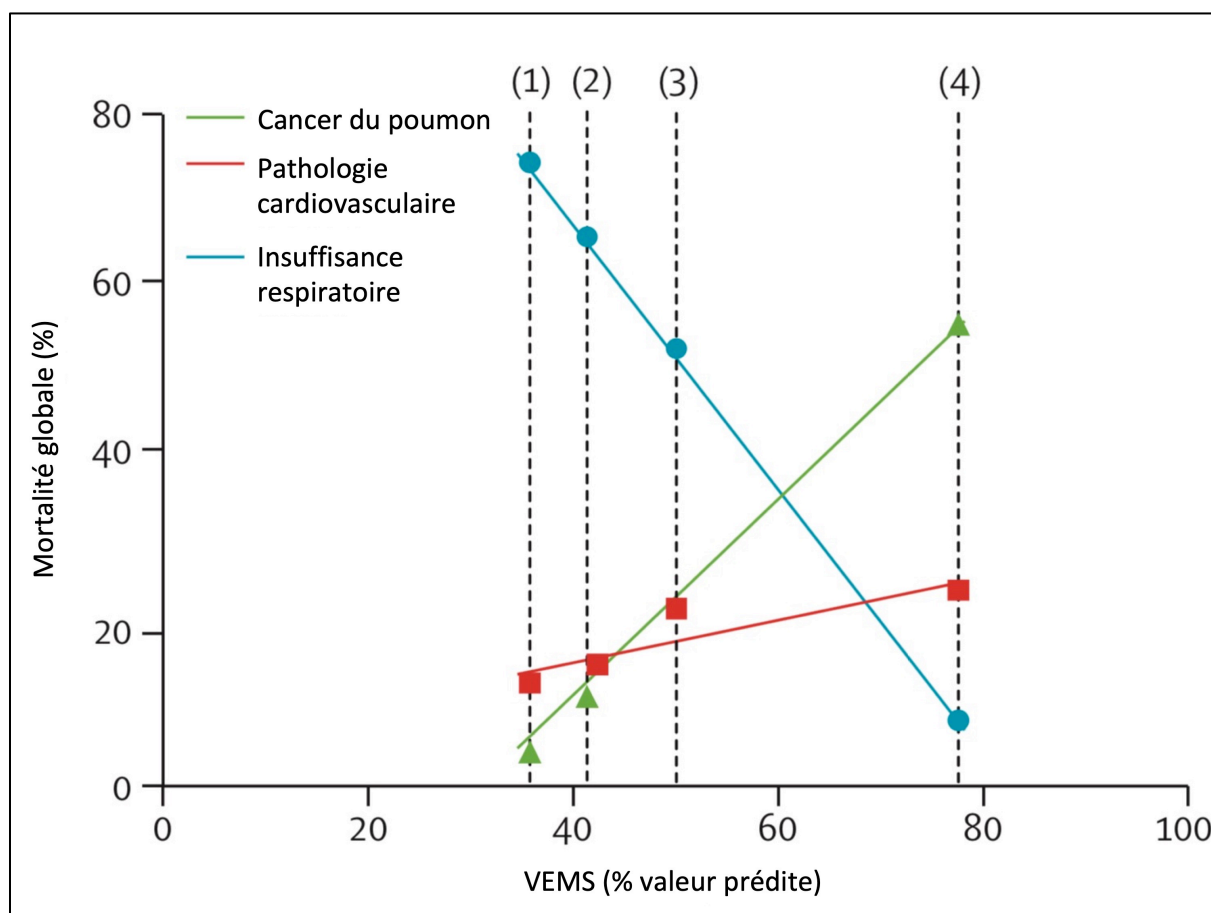
Certaines comorbidités comme la cardiopathie ischémique ou le diabète sont-elles mêmes associées à une inflammation systémique à l'origine d'un cercle vicieux : l'inflammation génère la comorbidité qui elle-même est source d'inflammation.

Chez les patients ayant une obstruction bronchique légère à modérée, les maladies cardiovasculaires et le cancer bronchique sont les principales causes de mortalité (74). Si les comorbidités peuvent être présentes dans les formes peu sévères de la BPCO, elles sont plus fréquentes lorsque l'obstruction bronchique est sévère. L'âge du patient est également un facteur de risque élevé d'avoir de multiples comorbidités (70, 72).

### 1. Pathologies cardiovasculaires

Les pathologies cardiovasculaires sont fréquentes chez les patients atteints de BPCO et contribuent à la morbi-mortalité (75) notamment dans les BPCO légère à modérée (figure 8)

(74). La prévalence des maladies cardiovasculaires est de 20 à 22 % chez les patients ayant une BPCO contre seulement 9 % chez les non-BPCO (76).



**Figure 8. Relation entre la fonction respiratoire et la mortalité due aux pathologies cardiovasculaires, à l'insuffisance respiratoire et au cancer broncho-pulmonaire dans la BPCO.** Adapté de Sin et al. (74)

L'association entre la BPCO et de la cardiopathie ischémique est fréquente (77). Plusieurs mécanismes physiopathologiques sont impliqués et imbriqués : l'inflammation systémique, le stress oxydatif, un état pro-thrombogène conduisant à l'athérosclérose. L'artériosclérose, avec rigidité artérielle et augmentation du rapport intima/média au niveau des carotides, est retrouvée même chez les patients ayant une obstruction bronchique modérée (78). Une étude menée sur la population britannique a montré que chez les patients ayant une BPCO, le risque d'infarctus du myocarde (IDM) était multiplié par 5,5 (79). L'insuffisance cardiaque systolique, d'origine ischémique et/ou hypertensive, est également fréquente ; sa prévalence était de 7 % dans une étude longitudinale britannique sur 3 ans, portant sur 2138 patients (80). La relation physiopathologique avec la BPCO est moins claire que celle avec les cardiopathies ischémiques. L'insuffisance cardiaque (diastolique) à fraction

d'éjection préservée et les dysfonctions ventriculaires gauches sont également courantes (81).

BPCO et cardiopathies forment une association délétère, souvent méconnue. Chez les patients ayant une BPCO de sévérité modérée à légère, ce sont les affections cardiovasculaires qui représentent la principale cause de mortalité (figure 8) et, à l'inverse, chez un patient coronarien ou insuffisant cardiaque, l'existence d'une BPCO est un facteur de mortalité et de morbidité qui aggrave le pronostic de la cardiopathie. Dans l'année suivant un IDM, les patients présentant une BPCO ont une mortalité deux fois plus élevée, sont plus souvent hospitalisés (+ 22 %), souffrent plus souvent d'angor résiduel (+ 22 %) et ont donc une moins bonne qualité de vie que les patients sans BPCO (82).

## **2. Accidents vasculaires cérébraux**

La prévalence des accidents vasculaires cérébraux (AVC) semble plus élevée chez les patients ayant une BPCO : elle serait de 9,9 % chez les patients atteints de BPCO contre 3,2 % dans la population générale (79). La physiopathologie des AVC et les facteurs de risque sont similaires à ceux des cardiopathies ischémiques. La fibrillation auriculaire avec la possibilité d'embolie cérébrale représente un risque supplémentaire (80).

## **3. Ostéoporose**

La prévalence de l'ostéoporose dans la BPCO varie de 9 à 59 %, en fonction des séries et de la sévérité de la maladie (83). L'ostéoporose est observée chez 30 % des femmes mais aussi chez 18 % des hommes de l'étude TORCH (84). Ostéoporose et ostéopénie seraient au minimum deux fois plus fréquentes dans la BPCO que dans la population témoin. Il existe un lien entre ostéoporose et sévérité de l'obstruction bronchique et de l'emphysème évalué par TDM (85).

L'inflammation systémique représente le mécanisme physiopathologique principal des troubles osseux : les cytokines pro-inflammatoires favorisent la résorption osseuse. Un déficit en vitamine D (dosage de 25-OHD < 20 ng/L) est constaté chez plus de 60 % des patients ayant une BPCO sévère (le déficit en vitamine D est une cause classique d'anomalies du métabolisme osseux) (83). Les corticoïdes oraux administrés chez les patients les plus graves ou exacerbateurs fréquents sont un facteur majeur de risque de fractures; par contre, le risque des corticostéroïdes inhalés à forte posologie n'est pas démontré (84).

#### **4. Comorbidités métaboliques**

La dénutrition et atteinte musculaire périphérique sont fréquentes et précoces dans la BPCO, à l'origine d'une limitation de l'activité physique dès les premiers stades de la maladie. Le diabète de type 2 est plus fréquent chez les patients ayant une BPCO de sévérité modérée à sévère que dans la population générale : sa prévalence est de l'ordre de 12 % et le risque relatif d'avoir un diabète est de 1,8 chez la femme (77, 86). L'existence d'un diabète est un facteur de mauvais pronostic à l'origine d'une plus forte mortalité et d'hospitalisations plus nombreuses (77). De même, chez les patients hospitalisés pour une exacerbation, l'existence d'une hyperglycémie est associée à une hospitalisation prolongée et à une évolution souvent péjorative (80).

L'origine de l'hyperglycémie est multiple : inflammation systémique, manque d'activité physique et aussi probablement cures de corticoïdes systémiques répétées chez les patients les plus sévères ou exacerbateurs fréquents. Limiter la corticothérapie systémique doit être un des objectifs à atteindre dans la prise en charge du patient BPCO.

L'obésité abdominale, quel que soit le sexe, semble significativement corrélée aux anomalies de la fonction respiratoire (87). La mesure du tour de taille, un bilan lipidique et une glycémie à jeun doivent faire partie du bilan initial chez un patient ayant une BPCO, surtout s'il est obèse.

#### **5. Anxiété et dépression**

Environ 50 % des patients souffrant de BPCO ont un trouble anxieux et 33 % une dépression. Cette prévalence est supérieure à celle observée dans d'autres pathologies chroniques (88, 89). Le mécanisme n'est pas univoque et incomplètement élucidé. On évoque toujours le rôle de l'inflammation systémique, de désordres neurobiologiques et celui de l'hypoxie bien que les troubles de l'humeur soient observés à tous les stades de sévérité de la BPCO ; la notion de maladie chronique irréversible, de handicap respiratoire lié à la dyspnée sont des facteurs sous-tendant anxiété et dépression.

L'anxiété est deux fois plus fréquente chez la femme que chez l'homme. Son intensité n'est pas corrélée à la sévérité de la BPCO. Son devenir au long cours reste mal précisé. La dépression, sévère à modérée, est présente également chez l'homme et la femme : elle est particulièrement fréquente chez les exacerbateurs fréquents, observée dans 58 % des cas. Anxiété et dépression ont un impact négatif sur la qualité de vie du patient : elles amplifient la perception de la dyspnée, sont un facteur de limitation précoce de l'activité physique, augmentent la fréquence des exacerbations et des hospitalisations ainsi que leur durée ; au même

stade de sévérité, les femmes ayant une BPCO ont, par rapport aux hommes, une dyspnée plus intense, une dépression plus fréquente et une qualité de vie plus altérée. La mortalité à 3 ans des patients BPCO dépressifs est majorée (72).

La dépression est sous-estimée et sous-diagnostiquée : seuls 31 % des patients BPCO déprimés sont traités pour leur dépression (88); elle doit être recherchée chez tout patient, en particulier de sexe féminin, gardant un tabagisme actif, ayant un handicap respiratoire important, avec des comorbidités et des conditions sociales difficiles (précarité, isolement). L'analyse des symptômes et l'utilisation d'un auto-questionnaire simple, comme l'hospitalization anxiety depression (HAD) doivent permettre un dépistage lors de la consultation.

### **C. BPCO et milieu agricole**

Malgré une prévalence de tabagisme plus faible qu'en population générale (90, 91), les personnes travaillant en milieu agricole présentent une prévalence de symptômes respiratoires et/ou des maladies respiratoires (BPCO, mais aussi maladie du poumon de fermier) plus élevée (92-95). Néanmoins, pour une même profession agricole, la prévalence de BPCO est très variable d'une étude à l'autre. De plus, les résultats concernant l'association entre certains métiers agricoles et le risque de BPCO sont discordants d'une étude à l'autre. Trois raisons principales peuvent expliquer ces différences de résultats : d'abord, les métiers de l'agriculture forment un ensemble complexe, composé d'un large éventail d'activités où les expositions professionnelles sont dictées par la taille et le type de ferme (allant de la ferme traditionnelle familiale aux grandes entreprises agricoles) ainsi que par le type d'activité agricole (96). De plus, une même profession est susceptible d'amener des expositions différentes selon la région d'exercice (93), notamment car les poussières organiques auxquelles sont exposés les agriculteurs n'ont pas la même composition d'une région à l'autre (97). Deuxièmement, la définition de la BPCO varie beaucoup d'une étude à l'autre : certaines études utilisent uniquement un critère clinique (bronchite chronique), alors que d'autres utilisent uniquement un critère spirométrique ( $VEMS/CVF < 70\%$  ou à la LIN). En revanche, très peu d'études utilisent les recommandations actuelles du GOLD, qui définissent la BPCO comme l'association d'au moins un symptôme respiratoire parmi la toux chronique, l'expectoration chronique et la dyspnée, et la présence d'un trouble ventilatoire obstructif persistant (correspondant à un rapport  $VEMS/CVF < 70\%$  après l'inhalation de bronchodilatateurs) (1).

## **1. Épidémiologie: les différents métiers agricoles et le risque de BPCO**

### **a. BPCO et milieu de production laitière**

Les prévalences de BPCO en milieu de production laitière observées dans la littérature s'étendent de 2,5 % à 10,7 % (93, 98-102). La plupart des études explorant les producteurs laitiers et faisant intervenir un groupe contrôle de sujets sans aucune exposition professionnelle mettaient en avant une prévalence de BPCO significativement plus élevée dans le groupe des producteurs laitiers, et cela malgré une proportion de fumeurs plus faible que dans le groupe contrôle (91, 93, 100, 103). Par ailleurs, une étude récente démontrait que les poussières organiques libérées pendant la distribution de la paille en milieu de production laitière étaient de taille assez petite pour être déposées dans l'épithélium des voies respiratoires basses et ainsi provoquer leur inflammation (104).

En milieu de production laitière, plusieurs éléments peuvent considérablement faire varier la prévalence de la BPCO. Une étude publiée par notre groupe a démontré que certaines tâches réalisées par les producteurs laitiers étaient susceptibles d'avoir un impact sur la fonction respiratoire (105). Par exemple, la manipulation de fourrage et/ou de paille de nourriture pour animaux était associée à des symptômes bronchiques ; la manipulation de nourriture pour animaux était également associée à un déclin de la fonction respiratoire. De plus, la modernité de la ferme, et donc les méthodes d'exercice, peuvent avoir un effet protecteur contre le développement d'une BPCO mais également sur le départ en retraite de façon anticipée (99). Jouneau et al. ont démontré que l'affouragement manuel et une durée élevée de paillage mécanisé étaient des marqueurs d'obstruction bronchique alors que l'utilisation d'une automotrice pour la distribution des aliments était un facteur protecteur (106). Une autre étude identifiait comme facteurs associés à une prévalence de BPCO plus faible une séparation entre le bâtiment d'habitation et l'étable (comparativement à une habitation et une étable dans le même bâtiment), une stabulation libre (par comparaison avec une stabulation entravée), la présence d'un couloir central dans l'étable et le fait d'avoir une grande exploitation (grand nombre d'hectares et cheptel important) (98). Des résultats similaires étaient retrouvés dans une étude américaine investiguant le risque de bronchite chronique selon le nombre d'animaux présents dans la ferme (101). Par ailleurs, une étude transversale concernant 1 638 producteurs laitiers a mis en évidence un "effet région" (93). En effet, les producteurs laitiers de Franche-Comté avaient une prévalence de BPCO 2,5 fois plus élevée que celle des producteurs laitiers de Bretagne (8,23 % versus 3,12 %, OR ajusté = 2,46 ; IC<sub>95</sub> : 1,49-4,06), ces derniers ayant une prévalence similaire à celle des témoins non exposés.

Enfin, l'association entre le fait d'être éleveur en milieu de production laitière et le tabagisme

peut avoir un effet additif, voire synergique, sur la fonction respiratoire (98).

De nombreuses études rapportent une prévalence importante de symptômes respiratoires (toux chronique, expectoration chronique, dyspnée, bronchite chronique) chez les éleveurs de bovins et les producteurs laitiers, et c'est bien souvent la présence de symptômes qui définit la BPCO dans ces études (107-109)

Néanmoins, la présence de symptômes respiratoires chroniques n'a que très rarement été mise en lien avec la présence d'une obstruction bronchique évaluée par un examen spirométrique. En d'autres termes, il est possible que les patients considérés comme étant porteurs d'une BPCO sur la foi de symptômes n'aient en fait qu'une bronchite chronique simple ou que leur dyspnée trouve son origine dans une autre cause que la BPCO. Des études plus récentes ont pratiqué des mesures spirométriques pour définir la BPCO ; néanmoins, la plupart de ces études s'arrêtent aux résultats obtenus et ne prennent pas en compte les symptômes.

#### **b. BPCO et élevage en espace confiné**

Alors que la plupart des activités agricoles se pratiquent en extérieur, certains élevages se font en espaces clos, avec une densité d'animaux parfois très élevée. Cette agriculture, souvent qualifiée d'"agriculture intensive", concerne principalement les élevages de porcs et de volailles. Les agriculteurs travaillant dans ces élevages sont quotidiennement exposés aux moisissures, poussières, bactéries, mais également à des particules d'endotoxine et d'ammoniac (57). Malgré cela, les prévalences d'asthme, d'allergie respiratoire et de rhinite y restent moins élevées qu'en population générale (109, 110). En revanche, les symptômes respiratoires, la bronchite chronique et la BPCO semblent fréquents dans ce milieu et leur sévérité est proportionnelle au temps passé dans les bâtiments d'élevage (108). L'élevage de porcs et l'élevage de volailles sont associés à des prévalences de BPCO atteignant jusqu'à 23,6 % et 31,7 %, respectivement (111, 112). Cependant, ces prévalences sont beaucoup plus modestes lorsque des critères spirométriques robustes sont utilisés pour le diagnostic de BPCO (VEMS/CVF postbronchodilatateur < 70 % ou à la LIN) tout en demeurant plus élevées que dans une population de sujets non exposés aux activités agricoles (93).

#### **c. BPCO et culture (céréaliers, maraîchers, etc.)**

Tous les agriculteurs ne pratiquant pas l'élevage seront considérés dans ce paragraphe comme cultivateurs. Les résultats concernant le risque de BPCO chez les cultivateurs sont discordants d'une étude à l'autre. Cela pourrait s'expliquer par la diversité des activités



professionnelles dans ce milieu. Alors que certaines études mettent en avant un surrisque de BPCO et un déclin accéléré de la fonction respiratoire chez les manutentionnaires de grains (113), d'autres études démontrent au contraire que les cultivateurs de certains fruits bénéficieraient d'une meilleure fonction respiratoire qu'une population de référence du même pays mais n'ayant jamais exercé cette profession (114). Plusieurs études transversales montrent une prévalence de symptômes respiratoires plus élevée et une accélération du déclin du VEMS chez les céréaliers par rapport à un groupe de sujets témoins non exposés, avec une relation dose-effet entre le niveau d'exposition ainsi que l'intensité des symptômes et la vitesse du déclin du VEMS (115). Dans ce milieu professionnel, la présence d'endotoxines semble être le principal facteur de risque d'altération de la fonction respiratoire (116). Comme dit précédemment, une même profession peut présenter des expositions et donc des risques de BPCO très différents selon le mode d'exercice. En particulier, les cultivateurs sous serre (*greenhouse workers* en anglais) sont exposés à des conditions climatiques difficiles (fort taux d'humidité et température élevée) et à diverses particules chimiques et organiques (pesticides, bactéries, moisissures, etc.) (117). Une récente étude chinoise s'est intéressée au risque de BPCO dans cette population. Pour cela, 5 420 travailleurs sous serre ont été inclus : 2 168 cultivaient des légumes, 1 084 des champignons, 1 355 des fleurs et 813 élevaient des volailles (sous serre également). La prévalence de la BPCO évaluée par un rapport VEMS/CVF postbronchodilatateur < 70 % était de 17,5 % (25,5 % chez les plus de 40 ans) et variable d'un type d'activité à l'autre (12,6 % chez les cultivateurs de légumes, 17,9% chez les cultivateurs de fleurs, 20,8% chez les éleveurs de volailles et 24,3 % chez les cultivateurs de champignons) et d'une région à l'autre (15,6 % dans les plaines, 19,4 % dans les zones côtières et 20,2 % en montagne) (118). En revanche, une autre étude incluant 411 cultivateurs a montré que ces derniers avaient une prévalence de BPCO similaire à celle de sujets témoins non exposés (OR = 1,02 ; IC<sub>95</sub> : 0,48-2,20) (93).

Par ailleurs, les cultivateurs sont souvent exposés aux pesticides (incluant les insecticides, raticides, fongicides et herbicides), qui sont utilisés pour lutter contre les animaux et plantes considérés comme nuisibles pour leurs exploitations (119). Les substances chimiques présentes dans les pesticides peuvent également avoir un effet délétère sur la santé. Le taux d'incidence pour les maladies causées par l'exposition aux pesticides est estimé à 1,17 pour 100 000 équivalents temps plein (120). Ces produits sont principalement administrés sous forme de spray, favorisant l'inhalation des particules toxiques par le cultivateur lors de la diffusion du produit et pouvant donc faciliter le développement de pathologies respiratoires. Deux revues de la littérature publiées récemment rapportent une

association significative entre l'utilisation de pesticides et la présence de symptômes respiratoires, le développement d'asthme, de bronchite chronique et de BPCO, même si les causes exactes restent à déterminer (121, 122). Enfin, une étude indienne publiée en 2009 comparait 348 témoins non exposés aux pesticides à 223 vaporisateurs occasionnels et 153 vaporisateurs réguliers de pesticides. Les témoins et les vaporisateurs occasionnels avaient un rapport VEMS/ CVF similaire ( $91,5 \pm 14,1$  et  $88,2 \pm 15,1$ , respectivement), alors que les vaporisateurs réguliers avaient un rapport VEMS/CVF significativement plus faible que ces 2 premiers groupes ( $84,6 \pm 16,1$ ,  $p = 0,027$  vs vaporisateurs occasionnels), suggérant ainsi une association dose-effet entre l'exposition aux pesticides et le déclin de la fonction respiratoire (123).

#### **d. BPCO et activités agricoles multiples**

Une grande partie des agriculteurs cumule plusieurs activités, soit en élevant divers types d'animaux, soit en associant élevage et culture, multipliant potentiellement les expositions. Plusieurs études ont donc investigué le risque de BPCO chez ces agriculteurs, afin de déterminer si la multiplicité des expositions induisait un effet additif, voire synergique, sur le risque de développer une BPCO.

Dans une étude publiée en 2004, Monso et al. ont recueilli des données spirométriques (avant et après la journée de travail) et des données concernant le lieu de travail (superficie, température, humidité et mesure de concentration en aérocontaminants), puis ont fait remplir des questionnaires portant sur les symptômes respiratoires à 105 agriculteurs non fumeurs travaillant en espace confiné (108). Ceux-ci élevaient des porcs (78,1 %), des bovins (30,5 %), des vaches laitières (21,9 %) et/ou des volailles (31,4 %). Chez cet échantillon d'agriculteurs, la température, la superficie et le taux d'humidité du lieu de travail n'étaient pas associés à un surrisque de BPCO. Parmi les 4 aérocontaminants mesurés (dioxyde de carbone, ammoniac, poussière et endotoxine), seules les poussières étaient associées à un surrisque de BPCO, et cela avec une relation dose-effet pour les troisième et quatrième quartiles (OR = 2,54 ; IC<sub>95</sub> : 0,32-20,27, et OR = 6,60 ; IC<sub>95</sub> : 1,10-39,54, respectivement), versus les premier et deuxième quartiles, après ajustement sur l'âge, le sexe et le type d'élevage.

Dans une étude norvégienne portant sur 4 735 agriculteurs, Eduard et al. ont montré que, parmi des agriculteurs atopiques, ceux qui pratiquaient une activité mixte d'élevage et de culture de céréales avaient une prévalence de BPCO 2,5 fois plus élevée que les cultivateurs exclusifs (14,8 % versus 6,0 %, respectivement ; OR = 5,5 [IC<sub>95</sub> : 1,4-21]) (124). En

revanche, les prévalences n'étaient pas différentes entre ces 2 groupes quand seuls les agriculteurs non atopiques étaient étudiés (12,2 % versus 10,5 %, respectivement ; OR = 1,4 [IC<sub>95</sub> : 0,92-2,1]). Parmi les aérocontaminants mesurés, ceux qui étaient associés à un surrisque de BPCO étaient les poussières organiques (OR = 1,2 ; IC<sub>95</sub> : 1,0-1,4), les endotoxines (OR = 1,2 ; IC<sub>95</sub> : 1,0-1,5) et les acariens (OR = 1,2 ; IC<sub>95</sub> : 1,0-1,3).

## **2. BPCO en milieu agricole : spécificités phénotypiques**

### **a. Symptômes**

Dès les années 1980, des études utilisant des critères robustes pour le diagnostic de BPCO ont suggéré que les symptômes (bronchite chronique, en particulier) étaient plus fréquents et plus sévères chez les patients atteints d'une BPCO consécutive à une exposition professionnelle que chez ceux atteints de BPCO post-tabagique sans exposition professionnelle (125), et ce, après ajustement sur le tabagisme (126-129). Cela est vrai aussi en milieu agricole, où nous avons montré que des symptômes tels que la toux, l'expectoration et la dyspnée chroniques étaient plus fréquentes chez des agriculteurs avec BPCO que chez des sujets non exposés porteurs d'une BPCO post-tabagique (93). Cet excès de symptômes est principalement retrouvé chez les cultivateurs et les éleveurs de bétail. Certaines expositions combinées induisent des symptômes plus fréquents, et on observe une relation dose-effet entre symptômes et durée d'exposition (130).

### **b. Allergies**

De façon générale, la prévalence des allergies est plus élevée parmi les patients présentant une BPCO associée à une exposition professionnelle que chez les patients dont la BPCO est d'origine tabagique (126, 131, 132). L'exposition professionnelle et l'atopie (définie par la présence d'un rhume des foins) sont même parfois considérées comme 2 facteurs indépendants de BPCO (133). Au sein d'une cohorte norvégienne de 4 735 fermiers, les liens entre les activités agricoles (et les expositions afférentes) et la BPCO étaient d'autant plus forts que les agriculteurs avaient une atopie (124). L'allergie pourrait donc également prédisposer au développement d'une "BPCO agricole". Plusieurs études menées dans le département du Doubs ont mis en évidence des arguments épidémiologiques, fonctionnels et immunologiques en faveur d'un possible mécanisme immunoallergique dans la genèse de la BPCO en milieu de production laitière. La présence d'une BPCO était notamment plus fréquente chez les agriculteurs ayant des antécédents de maladie de poumon de fermier ou de symptômes retardés liés à une exposition spécifique et également chez ceux ayant des taux de

précipitine sérique élevés. Il existait également dans cette population de producteurs laitiers une relation entre un taux élevé d'immunoglobulines (Ig) E et G, d'une part, et un déclin accéléré du VEMS, d'autre part (134).

### **c. Fonction respiratoire et imagerie thoracique**

La fonction ventilatoire semble s'altérer plus rapidement dans les BPCO professionnelles que dans la BPCO post-tabagique. L'exposition aux poussières agricoles est associée à un déclin accéléré du VEMS, principalement chez les patients fumeurs (135). Dans la cohorte SAPALDIA, la fonction respiratoire de 4 267 travailleurs suisses exposés à des poussières biologiques, des poussières minérales, des gaz, fumées et vapeurs a été évaluée : à tabagisme équivalent, la sévérité de la BPCO est corrélée, d'une part, à l'intensité de l'exposition, d'autre part, à sa durée (69). L'évaluation de l'emphysème pulmonaire en milieu agricole fait l'objet de peu d'études. Une étude multicentrique française a suggéré l'existence de phénotypes différents au sein des BPCO professionnelles selon le type d'exposition : la BPCO liée à une exposition aux poussières minérales semble être une maladie plus distale et pourvoyeuse d'emphysème alors que la BPCO agricole serait une maladie localisée au niveau des bronches (136). D'une manière générale, l'évaluation de la fonction respiratoire est d'une importance capitale dans la caractérisation et le suivi de la BPCO en milieu professionnel. L'ATS a d'ailleurs émis des recommandations spécifiques quant à la réalisation, l'interprétation et le suivi de la fonction respiratoire en milieu professionnel (137).

### **d. Risque cardiovasculaire**

Le rôle respectif de la BPCO et du tabagisme dans la genèse du risque cardiovasculaire reste débattu. L'évaluation de ce dernier demeure néanmoins confidentielle dans les BPCO professionnelles alors qu'elle permettrait d'estimer ce risque indépendamment du tabagisme. Chez les patients présentant une BPCO non tabagique, le risque de comorbidités cardiovasculaires semblent moins important que chez ceux présentant une BPCO tabagique (138).

## **3. Prise en charge thérapeutique de la BPCO en milieu agricole**

L'éviction des facteurs de risque (tabac, exposition professionnelle) est probablement la première mesure à prendre pour ralentir l'évolution de la maladie. Dans le contexte d'une exposition professionnelle, il est donc recommandé de porter un masque afin de limiter l'inhalation de particules toxiques. Cependant, même si les éleveurs en espace confiné ont

conscience de l'importance de se protéger, peu le font réellement. Une étude américaine a interrogé 30 éleveurs de volailles sur leur ressenti vis-à-vis des risques respiratoires engendrés par leur profession (139). Les résultats indiquaient que 75 % des éleveurs travaillaient dans un environnement avec une exposition à la poussière modérée à sévère. Parmi les 30 éleveurs, plus de 50 % considéraient que le port d'un masque était très important mais seuls 17 % en portaient un durant leurs tâches professionnelles. Par ailleurs, une étude randomisée réalisée dans 80 fermes (54 élevages de porcs et 26 élevages de bovins) montrait que, chez les éleveurs de porcs, le simple fait d'informer l'éleveur du niveau de concentration en poussière de sa porcherie et de lui donner des conseils pour éviter d'inhaler ces poussières (port d'un masque) réduisait de 20 à 30 % le niveau de poussières inhalées (140). Pour ce qui concerne les thérapies médicamenteuses, il n'existe actuellement aucune recommandation spécifique pour les BPCO d'origine professionnelle, et encore moins pour les BPCO d'origine agricole. Faute de mieux, ce sont donc les recommandations GOLD/ SPLF qui, pour l'instant, sont appliquées (1).

L'ensemble des professions agricoles sont associées à un surrisque de BPCO, et ce, après ajustement sur les facteurs de risque de cette maladie (âge, sexe et tabagisme). Il est donc crucial de proposer des mesures de prévention primaire et secondaire à cette population, qui représente près d'un million de personnes en France.

## II. OBJECTIFS

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L'objectif général de ce travail de thèse est de caractériser la BPCO secondaire à l'exposition aux poussières organiques. Les objectifs spécifiques des articles qui composent cette thèse sont de :

- faire un état des lieux des connaissances de la BPCO agricole ;
- dépister la BPCO parmi les agriculteurs producteurs laitiers afin de mesurer la prévalence de la BPCO « agricole » ;
- caractériser les patients présentant une BPCO agricole en termes de tabagisme, de dyspnée, de qualité de vie, de fonction respiratoire, d'inflammation systémique, de fonction vasculaire systémique et de capacité d'exercice) ;
- comparer ces caractéristiques avec celle de patients présentant une BPCO tabagique ;
- préciser les facteurs de risque étiologiques, individuels et professionnels (agricole et non agricole) de la BPCO, en comparant les caractéristiques des BPCO (agricoles et non agricoles) à des sujets contrôles non BPCO (agriculteurs et non agriculteurs).



### III. MATERIEL ET METHODE

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Afin de caractériser la BPCO en milieu agricole, l'étude BALISTIC-1 (BPCO en milieu rural : dépistage, caractérisation et constitution d'une cohorte) a initié en 2012. La 1<sup>ère</sup> partie de l'étude a constitué en un dépistage au sein de 2 populations distinctes (population composée de travailleurs agricoles et population générale). Parmi ces sujets dépistés, 400 patients ont été sélectionnés afin de constituer 2 groupes de sujets exposés aux poussières organiques (BPCO et témoins) et 2 groupes de sujets non exposés (BPCO et témoins). Ces 4 groupes ont permis de caractériser la BPCO secondaire à l'exposition aux poussières organiques sur le plan pulmonaire et systémique (2<sup>nde</sup> partie).

#### A. Modalités du dépistage et constitutions des groupes

##### 1. Populations concernées par le dépistage

Les sujets étudiés (BPCO et sujets témoins) ont été identifiés et sélectionnés au terme d'une campagne de dépistage. Deux populations étaient concernées par ce dépistage, sur une période s'étendant de septembre 2011 à novembre 2015 (141).

Pour ces deux populations, le seul critère d'inclusion retenu pour participer au dépistage était un âge compris entre 40 et 74 ans (annexe 1).

Les critères de non-inclusion étaient les suivants :

- une exposition professionnelle autre que la production laitière; en particulier, les agriculteurs d'autres filières professionnelles (céréales, viticulture, arboriculture, élevage de volailles, ...); cette éventuelle autre exposition professionnelle était recherchée grâce à un auto-questionnaire ;

- une BPCO de stade 4 de la classification du GOLD ;

- une grossesse en cours ;

- la prise de corticoïdes par voie générale ou d'immunosuppresseurs ;

- l'existence d'une asthme, de bronchectasies et/ou d'une pneumopathie d'hypersensibilité (type poumon de fermier) ;

- un pronostic vital estimé inférieur à un an.

La première population comprenait des personnes devant bénéficier (indépendamment de l'étude présentée ici) d'un bilan de santé organisé par la Mutualité sociale agricole (MSA) de Franche-Comté. Sur la période 2011 à 2013, environ 12 500 personnes âgées de 40 à 75



ans ont été convoquées à ces bilans de santé, parmi lesquelles environ 40 % se sont effectivement déplacées effectivement pour en bénéficier. Parmi les personnes réalisant les bilans de santé (soit environ 5000 sujets), 40% environ (soit 2000 sujets) étaient des producteurs laitiers. Un certain nombre de sujets affiliés à la MSA et non exposés à des aérocontaminants d'origine professionnelle (employés de la MSA, du Crédit Agricole, d'administrations agricoles,...) correspondaient également aux critères d'inclusion et bénéficiaient du dépistage de la BPCO. Ainsi, parmi les sujets se rendant à un bilan de santé organisé par la MSA, les producteurs laitiers et les sujets non exposés constituaient la population d'intérêt, respectivement pour le dépistage de la BPCO « agricole » et de la BPCO « non agricole ».

La seconde population était constituée de sujets dont le médecin traitant était adhérent à la fédération des maisons de santé comtoises (FeMaSaC). Huit maisons de santé pluri-professionnelles ont participé à l'étude. Deux modalités avaient été retenues pour inviter les patients à bénéficier du dépistage. Soit les médecins traitants envoyaient un courrier à certains de leurs patients répondant aux critères d'inclusion afin de leur proposer un rendez-vous au cours duquel un dépistage de la BPCO est réalisé; soit ils profitaient d'une consultation programmée pour leur proposer (voir ci-dessous pour les modalités de dépistage). Les sujets sollicités étaient pour l'essentiel des fumeurs ou des ex-fumeurs (bien que le tabagisme ne constitue pas un critère de sélection). L'objectif du dépistage dans cette population était de contribuer à sélectionner un groupe de patients atteints de BPCO « non agricole» qui soit le plus proche possible du groupe de patients atteints de BPCO « agricole ».

## **2. Modalités du dépistage**

Le dépistage de la BPCO était effectué par une courbe débit-volume permettant de mesurer le VEMS et la CVF selon les critères en vigueur (1). La courbe débit-volume était enregistrée avec un spiromètre (CPFS/D, Medical Graphics, Strasbourg, France). Le spiromètre était calibré au moins une fois par jour avec une seringue de 3L. La manœuvre était dirigée conformément aux recommandations de l'ATS/ERS par un technicien ayant reçu une formation appropriée (142). Les mesures étaient effectuées chez des sujets assis. Les critères de validité et de reproductibilité de la spirométrie étaient les suivants: réalisation d'au moins trois manœuvres, différence maximale observée de moins de 150mL entre la plus grande valeur de VEMS et de CVF et la valeur immédiatement inférieure, absence de toux pendant la première seconde de la mesure, différence de moins de 5 % entre la CVF mesurée et la CVF extrapolée à partir de la pente de la relation débit-volume.

Tous les sujets ayant un rapport VEMS/CVF inférieur à 70% bénéficiaient d'un test de réversibilité de l'obstruction bronchique, selon le protocole recommandé par l'ATS/ERS (142). Au total, quatre bouffées de 100 µg de salbutamol (soit 400 µg) étaient administrées à environ 30 secondes d'intervalle via une chambre d'inhalation (Volumatic spacer, GlaxoSmithKline, Uxbridge, Royaume-Uni). La manœuvre d'inhalation débutait par une expiration lente et complète suivie d'une inspiration rapide jusqu'à la CPT. Une apnée de cinq à dix secondes était réalisée à la CPT avant que le sujet ne reprenne une ventilation normale. Une nouvelle boucle débit-volume (BDV) était réalisée au moins dix minutes après et au plus 15 minutes après l'inhalation de salbutamol (142).

Les sujets ayant un rapport VEMS/CVF inférieur à 70% sur la mesure effectuée après salbutamol étaient considérés comme ayant une BPCO (critères GOLD 2007).

### **3. Composition des quatre groupes de l'étude**

À l'issue des visites de dépistage, quatre groupes ont été constitués :

- un groupe de patients ayant une BPCO « agricole » (producteurs laitiers) ;
- un groupe de patients ayant une BPCO « non agricole » ;
- un groupe de sujets témoins ayant une exposition « agricole » (producteurs laitiers)

et ayant une boucle débit—volume normale sans inhalation de bronchodilatateurs (rapport VEMS/CVF > 70% et rapport VEMS/CVF > limite inférieure de la valeur théorique et VEMS > 80% de la valeur théorique et CVF > 80% de la valeur théorique). Chaque sujet de ce groupe était apparié à un patient ayant une BPCO « agricole » (appariement pour l'âge, le sexe, le tabagisme et la localisation géographique) ;

- un groupe de sujets témoins « non agricole » ayant une BDV normale sans inhalation de bronchodilatateurs (rapport VEMS/CVF > 70% et rapport VEMS/CVF > limite inférieure de la valeur théorique et VEMS > 80% de la valeur théorique et CVF > 80% de la valeur théorique). Chaque sujet de ce groupe était apparié à un patient ayant une BPCO « non agricole » (appariement pour l'âge, le sexe, le tabagisme et la localisation géographique).

## **B. Questionnaires et examens d'exploration fonctionnelle**

Les méthodes décrites ci-dessous ont été appliquées aux quatre groupes inclus dans l'étude (annexe 2 à 7).

### **1. Questionnaires**

Chaque sujet remplissait un questionnaire médical adapté à partir de celui de

l'European Community Respiratory Health Survey (143, 144). Ce questionnaire évalue les symptômes associés à la BPCO (toux, expectoration, sifflements, dyspnée), à l'allergie et à l'asthme. Il comporte des questions destinées à dépister une maladie du poumon de fermier (145). Le tabagisme, les éventuelles comorbidités et la prise éventuelle de traitements médicamenteux sont pris en compte. Un questionnaire professionnel ciblait soit les métiers et expositions clairement associés à la BPCO autres que la production laitière, soit les producteurs laitiers. Pour ces derniers, on évaluait les pratiques professionnelles et leur évolution dans le temps, ainsi que des « indicateurs de modernité » de l'exploitation agricole ayant pu être associés à une meilleure santé respiratoire (98, 146).

Chez tous les sujets (patients BPCO et sujets normaux), la dyspnée était évaluée par l'échelle mMRC, le baseline dyspnea index (BDI) (147) et par le score de Borg (148).

Chez les patients porteurs de BPCO, la qualité de vie était mesurée par le questionnaire du Saint-George's Hospital (SGRQ) traduit et validé en français (149) et par l'auto-questionnaire CAT (150).

## **2. Mesure de l'exposition microbiologique domestique**

L'aérocontamination en micro-organismes fongiques et bactériens était mesurée dans les locaux domestiques de tous les sujets inclus. Cette mesure était réalisée à partir de lingettes électrostatiques déposées pendant dix semaines dans des zones prédéterminées, qui permettaient de quantifier par Q-PCR entre dix et 40 espèces, selon des procédures décrites par ailleurs (151, 152).

## **3. Dosages biologiques**

Un prélèvement sanguin sur veine périphérique (environ 35 mL) était réalisé chez tous les sujets inclus. Les échantillons étaient centrifugés de façon extemporanée; le sérum était prélevé, conditionné en aliquots de 500µL et conservé à -80°C jusqu'à ce que les dosages soient réalisés.

La présence de marqueurs spécifiques de l'inflammation pulmonaire dans la circulation sanguine plaide en faveur d'une dissémination systémique de l'inflammation. 49 cytokines, chimiokines et facteurs de croissance ont été quantifiés dans un échantillon de 32 patients (8 patients de chaque groupe) à l'aide de la multiplexée (Luminex) (Bio-Rad Laboratories, Hercules, USA). Parmi ceux-ci, 27 marqueurs ont été retenus: IL-1R $\alpha$ , IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17A, IL-2, IL-22, IL-23, IP-10, MCP-1, eotaxin, MCP-2, I-

TAC, IL-18BPA, sST2, VEGF, SP-D, TNF-R1, GRO $\alpha$ , MCP-4, hNGAL, SAA, sICAM-1, sVCAM-1) (R&D Systems Europe, Abingdon, UK). Un dosage ultrasensible de la protéine C réactive (hsCRP) a été réalisé par une technique d'immunonéphélométrie ultra-sensible.

Par ailleurs, des sérologies ont été réalisées chez tous les sujets; un des objectifs était de rechercher des arguments en faveur d'une origine immuno-allergique des anomalies pulmonaires constatées. Ces sérologies utilisent des méthodes d'immuno-diffusion (double diffusion selon Ouchterlony et confirmation en électrosynérèse). Dans les groupes de sujets producteurs laitiers (exposition «agricole»), le panel suivant d'antigènes était utilisé : *Absidia corymbifera*, *Wallemia sebi*, *Eurotium amstelodami*, *Saccharopolyspora viridis*. Dans les groupes de sujets n'ayant pas d'exposition agricole, il s'agissait du panel suivant : *Aspergillus versicolor*, *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, *Stachybotrys chartarum*, *Mucor racemosus*, *Alternaria alternata* (153, 154). Un dosage des IgE totales et une identification semi-quantitative des allergies aux pneumallergènes et trophallergènes courants étaient également réalisés chez tous les sujets inclus.

#### **4. Exploration fonctionnelle respiratoire**

L'exploration fonctionnelle respiratoire comportait la mesure des volumes pulmonaires statiques, des débits ventilatoires forcés et de la capacité de transfert (diffusion) pulmonaire du monoxyde de carbone. Les volumes étaient exprimés en litres à la température corporelle et à pression ambiante saturée en vapeur d'eau (conditions BTPS) (142). Les volumes et capacités pulmonaires étaient mesurés par pléthysmographie corporelle totale. La TLCO était mesurée pendant une apnée de neuf secondes, et exprimée en ml/min/mmHg; les valeurs de TLCO étaient interprétées en fonction de l'hémoglobinémie du patient au moment de la mesure, du pourcentage d'hémoglobine saturée en CO (HbCO) préalable à la mesure (en cas de tabagisme par exemple) et du volume alvéolaire mesuré au cours de la manœuvre (qui doit être égal à au moins 90 % de la CPT) (155). Au minimum, deux mesures étaient faites pour chaque sujet, et les meilleures valeurs étaient retenues. L'ensemble des valeurs numériques obtenues au cours de l'exploration fonctionnelle respiratoire était comparé aux valeurs théoriques du GLI (14, 156).

#### **5. Test de marche de six minutes**

Le test de marche était réalisé dans un couloir de 30 m de long selon une procédure décrite par ailleurs (29). On mesurait les grandeurs suivantes : distance parcourue ; fréquence

cardiaque (repos, toutes les minutes pendant l'exercice puis en récupération pendant deux minutes); saturation en oxygène (repos, toutes les minutes pendant l'exercice puis en récupération pendant deux minutes) ; dyspnée sur une échelle de Borg. La distance parcourue était comparée à une valeur théorique (157).

## **6. Épreuve fonctionnelle d'exercice métabolique (EFX)**

L'EFX était réalisée chez tous les sujets inclus, en dehors de la présence d'une ou plusieurs contre-indications (158). L'examen était pratiqué sur bicyclette ergométrique (Ergoline 200K), avec évaluation métabolique continue à partir des gaz expirés à la bouche (débit de ventilation [ $V'E$ ], débit d'oxygène consommé [ $V'O_2$ ] et débit de dioxyde de carbone rejeté [ $V'CO_2$ ] [Medical Graphics, logiciel Breeze]), de la saturation artérielle en oxygène et de l'électrocardiogramme 12 dérivations (General Electrics) (16). L'épreuve était « triangulaire » avec une période d'échauffement de trois minutes suivie d'un exercice progressif réalisé selon un protocole incrémental poursuivi jusqu'à épuisement. La puissance (exercice et créneaux) était adaptée à l'état clinique des patients. Des mesures de la gazométrie sur sang capillaire artérialisé prélevé à l'oreille et de la lactatémie étaient effectuées au repos, au seuil et au pic de l'exercice. Le seuil ventilatoire était déterminé conjointement par deux méthodes :

- la méthode des équivalents, dont le principe est de déterminer la valeur de  $V'O_2$  pour laquelle l'équivalent respiratoire en oxygène ( $V'E/V'O_2$ ) atteint son minimum avant d'augmenter alors que l'équivalent respiratoire en  $CO_2$  ( $V'E/V'CO_2$ ) diminue ou reste constant ;

- l'identification de la valeur de  $V'O_2$  à partir de laquelle les pentes de  $V'E$  et de  $V'CO_2$  deviennent plus élevées que celle de la  $V'O_2$ .

Une mesure de la capacité inspiratoire est réalisée à chaque palier d'exercice afin de juger de l'apparition d'une distension dynamique.

## **7. Étude de la rigidité artérielle**

Les patients atteints d'une BPCO ont un risque cardiovasculaire augmenté par rapport aux sujets sains. Il existe une corrélation entre un VEMS bas et un risque élevé de mortalité par événement cardiovasculaire (159). Les mécanismes qui peuvent expliquer le lien entre mortalité cardiovasculaire et BPCO sont l'hypoxie tissulaire, l'inflammation systémique, le stress oxydant et l'activation du système orthosympathique (160). La rigidité artérielle peut être étudiée par la mesure de la vitesse d'onde de pouls (VOP). Plus la paroi d'une artère est

rigide, plus la vitesse de propagation est élevée et inversement. La rigidité d'une artère de gros calibre augmente avec la dysfonction endothéliale, la modification de la fonction contractile du muscle lisse et la perte de fibres élastiques. Dans la BPCO, la rigidité artérielle est d'autant plus élevée que l'obstruction bronchique et l'inflammation systémique sont sévères (161-163). La rigidité artérielle est améliorée par la réhabilitation respiratoire (164). Une VOP élevée est associée à une augmentation significative du risque de survenue « d'évènements cardiovasculaires » (infarctus du myocarde, accident vasculaire cérébral, ...).

La VOP était mesurée chez tous les sujets de l'étude par tonométrie d'aplanation, technique qui consiste à utiliser une sonde ultrasensible aux variations de pression et aux mouvements des tissus entourant l'artère étudiée (Millar Instruments, Houston, Texas). Deux enregistrements successifs sur deux zones anatomiques étaient nécessaires afin d'évaluer une vitesse. La rigidité du tronçon carotide-fémorale était évaluée avec le système SphygmoCor (AtCor Medical, Paris, France).

## **8. Étude de l'activité du système nerveux végétatif au repos**

L'analyse de la variabilité de la fréquence cardiaque (VFC) et de pression artérielle ainsi que l'étude du baroréflexe spontané sont les principales méthodes d'exploration non vulnérante de l'activité neurovégétative à destinée cardiovasculaire. De nombreux travaux ont montré que la réduction de la variabilité de la fréquence cardiaque et/ou la modification de certains paramètres extraits de l'analyse spectrale constituent des facteurs pronostiques d'un risque cardiovasculaire notamment dans l'infarctus du myocarde, l'insuffisance cardiaque, l'hypertension artérielle et le diabète (165). L'inflammation systémique associée à la BPCO, induit un déséquilibre péjoratif de l'activité du système nerveux végétatif, et une réduction de la variabilité de fréquence cardiaque a été décrite (166).

Le système baroréflexe dont l'action ajuste en permanence l'activité cardiaque et la vasomotricité permet l'optimisation à court terme de la pression artérielle et du débit cardiaque. Son exploration est réalisée de façon non vulnérante à l'aide de calculs issus des enregistrements cycle par cycle de la pression artérielle digitale et de la durée de l'intervalle RR de l'ECG (167). Chez tous les sujets, un enregistrement de la pression artérielle instantanée et des intervalles RR, était effectué pendant dix minutes en décubitus, après un repos de 20 minutes dans des conditions de calme et de neutralité thermique.

La VFC était étudiée conjointement par deux méthodes décrites par ailleurs (168) :

- la méthode temporelle repose sur le calcul d'indices statistiques renseignant sur la VFC globale et sur la réponse du nœud sinusal à l'activité parasympathique ;

- la méthode fréquentielle fondée sur l'analyse spectrale appliquée à une série de mesures de l'intervalle RR (enregistrement de cinq minutes). Elle permet d'obtenir des spectres représentatifs des activités parasympathique et orthosympathique à destinée cardiovasculaire.

## **C. Plan statistique**

### **1. Nombre de sujets à inclure**

L'objectif principal de notre étude était d'estimer avec une bonne précision la prévalence de BPCO en milieu agricole de production laitière en Franche-Comté. Si l'on considère que la prévalence attendue de BPCO dans ces populations est d'environ 9% (résultats d'études européennes) et que l'on souhaite obtenir un résultat avec une précision de 1,5 % et un risque de première espèce à 5 %, le nombre de sujet nécessaire pour obtenir une puissance de 80 % est d'environ 1398 individus. Nous avons donc choisi de cibler une population de 2000 individus afin d'avoir une puissance d'étude suffisante, en prenant en compte d'éventuels non répondants. Les données de la littérature montrent en fait que le taux de réponse des études de population avoisine 70 % (1400 correspond à 70 % de 2000). Un nombre égal de sujets témoins sera inclus.

### **2. Critères de jugement**

Plusieurs critères de jugement ont été retenus: la présence d'une BPCO, la dyspnée, la fonction respiratoire (distension, diffusion du CO, réversibilité de l'obstruction et/ou de la distension sous traitement), l'inflammation systémique, la fonction vasculaire systémique, la capacité d'exercice (distance de marche de six minutes et épreuve d'effort) et enfin la qualité de vie.

### **3. Analyse principale**

Les analyses consistaient tout d'abord à estimer la prévalence de BPCO en milieu de production laitière (BPCO « agricole »), à caractériser les patients atteints de BPCO «agricole» en termes de dyspnée, de qualité de vie, de fonction respiratoire, d'inflammation systémique, de fonction vasculaire systémique, de capacité d'exercice et à comparer leurs caractéristiques avec celles d'un groupe de BPCO « non agricole ». La statistique descriptive, à savoir les moyennes et les distributions dans le cas des variables continues et les pourcentages dans le cas des variables catégorielles et les techniques de comparaison des moyennes (test de Student, Anova) et des pourcentages (test du Chi2) ont été utilisées pour

mettre en évidence l'existence de différences statistiquement significatives entre les différents groupes (BPCO « agricole », BPCO « non agricole » et témoins).

Des analyses univariées ont été réalisées afin de mettre en évidence des associations entre la BPCO « agricole » et « non agricole », les facteurs de jugement choisis et les facteurs de risque potentiels. Enfin les analyses multivariées ont permis de mettre en évidence les variables d'exposition et des facteurs de risque associés avec les deux formes de BPCO après ajustement sur les facteurs potentiels de confusion (âge, sexe, tabagisme ...).

#### **4. Analyses secondaires**

Les analyses secondaires étaient de trois types :

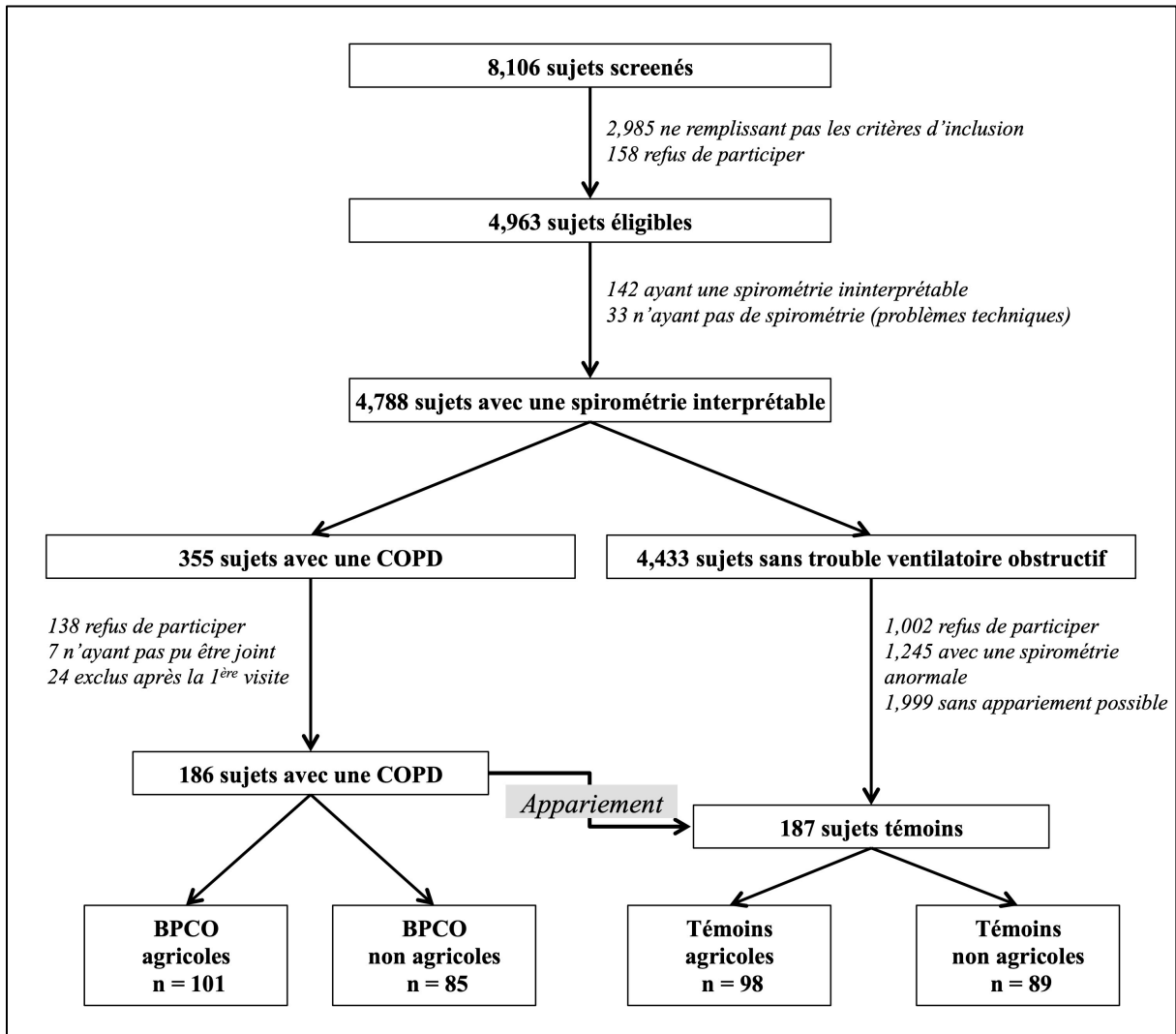
- comparaison entre les BPCO « agricoles » et les contrôles « agricoles » :
  - des pratiques agricoles et des caractéristiques des fermes,
  - de l'exposition microbiologique domestique,
  - de la sensibilisation (dosage des IgE et IgG). Ces analyses nous ont permis d'étudier les mécanismes potentiels impliqués dans le développement de la BPCO en milieu agricole.
- analyses de la qualité de vie des patients souffrant d'une BPCO « agricole » et d'une BPCO « non agricole ». Un score de qualité de vie a été calculé. Il s'agit d'une variable qualitative ordinaire dont l'analyse reposera sur la description de la répartition des valeurs et sur des comparaisons à l'aide du test du Chi<sup>2</sup>
- analyse comparative de l'inflammation pulmonaire, vasculaire et systémique entre sujets témoins « agricoles » et « non agricoles » afin de mesurer l'influence de l'exposition agricole sur ces paramètres.

#### **5. Logiciels utilisés**

Les logiciels utilisés pour l'analyse des données seront les logiciels SAS et R.



## D. Flow chart de la population



## IV. RESULTATS

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### A. Etat de l'art de la BPCO secondaire à l'exposition aux poussières organiques

#### 1. Article 1 : BPCO professionnelles et BPCO post-tabagique : similarités et différences

##### *a. Contexte et objectif*

Le tabagisme est la principale cause de BPCO et le principal facteur de risque identifié de BPCO. Cependant, on estime que près de 15% des cas de BPCO sont consécutifs à une exposition professionnelle.

Cela concerne l'industrie du papier, le nettoyage, les industries de transformation des aliments et bois, l'extraction minière (charbon, or, potasse), les travaux publics et du bâtiment (asphaltage et ouvriers tunneliers), les secteurs des fonderies et de la sidérurgie, la cimenterie, la production laitière, l'élevage de porcs et de volailles, le travail du bois, l'usinage des métaux et l'activité de soudage.

Les caractéristiques cliniques et la fonction pulmonaire des BPCO professionnelles restent assez mal connues bien que la littérature ait été enrichie de nombreuses études ces cinq dernières années. Cette revue propose d'inventorier les différences et les similarités entre la BPCO post-tabagique et la BPCO d'origine professionnelle.

##### *b. Manuscrit*

Article "BPCO professionnelles et BPCO post-tabagique : similarités et différences". <b>Soumagne T</b> , Caillaud D, Degano B, Dalphin JC. <i>Revue des maladies respiratoires</i> 2017.
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REVUE GÉNÉRALE

# BPCO professionnelles et BPCO post-tabagique : similarités et différences



*Differences and similarities between occupational and tobacco induced COPD*

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## MOTS CLÉS

BPCO ;  
Profession ;  
Épidémiologie ;  
Fonction  
respiratoire ;  
Symptômes

**Résumé** Le développement d'une bronchopneumopathie chronique obstructive (BPCO) est le plus souvent secondaire à l'exposition à des substances toxiques inhalées. Le tabagisme en reste la principale cause mais l'existence de BPCO liées à des agents d'origine professionnelle est maintenant clairement établie. Après un bref aperçu de l'épidémiologie de cette « autre BPCO », les caractéristiques cliniques et fonctionnelles des BPCO professionnelles sont résumées, en tenant compte de l'avancée très récente des connaissances. Les effets combinés de l'exposition professionnelle et du tabagisme sont également abordés et témoignent de la nécessité de renforcer les campagnes d'éducation et de prévention sur les BPCO professionnelles. © 2017 SPLF. Publié par Elsevier Masson SAS. Tous droits réservés.

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**KEYWORDS**

COPD;  
Occupation;  
Epidemiology;  
Lung function;  
Symptoms

**Summary** Chronic obstructive pulmonary disease (COPD) most often results from the inhalation of toxic agents. Cigarette smoking still remains the principal cause but the pertinence of occupational COPD is now clearly established. After a brief overview of the epidemiology of this "other COPD", the clinical and functional characteristics are summarized, taking into account recent advances in this field. The combined effects of occupational exposure and tobacco are also considered, providing evidence of the need to continuously reinforce campaigns of education and prevention in occupational COPD.

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## Introduction

La bronchopneumopathie chronique obstructive (BPCO) est une maladie respiratoire chronique définie par une obstruction permanente et progressive des voies aériennes. Cette obstruction est causée par l'association, variable selon les patients, d'une diminution du calibre des bronchioles du fait de modifications anatomiques (remodelage) et d'une destruction pulmonaire en aval des bronchioles terminales (emphysème) [1].

Le diagnostic de BPCO repose sur l'existence d'un trouble ventilatoire obstructif ; il est donc fondé sur la réalisation d'une spirométrie qui permet la mesure du volume expiratoire maximal à la première seconde (VEMS) et de la capacité vitale forcée (CVF). La définition la plus consensuelle du trouble ventilatoire obstructif de la BPCO est actuellement celle du *global initiative for chronic obstructive lung disease* (GOLD), à savoir un rapport VEMS/CVF < 70 % après administration d'un bronchodilatateur. Cette définition ne précise pas toutefois si l'obstruction s'associe à une distension, ni s'il existe une composante réversible en termes d'obstruction et/ou de distension. Par ailleurs, la définition LIN (rapport VEMS/CVF < limite inférieure de la normale) semble plus pertinente, notamment pour le dépistage, puisqu'elle est spécifique à l'âge et non fixe. En effet, la présence de symptômes fait partie de la définition de la BPCO mais des patients présentant une obstruction bronchique définie par un rapport VEMS/CVF < LIN, qui ne rapportent pas de symptômes, peuvent révéler des anomalies physiologiques à l'exercice [2,3]. La présence de symptômes n'est donc pas indispensable pour que soit faite une spirométrie de dépistage de BPCO, si tant est que la définition ATS/ERS (VEMS/CVF < LIN) soit appliquée pour définir l'obstruction bronchique persistante.

Le développement d'une BPCO est le plus souvent secondaire à l'exposition à des substances inhalées. C'est une affection d'installation lentement progressive, non réversible après l'arrêt de l'exposition. Sa gravité, chez les sujets sensibles à une ou plusieurs substances inhalées, est proportionnelle à l'exposition (durée et intensité) [4]. La fumée de tabac reste le principal facteur de risque (responsable d'environ 80 % des BPCO) bien que des causes génétiques, environnementales et professionnelles aient été identifiées. En effet, certains milieux professionnels où les sujets sont exposés à des vapeurs, des gaz, des poussières et/ou des fumées (*vapor, gases, dust and fumes* [VGDF]) sont associés à un risque accru d'aggravation et/ou de survenue de BPCO. Ceci concerne l'industrie du papier, le nettoyage, les industries de transformation des aliments et bois, l'extraction

**Tableau 1** Professions à risque de BPCO.

Profession	Type d'exposition
Élevage d'animaux (production laitière, élevage de porcs)	Poussières organiques, ammoniac, bactéries
Culture céréalière, maraîchage	Poussières organiques et inorganiques, pesticides
Secteur minier (charbon, or, potasse), bâtiment-travaux publics, cimenterie	Poussières minérales
Industries plastiques, textiles, cuir, caoutchouc	Produits chimiques, noir de carbone
Usinage des métaux et activité de soudage	Particules métalliques et gazeuses (ozone, oxyde d'azote)
Travail du bois	Poussières
Transport, réparation automobile	Particules diesel
Entretien (balayage)	Poussières des routes

minière (charbon, or, potasse), les travaux publics et du bâtiment (asphaltage et ouvriers tunneliers), les secteurs des fonderies et de la sidérurgie, la cimenterie, la production laitière, l'élevage de porcs et de volailles, le travail du bois, l'usinage des métaux et l'activité de soudage (Tableau 1) [4,5].

Les caractéristiques cliniques et la fonction pulmonaire des BPCO professionnelles restent assez mal connues bien que la littérature ait été enrichie de nombreuses études ces cinq dernières années. Cette revue propose d'inventorier les différences et les similarités entre la BPCO post-tabagique et la BPCO d'origine professionnelle.

## Épidémiologie

La responsabilité des facteurs professionnels dans la genèse ou l'aggravation des BPCO est restée longtemps méconnue, occultée en particulier par le poids prépondérant du tabagisme. Néanmoins, la prévalence de la BPCO chez des patients sans aucune exposition tabagique est estimée entre 2,5 % à 6,5 %, selon de récentes études [6–8]. Par ailleurs,

25 à 45 % des sujets ayant une BPCO n'auraient jamais fumé [9]. Il existe désormais un corpus de données expérimentales et surtout épidémiologiques qui attestent du rôle important des expositions professionnelles en tant que facteur étiologique des BPCO. Dans une revue générale récente, Blanc et al. ont estimé que la fraction des BPCO attribuable à l'exposition professionnelle (qui correspond au pourcentage de BPCO qui ne serait pas survenue s'il n'y avait pas eu d'exposition professionnelle) était d'environ 15 %, chiffre corroborée par un récent consensus de l'American Thoracic Society [4,10]. Cette fraction, qui est néanmoins très variable (de 1 à 40 %) entre les études, dépend des facteurs suivants :

- le type, la durée et le mode de recueil de l'exposition : simple déclaration ou utilisation d'une matrice emploi-exposition ;
- les critères utilisés pour le diagnostic de BPCO : simple déclaration, existence d'une bronchite chronique, définition spirométrique GOLD ou limite inférieure de la normale (LIN) ;
- la population étudiée : étude en population générale ou au sein d'une catégorie socioprofessionnelle donnée ;
- le fait que l'exposition soit combinée ou pas au tabagisme.

Par rapport à des sujets non exposés, on estime que la probabilité de développer une BPCO est 1,7 à 3,8 fois plus élevée chez les travailleurs exposés à des matières inorganiques (fumée de soudure, ciment, charbon...) et 1,2 à 8,9 fois plus élevée chez les travailleurs exposés à des matières organiques (coton, laine, grain, poussières de fermes...) [11]. Néanmoins, beaucoup d'études épidémiologiques se heurtent à des difficultés méthodologiques qui contribueraient à sous-estimer l'importance des facteurs professionnels. Un des principaux biais est celui de l'effet « travailleur sain » dans les études réalisées en population sélectionnée. Les études épidémiologiques en population générale, permettent en partie de s'affranchir de ce biais (Fig. 1) [8,12–26].

La question de l'influence du sexe dans l'association BPCO et exposition professionnelle n'est pas tranchée. Matheson et al. [15] ont montré qu'il existait une association entre BPCO et exposition professionnelle plus forte chez les femmes que chez les hommes, ce que réfute une étude plus récente de Paulin et al. [27]. Néanmoins, dans une étude transversale sur 5176 sujets (dont 60 % de femmes), Tan et al. estiment que la prévalence de la BPCO serait plus élevée chez les femmes (7,4 %) que chez les hommes (5,4 %) au sein d'une population de non-fumeurs alors qu'elle serait équivalente (15 %) chez les fumeurs, suggérant une plus grande sensibilité des femmes à des facteurs autres que le tabac [7].

Enfin, les connaissances actuelles sur les causes de la BPCO sont généralement basées sur des données issues de populations âgées où la pathologie est fréquente. Les facteurs de risques intervenant dans la genèse de la BPCO sont de ce fait mal connus puisqu'il n'existe qu'un nombre limité d'études sur de jeunes populations. Dans une population de 4636 sujets âgés de 20 à 44 ans, de Marco et al. n'ont pas montré d'association entre l'exposition professionnelle et la BPCO, vraisemblablement du fait d'une exposition cumulée trop faible [28]. Néanmoins, il existerait une prévalence plus importante de bronchite chronique dans ce

type de population, sous-tendant l'hypothèse d'une BPCO en devenir chez les sujets ayant une exposition professionnelle [29].

- Les principaux secteurs professionnels à risque de BPCO sont l'industrie du papier, le nettoyage, les industries de transformation des aliments, l'extraction minière (charbon, or, potasse), les travaux publics et du bâtiment (asphaltage et ouvriers tonneliers), les secteurs des fonderies et de la sidérurgie, la cimenterie, la production laitière, l'élevage de porcs et de volailles, le travail du bois, l'usinage des métaux et l'activité de soudage.
- La fraction des BPCO attribuable à l'exposition professionnelle est de 15 %, variant suivant le type d'exposition et selon l'association à une exposition tabagique.
- La prévalence des BPCO professionnelles reste moins élevée que celle de la BPCO post-tabagique.

## Symptômes

Des études datant des années 1980 ont mis en évidence un excès de symptômes chez les sujets présentant une exposition professionnelle antérieure [30]. Plus récemment, des études avec des critères « robustes » de BPCO ont suggéré que les symptômes étaient plus fréquents et plus sévères chez les patients atteints d'une BPCO d'origine professionnelle que chez les patients avec une BPCO post-tabagique (Tableau 2). De nombreuses études ont conclu que la bronchite chronique était deux fois plus fréquente chez des patients atteints d'une BPCO suite à une exposition antérieure aux VGDF que chez des sujets BPCO non exposés et ce indépendamment du tabagisme [27,31–34]. La fréquence des symptômes est identique qu'il s'agisse d'une exposition aux poussières minérales ou d'une exposition aux poussières organiques [15,32,35]. Rodriguez et al. ont mis en évidence que certaines expositions combinées induisaient des symptômes plus fréquents et qu'il existait une relation dose–effet entre symptômes et durée d'exposition [36]. En milieu agricole, Guillien et al. ont mis en évidence que des symptômes tels que la toux, l'expectoration et la dyspnée chronique étaient plus fréquents chez des fermiers avec BPCO que chez des sujets non exposés porteurs d'une BPCO post-tabagique [26]. Cet excès de symptômes était principalement retrouvé chez les cultivateurs et les éleveurs de bétail.

- La toux, l'expectoration, la bronchite chronique, la dyspnée et les sibilants semblent plus fréquents dans les BPCO professionnelles que dans la BPCO post-tabagique, notamment avec l'exposition aux poussières agricoles et aux fumées et gaz.
- Il existe une relation dose–effet entre la durée cumulée d'exposition professionnelle et ces symptômes.

**Tableau 2** Association entre exposition professionnelle et symptômes respiratoires.

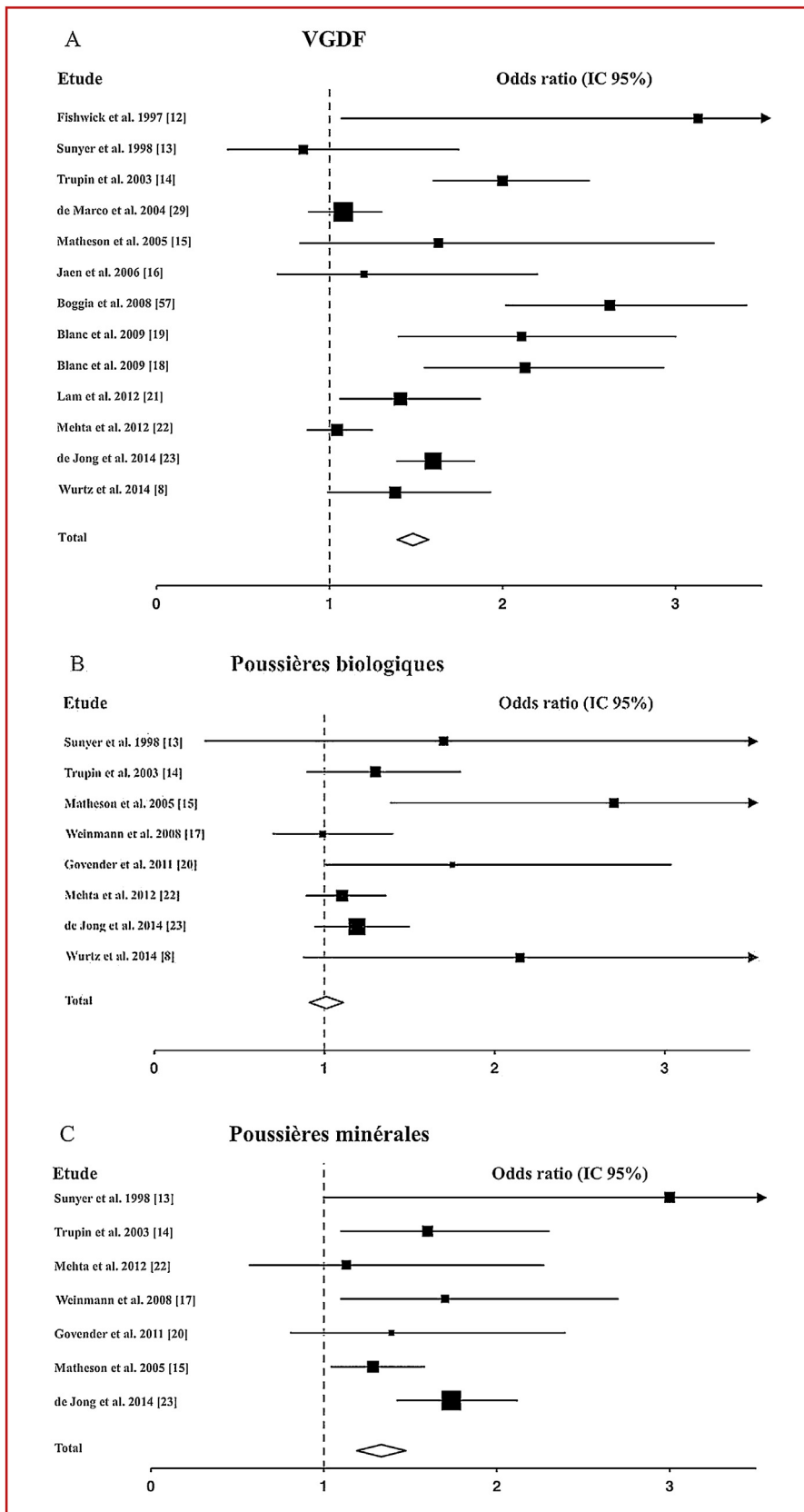
Référence	Type d'étude	Population	Type de sujets	âge (années)	Nombre de sujets	Type d'exposition	Évaluation de l'exposition	Diagnostic de BPCO	Test de réversibilité	Symptômes <sup>a</sup> (vs non exposés)
Caillaud et al., 2012 [32]	Transversale	Population sélectionnée	BPCO	55–75	591	VGDF	Auto-évaluation (ECRHS)	GOLD, VEMS/CVF < 0,70	Oui	Expectorations et wheezing plus fréquents NA
Sunyer et al., 1998 [13]	Transversale	Population générale	Sujets présumés sains	20–44	1754	Gaz et fumées, poussières biologiques et minérales	Auto-évaluation (ECRHS) et MEE	GOLD, VEMS/CVF < 0,70	Oui	Toux : OR 2,1 (1,5–3,0) (AE) et OR 1,5 (1,0–2,2) Expectorations : OR 1,5 (1,2–2,0) (AE) et 1,5 (1,7–2,0) Bronchite chronique : OR : 1,0 (0,4–2,1) (non-fumeurs) OR : 1,7 (1,2–2,4) (fumeurs actifs) Bronchite chronique : RRR : 1,47 (1,31–1,65) Bronchite chronique Poussières biologiques : OR : 3,19 (1,27–7,97) Poussières minérales : OR : 1,40 (0,56–3,51) Gaz et fumées : OR : 1,31 (0,72–2,40)
Zock et al., 2001 [31]	Transversale	Population générale	Sujets présumés sains	20–44	13 253	VGDF	Auto-évaluation (ECRHS) et MEE	NA	NA	NA
de Marco et al., 2004 [29]	Transversale	Population générale	Sujets présumés sains	20–44	14 855	VGDF	Auto-évaluation	GOLD, VEMS/CVF < 0,70	Non	NA
Matheson et al., 2005 [15]	Transversale	Population générale	Sujets présumés sains	45–70	1213	Gaz et fumées, poussières biologiques et minérales	MEE	GOLD, VEMS/CVF < 0,70	Non	NA

Tableau 2 (Suite)

Référence	Type d'étude	Population	Type de sujets	âge (années)	Nombre de sujets	Type d'exposition	Évaluation de l'exposition	Diagnostic de BPCO	Test de réversibilité	Symptômes <sup>a</sup> (vs non exposés)
Rodriguez et al., 2008 [36]	Transversale	Population sélectionnée	BPCO	40–90	185	Gaz et fumées, poussières biologiques et minérales	Auto-évaluation et MEE	GOLD, VEMS/CVF < 0,70	Oui	Toux : OR : 1,1 (0,9–1,3) Expectorations : OR : 1,2 (1,0–1,4) Dyspnée : OR : 1,2 (1,0–1,4) NA Toux : OR : 2,0 (1,6–2,1) Expectorations : OR : 2,2 (1,8–2,8) Dyspnée : OR : 1,9 (1,5–2,3) NA
Melville et al., 2010 [39]	Transversale	Population générale	Sujets présumés sains	45–69	845	Exposition professionnelle	Auto-évaluation	GOLD, VEMS/CVF < 0,70	Oui	Toux chronique : OR : 1,78 (1,59–1,98) Expectorations chroniques : OR : 1,19 (1,03–1,38)
Marchetti et al., 2014 [48]	Transversale	Population sélectionnée	Sujets fumeurs	45–80	9614	Poussière et fumées	Auto-évaluation	GOLD, VEMS/CVF < 0,70	Oui	Toux chronique : OR : 1,78 (1,59–1,98) Expectorations chroniques : OR : 1,19 (1,03–1,38)

MEE : matrice emploi-exposition ; NA : non applicable ; OR : odds ratio ; VA : voies aériennes ; VGDF : vapeur, gaz, poussières et fumées.  
<sup>a</sup> Odds ratio ajustés pour l'âge, le sexe et le tabagisme.





**Figure 1.** Association entre la présence d’une BPCO et l’exposition aux vapeurs, gaz, poussières et/ou fumées (A), aux poussières biologiques (B) et aux poussières minérales (C). Odds ratio ajustés pour l’âge, le sexe et le tabagisme (sauf pour le total).

## Allergies

La prévalence des allergies est plus élevée parmi les patients présentant une BPCO associée à une exposition professionnelle que chez les patients avec BPCO tabagique [7,32,37]. Au sein d'une cohorte norvégienne de 4735 fermiers, Eduard et al. ont montré que les effets des activités agricoles et des expositions spécifiques sur la BPCO étaient exacerbés chez les fermiers ayant une atopie [38]. En population générale, Melville et al. concluaient que l'exposition professionnelle et l'atopie (définie par la présence d'un rhume des foins) étaient deux facteurs indépendants de BPCO [39]. L'allergie pourrait donc prédisposer au développement des BPCO professionnelles. Par ailleurs, différentes études menées dans le Doubs ont mis en évidence des arguments épidémiologiques, fonctionnels et immunologiques en faveur d'un possible mécanisme immuno-allergique dans la genèse de la BPCO [40]. La présence d'une BPCO était notamment plus fréquente chez les agriculteurs ayant des antécédents de maladie de poumon de fermier ou de symptômes retardés liés à une exposition spécifique et également chez ceux ayant des taux de précipitines sériques élevés. Il existait également dans cette population de producteurs laitiers une relation entre un taux élevé d'IgE et d'IgG et un déclin accéléré du VEMS [41].

Ceci relance une hypothèse vieille de 50 ans, l'hypothèse hollandaise, selon laquelle l'asthme et la BPCO seraient des manifestations différentes d'une seule maladie, la « bronchopneumopathie chronique non spécifique » du fait de facteurs de risques génétiques et environnementaux communs (allergies, infections, tabagisme...) [42]. En effet, de nombreux aéro-contaminants professionnels ont des propriétés allergisantes, d'où la possibilité du développement possible d'une obstruction bronchique chez des sujets prédisposés (allergie). L'asthme chronique pourrait entraîner une obstruction bronchique non réversible, parfois qualifié d'*asthma-COPD overlap syndrome* (ACOS). Postma et al. proposent que des facteurs génétiques pourraient conduire soit vers un asthme, soit vers une BPCO en fonction de la période de la vie où l'exposition a lieu et des facteurs environnementaux rencontrés [43].

- Les allergies semblent plus fréquentes chez les patients atteints d'une BPCO suite à une exposition antérieure à des vapeurs, gaz, poussières et/ou fumées.
- Des données récentes, notamment génétiques, relancent en partie « l'hypothèse hollandaise » selon laquelle l'asthme et la BPCO seraient des manifestations différentes d'une seule maladie, la « bronchopneumopathie chronique non spécifique ».

## Fonction respiratoire et imagerie thoracique

La fonction ventilatoire semble s'altérer plus rapidement dans les BPCO professionnelles que dans la BPCO

post-tabagique. Sur une cohorte de 2579 mineurs, Montes et al. ont montré que l'exposition aux poussières minérales est associée à une déclin accéléré du VEMS, principalement chez les patients fumeurs [44]. Des résultats similaires sont retrouvés dans des cohortes où les sujets ont une exposition aux poussières agricoles ou aux fumées de soudage [45,46]. Mehta et al., dans la cohorte SAPALDIA ont évalué la fonction respiratoire de 4267 travailleurs suisses exposés à des poussières biologiques, des poussières minérales, des gaz, fumées et vapeurs. À tabagisme équivalent, les auteurs ont montré que la sévérité de la BPCO était corrélée, d'une part, à l'intensité de l'exposition et, d'autre part, à sa durée [22]. Les sujets ayant une exposition de niveau élevé, quelle qu'elle soit, avait 2 à 5 fois plus de risques de développer une BPCO de stade GOLD II+.

L'évaluation de l'emphysème pulmonaire fait l'objet d'études plus récentes. Sur une série autopsique de 722 sujets (mineurs et non mineurs), Kuempel et al. ont démontré que la sévérité de l'emphysème était plus importante chez les mineurs de charbon quelle que soit l'exposition tabagique [47] et qu'il existait une relation dose-effet entre l'exposition à la poussière de charbon et la présence d'un emphysème. Au sein de la cohorte COPD-Gene, Marchetti et al. ont évalué l'emphysème pulmonaire par tomodensitométrie thoracique chez 9614 sujets issus de la population générale. Dans cette étude, l'exposition concomitante aux fumées et poussières était associée à un volume d'emphysème pulmonaire plus important que chez les sujets non exposés, même chez ceux ayant une BPCO tabagique [48]. Chez ces sujets ayant un emphysème tomodensitométrique, il existait une diminution du transfert du monoxyde de carbone (TLCO) [34].

Enfin, une étude multicentrique française suggérait qu'il existait des phénotypes différents au sein de BPCO professionnelles suivant le type d'exposition : la BPCO liée à une exposition à des poussières minérales semblerait être une maladie plus distale et pourvoyeuse d'emphysème alors que la BPCO agricole serait une maladie localisée au niveau des bronches [49,50].

D'une manière générale, l'évaluation de la fonction respiratoire est d'une importance capitale dans la caractérisation et le suivi de la BPCO en milieu professionnel. L'American Thoracic Society a d'ailleurs récemment émis des recommandations spécifiques quant à la réalisation, l'interprétation de la spirométrie et le suivi de la fonction respiratoire en milieu professionnel [51].

- La sévérité de la BPCO est corrélée à l'intensité et à la durée de l'exposition professionnelle.
- De manière similaire à l'exposition à la fumée de cigarette, il existe une atteinte des petites bronches dans l'exposition aux vapeurs, gaz, poussières et/ou fumées.
- La prévalence de l'emphysème semble plus importante chez les patients ayant une BPCO professionnelle aux poussières minérales, aux gaz et aux vapeurs que chez ceux ayant une BPCO tabagique.

## Retentissement professionnel, qualité de vie et mortalité

L'absentéisme professionnel est un problème commun dans les pathologies respiratoires mais il semble bien plus important parmi les personnes chez qui l'apparition de la pathologie est directement liée à une exposition professionnelle. Dans une étude transversale française, Caillaud et al. ont comparé 591 sujets BPCO fumeurs ou anciens fumeurs ayant ou non une exposition antérieure aux VGDF. L'exposition aux VGDF était associée avec une incapacité au travail plus importante que chez les sujets non exposés [32]. Blanc et al. ont par ailleurs montré, dans une étude transversale portant sur 234 sujets rapportant une BPCO, que le fait d'avoir une incapacité au travail induite par une exposition aux VGDF était associé à une restriction d'activité et à une fréquence de consultation aux urgences ou d'hospitalisation plus élevée que chez les sujets ayant une incapacité de travail liée à une BPCO post-tabagique [52]. Cette incapacité semblerait en partie corrélée avec l'intensité et la durée d'exposition professionnelle, notamment dans le cadre de poussières biologiques [36].

Par ailleurs, parmi les sujets présentant une BPCO, ceux ayant une exposition professionnelle significative auraient un retentissement plus important sur leur qualité de vie (évaluée par différents scores SGRQ, SF-12, CAT) du fait d'une symptomatologie plus sévère [27]. De plus, le nombre d'exacerbation requérant une hospitalisation serait également plus élevé dans cette population. Ces différentes études témoignent donc que la morbidité est plus élevée chez les sujets ayant une BPCO secondaire à une exposition professionnelle et ce indépendamment du tabagisme.

L'évaluation de la mortalité liée aux expositions professionnelles reste basée sur des cohortes de travailleurs. Dans une cohorte de 354 718 travailleurs du bâtiment, sur une période de suivi de 40 ans, Tóren et al. ont estimé que la mortalité des patients présentant une BPCO était jusqu'à deux fois plus élevée chez ceux qui présentaient une exposition professionnelle (notamment aux poussières inorganiques et aux fumées) par rapport à des sujets non exposés [53]. Celle-ci était néanmoins plus faible que celle induite par l'exposition tabagique. Par ailleurs, deux autres études ont démontré que la mortalité chez ces sujets était corrélée à la durée d'exposition [54,55]. En effet, Hart et al. ont montré que, chez les cheminots, chaque année supplémentaire d'exposition aux particules diesel entraînait une augmentation de 2,5 % de la mortalité liée à la BPCO, ceci témoignant une nouvelle fois de l'importance des mesures de prévention collectives et individuelles dans les BPCO professionnelles.

Les aspects médicolégaux et la conduite à tenir devant une BPCO professionnelle ont fait l'objet d'un autre article dans la *Revue des maladies respiratoires* [56].

- L'incapacité au travail est fréquente dans la BPCO, et l'est d'autant plus qu'elle est liée à une exposition professionnelle durable.
- L'exposition à des agents professionnels est liée à une qualité de vie moins bonne et à une consommation de soins médicaux plus élevée.

- La mortalité liée aux BPCO professionnelles est plus élevée que dans la population générale mais de moindre magnitude que celle liée à l'exposition tabagique.

## Poids du tabagisme

Le poids du tabagisme est une question récurrente dans la BPCO puisque la plupart des patients présentant une BPCO d'origine professionnelle sont tabagiques, en dehors des professions agricoles. Dans une revue récente, Szram et al. ont examiné le lien entre le déclin de la fonction respiratoire, le tabagisme et le métier de soudeurs [46]. Ils mettent en évidence que les sujets fumeurs et exerçant le métier de soudeur ont un déclin accéléré du VEMS. Les soudeurs non tabagiques n'ont quant à eux pas de déclin du VEMS statistiquement plus élevé que la population générale. Les auteurs concluent donc qu'il doit exister des effets additifs voire synergiques entre ces deux sources d'irritation bronchique simultanée. De nombreuses autres études ont également démontré cet effet combiné et additif entre l'exposition professionnelle et le tabagisme sur le développement de la BPCO [18,22,57,58].

Cette double exposition a également un impact en termes de mortalité. Tóren et al. concluaient que le fait d'avoir une exposition professionnelle et un tabagisme était associé à une mortalité plus importante que chez les sujets ayant un seul de ces facteurs [53].

Ce rôle combiné du tabagisme et de l'exposition professionnelle est également impliqué dans la genèse de l'emphysème. Marchetti et al. ont mis en évidence que l'emphysème pulmonaire évalué par tomodynamométrie était plus fréquent en cas d'exposition combinée, bien que cet effet ne soit pas additif [48].

Enfin, dans une étude écologique, Blanc et al. ont démontré que pour réduire de 20 % la prévalence de la BPCO, il fallait réduire de 5,4 % le tabagisme ou de 8,8 % l'exposition professionnelle [59]. Bien que le tabac reste la principale cause de BPCO, la contribution de l'exposition professionnelle ne doit donc pas être ignorée et doit inciter à la mise en place de mesures de prévention collective et individuelle en milieu de travail accompagnée d'un sevrage tabagique le cas échéant.

- L'exposition combinée au tabagisme et à des agents professionnels (poussières biologiques, minérales, vapeurs, gaz, poussières et/ou fumées) a un effet additif voire synergique sur le risque de développement d'une BPCO et de la mortalité induite par la BPCO.

## Risque cardiovasculaire

Le rôle respectif de la BPCO et du tabagisme dans la genèse du risque cardiovasculaire reste débattu. L'évaluation du risque cardiovasculaire reste néanmoins confidentielle dans les BPCO professionnelles bien qu'elle permettrait d'estimer

ce risque indépendamment du tabagisme. Claudé et al. ont étudié ce risque via l'étude de la rigidité artérielle par mesure de l'onde de pouls chez des patients BPCO fumeurs et non fumeurs comparativement à des sujets témoins [60]. Le tabagisme semblait jouer un rôle dans le risque cardiovasculaire de la BPCO, au moins en partie via une anomalie des rapports ventilation/perfusion induisant une hypoxie intermittente d'effort. Ces résultats restent néanmoins à confirmer dans une population plus importante. Paulin et al. quant à eux, ne mettaient pas en évidence de différence sur les comorbidités cardiovasculaires chez les sujets présentant une BPCO ayant ou non une exposition professionnelle [27].

- Le rôle respectif de la BPCO et de l'exposition professionnelle reste à définir dans le risque cardiovasculaire.

## Conclusion

L'exposition professionnelle contribue pour une part non négligeable dans la prévalence de la BPCO et ce d'autant plus chez les sujets non tabagiques. Les BPCO professionnelles sont associées à un excès de symptômes et à un déclin accéléré de la fonction respiratoire qui reste néanmoins de plus faible magnitude que celle de la BPCO post-tabagique. Il existe par ailleurs un effet additif entre l'exposition professionnelle et le tabagisme. La physiopathologie des BPCO professionnelles reste mal connue bien que des mécanismes immuno-allergiques aient été suggérés. Les effets et le lien de causalité entre les expositions professionnelles et la BPCO sont maintenant clairement établis, et il est donc temps de mettre l'accent sur le devenir, les phénotypes et les mécanismes des BPCO d'origine professionnelle comme cela a pu être fait dans d'autres études cliniques sur la BPCO tabagique [61].

Une étude prospective en milieu agricole menée par notre équipe visant à caractériser cette « autre BPCO » devrait permettre de répondre à certaines de ces interrogations [62]. Aucune étude ne s'est pour l'instant intéressée aux traitements médicamenteux des BPCO professionnelles. En l'absence de données, le traitement de ces BPCO est calqué sur celui des BPCO post-tabagiques. Néanmoins, la mise en évidence de mécanismes immuno-allergiques propres aux BPCO professionnelles devrait modifier les pratiques thérapeutiques dans ce domaine, notamment en faveur de traitements immuno-modulateurs ciblés. La mise en place de registres voire d'études prospectives devrait aider à répondre à ces questions thérapeutiques.

Enfin, bien que des campagnes de dépistage dans des secteurs professionnels ciblés aient vu le jour, la BPCO reste largement sous diagnostiquée. La mise en place de mesures visant à un diagnostic précoce ainsi que des campagnes de d'éducation et de prévention de cette pathologie en milieu professionnel accompagné d'un sevrage tabagique le cas échéant, doivent devenir une priorité.

### Points essentiels

- Bien que longtemps méconnue, il est maintenant communément admis que l'exposition professionnelle contribue pour 15 % dans le poids global de la BPCO, voire 40 % chez les sujets non fumeurs.
- L'exposition aux vapeurs, gaz, poussières et/ou fumées, aux poussières organiques et inorganiques ont été continuellement associées à un risque accru de BPCO.
- Il existe un excès de symptômes et un déclin de la fonction respiratoire plus important chez les patients BPCO présentant une exposition professionnelle.
- La mortalité reste néanmoins de plus faible magnitude que chez les patients atteints de BPCO post-tabagiques.
- Le tabagisme et l'exposition professionnelle ont un effet combiné et additif voire synergique sur ces différents paramètres.

## Déclaration de liens d'intérêts

Au cours des 5 dernières années, J.C. Dalphin a perçu des honoraires ou financements de la part de Novartis Pharma, Chiesi, GSK, Boehringer Ingelheim, Intermune, AstraZeneca Participation à un congrès Communications Travaux de recherche Participation à des groupes d'experts.

Thibaud Soumagne, Denis Caillaud, Bruno Degano déclarent ne pas avoir de liens d'intérêts.

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## 2. Article 2: COPD, airflow limitation and chronic bronchitis in farmers: a systematic review and meta-analysis.

### *a. Contexte et objectif*

La fumée du tabac, responsable d'environ 80% des BPCO actuellement, est le principal facteur de risque identifié de la BPCO. Cependant, plusieurs études ont mis en évidence une prévalence de BPCO anormalement élevée dans des populations de sujets non-fumeurs. Parmi les facteurs de risque associés au développement de la BPCO dans ces populations étaient retrouvés : une prédisposition génétique, le statut socio-économique, la pollution (intérieure et extérieure), les antécédents de maladies respiratoires, mais également des expositions environnementales et professionnelles.

Plusieurs secteurs professionnels comme l'extraction (mines, travaux publics, puits...), la boulangerie, la cimenterie, le travail du bois et les professions agricoles ont été démontré comme présentant un risque accru de BPCO, et cela après ajustement sur les facteurs de confusion comme le tabagisme. Le milieu agricole fait d'ailleurs l'objet de nombreuses études épidémiologiques depuis les années 90. Cependant, les prévalences rapportées sont très variables d'une étude à l'autre.

Cette revue systématique avait pour but de recenser tous les articles rapportant une prévalence de BPCO, de TVO et/ou de bronchite chronique dans un groupe de sujets ayant une activité agricole, et la comparant à un groupe de témoins sans exposition agricole. Une méta-analyse était ensuite réalisée afin d'obtenir une prévalence globale selon le type d'activité agricole exercé et selon le type de critère diagnostique utilisé.

### *b. Manuscrit*

Article "COPD, airflow limitation and chronic bronchitis in farmers: a systematic review and meta-analysis". Guillien A, Soumagne T, Dalphin JC, Degano B. Occupational and Environmental Medicine 2019.
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# COPD, airflow limitation and chronic bronchitis in farmers: a systematic review and meta-analysis

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## ABSTRACT

**Introduction** The current definition of chronic obstructive pulmonary disease (COPD) associates persistent airflow limitation and chronic respiratory symptoms. Agricultural work has been associated with an increased risk of developing COPD, but the prevalence and definition of the disease vary greatly between studies. This meta-analysis aimed to assess the association between agricultural work and COPD using the most widely used definitions of the disease.

**Methods** Inclusion criteria were: (1) design: cross-sectional or longitudinal, (2) groups: at least one group of farmers and a control group of non-farmers, (3) outcome: prevalence or unadjusted OR of COPD, airflow limitation and/or chronic bronchitis, (4) study subjects: groups of exposed subjects comprising  $\geq 30$  individuals and with a mean age  $\geq 40$  years and (5) language: English and French language, full-length, original publications in peer-reviewed journals.

**Results** In total, 22 manuscripts were included in the meta-analysis. Eight studies assessed only the prevalence of airflow limitation, nine assessed only the prevalence of chronic bronchitis and four assessed the prevalence of both these parameters. Only one assessed the prevalence of COPD according to its current definition, and this study also provided the prevalence of airflow limitation. Ten studies showed a positive association between farming exposure and airflow limitation or chronic bronchitis, and 12 showed no association (OR (95% CI)=1.77 (1.50 to 2.08),  $p < 0.001$ ). Cattle, swine, poultry and crop farming were associated with either airflow limitation or chronic bronchitis.

**Conclusion** Although some features of COPD are associated with some agricultural work, well-designed studies with appropriate diagnostic criteria should be conducted to draw strong conclusions about the relationship between COPD and farming.

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common disease with high global morbidity and mortality.<sup>1</sup> COPD affects between 2.5% and 19% of subjects aged 40 years or more depending on the definition of the disease and on the country,<sup>2,3</sup> and is now the third leading cause of mortality, accounting for 3 million deaths in 2010.<sup>4</sup> The last Global Initiative for Chronic Obstructive Lung Disease (GOLD) statement defines COPD as the association of persistent airflow limitation (postbronchodilator (post-BD) forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio  $< 0.70$ ) with chronic respiratory symptoms (chronic dyspnoea, chronic cough, chronic wheeze and/or chronic sputum) in subjects with a history of exposure to risk factors for the disease.<sup>5</sup> As such,

## Key messages

### What is already known about this subject?

- Agriculture and farming have been associated with increased prevalence of chronic obstructive pulmonary disease (COPD).
- This association varies from one agricultural job to another, and data for some agricultural jobs are conflicting.

### What are the new findings?

- Only a few epidemiological studies use the appropriate definition of COPD for studying the relationship between farming and COPD.
- This meta-analysis shows that cattle farming, swine farming and poultry farming are strongly associated with airflow limitation and chronic bronchitis.
- Crop/grain farmers have also a higher prevalence of airflow limitation and of chronic bronchitis compared with unexposed controls.

### How might this impact on policy or clinical practice in the foreseeable future?

- New epidemiological studies investigating different farming groups and using the appropriate definition of COPD are needed.

COPD may be regarded as a syndrome with several underlying mechanisms and aetiologies.<sup>6</sup> Although the most frequent aetiological factor for COPD in developed countries is tobacco smoking, some studies strongly support the association between several workplace exposures and COPD, with a global population-attributable occupational risk for COPD estimated at 15%–20%.<sup>7,8</sup>

Beside mining and some non-mining industrial sectors including welding,<sup>9</sup> agriculture and farming are among the occupations that have been associated with increased prevalence of COPD.<sup>9,10</sup> Nevertheless, this association varies from one agricultural job to another, and data for some agricultural jobs are conflicting. There are at least three possible reasons for these conflicting results. First, agricultural and farming jobs cover a wide spectrum of activities, and for a given farming job, occupational exposures are dictated by the size and type of the farm, differing between traditional family farms and large and modern farm businesses.<sup>11,12</sup> Furthermore, recent data suggest that a given farming job (eg, cattle raising) may lead to different exposures depending on the region of exercise.<sup>2</sup> Second, the definition of COPD varies widely between studies: in general, older studies use only clinical criteria of chronic bronchitis to define COPD, and only more



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recent ones use spirometric criteria of airflow limitation (either pre-BD or post-BD).<sup>13</sup> Notably, the definition of airflow limitation may also vary between studies, depending on the cut-off used to define an abnormally low FEV1/FVC ratio.<sup>14</sup> Thirdly, results may depend on study designs and on methods of population sampling. For example, several cross-sectional studies found that farming jobs exposing workers to organic dusts were associated with increased prevalence of COPD,<sup>15 16</sup> whereas a recent meta-analysis including only longitudinal studies concluded that there was limited evidence for a causal association between occupational exposure to organic dust and decline of lung function.<sup>17</sup> In addition, many studies did not include a control group of subjects unexposed to any agricultural environment, which may also lead to some bias.

From a workplace health perspective in the agricultural setting, it is very important to identify those jobs that are associated with increased prevalence of COPD. Identification will help to organise both primary and secondary prevention for workers employed in jobs identified as 'at risk' of COPD. In view of current knowledge regarding the relationship between COPD and agriculture, we believe that there is a need for a systematic review and meta-analysis aiming to study the association between agricultural work and COPD, taking into account the various definitions of COPD used in the available literature.

## METHODS

### Search strategy

The literature search of published population-based studies was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), as currently recommended.<sup>18</sup> NCBI PubMed, Embase and the Cochrane Library were searched for English and French-language peer-reviewed publications from January 1980 to July 2018. The research equation was constructed using the PubMed Search Builder and was then translated into Embase and Cochrane languages. The terms used to identify COPD were: 'chronic obstructive disease', 'COPD', 'chronic airway disease', 'airway obstruction', 'chronic respiratory disease' and 'lung function'. The terms used to identify agricultural employment and associated exposures were: 'agriculture', 'farm', 'occupational exposure', 'rural health' and 'dust'.

Once the list of articles was available, duplicates were eliminated and the relevant studies were identified by reading first the title, then the abstract and finally the full articles.

### Criteria for study selection

The criteria for study selection were the following: (1) design: either cross-sectional or longitudinal. When more than one article analysed the same population (for longitudinal cohorts), only the last published article was retained in the final analysis; (2) groups: at least one group of farmers (a priori considered as the exposed group) and a control group. The control groups had to comprise subjects who worked and/or had worked only in non-agricultural jobs and who had had no occupational exposure of any kind. The exposed group had to be composed of subjects who reported at least one farming activity; (3) outcome: prevalence or unadjusted OR of COPD, airflow limitation and/or chronic bronchitis. Prevalence for both exposed and control groups and/or unadjusted ORs had to be clearly presented in the Results sections of the articles. As most articles that report data on airflow limitation in farmers use the GOLD criterion (FEV1/FVC<0.70), when an article presented prevalence of airflow limitation with both the GOLD and the American

Thoracic Society/European Respiratory Society (ATS/ERS) (FEV1/FVC<lower limit of normal (LLN)) criteria, we used only the prevalence defined with the GOLD criterion; (4) study subjects: groups of exposed subjects composed of at least 30 individuals and a mean age of at least 40 years (in order to avoid the risk of confusion between COPD and asthma); (5) language: English and French language, full-length, original publications in peer-reviewed journals.

The quality of the included studies was assessed using the Newcastle-Ottawa Quality Assessment Scale for cohort and cross-sectional studies.<sup>19</sup>

For all analyses, the item 'airflow limitation' was retained according to spirometric criteria (either GOLD or ATS/ERS); the item 'chronic bronchitis' was defined by the presence of chronic cough and sputum production for at least 3 months a year for two consecutive years; COPD was a priori defined by the association of persistent airflow limitation with chronic respiratory symptoms (chronic dyspnoea, chronic cough, chronic wheeze and/or chronic sputum). Prevalences of airflow limitation, chronic bronchitis or COPD were collected for farmers and controls, and ORs for each eligible study were computed.

We found only one article in which COPD was defined as the association of persistent airflow limitation with chronic respiratory symptoms.<sup>20</sup> In all other selected articles, COPD was defined either according to a spirometric criterion (airflow limitation, either pre-BD or post-BD) or according to a clinical criterion (chronic bronchitis). In some articles, both prevalence of airflow limitation and prevalence of chronic bronchitis were given, but not the prevalence of the combination of persistent airflow limitation with chronic bronchitis.

We performed a first analysis assessing the OR of either COPD or airflow limitation or chronic bronchitis in farmers compared with non-exposed subjects. For this first analysis, in studies providing prevalence of airflow limitation and of chronic bronchitis, only the prevalence of airflow limitation was used, and for the study who provided both prevalence of COPD and airflow limitation, we used the prevalence of COPD. We performed a second and a third analysis assessing the ORs of airflow limitation and of chronic bronchitis in farmers compared with non-exposed control subjects, respectively. These three analyses were displayed using forest plots. Pooled ORs were computed using random effect models. For each analysis, sensitivity analyses were performed for the following types of activities: cattle farmers, poultry farmers, swine farmers, livestock farmers (this term was retained for studies where no details on the animals raised were provided or for studies where farmers raised several types of animals), crop/grain farmers (including farmers exposed to crop protection products) and other farmers (for studies where farming activities were not specified or where subjects had multiple farming activities). As only one study provided prevalence of COPD according to the GOLD definition, this result was not used for the sensitivity analyses.

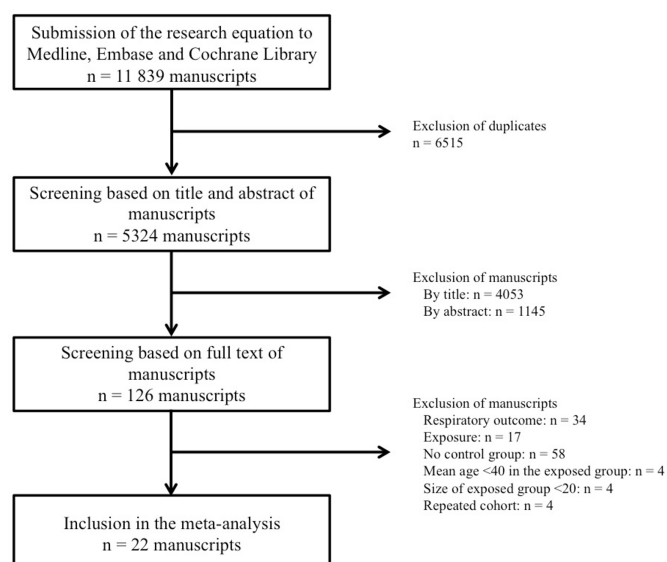
We assessed heterogeneity between studies using Higgins I<sup>2</sup> statistics and publication bias by funnel plots.<sup>21 22</sup>

All analyses were performed using Review Manager V.5.3 software.

## RESULTS

### Study selection

Submission of the research equation to the three databases (Medline, Embase and the Cochrane Library) identified 11 839 manuscripts. After exclusion of 6515 duplicates, the 5324 remaining publications were screened based on title and abstract,



**Figure 1** Flow chart of the studies included.

leading to exclusion of 4053 based on their title and 1145 based on their abstract. Based on the full-text review of the remaining 126 articles and on the inclusion criteria applied, 22 manuscripts were included in the final meta-analysis: eight studies assessed only the prevalence of airflow limitation, nine assessed only the prevalence of chronic bronchitis and four assessed the prevalence of both these parameters. Only one assessed the prevalence of COPD according to its current definition, and this study also provided the prevalence of airflow limitation (figure 1).

### Study characteristics

The main characteristics of the 22 included manuscripts are presented in table 1 in chronological order and the quality of each is assessed in online supplementary table 1. Publication bias of each analysis is presented in online supplementary figure 1 and sensitivity analysis by quality score is presented in online supplementary figure 2. The mean $\pm$ SD year of publication of the 22 included studies was 2004 $\pm$ 11, ranging from 1986 to 2017. Studies in which COPD was defined as chronic bronchitis were published much earlier than those defining COPD as airflow limitation (1998 $\pm$ 10 vs 2009 $\pm$ 9, respectively;  $p=0.01$ ). The majority of studies were performed in Europe ( $n=14$ ; 64% of the total), while others were performed in the USA ( $n=3$ ), Canada ( $n=2$ ), Nigeria ( $n=1$ ), India ( $n=1$ ) and New Zealand ( $n=1$ ).

Sample sizes of the included studies ranged from 77 to 34 616 subjects, resulting in a total sample size of 63 572 subjects. Seven studies had a group of cattle farmers,<sup>2 20 23–27</sup> 4 had a group of swine farmers,<sup>2 28–30</sup> 2 had a group of poultry farmers,<sup>2 31</sup> 2 had a group of livestock farmers,<sup>2 32</sup> 6 had a group of crop/grain farmers or farmers exposed to crop protection products<sup>2 20 30 33–35</sup> and 10 had a group of mixed farming activities or gave no detail about farming activities.<sup>2 25 27 36–42</sup> All studies had a control group of subjects unexposed to any farming activity, the size of these control groups ranging from 39 to 31 935.

### Prevalence of COPD defined either by airflow limitation or by chronic bronchitis

After pooling of the 22 studies, the prevalence of COPD defined either by airflow limitation or by chronic bronchitis was 7.5% (948/12 702) among farmers and 2.3% (1149/50 870) among

controls (OR (95% CI)=1.77 (1.50 to 2.08),  $p<0.001$ ). Ten studies showed a positive association between farming exposure and presence of COPD/airflow limitation/chronic bronchitis, and 12 showed no association (see online supplementary figure 3).

COPD defined by airflow limitation/chronic bronchitis was significantly associated with the following: cattle farmers, swine farmers, poultry farmers, crop farmers and mixed groups of farmers, with ORs (95% CI) ranging from 1.59 (1.30 to 1.95) to 2.64 (1.95 to 3.58) (figure 2). In contrast, livestock farming were not associated with either persistent airflow limitation or chronic bronchitis (figure 2).

Overall, heterogeneity between the 22 studies, assessed by  $I^2$ , was high (44%;  $p=0.01$ ), but was significant only for studies dealing with livestock farmers ( $I^2=77%$ ,  $p=0.04$ ).

### Prevalence of COPD defined by airflow limitation

In the 13 studies that defined COPD according to spirometric criteria, the following different definitions of airflow limitation were used: post-BD FEV1/FVC<0.70 in three studies<sup>20 26 41</sup>; post-BD FEV1/FVC<LLN in two studies<sup>34 42</sup>; pre-BD FEV1/FVC<0.70 in two studies<sup>31 33</sup>; pre-BD FEV1/FVC<0.70 and FEV1<80% of the predicted value in one study<sup>40</sup>; post-BD FEV1/FVC<90% of the predicted value in one study<sup>24</sup>; pre-BD FEV1<85% of the predicted value in one study.<sup>28</sup> Moreover, two studies gave no details of the spirometric definition of COPD,<sup>35 38</sup> and one study used two different definitions of COPD (post-BD FEV1/FVC<0.70 and post-BD FEV1/FVC<LLN).<sup>2</sup>

The prevalence of airflow limitation was 8.5% (579/6799) among farmers and 4.0% (602/14 981) among controls ( $p<0.001$ ). The pooled OR of these 13 studies was statistically significant (OR (95% CI)=1.82 (1.53 to 2.16),  $p<0.001$ ) and heterogeneity between studies was relatively low ( $I^2=18%$ ,  $p=0.26$ ) (see online supplementary figure 4).

All categories of farming activities had increased risk of airflow limitation in comparison with non-exposed controls: OR (95% CI) ranged from 1.70 (1.34 to 2.15) in mixed groups of farmers to 2.22 (1.39 to 3.56) in swine farmers (figure 3).

### Prevalence of COPD defined by chronic bronchitis

A total of 13 included studies defined COPD as chronic bronchitis, with the following definitions for chronic bronchitis: cough and/or expectoration/phlegm/sputum for 3 months of the year or more for at least two consecutive years in 11 studies<sup>23–25 27–30 33 34 37 39</sup>; self-reported doctor-diagnosed chronic bronchitis<sup>32</sup>; one study gave no details of the definition of chronic bronchitis.<sup>36</sup>

The prevalence of chronic bronchitis was 7.3% (497/6815) among farmers and 1.7% (612/37 027) among controls (OR (95% CI)=1.94 (1.46 to 2.56),  $p<0.001$ ). Heterogeneity between these 13 studies was  $I^2=65%$  ( $p<0.001$ ) (see online supplementary figure 5).

Among the different farming activities, exposure was significantly associated with chronic bronchitis in cattle farmers, swine farmers and crop/grain farmers (OR (95% CI)=2.38 (1.75 to 3.25), 2.62 (1.86 to 3.70), 2.41 (1.29 to 4.47), respectively) but not in mixed groups of farmers (figure 4).

The great variability that exists from one epidemiological study to another regarding the definition of COPD in farmers may lead to some misinterpretation of the relationship between farming and risk of the disease. We present for the first time a systematic review and meta-analysis that takes into account these different definitions of COPD used in the literature. We were

**Table 1** Description of studies included in this meta-analysis

Study	Study design	Studied sample size* (exposed–controls)	Age (year)	Smoking habits	Type of exposure	Description of controls	AL		CB prevalence among controls (%)	
							Prevalence among exposed (%)	Prevalence among controls (%)		
Milosevic <sup>25</sup> 1986	Cross-sectional	824 (311–513)	MD	Non-smokers (%): farmers=36 controls=36	Farmers and cattle farmers	Tractor drivers and crabmen	–	–	Farmers=36 Cattle farmers=39	22
Dosman et al <sup>26</sup> 1988	Cross-sectional	376 (183–193)	Mean±SD: farmers=42.2±12.9 controls=39.7 ± 12.9	Mean pack-years:SD: farmers=13.6±16.6 controls=13.2±15.8	Swine farmers	Non-farming rural-dwelling	17.5	7.8	13.1	7.6
Dalphin et al <sup>27</sup> 1989	Cross-sectional	500 (250–250)	Mean±SD: farmers=40.07±9.38 controls=40.16±8.99	Never-smokers (%): farmers=60 controls=60	Dairy farmers	Administrative employees	–	–	12	6
Heederik et al <sup>28</sup> 1990	Longitudinal	399 (30–369)	Range: 40–89	MD	Farmers	White collar workers	33.3	21.7	–	–
Zejda et al <sup>29</sup> 1993	Cross-sectional	763 (500–263)	Mean±SD: farmers=41.2±11.7 controls=40.7 ± 11.2	Never-smokers (%): farmers=56 controls=43	Swine farmers and grain farmers	Non-farming subjects	–	–	Swine farmers=15.3, grain farmers=7.2	5.7
Brackbill et al <sup>30</sup> 1994	Cross-sectional	34 616 (2 681–31 935)	Mean age of farmers=44.3	MD	Farmers	White collar workers	–	–	0.34	0.54
Dalphin et al <sup>31</sup> 1998	Cross-sectional	414 (265–149)	Mean±SD: farmers=45.9±11.3 controls=37.4±10.3	Never-smokers (%): farmers=58 controls=48	Dairy farmers	Non-exposed administrative workers	21.2	14.5	6.4	0.7
Vogelzang et al <sup>32</sup> 1999	Cross-sectional	550 (239–311)	Mean: farmers=41.0 controls=41.9	Mean pack-years: farmers=6.8 controls=9.1	Swine farmers	Non-agricultural workers	–	–	20.2	7.7
Danuser et al <sup>33</sup> 2001	Cross-sectional	1902 (442–1460)	Mean±SD: farmers=48.9±13.0 controls=MD	Never-smokers (%): farmers=58 controls=MD	Farmers	Swiss population	–	–	12.0	6.8
Hnizdo et al <sup>34</sup> 2002	Cross-sectional	2869 (659–2277)	Range: 30–75	Total population (n=982): men=47%	Farmers	Office workers	10.1	5.2	–	–
Lamprecht et al <sup>35</sup> 2007	Cross-sectional	1258 (288–970)	Median (range): farmers=59 (40–90) controls: 56 (40–98)	Never-smokers (%): farmers=54 controls=45	Farmers	No farming	30.2	20.1	–	–
Sigurdsson et al <sup>36</sup> 2008	Cross-sectional	1796 (1107–689)	>50: farmers=51%, controls=39%	Current smokers (%): farmers=13 controls=20	Livestock farmers	General population	–	–	3.9	4.3
Chakraborty et al <sup>37</sup> 2009	Cross-sectional	724 (376–348)	Median (QR): farmers=41 (24–52) controls=40 (25–43)	All non-smokers	Crop workers	Individuals not engaged in agriculture	18.1	6.9	19.1	6.0
Rimac et al <sup>38</sup> 2010	Cross-sectional	86 (41–45)	Mean±SD: farmers=46.0±8.7 controls=44.9±8.7	Non-smokers (%): farmers=49 controls=62	Poultry farmers	Office workers	7.89	2.56	–	–
Thaon et al <sup>39</sup> 2017	Longitudinal	448 (349–99)	Mean±SD: dairy farmers=58.0±11.1 non-dairy agricultural workers=52.0 ± 11.2	Never-smokers (%): farmers=63 controls=53	Dairy farmers and non-dairy agricultural workers	Administrative employees from agricultural companies	Dairy farmers=3.8; non-dairy agricultural workers=2.3	1.0	–	–
Hansell et al <sup>40</sup> 2014	Cross-sectional	AL: 391 (71–320) CB: 537 (89–448)	Total population (n=1077): 53.2±12.9	Total population: never-smokers=52%	Crop farmers	Subjects with no exposure to VGDF	11.3	10.3	14.6	6.0
Stolecki et al <sup>41</sup> 2015	Cross-sectional	150 (75–75)	Mean±SD: farmers=51.4±7.3 controls=52.7±7.6	Male/female ratio: farmers=1.4 controls=1.3	Crop farmers	Office workers	16.0	6.7	–	–
Stolecki et al <sup>42</sup> 2015a	Cross-sectional	104 (52–52)	Mean±SD: farmers=49.4±5.1 controls=48.7±4.9	Never-smokers only	Dairy farmers	Office workers	10.7	2.7	–	–
Earle-Richardson et al <sup>43</sup> 2015	Cross-sectional	9612 (636–9076)	Median: farmers=55.2 controls=51.2	OR (95% CI) of current smoking (farmers vs controls)=0.60 (0.40–0.89)	Farmers	Rural non-farmers	1.87	1.32	–	–

continued

Table 1 continued

Study	Study design	Studied sample size* (exposed–controls)	Age (year)	Gender	Smoking habits	Type of exposure	Description of controls	AL		CB prevalence among controls (%)
								Prevalence among exposed (%)	Prevalence among controls (%)	
Guillien <i>et al</i> <sup>4</sup> 2016	Cross-sectional	4704 (917–3787)	Farmers: 40–49=32% 50–59=31% >60=37% Controls: 40–49=34% 50–59=31% >60=35%	Men (%): farmers=63 controls=46	Never-smokers (%): farmers=88 controls=59	Cattle farmers, swine farmers, poultry farmers, livestock farmers, crop farmers and mixed groups of farmers	Subjects who had worked only in non-agricultural jobs with no known exposure	Farmers=5.12, cattle farmers=4.82, swine farmers=5.98, poultry farmers=5.73, livestock farmers=5.09, crop farmers=2.92, mixed groups of farmers=6.12	2.94	–
Obaseki <i>et al</i> <sup>45</sup> 2016	Cross-sectional	875 (386–489)	Total: 40–49=37% 50–59=26% 60–69=24% ≥70=12%	Total: men=39%	Total: never-smokers=90%	Farmers	Subjects never engaged in farming	7.7	7.7	–
Stoleski <i>et al</i> <sup>46</sup> 2017	Cross-sectional	250 (170–80)	Means±SD, crop farmers=53.4±7.8 dairy farmers=52.6±8.7 controls=52.7±8.2	Male/female ratio: crop farmers: 3.6 dairy farmers: 2.6 controls: 2.7	Active smokers: crop farmers=51.7% dairy farmers=46.9% controls=48.7%	Crop farmers and dairy farmers	Office controls	COPD: crop farmers=6.9; dairy farmers=8.4; AL: crop farmers=8.1; dairy farmers=8.4	COPD: 3.8; AL: 5	–

\*Subjects not belonging to the exposed group or the control group were not included in the sample size.

AL, airflow limitation; CB, chronic bronchitis; COPD, chronic obstructive pulmonary disease; MD, missing data; VGD, vapours, gases, dusts or fumes.

unable to find a single study that used the current appropriate definition of COPD for investigating this question. We found that most studies published during the 80s and the 90s used only clinical criteria to define COPD, while most recent studies used only spirometric criteria. Overall, we found a significant association between the presence of at least one criterion of COPD (either clinical criteria or spirometric criteria) and some farming activities (cattle farmers, swine farmers and poultry farmers). This finding is based on results that were homogeneous from one study to another. In contrast, we found discordant results depending on the definition of COPD for crop/grain farmers and for mixed groups of farmers. Finally, we found that livestock farmers (farmers who raised multiple types of animals or farmers who raised unspecified animal types) had the same prevalence of chronic bronchitis and of airflow limitation as unexposed controls.

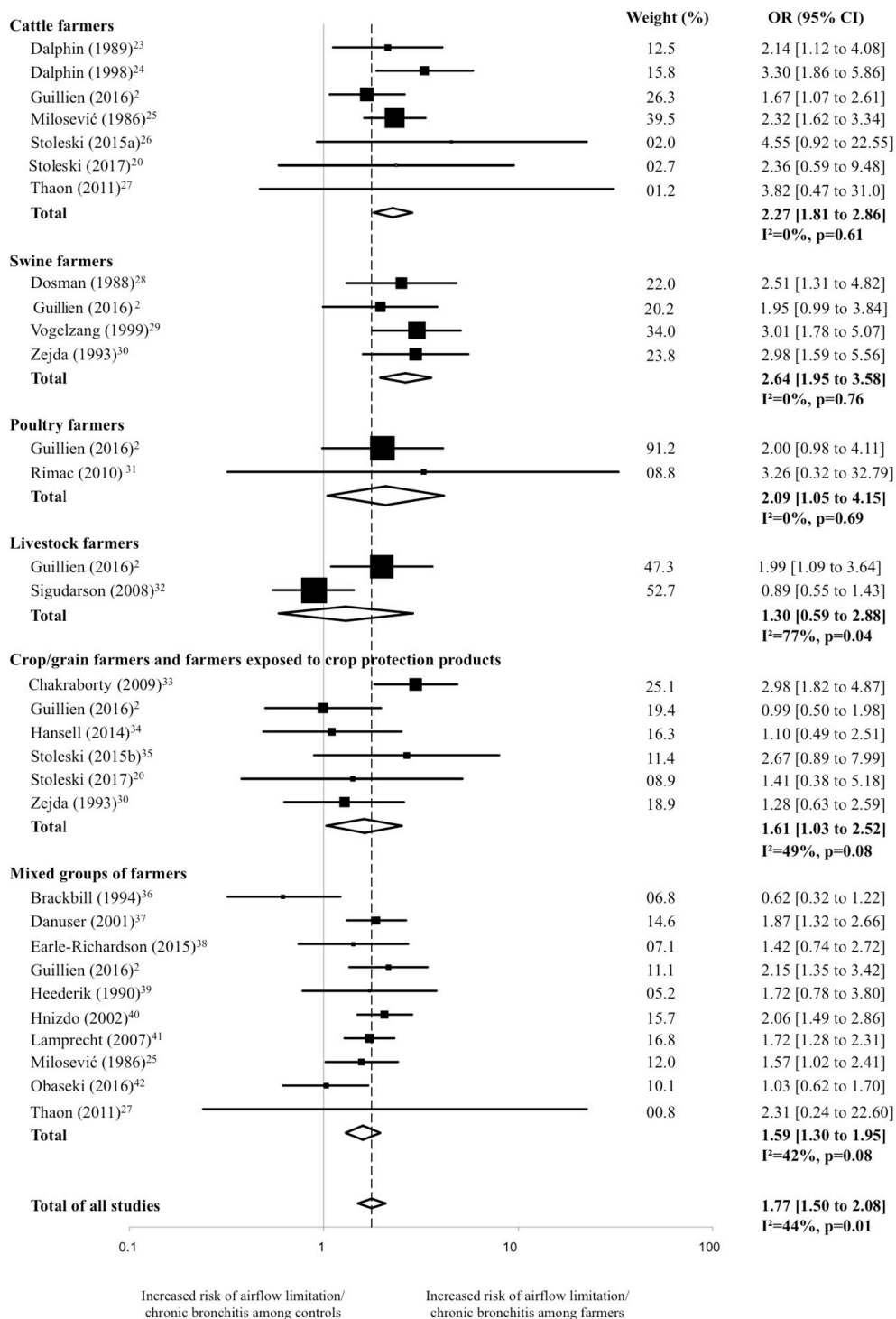
## DISCUSSION

### Definitions of COPD

Our results highlight the fact that one of the challenges faced in interpreting the currently available literature investigating the relationships between farming and COPD relates to the definitions of the disease. As mentioned above, the most recent GOLD statement defines COPD as the association of persistent airflow limitation (assessed by a post-BD FEV1/FVC ratio < 0.70) with at least one of the following respiratory symptoms: dyspnoea, chronic cough and/or chronic sputum production.<sup>5</sup> To the best of our knowledge, only one of the currently published studies carried out in farmers used this definition.<sup>20</sup> Many studies used an exclusive clinical criterion of chronic bronchitis to define COPD. Unfortunately, it is well established that the main COPD symptoms have poor specificity for the diagnosis of persistent airflow limitation.<sup>43</sup> In agreement with this statement, we have recently shown that the items ‘chronic cough’ and ‘chronic sputum’ were not discriminant enough to be retained in a questionnaire aiming to screen farmers for persistent airflow limitation.<sup>44</sup> Equally, persistent airflow limitation may develop without any history of the main COPD symptoms.<sup>5</sup> The reasons for this are multiple. Some subjects with persistent airflow limitation may be truly asymptomatic if they adopt a sedentary lifestyle, while others may attribute progressively increasing symptoms to growing older.<sup>45</sup> In other words, it is likely that a purely clinical definition of COPD, as used in about half the studies included here, is neither specific nor sensitive for the diagnosis of COPD according to the current definition of the disease.

Although persistent (ie, post-BD) airflow limitation is a prerequisite for the diagnosis of COPD, only a minority of studies defining COPD with a spirometric criterion included a post-BD test. This is another major limitation of the available literature. A BD test is necessary to distinguish COPD from asthma, particularly among younger adults.<sup>46–47</sup> In addition, some subjects with a pre-BD FEV1/FVC ratio above 0.70 and/or below LLN may have a post-BD value under these thresholds due to an increase in FVC without any further change in FEV1 with BDs.<sup>48</sup>

Another factor that may influence the epidemiology of COPD in farmers is related to the definition of airflow limitation used in the literature in terms of cut-off for the FEV1/FVC ratio. It is obvious that the fixed cut-off of 0.70 that is recommended by the GOLD committee may be regarded as inappropriate as it underestimates prevalence of airflow limitation in younger people (age 45 years or less) and



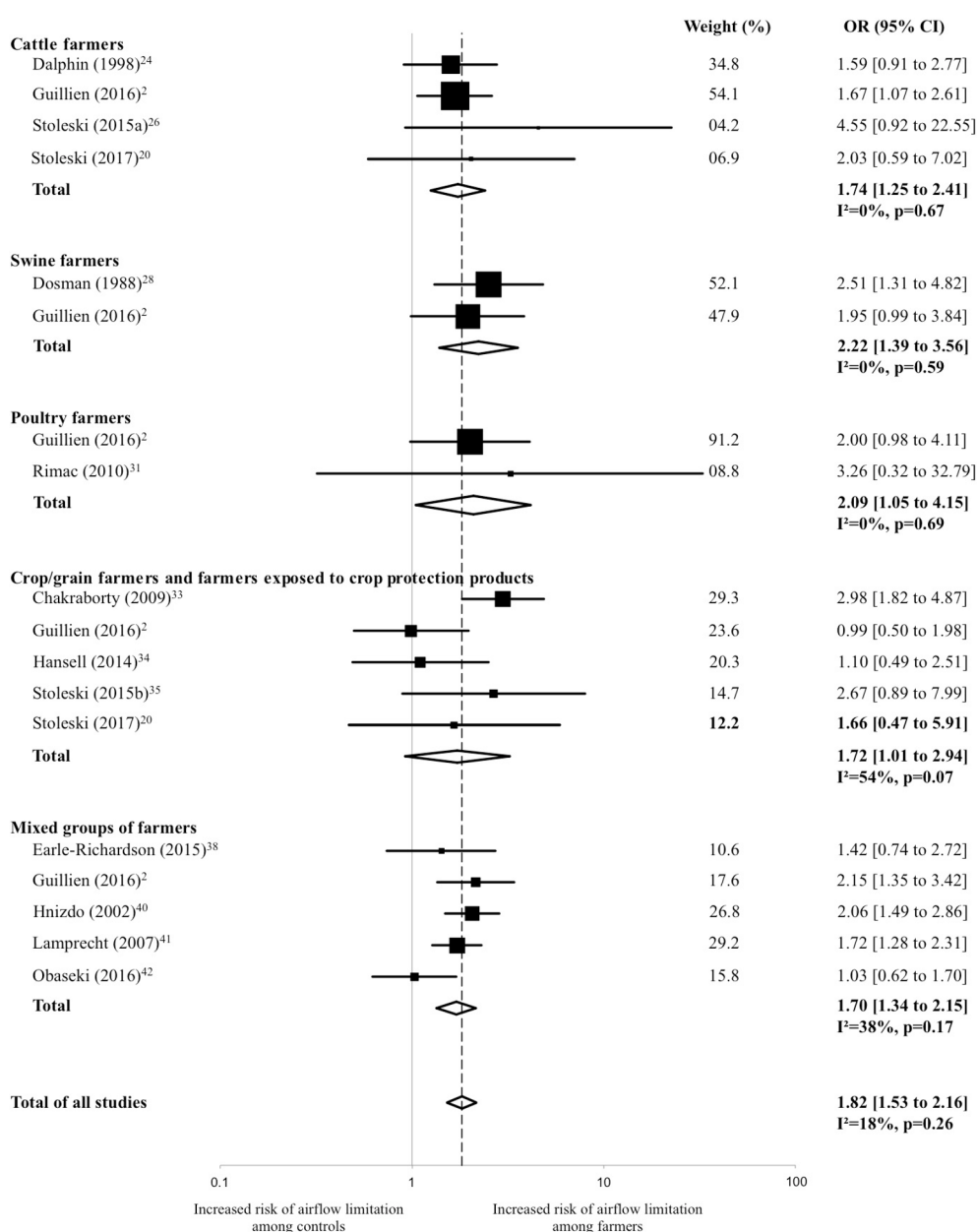
**Figure 2** Risk of chronic obstructive pulmonary disease/airflow limitation/chronic bronchitis according to farm activity.

overestimates prevalence in older people.<sup>14 49</sup> In the current review and meta-analysis, we included only subjects aged 40 years or older. It is therefore likely that studies that used the fixed cut-off of 0.70 overestimated the true prevalence of airflow limitation, as the true prevalence is better reflected by the age-dependent LLN cut-off.<sup>49</sup>

For all the above reasons, we need to keep in mind that the epidemiology of COPD in farmers is currently impossible to determine, and only the prevalence/risk of either clinical or spirometric patterns that may be associated with COPD can be determined with the current meta-analysis.

### Respiratory outcomes and farming activities

The increased prevalence of chronic bronchitis and of airflow limitation found in our meta-analysis in farmers raising cattle, swine or poultry is consistent with pathophysiological considerations. Despite a lower prevalence of asthma and allergic rhinitis reported in these populations,<sup>50 51</sup> farmers working inside confined buildings, especially poultry and swine workers, are exposed to indoor air contaminants (carbon dioxide, ammonia, dust and endotoxin) which are determinants of COPD traits, with a clear dose-response relationship with the number of hours



**Figure 3** Risk of airflow limitation according to farm activity.

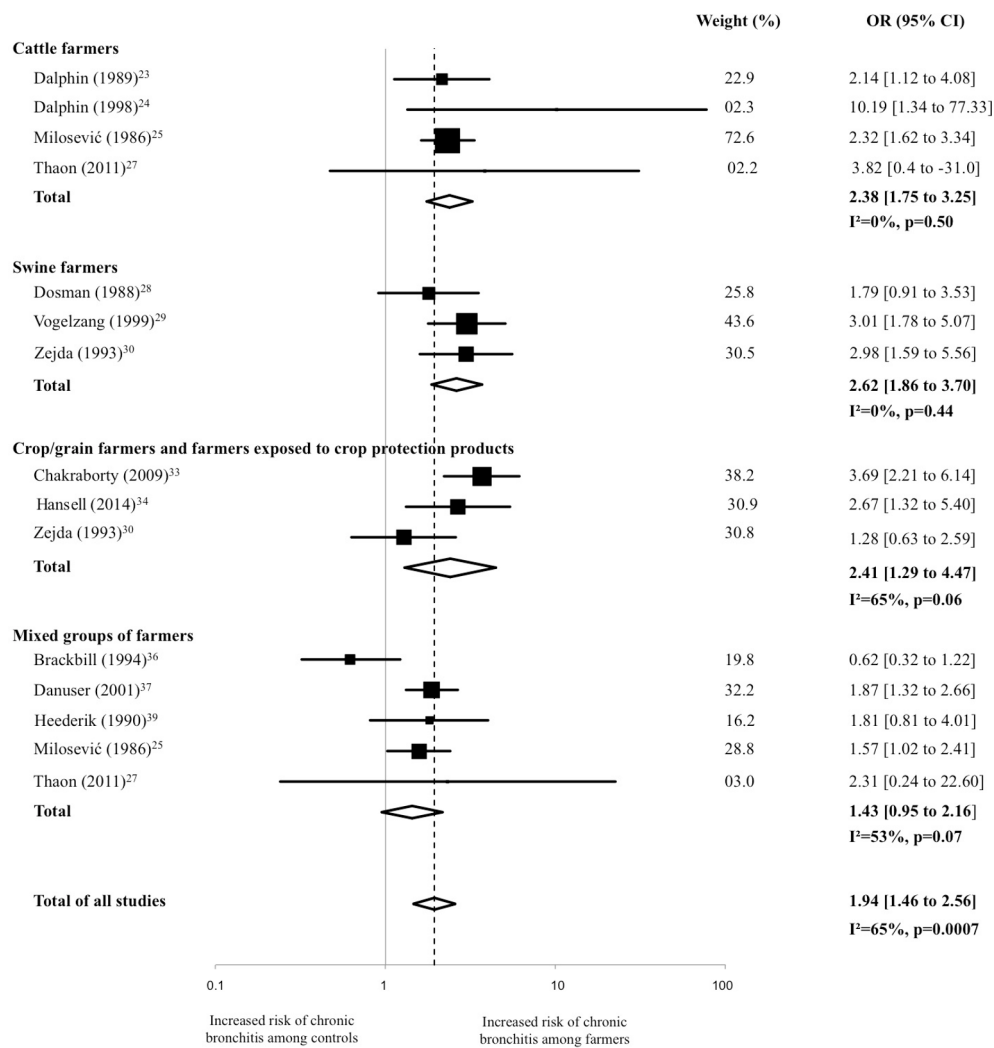
working inside the buildings.<sup>52-53</sup> For example, in a longitudinal study investigating 63 poultry workers, Guillam *et al* concluded that exposure to only 0.1 mg/m<sup>3</sup> of respirable dust present in poultry houses induces a fourfold increase in the risk of chronic bronchitis.<sup>54</sup> Moreover, a Danish study comparing decline of lung function between swine workers and dairy farmers showed that working in swine-confinement buildings causes a mean annual decline of 53 mL of FEV<sub>1</sub>, a value that was greater than in the group of dairy farmers (36 mL; p=0.02 between groups).<sup>55</sup>

Although their working conditions and exposure differ from those of farmers working in confined spaces, dairy and cattle farmers also have an increased risk of impaired lung function and respiratory symptoms compared with unexposed controls.<sup>56-59</sup> The seven studies of cattle farming included in this meta-analysis reported homogeneous results (I<sup>2</sup>=0%, p=0.61). The four studies with the highest weight showed a significant association between cattle farming and prevalence of either chronic bronchitis or airflow limitation (total weight=94.1%) while the two

other studies only showed a trend towards significant association, with a global OR (95% CI) as high as 2.27 (1.81 to 2.86). These results remained unchanged when the prevalence of chronic bronchitis and of airflow limitation were analysed separately. It is noteworthy that this increased risk of COPD traits among dairy farmers may have decreased during the last few years. Our group recently investigated the impact of farm modernity on respiratory outcomes such as airflow limitation and chronic bronchitis in dairy farmers.<sup>12</sup> This study demonstrated that working in a modern farm (with a separation between the house and the cowshed and using a loose housing system) decreased the risk of airflow limitation twofold after adjustment for age and for smoking habits.<sup>12</sup>

Although there are homogeneous data suggesting that raising one specified type of animal increases the risk of COPD traits, we found here that raising more than one type of animal or raising unspecified types of animals was not associated with increased risk of chronic bronchitis and/or airflow limitation. This finding





**Figure 4** Risk of bronchitis according to farm activity.

is in contradiction with the conclusions of studies that were not included in the current meta-analysis. In a cross-sectional study of Norwegian farmers, Eduard *et al* assessed respiratory symptoms and pre-BD lung function in 994 exclusive plant producers and 3741 livestock farmers.<sup>60</sup> The authors reported an increased prevalence of chronic bronchitis, airflow limitation and reduced FEV1 among livestock farmers. In another study performed in the Netherlands, living in an area with high density of livestock was shown to be a major risk factor for exacerbations in patients with COPD.<sup>61</sup> The lack of significant association found in our meta-analysis between COPD traits and raising more than one animal type or raising unspecified types of animals could be explained by the very small number of studies included in this group (n=2) and the fact that these two studies used different respiratory outcomes. In addition, between-studies heterogeneity was as high as 77% (p=0.04), indicating that these results must be interpreted with caution.

In the current literature, the group of grain/crop farmers is the most controversial regarding the prevalence of airflow limitation and/or chronic bronchitis.<sup>62, 63</sup> Among the studies included in the current meta-analysis for this group, one study showed a strong association, one study reported a weak and non-significant association and four showed no association between crop/grain farming and prevalence of airflow limitation and/or chronic bronchitis in comparison with non-exposed subjects.

The heterogeneity of results between these farmers could be explained by the wide range of activities (grain elevator workers, fruit growers, etc), working methods (use of greenhouse, use of closed and well-ventilated tractors, etc) and the type of climate. When analyses were stratified on respiratory outcome, crop/grain farmers showed a weak but significant association with prevalence of airflow limitation (OR (95% CI)=1.72 (1.01 to 2.94), p=0.049) and heterogeneity between studies was high but not significant (I<sup>2</sup>=54%, p=0.07). Similarly, crop/grain farming was strongly associated with prevalence of chronic bronchitis (OR (95% CI)=2.41 (1.29 to 4.47), p=0.006). This finding is of importance as some studies used crop/grain farmers as a control group, considering that the risk of COPD in this group was similar to that of unexposed subjects.<sup>60</sup> Our observation suggests that crop/grain farmers should be investigated more closely in order to understand the pathophysiology of chronic bronchitis and of airflow limitation found in this group.

In the current meta-analysis, we reviewed the risk of airflow limitation and/or chronic bronchitis among mixed, unspecified groups of farmers. A large number of studies included groups of farmers working in more than one activity, whereas other studies did not describe their type of activities. Among mixed, unspecified groups of farmers, we found a significantly increased prevalence of either airflow limitation or chronic bronchitis (OR (95% CI)=1.59 (1.30 to 1.95)), or airflow limitation alone (OR

(95% CI)=1.70 (1.34 to 2.15)), but not of chronic bronchitis alone (OR (95% CI)=1.43 (0.95 to 2.16)). However, it is difficult to interpret this result, given that the definition of exposed groups varies greatly from one study to another.

### Quality of the studies included

The sensitivity analysis of all the studies included, ranked according to their quality score, revealed that the OR estimated with the five studies with the best quality scores (OR (95% CI)=1.72 (1.39 to 2.14)) was very similar to the global OR of our meta-analysis (OR (95% CI)=1.77 (1.50 to 2.08)) (see online supplementary figure S2). This result is reassuring since it means that the global OR is mainly based on high-quality studies, rather than on studies whose quality could skew the results obtained.

### Additional value to the literature

A review paper recently published by Fontana *et al* shares some conclusions with ours, especially regarding the negative effects of farming on respiratory health.<sup>15</sup> Nevertheless, we believe that our study adds new findings on respiratory health in farmers, for the following reasons. First, our meta-analysis includes a greater number of articles than the review published by Fontana *et al* (22 instead of 14) and the literature search has been completed more recently (July 2018 instead of December 2016). Second, the major added value of our current study comes from the subanalyses of the relationship between COPD and farming considering different definitions of COPD. As stated in the 'Introduction' section, the definition of COPD varies widely between studies and in general, older studies use only clinical criteria of chronic bronchitis to define COPD, and only more recent ones use spirometric criteria of airflow limitation. In addition, we performed a meta-analysis instead of a systematic review. Combining these two approaches allowed us to demonstrate for the first time a significant (although weak) relationship between crop farming on the one hand, and airflow limitation and chronic bronchitis on the other hand.

### Limitations of the study

We acknowledge that our meta-analysis carries several limitations. First, the between-studies heterogeneity could have biased interpretation of some of our results. The global effects of all included studies are significant for the analyses of the criteria 'either airflow limitation or chronic bronchitis' and 'chronic bronchitis', but both analyses had significant between-studies heterogeneity. This could be due to the large number of studies included and the wide range of exposure and respiratory outcome definitions. Nevertheless, for the criterion 'airflow limitation', the pooled OR did not have significant between-studies heterogeneity. This could be explained by the fact that studies using a spirometric criterion to define COPD are more recent, better designed and/or with a more robust outcome than studies using a clinical criterion. Second, some inclusion criteria, in particular, age limit over 40 years and the number of 30 individuals per group, can be regarded as arbitrary. Nevertheless, the age limit of 40 was chosen in order to avoid confusion between COPD and asthma. This confusion may exist when COPD is defined by a pre-BD FEV1/FVC ratio below either 0.70 or age-dependent LLN. In addition, COPD is very infrequent before the age of 40 years. The number of individuals was chosen in order to avoid the inclusion of non-representative studies. The limit of 30 was taken by analogy statistical practice, where a sample of 30 individuals is considered to be representative of the whole

group. Third, we excluded many studies that did not fulfil the inclusion criteria of our meta-analysis. Many studies were not included because of the lack of a control group of non-exposed subjects.<sup>60 64–70</sup> Others were not included because of the respiratory outcome: a large number of studies assessing respiratory status presented only the mean FEV1/FVC values instead of the proportion of subjects with a FEV1/FVC ratio below 0.70 or below LLN, and some studies reported 'respiratory symptoms' with no further detail, so that it was not possible to estimate the proportion of subjects with chronic bronchitis.<sup>50 71 72</sup> Fourth, we used only crude OR for respiratory outcomes, without taking into account confounding factors such as gender, age or smoking status. We did so because several studies that we included did not provide ORs adjusted for these confounding factors. We acknowledge that this choice could introduce a confounding bias, particularly when the population of agricultural workers was different to the controls for these factors. Nevertheless, for better interpretation of our results, in this paper we present the distribution of all confounding factors in all the groups of agricultural workers and controls included.

### CONCLUSION

This meta-analysis demonstrates that cattle farming, swine farming and poultry farming are strongly associated with airflow limitation and chronic bronchitis. Our analysis also demonstrates for the first time that crop/grain farmers have a higher prevalence of airflow limitation and of chronic bronchitis compared with unexposed controls. However, the results concerning farmers breeding undefined livestock depend on the chosen respiratory outcome and, perhaps, on the real exposure of the different groups of farmers studied. The current meta-analysis highlights the fact that at this stage, it is not possible to draw firm conclusions regarding the relationships between farming and COPD, as there are very few studies in which COPD has been defined according to appropriate criteria. It seems therefore crucial to conduct new epidemiological studies investigating different farming groups (by job titles and with details regarding type of farming, working activities as well as qualitative and quantitative measurement of airborne exposure) and using the appropriate definition of COPD (ie, persistent airway obstruction according to age-dependent LLN threshold along with symptoms).

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**Competing interests** None declared.

**Patient consent** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

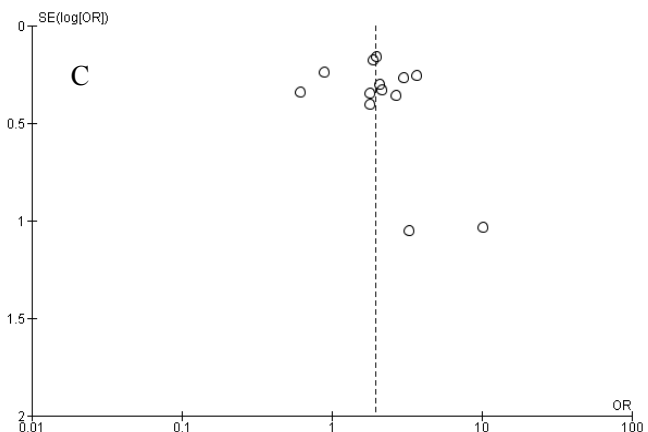
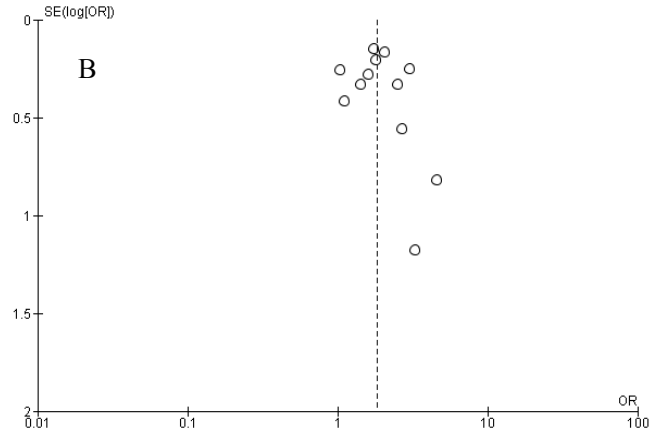
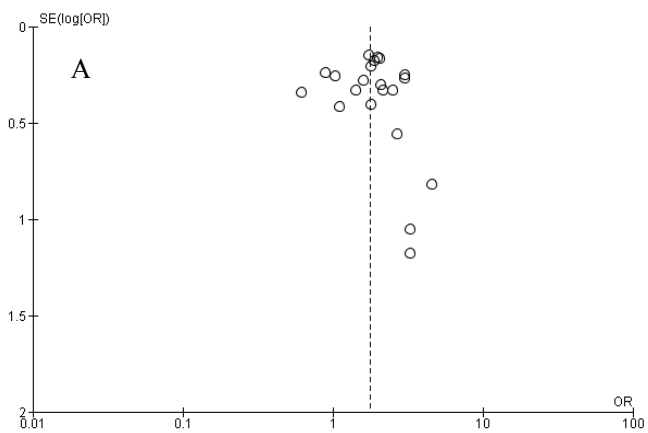
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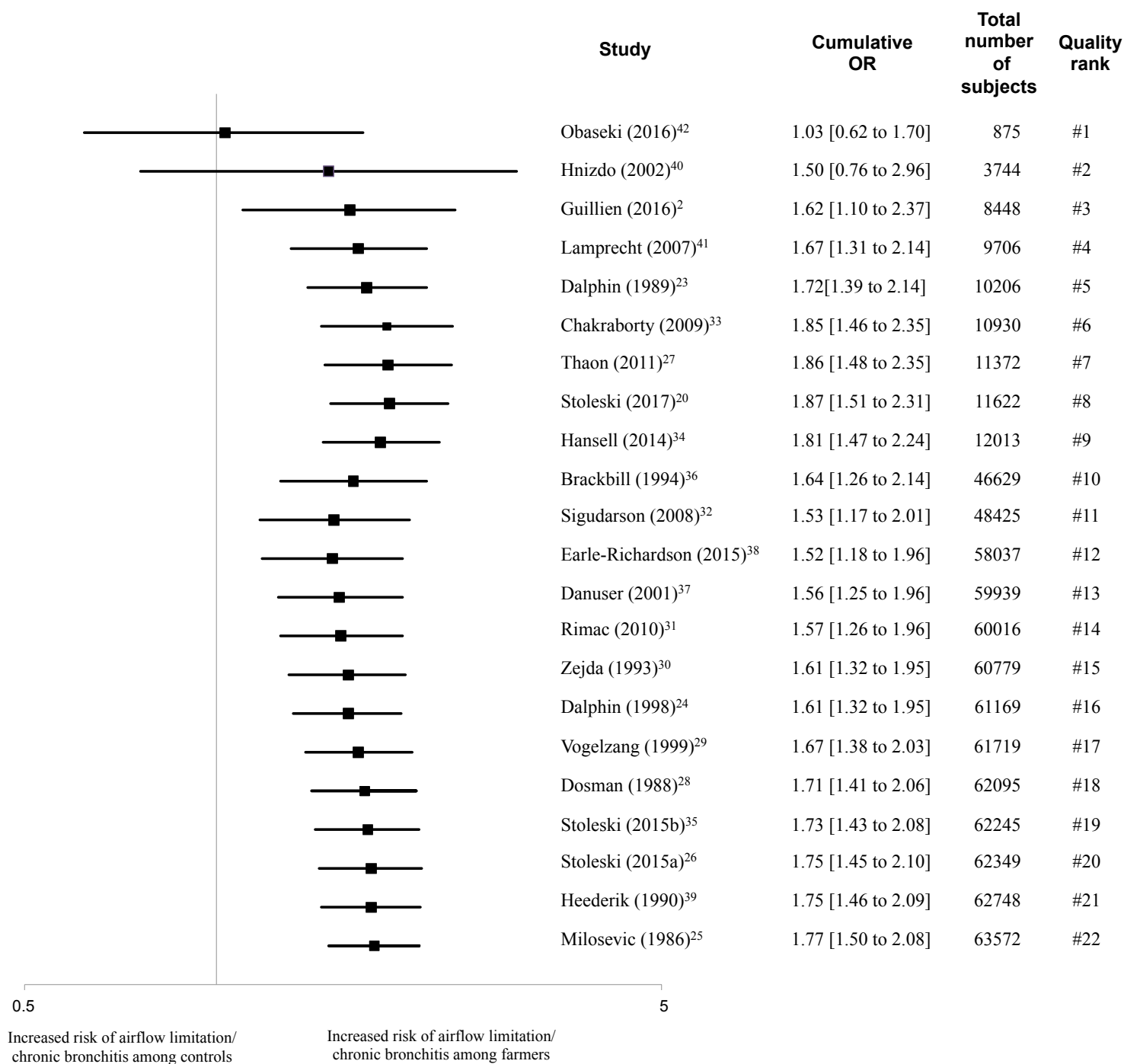
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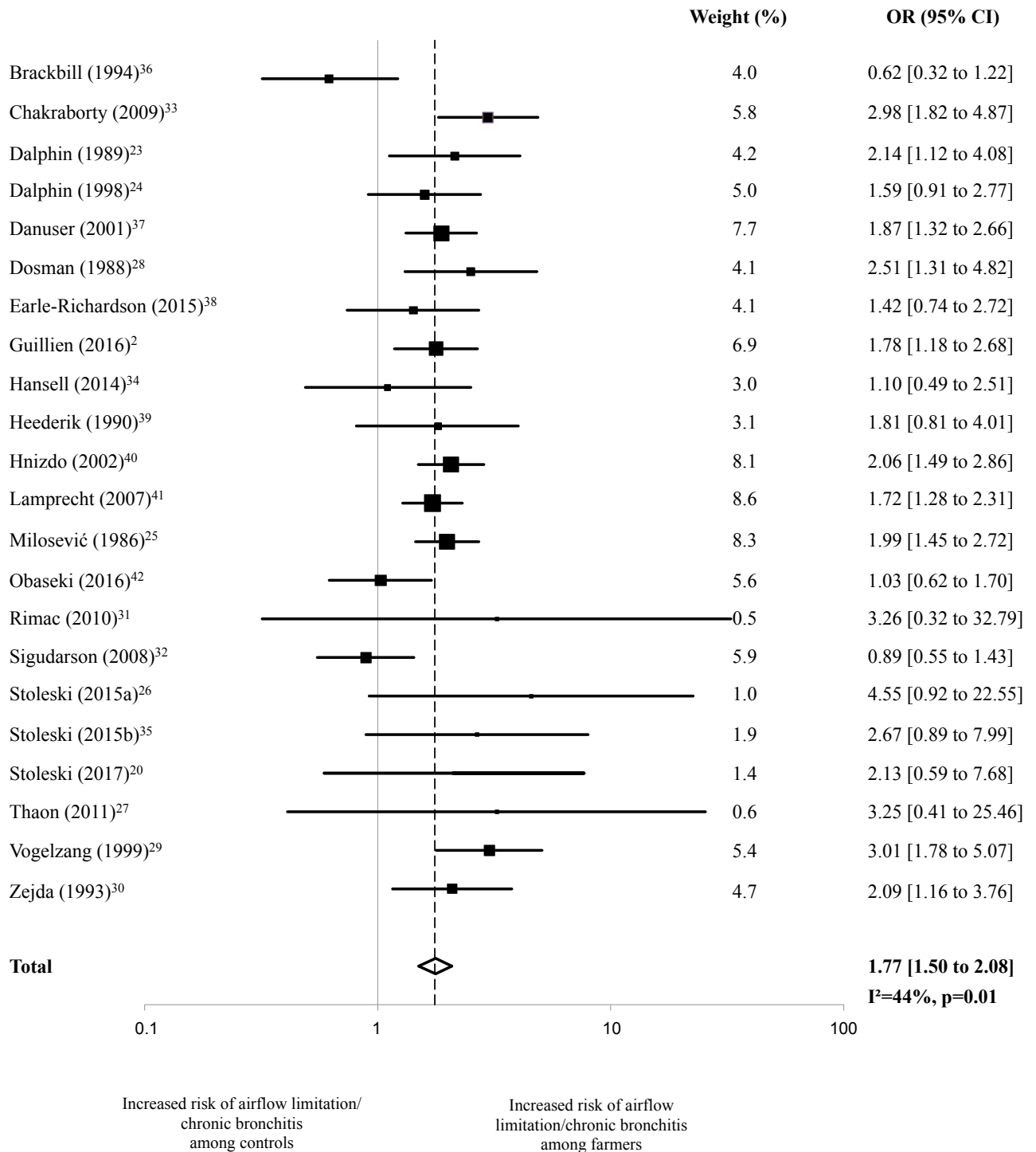
**Figure S1.** Funnel plots of studies assessing association between farming activity and (A) prevalence of airflow limitation/chronic bronchitis; (B) prevalence of airflow limitation; (C) prevalence of chronic bronchitis.



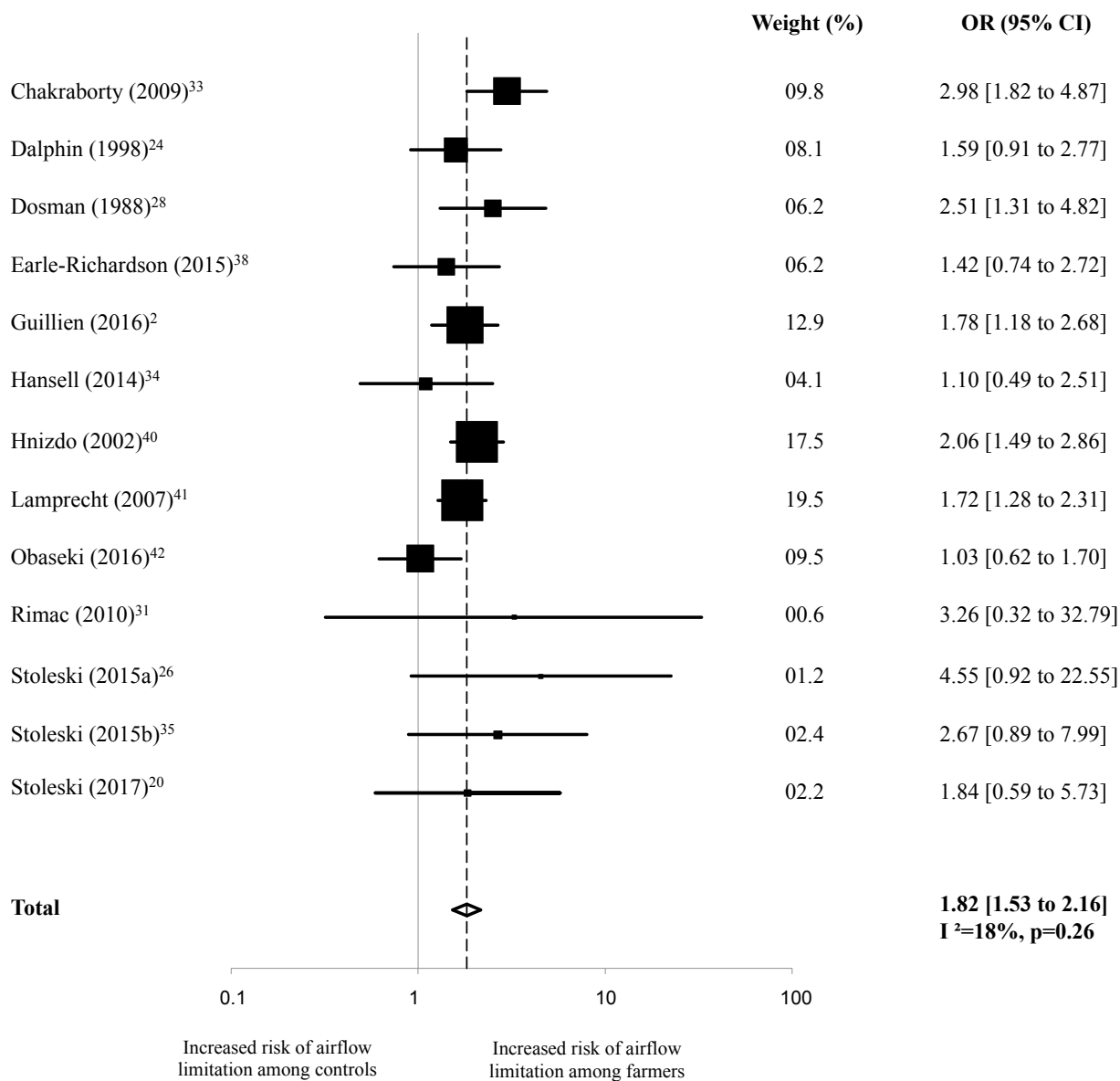
**Figure S2.** Sensitivity analysis according to quality score.



**Figure S3.** Risk of COPD/airflow limitation/chronic bronchitis among farmers.

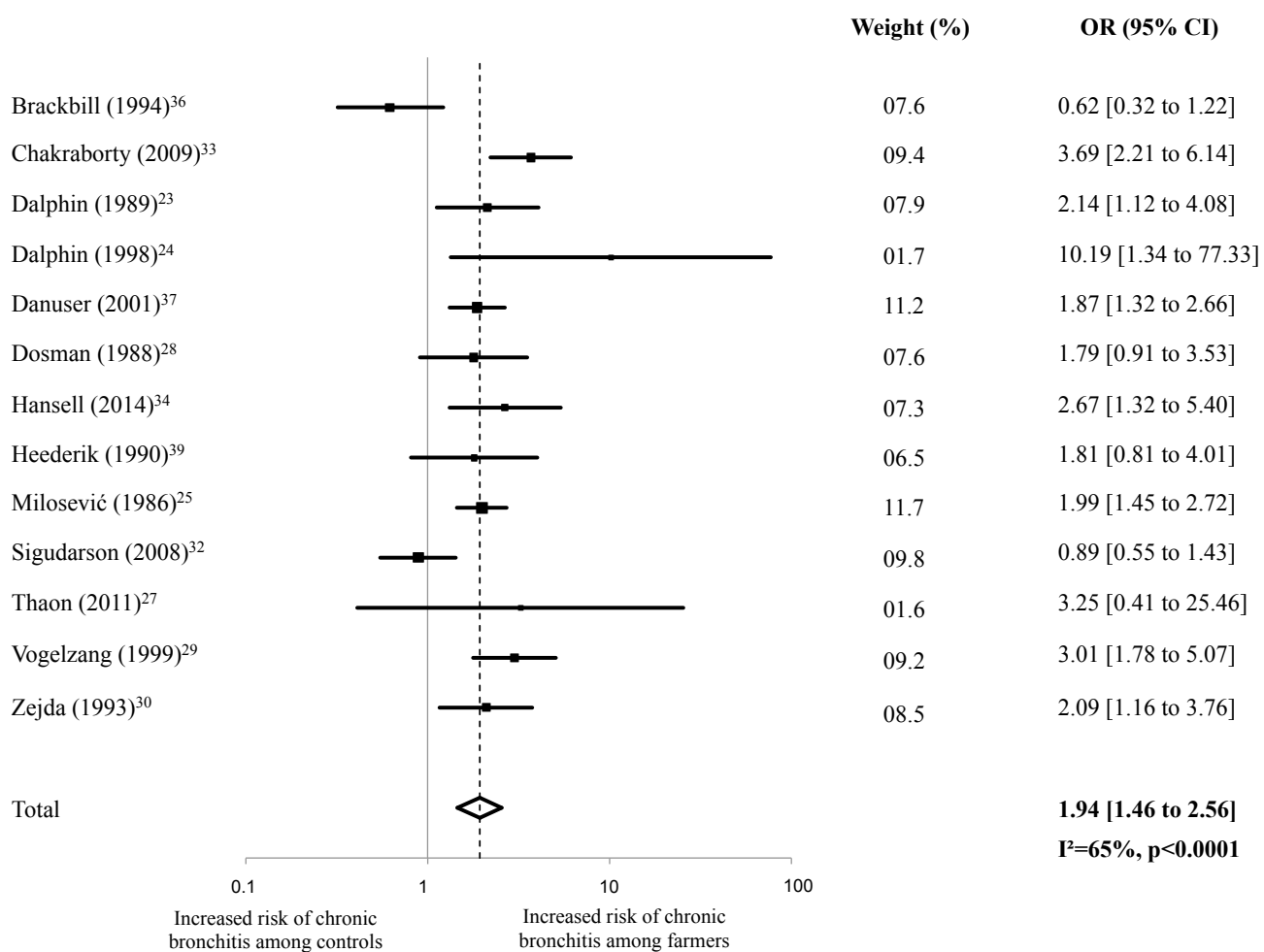


**Figure S4. Risk of airflow limitation among farmers.**





**Figure S5.** Risk of chronic bronchitis among farmers.



### 3. Article 3: Gender Differences in Respiratory Health Outcomes among Farming Cohorts around the Globe: Findings from the AGRICOH Cohort Consortium.

#### *a. Contexte et objectif*

L'association entre le milieu agricole et les maladies respiratoires est maintenant bien connue. La prévalence des différentes pathologies respiratoires varie néanmoins considérablement selon l'activité agricole et les régions géographiques.

Bien que de nombreuses études de cohortes aient été menées chez les agriculteurs dans de nombreux pays, il n'existe à ce jour aucune étude internationale estimant l'importance des pathologies respiratoires au niveau mondial. Le consortium international AGRICOH a été créé en 2006 dans le but de rassembler les données de ces différentes cohortes afin d'évaluer l'impact sur la santé des expositions agricoles à l'échelle internationale. Actuellement, 30 cohortes participent et comprennent un large éventail d'activité agricole issu de tous les continents.

Le but de la présente étude était d'évaluer la prévalence des symptômes et des différentes pathologies respiratoires chez plus de 200 000 sujets issus de 11 pays.

#### *b. Manuscrit*

<p>Article "Gender Differences in Respiratory Health Outcomes among Farming Cohorts around the Globe: Findings from the AGRICOH Cohort Consortium." Fix J, Annesi-Maesano I, Boulanger M, Cheng S, Cortes S, Dalphin JC, Dalvie A, Degano B, Douwes J, Eduard W, Elholm G, Ferreccio C, Frost G, Harding AH, M, Kelly KM, Kromhout H, Lebailly P, MacFarlane E, Maesano CN, Mitchell D, Naidoo S, Negatu B, Ngajilo D, Nordby KC, Parks C, Sandler DP, Schenker M, Shin A, Sigaard T, Sim M, <b>Soumagne T</b>, Taylor M, Thorne PS, Yoo KY, Hoppin JA. <i>En cours de soumission</i>.</p>
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## **Gender Differences in Respiratory Health Outcomes among Farming Cohorts around the Globe: Findings from the AGRICOH Cohort Consortium**

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## **Abstract**

Respiratory hazards of farming have been identified for centuries, but with little focus on gender differences. We used data from the AGRICOH consortium, a collective of prospective cohorts of agricultural workers, to assess respiratory disease prevalence among adults in 18 cohorts representing over 200,000 farmers, farm workers, and their spouses from six continents. Cohorts collected data between 1992 and 2016 and ranged in size from 200 to >128,000 individuals; farming practices varied from subsistence farming to large scale industrial agriculture. All cohorts provided some respiratory outcome information (cough, phlegm, wheeze, asthma, allergic/non-allergic asthma, and chronic obstructive pulmonary disease (COPD)) for their cohort using their study definition. The majority of outcomes were based on self-report using standard respiratory questionnaires; the greatest variability was associated with COPD. For all three respiratory symptoms (cough, phlegm, and wheeze), the prevalence in men was higher than in women, with the greatest difference for phlegm. For asthma, women had a higher overall prevalence with the difference associated with more allergic asthma. We observed differences among the cohorts with regard to the relative proportion of allergic asthma. In two of eight cohorts for women and two of seven cohorts for men, allergic asthma was more common than non-allergic asthma. These findings indicate the variability, yet similarity, of respiratory symptoms and outcomes among farmers around the world despite the differences in agricultural production. As in the general population, women are at higher risk for asthma and should be included in occupational studies of respiratory diseases for agricultural workers.

## Introduction

Agricultural work is an occupation represented by both men and women, though little formal comparison of respiratory disease prevalence by gender has been conducted. Women have an active role in farm production activities but frequently are not regarded as farmers, and as such, the impact of their occupational exposures are under-represented. Agricultural workers include both farmers who are owners and operators and farm workers (local and migrant). Agricultural work remains an occupation that people begin at young ages.

Work in agriculture has been associated with respiratory diseases as early as the mid-1500's<sup>1</sup>. Even as farm work becomes more industrialized, we continue to see evidence of increased respiratory risk for agricultural workers. Factors contributing to increased risk of respiratory diseases and symptoms include frequent exposure to dusts, microorganisms, toxic gases (e.g. diesel motor exhaust, welding fumes, and asbestos), and pesticides<sup>2</sup>. While respiratory diseases have been a concern among farming populations for many centuries, recent studies have focused on differences between allergic vs. non-allergic phenotypes<sup>3-7</sup> due to the reduced risk of allergy associated with growing up on a farm<sup>8</sup>.

While many papers have been published related to the respiratory health of agricultural workers, few, if any, have attempted to integrate data from across continents and farming practices to characterize the global respiratory health implications of agricultural work. In 1998, the American Thoracic Society published a research statement on Respiratory Hazards of Agriculture<sup>1</sup>. In this seminal work, extensive detail was provided on the specific respiratory outcomes, as well as the range of exposures experienced by agricultural workers primarily in developed countries. However, respiratory hazards of women and agricultural work in low and middle income countries were not well represented. Although there has not been a multi-country study to estimate the global burden of respiratory disease among farmers, the numerous cohort studies that have been conducted around the world can help start to address this gap in knowledge. The AGRICOH consortium was created in 2006 as a collaborative effort to assess relationships between farming exposures and health outcomes. Currently 30 cohorts are participating and they include a diverse range of types of farming from all continents<sup>9</sup>. To assess the prevalence of respiratory disease and symptoms among diverse farming populations and to explore differences between men and women, we focused on common respiratory endpoints from AGRICOH cohorts with respiratory outcome information.

## **Methods**

### *Study Population*

The AGRICOH consortium consists of 30 cohorts from around the world (<http://agricoh.iarc.fr/>). We focused on adults for this analysis because we were interested in occupational exposures. We excluded 11 cohorts from this analysis: four did not include adults and seven did not collect respiratory outcome information. All eligible AGRICOH cohorts agreed to participate.

Additionally, three farming cohorts outside of AGRICOH, but that included AGRICOH investigators, were also identified for inclusion, resulting in 18 cohorts for this analysis<sup>10-26</sup>. These cohorts cumulatively represent 211,232 people. The data presented includes farming populations around the world, including those of low and middle income countries with different socioeconomic settings, spanning from 1992 to 2016.

### *Data Collection*

For this project, we developed a structured reporting form to collect standardized demographic and outcome information (see supplement 1). All cohorts were asked to provide summary statistics on their cohorts; raw data were not collected. All cohorts except the Agricultural Health Study (AHS) provided prevalence estimates for data collected at enrollment. For the AHS, data from the most recent AHS interview were included because they were more complete than enrollment.

### *Cohort Demographic Information*

The data collection form requested information on cohort: sample size and calendar year of data collection, as well as summary statistics regarding age distribution, gender, smoking status (current/past/never), body mass index (BMI, <20, 20-<25, 25 - <30, and  $\geq$ 30). We also collected data on the participant types (farmer, farm worker, subsistence farmer), role of women in cohort (full time farmers, help on farm, spouses), type of livestock raised (poultry, beef cattle, dairy cattle, pork, other), type of farming (row crops, vegetables, orchards, other), and grain handling. We applied the World Bank Atlas method to determine country economy classifications, categorized by gross national income (GNI) per capita: low-income ( $\leq$ \$1,025),



lower-middle-income (\$1,026-\$4,035), upper-middle-income (\$4,036-12,475), and high income ( $\geq$ \$12,476).<sup>27</sup>

### *Respiratory Outcome Information*

Cohorts were asked to provide prevalence and 95% confidence interval information for three respiratory symptoms (cough, phlegm, and wheeze) and two respiratory diseases (asthma and chronic obstructive pulmonary disease (COPD)). Asthma was further classified as either allergic or non-allergic, based on each cohort's definition.

Outcome definitions varied by cohort with most using standard respiratory questionnaires (e.g., ATS and ECRHS). Some studies collected only self-reported outcome information while others incorporated clinical measurements as well. All symptom information was self-reported. Asthma was reported as either ever asthma or asthma in the past 12 months. All but one study relied on self-reported doctor's diagnosis of asthma, while the remaining study included a broader definition of having had an asthma attack in the past year, using asthma medication in the past year, or having a positive bronchodilator test conducted by study staff<sup>28</sup>.

COPD definition was most variable among the cohorts (supplement 1). Fifteen cohorts provided information on COPD prevalence. This information was based on self-report (67%, 10/15 cohorts), spirometry (27%, 4/15 cohorts), or an inclusive definition of either spirometry or self-report (7%, 1/15 cohorts). Among the 11 studies that used self-report to ascertain COPD status, definitions for COPD included: doctor diagnosis of COPD alone (27%); doctor diagnosis of chronic bronchitis alone (45%); the combination of doctor diagnosis of COPD, chronic bronchitis, or emphysema (18%); or self-reported history of COPD alone (9%).

Among eight cohorts reporting allergic and non-allergic asthma, definitions for allergic asthma included: asthma with hay fever (29%), asthma with hay fever and/or eczema (14%), asthma with rhinitis and other allergic diseases (14%), asthma with positive Phadiatop test (14%), asthma with atopy or fractional exhaled nitric oxide (FeNO) >50 ppb (14%), or asthma with three or more positive responses to a skin-prick test (14%).

### *Statistical Analysis*

Our statistical analysis focused on descriptive statistics of health outcomes. We integrated the summary statistics from all cohorts to create summary tables. To describe the distribution of respiratory outcomes among the cohorts, we calculated the median, interquartile range (IQR), and total range for prevalence of respiratory symptoms and diseases, for cohorts as a whole and then by gender. Reported medians were not weighted by cohort size, and reflect the 50<sup>th</sup> percentile of individual cohort prevalence estimates. To assess the relative prevalence of allergic to non-allergic asthma, gender-stratified prevalence ratios and corresponding 95% confidence intervals were calculated for each cohort. Analysis was completed in SAS 9.4 and figures developed in R.

## **Results**

### *Cohort Characteristics*

A total of 18 cohorts from 11 countries participated, including 118,520 men and 92,712 women (N=211,232) from both crop and livestock farming populations. Cohorts had a wide geographic spread, including populations from six continents (Figure 1). Participants include farmers, farm workers, subsistence farmers, and rural residents. Agricultural activities range from large scale industrial agriculture to rural fruit and farmworkers in Africa (supplement 2). The majority of the cohorts were conducted in high-income countries (14/18, 78%), while three (17%) were in upper-middle-income, and one (6%) in a low-income country.

The cohorts ranged in size from 89 to 128,388 participants: six (33%) included fewer than 1,000 participants; ten studies (56%) included 1,000-10,000 participants; and two (11%) included greater than 10,000 participants (Table 1). The majority of cohorts featured a greater proportion of males; however, two cohorts (KwaZulu-Natal Crop Farmers <sup>11</sup> and Western Cape Fruit Farm Workers <sup>12</sup>) included only female farm workers. The median proportion of males among cohorts was 60% (Interquartile Range (IQR): 44-78%). Among all cohorts, ages ranged from 15 to 105 years, with a median cohort-specific age of 51 years (IQR: 40.7-55.5).

### *Covariates*

We collected data on two common risk factors for respiratory outcomes: smoking and body mass index (BMI). The prevalence of smoking varied among the cohorts. The prevalence of current smoking ranging between 0% and 50%, with a median of 15% (IQR: 9%-30%) (Table 1 and supplement 3). There were no apparent differences in smoking prevalence between industrialized and non-industrialized countries or by geographic region. Smoking varied by gender, with current smoking prevalence consistently higher among males (median = 17% [IQR: 9%-30%]) compared to females (median = 11% [IQR: 7%-22%]). For BMI, the proportion of obese individuals ranged from 1% to 40% (median = 17% [IQR: 13%-33%]), and overweight individuals ranged from 11% to 44% (median = 31% [IQR: 21%-44%]). Higher proportions of underweight participants were more common among KMCC<sup>14</sup> and African cohorts<sup>10-13</sup> (Table 1 and supplement 4).

### *Respiratory Symptoms*

We collected data on cough, phlegm, and wheeze. Most cohorts collected data on all these outcomes (10 of 18); 72% of cohorts provided data cough, 61% phlegm, and 61% wheeze. Respiratory symptoms were common in all cohorts (Table 2a). The median prevalence was 18.6% (IQR: 14.4-30.0%) for cough, 13.3% (IQR: 7.8-18.8%) for phlegm, and 15.0% (IQR: 8.8-16.1%) for wheeze. Farmworkers from South Africa (Western Cape Fruit Farm Workers<sup>12</sup> and North West Poultry Workers<sup>13</sup>) and France (FERMA)<sup>17</sup> had the highest prevalence of cough and phlegm among all cohorts. Western Cape Fruit Farm workers also had the highest prevalence of wheeze (31%), while the farmers in the AHS in the United States had the second highest prevalence of wheeze (22%). When the data were stratified by gender (Table 2b, Figures 2a, 2b, 2c), males were more likely to report respiratory symptoms than females, though the IQR for the median values overlapped between males and females.

### *Respiratory Diseases*

We collected data on asthma, allergic asthma, non-allergic asthma, and COPD based on each cohort's definitions (table 3a). All cohorts provided data on asthma (KMCC<sup>14</sup> only collected information on allergic asthma), and 44% of these provided information on allergic phenotypes. 83% of cohorts provided information on COPD.

The median prevalence of asthma was 7.2% (IQR 5.5%-11.1%) with individual study values ranging from 0.5% in the Ethiopian<sup>10</sup> cohort to 16.0% among Victorian Grain Farmers<sup>25</sup>. Differences in asthma prevalence by gender did not follow the same trend as observed for symptoms (figures 3a and 3b). The median prevalence was slightly higher among females (7.8% [IQR:6.4%-10.9%]) compared with males (6.5% [IQR: 3.8%-11.6%]).

Allergic asthma (5.0% [IQR:3.4%-5.3%]) was more common than non-allergic asthma (2.0% [IQR:1.9%-7.0%]). This trend persisted after stratifying by gender; the median prevalence of allergic and non-allergic asthma among females was 5.5% and 3.5%, respectively, while among men, the median prevalence of allergic and non-allergic asthma was 4.3% and 3.6%, respectively. Among the gender stratified results, we also observed that women had higher prevalence ratios (PRs) than men, comparing allergic to non-allergic asthma (Figure 4). Upon aggregating data across all cohorts, women (PR: 1.01 [95% CI: 0.95-1.06]) had a significantly higher prevalence ratio of allergic to non-allergic asthma compared to men (PR: 0.76 [95% CI: 0.72-0.82]), indicating that allergic asthma and non-allergic asthma prevalence were similar in women, but that men were less likely to have allergic asthma.

Information on COPD was provided by 14 of 18 cohorts. One third of the cohorts used spirometry to classify COPD; the remaining two thirds used self-reported doctor diagnosis of at least one of these three outcomes (chronic bronchitis, emphysema, or COPD). The median prevalence of COPD among all cohorts was 4.5% [IQR: 2.8%-10.0%]) with COPD prevalence ranging from 0.5% in the MAUCO<sup>26</sup> cohort to 14.6% among Norwegian<sup>19</sup> farmers. The studies that reported using spirometry to estimate COPD prevalence had values ranging from 1.9% to 14.6% [median = 10.0% [IQR: 4.5%-11.1%]; studies that used self-reported prevalence had values ranging from 0.5 to 11.9% for COPD [median = 3.7% [IQR: 2.8%-10.0%]]. When we stratified the COPD data by gender, the median prevalence was similar between males (5.5% [IQR: 1.9%-12.2%]) and females (4.0% [IQR: 2.0%-7.9%]) (table 3b).

## **Discussion**

Using the data from 18 agricultural cohorts from around the world, we sought to characterize the burden of respiratory symptoms and diseases among the AGRICOH consortium as an indicator of the potential indicator of respiratory health among farmers worldwide. Respiratory outcomes among farmers remain a concern due to risk of occupational and environmental exposures. In this analysis, respiratory symptoms were relatively common and higher among men; while respiratory diseases were less common and women had a higher burden of asthma and men had higher prevalence of COPD. Although farming practices differ around the world, we found that the prevalence of these respiratory outcomes did not vary substantially between cohorts with any notable trends by region.

The eighteen cohorts included here represent a small subset of agricultural workers worldwide. No comprehensive study of agricultural workers around the world has been conducted. While large, our sample is by no means comprehensive and our cohorts are more representative of agriculture in developed countries. Some of the cohorts included a relatively small number of farmers and specific types of farming such as the all-female Western Cape Fruit Farmers<sup>12</sup> cohort in South Africa, while others featured much larger populations that included a wide variety of types of farming activities as well as spouses of farmers (e.g., AHS, AGRICAN). Overall, the average age among our cohort members (56.8 years) is similar to the average age of farmers in both developed countries and across Africa (60 years), as reported by the Food and Agriculture Organization of the United Nations in 2014<sup>29</sup>. Thus, our findings may be relevant to current agricultural workers around the world.

Overall, males tended to report more respiratory symptoms than women, but this was not always the case. In general population samples, such as the NHANES survey in the United States, the prevalence of wheeze and cough is similar in men and women while the prevalence of phlegm is much more common in men<sup>30</sup>. Here we observe greater differences between men and women particularly for activities with a high potential for exposure to respiratory irritants such as poultry workers. Occupational exposures and the types of tasks that men are more likely to engage in may contribute to this higher outcome prevalence. Smoking is more common in men, and that may also contribute to the some of the observed differences. However, in our study sample,

there is more variability in the prevalence of symptoms by cohort rather than between men and women, suggesting different baseline rates of symptoms in different populations as well as differences in exposure prevalence.

Asthma prevalence was higher in women agricultural workers, due to the higher prevalence of allergic asthma in women. Similarly, women around the world have a higher prevalence of asthma than men. In an earlier analysis of AHS data from 2005-2010, women in the AHS had more asthma than men in the AHS, but lower prevalence of asthma compared to the US population<sup>31</sup>. This may be due to factors associated with who becomes a farmer (for example, people with a history of asthma may choose a different profession) as well as agricultural exposures that appear to reduce allergic outcomes in farmers and their families<sup>3, 8, 32</sup>. The prevalence of asthma among our cohorts ranged from <1% to 16% with a median prevalence of 7.2%. Globally, asthma prevalence in adults aged 18-45 from 70 countries in 2002-2003 ranged from 0.2 (China) to 21% (Australia) with an overall prevalence of 4.3%<sup>33</sup>. While that study did not include all the same countries included here, it suggests that farmers may have higher prevalence of asthma than the general population.

The COPD results highlight the challenge of combining data across cohorts when disease definitions vary greatly, as has been reported by others<sup>34</sup>. Among the 15 cohorts that evaluated COPD prevalence, six different definitions of COPD were employed. COPD is classified based both on symptoms (chronic cough and phlegm) as well as airway obstruction as assessed through spirometry<sup>35</sup>. COPD encompasses the spectrum of obstructive diseases and includes chronic bronchitis and emphysema. Not all cohorts used spirometry to diagnosis COPD, but rather relied on doctor diagnosis of COPD. This will capture individuals who have been diagnosed accurately by their physician, but will miss those who have not had this detailed clinical work up; this suggests a sensitive but not specific outcome. Additionally it will capture those who received a diagnosis but did not meet the clinical criteria, creating false positives<sup>36</sup>. Within an individual cohort, these biases probably behave in the same manner, but between cohorts the factors that influence diagnosis and over reporting may differ making it almost impossible to combine across these studies.

Although this study importantly characterizes farming cohorts around the world, it does feature some limitations. Comparability between cohorts may be limited due to differences in study year, ranging from 1992 to 2016. AGRICOH cohorts range in from small focused cohorts with detailed information on a specific type of agriculture to large studies that includes farmers engaged in a wide variety of activities. In choosing to include as many cohorts as possible, we lost the ability to look at specific types of agriculture or agricultural practices. Participating cohorts provided demographic and symptom and disease metadata, but did not share participant-level data. As a result, the prevalence estimates reported are not adjusted or standardized to account for important confounding variables. While our study includes cohorts from around the world, the analysis was limited to AGRICOH cohorts that evaluated respiratory symptoms and diseases around the world. Consequently, our descriptive analysis only features study populations from the 11 countries in which AGRICOH cohorts were conducted, and more work is needed to truly characterize the global burden of respiratory outcomes.

Collaborative research provides valuable opportunities to investigate symptom and disease burden across many regions and geographic settings and diverse study populations. Future research would greatly benefit from sharing of participant-level data, allowing estimates to be standardized or adjusted for important confounders, improving comparability between cohorts. Data harmonization and standardization are not trivial tasks and we were unable to perform these at this time. While participating cohorts did well to include women, more investment must be made in research that characterizes the burden of disease among women in farming settings, whether they are farm owners/operators, farm workers, or spouses living on farms in order to identify sub-groups at higher risk of disease.

Asthma and COPD are important contributors to morbidity and mortality worldwide (GBD). Our analysis shows that farmers and agricultural workers are impacted by these health outcomes. The United Nation's International Labour Organization estimated in 2009 that there were about 1.07 billion people employed in agriculture around the world, accounting for nearly 35% of the global workforce <sup>37</sup>. Therefore understanding the impacts of agricultural production on respiratory health is critical.

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**Table 1: Characteristics of the AGRICOH cohorts that included respiratory outcomes**

Cohort	Country	Sample Size	Year <sup>‡</sup>	Male (%)	Mean Age (std)	Age Range	Smoking (%) <sup>+</sup>			BMI (%) <sup>+</sup>			
							Never	Past	Current	<20	20 - <25	25 - <30	≥30
Africa													
Ethiopia <sup>1</sup>	Ethiopia	206	2014	69	27 (7)	15-57	95	5	0	32	56	11	1
KwaZulu-Natal Crop Farmers <sup>2</sup>	South Africa	911	2006	0	42 (13)	18-82	94	0	6	NR	NR	NR	NR
Western Cape Fruit Farm Workers <sup>3</sup>	South Africa	211	2009	0	37 (12)	17-73	50	0	50	17	28	22	33
North West Poultry Workers <sup>4</sup>	South Africa	230	2012	68	37 (9)	21-68	56	1	43	17	48	17	17
Asia													
KMCC <sup>5</sup>	Korea	8,431	2004	44	58 (10)	19-91	60	11	29	14	65	18	3
Europe													
AGRICAN <sup>6</sup>	France	128,388	2007	56	65 (15)	20-105	65	27	9	4	37	44	15
BM3R <sup>7</sup>	France	5,095	2013	59	57 (9)	40-75	65	22	14	4	46	31	19
FERMA <sup>8</sup>	France	473	2012	71	47 (12)	18-78	55	24	21	8	45	29	17
Franche-Comte Farmers <sup>9</sup>	France	915	2006	74	55 (11)	30-80	62	24	15	2	49	32	17
Norwegian Farmers <sup>10</sup>	Norway	4,735	2009	60	49 (11)	21-69	50	22	28	2	43	44	11
PIPAH <sup>11</sup>	United Kingdom	4,536	2014	98	54 (12)	19-88	65	26	9	2	41	44	13
SUS Study <sup>12</sup>	Denmark	1,964	1992	88	19 (3)	17-49	70	0	30	NR	NR	NR	NR
North America													
AHS*	USA	39,464	2016	53	65 (11)	32-104	68	27	5	3	22	40	35
Farmer Health Study <sup>13</sup>	USA	1,947	1993	90	54 (13)	21-90	55	32	12	NR	NR	NR	NR
KCRHS <sup>14</sup>	USA	1,256	1997	44	52 (16)	18-92	62	24	15	3	31	31	36
Oceania													
New Zealand <sup>15</sup>	New Zealand	4,288	2005	52	49 (11)	20-93	58	30	12	4	36	44	17
Victorian Grain Farmers <sup>16</sup>	Australia	1,102	1995	100	49 (13)	17-89	57	13	30	NR	NR	NR	NR
South America													
MAUCO <sup>17</sup>	Chile	7,080	2015	39	54 (10)	36-77	45	23	31	1	16	44	40

\* Data not yet published

+ Percentages may not add up to 100% due to rounding

‡ If multiple years included in cohort, the last year of data collection is provided

BMI, body mass index; NR, not reported

KMCC, Korean Multi-center Cancer Cohort ; AHS, Agricultural Health Study; GDMSP, Grain Dust Medical Surveillance Program; KCRHS, Keokuk County Rural Health Study

**Table 2a: Self-reported respiratory symptom prevalence and 95% confidence intervals (CI) among AGRICOH cohorts**

Cohort	Cough (95% CI)	Phlegm (95% CI)	Wheeze (95% CI)
<b>Africa</b>			
Ethiopia	13.0 (8.0, 17.0)	6.0 (3.0, 9.0)	13.0 (8.0, 17.0)
Kwazulu-Natal Crop Farmers	NR	NR	NR
Western Cape Fruit Farm Workers	37.0 (30.0, 44.0)	NR	31.0 (25.0, 37.0)
North West Poultry Workers	36.0 (29.0, 42.0)	25.0 (20.0, 31.0)	15.0 (11.0, 21.0)
<b>Asia</b>			
KMCC	NR	NR	NR
<b>Europe</b>			
AGRICAN	11.1 (10.9, 11.3)	NR	NR
BM3R	17.0 (16.0, 18.0)	13.3 (12.4, 14.2)	NR
FERMA	36.5 (32.2, 40.9)	24.0 (19.9, 28.2)	16.0 (12.6, 19.4)
Franche-Comte Farmers	19.7 (16.3, 23.1)	18.2 (14.9, 21.5)	8.8 (6.7, 10.9)
Norwegian Farmers	23.9 (22.7, 25.1)	18.8 (17.7, 19.9)	16.0 (15.0, 17.1)
PIPAH	NR	NR	NR
SUS Study	20.3 (18.6, 22.2)	13.1 (11.7, 14.7)	9.7 (8.4, 11.1)
<b>North America</b>			
AHS	15.7 (15.5, 15.8)	14.4 (14.2, 14.7)	22.3 (22.0, 22.5)
Farmer Health Study	4.2 (3.4, 5.2)	3.9 (3.0, 4.8)	8.6 (7.4, 10.0)
KCRHS	18.6 (16.4, 20.7)	12.9 (11.0, 14.8)	6.8 (5.4, 8.4)
<b>Oceania</b>			
New Zealand	16.1 (15.0, 17.2)	7.8 (7.0, 8.6)	16.1 (15.0, 17.2)
Victorian Grain Farmers	NR	NR	NR
<b>South America</b>			
MAUCO	NR	NR	NR
<b>Overall Median (IQR)</b>	<b>18.6 (14.4, 30.0)</b>	<b>13.3 (7.8, 18.8)</b>	<b>15.0 (8.8, 16.1)</b>

NR, not reported; IQR, inter-quartile range  
 KMCC, Korean Multi-center Cancer Cohort; AHS, Agricultural Health Study; GDMSP, Grain Dust Medical Surveillance Program; KCRHS, Keokuk County Rural Health Study

**Table 2b: Gender stratified respiratory symptom prevalence and 95% confidence intervals (CI)**

Cohort	Cough		Phlegm		Wheeze	
	Female (95% CI)	Male (95% CI)	Female (95% CI)	Male (95% CI)	Female (95% CI)	Male (95% CI)
Africa						
Ethiopia	11.0 (3.0, 19.0)	13.0 (8.0, 19.0)	3.0 (0.0, 8.0)	7.0 (3.0, 11.0)	9.0 (2.0, 17.0)	14.0 (8.0, 20.0)
KwaZulu-Natal Crop Farmers	NR	NA	NR	NA	NR	NA
Western Cape Fruit Farm Workers	37.0 (30.0, 44.0)	NA	NR	NA	31.0 (25.0, 37.0)	NA
North West Poultry Workers	27.0 (17.0, 39.0)	40.0 (32.0, 48.0)	16.0 (8.0, 27.0)	29.0 (22.0, 37.0)	12.0 (6.0, 22.0)	17.0 (11.0, 23.0)
Asia						
KMCC	NR	NR	NR	NR	NR	NR
Europe						
AGRICAN	9.7 (9.4, 10.0)	12.3 (12.0, 12.5)	NR	NR	NR	NR
BM3R	16.1 (14.5, 17.7)	17.7 (16.3, 19.1)	9.6 (8.3, 10.9)	15.8 (14.5, 17.1)	NR	NR
FERMA	36.6 (28.3, 44.8)	36.5 (31.3, 41.7)	19.4 (12.5, 26.3)	26.1 (21.0, 31.3)	17.7 (11.0, 24.3)	15.3 (11.3, 19.2)
Franche-Comte Farmers	9.2 (3.5, 14.9)	22.0 (18.2, 25.8)	11.2 (4.9, 17.5)	19.8 (16.0, 23.8)	6.0 (2.4, 9.6)	9.6 (7.1, 12.1)
Norwegian Farmers	21.6 (19.8, 23.5)	25.4 (23.9, 27.0)	16.8 (15.1, 18.5)	20.2 (18.7, 21.7)	13.9 (12.4, 15.5)	17.4 (16.1, 18.8)
PIPAH	NR	NR	NR	NR	NR	NR
SUS Study	30.9 (25.0, 37.3)	18.9 (17.1, 20.8)	20.9 (15.8, 26.7)	12.1 (10.6, 13.7)	18.7 (13.9, 24.3)	8.5 (7.2, 9.9)
North America						
AHS	13.8 (13.6, 14.1)	17.2 (16.9, 17.4)	10.0 (9.8, 10.2)	18.0 (17.7, 18.3)	18.8 (17.5, 20.1)	25.2 (24.9, 25.5)
Farmer Health Study	3.1 (1.1, 6.5)	4.3 (3.4, 5.4)	2.0 (0.6, 5.1)	4.1 (3.2, 5.1)	4.1 (1.8, 7.9)	9.1 (7.8, 10.6)
KCRHS	17.9 (15.0, 20.7)	19.5 (16.2, 22.8)	9.6 (7.5, 11.8)	17.1 (13.9, 20.2)	5.8 (4.1, 7.5)	8.2 (5.9, 10.5)
Oceania						
New Zealand	15.4 (13.8, 16.9)	16.8 (15.2, 18.3)	6.5 (5.4, 7.5)	9.0 (7.8, 10.2)	15.3 (13.8, 16.9)	16.8 (15.3, 18.4)
Victorian Grain Farmers	NA	NR	NA	NR	NA	NR
South America						
MAUCO	NR	NR	NR	NR	NR	NR
Overall Median (IQR)	16.1 (10.3, 29.0)	18.3 (14.0, 24.6)	10.0 (6.5, 16.8)	17.1 (9.0, 20.2)	13.9 (6.0, 18.7)	14.7 (9.0, 17.1)

NR, not reported; NA, not applicable; IQR, inter-quartile range

KMCC, Korean Multi-center Cancer Cohort ; AHS, Agricultural Health Study; GDMSP, Grain Dust Medical Surveillance Program; KCRHS, Keokuk County Rural Health Study

**Table 3a: Respiratory disease prevalence and 95% confidence intervals (CI) among AGRICOH cohorts**

Cohort	Asthma				COPD* (95% CI)
	Overall Asthma (95% CI)	Allergic Asthma (95% CI)	Non-Allergic Asthma (95% CI)		
<b>Africa</b>					
Ethiopia	0.5 (0.0, 1.4)	NR	NR	NR	1.9 (0.0, 4.0) <sup>a</sup>
Kwazulu-Natal Crop Farmers	9.0 (7.3, 11.0)	NR	NR	NR	2.9 (1.9, 4.2) <sup>b</sup>
Western Cape Fruit Farm Workers	6.0 (3.0, 9.0)	5.0 (2.0, 8.0)	1.0 (0.0, 2.0)	NR	NR
North West Poultry Workers	12.0 (8.0, 16.0)	5.0 (2.0, 8.0)	7.0 (4.0, 11.0)	NR	10.0 (6.0, 14.0) <sup>a,b</sup>
<b>Asia</b>					
KMCC	NR	6.1 (5.5, 6.8)	NR	NR	2.8 (2.4, 3.2) <sup>b</sup>
<b>Europe</b>					
AGRICAN	7.9 (7.8, 8.1)	3.0 (2.9, 3.1)	4.9 (4.8, 5.0)	NR	11.9 (11.7, 12.1) <sup>c</sup>
BM3R	6.7 (6.6, 6.8)	NR	NR	NR	4.5 (3.9, 5.1) <sup>a</sup>
FERMA	12.8 (9.8, 15.9)	5.4 (3.3, 7.4)	7.5 (5.1, 9.9)	NR	4.8 (2.8, 6.7) <sup>d</sup>
Franche-Comte Farmers	6.8 (4.3, 9.3)	NR	NR	NR	11.1 (8.7, 13.5) <sup>a</sup>
Norwegian Farmers	3.6 (3.1, 4.2)	NR	NR	NR	14.6 (13.6, 15.6) <sup>a</sup>
PIPAH	10.3 (9.4, 11.2)	NR	NR	NR	0.9 (0.7, 1.2) <sup>d</sup>
SUS Study	4.9 (4.0, 5.9)	2.9 (2.2, 3.7)	2.0 (1.4, 2.7)	NR	8.2 (7.0, 9.5) <sup>b</sup>
<b>North America</b>					
AHS	7.1 (6.9, 7.2)	5.1 (5.0, 5.2)	1.9 (1.8, 2.0)	NR	3.1 (3.0, 3.2) <sup>e</sup>
Farmer Health Study	7.8 (6.6, 9.0)	NR	NR	NR	NR
KCRHS	7.2 (5.8, 8.7)	4.4 (3.3, 5.6)	2.0 (1.2, 2.8)	NR	3.7 (2.6, 4.8) <sup>b</sup>
<b>Oceania</b>					
New Zealand	15.1 (14.0, 16.1)	NR	NR	NR	NR
Victorian Grain Farmers	16.0 (14.0, 18.0)	NR	NR	NR	10.0 (9.0, 12.0) <sup>f</sup>
<b>South America</b>					
MAUCO	2.9 (2.5, 3.3)	NR	NR	NR	0.5 (0.4, 0.7) <sup>d</sup>
<b>Overall Median (IQR)</b>	<b>7.2 (5.5, 11.1)</b>	<b>5.0 (3.4, 5.3)</b>	<b>2.0 (1.9, 7.0)</b>	<b>NR</b>	<b>4.5 (2.8, 10.0)</b>

COPD, chronic obstructive pulmonary disease; NR, not reported; IQR, inter-quartile range

COPD definitions: <sup>a</sup> Spirometry test threshold, <sup>b</sup> Doctor-diagnosed chronic bronchitis only, <sup>c</sup> Doctor-diagnosed chronic bronchitis or emphysema

<sup>d</sup> Doctor-diagnosed COPD only, <sup>e</sup> Doctor-diagnosed COPD, emphysema, or chronic bronchitis, <sup>f</sup> Self-reported history of COPD.

COPD definitions provided in supplement 1

KMCC, Korean Multi-center Cancer Cohort; AHS, Agricultural Health Study; GDMSP, Grain Dust Medical Surveillance Program; KCRHS, Keokuk County Rural Health Study

**Table 3b: Gender stratified respiratory disease prevalence and 95% confidence intervals (CI)**

Cohort	Asthma										COPD	
	Overall Asthma				Allergic Asthma				Non-Allergic Asthma		Female (95% CI)	Male (95% CI)
	Female (95% CI)	Male (95% CI)	Female (95% CI)	Male (95% CI)	Female (95% CI)	Male (95% CI)	Female (95% CI)	Male (95% CI)				
Africa												
Ethiopia	NR	0.7 (0.0, 1.4)	NR	NR	NR	NR	NR	NR	NR	NR	3.6 (0.0, 8.6)	1.0 (0.0, 2.9)
KwaZulu-Natal Crop Farmers	NR	NA	NR	NA	NR	NA	NR	NR	NA	NA	NR	NA
Western Cape Fruit Farm Workers	6.0 (3.0, 9.0)	NA	5.0 (2.0, 8.0)	NA	NA	1.0 (0.0, 2.0)	NA	NA	NA	NA	NR	NA
North West Poultry Workers	12.0 (6.0, 22.0)	12.0 (7.0, 18.0)	4.0 (0.8, 11.0)	5.0 (2.0, 10.0)	8.0 (3.0, 17.0)	7.0 (4.0, 12.0)	4.0 (0.8, 11.0) <sup>a</sup>	12.0 (7.0, 18.0) <sup>a,b</sup>				
Asia												
KMCC	NR	NR	6.6 (5.7, 7.4)	5.6 (4.7, 6.5)	NR	NR	NR	NR	NR	NR	2.1 (1.6, 2.6) <sup>b</sup>	3.7 (3.0, 4.4) <sup>b</sup>
Europe												
AGRICAN	7.8 (7.5, 8.0)	8.1 (7.9, 8.1)	3.1 (3.0, 3.3)	3.0 (2.8, 3.0)	4.7 (4.5, 4.8)	5.1 (4.9, 5.1)	10.3 (10.0, 10.6) <sup>c</sup>	13.1 (12.8, 13.4) <sup>c</sup>				
BM3R	6.7 (5.6, 7.8)	6.5 (5.6, 7.4)	NR	NR	NR	NR	1.9 (1.3, 2.5) <sup>a</sup>	6.2 (5.3, 7.1) <sup>a</sup>				
FERMA	15.9 (9.6, 22.2)	11.6 (8.2, 15.1)	6.8 (2.5, 11.2)	4.8 (2.5, 7.1)	9.1 (4.1, 14.1)	6.7 (4.1, 9.6)	4.5 (0.9, 8.1) <sup>d</sup>	4.8 (2.5, 7.2) <sup>d</sup>				
Franche-Comte Farmers	7.8 (3.7, 11.9)	6.1 (2.9, 9.3)	NR	NR	NR	NR	5.4 (2.0, 8.8) <sup>a</sup>	12.9 (10.0, 15.8) <sup>a</sup>				
Norwegian Farmers	3.3 (2.6, 4.2)	3.7 (3.1, 4.5)	NR	NR	NR	NR	10.4 (9.1, 11.9) <sup>a</sup>	17.4 (16.0, 18.8) <sup>a</sup>				
PIPAH	10.5 (5.3, 19.7)	10.2 (9.4, 11.2)	NR	NR	NR	NR	1.3 (0.2, 8.9) <sup>d</sup>	0.9 (0.6, 1.2) <sup>d</sup>				
SUS Study	6.5 (3.7, 10.5)	4.7 (3.7, 5.8)	3.0 (1.2, 6.2)	2.9 (2.1, 3.8)	3.5 (1.5, 6.7)	1.8 (1.2, 2.5)	13.5 (9.3, 18.6) <sup>b</sup>	7.5 (6.3, 8.8) <sup>b</sup>				
North America												
AHS	7.8 (7.6, 8.0)	6.4 (6.2, 6.6)	6.1 (6.0, 6.2)	4.3 (4.2, 4.4)	1.7 (1.6, 1.8)	2.1 (2.0, 2.2)	2.3 (2.2, 2.4) <sup>e</sup>	3.8 (3.6, 3.9) <sup>e</sup>				
Farmer Health Study	9.2 (5.6, 14.1)	7.6 (6.4, 8.9)	NR	NR	NR	NR	NR	NR				
KCRHS	9.9 (7.7, 12.1)	3.8 (2.2, 5.4)	5.9 (4.1, 7.6)	2.6 (1.2, 3.9)	2.7 (1.5, 3.9)	1.1 (0.2, 2.0)	4.8 (3.2, 6.4) <sup>b</sup>	2.2 (1.0, 3.4) <sup>b</sup>				
Oceania												
New Zealand	16.0 (14.4, 17.6)	14.2 (12.7, 15.6)	NR	NR	NR	NR	NR	NR				
Victorian Grain Farmers	NA	16.0 (14.0, 18.0)	NA	NR	NA	NR	NA	10.0 (9.0, 12.0) <sup>f</sup>				
South America												
MAUCO	3.6 (3.1, 3.9)	1.9 (1.6, 2.2)	NR	NR	NR	NR	0.7 (0.5, 0.9) <sup>d</sup>	0.3 (0.2, 0.5) <sup>d</sup>				
Overall Median (IQR)	7.8 (6.4, 10.9)	6.5 (3.8, 11.6)	5.5 (3.3, 6.5)	4.3 (2.9, 5.0)	3.5 (1.7, 8.0)	3.6 (1.6, 6.8)	4.0 (2.0, 7.9)	5.5 (1.9, 12.2)				

COPD, chronic obstructive pulmonary disease; NR, not reported; NA, not applicable; IQR, inter-quartile range

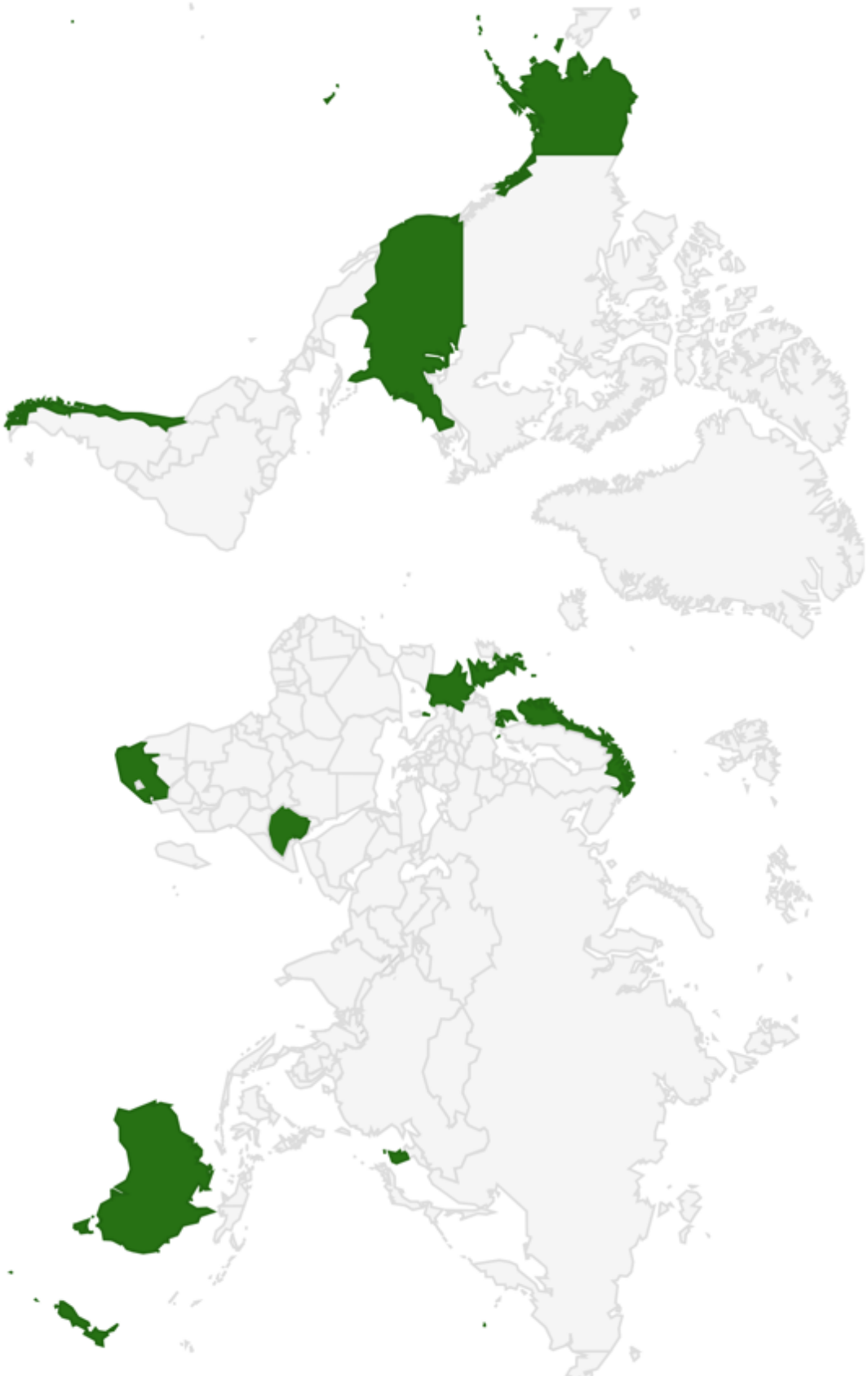
COPD definitions: <sup>a</sup> Spirometry test threshold, <sup>b</sup> Doctor-diagnosed chronic bronchitis only, <sup>c</sup> Doctor-diagnosed chronic bronchitis or emphysema

<sup>d</sup> Doctor-diagnosed COPD only, <sup>e</sup> Doctor-diagnosed COPD, emphysema, or chronic bronchitis, <sup>f</sup> Self-reported history of COPD.

COPD definitions provided in supplement 1

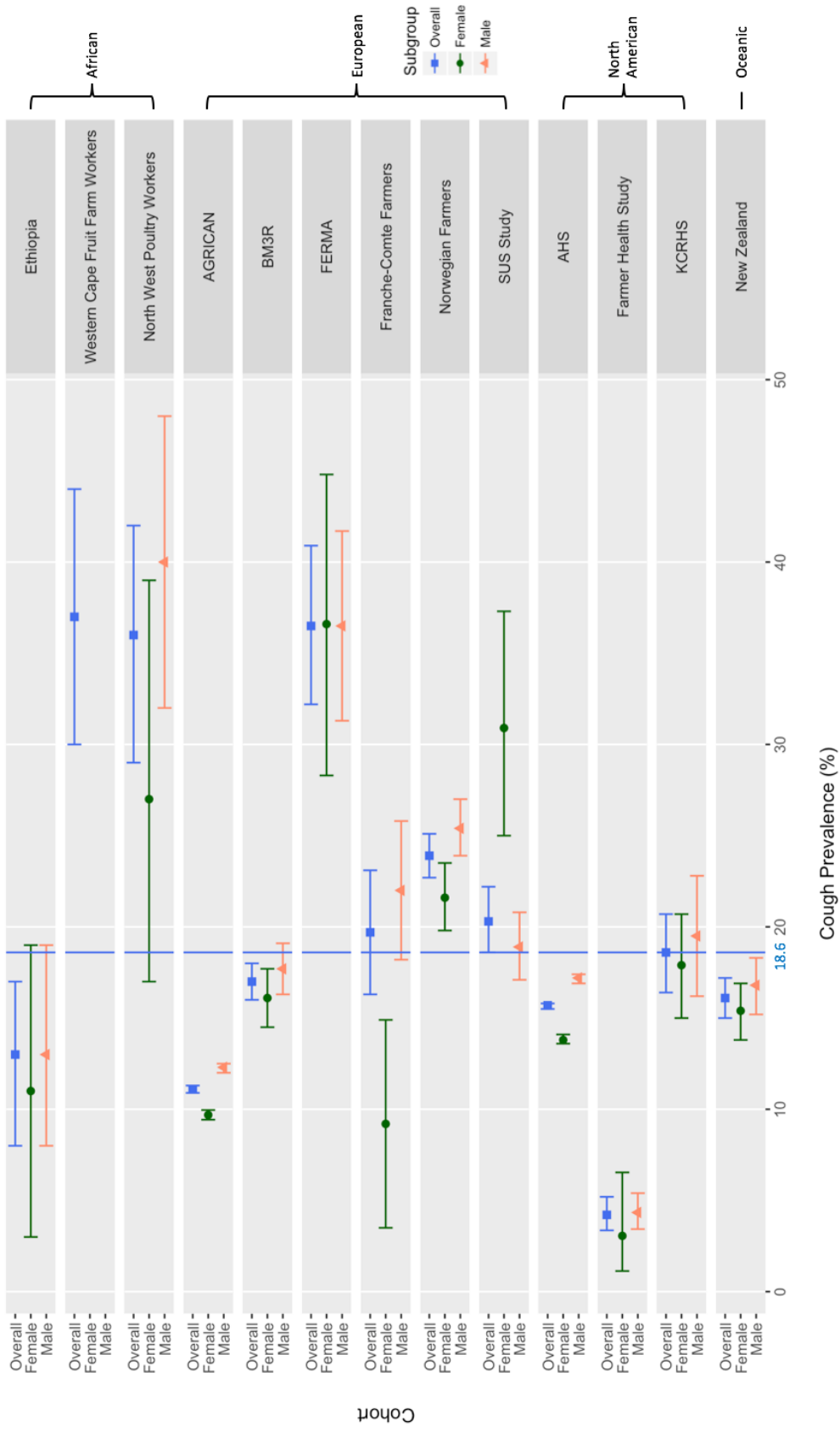
KMCC, Korean Multi-center Cancer Cohort ; AHS, Agricultural Health Study; GDMSP, Grain Dust Medical Surveillance Program; KCRHS, Keokuk County Rural Health Study

Figure 1: World map of included AGRICOH Countries and U.S. States

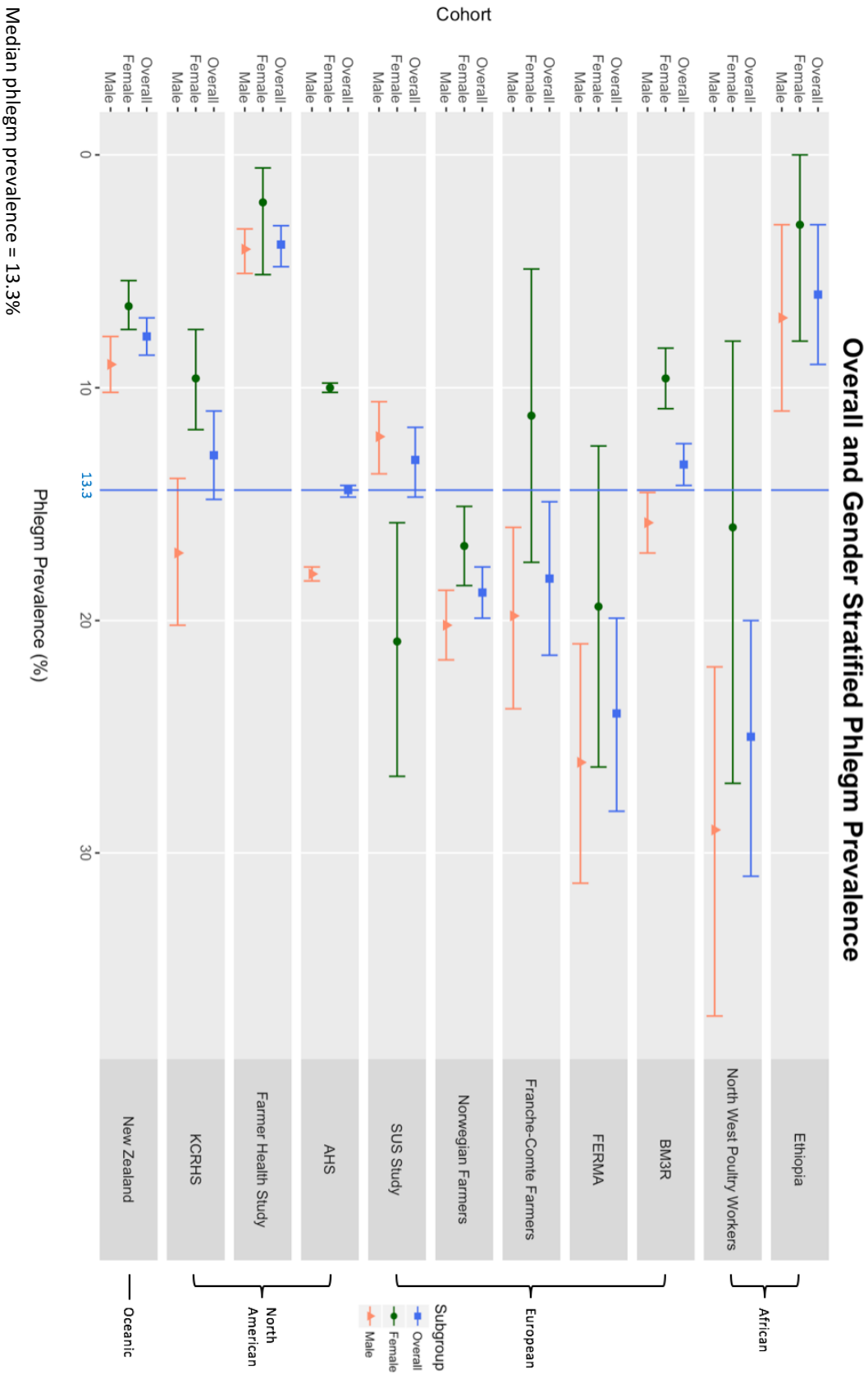




**Figure 2a: Overall and gender-stratified cough prevalence among AGRICOH cohorts**



**Figure 2b: Overall and gender-stratified phlegm prevalence among AGRICOH cohorts**



**Figure 2c: Overall and gender-stratified wheeze prevalence among AGRICOH cohorts**

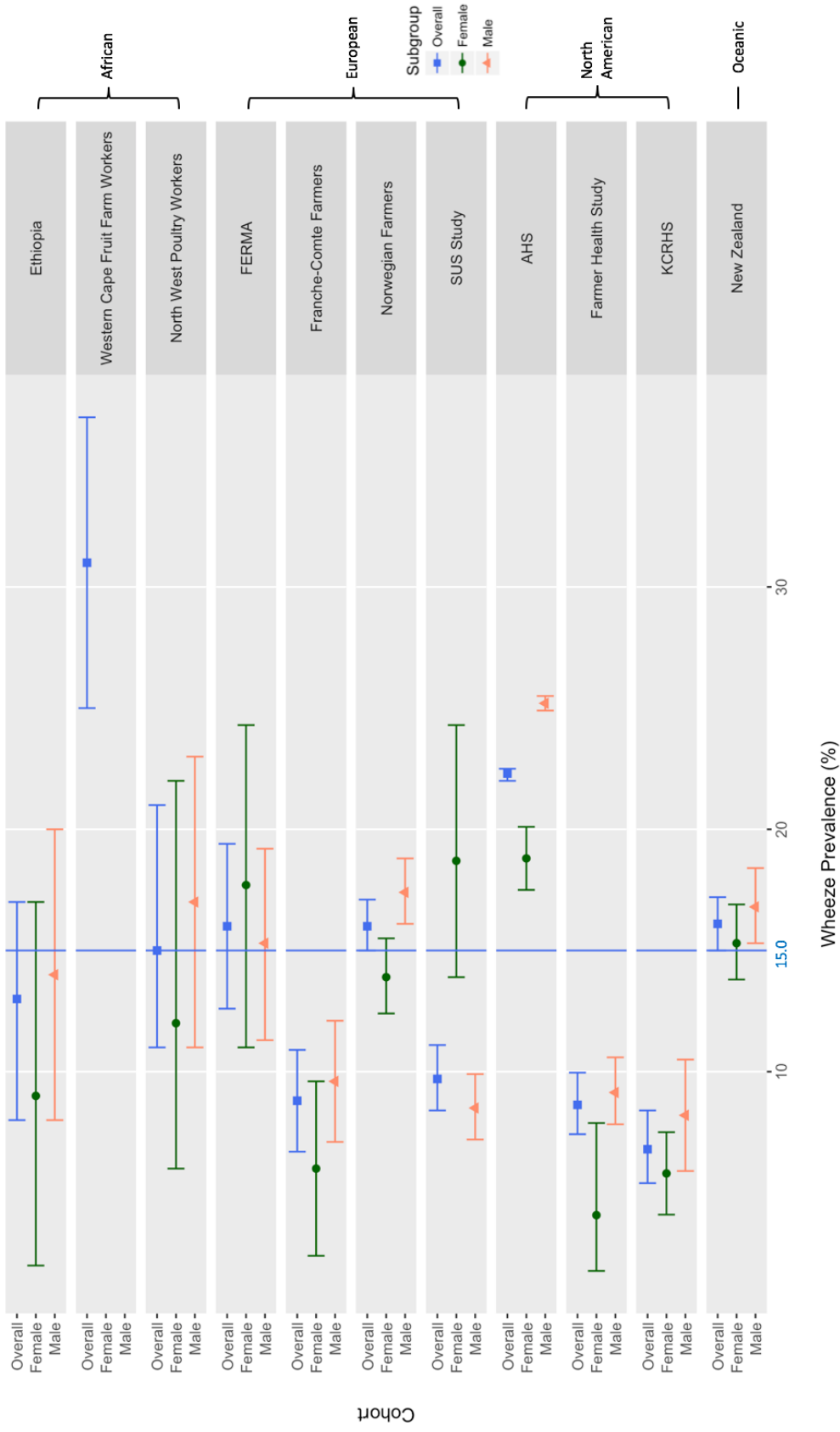
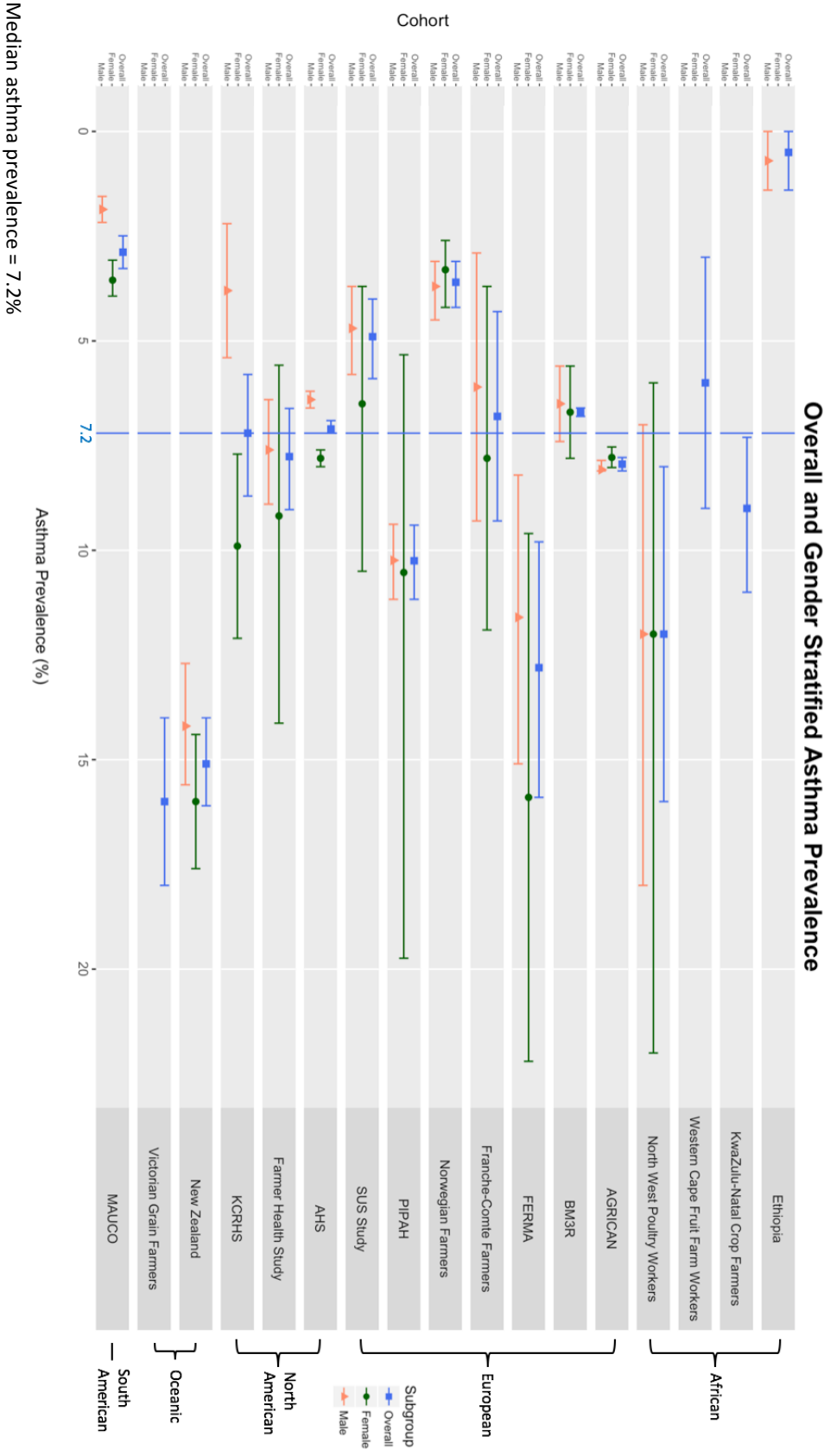
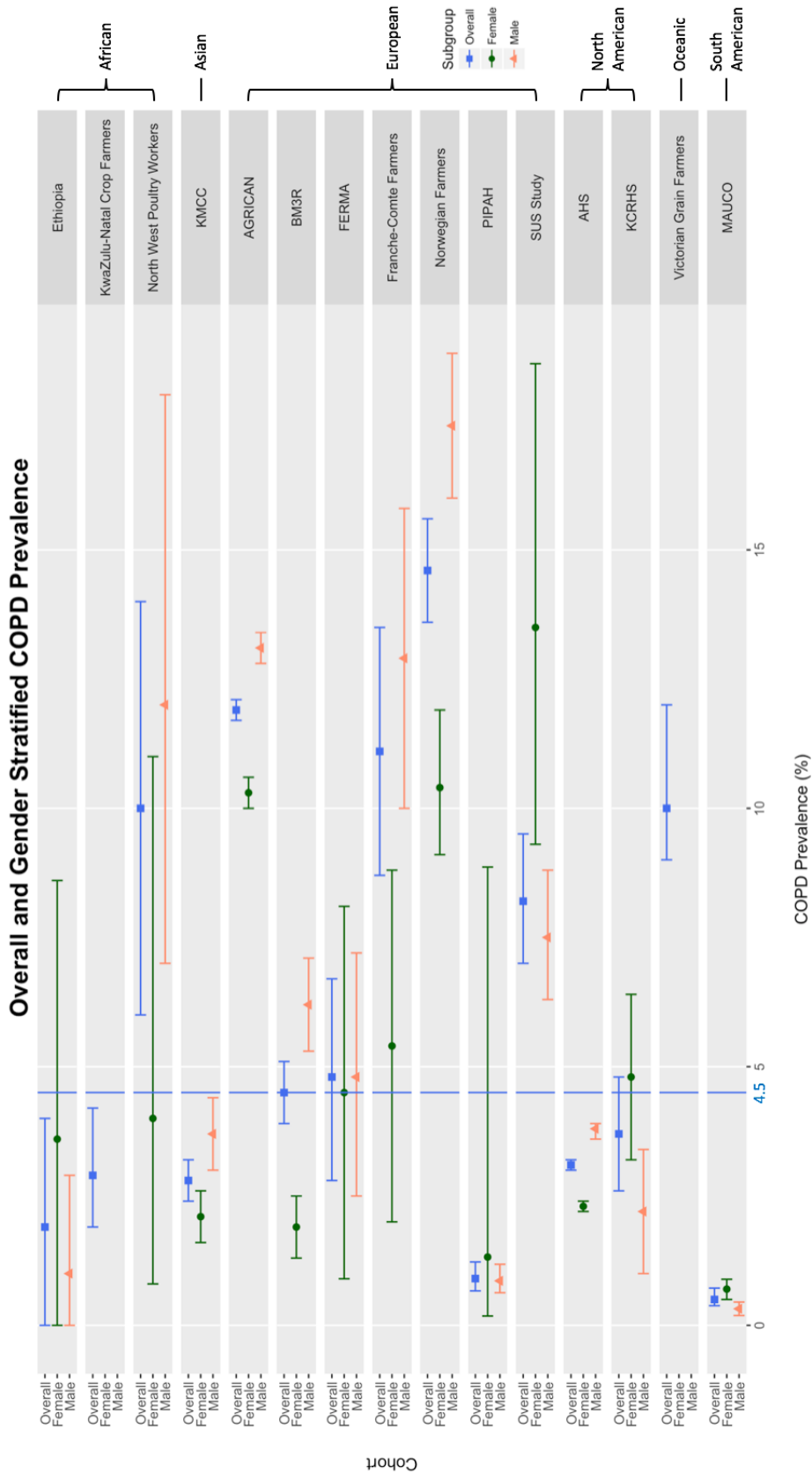


Figure 3a: Overall and gender-stratified asthma prevalence among AGRICOH cohorts

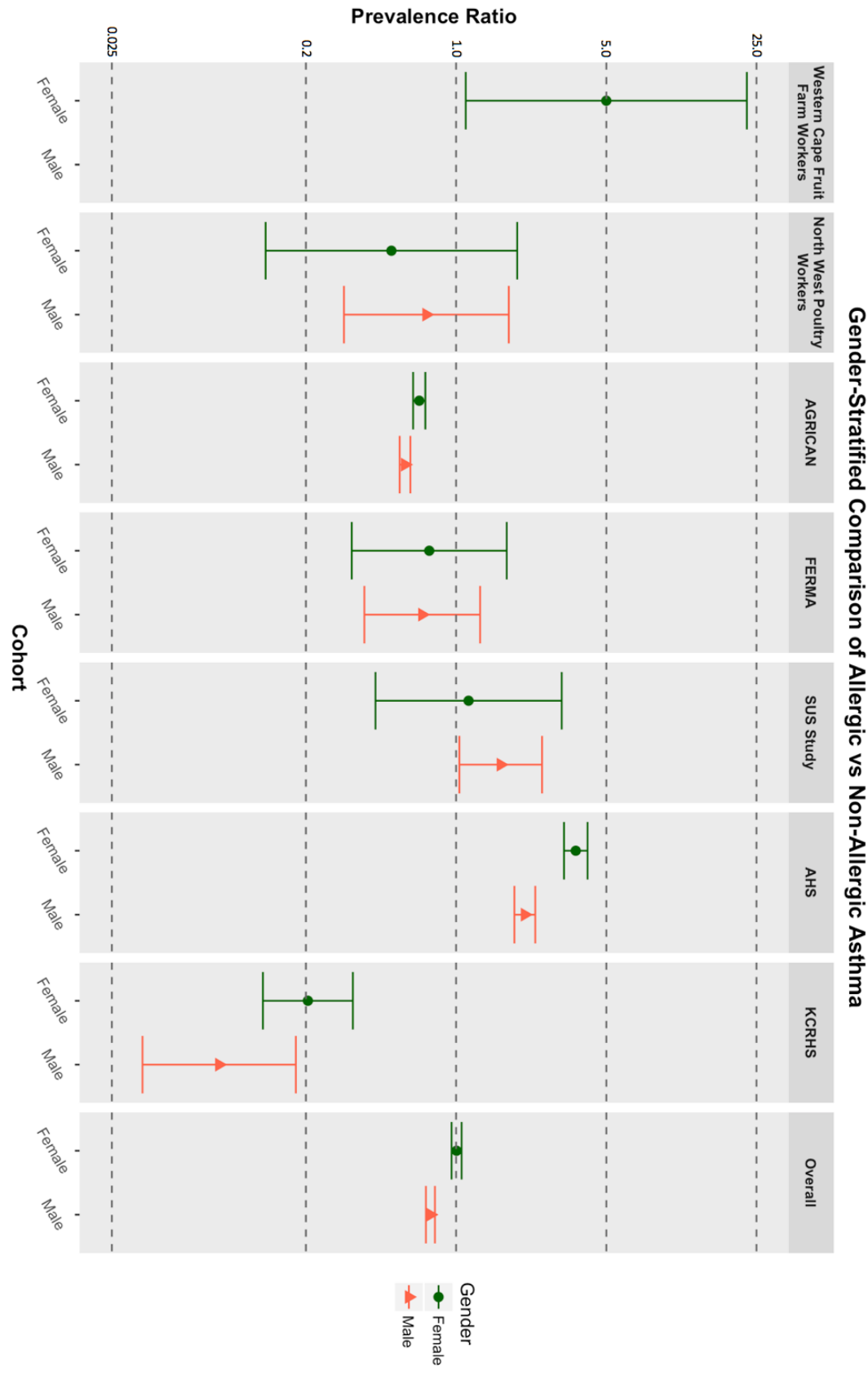


**Figure 3b: Overall and gender-stratified COPD prevalence among AGRICOH cohorts**



COPD, chronic obstructive pulmonary disease  
 COPD definitions included in supplement 1  
 Median COPD prevalence = 4.5%

**Figure 4: Gender-stratified ratio of allergic vs. non-allergic asthma within AGRICOH cohorts**



Values >1 indicate a higher prevalence of allergic asthma  
 Definitions of allergic vs non-allergic asthma provided in supplement 1

**Supplement 1: Outcome definitions for all investigated respiratory diseases and symptoms for each AGRICOH cohort.**

Cohort	Asthma				Cough	Phlegm	Wheeze	COPD
	Overall	Allergic	Non-Allergic					
Ethiopia	Self-reported doctor diagnosis of asthma				Self-reported usual cough for at least three months in past year	Self-reported usual cough producing phlegm for at least three months in past year	Self-reported wheeze, without cold, in past year	Mild airflow limitation in which FEV1 / FVC is < 70% and FEV1 is ≥ 80% of the predicted value
KwaZulu-Natal	Self-reported doctor diagnosis of asthma							Self-reported treatment of chronic bronchitis
Western Cape Fruit Farm Workers	Self-reported history of asthma attack in past year or currently taking asthma medication	Self-reported history of asthma attack in past year or currently taking asthma medication and positive Phadiatop test (>0.35 KU/L)	Self-reported history of asthma attack in past year or currently taking asthma medication and negative Phadiatop test (>0.35 KU/L)		Self-reported being woken by an attack of cough in past year		Self-reported wheeze or whistling in past year	
North West Poultry Workers	Self-reported history of asthma attack in past year or currently taking asthma medication or positive bronchodilator test (increase in FEV1 ≥ 12% and ≥ 200ml increase compared to baseline)	Self-reported history of asthma attack in past year or currently taking asthma medication or positive bronchodilator test (increase in FEV1 ≥ 12% and ≥ 200ml increase compared to baseline) and presence of atopy or FeNO > 50ppb	Self-reported history of asthma attack in past year or currently taking asthma medication or positive bronchodilator test (increase in FEV1 ≥ 12% and ≥ 200ml increase compared to baseline) and absence of atopy or FeNO > 50ppb		Self-reported usual cough	Self-reported usual phlegm	Self-reported wheeze or whistling in past year	FEV1/FVC ratio post-bronchodilator increase or below 5th percentile or reporting symptoms suggestive of bronchitis (defined as “cough and phlegm on most days/nights for 3 months or more per year during the last 2 years”)
KMCC		Self-reported history of asthma, rhinitis, and other allergic diseases						Self-reported doctor diagnosed chronic bronchitis

Cohort	Asthma						Cough	Phlegm	Wheeze	COPD
	Overall	Allergic	Non-Allergic							
AGRICAN	Self-reported doctor diagnosis of asthma	Self-reported doctor diagnosis of asthma with hay fever and/or eczema	Self-reported doctor diagnosis of asthma without hay fever and/or eczema	Self-reported doctor diagnosis of chronic bronchitis					Self-reported doctor diagnosis of chronic bronchitis or emphysema	
BM3R	Self-reported doctor diagnosis of asthma			Self-reported usual morning cough and/or being woken by an attack of cough in past year	Self-reported usual sputum			FEV1/FVC < 0.70 post bronchodilator test at spirometry associated with either dyspnea, chronic cough or chronic sputum		
FERMA	Self-reported doctor diagnosis of asthma	Self-reported doctor diagnosis of asthma with hay fever	Self-reported doctor diagnosis of asthma without hay fever	Self-reported usual cough in the past year without cold	Self-reported phlegm in past year	Self-reported wheeze in past year		Self-reported doctor diagnosis of COPD		
Franche-Comte Farmers	Self-reported history of asthma			Self-reported usual morning cough and/or being woken by an attack of cough in past year	Self-reported usual sputum	Self-reported wheeze in past year		FEV1/FVC < 0.70 post bronchodilator test at spirometry associated with either dyspnea, chronic cough or chronic sputum		
Norwegian Farmers	Self-reported doctor diagnosis of asthma			Self-reported usual cough	Self-reported usual phlegm	Self-reported ever wheeze		At or below 5% lower limit of normal spirometry test result		
PIPAH	Self-reported doctor diagnosis of asthma							Self-reported doctor diagnosis of COPD		
SUS Study	Self-reported doctor diagnosis of asthma	Self-reported doctor diagnosis of asthma and positive response to skin prick test	Self-reported doctor diagnosis of asthma without positive response to skin prick test	Self-reported usual cough	Self-reported usual phlegm	Self-reported ever wheeze				
Agricultural Health Study	Self-reported doctor diagnosis of asthma	Self-reported doctor diagnosis of asthma with hay fever	Self-reported doctor diagnosis of asthma without hay fever	Self-reported usual cough	Self-reported usual phlegm	Any episode of wheeze in the past 12 months		Self-reported doctor diagnosis of COPD, emphysema, or chronic bronchitis		



Cohort	Asthma		Cough	Phlegm	Wheeze	COPD
	Overall	Allergic				
Farmer Health Study	Self-reported history of asthma		Self-reported usual cough most days for the week for at least three months in the past year	Usual phlegm for at least three consecutive months of the year for greater than two years or your life	Self-reported wheeze most days or nights, with or without cold	
Keokuk County	Self-reported doctor diagnosis of asthma	Self-reported doctor diagnosis of asthma and three or more positive responses to a battery of allergens administered in a skin prick test.	Self-reported usual cough	Self-reported usual phlegm	Self-reported wheeze most days or nights	Self-reported doctor diagnosis of chronic bronchitis
New Zealand	Self-reported history of asthma		Self-reported usual cough in past year	Self-reported usual phlegm in past year	Self-reported wheeze or whistling in past year	Self-reported history of chronic bronchitis
Victorian Grain Farmers	Self-reported history of asthma					Self-reported history of COPD
MAUCO	Self-reported doctor diagnosis of asthma					Self-reported doctor diagnosis of COPD

FEV1, forced expiratory volume in one second  
FEC, forced vital capacity  
FeNO, fractional exhaled nitric oxide  
COPD, chronic obstructive pulmonary disease

Supplement 2: Cohort Characteristics Summary Table

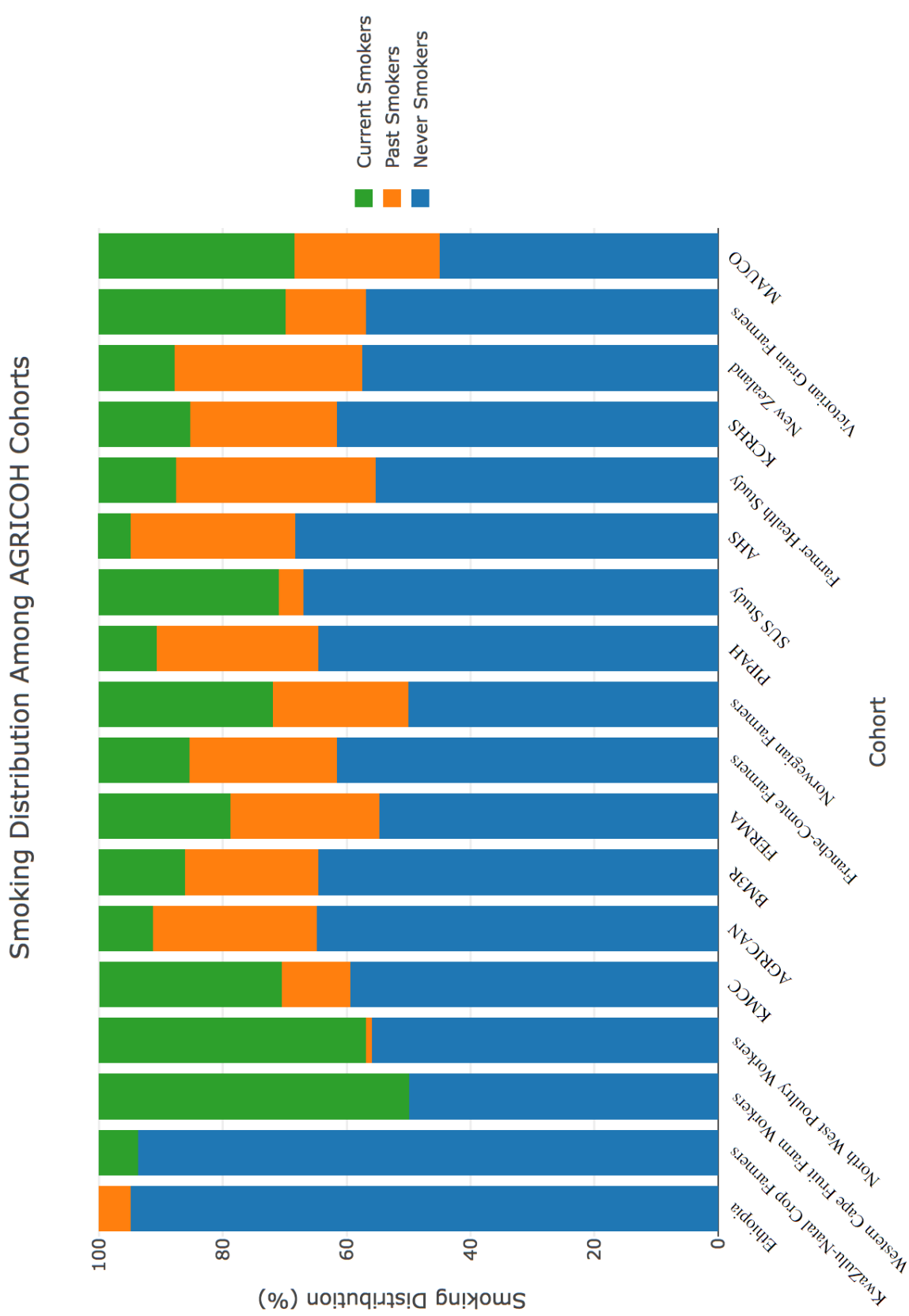
Cohort	Participant Types				Women in Cohort				Livestock							Type of Farming				
	Farm Owner/ Operator	Farm Workers	Subsistence Farmers	Rural Residents	Full time Farmers	Help on Farm	Spouses	Poultry	Beef Cattle	Dairy Cattle	Pork	Other	Crops				Grain Handling			
													Row Crops	Vegetables	Orchard	Other				
Ethiopia	Y	Y	N	Y	N	N	N	N	N	N	N	N	N	Y	N	N	Y	N		
Kwazulu-Natal Crop Farmers	Y	Y	N	Y	Y	Y	Y	N	N	N	N	N	N	Y	Y	N	N	N		
Western Cape Fruit Farm Workers	N	Y	N	Y	Y	N	Y	N	N	N	N	N	N	N	N	Y	N	N		
North West Poultry Workers	N	Y	N	N	Y	N	N	N	N	N	N	Y	N	N	N	N	N	N		
KMCC	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
AGRICAN	Y	Y	N	N	Y	N	N	Y	NR*	NR*	Y	Y	Y	Y	Y	Y	Y	Y		
BM3R	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		
FERMA	Y	Y	N	Y	Y	NR	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N		
Franche-Comte Farmers	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	N	N	N	N	N	N	Y		
Norwegian Farmers	Y	N	N	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		
PIPAH	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		
SUS Study	Y	Y	NR	Y	Y	NR	NR	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y		
AHS	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		
Farmer Health Study	Y	N	N	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		
KCRHS	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		
New Zealand	Y	N	N	N	Y	N	Y	N	Y	N	N	Y	Y	Y	Y	N	N	N		
Victorian Grain Farmers	Y <sup>+</sup>	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	NR	NR	NR	Y		
MAUCCO	Y	Y	N	Y	Y	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y		
Proportion with Characteristic	83%	67%	14%	56%	83%	38%	65%	61%	59%	65%	56%	67%	67%	65%	59%	59%	59%	61%		

Y, yes; N, no; NR, information not requested

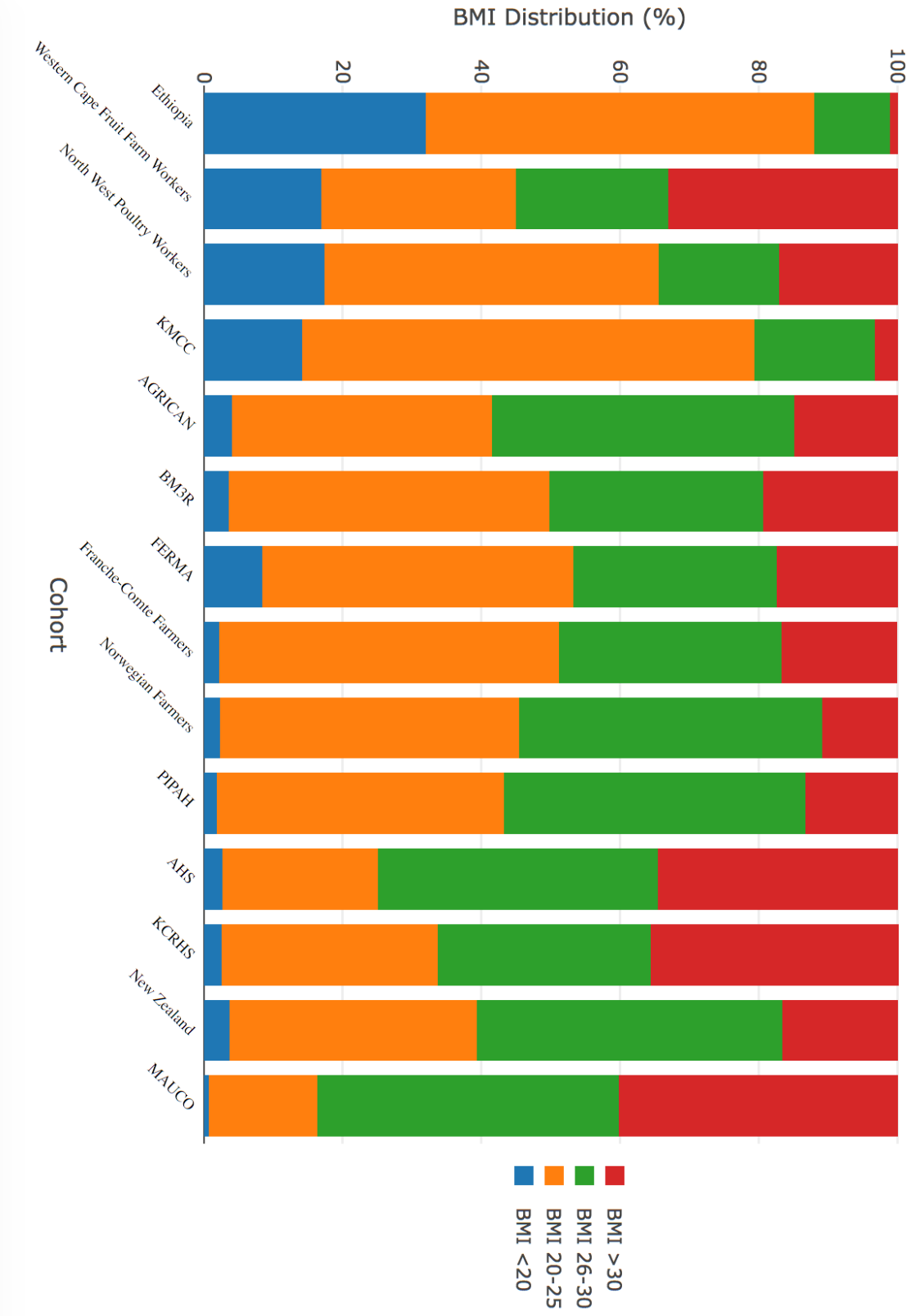
\* Cattle farming reported indistinctly

<sup>+</sup> All VGF cohort members were male financial members of the Victorian Farmers Federation Grain Commodities Interest Group

Supplement 3: Smoking distribution among AGRICOH cohorts



BMI Distribution Among AGRICOH Cohorts



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## **B. BPCO secondaire à l'exposition aux poussières organiques: caractérisation pulmonaire**

### **1. Article 4 : Quantitative and qualitative evaluation of spirometric testing for COPD screening in general practice**

#### *a. Contexte et objectif*

Le diagnostic de la BPCO reste un problème de santé public majeur. En effet, on considère que seulement 20 à 30% des sujets atteints de BPCO ont été effectivement identifiés et qu'à l'inverse, plus de la moitié des sujets chez qui un diagnostic de BPCO a été retenu ne sont en réalité pas atteints. L'identification d'un trouble ventilatoire obstructif persistant par la réalisation d'une spirométrie est une étape essentielle au diagnostic de BPCO. Néanmoins, des difficultés liées à la réalisation et à l'interprétation de la spirométrie limite son utilisation en soins primaires et notamment en médecine générale. La réalisation d'un examen de qualité est d'ailleurs un prérequis pour une bonne interprétation de celui-ci.

Dans le cadre de l'étude BALISTIC-1, nous avons mené une campagne de dépistage de BPCO incluant la réalisation d'une spirométrie par des infirmières, des techniciens et des médecins généralistes ayant reçu une formation préalable. Parmi les sujets dépistés, environ 400 ont bénéficié d'une spirométrie de confirmation dans le service d'exploration fonctionnelle respiratoire

L'objectif de cette étude était d'évaluer la concordance entre les résultats de spirométrie obtenue lorsqu'elle était réalisée par une infirmière, un technicien ou un médecin généraliste et de celle réalisée dans un service d'explorations fonctionnelles respiratoires.

#### *b. Manuscrit*

Article "Quantitative and qualitative evaluation of spirometric testing for COPD screening in general practice". <b>Soumagne T</b> , Guillien A, Roux P, Laplante JJ, Botebol M, Laurent L, Roche N, Dalphin JC, Degano B. <i>Respiratory Medicine and Research</i> - accepté.
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## **Quantitative and qualitative evaluation of spirometric testing for COPD screening in general practice**

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## **Abstract**

**Introduction:** Proper diagnosis of COPD remains a challenge. Performing spirometry in primary care may help to improve COPD misdiagnosis. Assessment of the reliability of spirometry is a prerequisite for its use as an instrument for diagnosis.

**Objectives:** To investigate (1) the validity of spirometries performed in primary care and (2) the accuracy of the diagnostic of airflow limitation obtained through these spirometries.

**Methods:** Subjects attending a COPD screening program had a “screening spirometry” performed either by general practitioners (GPs) or by trained nurses or technicians, all having received two 3-hours training sessions. Subjects with airflow limitation and a subset of subjects with normal spirometry at screening were invited to have a “confirmatory spirometry” performed by certified nurses in a pulmonary function laboratory.

**Results:** Among the 4610 subjects who attended the “screening” sessions, 96.5% had a valid “screening spirometry”. A total of 392 subjects attended the “confirmation” sessions. Values measured by “screening spirometries” were satisfactory compared with “confirmatory spirometries” ( $\rho_c = 0.83$ ). With “confirmatory spirometry” taken as reference, the positive predictive value of “screening spirometries” for the diagnosis of persistent airflow limitation was 93% and a specificity of 95%. Agreement for the diagnosis of persistent airflow limitation was substantial ( $\kappa = 0.80$ ).

**Conclusion:** A strategy consisting in performing spirometries in primary care by trained caregivers allows identification of persistent airflow limitation with a good performance. This may encourage pulmonologists to collaborate with primary care settings with the aim to improve an appropriate diagnosis of COPD.

**Key words:** COPD, airflow limitation, screening, spirometry, nurses, general practitioners

**Highlights:**

- Approximately 70% of COPD worldwide may be under-diagnosed. Conversely, between 30-60% of patients with a previous physician diagnosis of COPD do not actually have the disease and hence have been over-diagnosed.
- Performing spirometries in primary care allows identification of persistent airflow limitation with a good performance.
- Collaboration with primary care settings may improve the appropriate diagnosis of COPD.

**Author Contributions:**

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

- BD and JCD made substantial contributions to the conception and design of the work.
- AG and TS performed all analyses.
- JCD and BD obtained study funding.
- All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work.
- BD wrote the first draft of the manuscript. BD, TS, AG, PR, JLL, MB, LL, NR and JCD revised the manuscript for important intellectual content.
- All authors approved the manuscript.

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## Introduction

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease that develops in a subset of subjects exposed to noxious particles or gases [1, 2]. COPD is one of the leading causes of morbidity and mortality worldwide and results in an increasing economic and social burden [3]. Appropriate diagnosis of COPD is a prerequisite that allows modifying exposure to risk factors and initiating appropriate therapy among those who have the disease on the one hand, and avoiding unnecessary costs and potential side effects of treatments among those who do not on the other [4, 5]. Nevertheless, diagnosis of COPD remains challenging, and as few as 20-30% of those who have COPD are actually identified, whereas more than half of patients who receive a diagnosis of COPD do not actually have the disease [6-8].

Spirometry is the cornerstone of the COPD diagnosis, and appropriate application of spirometric testing may reduce the number of undetected COPD cases as well as diagnostic misclassification [9]. However, even in countries with developed health services, it is not feasible to refer all at-risk subjects to a respiratory specialist for performing high-quality spirometry [10]. Other options should therefore be considered, such as performing spirometry in primary care, either by general practitioners (GPs) or by trained technicians or nurses. In all cases, the reliability of spirometric tests is a prerequisite for their use as an instrument for diagnosis for a patient's perspective as well as for epidemiological purposes. Some concerns have been raised regarding this reliability. Schermer *et al.* have reported a small but statistically significant difference in lung function values between spirometry performed in general practice and those performed in a pulmonary function laboratory [11]. Lusuardi *et al.* have shown that a proportion as high as one third of patients diagnosed with COPD with office-based spirometry in primary care were in fact found to have normal spirometries when tested in a pulmonary function laboratory [12].

From September 2011 to December 2014, we performed a large COPD screening program among subjects exposed either to tobacco smoking and/or to organic dusts (BalistiC study; ClinicalTrials.gov Identifier: NCT02540408) [13]. Screening spirometries were performed either by trained technicians/nurses or by trained GPs, and a subset of subjects who underwent screening had spirometries repeated in a pulmonary function laboratory (confirmatory spirometries). The main objective of the current study was to assess the extent to which the results of screening spirometries corresponded with the results of the same tests performed in a pulmonary function laboratory. We also aimed to compare reproducibility of spirometries performed by technicians/nurses with those performed by GPs. Finally, we compared the diagnostic accuracy for the diagnosis of airflow limitation between screening spirometries and confirmatory spirometries.

## **Methods**

### ***Study population***

Data for this study were collected as part of the BalistiC project [13] which was conducted at the University Hospital of Besançon in collaboration with the French national social security system for all the agricultural workers (Mutualité Sociale Agricole, MSA) and with the care homes federation of Franche-Comté (Fédération des Maisons de Santé Comtoises, FeMaSaC). This study was set up primarily to obtain data on the prevalence and on a number of specific characteristics of COPD occurring in dairy farmers (COPD secondary to organic dusts exposure) by comparing these subjects to COPD unexposed to organic dusts.

Every five years, all MSA affiliated members are invited to attend a free health check-up, and around 20 % of these invited subjects attend this health check-up [14]. Participation in the screening phase of the BalistiC study was offered to all subjects aged 40-74 years attending the health check-up and exposed either to organic dusts (dairy farmers) and/or to tobacco

smoking. Exclusion criteria were an established diagnosis of asthma, bronchiectasis, hypersensitivity pneumonitis and/or COPD, and having/having had a history of occupational exposure other than dairy farming. For these MSA affiliated members, spirometries were performed by a trained nurse or by a trained technician.

In parallel, GPs of the FeMaSaC offered to their patients fulfilling the above inclusion and exclusion criteria to take part in the screening phase of the BalistiC study. These patients were invited (for free) to attend screening sessions during which a spirometry was performed by their GPs.

A confirmatory spirometry (performed by certified nurses at the pulmonary function laboratory of the University Hospital of Besançon) was proposed to all subjects with persistent airflow limitation according to the GOLD criterion (i.e., post-bronchodilator  $FEV_1/FVC < 0.70$ ) detected during the screening phase of the BalistiC study. A confirmatory spirometry was also proposed to a subset of subjects without persistent airflow limitation and with a  $FEV_1 > 80\%$  predicted; these subjects were matched to subjects with persistent airflow limitation depending on occupational exposure, gender, age and smoking status.

Approval was received from the local Ethics Committee (CPP Est; P-2011-119), and written consent was obtained from all subjects.

### ***Spirometry***

Nurses, technicians and GPs who performed the screening spirometries all received the same training consisting in two 3-hour sessions that took place in the pulmonary function laboratory of the University Hospital Besançon. This training comprised a theoretical part on spirometry (validity and reproducibility criteria of flow-volume loop according to the ATS recommendations; identification of non-interpretable loops; analysis and interpretation of spirometric data; use of the MedGraphics software) and a practical part (use of the software,

calibration of the equipment, appropriate realization of flow-volume loops, appropriate selection of the loops, interpretation of the results).

Screening and confirmatory spirometries were performed using the same type of pneumotachograph (MedGraphics; MSE Medical, Strasbourg, France). All spirometers were calibrated at least once daily using a 3-L syringe. At the time of screening, a bronchodilator test was proposed only to subjects with a baseline airflow limitation defined by a FEV1/FVC ratio  $<0.70$ . During the screening procedures, bronchodilation was performed by administering 400  $\mu\text{g}$  of the short-acting  $\beta_2$ -agonist salbutamol (Ventoline; GlaxoSmithKline, Marly-le-Roi, France) and the spirometry was repeated after a 10- to 15-min delay, as recommended (7). For confirmation, bronchodilation was performed with nebulisation of terbutaline (5 mg/2 mL) and ipratropium bromide (0.5 mg/2 mL) for 15-min, and the spirometry being repeated after a 15- to 20-min delay. All subjects who attended the confirmatory sessions (i.e., those with and those without airflow limitation) had a bronchodilator test.

Technicians, nurses, GPs and pulmonary function specialists were asked to perform at least 3 reproducible forced manoeuvres, that is, reproducibility of  $<5\%$  and  $<200$  ml between the two highest FEV1 values [15]. Validity of the spirometries according to these criteria was reviewed on actual traces by a pulmonologist (PR, TS or BD). Only subjects with valid spirometries at screening were further included in the BalistiC study and therefore had a confirmatory spirometry.

### ***Statistical analysis***

Data are presented as size group (percent) and mean  $\pm$  standard deviation for qualitative and quantitative variables, respectively.

For chosen spirometric parameters (FEV1, FVC, FEV1/FVC ratio, peak expiratory flow

[PEF] and mean expiratory flow between 25 % and 75% of FVC [MEF25-75]) measured pre-bronchodilator, agreement between screening and confirmatory spirometries was assessed using Bland-Altman plots and Lin's concordance coefficient ( $\rho_c$ ). In addition, paired t tests were performed to analyse the within-subjects' differences for each parameter between the two measurements (screening vs. confirmation). Finally, Pearson correlation coefficients were calculated to assess association between time elapsed between the two measurements on the one hand and the discrepancy between the two measurements on the other.

For the diagnosis of airflow limitation, comparison between the two measurements (screening vs. confirmation) was performed using the Chi-squared test. Diagnosis of airflow limitation made at confirmation was taken as reference, and positive predictive value and specificity of screening for the diagnosis of airflow limitation were calculated. Agreement between diagnosis of airflow limitation at screening and at confirmation was analysed using Cohen's kappa ( $\kappa$ ).

A *p*-value less than 0.05 was considered statistically significant. All data were analysed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

## **Results**

### ***Subjects' enrolment***

The flowchart of the participating population is displayed in Figure 1. Of the 4610 subjects who underwent COPD screening, 4447 (96.5%) had a spirometry of acceptable quality. Among these 4447 subjects, 392 (8.8% of those who had a valid spirometry) were invited to perform a confirmation spirometry, including 197 with pre-bronchodilator FEV1/FVC<0.70 and 195 frequency-matched controls without pre-bronchodilator airflow limitation at screening.

### ***Population characteristics***

The main characteristics of the 392 participants are shown in Table 1. Compared to those who entered the study *via* the MSA, subjects who entered the study *via* the FeMaSaC were more frequently current smokers, and were less frequently men.

Among subjects with pre-bronchodilator FEV1/FVC<0.70 at screening, those who entered the study *via* the MSA had higher FEV1 (80 ± 15 % predicted *vs.* 70 ± 14 % predicted, respectively, p<0.001) and had more often a FEV1 > 80 % predicted (61 % *vs.* 30 %, respectively, p<0.001) than those who entered the study *via* the FeMaSaC. However, the frequency-matched control subjects without airflow limitation at screening who entered the study *via* the MSA had similar FEV1 compared to those who entered the study *via* the FeMaSaC (104 ± 11 % predicted *vs.* 104 ± 13 % predicted, respectively, p=0.96).

### ***Comparison of spirometries performed at screening versus confirmation***

Comparisons of spirometric values measured pre-bronchodilator at screening and at confirmation are shown in Table 2. Peak expiratory flow (PEF) and FVC were similar at screening and at confirmation, while FEV1 was higher at screening than at confirmation, resulting in a mean value of FEV1/FVC ratio that was lower by a value as high as 3.3 % at confirmation than at screening. These differences between screening and confirmation were found similar in subjects recruited *via* the FeMaSaC and *via* the MSA. Agreements between the two measurements for all spirometric values are illustrated in Figure 2. Lin's concordance coefficient was almost perfect ( $\rho_c = 0.83$ ).

Differences between screening and confirmation spirometries were not significantly correlated with the time elapsed between the two measurements ( $r = -0.13, -0.11, 0.03, 0.01$  and  $-0.18$  for pre-bronchodilator  $\Delta$ FVC,  $\Delta$ FEV1,  $\Delta$ FEV1/FVC,  $\Delta$ PEF and  $\Delta$ FEF25-75, respectively).



As shown in Table 3, differences between screening and confirmation were similar for subjects recruited *via* the MSA (for whom screening spirometries were performed by nurses or by technicians) and for subjects recruited *via* the FeMaSaC (for whom screening spirometries were performed by general practitioners).

### ***Agreement for the diagnosis of airflow limitation***

All 392 subjects had pre-bronchodilator spirometry both at screening and at confirmation. Among them, 197 had pre-bronchodilator airflow limitation at screening and 209 had pre-bronchodilator airflow limitation at confirmation spirometry. With confirmation spirometry taken as reference, 86% of subjects received an appropriate diagnosis of pre-bronchodilator airflow limitation at screening, with a positive predictive value of 91% and a specificity of 91%. Agreement for the diagnosis of pre-bronchodilator airflow limitation was substantial ( $\kappa = 0.77$ ).

Unfortunately, 31 subjects who had a pre-bronchodilator airflow limitation at screening did not have a post-bronchodilator spirometry performed during the screening sessions. Among the 361 remaining subjects, 158 had post-bronchodilator (i.e., persistent) airflow limitation at confirmation spirometry and 203 did not. With confirmation spirometry taken as reference, the positive predictive value of screening spirometry for the diagnosis of persistent airflow limitation was 93%, with a specificity of 95%. Agreement for the diagnosis of persistent airflow limitation was substantial ( $\kappa = 0.80$ ).

## **Discussion**

The results of the current study indicate that values measured by spirometries performed for COPD screening in primary care were satisfactory in comparison with spirometries performed in a reference pulmonary function laboratory. The current results also indicate that

measurements made for screening purpose by nurses/technicians on the one hand, and by GPs on the other (all having the same training programme for spirometry) were equivalent. Finally, with the strategy that consisted in performing a bronchodilator test at screening only in subjects with pre-bronchodilator airflow limitation, there was a good agreement for the diagnosis of persistent airflow limitation between screening and confirmation.

Diagnosis of COPD remains challenging, with a minority of patients who have COPD who are actually identified and a vast proportion of subjects who receive a diagnosis of COPD who are misdiagnosed [6, 7]. Appropriate identification of persistent airflow limitation is essential test for making a diagnosis of COPD [16]. An important factor contributing to COPD misdiagnosis (that is, either under-diagnosis or over-diagnosis) is the under-utilization of spirometry, particularly in the primary care setting [7, 16]. In addition, lack of expertise in performing and interpreting spirometries limits their use and their usefulness in primary care [16]. Nevertheless, given the burden of COPD, most diagnosis are likely to take place in the primary care setting, thus reinforcing the need for an appropriate evaluation of spirometries performed outside of a pulmonary function laboratory.

A prerequisite for interpretation of spirometry is evaluation of test quality. Some studies conducted over the last decade found that the quality of spirometry in primary care was low, with up to one third of tests of unacceptable quality [10, 17]. By contrast, in a study recently conducted in the UK, the quality of spirometries undertaken in primary care was high, with more than 96% of tests of acceptable quality [18]. Here, we also found that a vast majority (96.5%) of the screening spirometries (performed by non-specialists) were of acceptable quality.

There are no legal restrictions on who can perform spirometries [19]. At least in France, the labour cost (for training and for testing) is lower when spirometries are performed by nurses or technicians rather than by physicians. With the aim to diminish the cost of spirometry

testing, we aimed to investigate whether or not nurses or technicians on the one hand, and general practitioners on the other hand, all with the same training courses, would perform spirometries of the same technical quality. We report here for the first time that it was obviously the case.

Differences between spirometries performed in two different settings in a single subject may be due to the use of different types of spirometers at these two occasions [20]. As we wished to minimise any potential bias in the comparison between screening and confirmatory tests, we chose to equip screening sites and laboratories with the same type of spirometers. These spirometers were pneumotachographs. Although pneumotachographs have to be calibrated at least once daily (and that could be considered as a disadvantage), they give highly reproducible measurements, contrarily to turbine spirometers that may produce spirometric values which diverge from the advanced equipment used in pulmonary function laboratories [21]. Nevertheless, values measured by “screening spirometries” (in primary care) were not interchangeable with values measured by “confirmatory spirometries”, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and MEF<sub>25-75</sub> being all significantly lower (by 3-5 %) at confirmation than at screening; this has resulted in the identification of persistent airflow limitation at confirmatory spirometry in subjects primarily considered to have normal spirometry. By contrast, higher values of FEV<sub>1</sub>, but also of FVC, have previously been reported for measurements made in general practice compared with pulmonary function laboratories measurements; this had been interpreted as a flaw of pulmonary function laboratories [22]. In our hands, as volumes (especially FVC) were similar at the two measurements (screening and confirmation), calibrations of the spirometers were very unlikely to account for the differences found in flows. One possible explanation may relate to a “more forced” expiratory manoeuvres for the spirometries performed in pulmonary function laboratories than by nurses or technicians – this “more forced” manoeuvre being considered “more appropriate” and not “flawed”. Of note, in our hands,

differences between screening and confirmation were similar for screening spirometries performed by nurses/technicians and by general practitioners.

To perform or not a bronchodilator test for every subject at screening spirometry is questionable. Keeping in mind that only results of post-bronchodilator spirometries are appropriate for the diagnosis of COPD, this question is of importance. We have shown elsewhere that among non-selected subjects who had a systematic spirometry performed for COPD screening,  $\approx 8\%$  had pre-bronchodilator airflow limitation with the GOLD criterion (fixed cut-off of 0.70 for the FEV1/FVC ratio) and only  $\approx 4\%$  had persistent airflow limitation [2]. Here, although our strategy that consisted in performing a bronchodilator test only in subjects with pre-bronchodilator airflow limitation resulted in a specificity of 95%; it avoided at least 90% of bronchodilator tests that would have been inappropriate.

### **Limitations of the study**

We acknowledge that our study has some limitations. First, randomisation of the order in which screening and laboratory tests took place would have been the preferred methodological approach. However, because the current analysis is ancillary to the BalistiC study, the order of the tests was dictated by the study protocol who was designed to identify subjects with persistent airflow limitation close to their home. It is therefore not possible to rule out the possibility of a systematic bias due to natural variability in lung function. In addition, short term intra-individual variability such as airway reactivity and diurnal variation in lung function may also have influenced our findings. Second, subjects who had a confirmatory spirometry were not randomly selected: a confirmatory spirometry was performed in the subset of subjects with post-bronchodilator FEV1/FVC $<0.70$  at screening who accepted to attend a visit at our PFTs lab and in a selected subset of matched subjects without persistent airflow limitation (matched to subjects with persistent airflow limitation

depending on occupational exposure, gender, age and smoking status). The selection of matched subjects was also based on the presence of a “normal” FEV1 (i.e., > 80% predicted). Therefore, our analysis cannot rule out a poor reproducibility of spirometry in subjects with a restrictive pattern (i.e., FEV1/FVC>0.70 along with FEV1<80% predicted). In addition, it did not allow us to estimate sensitivity or negative predictive value for the diagnosis of airflow limitation. Third, the BalistiC study was designed in 2011, before the publication of the reference equations of the Global Lung function Initiative (GLI) [23]. Although these equations and the related age-dependant lower limit of normal are much more appropriate especially for the identification of airflow limitation, we have decided to performed the current analyses with the equations by the European Community for Steel and Coal [24] and with the fixed cut-off of 0.70 for the definition of airflow limitation.

## **Conclusion**

The current analysis shows that a strategy consisting in performing spirometries in primary care (by nurses, technicians or general practitioners) allows identification of persistent airflow limitation with a good performance. In addition, we report for the first time that nurses/technicians and general practitioners, all having had the same 2 courses of 3-hour for spirometry teaching, performed spirometries with a similar good quality. As misdiagnosis of COPD is a frequent and harmful problem that is mainly due to underuse and misuse of spirometry, the current results may encourage pulmonologists to collaborate with primary care settings with the aim to improve an appropriate diagnosis of COPD.

## **Acknowledgement**

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**Table 1.** Main characteristics of the studied population.

	Total (n=392)	MSA (n=271)	FeMaSaC (n=121)
Age, years	60.0 ± 8.4	59.8 ± 8.7	60.7 ± 7.9
Men, n (%)	322 (82)	233 (86)	89 (74)
BMI, kg/m <sup>2</sup>	26.5 ± 4.1	26.8 ± 4.1	26.0 ± 4.2
Smoking status			
Never, n (%)	132 (34)	123 (45)	9 (7)
Former, n (%)	149 (38)	99 (37)	50 (41)
Current, n (%)	111 (28)	49 (18)	62 (51)
Number of pack-years	17.7 ± 19.9	11.8 ± 15.8	30.9 ± 21.7
Occupational exposure, n (%)	214 (55)	214 (79)	0

Data are expressed as n (%) or mean ± SD.

BMI: body mass index.

**Table 2.** Comparison of screening and confirmatory pre-bronchodilator spirometries for the 392 studied subjects.

	Screening	Confirmation	Delta	p
FEV1 (L)	3.06 ± 0.84	2.93 ± 0.80	-0.12 ± 0.27	< <b>0.001</b>
FVC (L)	4.29 ± 1.03	4.32 ± 1.00	0.03 ± 0.35	0.06
FEV1/FVC (%)	71.1 ± 9.4	67.8 ± 10.0	-3.3 ± 4.9	< <b>0.001</b>
PEF (L.s <sup>-1</sup> )	7.80 ± 2.27	7.71 ± 2.14	-0.09 ± 1.19	0.12
FEF25-75 (L.s <sup>-1</sup> )	2.35 ± 1.21	1.98 ± 1.01	-0.37 ± 0.55	< <b>0.001</b>

Data are expressed as mean ± SD.

FEV1: forced expiratory volume in 1s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF25-75: forced expiratory flow at 25-75% of FVC.

Delta = confirmatory value - screening value

**Table 3.** Comparison of differences between screening and confirmatory pre-bronchodilator values between the “MSA group” (spirometries performed by technicians or nurses) and the “FeMaSaC group” (spirometries performed by general practitioners)

	MSA (n=271)	FeMaSaC (n=121)	p
$\Delta$ FEV1 (L)	-0.11 $\pm$ 0.24	-0.15 $\pm$ 0.32	0.21
$\Delta$ FVC (L)	0.04 $\pm$ 0.34	0.03 $\pm$ 0.39	0.81
$\Delta$ FEV1/FVC (%)	-2.97 $\pm$ 4.61	-4.03 $\pm$ 5.37	0.06
$\Delta$ PEF (L.s <sup>-1</sup> )	-0.17 $\pm$ 1.17	0.07 $\pm$ 1.21	0.07
$\Delta$ MEF25-75 (L.s <sup>-1</sup> )	-0.36 $\pm$ 0.53	-0.41 $\pm$ 0.57	0.45

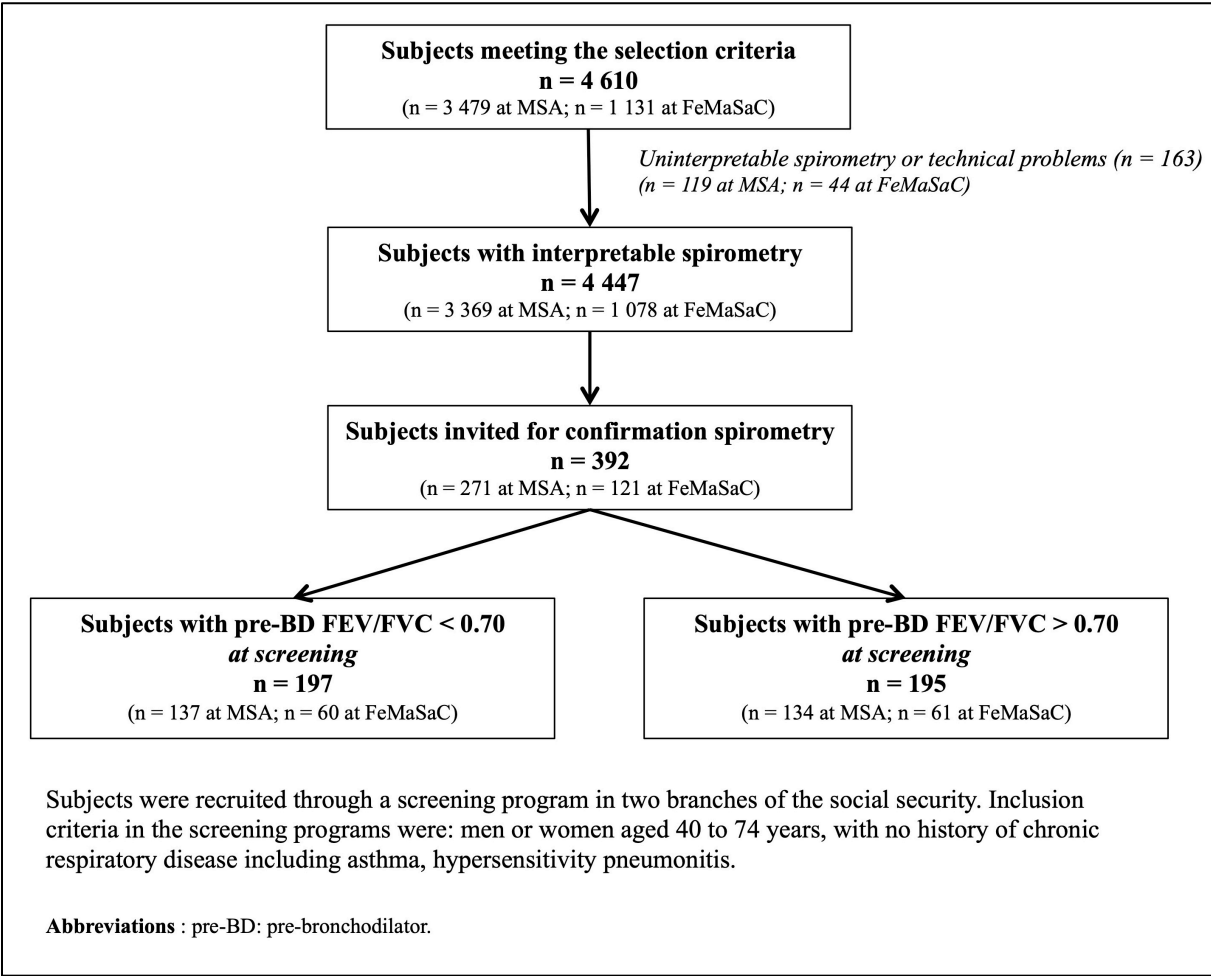
Data are expressed as mean  $\pm$  SD.

FEV1: forced expiratory volume in 1s; FVC: forced vital capacity; PEF: peak expiratory flow;

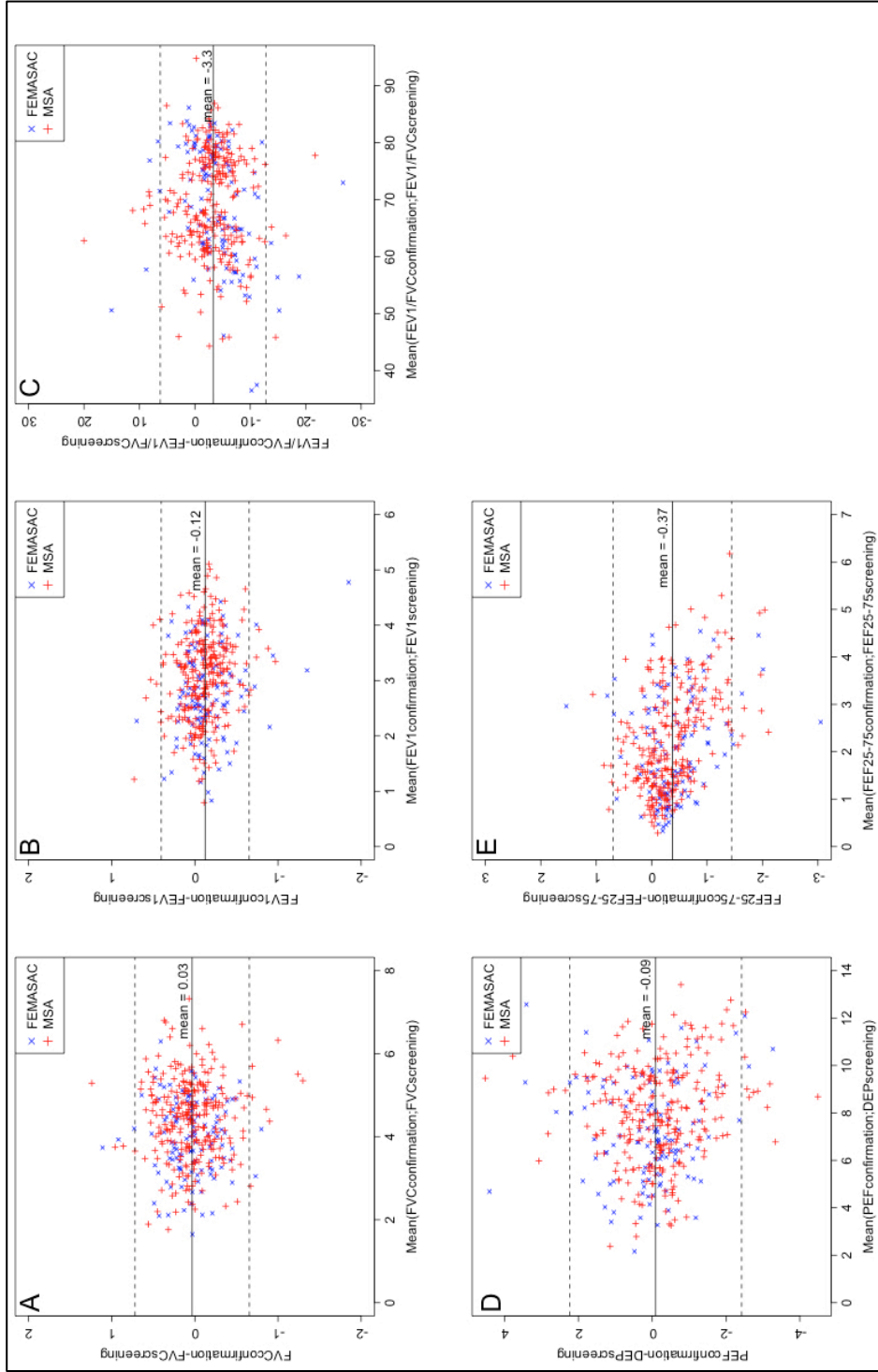
MEF25-75: forced expiratory flow at 25-75% of FVC

$\Delta$  = confirmatory value - screening value

**Figure 1. Flow chart of participants included in the study.**



**Figure 2. Bland-Altman plots of pre-bronchodilator spirometries at screening and at confirmation.**



Plots were comparing FVC (A), FEV<sub>1</sub> (B), FEV<sub>1</sub>/FVC (C), PEF (D) and FEF 25-75 (E). Dotted line represents 95% confidence interval of mean. Abbreviations: FEV<sub>1</sub>: forced expiratory volume in 1s; FVC: forced vital capacity; PEF: peak expiratory flow; FEF25-75: forced expiratory flow at 25-75% of FVC.

## 2. Article 5 : Characterization of chronic obstructive pulmonary disease in dairy farmers exposed to organic dust

### *a. Contexte et objectif*

Le développement d'une BPCO est le plus souvent secondaire à l'exposition à des substances inhalées. Bien que le tabac reste le principal facteur de risque (responsable d'environ 80 % des BPCO), on considère qu'environ 20% des cas sont attribuables à une exposition professionnelle. Plusieurs études pilotes ont indiqué que la prévalence de la BPCO était plus élevée chez les producteurs laitiers que dans la population générale bien que la prévalence du tabagisme soit plus faible. Cependant, de multiples questions demeurent quant à l'épidémiologie et à la caractérisation de cette BPCO professionnelle en milieu de production laitière.

L'objectif de cette étude était de mesurer la prévalence de la BPCO dans ce milieu professionnel spécifique, de caractériser les patients (en termes de tabagisme, de dyspnée, de qualité de vie, de fonction respiratoire, d'inflammation systémique) et de préciser les caractéristiques de leur exposition.

### *b. Manuscrit*

Article "Characterization of chronic obstructive pulmonary disease in dairy farmers exposed to organic dust" <b>Soumagne T</b> , Degano B, Guillien A, Annesi-Maesano I, Andujar P, Hue S, Jouneau S, Botebol M, Laplante JJ, Roche R, Dalphin JC. <i>En cours de soumission.</i>
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**Characterization of chronic obstructive pulmonary disease  
in dairy farmers exposed to organic dust**

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## **Abstract**

**Background:** Although farming is often considered as risk factor for COPD, there are few and conflicting data regarding prevalence and COPD characteristics in dairy farming.

**Objectives:** to assess the prevalence and to characterize COPD in dairy farmers.

**Methods:** 4788 were included in the screening phase of the study. All subjects with COPD detected during the screening phase were invited to participate in the characterization phase of the study. Those who accepted were included in two subgroups, namely dairy farmers with COPD (DF-COPD) (n = 101) and non-farmers with COPD (NF-COPD) (n = 85). Patients with COPD were matched 1:1 with subjects with normal spirometry in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and sex (namely DF-controls (n = 98) and NF-controls (n = 89)). All subjects underwent lung function and exercise testing, questionnaires and laboratory analysis.

**Results:** The prevalence of COPD in dairy farmers was of 8.0% using the GOLD criterion and 6.2% using the LLN criterion and was similar than in non-farming subjects (screening phase); DF-COPD had greater pulmonary function, exercise capacity and quality of life and less symptoms, comorbidities and systemic inflammation than NF-COPD (characterization phase). In addition, in farmers there were no relationship between COPD and occupational factors, supporting the role of host factors.

**Conclusion:** COPD secondary to organic dust exposure (*i.e.* dairy farming) might be associated with a less damaging effect on lung parenchyma and less systemic consequences than COPD secondary to tobacco smoking.

Chronic obstructive pulmonary disease (COPD) is a major health-care burden due to high morbidity and mortality and health costs it generates (1, 2). Screening, early diagnosis and appropriate management are encouraged to reduce mortality and respiratory disability and to improve the quality of life and socio-professional integration of patients (3). The prevalence of COPD is however projected to increase over the coming decades due to continued exposure to COPD risk factors and aging of the population (2). Prevention strategies are therefore warranted including first and foremost smoking cessation but also a reduction in exposure to occupational airborne contaminants (3).

It is now admitted that up to 50% of cases of COPD can be attributed to non-smoking risk factors such as indoor and outdoor air pollutants and occupational causes such as dairy farming (4, 5). Several pilot studies have indicated that the prevalence of COPD is higher among dairy farmers than in the general population (6-9), although the prevalence of tobacco smoking in dairy farmers is lower (10). An immuno-allergic and inflammatory reaction following the inhalation of organic dust contained in hay has been proposed to explain this phenomenon (11, 12). The existence of bronchial hyper-reactivity during exposure to organic particles could be an indicator associated with the onset, in the more or less long term, of COPD (11, 13). However, the current knowledge on occupational COPD in dairy farming remains scarce.

We therefore conducted a prospective cross-sectional study to (i) evaluate the prevalence of COPD in this specific professional environment, (ii) to characterize patients in terms of smoking habits, dyspnea, quality of life, pulmonary function and exercise capacity and (iii) to propose hypotheses regarding the pathophysiology and etiologic factors of COPD in dairy production.

## **METHODS**

### **Screening programs**

The BALISTIC project (COPD in dairy farmers: screening, characterization and constitution of a cohort; ClinicalTrials.gov Identifier: NCT02540408) was conducted from 2011 to 2015 at the University Hospital of Besançon in collaboration with the French national social security system for agricultural workers (Mutualité Sociale Agricole, MSA) and the federation of community health practices of Franche-Comté (Fédération des Maisons de Santé Comtoises, FeMaSaC) (14).

Subjects with COPD and matched controls were recruited through 2 parallel screening programs (the screening phase of the BALISTIC study). Inclusion criteria in the screening programs were: men or women aged 40-74 years, with no previous diagnosis of asthma or hypersensitivity pneumonitis and who were either dairy farmers (“dairy farmers” subgroups) or who were unexposed to any occupational hazard associated with COPD (“non-farmers” subgroups). One of these two screening programs was conducted during free health check-ups organized by the MSA for their affiliated members, where COPD screening was proposed to all subjects who attended the health check-up and fulfilled the inclusion criteria. In parallel, general practitioners of the FeMaSaC conducted a second COPD screening program targeting all their patients who met the same inclusion criteria. For COPD screening, the two programs used the same material, that is, a pneumotachograph (MedGraphics; MSE Medical, Strasbourg, France) calibrated at least once daily using a 3-L syringe, and the same protocol.

### **Study groups**

A diagnosis of post-bronchodilator airway obstruction was retained when the post-bronchodilator FEV1/FVC ratio was less than 0.70 (15) according to the GLI-2012 equations

(3, 16). Spirometry was considered normal when the FEV1/FVC ratio was  $> 0.70$  and FEV1 was  $> 80\%$  of the predicted value before bronchodilator administration.

During the study period, all subjects with COPD detected during the screening phase of the BALISTIC study were invited to participate in the characterization phase of the study. Those who accepted were included in two subgroups, namely dairy farmers with COPD (DF-COPD) and non-farmers with COPD (NF-COPD). Patients with COPD were matched 1:1 with subjects with normal spirometry (as defined above) in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and sex (namely DF-controls and NF-controls). Patients were in stable condition (no exacerbation of COPD during the previous 6 weeks) and were not taking any oral or inhaled anti-inflammatory drugs, including oral corticosteroids. Subjects were asked to interrupt short and long-acting bronchodilators 72 hours prior to the visit, if required. Exclusion criteria were the following: long-term oxygen therapy; a history of asthma, pulmonary fibrosis, tuberculosis and bronchiectasis.

All subjects who participated in the characterization phase attended a visit at the University Hospital of Besançon, during which examinations and questionnaires were performed.

Ethical approval was received from the local Ethics Committee (CPP Est; 11/617), and written consent was obtained from all subjects.

## **Procedures**

*Lung function and exercise testing.* Routine spirometry, constant-volume body plethysmography and single breath lung transfer for carbon monoxide (TLCO) were performed in accordance with recommended techniques (Platinum Elite; MGC Diagnostics Corporation, Saint Paul, Minnesota, USA). Subjects with post-bronchodilator airway obstruction were rated either as stage 1 (FEV1  $> 80\%$  of the predicted value) or stage 2+ (FEV1  $\leq 80\%$  of the predicted value). Symptom-limited incremental CPET was performed on

an electronically braked cycle ergometer (Ergometrics 900; Ergoline; Bitz, Germany) as previously described (17).

*Laboratory analysis.* For each patient, blood gases were measured from samples drawn from the arterialized earlobe, and measurements were corrected in order to estimate arterial oxygen partial pressure (PaO<sub>2</sub>). (18) In addition, a venous blood sample was collected in the fasting state, and serum samples were stored at -80°C for further analyses. Measurement of several inflammatory markers was carried out using a bead-based cytometric immunoassay (R&D Systems Europe, Abingdon, UK). Serum high-sensitivity C-reactive protein (hsCRP) concentrations were measured using a highly sensitive immunonephelometric assay.

*Questionnaires* A medical questionnaire (completed by the physician) collected data on demographic characteristics, medical history, treatments, symptoms and smoking history. In addition, an adapted French translation of the long version of the European Community Respiratory Health Survey questionnaire was completed by the subjects (19). An occupational questionnaire was sent to the subjects 10 days before their medical examination, collected during the medical examination, and reviewed in the presence of the subject (8, 20).

### **Statistical Analysis**

One of the main objectives of our study was to assess the prevalence of COPD in dairy farmers. A sample size of 1400 subjects in each group (dairy farmers and non dairy farmers) was estimated to provide 80% power for an expected prevalence of COPD of 9% with a precision of 1.5% and  $\alpha=0.05$ . As response rate is estimate at about 70%, 2000 subjects were included in each group.

Data are presented as number (percent) and mean  $\pm$  standard deviation for qualitative and quantitative variables, respectively. Quantitative variables were compared with the Student t

test. Qualitative variables were compared with the Chi-square ( $\chi^2$ ) test. To identify factors associated with pulmonary function, exercise capacities and symptoms, bivariate linear regressions for all candidate factors were performed.

A  $p$  value  $< 0.05$  was considered statistically significant. Analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

## **Results**

### *Prevalence of COPD in dairy farmers*

Among the 8 106 subjects who underwent screening, 4788 met the inclusion criteria for the study and had an interpretable spirometry (59.1%). In total, 8.0% of the dairy farmers had a post-bronchodilator FEV1/FVC  $< 0.70$  and 6.2% had a post-bronchodilator FEV1/FVC $<$ LLN (figure 1). All subjects who had COPD according to the LLN definition also had COPD according to the GOLD definition. The prevalence of COPD, either defined by GOLD or LLN, was similar between dairy farmer and non-dairy farmers. Nevertheless, prevalence of tobacco smoking was much lower in dairy farmers with COPD (respectively 21% of current smokers and 34% of subjects smoking more than 15 pack-years) compared to non-farmers with COPD (52% of current smokers and 72% of subjects smoking more than 15 pack-years,  $p < 0.0001$ ).

### *Population characteristics*

A total of 186 subjects with COPD and 187 matched controls with normal spirometry were included in the characterization phase of the study. By design, all groups had similar sex distribution, age and BMI (table 1). The number of former and current smokers was much lower among dairy farmers than among non-farmers. In addition, adequate matching on smoking status was not possible between COPD and control subjects. In non-farming

subjects, 61% were working in tertiary sector (mainly financial and professional services), 23% in secondary sector (mainly car and watch manufacturing) and 16% were unemployed (supplemental figure 1). About 40% of patients with COPD had mild disease and 13% were taking long-term bronchodilators at the time of screening (online supplemental table 1).

### *Pulmonary function*

Pulmonary function of patients with COPD was significantly different than that of healthy controls (table 2). A large majority of subjects with COPD had preserved FEV<sub>1</sub>. Nevertheless, post-bronchodilator (BD) FEV<sub>1</sub> and TLCO were significantly higher in DF-COPD than in the NF-COPD. After adjustment on post-BD FEV<sub>1</sub>, TLCO remained significantly higher in DF-COPD. Total lung capacity (TLC) was similar in the all groups. Analysis of NF-COPD and non-smoking DF-COPD showed similar results (supplemental table 2).

### *Symptoms and quality of life*

Chronic bronchitis and wheezing were more frequent in DF-COPD compared to DF-controls but were similar with NF-COPD (table 3). Dyspnoea (i.e. BDI and mMRC) and quality of life score (CAT and SGRQ) were better among DF-COPD than NF-COPD. After adjustment on TLCO, post-BD FEV<sub>1</sub> or both, only BDI remained significantly better in DF-COPD ( $p < 0.0001$ ). In addition, frequency and severity of exacerbation were similar in DF-COPD and NF-COPD.

### *Comorbidities*

The main comorbidities in dairy farmers were hypertension, dyslipidaemia and gastro-oesophageal reflux and were similar between DF-COPD and DF-controls (figure 2). Diabetes mellitus and osteoporosis were less frequent in DF-COPD than in NF-COPD. In addition, DF-

COPD were taking less medication especially anti-aggregates and proton pump inhibitors (supplemental table 1).

#### *Exercise capacities*

All patients performed a maximal exercise (respiratory exchange ratio >1.1). Patients with COPD had significantly lower exercise capacities than controls (table 4). DF-COPD had higher peak work rate (WR) and higher peak oxygen consumption (VO<sub>2</sub>) than NF-COPD (table 4). After adjustment on both post bronchodilator FEV1 and TLCO, only peak VO<sub>2</sub> remained higher in DF-COPD compared to NF-COPD. Finally no difference was found in 6MWT between COPD patients.

#### *Systemic inflammation*

The crude comparison of serum level biomarkers showed that DF-COPD had similar levels of inflammatory markers than DF-controls but significantly lower concentrations of leucocytes, fibrinogen, tumor necrosis factor receptor 1 (TNF-R1), interleukin (IL) 6 and 8, IL18 binding protein (IL18BP), human neutrophil gelatinase-associated lipocalin (hNGAL) and monocyte chemoattractant protein 1 (MCP-1) than NF-COPD (supplemental table 3). In addition, some Th2-related markers (periostin and MCP-2) were found at higher level in DF-COPD compared to other subjects (figure 3).

#### *Occupational characteristics of dairy farmers*

Several occupational characteristics among dairy farmers were assessed (supplemental table 4, 5 and 6). Activity level, farm characteristics, hay conditioning, exposure to organic dusts and chemical products were similar between DF-COPD and DF-controls.



## **Discussion**

The analysis of this study issued from a large screening population allows us to show that (i) the prevalence of COPD in dairy farmers was of 8.0% using the GOLD criterion and 6.2% using the LLN criterion. This prevalence was similar than in non farming working subjects but was associated with a lower prevalence of tobacco consumption; (ii) DF-COPD have greater pulmonary function, exercise capacity and quality of life and less symptoms, comorbidities and systemic inflammation than matched patients with NF-COPD mainly due to tobacco; (iii) COPD in dairy-farmers seems to be associated with a predominantly Th2-phenotype. In addition, (iv) the absence of relationship between COPD and occupational exposure factors in farmers supports the role of host factors in the pathogenesis of COPD.

Although farming is often considered a risk factor for COPD, there are few and conflicting data regarding prevalence and the magnitude of the risk in dairy farmers. In a study performed in Macedonia, Stoleski et al. reported a COPD prevalence of 10.7% in dairy farmers whereas in a study performed in Brittany, Jouneau et al. found a COPD prevalence of only 1.3% (9, 21). In our study, we observed a prevalence of COPD among farmers that is in the range of previous studies (9, 12, 21). The differences in COPD prevalence could be due to difference in the level of organic dusts exposure in these different regions. Indeed, in a previous study from our team, we demonstrated that prevalence of COPD in farmers varied from one region to another due to differences of farming activity (livestock, dairy production), meteorological data (climate, altitude) and farm size and modernity. In our region, where farms are mainly traditional (22), prevalence of COPD among farmers is higher than in Brittany (6). However, characteristics of the farm and level of organic dust exposure might not explain alone the occurrence of COPD in dairy farmers as we did not find any differences between DF-COPD and controls. In addition, data issued from the same cohort showed similar exposure pattern among dairy farmers and therefore exposure could not

explain the occurrence of COPD (23). We therefore aimed to evaluate their clinical characteristics.

To our knowledge, this is the first article comparing clinical, functional and blood parameters among DF-COPD patients and tobacco smoking COPD patients and healthy dairy farmers and controls. Several studies have shown that dairy farming is associated with an excess of respiratory symptoms including chronic bronchitis and related symptoms (cough, phlegm, wheezing) compared to general population (7, 19, 24). In the current study we found a similar prevalence of respiratory symptoms between dairy farmers and non-farmers.

Compared to NF-COPD, DF-COPD developed less severe airway obstruction even though duration of exposure to toxic inhalants was similar to NF-COPD. In addition, TLCO was significantly higher in DF-COPD even after adjustment on FEV1. Low TLCO has been clearly linked to structural markers of early emphysema (25). We can therefore suggest that organic dust exposure might be associated with a less damaging effect on lung parenchyma than tobacco smoking. HRCT was however not available for emphysema quantification, but is planned to be done in the second part of the BALISTIC project (NCT03468101). Nevertheless, these results are in line with those found COPD induced by exposure to biomass smoke (which is could be close to dairy farming COPD) where emphysema is rarely found in this type of COPD (26, 27).

COPD is in an inflammatory lung disease associated with systemic inflammation. It has been shown that levels of inflammatory markers such as TNF $\alpha$ , fibrinogen, IL6 and CRP are higher than in non-COPD smokers, regardless to the severity of airway obstruction. Mechanisms of systemic inflammation are multifactorial and remain incompletely understood: one hypothesis is that chronic inflammation is coming from the spillover of inflammatory molecules from the lungs into the systemic circulation; other mechanisms have been proposed, including a direct effect of tobacco smoke on the activation of neutrophils or a

passage in the circulation of toxic gases or inhaled particles. In non-smoking COPD, it has been shown that patients exhibit higher levels of inflammatory biomarkers than healthy subjects (28, 29). Systemic inflammation is somewhat lower than COPD secondary to tobacco smoking (28, 29). We did not find difference between DF-COPD and DF-controls regarding systemic inflammation. However, COPD in dairy farming was associated with a lower systemic inflammatory status than in NF-COPD. This could in part explain the lower prevalence of comorbidities in DF-COPD. Indeed, a pathophysiological link between persistent low-grade systemic inflammation and comorbidities in COPD has been suggested (30, 31). Similar results were found in COPD induced by biomass exposure regarding this lower prevalence of comorbidities (32). It is plausible that the nature of the noxious agents (tobacco or organic dust exposure) could have a different impact on the occurrence of these comorbidities. In addition, it has been shown that people living and working on dairy farms have a rich and distinct nasal microbiome compared to that of non-farmers (33). This increased microbial diversity could be contributing to protect from chronic inflammatory conditions (34). Finally, we found that DF-COPD patients might have a predominantly Th2 phenotype as it has been shown in biomass COPD (26). Indeed, periostin and MCP-2/CCL8 which are known to mediate Th2 inflammation with a specific potential to drive chronic eosinophilic inflammation, were found at higher level in DF-COPD (35, 36). In addition, we found in the same population that IgE-mediated reactions (atopy) may play a role in COPD development in dairy farming (37).

It is worth to note that all reasonable precautions were taken to verify the absence of asthma including spirometries performed by trained nurses and careful medical examination. All subjects with self-reported asthma were excluded. Our findings therefore reinforce the so-called “Dutch hypothesis” that postulates that chronic obstructive airway disease may occur on a continuum, with “pure” forms of asthma and COPD representing the extremes of the

same disease and the various forms of chronic obstructive airway disease being different expressions of a single disease entity (38, 39).

### Limitations

Our study includes limitations inherent to a large screening program. Only subjects who accepted to undergo a health check-up were included in the COPD screening program. In addition, only half the subjects who had COPD detected by screening accepted to attend the hospital visit for “characterization”. It has previously been reported that among all subjects who were invited to health check-ups organized by the French agricultural health insurance system, those who attend these check-ups and those who do not have different health characteristics (6). It is therefore possible that our population was not representative of the whole population of dairy farmers (target population) regarding health status. Our studied population, however, is not different from the target population in terms of smoking habits. Finally, our outcomes can only be considered robust if we are sure that the diagnosis of COPD was accurate, and that patients with asthma were truly excluded.

### Conclusion

In conclusion, this study demonstrates that dairy farmers have a similar prevalence of COPD (8%) than unexposed subjects concurrently with a lower prevalence of tobacco smoking. Patients with COPD secondary to dairy farming have better respiratory function and quality of life and less comorbidities compared to patients with COPD secondary to tobacco smoking. This may suggest that COPD secondary to organic dust exposure (ie dairy farming) might be associated with a less damaging effect on lung parenchyma than tobacco smoking. In addition, the inflammatory response induced by exposure to organic dust differs greatly from that caused by tobacco smoking.

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**Author Contributions:**

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

JCD and BD made substantial contributions to the conception and design of the work.

TS and AG performed all analyses.

JCD and BD obtained study funding.

All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work. TS wrote the first draft of the manuscript. BD, TS, AG, NR, IAM, PA, LL, SH, SJ, MB, JJJ and JCD revised the manuscript for important intellectual content. All authors approved the manuscript.

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**Table 1. Main characteristics of the population**

	Dairy farmers		Non farmers		Dairy farmers vs non farmers	
	COPD n=101	Control n=98	COPD n=85	Control n=89	p	p
<b>Demographics</b>						
Male	86 (85)	86 (88)	66 (78)	65 (73)	0.48	NS
Age, year	60.3 ± 9.1	59.3 ± 8.9	61.4 ± 7.6	60.2 ± 6.5	0.24	NS
Body mass index, kg.m <sup>-2</sup>	26.5 ± 4.1	27.0 ± 4.1	26.1 ± 4.1	26.8 ± 3.8	0.24	NS
<b>Exposure</b>						
Smoking status						
Non smoker	48 (48)	54 (55)	7 (8)	12 (13)	0.31	< <b>0.0001</b>
Former smoker	33 (33)	32 (33)	36 (42)	42 (47)		
Current smoker	20 (20)	12 (12)	42 (49)	35 (39)		
Tobacco, pack-year	12.5 ± 16.8	8.1 ± 12.1	31.8 ± 23.8	24.7 ± 17.7	< <b>0.05</b>	< <b>0.0001</b>
Exposure to organic dust, years	37.9 ± 6.1	36.7 ± 7.1	-	-	-	-
<b>Severity of airway obstruction</b>						
Stage I	44 (44)		33 (39)			NS
Stage II +	57 (56)		52 (61)			

Values are means ± SD or number (percent)

**Table 2. Pulmonary function tests**

	Dairy farmers			Non farmers			Dairy farmers vs non farmers COPD p
	COPD n=101	Control n=98	p	COPD n=85	Control n=89	p	
<b>Post-BD FEV1, L</b>	2.84 ± 0.72	3.46 ± 0.69	< 0.0001	2.58 ± 0.76	3.42 ± 0.68	< 0.0001	< 0.05
z-score	-0.99 ± 0.98	0.38 ± 0.77	< 0.0001	-1.31 ± 1.05	0.58 ± 0.85	< 0.0001	< 0.05
<b>Post-BD FEV1/FVC, %</b>	63 ± 7	79 ± 4	< 0.0001	61 ± 8	80 ± 4	< 0.0001	NS
z-score	-1.95 ± 0.78	0.07 ± 0.64	< 0.0001	-2.18 ± 0.86	0.27 ± 0.59	< 0.0001	NS
<b>Pre-BD pulmonary function</b>							
FEV1, L	2.63 ± 0.72	3.37 ± 0.66	< 0.0001	2.41 ± 0.73	3.31 ± 0.66	< 0.0001	NS
z-score	-1.39 ± 1.03	0.18 ± 0.74	< 0.0001	-1.66 ± 1.04	0.34 ± 0.83	< 0.0001	NS
FVC, L	4.36 ± 1.11	4.48 ± 0.87	NS	4.08 ± 1.09	4.31 ± 0.9	NS	NS
z-score	0.12 ± 1.15	0.43 ± 0.77	< 0.05	-0.13 ± 1.11	0.42 ± 0.82	< 0.001	NS
FRC, L	4.07 ± 0.81	3.71 ± 0.78	< 0.01	4.13 ± 0.88	3.55 ± 0.83	< 0.001	NS
z-score	1.14 ± 1.24	0.54 ± 1.12	< 0.0001	1.32 ± 1.15	0.45 ± 1.18	< 0.001	NS
IC, L	3.31 ± 0.79	3.38 ± 0.7	NS	3.01 ± 0.83	3.34 ± 0.72	< 0.01	< 0.05
RV, L	2.87 ± 0.71	2.47 ± 0.58	< 0.01	2.89 ± 0.81	2.47 ± 0.57	< 0.01	NS
z-score	1.42 ± 1.78	0.47 ± 1.29	< 0.0001	1.48 ± 1.79	0.57 ± 1.85	< 0.001	NS
TLC, L	7.38 ± 1.27	7.10 ± 1.16	NS	7.14 ± 1.33	6.90 ± 1.25	NS	NS
z-score	1.13 ± 1.40	0.87 ± 1.05	NS	0.99 ± 1.28	0.89 ± 1.33	NS	NS
RV/TLC, %	39.2 ± 8.5	34.8 ± 6.1	< 0.0001	40.7 ± 8.8	35.8 ± 8.9	< 0.0001	NS
TLCO, mmol/min/kPa	8.45 ± 2.35	9.47 ± 2.12	< 0.01	6.74 ± 2.37	8.33 ± 2.39	< 0.0001	< 0.0001
z-score	-0.26 ± 1.33	0.56 ± 1.16	< 0.0001	-1.45 ± 1.40	-0.04 ± 1.42	< 0.0001	< 0.0001
KCO, mmol/min/kPa/L	1.34 ± 0.25	1.47 ± 0.23	< 0.001	1.13 ± 0.25	1.37 ± 0.26	< 0.0001	< 0.0001
z-score	-0.47 ± 1.13	0.08 ± 1.02	< 0.001	-1.44 ± 1.13	-0.35 ± 1.13	< 0.0001	< 0.0001
<b>Arterial blood gases</b>							
PeapO2, kPa	81.0 ± 9.8	85.5 ± 9.8	< 0.01	79.5 ± 9.8	84.0 ± 9.8	< 0.01	NS
PeapCO2, kPa	36.8 ± 3.8	36.8 ± 3.0	NS	37.5 ± 3.8	37.5 ± 3.8	NS	NS

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; BD = bronchodilator; FEV1 = forced expiratory volume in 1 second; z-score = standardized residual; FVC = forced vital capacity; FRC = functional residual capacity; IC = inspiratory capacity; RV = residual volume; TLC = total lung capacity; TLCO = lung transfer for carbon monoxide; KCO = carbon monoxide transfer coefficient.  
Values are means ± SD or number (percent)

**Table 3. Symptoms and quality of life**

	Dairy farmers		Non farmers		Dairy farmers vs non farmers COPD	
	COPD n=101	Control n=98	COPD n=85	Control n=89	p	p
<b>Dyspnea</b>						
mMRC	38 (38)	15 (15)	39 (46)	19 (21)	< 0.001	NS
BDI	0.41 ± 0.55	0.15 ± 0.36	0.61 ± 0.77	0.25 ± 0.57	< 0.001	< 0.05
	10.8 ± 1.4	11.5 ± 1.0	10.1 ± 2.1	11.3 ± 1.4	< 0.0001	< 0.01
<b>Wheezing</b>						
Ever	32 (32)	6 (6)	25 (29)	10 (11)	< 0.0001	NS
In the last 12 months	30 (30)	11 (11)	28 (33)	15 (17)	< 0.01	NS
<b>Chronic bronchitis, n (%)</b>	35 (35)	9 (9)	32 (38)	12 (13)	< 0.0001	NS
<b>Exacerbation, n (%)</b>	23 (23)		23 (27)			NS
In the last 12 months	1.6 ± 1.2		1.4 ± 0.9			NS
<b>Naso-sinus symptoms</b>						
Hay fever	27 (27)	17 (18)	31 (36)	19 (21)	NS	NS
	9 (9)	10 (10)	12 (14)	11 (13)	NS	NS
<b>Quality of life/prognosis</b>						
SGRQ	15.1 ± 12.9	7.3 ± 7.2	19.4 ± 14.9	9.3 ± 9.3	< 0.0001	< 0.05
CAT	9.8 ± 5.7		11.7 ± 5.7			< 0.05
BODE index	0.2 ± 0.6		0.4 ± 0.5			< 0.05

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; mMRC = modified medical research council; BDI = baseline dyspnoea index; SGRQ: St. George's Respiratory Questionnaire; CAT: COPD Assessment Test.  
 Values are means ± SD or number (percent)

**Table 4. Exercise capacities**

	Dairy farmer		p	Non farmer		p	Dairy farmer vs non farmer
	COPD n=101	Control n=98		COPD n=85	Control n=89		
<b>CPET (measurements at peak exercise)</b>							
Work rate, W	159 ± 46	171 ± 46	NS	140 ± 47	161 ± 46	< 0.01	< 0.01
% predicted	85 ± 16	92 ± 17	< 0.01	77 ± 19	89 ± 19	< 0.01	< 0.01
V̇O <sub>2</sub> , L/min	2.18 ± 0.57	2.36 ± 0.56	< 0.05	1.92 ± 0.56	2.23 ± 0.57	< 0.001	< 0.01
% predicted	99 ± 17	108 ± 19	< 0.001	90 ± 19	104 ± 20	< 0.0001	< 0.01
HR, % predicted maximum	97 ± 10	99 ± 7	NS	95 ± 10	99 ± 10	NS	NS
PcapO <sub>2</sub> , kPa	11.8 ± 1.3	12.4 ± 1.1	< 0.001	11.5 ± 1.4	12.6 ± 1.3	< 0.0001	NS
PcapCO <sub>2</sub> , kPa	4.8 ± 0.6	4.7 ± 0.5	NS	4.8 ± 0.6	4.7 ± 0.6	NS	NS
Reason for stopping*							
Leg discomfort	41 (56)	52 (68)	NS	27 (46)	49 (62)	NS	NS
Breathing discomfort	18 (25)	6 (8)	< 0.01	12 (21)	22 (28)	NS	NS
Both breathing and leg discomfort	5 (7)	7 (9)	NS	11 (19)	4 (5)	< 0.05	< 0.01
Other reason	9 (12)	11 (15)	NS	8 (14)	4 (5)	NS	NS
<b>6MWT</b>							
Distance, m	603 ± 85	617 ± 70	NS	604 ± 85	620 ± 85	NS	NS
% of predicted distance	88 ± 9	91 ± 10	NS	90 ± 10	93 ± 10	NS	NS
SpO <sub>2</sub> at rest, %	96 ± 2	96 ± 2	NS	96 ± 2	96 ± 2	NS	NS
Minimal SpO <sub>2</sub> during walk test, %	93 ± 3	94 ± 3	< 0.01	93 ± 3	94 ± 3	< 0.01	NS

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; CPET: cardiopulmonary exercise testing; V̇O<sub>2</sub> = oxygen uptake; HR = heart rate; PcapO<sub>2</sub> = arterialized partial pressure of oxygen; PcapCO<sub>2</sub> = arterialized partial pressure of carbon dioxide; respectively; 6MWT: 6 minutes walk test.

**Figure 1. Flow chart of participants included in the study.** Subjects were recruited through a screening program in two branches of the social security. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma, hypersensitivity pneumonitis.

Abbreviations: COPD: chronic obstructive lung disease, DF: dairy farmer, NF: non-farmer.

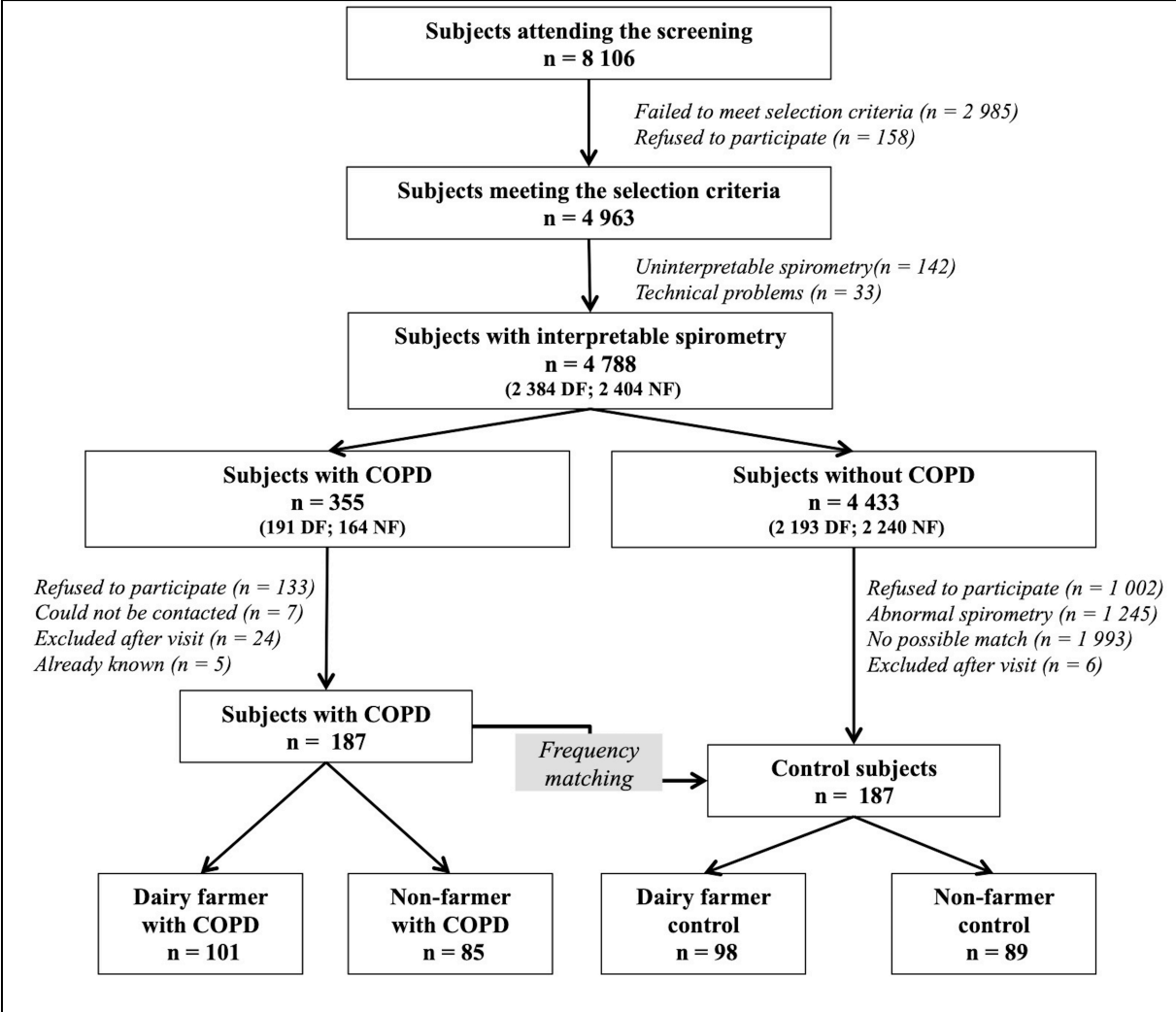
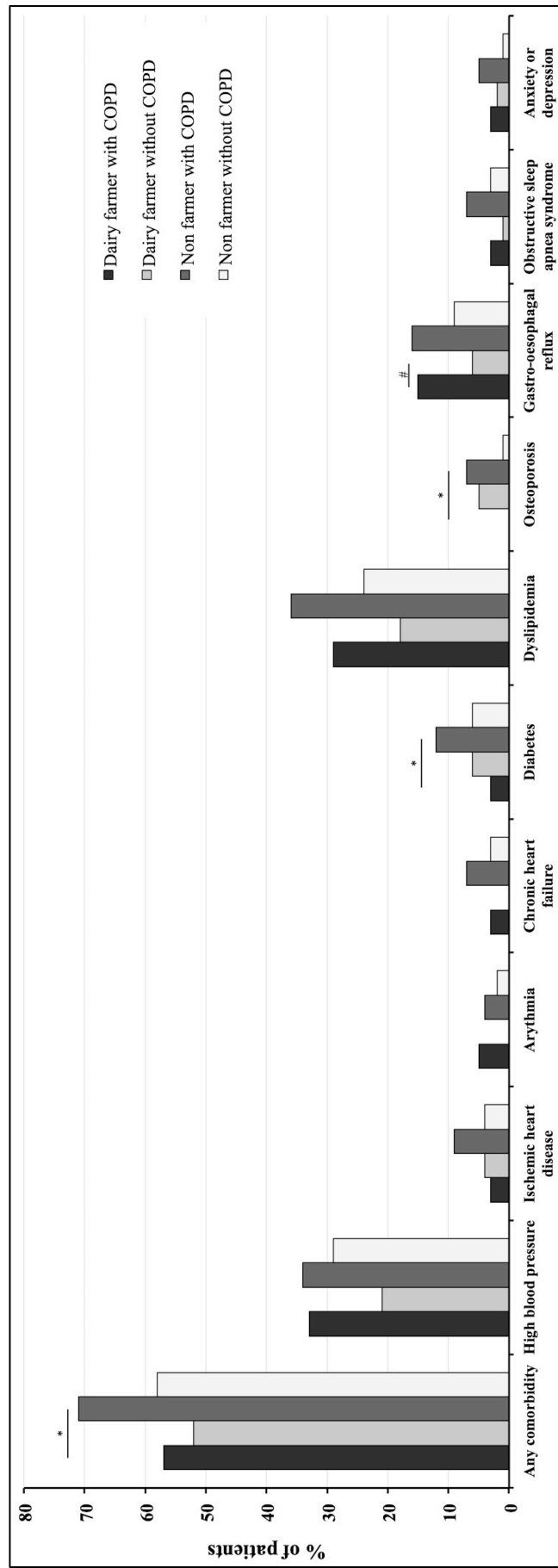
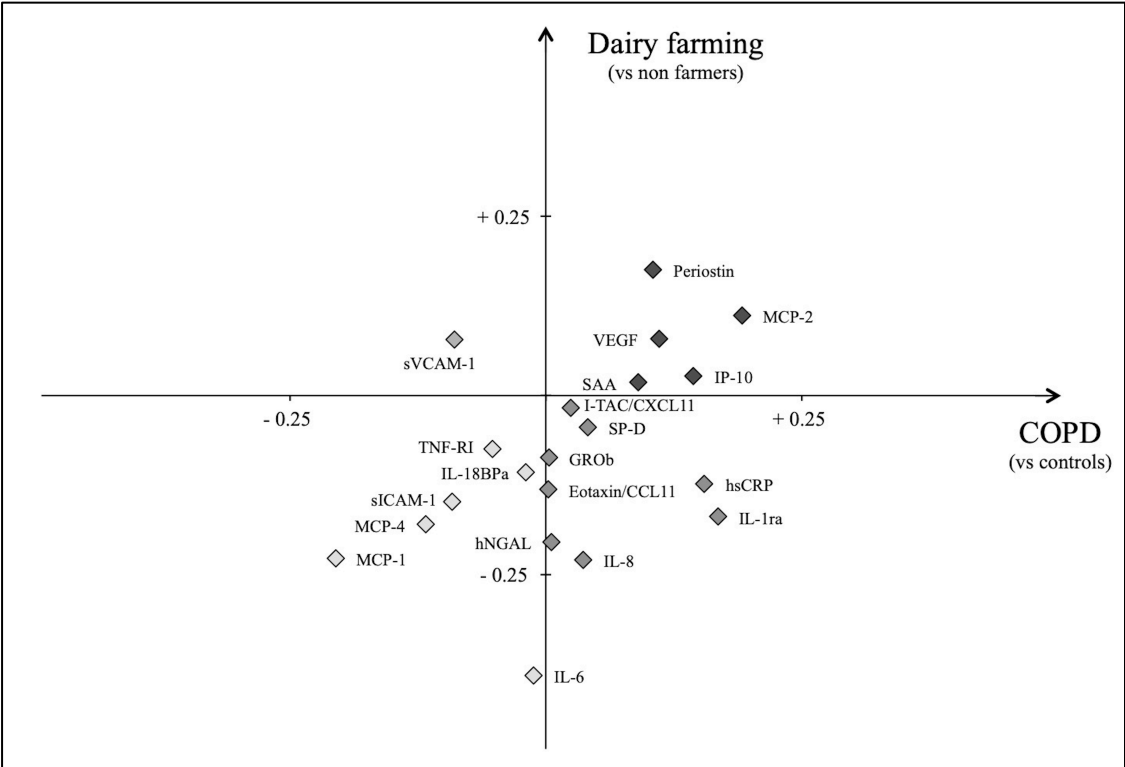


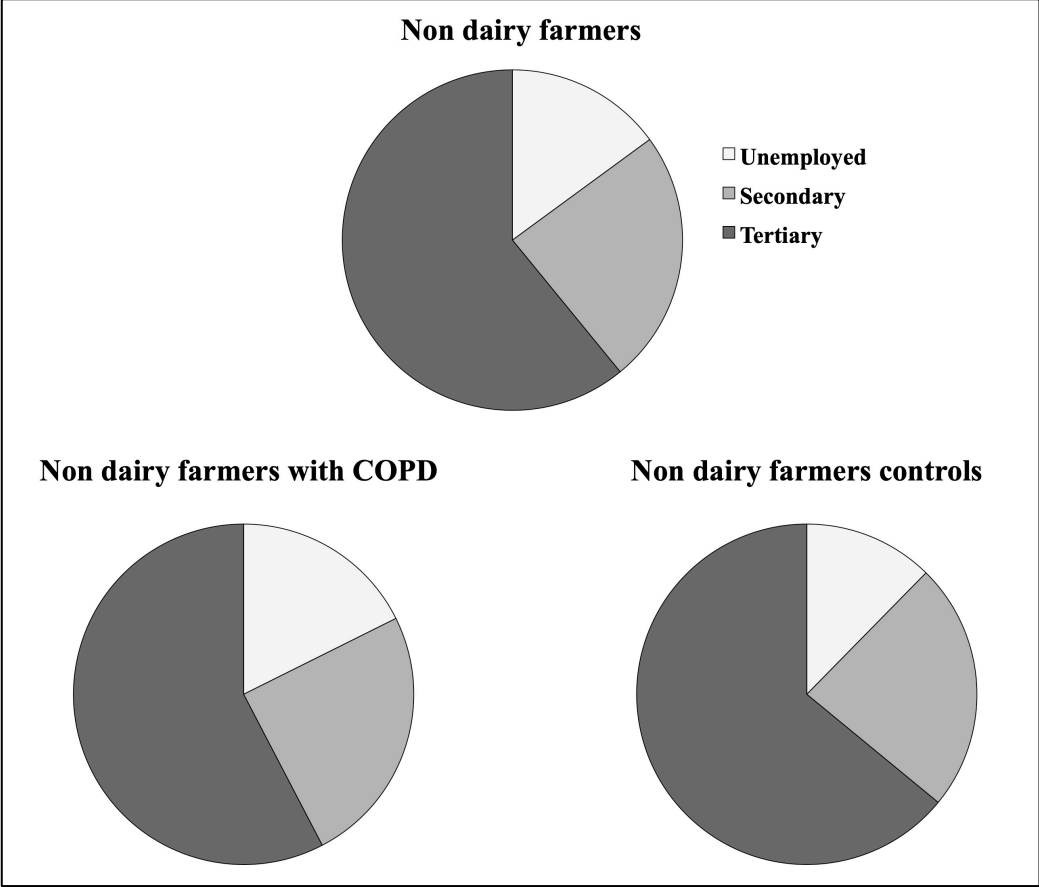
Figure 2. Prevalence of comorbidities in patients with COPD and controls subjects. \*  $p < 0.05$  vs non farmers COPD; #  $p < 0.05$  vs dairy farmers control



**Figure 3. Scatter plot** showing the relationship between difference of mean z score of several cytokines between COPD and controls (X-axis) and dairy farmers and non-farmers (Y-axis).



**Supplemental figure 1.** Occupation of patient with COPD and control subjects among non-farmers





**Supplemental Table 1. Use of medication among subjects**

	Dairy farmer		Non farmer		Dairy farmer vs non farmer	
	COPD n=101	Control n=98	COPD n=85	Control n=89	p	p
<b>Use of pulmonary medication*, n (%)</b>	11 (11)	3 (3)	13 (15)	1 (1)	<0.05	<0.001
Short-acting β2 agonists, n (%)	0	0	0	0		
Long-acting β2 agonists (LABA), %	3 (3)	1 (1)	4 (5)	0	NS	NS
Combined LABA and inhaled corticosteroids,	4 (4)	1 (1)	5 (6)	0	NS	<0.05
Short-acting anticholinergics, %	1 (1)	0	0	0	NS	NS
Long-acting anticholinergics, %	1 (1)	0	7 (8)	0	NS	<0.01
Theophyllin, %	0	0	0	0		
Inhaled corticosteroids, %	0	1 (1)	1 (1)	0	NS	NS
Oral corticoids, %	0	0	0	0		
Antileukotrienes, %	0	0	0	0		
Roflumina <sup>s</sup> , n(%)	0	0	0	0		
Long-term oxygen therapy, %	0	0	0	0		
CPAP, n (%)	1 (1)	1 (1)	2 (2)	2 (2)	NS	NS
<b>Use of cardiovascular and other medication, %</b>	43 (43)	44 (45)	55 (65)	48 (54)	NS	<0.01
Anti-hypertensive treatment	28 (28)	21 (21)	27 (32)	26 (29)	NS	NS
ACE, %	15 (15)	9 (9)	12 (14)	10 (11)	NS	NS
β-blocker, %	12 (12)	3 (3)	13 (15)	7 (8)	<0.05	NS
ARB, %	10 (10)	9 (9)	10 (12)	6 (7)	NS	NS
Diuretic, %	10 (10)	4 (4)	3 (4)	9 (10)	NS	NS
Calcium antagonist, %	3 (3)	0	3 (4)	5 (6)	NS	NS
Anti-arrhythmica, %	5 (5)	2 (2)	5 (6)	0	NS	<0.05
Antiaggregates, %	8 (8)	9 (9)	20 (24)	9 (10)	NS	<0.05
Anticoagulants, n(%)	6 (6)	1 (1)	4 (5)	2 (2)	NS	NS
Oral antidiabetica, %	2 (2)	5 (5)	5 (6)	3 (3)	NS	NS
Statins, %	21 (21)	15 (15)	24 (28)	15 (17)	NS	NS
Proton pump inhibitors, %	2 (2)	4 (4)	8 (9)	5 (6)	NS	NS
Anxiolytics, %	2 (2)	2 (2)	6 (7)	0	NS	<0.05
Antidepressives, %	3 (3)	3 (3)	6 (7)	1 (1)	NS	NS
Sleep medication, %	2 (2)	1 (1)	4 (5)	1 (1)	NS	NS

\* Pulmonary medications were interrupted 72h prior to the visit.

Definition of abbreviations: ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blockers; CPAP: continuous positive airway pressure; COPD = chronic obstructive pulmonary disease.

Supplemental table 2

	Never-smoker dairy farmer with COPD n= 48	Non farmer with COPD n=98	p
<b>Male, n (%)</b>	60.0 ± 9.2	66 (78)	NS
<b>Age, year</b>	40 (83)	61.4 ± 7.6	
<b>mMRC</b>	0.28 ± 0.45	0.61 ± 0.77	< 0.01
<b>BDI</b>	11.1 ± 1.3	10.1 ± 2.1	< 0.01
<b>Post-BD FEV1, L</b>	2.89 ± 0.74	2.58 ± 0.76	< 0.05
z-score	-0.89 ± 0.97	-1.31 ± 1.05	< 0.05
<b>Post-BD FEV1/FVC, %</b>	63.7 ± 6.5	61 ± 8	< 0.05
<b>Pre-BD pulmonary function</b>			
TLC, z-score	1.14 ± 1.18	0.99 ± 1.28	NS
TLCO, z-score	-0.39 ± 1.16	- 1.45 ± 1.40	< 0.0001
FEV1, L	2.68 ± 0.74	2.41 ± 0.73	< 0.05
FEV1, z-score	-1.28 ± 0.99	-1.66 ± 1.04	< 0.05
<b>Work rate, % predicted</b>	88 ± 18	77 ± 19	< 0.01
<b>VO2, % predicted</b>	101 ± 19	90 ± 19	< 0.01

**Supplemental Table 3. Complete blood count and inflammatory markers**

	Dairy farmers			Non farmers			Dairy farmer vs non farmer COPD	
	COPD n=101	Control n=98	P	COPD n=85	Control n=89	P	P	P
Fibrinogen, g/L	3.19 ± 0.70	3.10 ± 0.73	NS	3.48 ± 0.82	3.23 ± 0.82	< 0.05	< 0.05	< 0.05
Hemoglobin, g/dL	15.3 ± 1.1	15.2 ± 1.1	NS	15.2 ± 1.2	14.9 ± 1.2	NS	NS	NS
Platelets G/L	249.3 ± 72.9	247.5 ± 61.2	NS	256.7 ± 76.0	252.2 ± 77.7	NS	NS	NS
WBC, 10 <sup>9</sup> /L	6.12 ± 1.71	6.07 ± 1.84	NS	7.04 ± 1.77	6.72 ± 1.78	NS	NS	< 0.05
hsCRP, mg/L	2.73 ± 3.23	2.39 ± 2.56	NS	3.18 ± 2.69	2.63 ± 2.50	NS	NS	NS
MCP-2/CCL8, pg/mL	76.0 ± 112.1	57.0 ± 24.9	NS	61.6 ± 25.0	58.1 ± 22.2	NS	NS	NS
Periostin, ng/mL	37.1 ± 27.0	35.4 ± 17.4	NS	33.9 ± 24.5	31.1 ± 14.4	NS	NS	NS
IP-10/CXCL10, pg/mL	24.2 ± 14.1	25.3 ± 17.6	NS	27.7 ± 27.7	20.4 ± 11.0	< 0.05	< 0.05	NS
MCP-1/CCL2, pg/mL	288.4 ± 117.2	323.7 ± 166.6	NS	327.2 ± 107.9	342.5 ± 117.3	NS	NS	< 0.05
I-TAC/CXCL11, pg/mL	51.9 ± 22.9	55.6 ± 19.1	NS	56.9 ± 21.6	51.2 ± 17.5	NS	NS	NS
IL-18BPA, pg/mL	412.6 ± 182.7	453.9 ± 197.1	NS	474.6 ± 224.6	433.7 ± 181.1	NS	NS	< 0.05
IL-1ra/IL-1F3, pg/mL	694.5 ± 636.3	609.0 ± 249.3	NS	763.7 ± 406.7	695.5 ± 377.9	NS	NS	NS
VEGF, pg/mL	65.9 ± 46.7	67.8 ± 49.3	NS	69.6 ± 45.6	56.0 ± 33.5	NS	NS	NS
SP-D, ng/mL	13.6 ± 7.0	14.4 ± 8.6	NS	15.1 ± 7.8	13.5 ± 6.5	NS	NS	NS
TNF RI, pg/mL	4084.2 ± 885.4	4390.5 ± 1749.2	NS	4441.9 ± 1226.0	4213.5 ± 851.3	NS	NS	< 0.05
Eotaxin/CCL11, pg/mL	103.0 ± 52.9	103.9 ± 45.2	NS	110.7 ± 43.1	109.2 ± 56.8	NS	NS	NS
GROB/CXCL2, pg/mL	384.7 ± 235.2	383.7 ± 247.7	NS	406.4 ± 260.1	405.5 ± 268.7	NS	NS	NS
MCP-4/CCL13, pg/mL	87.9 ± 48.9	91.1 ± 48.8	NS	93.7 ± 41.3	101.5 ± 41.1	NS	NS	NS
hNGAL, ng/mL	57.5 ± 22.8	59.7 ± 25.8	NS	65.5 ± 30.2	62.5 ± 25.9	NS	NS	< 0.05
SAA, ng/mL	598.7 ± 1045.6	799.7 ± 2517.8	NS	677.4 ± 824.4	435.8 ± 440.8	< 0.05	< 0.05	NS
sICAM-1, ng/mL	881.6 ± 367.1	958.2 ± 444.7	NS	985.1 ± 394.9	976.0 ± 448.4	NS	NS	NS
sVCAM-1, ng/mL	382.5 ± 118.2	415.1 ± 155.3	NS	394.9 ± 107.8	382.5 ± 124.1	NS	NS	NS
CXCL8/IL-8, pg/mL	11.6 ± 5.0	13.7 ± 11.8	NS	15.1 ± 9.6	13.1 ± 5.5	NS	NS	< 0.01
Detectable IL-6, n(%)	19 (19)	8 (8)	NS	29 (34)	18 (20)	NS	NS	< 0.05
IL-6, pg/mL	2.5 ± 0.8	3.0 ± 1.4	NS	3.2 ± 1.4	2.96 ± 1.28	NS	NS	< 0.05
Detectable IL-10, n(%)	5 (5)	8 (8)	NS	4 (5)	6 (7)	NS	NS	< 0.05
Detectable IL-4, n(%)	0	1 (1)	NS	0	0	NS	NS	< 0.05
Detectable IL-5, n(%)	5 (5)	0	NS	8 (9)	1 (1)	NS	NS	< 0.05
Detectable IL-13, n(%)	0	0	NS	0	0	NS	NS	< 0.05
Detectable IL-17A, n(%)	0	0	NS	0	0	NS	NS	< 0.05
Detectable IL-2, n(%)	0	0	NS	0	0	NS	NS	< 0.05
Detectable IL-22, n(%)	1 (1)	0	NS	0	0	NS	NS	< 0.05
Detectable IL-23, n(%)	0	0	NS	1 (1)	0	NS	NS	< 0.05

Abbreviation: WBC: white blood count; hsCRP: high-sensitivity C-reactive protein; MCP: Monocyte chemoattractant protein; CCL or CXCL: chemokines; IL: Interleukin, monocyte chemoattractant protein, I-TAC: Interferon-induced T Cell Alpha Chemoattractant; IP: interferon  $\gamma$ -induced protein; sST: soluble toll-like receptor; VEGF: Vascular endothelial growth factor; SP-D: surfactant protein D; Gro: Growth-regulated protein; hNGAL: human Neutrophil Gelatinase-Associated Lipocalin; SAA: Serum amyloid A; sTNFR: soluble tumour necrosis factor receptor; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1

**Supplemental table 4.** Activity level and farm characteristics

	Dairy farmers		p
	COPD n=101	Control n=98	
<b>Activity level</b>			NS
Active	47 (47)	51 (52)	
Retired	44 (43)	46 (48)	
still living on a farm	16 (16)	18 (18)	
still working on a farm	22 (22)	22 (22)	
<b>Farm characteristics</b>			
Separation between house and cowshed	74 (73)	71 (72)	NS
duration since beginning, years	24.4 ± 24.2	22.1 ± 17.1	NS
Central corridor	75 (74)	80 (82)	NS
duration since beginning, years	28.5 ± 13.6	25.8 ± 11.7	NS
Loose housing system	50 (51)	55 (59)	NS
duration since beginning, years	22.4 ± 13.3	25.5 ± 10.9	NS
Loading grippers	28 (28)	29 (30)	NS
duration since beginning, years	7.1 ± 12.9	7.6 ± 12.6	NS
Food grippers	15 (15)	17 (18)	NS
duration since beginning, years	2.9 ± 8.9	3.5 ± 8.8	NS
Straw blower	21 (21)	23 (24)	NS
duration since beginning, years	2.8 ± 6.8	3.6 ± 7.6	NS
Milking parlour	54 (53)	59 (60)	NS
since	20.3 ± 12.1	23.7 ± 11.6	NS
Ventilation	44 (44)	37 (38)	NS
since	31.2 ± 15.3	27 ± 11.4	NS
<b>Size of the farm, mean ± SD</b>			
Total size, hectares			
in the past 10 years	94.9 ± 56.3	97.3 ± 58.4	NS
currently	64.5 ± 72.9	94.3 ± 98	< 0.05
Size of fodder lands, hectares			
in the past 10 years	52.3 ± 34.1	56.6 ± 34.3	NS
currently	33.2 ± 42.3	51.2 ± 51.5	< 0.05
Size of cereal crop lands, hectares			
in the past 10 years	24.1 ± 34.7	28.9 ± 42.1	NS
currently	13.3 ± 31.2	27.9 ± 53.9	< 0.05
Size of cattle			
in the past 10 years	108.9 ± 69.1	106.8 ± 72.2	NS
currently	71.8 ± 84.3	99.2 ± 110.6	< 0.05
Number of cows			
in the past 10 years	43.2 ± 21.6	44.3 ± 23.4	NS
currently	27.9 ± 30.8	35.6 ± 37.2	NS

**Supplemental table 5.** Activity level and farm characteristics

	Dairy farmer		p
	COPD n=101	Control n=98	
<b>Hay conditioning</b>			
Loose stacked hay	15 (15)	19 (19)	NS
duration since beginning, years	3.0 ± 8.7	3.5 ± 8.7	NS
Medium density bales	41 (41)	41 (42)	NS
duration since beginning, years	8.0 ± 11.9	8.1 ± 11.4	NS
Hay bales	85 (85)	76 (78)	NS
duration since beginning, years	20.9 ± 9.2	16.9 ± 11.7	0.01
Barn hay drying	19 (19)	20 (20)	NS
duration since beginning, years	20.2 ± 11.2	5.4 ± 10.6	<0.01
Barn drying system	18 (18)	21 (21)	NS
duration since beginning, years	23.2 ± 14.2	10.1 ± 14.9	<0.01
Drying quality (assessed by the	3.1 ± 0.7	3.2 ± 0.6	NS
<b>Food supplements for the cattle</b>			
Feed grains or meat meal			NS
Never	11 (11)	14 (15)	
Former	46 (47)	37 (39)	
duration since beginning, years	31.8 ± 11.8	30.6 ± 10	NS
Current	41 (42)	44 (46)	
duration since beginning, years	30.6 ± 8.8	30.1 ± 7.9	NS
Frequency			NS
less than one time a week	3 (3)	1 (1)	
more than one time a week	7 (7)	3 (3)	
every day	65 (66)	69 (73)	
Silage	70 (70)	57 (58)	NS

**Supplemental table 6.** Exposure to organic dusts or chemical products

	Dairy farmers		P
	COPD n=101	Control n=98	
Hour spent on the farm during cow stalling per day			
in the past 10 years	5.4 ± 1.9	5.2 ± 1.7	0.31
currently	2.7 ± 2.9	3.1 ± 2.8	0.36
Duration of cow stalling per year, months			
in the past 10 years	6.4 ± 2.1	6.6 ± 2.3	0.73
currently	3.5 ± 3.3	4.1 ± 3.7	0.21
Milking in cowshed			0.29
Never	18 (18)	21 (21)	
Former	65 (66)	54 (55)	
Current	16 (16)	23 (23)	
Duration of milking per day, minutes	120 ± 68	120 ± 56	0.99
Duration since beginning, years	24.2 ± 16	20.5 ± 15.4	0.10
Milking in parlour			0.71
Never	50 (51)	44 (45)	
Former	18 (18)	21 (21)	
Current	31 (31)	33 (34)	
Time spent in parlour per day, minutes	127 ± 62	124 ± 57	0.86
Duration since beginning, years	8.9 ± 12.2	12.0 ± 13.9	0.10
Manual handling of bedding			0.57
Never	6 (6)	9 (9)	
Former	52 (53)	45 (46)	
Current	41 (41)	43 (44)	
Time spent per day, minutes	55 ± 34	70 ± 85	0.28
Duration since beginning, years	31.7 ± 9.6	32.7 ± 9.1	0.62
Mechanized feeding distribution			0.09
Never	74 (75)	60 (61)	
Former	12 (12)	14 (14)	
Current	13 (13)	24 (24)	
Time spent per day, minutes	41 ± 18	44 ± 40	0.75
Duration since beginning, years	5.3 ± 12	8.5 ± 12.9	0.07
Manure removal			0.10
Never	9 (9)	10 (10)	
Former	52 (53)	37 (38)	
Current	37 (38)	50 (52)	
Time spent per day, minutes	40 ± 27	31 ± 27	0.18
Duration since beginning, years	30.3 ± 13.7	28.5 ± 14	0.38
Distribution of bedding			0.42
Never	4 (4)	7 (7)	
Former	46 (47)	38 (39)	
Current	48 (49)	52 (54)	
Time spent per day, minutes	33.5 ± 18.1	23.3 ± 12.8	<0.01
Duration since beginning, years	32.4 ± 11.1	31 ± 12.6	0.40
Use of fertilizers			0.81
Never	18 (18)	16 (16)	
Former	43 (44)	40 (41)	

Current	37 (38)	41 (42)	
Time spent daily using fertilizers, minutes	5.5 ± 5.3	6.6 ± 8.7	0.54
Duration of use, years	26.9 ± 15	26.8 ± 14.4	0.95
Use of pesticides			0.65
Never	45 (45)	39 (41)	
Former	31 (31)	36 (38)	
Current	23 (23)	21 (22)	
Time spent daily using pesticides, minutes	2.6 ± 2.6	2.1 ± 1.6	0.42
Duration of use, years	18 ± 18.1	16.6 ± 16.1	0.57
Use of herbicides			0.74
Never	32 (32)	36 (38)	
Former	38 (38)	33 (34)	
Current	29 (29)	27 (28)	
Time spent daily using herbicides, minutes	3.8 ± 2.4	4.3 ± 4.2	0.63
Duration of use, years	21.6 ± 17	18 ± 15.9	0.13
Use of fungicides			0.98
Never	40 (40)	40 (42)	
Former	33 (33)	31 (32)	
Current	26 (26)	25 (26)	
Time spent daily using fungicide, minutes	2.4 ± 1.9	3.1 ± 3.4	0.40
Duration of use, years	17.6 ± 16.8	16.5 ± 15.9	0.64
Use of protection mask			0.58
Never	64 (65)	54 (56)	
Sometimes	25 (25)	32 (33)	
Often	9 (9)	9 (9)	
Always	1 (1)	2 (2)	
Chemical treatment of fodder			0.48
Never	95 (96)	89 (92)	
Sometimes	3 (3)	7 (7)	
Often	1 (1)	1 (1)	
Drying hay			0.08
Never	78 (79)	71 (73)	
Sometimes	0 (0)	6 (6)	
Often	7 (7)	8 (8)	
Always	14 (14)	12 (12)	
Salt drying hay			0.25
Never	73 (74)	61 (63)	
Sometimes	16 (16)	27 (28)	
Often	7 (7)	6 (6)	
Always	3 (3)	3 (3)	
Exposure to organic dust in the last 10 years (self assessment)			0.74
has increased	13 (13)	10 (10)	
remain stable	61 (62)	58 (60)	
has decreased	25 (25)	28 (29)	

### 3. Article 6 : Microbial exposure to dairy farmers' dwellings and COPD occurrence

#### *a. Contexte et objectif*

La prévalence de la BPCO est deux fois plus élevée chez les agriculteurs que dans la population générale et ce malgré une moindre prévalence du tabagisme. Il a été montré que l'importance de l'exposition aux poussières organiques et aux endotoxines était corrélée à la prévalence de la BPCO. Néanmoins, la précédente étude (article 5) n'a pas permis d'identifier un lien entre les caractéristiques de l'exposition aux poussières organiques et l'existence d'une BPCO.

L'objectif de la présente étude était de mesurer plus précisément l'exposition en identifiant les micro-organismes (bactéries et champignons) présent dans les logements des sujets (BPCO et témoins) exposés aux poussières organiques et des sujets non exposés. Par ailleurs, la sensibilisation des sujets à ces différents micro-organismes était également étudiée

#### *b. Manuscrit*

Article "Microbial exposure to dairy farmers' dwellings and COPD occurrence". Barrera C, Rocchi S, Degano B, **Soumagne T**, Laurent L, Bellanger AP, Laplante JJ, Millon L, Dalphin JC, Reboux G. *International Journal of Environmental Health Research* 2018.







## Microbial exposure to dairy farmers' dwellings and COPD occurrence

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### ABSTRACT

Dairy farming is a risk factor for chronic obstructive pulmonary disease (COPD). The aim was to determine predictive markers either in blood samples or in dwelling dust samples by comparing COPD and healthy controls with or without farming activity.

Dust was collected and analyzed by real-time quantitative PCR. ELISA and DELFIA<sup>®</sup> were performed to assay the level of specific IgG and IgE of 10 targeted microorganisms.

The dwelling exposure of farmers was higher than in the non-farmers (Especially *Eurotium amstelodami* and *Lichtheimia corymbifera*). The IgG response against *Wallemia sebi* and *Saccharopolyspora rectivirgula* was more often higher in the farmers than the non-farmers. However, exposure and sensitization to the microorganisms tested cannot explain the occurrence of COPD in the dairy farmers' population. COPD development is probably caused by multiple factors associated with exposure over a period of several years.

### ARTICLE HISTORY

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### KEYWORDS

COPD (Chronic Obstructive Pulmonary Disease); farmers; dwellings; microorganisms; IgE (Immunoglobulin E); IgG (Immunoglobulin G)

## Introduction

It is now well known that dairy farmers (DF), exposed to high levels of dust, have an increased risk of developing a respiratory disease (Eduard et al. 2009; Thaon et al. 2011). Chronic Obstructive Pulmonary Disease (COPD) is a common, preventable and treatable disease, characterized by persistent airflow limitation due to airway and/or alveolar abnormalities (Vogelmeier et al. 2017). The most important etiological factor of COPD is tobacco smoking but environmental exposures may contribute (exposure to vapors, gas, dust or fumes). The prevalence of COPD is about two times higher in DF than in the general population (Guillien et al. 2016) although the prevalence of smoking is lower in DF (Roux et al. 2017). High levels of dust and endotoxins were correlated with the prevalence of COPD in a never-smoking animal farmer population (Monso et al. 2004). Exposure to high levels of total dust, organic dust silica, ammonia and hydrogen sulfide were also considered as risk factors for farmers with an atopic status (Eduard et al. 2009). The microorganisms of dust composition on farms in Franche-Comté are well documented. Several investigations have been conducted due to a high prevalence of farmer's

lung disease in this region (Dalphin et al. 1991; Reboux et al. 2001; Roussel et al. 2004, 2005). Specific microflora of farms is transferred into dwellings (Normand et al. 2011), consequently bedroom airborne dust can be considered as the consequence of the kind of occupational activity. Repeated inhalation of fungi and bacteria antigens contained in dust induces an immunological response, and high levels of immunoglobulins G (IgG) or precipitins against farm-specific microorganisms were used as markers of farmer's lung disease (Fenoglio et al. 2007). Moreover a positive level of immunoglobulins E (IgE) against *Aspergillus fumigatus* could predict a risk for tobacco COPD patients of developing bronchiectasis (Everaerts et al. 2017) or allergic bronchopulmonary aspergillosis (Agarwal et al. 2015). The importance of a high level of IgE was also shown with anti-IgE therapy on an asthma COPD patient whose symptoms improved (Yalcin et al. 2016). While it is currently observed that farmers and agricultural workers have a low prevalence of asthma and atopy (Eduard et al. 2009), a recent French study showed that atopy was similar in agricultural workers and controls (Guillien et al. 2016). To our knowledge, in COPD farmers, a population particularly exposed to fungi and bacteria, levels of specific IgE or IgG have never been assessed.

This study aims to highlight a biological marker to screening COPD in farmers by comparing specific IgG and IgE levels between four different subject groups: dairy farmers with COPD (DF COPD), non-farmers with COPD (NF COPD), dairy farmers without COPD (DF controls) and non-farmers without COPD (NF controls). The first step of this study was to identify the microorganisms (bacteria, fungi and mites) present in the organic dust from dwellings of COPD patients and control populations to adapt the panel of antigenic extracts. Dust from bedroom of participants was chose for the sampling instead the dust of the workplace because we spend the majority of a day in the bedroom, and according to the jobs of NF participants, sampling in the workplace is not possible. The second step was to use new homemade specific antigens to test in Enzyme linked immunosorbent assay (ELISA) for specific IgG levels and Dissociated Enhanced lanthanide fluorescent immune Assay (DELFI<sup>®</sup>, PerkinElmer<sup>®</sup>, Waltham, MA, USA) for specific IgE levels in participants' sera.

## Materials and methods

### Recruitment

Patient recruitment was conducted by the Pneumology department of Besançon University Hospital for the BALISTIC cohort (ClinicalTrials.gov Identifier: NCT02540408). The department screened DF and the general population for COPD according to the standard guidelines (Vogelmeier et al. 2017) and covered the entire Franche-Comté region (Eastern France) (Guillien et al. 2018). During his/her consultation, each patient have a health check-up, respond to an environmental and a health questionnaires (details in previous study (Guillien et al. 2016)) and received an electrostatic dust collector (EDC) for environmental investigation, and blood samples were taken for serological analysis. Patients were males or females between 40 and 70 years old. The criteria for exclusion were pregnancy, stage 4 of the GOLD classification (Vogelmeier et al. 2017), corticoid and immunosuppressive treatments, individuals with asthma (self-reported or confirmed by a doctor) or hypersensitivity pneumonitis, individuals with vital prognosis less than 1 year.

### Environmental investigations

#### Dust sampling

One EDC (Noss et al. 2008; Frankel et al. 2012) was placed in the bedroom of each dwelling for 10 weeks. EDCs are sterilized electrostatic wipes that collect dust passively. This device had already been used for analysis with real-time quantitative PCR (qPCR) (Scherer et al. 2014;

Rocchi et al. 2015b). Once they were returned by mail, the EDC wipes were placed in a plastic bag with 20 ml of a solution of Tween 80 to 0.1% and washed in Stomacher™ (AES Chemunex, BioMérieux, Marcy L'Etoile, France) for 10 min. Nine milliliters of rinsing liquid was then collected.

## **Microbiological analysis and identification**

### **Culture**

Cultures were carried out for 104 homes to investigate the culturable microorganisms present in the indoor environment to choose species targeted for DNA quantification by qPCR. Culturing was done on four media: 3% Malt Agar (AES, Bruz, France) 10% salt and 0.5 % chloramphenicol incubated at 20°C, Dichloran glycerol 18% (DG18) (Oxoid, Basingstoke, Hampshire, United Kingdom) at 30°C, Difco actinomycetes isolation agar (BD®, Le Pont-de-Claix, France) at 30°C and R8 at 52°C (Amner et al. 1989). Fifty microliters of liquid samples from the EDC wipes were plated onto the four media. Cultures were checked after 3 and 7 days of incubation. Fungal and actinomyces species were identified by macroscopic and microscopic examination.

### **qPCR**

DNA extraction was performed from the centrifuged washing liquid (8000 g, 10 min) using mechanical and thermal lysis as previously described by Scherer et al. (2014). In each extraction series, a sterile distilled water sample was included as a negative control, and negative and positive controls were added to each qPCR reaction.

Thirteen species were chosen for quantitative measurement by qPCR for the following reasons: (i) presence in dwellings revealed by culture: two fungi *Eurotium amstelodami*, *Penicillium chrysogenum*, and three actinomycetes *Thermoactinomyces vulgaris*, mesophilic *Streptomyces* and *Saccharopolyspora rectivirgula*; (ii) because of their infectious, allergenic and/or toxic pathogenic effects and/or because they are commonly detected in indoor air: *Aspergillus fumigatus*, *Aspergillus versicolor*, *Cladosporium sphaerospermum*., *Stachybotrys chartarum* and *Alternaria alternata* (Reboux et al. 2009) (Kuhn and Ghannoum 2003); (iii) because of their implication in farmer's lung in the Franche-Comté area: *Wallemia sebi* and *Lichtheimia corymbifera* (Reboux et al. 2001). One mite (*Acarus siro*), very common in stored plants, was also targeted.

DNA of the 13 species was quantified by qPCR using primers and Taqman™ (Applied Biosystems, Foster City, CA) probes. qPCR results obtained for *W. sebi* were not interpretable, and finally 12 species were targeted. Eight targeted fungi species were interpretable: *A. alternata*, *A. fumigatus*, *E. amstelodami*, *A. versicolor*, *C. sphaerospermum*, *L. corymbifera*, *P. chrysogenum* and *S. chartarum* (Haugland et al. 2004), as well as three actinomycetes species: mesophilic *Streptomyces*, *S. rectivirgula* and *T. vulgaris* (Schäfer et al. 2011; Betelli et al. 2013). One mite genus (*Acarus siro*) was also targeted (Roussel et al. 2013).

### **Serology**

The levels of specific IgG or IgE antibodies of 10 antigens were measured, respectively, by ELISA or DELFIA® for each individual of the 4 groups. The 10 antigens selected were: *E. amstelodami*, *L. corymbifera*, *A. alternata*, *T. vulgaris*, *S. rectivirgula*, *W. sebi*, *A. fumigatus*, *A. versicolor*, *C. sphaerospermum* and *S. chartarum*. Unfortunately, the *A. siro* mite had to be excluded due to the difficulty of producing homemade antigens.

### **Collection of sera**

From blood samples ( $n = 365$ ), sera were obtained by centrifugation after 10 min at 3000 g, and saved at -80°C.

### Production of antigens

The preparation of protein fraction was made from ten reference strains of molds and actinomycetes from dairy farms. Strains were deposited in the fungal BBCM/IHEM collection (Scientific Institute of Public Health, Brussels, Belgium): *A. alternata* (IHEM 22669), *A. fumigatus* (IHEM 22670), *E. amstelodami* (IHEM 16286), *A. versicolor* (IHEM 22671), *C. sphaerospermum* (IHEM 6976), *L. corymbifera* (IHEM 3809), *S. chartarum* (IHEM 20352), *W. sebi* (IHEM 16284) and in the microorganism collection (Leibniz Institute DSMZ, Leibniz, Germany): *S. rectivirgula* (DSMZ 43747), *T. vulgaris* (DSMZ 43016). Antigens were obtained as previously described by Roussel et al. (2010) and protein profiles were controlled by electrophoresis (SDS-PAGE).

### Enzyme linked immunosorbent assay (ELISA)

Specific Immunoglobulin G (IgG) antibodies were detected by indirect ELISA as described by Roussel et al. (2011a). Sera were diluted at 1/100 and the 10 antigens described above were used at different concentrations: 0.5 µg/ml (*T. vulgaris*), 1 µg/ml (*A. versicolor*, *A. alternata*, *S. chartarum*, *S. rectivirgula*), 5 µg/ml (*C. sphaerospermum*, *W. sebi*) and 10 µg/ml (*A. fumigatus*, *E. amstelodami*, *L. corymbifera*).

On each ELISA plate, six sera from the DF COPD group of the BALISTIC cohort were tested as a reference. A serum with an OD value three times higher than the OD blank value was considered positive. The three optical density values (OD) were blank-corrected and averaged, and a standard deviation and variation coefficient were calculated. If the variation coefficient was higher than 20%, outliers were deleted. The values were normalized based on the mean of the OD values obtained for the 12 reference sera to obtain an IgG index value.

### Dissociation Enhanced Lanthanide Fluorescent Immunoassay (DELFI<sup>®</sup>)

Specific IgE antibodies were detected by DELFIA<sup>®</sup> as described by Barrera et al. (2016) also with the 10 antigens mentioned above. Briefly, 96 well plates were coated with 100 µL of antigen solution, used at different concentrations: 0.1 µg/ml (*S. rectivirgula*), 0.5 µg/ml (*C. sphaerospermum*, *A. versicolor*, *A. fumigatus*), 1 µg/ml (*L. corymbifera*), 2.5 µg/ml (*E. amstelodami*, *S. chartarum*, *T. vulgaris*) and 5 µg/ml (*A. alternata*, *W. sebi*). Then plates were blocked with bovine serum albumin. Participants' sera were incubated for 2 hours, and specific IgE were then detected with a biotinylated anti-human IgE. The final step consisted in adding an enhancement solution after incubation with a europium-labeled streptavidin. The fluorescent read was performed with the Victor 2 multilabel counter<sup>™</sup> (PerkinElmer<sup>®</sup>, Waltham, MA, USA). Only the smallest of the three count values obtained was kept for analysis to avoid hotspot values. An index value was calculated with this smallest value divided by the smallest value of the blank. If the index was >3, the result was considered positive.

### Statistical analysis

Data were analyzed using the statistical program R (version 3.2.2) (R Development Core Team, Vienna, Austria).

Microorganism concentrations were expressed in colony-forming units (CFU) per EDC for those obtained by culture and in DNA equivalents (fg/µl) for those obtained by qPCR. For culture results ( $n = 104$ ), general linear models (glm) with logit-link function for binomial distribution were used to compare the exposure of COPD patients vs controls and DF vs NF. Species richness was also calculated and compared between the different environments (farmer vs non-farmer with a Wilcoxon test) and between the different categories of individuals (DF COPD, DF controls, NF COPD, NF controls with a Kruskal Wallis test). A spearman test was also performed to estimate the influence of the distance between the farm and the house on the microbial contamination.

For qPCR results, a single redundancy analysis (RDA) was performed to assess the structuring effects of month of sampling on microorganism populations. Then, to compare microbial

community composition between the different categories of participants (DF COPD, DF controls, NF COPD, NF controls), partial redundancy analysis (pRDA) was performed (month of sampling considered as covariable to remove its influence).

To estimate the association between qPCR results and sensitization of patients (specific IgG and IgE), tests of correlation (Pearson's correlation coefficient) were performed.

IgG indexes were used for statistical analysis by receiver operating characteristic curves (ROC). ROC allowed antigens to be compared by examining the area under the curve (AUC) to determine if the level of specific IgG was a biological marker of COPD. A chi-square test was used to compare the number of subjects with a positive result for IgG level, and an analysis of variance was used for other quantitative/continuous variables.

## Results

### Subject characteristics

A total of 373 participants were recruited in the BALISTIC study between 2011 and 2015. They were uniformly recruited during the four seasons. Only 365 sera were available for the analyses (100 DF COPD, 84 NF COPD, 93 DF controls and 88 NF controls) and among them only 314 EDC were available for environmental analyses (89 DF COPD, 68 NF COPD, 81 DF controls and 76 NF controls). Subject characteristics are summarized in Table 1. All groups were mainly made up of males. No statistical differences in age, sex ratio or smoking were found between DF COPD and DF controls or between NF COPD and NF controls. Fewer DF were smokers as compared to NF. Lower pack/year was reported for DF smokers in comparison with NF smokers (27.2 pack/year instead of 36.6, respectively, for male COPD).

For farmers, 28% have their dwellings in the farm, for others the house was separated of the farm of 346 m in mean. Forty-one percent of the non-farmers have a job in the tertiary sector (education, accounting, secretariat, etc.), 45% in the secondary sector (building worker, industry, etc.) and 14% in the primary sector other than farmer (cheesemonger, florist, etc.).

### Dwelling microbial balance by culture

Twenty-five different species of molds, actinomycetes and yeasts were detected with EDC by culture in the 104 dwellings (the 13 most common species are presented in Table 2). *E. amstelodami* and *Penicillium* spp. were the two mold species most commonly found in dwellings and in high concentrations as well as three actinomycete species (mesophilic *Streptomyces*, *S. rectivirgula* and *T. vulgaris*) (Table 2).

The comparison of microorganisms (in terms of global contamination, species richness or individual species concentrations) between homes of DF COPD and DF controls or between NF

**Table 1.** Subject characteristics.

	DAIRY FARMERS		NON-FARMERS	
	DF COPD (n = 100)	DF Controls (n = 93)	NF COPD (n = 84)	NF Controls (n = 88)
Age (mean years) [Min, Max]	60.4 [40–75]	59.2 [39–74]	61.5 [43–75]	60.1 [40–73]
Men, n (%)	85 (85%)	81 (87%)	65 (77%)	65 (74%)
<b>Smoking habits</b>				
Non-smoker, n (%)	48 (48%)	50 (54%)	7 (8%)	12 (14%)
Former smoker, n (%)	33 (33%)	31 (33%)	35 (42%)	42 (48%)
Active smoker, n (%)	19 (19%)	12 (13%)	42 (50%)	34 (39%)
Number of packs-year	27.5 ± 16.1	22.5 ± 12.1	39.3 ± 23.9	31.9 ± 17.5

DF COPD: Dairy farmers with chronic obstructive pulmonary disease;

DF controls: Dairy farmer controls;

NF COPD: Non-farmers with chronic obstructive pulmonary disease;

NF controls: Non-farmer controls.

**Table 2.** Microorganisms detected by culture analyses from Electrostatic Dust Collector (EDC) (340 cm<sup>2</sup>) after 10 weeks of exposure in participants' dwellings (n = 104).

Microorganisms	DAIRY FARMERS DWELLINGS						NON-FARMERS DWELLINGS					
	Presence (%)			Mean of CFU/EDC			Presence (%)			Mean of CFU/EDC		
	DF COPD	DF Controls	DF Controls of the studied area	DF COPD	DF Controls	DF Controls	NF COPD	NF Controls	NF Controls	NF COPD	NF Controls	All NF (n = 53)
Species known to be present in farms of the studied area												
<i>Eurotium amstelodami</i>	100	92	316,2	224,8	96	270,5	67	62	20,7	146,9	64	83,8
<i>Lichtheimia corymbifera</i>	50	28	36,9	13,6	39	25,25	0	8	0	3,1	4	1,55
<i>Wallemia sebi</i>	31	28	12,3	12	29	12,15	7	4	2,2	1,5	6	1,85
<i>Thermoactinomyces vulgaris</i>	69	72	168,5	78,4	71	123,45	78	62	79,3	81,5	70	80,4
<i>Saccharopolyspora rectivirgula</i>	85	68	896,2	642,4	76	769,3	41	42	20,7	70	42	45,35
<i>Saccharomonospora viridis</i>	69	56	105,4	114,4	63	109,9	26	46	8,1	83,1	36	45,6
<b>Mesophilic Streptomyces</b>	88	84	497,7	497,6	86	497,65	44	58	37	145,4	51	91,2
Others common species												
<i>Penicillium</i> spp.	65	72	84,6	48,8	69	66,7	52	85	42,2	70	68	56,1
<i>Aspergillus versicolor</i>	27	32	35,4	41,6	29	38,5	15	50	5,9	40	32	22,95
Thermophilic <i>Streptomyces</i>	31	24	37,7	71,2	27	54,45	22	19	6,7	9,2	21	7,95
<i>Aspergillus Aspergillus niger</i>	23	8	33,1	2,4	16	17,75	7	12	2,2	13,8	9	8
<i>Aspergillus fumigatus</i>	0	4	0	0,8	2	0,4	7	0	1,5	0	4	0,75
<i>Cladosporium</i> sp.	8	0	1,5	0	4	0,75	0	0	0	0	0	0

Species in bold were chosen for qPCR analyses.

CFU/EDC: colony forming unit per Electrostatic Dust Collector;

DF COPD: Dairy farmers with chronic obstructive pulmonary disease (n = 26);

DF controls: Dairy farmer controls (n = 25);

NF COPD: Non-farmers with chronic obstructive pulmonary disease (n = 27);

NF controls: Non-farmer controls (n = 26).

COPD and NF controls showed no significant statistical differences. Only differences between types of environmental exposure (farmers vs non-farmers) were found. Species richness was greater for farmers' dwellings ( $p$ -value  $<0.01$ ). Farmers' environments were more contaminated (in total 1993 CFU per EDC for farmer dwellings vs a total of 442 CFU per EDC for non-farmer dwellings), and the *S. rectivirgula* contamination difference between all DF homes and all NF homes was determined (glm  $p$ -value  $<0.05$ ).

### Microbial quantification by qPCR

According to the RDA on qPCR results of 12 species, the microorganism distribution differed significantly among the various months of sampling ( $p$ -value = 0.001). The mold *A. alternaria* was more present during the summer season (June–September). However, *L. corymbifera*, *A. versicolor*, *E. amstelodami* and to a lesser degree *T. vulgaris* were more abundant from October to January, a period when cows are fed with stored hay. *E. amstelodami* was more frequently observed when the farm is closer to the dwellings ( $p$ -value = 0.006).

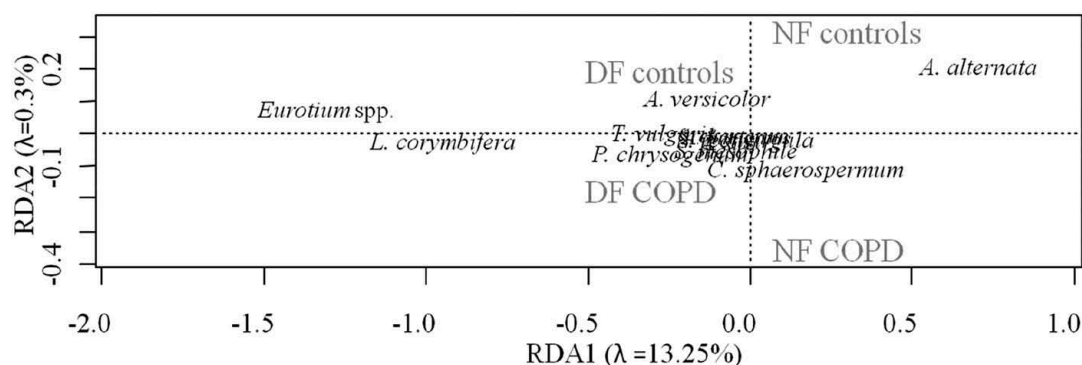
In the pRDA (with months of sampling as covariates explaining 7.3% of the variance), the microorganism distribution differed significantly among the four groups (DF COPD, DF controls, NF COPD and NF controls,  $p$ -value = 0.002). These differences were significant and account for 12.7% of the microorganism variability. Dwelling dust of DF COPD and DF controls were better correlated with the abundance of *E. amstelodami* and *L. corymbifera*, whereas dwelling dust of NF COPD and NF controls were correlated with *A. alternaria* (Figure 1). Thus, in accordance with this analysis of microorganism communities, a microflora specific to farmers' dwellings can be described. qPCR results expressed in DNA equivalents are also shown in Table 3.

### Immunological responses

Participants were recruited throughout the year and exposure to microorganisms varied according to the season, but no 'month effect' was statistically detected on the immunological response ( $p$ -value = 0.071).

### Specific IgG response to fungi and bacterial species

Few differences in IgG-specific responses of the 10 species tested were observed between the 4 groups (Figure 2). IgG level for *C. sphaerospermum* was particularly elevated unlike global IgG levels for *A. alternaria*, *A. fumigatus* and *A. versicolor* (Figure 2). The number of participants with a positive IgG response (three times higher to the background) was significantly higher for both DF groups than NF groups for *W. sebi* ( $\chi^2 = 32.6$ ,  $p$ -value  $<0.001$ ) and *S. rectivirgula* ( $\chi^2 = 7.9$ ,



**Figure 1.** Partial redundancy analysis (pRDA) plot showing target microorganisms (in black) and the distribution of patient groups (in grey).

DF COPD: Dairy farmers with chronic obstructive pulmonary disease; DF controls: Dairy farmer controls; NF COPD: Non-farmers with chronic obstructive pulmonary disease; NF controls: Non-farmer controls.



**Table 3.** Mean microorganism concentrations quantified by qPCR from Electrostatic Dust Collector (EDC) among the four groups.

qPCR targets in fg/ $\mu$ L eq DNA	Dairy farmers dwellings		Non-farmers dwellings	
	DF COPD (n = 89)	DF Controls (n = 81)	NF COPD (n = 68)	NF Controls (n = 76)
<i>Alternaria alternata</i> . $10^2$	0.90 $\pm$ 2.15	0.90 $\pm$ 2.47	1.79 $\pm$ 3.68	1.71 $\pm$ 3.8
<i>Aspergillus fumigatus</i> . $10^{-2}$	0.95 $\pm$ 3.42	0.95 $\pm$ 2.57	0.10 $\pm$ 0.31	0.47 $\pm$ 2.04
<i>Acarus siro</i> . $10^{-2}$	11.3 $\pm$ 29.15	5.58 $\pm$ 15.64	0.77 $\pm$ 2.11	0.93 $\pm$ 2.92
<i>Cladosporium sphaerospermum</i>	0.54 $\pm$ 2.04	0.20 $\pm$ 0.33	0.53 $\pm$ 0.72	0.32 $\pm$ 0.68
<i>Eurotium amstelodami</i> . $10^1$	6.10 $\pm$ 16.54	4.48 $\pm$ 9.76	0.24 $\pm$ 0.55	0.40 $\pm$ 1.2
<i>Lichtheimia corymbifera</i>	14.69 $\pm$ 45.23	10.06 $\pm$ 20.93	0.38 $\pm$ 1.06	0.31 $\pm$ 0.9
<i>Stachybotris chartarum</i> . $10^{-2}$	9.22 $\pm$ 32.49	7.63 $\pm$ 25.6	2.88 $\pm$ 12.04	8.56 $\pm$ 30.56
<i>Penicillium chrysogenum</i>	4.69 $\pm$ 6.91	6.99 $\pm$ 14.78	4.32 $\pm$ 8.78	3.01 $\pm$ 5.01
Mesophilic <i>Streptomyces</i> . $10^{-1}$	3.50 $\pm$ 26.93	1.03 $\pm$ 1.99	28.86 $\pm$ 243.01	0.49 $\pm$ 1.85
<i>Saccharopolyspora rectivirgula</i> . $10^{-3}$	15.16 $\pm$ 68.84	16.89 $\pm$ 43.92	0.66 $\pm$ 3.99	0.90 $\pm$ 5.55
<i>Thermoactinomyces vulgaris</i>	1.15 $\pm$ 2.68	1.51 $\pm$ 3.76	0.21 $\pm$ 0.49	0.23 $\pm$ 0.73
<i>Aspergillus versicolor</i>	3.12 $\pm$ 5.47	5.45 $\pm$ 12.08	2.39 $\pm$ 4.37	4.45 $\pm$ 12.49

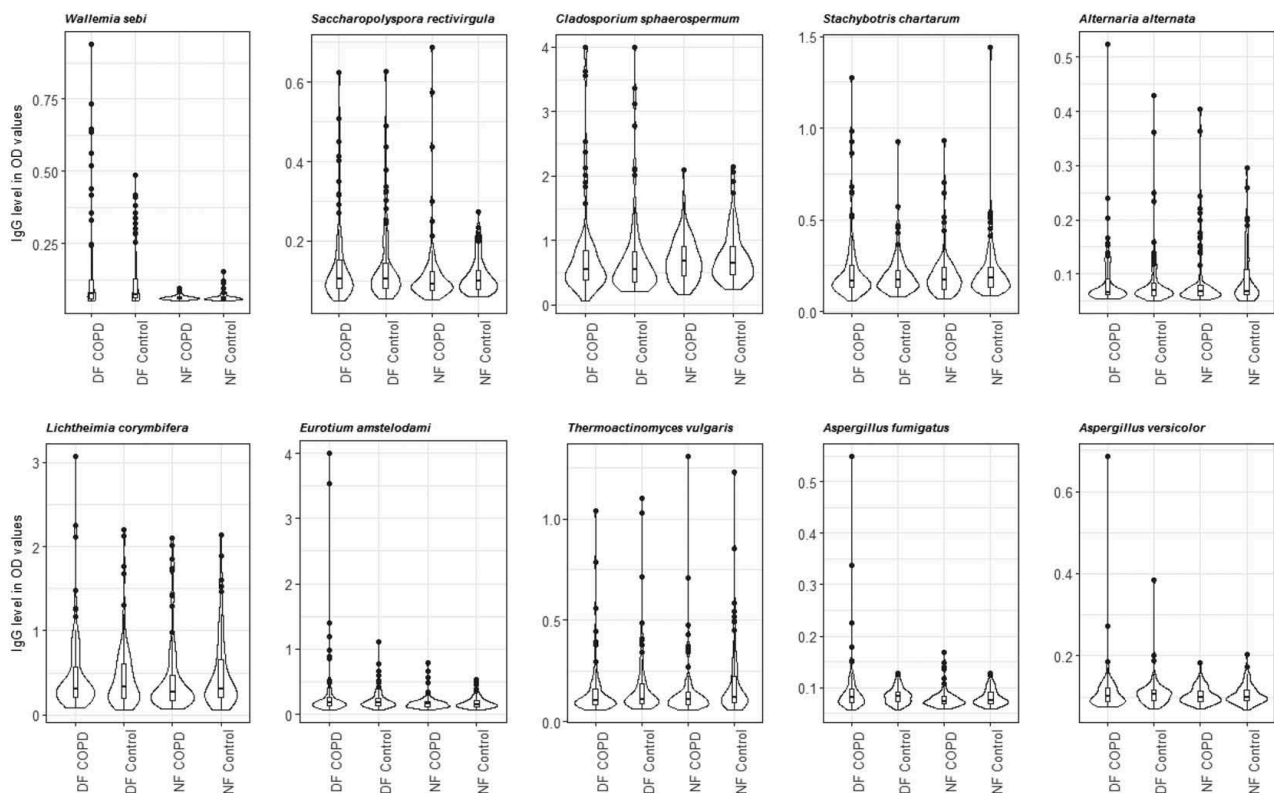
Microorganism concentrations are expressed in DNA equivalents (fg/ $\mu$ L). NB: the species cannot be compared with each other because the number of repeats of DNA copies is not the same for each species.

DF COPD: Dairy farmers with chronic obstructive pulmonary disease;

DF controls: Dairy farmer controls;

NF COPD: Non-farmers with chronic obstructive pulmonary disease;

NF controls: Non-farmer controls.

**Figure 2.** Violin plot of participants' IgG response in OD values to the 10 species.

DF COPD: Dairy farmers with chronic obstructive pulmonary disease; DF controls: Dairy farmer controls; NF COPD: Non-farmers with chronic obstructive pulmonary disease; NF controls: Non-farmer controls.

$p$ -value = 0.048) even if less than 25% of the two DF groups showed a positive IgG response to these two antigens. For the species *W. sebi*, the comparison of IgG response by statistical analysis of the ROC curves shows an AUC of 0.74 between DF controls and NF controls and an AUC of 0.70 between DF COPD and NF COPD. These results reflect a particular sensitization to *W. sebi* among DF independently of the occurrence of COPD (AUC of 0.50 between DF COPD and DF

controls). Thus, none of the species was highlighted as a biological marker in terms of level of IgG specific for the COPD.

### Specific IgE response to fungi and bacterial species

Among all participants, 24 (6.6%) had a positive IgE response to at least one antigen (Index value >3) (Table 4), thus indicating a prevalence of 7.6% for fungal IgE sensitization in COPD patients and 5.5% for controls. Only one, a DF control, had a positive IgE response to two antigens, *A. fumigatus* and *E. amstelodami*. The prevalence of IgE sensitization to *A. fumigatus* was 2.7% in the COPD population (3 DF COPD and 2 NF COPD; Table 4). None of the participants developed a positive IgE response to *L. corymbifera*, *T. vulgaris* and *W. sebi*. Among the 10 DF COPD with a positive IgE response, 8 had never smoked, 1 was a former smoker and the remaining 1 was a current smoker. On the whole, no difference was observed between the four groups for specific microorganism IgE sensitivity.

### Specific IgG or IgE response and levels of aerocontamination

No correlation was found between the IgG- or IgE-specific level and qPCR results ( $p$ -values >0.05 in correlation tests). So, a high level of contamination for one species does not induce a high level of IgG- or IgE-specific antibodies in our studied population.

### Influence of tobacco consumption

Sub-groups were created to study the smoking habit effect on COPD participants (smokers and participants who had never smoked). Former smoker participants were excluded from the analyses because of their ambiguous status. In the remaining population, according to their smoking habits, COPD and controls were similarly sensitized. If we considered only current smokers NF COPD (caused by tobacco,  $n = 42$ ) and DF COPD who declared that they had never smoked (for whom the cause of COPD was unclear,  $n = 48$ ), IgG sensitization was similar. Tobacco consumption does not influence the level of IgG-specific antibodies.

## Discussion

Results on housing dust samples, by culture ( $n = 104$ ) and by qPCR ( $n = 314$ ), showed higher mold and actinomycete concentrations in the dwellings of farmers with and without COPD (DF COPD and DF controls). However, microorganism concentrations were not significantly higher in dwellings of COPD patients compared to their respective control categories. The immunological response of all farmers showed a higher frequency of positive IgG response to the antigens of

**Table 4.** Number of participants with a specific IgE positive response among the 365 participants.

	Dairy farmers		Non farmers	
	DF COPD ( $n = 100$ )	DF controls ( $n = 93$ )	NF COPD ( $n = 84$ )	NF controls ( $n = 88$ )
Total Fungal sensitized <sup>a</sup>	10 (10%)	6 (6.4%)	4 (4.7%)	4 (4.5%)
<i>Aspergillus fumigatus</i>	3 (3%)	3 (3.2%)	2 (2.4%)	2 (2.3%)
<i>Aspergillus versicolor</i>	3 (3%)	2 (2.1%)	2 (2.4%)	1 (1.1%)
<i>Alternaria alternata</i>	2 (2%)	1 (1%)	0 (0%)	0 (0%)
<i>Stachybotrys chartarum</i>	1 (1%)	0 (0%)	0 (0%)	0 (0%)
<i>Cladosporium sphaerospermum</i>	0 (0%)	0 (0%)	0 (0%)	1 (1.1%)
<i>Saccharopolyspora rectivirgula</i>	1 (1%)	0 (0%)	0 (0%)	0 (0%)
<i>Eurotium amstelodami</i>	0 (0%)	1 (1%)	0 (0%)	0 (0%)

Data are presented as  $n$  (%); Positivity was defined by an IgE index value >3. *W. sebi*, *L. corymbifera* and *T. vulgaris* do not appear in the table because none of the participants has a positive IgE level.

DF COPD: Dairy farmers with chronic obstructive pulmonary disease;

DF controls: Dairy farmer controls;

NF COPD: Non-farmers with chronic obstructive pulmonary disease;

NF controls: Non-farmer controls;

<sup>a</sup>sensitization to at least one of the antigens.

*W. sebi* and *S. rectivirgula* than the entire NF population. No correlation was found between the level of immunological response and the level of microorganisms according to qPCR results. With regard to smoking habits, no IgG response difference was observed among participants.

Settled dust from EDC is recognized to be a standardized method, the most representative of all airborne dust (Frankel et al. 2012; Cox et al. 2017) and relevant for large-scale study (Rocchi et al. 2015a). Culture assays, although biased by the development of only viable microorganisms and the competition between species (Crawford et al. 2015), were useful in our study to assess species commonly encountered in recruited dwellings. Thus, the qPCR target selection was partly based on the culture results as well as the high allergenicity potential of some species (such as *A. alternata* for example). In contrast to culture methods, qPCR analyses detect viable and unviable fragments that may be involved in clinical manifestations (Tischer et al. 2011). Another advantage of qPCR is the use of the same standard operational procedure for different microbial agents (molds, actinomycetes and mites).

We chose to analyze dwelling dust rather than that of professional exposure in order to compare with NF participants who do different jobs (especially for jobs with outdoor tasks). COPD patients were not more exposed than healthy subjects of their respective categories (DF controls or NF controls). Not surprisingly, the high incidence and concentrations of *E. amstelodami*, *L. corymbifera* and some mesophilic (mesophilic *Streptomyces*) or thermophilic (*S. rectivirgula*, *T. vulgaris*) actinomycetes were observed in Franche-Comté DF dwellings. These microorganisms are already known to be abundant on Franche-Comté farms and are involved in the occurrence of Farmers' Lung Disease (Reboux et al. 2001). This observation suggests the transfer of a large number of microorganisms from the barn to the dwellings (Normand et al. 2011). This transfer may potentially be caused by air flow in dwellings close to barns, by farmers going in and out of their homes or by bringing contaminated clothes inside. This flow or farmer's lifestyle may modify the microbial flora and therefore change the microbial exposure of the DF in their dwellings (Roussel et al. 2011b).

Tools used in our study confirmed the presence of a special microbial community within farmers' dwellings but they did not identify a special microbial community that could be responsible for the occurrence of COPD in the DF population. No correlation between exposure and disease was found, and it is probably because COPD is caused by multiple factors including individual characteristics, smoking or by exposure to some chemicals like biocides or toxic and inflammatory substances secreted by the microorganisms (mycotoxins) (Garon et al. 2006; Seltzer and Fedoruk 2007) or coming from them (endotoxins) (Barnig et al. 2013). Agricultural practices are highly similar in our farmer population because of a strictly control of cow milk for Comté Cheese production. It is supposed that occurrence of COPD could not be explained by differences of working activity.

High exposure to microorganisms does not necessarily lead to strong sensitization, and NF participants are also sensitized. Among the 10 representative species tested, specific IgG and IgE responses were similar in both the DF and NF populations, respectively (COPD patients and controls). Frequency of positive IgG response was higher to *W. sebi* and *S. rectivirgula* for DF COPD and DF controls than both NF COPD and NF controls. *S. rectivirgula* and *W. sebi* were also very common in the indoor environment (Desroches et al. 2014). Farmers potentially cumulate indoor exposure in their dwellings with high professional microbial exposure because these species are very common in straw or hay (Reboux et al. 2001; Roussel et al. 2005). However, this positive IgG response was observed only in a minority of the DF population (less than 30% of DF COPD and DF controls for *S. rectivirgula* and less than 20% for *W. sebi*). The comparison of Ig responses in the two sub-populations was also similar: NF COPD (due to tobacco consumption because they were current smokers) and DF COPD (who never smoked and the cause of their illness remained unclear). Allergic IgE response against microbes was low in our population. Only 24 participants presented high levels of specific IgE, and less than 5% of COPD had specific IgE to *A. fumigatus*. A mono-sensitization was

observed (except for one control) like in other studies cited in the review by Pashley (2014). Bafadhel et al. (2014) reported that 13% of COPD had an allergic response to *A. fumigatus* (demonstrated by skin prick test or specific IgE by ImmunoCAP system®, Thermo Scientific Phadia®, Uppsala, Sweden) and Jin et al. (2014) showed that 41 out of a total of 273 study participants had *A. fumigatus* sensitization (15%). The difference we observed in our study may be due to our recruitment, which focused on COPD in the agricultural context, but also our study technique. We used an in-house DELFIA® technique and not the ImmunoCAP system, as was the case in other studies, because the former allowed us to adapt the antigen panel to the specific exposure of our study population.

## Conclusion

The occurrence of COPD in DF cannot be explained by the tools used in this study (i.e. microbe exposure and specific IgG and IgE levels). Both healthy and COPD farmers have a higher positive IgG response for *S. rectivirgula* and *W. sebi* than the NF population. Farmers were far more exposed to microbes in dwellings than the general population and were also highly exposed in their professional environment. Consequently, they cumulate the risks of developing respiratory pathologies linked to airborne microbes. No specific microbial community of agricultural COPD patients' dwelling could be updated, nor did specific biomarkers such as specific IgG or IgE linked to the microflora of COPD patients' dwelling dust, nor were we able to identify a predictive marker related to the smoking habits of the study population. Mycotoxins and endotoxins have already been measured in environmental samples and these investigations show that they cause respiratory disorders. Further research is needed to identify a new biomarker of the COPD caused by the farm environment, and a measurable fraction of toxins in biological fluid samples (blood or urinary samples) in farmers' COPD could also be studied.

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#### 4. Article 7 : Is atopy a risk indicator of chronic obstructive pulmonary disease in dairy farmers?

##### *a. Contexte et objectif*

Bien que la physiopathologie de la BPCO tabagique soit maintenant bien connue, peu d'études se sont intéressées à celle de la BPCO secondaire aux poussières organiques. L'exposition aux poussières organiques, évaluée soit sous forme de questionnaire (caractéristiques de la ferme et de l'exposition), soit de manière quantitative, ne semble pas suffisante pour expliquer à elle seule la survenue de la BPCO (article 5 et 6). Plus généralement, on considère que seuls 10 à 20 % des fumeurs développent une BPCO. Ceci suggère des facteurs liés à l'hôte. Un lien entre l'atopie et un VEMS bas a d'ailleurs été proposé chez les sujets exposés aux poussières organiques.

L'objectif de cette étude était d'évaluer la relation entre l'existence d'une BPCO et la présence de marqueurs cliniques et biologiques d'atopie chez les sujets agriculteurs et non agriculteurs.

##### *b. Manuscrit*

Article "Is atopy a risk indicator of chronic obstructive pulmonary disease in dairy farmers?" Veil-Picard M, <b>Soumagne T</b> , Vongthilath R, Annesi-Maesano I, Guillien A, Laurent L, Andujar P, Roche R, Jouneau S, Cypriani B, Laplante JJ, Degano B, Dalphin JC. <i>Respiratory Research - Accepted.</i>
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# Is atopy a risk indicator of chronic obstructive pulmonary disease in dairy farmers?

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**Abstract:** Allergic mechanisms related to environmental and occupational exposure have been suggested to contribute to the development of chronic obstructive pulmonary disease (COPD).

**Objectives:** To investigate the relationships between atopy markers, persistent airflow limitation (PAL) and occupational exposure in dairy farmers.

**Methods:** Clinical and biological (total IgE and 21 allergen specific IgE) markers of atopy were assessed in 101 dairy farmers with PAL (DF-PAL), 85 non-farmers with PAL (NF-PAL) (both groups were prospectively included from a screening program performed between 2011 and 2015), and matched controls, i.e. 98 farmers without PAL (DF-controls) and 89 non-farming subjects without PAL (NF-controls). Occupational exposure in farmers was estimated using a validated questionnaire.

**Results:** Prevalence of allergy history was significantly higher in DF-PAL and in NF-PAL than in controls. Polysensitization, and sensitization to seasonal and food allergens were more frequent in DF-PAL than in DF-controls, respectively: 13.8% vs 1% (adjusted odds ratio (aOR): 17.5 (2.2–134), 11.9% vs 3.1% (aOR: 4.4 (1.2–7.2) and 16.8% vs 4.1% (aOR: 5.2 (1.7–7.2)). The prevalence of atopy markers was similar between NF-PAL patients and NF-controls.

**Conclusions:** PAL in farmers is associated with a high rate of markers of atopy, supporting atopy as a risk indicator.

Clinical trial registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02540408) (NCT02540408).

**Keywords:** Atopy, Chronic obstructive pulmonary disease, Specific IgE, Farmers, Occupational exposure, Matthieu Veil-Picard and Thibaud Soumagne contributed equally to the work.

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease, characterized by persistent airflow limitation, which is usually progressive [1]. The most important etiological factor for COPD is tobacco smoking, but many other factors can trigger the lung inflammation leading to COPD. Occupational

exposure to vapors, gas, dust or fumes ranks high among these factors, and accounts for 20% of COPD in developed countries [2, 3]. Biological dusts may be associated with a higher risk of occupational COPD than mineral dusts [3]. Exposure to organic dusts has been shown to be associated with an increased risk of obstructive lung diseases, especially in dairy farmers [4, 5].

Although the pathophysiological traits of tobacco-related COPD are now well described, there are very few studies that have specifically assessed the pathophysiology of COPD related to organic dust exposure. Inflammatory response in tobacco-related COPD is dominated by Th1-type lymphocyte response, along with Th17

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cytokine production [6]. By contrast, COPD induced by inhalation of biomass fumes could be associated with a predominantly Th2-type lymphocyte production profile, in addition to an increase in IL-4 [7].

Although there is some evidence suggesting a link between atopy and a lower FEV1 [8–10] related to organic dust exposure, especially in non-smokers, a comprehensive study of atopic response and the presence of PAL in dairy farmers using non-PAL dairy farmers as controls, as well as in subjects with or without PAL in the general population, is lacking. Since we speculate that PAL in dairy farmers may involve IgE-mediated reactions, we aimed to analyze the relationship between PAL, clinical and biological markers of atopy in farming and non-farming subjects.

## Methods

### Study design and subjects

Data for this study were collected as part of the BALISTIC project (COPD in dairy farmers: screening, characterization and constitution of a cohort; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02540408) Identifier: [NCT02540408](https://clinicaltrials.gov/ct2/show/study/NCT02540408)) (see additional file 1) [11].

A diagnosis of PAL was retained when the FEV1/FVC ratio post-bronchodilator was less than 0.70. PAL patients were rated either as stage 1 (i.e., FEV1 was >80% of the predicted value) or stage 2+ (i.e., FEV1 was ≤80% of the predicted value). Spirometry was considered normal when the FEV1/FVC ratio was >0.70 and FEV1 was >80% of the predicted value before bronchodilator administration. Predicted values were based on the GLI equations [12].

All subjects in whom PAL was detected during the “screening phase” were invited to participate in the “characterization” phase of the study. Those who accepted to participate in this “characterization” phase were included in two subgroups, namely dairy farmers with PAL (DF-PAL) and non-farmers with PAL (NF-PAL). Subjects with normal spirometry who had participated in the “screening” phase of the study were frequency matched with the PAL subjects in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and sex; these subjects with normal spirometry constituted 2 additional subgroups, namely, dairy farmers without PAL (DF-Controls) and non-dairy farmers without PAL (NF-Controls). Ethical approval was received from the local Ethics Committee (CPP Est; 11/617), and written consent was obtained from all subjects.

### Procedures

During the “characterization” hospital visit, subjects had a second spirometry test performed by a physician specialized in physiology, as well as several standardized questionnaires and allergological examinations. The medical questionnaire was an adapted French translation

of the long version of the European Community Respiratory Health Survey questionnaire [13, 14]. The occupational questionnaire was sent to the subjects 10 days before their medical examination, collected during the medical examination, and reviewed in the presence of the subject [5, 15].

Total IgE concentration in the blood and allergen-specific IgE against 21 common food and inhalant allergens were assessed. Allergen mix was performed as the first-line. If the allergen mix was positive, then allergen-specific IgE were determined.

Blood assays were performed using enzyme-linked immunosorbent assay (ELISA), ImmunoCAP® (ThermoFisher Scientific/Phadia, Uppsala, Sweden), in the Biochemistry Laboratory at the University Hospital of Besançon, France. The total and allergen-specific IgE concentration in the blood were dichotomized at the detection limit of 100 kIU/L and 0.35 kUA/L, respectively [16, 17]. A polysensitized patient was defined as a subject having at least three positive tests for allergen-specific IgE.

In addition, analyses of dust from each patient dwelling and specific patient sensitization to his environment have been previously published [18].

### Statistical analysis

Qualitative variables are presented as number and percentage, and quantitative variables as mean ± standard deviation (SD) or median and interquartile range (IQR) (Q1-Q3).

Characteristics of PAL and control subjects were compared using the Student t-test or Wilcoxon test as appropriate; and the Chi-square or Fisher’s exact test as appropriate, for quantitative and qualitative variables, respectively.

Bivariate analyses were used to compare the results of clinical and biological markers of atopy in DF-PAL vs DF-controls, and in NF-PAL vs NF controls. The association between these atopy markers and PAL in farmers and PAL in non-farmers was assessed by using logistic regressions with adjustment for age, smoking status (< 1 pack-year (reference), 1–15 pack-years and > 15 pack-years) and sex (female as reference). Odds ratios are presented using forest plots. We used different multivariate regression models to explain PAL in the whole population in order to test the relevant atopy markers with adjustment for farming status, age, sex and smoking. Odds ratios are given with 95% confidence intervals (CI).

A *p*-value < 0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

## Results

Among the 8106 subjects who underwent screening, 6704 were affiliated to the Mutualité Sociale Agricole

(MSA), i.e. approximately 40% of the regional target population for the free health check-up. The remaining 60% of subjects were sampled to assess the representativeness of our study population. Among the 8106 subjects, 4963 met the inclusion criteria for the study (60.8%). Among the 2384 farmers with interpretable spirometry, 191 (8.01%) suffered from PAL. In total, 355 patients with PAL (191 dairy farmers and 164 non-farmers) and 3188 patients with normal spirometry were identified. 2 hundred and 10 PAL patients and 193 non-PAL matched controls agreed to take part in the “characterization” phase of the study during which 30 subjects had to be excluded, mainly owing to the existence of a history of asthma or to occupational exposure other than dairy farming. Finally, a total of 373 subjects were included: 101 DF-PAL, 98 DF-Controls, 85 NF-PAL and 89 NF-Controls (Table 1 and Fig. 1).

A total of 365 subjects affiliated to the MSA and not present at the screening were successfully contacted. They were older on average ( $61.9 \pm 10.0$  vs  $60.2 \pm 9.7$  years,  $p = 0.0014$ ) and more often male (72.6% vs 57.5%,

$p < 0.0001$ ) than subjects who participated in the screening, but their smoking status was not statistically different. In addition, patients who participated in the hospital “characterization” visit were not different from those screened (PAL and non-PAL groups) in terms of smoking status, sex-ratio, spirometry (% of theoretical values), but they were younger (60.3 vs 64.2 years,  $p = 0.0047$ ).

The main features of the four groups are summarized in Table 1. Both PAL groups were composed mainly of mild PAL with preserved FEV1. The proportion of current-smokers and former-smokers was lower among farmers than in non-farmers. The proportion of men was higher among farmers than among non-farmers, but the difference was not statistically significant.

The mean level of bronchodilation during the bronchodilation test was similar in DF-PAL and NF-PAL:  $8.2\% \pm 8.2$  vs  $7.8\% \pm 7.5$ ,  $p = 0.7202$ , respectively.

The atopy markers among the groups are shown in Table 2. There was a statistically significant relation between a self-reported personal history of allergy and PAL. In dairy farmers, the frequency of sensitizations to

**Table 1** Characteristics of the study patients ( $n = 373$ )

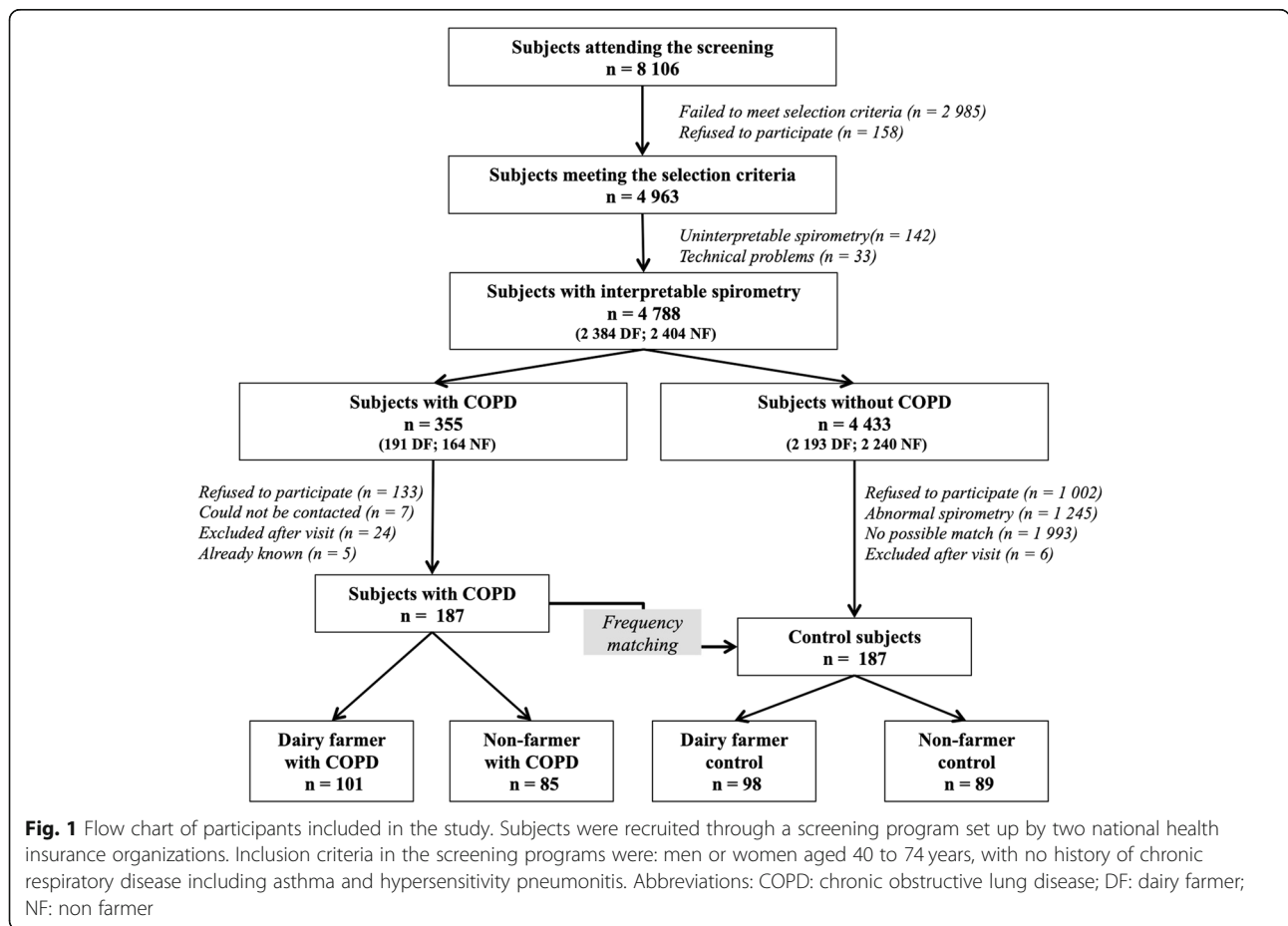
	DF- COPD $n = 101$	DF-Control $n = 98$	<i>p</i> -value	NF- COPD $n = 85$	NF-Control $n = 89$	<i>p</i> -value
Age, years	$60.3 \pm 9.1$	$59.3 \pm 8.9$	NS	$61.4 \pm 7.6$	$60.2 \pm 6.5$	NS
Men	86 (85.1)	86 (87.8)	NS	66 (77.6)	65 (73.0)	NS
BMI	$26.5 \pm 4.1$	$27.0 \pm 4.1$	NS	$26.1 \pm 4.1$	$26.8 \pm 3.8$	NS
Smoking status			NS			NS
Non-smoker	48 (47.5)**	54 (55.1)		7 (8.2)	12 (13.5)	
Former-smoker	33 (32.7)**	32 (32.7)		36 (42.4)	42 (47.2)	
Currentsmoker	20 (19.8)**	12 (12.2)		42 (49.4)	35 (39.3)	
Smoking pack years			NS			NS
< 1	49 (48.5)**	55 (56.1)		7 (8.2)	13 (14.6)	
1–15	18 (17.8)**	21 (21.4)		13 (15.3)	15 (16.9)	
> 15	34 (33.7)**	22 (22.5)		65 (76.5)	61 (68.5)	
Exacerbation	23 (22.8)			23 (27.1)		
At least one respiratory symptom	54 (53.1)	21 (21.3)	<b>&lt; 0.001</b>	51 (60.0)	23 (25.9)	<b>&lt; 0.001</b>
Dyspnea, mMRC > 0	38 (38.0)	15 (15.3)	<b>&lt; 0.001</b>	39 (45.9)	19 (21.4)	<b>&lt; 0.001</b>
SGRQ	$15.1 \pm 12.9^*$	$7.3 \pm 7.2$	<b>&lt; 0.001</b>	$19.4 \pm 14.9$	$9.3 \pm 9.3$	<b>&lt; 0.001</b>
FEV1 /FVC post-BD, %pred	$63.0 \pm 6.8^*$	$78.5 \pm 4.3$	<b>&lt; 0.001</b>	$60.6 \pm 8.0$	$79.9 \pm 4.1$	<b>&lt; 0.001</b>
FEV1 post-BD, %pred;	$85.2 \pm 14.8^*$	$105.5 \pm 10.9$	<b>&lt; 0.001</b>	$80.0 \pm 16.4$	$108.3 \pm 12.7$	<b>&lt; 0.001</b>
FVC post-BD, %pred;	$105.3 \pm 15.7$	$104.8 \pm 11.4$	NS	$102.1 \pm 14.8$	$105.7 \pm 12.0$	NS
Severity of airway obstruction, n (%)						
Stage I	44 (44)			33 (39)		
Stage II +	57 (56)			52 (61)		

Data are presented as n, n (%) or mean  $\pm$  SD, unless otherwise stated

COPD chronic obstructive pulmonary disease, DF-COPD dairy farmers with COPD, NF-COPD non-dairy farmers with COPD, SD standard deviation, BMI body mass index, mMRC modified Medical Research Council, SGRQ St George's Respiratory Questionnaire, FVC forced vital capacity, FEV1 forced expiratory volume in 1 s

\*  $p < 0.05$ , vs NF- COPD, \*\*  $p < 0.01$ , vs NF- COPD

Significant *P* values ( $< 0.05$ ) are in bold



**Table 2** History of atopy, specific IgE level and total IgE amount among dairy-farmers and non-dairy-farmers

	DF-COPD n = 101	DF-Control n = 98	p-value	NF- COPD n = 85	NF-Control n = 89	p-value
Personal history of hay fever, %	8.9	10.2	NS	14.1	12.8	NS
Personal history of atopic dermatitis, %	5.0	5.1	NS	9.6	4.6	NS
Personal history of allergy, %	60.0	44.3	<b>0.0277</b>	70.2	52.8	<b>0.0187</b>
Family history of allergy, %	46.4	32.9	NS	42.6	41.1	NS
Wheezing, %	31.7	6.3	<b>&lt;0.0001</b>	29.4	11.2	<b>0.0028</b>
Total IgE amount						
median (Q1-Q3)	40 (17–125)	37 (18–81)	NS	42 (13–114)	28 (15–75)	NS
> 100 IU/mL, %	30.3	24.7	NS	31.7	17.1	<b>0.0256</b>
Positive specific IgE (> 0.35 kUA/L)						
At least one, %	21.8	14.4	NS	28.2	23.9	NS
Food allergens, %	16.8*	4.1	<b>0.0037</b>	7.1	5.6	NS
Perennial inhalant allergens, %	10.9	9.3	NS	10.6	12.4	NS
Seasonal inhalant allergens, %	11.9	3.1	<b>0.0195</b>	21.2	11.4	NS
Polysensitized (at least 3 IgE)	13.8	1.0	<b>0.0006</b>	8.2	6.7	NS

COPD chronic obstructive pulmonary disease, DF-COPD dairy farmers with COPD, NF-COPD non-dairy farmers with COPD, SD standard deviation, allergy was self reported, history of allergy covered nasal allergies including hay fever, eczema or any kind of skin allergy, or allergy to insect stings or bites. \* p < 0.05, vs NF- COPD

Significant P values (< 0.05) are in bold

food allergens and seasonal inhalant allergens was significantly higher in DF-PAL. No significant relationship was found between atopy markers and PAL in non-farmers. Overall, specific IgEs were higher in the DF-PAL group than in the DF-controls for all allergens (Fig. 2). No significant relationship was found between occupational exposure in dairy farming and PAL in farmers (additional file 2).

As there were slight, albeit non-statistically significant differences between PAL groups and their controls in terms of age, gender and smoking, we adjusted for these variables when comparing the distribution of biological markers of atopy in PAL and controls separately in farmers and in non-farmers (Fig. 3). Results showed a higher prevalence of IgE sensitization only in PAL in farmers (Fig. 3).

Then, we constructed three different models to determine factors associated with PAL in the whole population. After adjustment for potential confounders, PAL was mainly explained by the presence of biological atopy markers and smoking (> 15 pack-years) (Table 3). The use of the same models replacing PAL by FEV1 as the outcome variable showed the same results. Models using perennial inhalant allergens or at least one IgE were not relevant. Regarding atopy markers, we did not find an association with occupational exposure after adjustment except for seasonal inhalant allergens which were inversely associated with dairy farming (additional file 3).

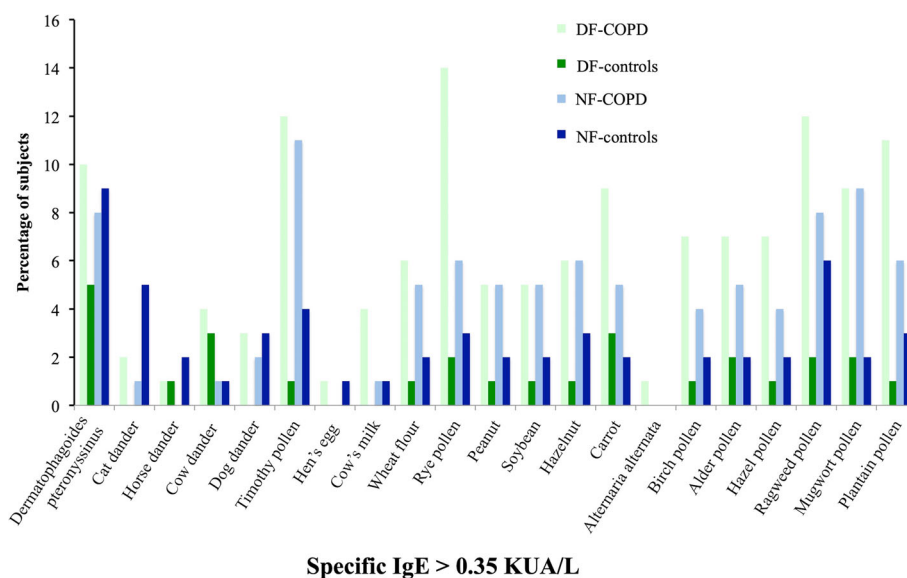
Finally, non-smoker DF-PAL were compared to smoker DF-PAL (current and former) for age, sex, clinical and biological atopy markers. No differences were identified.

**Discussion**

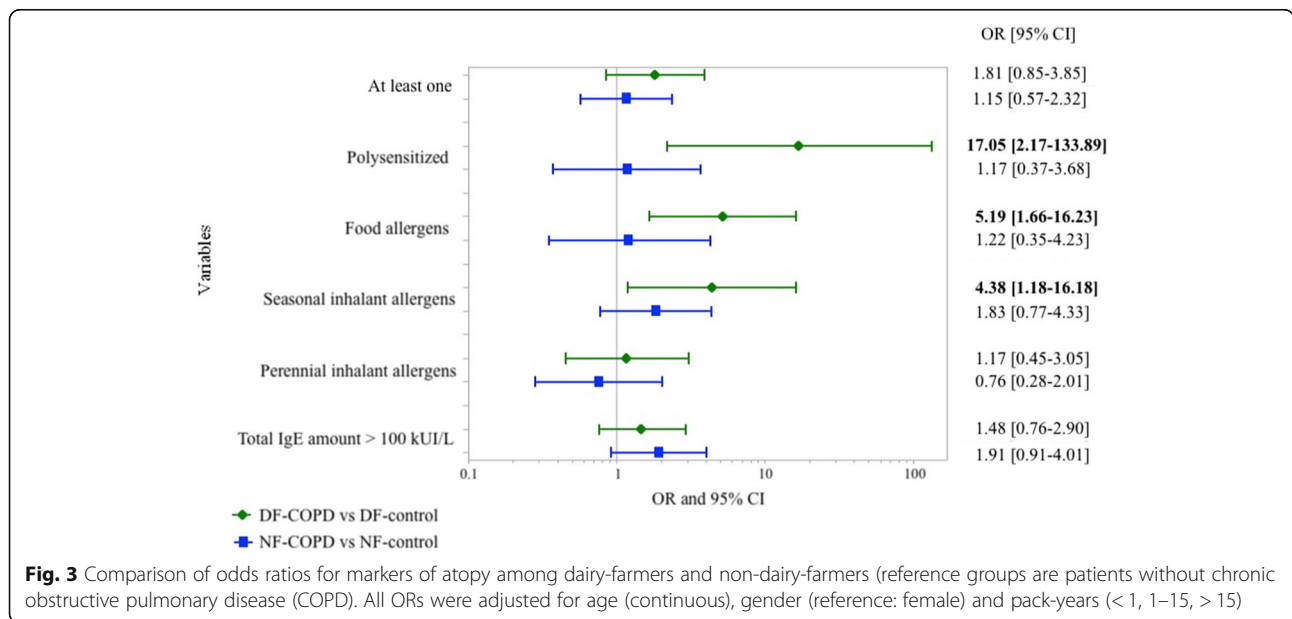
In this study, the frequency of atopy markers was higher in PAL-groups than among controls, but the difference was driven by a significantly higher rate among dairy farmers only. Our study suggests that IgE-mediated reactions may play a role in PAL development in dairy farming. This hypothesis is reinforced by the fact that in farmers, there was no relationship between occupational exposure and either PAL or atopy markers. Indeed, data issued from the same cohort showed similar exposure pattern among dairy farmers and therefore exposure could not explain the occurrence of COPD [18]. This may suggest a key role of individual factors in the genesis of COPD in dairy farming.

Since atopy is not a usual characteristic for subjects with COPD, this supports the hypothesis that COPD in dairy farmers is different than COPD associated with tobacco smoking. Our results suggest that IgE-mediated reactions (therefore possibly a Th2 cytokine production profile) are involved in the development of COPD in dairy farming. COPD due to tobacco smoking is dominated by Th1-type lymphocyte response [6] and is associated with Th17 cytokine production [7]. Conversely, COPD induced by exposure to biomass smoke might be associated with a predominantly Th2-type lymphocyte production profile, as in asthma, and an increase of IL-4 [7, 19]. Moreover, emphysema is rarely found in this type of COPD [7]. COPD in dairy farming could be close to COPD induced by exposure to biomass smoke [19].

The involvement of atopy in COPD has never been extensively studied, to the best of our knowledge. Eduard



**Fig. 2** Frequency histogram of specific IgE among dairy-farmers and non-dairy-farmers' patientsCOPD: chronic obstructive pulmonary disease; DF-COPD: dairy farmers with COPD; DF-control: dairy farmers in control group; NF- COPD: COPD in non-dairy farmers; NF-control: non-dairy farmers in control group.



et al. reported that Norwegian farmers with atopy had a significantly lower FEV1 than non-atopic counterparts [8]. However, in their study, no bronchodilation test was performed, and the relationship between FEV1 and atopy disappeared after the exclusion of asthmatics. In addition, atopy was defined as a positive ImmunoCap® result [8]. In our study, measurements of allergen-specific IgEs against 21 common food and inhalant allergens were performed. Another study in farmers showed that a total IgE concentration > 180 kIU/L was associated with lower FEV1 [20]. More recently, Cushen et al. observed that among non-smoker Irish farmers, those with lung obstruction more often had histories of hay fever or allergies [21]. However, according to the authors, the presence of asthmatics at least partly explains these results. Nonetheless, in previous studies, no significant relationships emerged between atopy markers and COPD. Ours seems to be the first study showing a strong link between markers of atopy and COPD. In contrast with previous studies performed in the Franche-Comté region [5], we did not find any link between the level or type of occupational exposure and agricultural COPD prevalence. The most likely explanation is a progressive homogenization of occupational exposure, with modernization of dairy farms over time.

The associations observed in our study between COPD in dairy farmers and IgE sensitization concerns mainly seasonal allergens. These results are in line with the excess of prior history of personal allergy, and the higher degree of wheezing observed in DF-COPD. However, the absence of any difference between groups of farmers regarding hay fever is unusual (Table 2). This is possibly due to the fact that we excluded asthmatics and

consequently, allergic subjects. The relationship between COPD in farmers and food allergens may be partially explained by cross-reactions between food and seasonal inhalant allergens. It is noteworthy that the protection against allergy conferred by dairy farming observed in the large PASTURE European birth cohort that included farmers living in the same region as the present study, also concerned seasonal and food allergens, but not perennial ones [22]. Dairy farming may be associated with a reduced risk of lung cancer [23] and is known to protect against allergy in children, but possibly also in adults [9]. Therefore, those who still develop atopy even with an early-life farm exposure are those who may be at risk of COPD. Besides, this occupational environment is also known to generate COPD, especially in regions where organics dusts and microorganisms constitute the main exposure [4, 5]. We can therefore hypothesize that this exposure in atopic subjects causes a bronchial disease different from asthma, which is characterized by a FEV1/FVC ratio after bronchodilation < 0.7. Analyses of cytokine measurements as well as IgE and IgG-mediated reactions against microorganisms present in the home of the studied subjects are planned and could be of substantial interest in understanding the mechanisms of COPD in dairy farmers.

Our study includes limitations inherent to a large screening program. Only subjects who accepted to undergo a health check-up were included in the COPD screening program. In addition, only half the subjects who had COPD detected by screening accepted to attend the hospital visit for “characterization”. It has previously been reported that among all subjects who were invited to health check-ups organized by the French

**Table 3** Adjusted odds ratios for chronic obstructive pulmonary disease in the whole population considering three tested indicators of atopy

	COPD		
	OR	95% CI	p-value
<b>Model 1</b>			
Food allergens; ref.: no	<b>2.71</b>	<b>1.23–6.46</b>	<b>0.0176</b>
Nasal allergies; ref.: no	<b>2.17</b>	<b>1.34–3.56</b>	<b>0.0019</b>
Farmers; ref.: non-farmer	1.41	0.86–2.31	0.1725
Age; continuous	1.02	0.99–1.05	0.1259
Sex; ref.:female	1.06	0.61–1.84	0.8370
Pack-years; ref. < 1	1		
1–15	1.13	0.60–2.13	0.7039
> 15	1.69	0.98–2.92	0.0599
<b>Model 2</b>			
Seasonal inhalant allergens; ref.: no	<b>2.57</b>	<b>1.29–5.39</b>	<b>0.0091</b>
Nasal allergies; ref.: no	<b>2.21</b>	<b>1.36–3.63</b>	<b>0.0015</b>
Farmers; ref.: non-farmer	1.57	0.96–2.60	0.0746
Age; continuous	1.02	0.99–1.05	0.1681
Sex; ref.:female	1.04	0.60–1.82	0.8861
Pack-years; ref. < 1	1		
1–15	1.07	0.57–2.01	0.8352
> 15	1.64	0.96–2.85	0.0737
<b>Model 3</b>			
Polysensitized (at least 3 IgE); ref.: no	<b>3.45</b>	<b>1.46–9.12</b>	<b>0.0073</b>
Nasal allergies; ref.: no	<b>2.25</b>	<b>1.38–3.70</b>	<b>0.0011</b>
Farmers; ref.: non-farmer	1.44	0.89–2.37	0.1427
Age; continuous	1.02	0.99–1.05	0.1081
Sex; ref.:female	1.15	0.66–2.02	0.6178
Pack-years; ref. < 1	1		
1–15	1.14	0.61–2.14	0.6849
> 15	1.67	0.97–2.89	0.0669

Logistic regressions of chronic obstructive pulmonary disease (COPD) for atopy markers and covariates that are known to be independent risk factors for COPD; models using perennial inhalant allergens and at least one IgE like indicators of atopy have shown no significant difference  
Significant *P* values (< 0.05) are in bold

agricultural health insurance system, those who attend these check-ups and those who do not have different health characteristics [4]. It is therefore possible that our population was not representative of the whole population of dairy farmers (target population) regarding health status. Our studied population, however, is not different from the target population in terms of smoking habits. There are significant differences for age and sex ratio, but neither of these factors were associated with atopy markers in the present analysis. Finally, our outcomes can only be considered robust if we are sure that the diagnosis of COPD was accurate, and that patients with asthma were truly excluded. All reasonable precautions

were taken to verify the diagnosis of COPD. Spirometries were performed by trained nurses during the “screening” phase, followed by diagnostic confirmation in the Department of Respiratory Medicine & Physiology of our university hospital. Subjects with self-reported asthma were excluded. Therefore, we can rule out an asthma-COPD-overlap syndrome, which would have been more frequent in DF-COPD. Farmer’s lung disease, another possible differential diagnosis in our farming region, was also ruled out by a question in the medical questionnaire and thanks to local expertise in this disease [24].

### Conclusion

Dairy farmers with COPD are more polysensitized than non-farming patients with COPD, and more polysensitized, sensitized to food and seasonal inhalant allergens than non-COPD subjects. These results expand the hypothesis that atopy may be a risk indicator for COPD in dairy farming, distinguishing it from COPD associated with tobacco smoking.

### Additional files

**Additional file 1:** Supplementary methods (DOCX 25 kb)

**Additional file 2:** Occupational characteristics of dairy farming COPD and controls. (DOCX 20 kb)

**Additional file 3:** Adjusted odds ratios for markers of atopy in the whole population considering three tested indicators (DOCX 17 kb)

### Abbreviations

BMI: Body mass index; CI: Confidence intervals; COPD: Chronic obstructive pulmonary disease; DF: Dairy farmers; ELISA: Enzyme-linked immunosorbent assay; FEV1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; IQR: Interquartile range; mMRC: modified Medical Research Council; MSA: Mutualité Sociale Agricole; NF: Non-dairy farmers; SD: Standard deviation; SGRQ: St George’s Respiratory Questionnaire.

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### Authors’ contributions

All authors contributed to the content and writing of the manuscript. In addition: J-CD was the principal investigator and contributed the original idea for the study; MVP, RV, BD and J-CD made substantial contributions to the conception and design of the work; MVP, RV and AG performed all analyses; J-CD and BD obtained study funding; IAM, PA, NR, SJ, BC and J-JL made substantial contributions to the acquisition or interpretation of data for the work; MVP wrote the first draft of the manuscript. MVP, TS and J-CD revised the manuscript for important intellectual content. All authors have read and approved the final manuscript.

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### Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.



**Ethics approval and consent to participate**

Ethical approval was received from the local Ethics Committee (CPP Est; 11/617) and written consent was obtained from all subjects.

**Consent for publication**

Written consent was obtained from all subjects.

**Competing interests**

Dr. Roche reports grants and personal fees from Boehringer Ingelheim, Pfizer and Novartis, personal fees from Teva, GSK, AstraZeneca, Chiesi, Mundipharma, Cipla, Sano, Sandoz, 3 M, Zambon, outside the submitted work. Dr. Dalphin reports grants, personal fees and non-financial support from Novartis Pharma, GSK, Chiesi, Intermune, AstraZeneca, Boehringer Ingelheim and non-financial support from Stallergenes, outside the submitted work.

Dr. Dalphin reports grants, personal fees and non-financial support from Novartis Pharma, personal fees and non-financial support from GSK, personal fees and non-financial support from Chiesi, personal fees and non-financial support from Intermune, personal fees and non-financial support from AstraZeneca, personal fees and non-financial support from Boehringer Ingelheim, non-financial support from Stallergenes, outside the submitted work. The other authors declare that they have no conflict of interest for the submitted work.

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## **Supplementary methods**

### **METHODS**

#### **Screening program**

The BALISTIC project (COPD in dairy farmers: screening, characterization and constitution of a cohort; ClinicalTrials.gov Identifier: NCT02540408) which was conducted from 2011 to 2015 at the University Hospital of Besançon in collaboration with the French national social security system for agricultural workers (Mutualité Sociale Agricole, MSA) and the federation of community health practices of Franche-Comté (Fédération des Maisons de Santé Comtoises, FeMaSaC). This study was set up to assess the prevalence, and specific characteristics of COPD in dairy farmers (COPD secondary to organic dust exposure) in comparison with COPD in patients without any occupational exposure, and compared to matched controls without COPD [1].

COPD patients and controls were recruited through a screening program (“screening” phase of the BALISTIC study) in two branches of the social security. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma, hypersensitivity pneumonitis, and who were either a dairy farmer (“dairy farmers” subgroups) or unexposed to any occupational hazard associated with COPD (“non-farmers” subgroups). COPD screening was proposed to all invited subjects who attended the health check-up organized by the MSA or were invited by the general practitioners (GP) of the FeMaSaC and who fulfilled the inclusion criteria. A random sample of 5% of the non-respondent subjects (i.e. farmers who did not attend the health check-up) was drawn to compare participants and non-participants with regard to age, sex and smoking habits.

For the screening, spirometry was performed as previously described [2]. Spirometry outcomes included forced expiratory volume in 1 second (FEV1) and forced vital capacity

(FVC). A bronchodilation test was applied when the FEV1/FVC ratio was less than 0.70 [3]. Predicted values were based on the GLI equations [4].

### **Outcome definitions**

A never-smoker was defined as a subject having smoked less than one cigarette, one cigar or one pipe a day for one year. An ex-smoker was defined as a subject having stopped smoking for at least one month before completing the questionnaires. Chronic bronchitis was defined as cough and daily sputum three months a year for two consecutive years.

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**Additional file 2.** Occupational characteristics of dairy farming COPD and controls.

	DF-COPD n = 101	DF-Control n = 98	p
Activity level			NS
Retired, no longer working on a farm	28 (29)	25 (26)	
Retired, still working on a farm	22 (23)	22 (22)	
Active	47 (47)	51 (52)	
Farm's characteristics for at least 10 years (presence of)			
Separation between house and cowshed	74 (73)	71 (72)	NS
Loading grippers	28 (28)	29 (30)	NS
Food grippers	15 (15)	17 (18)	NS
Straw blower	21 (21)	23 (24)	NS
Central corridor	75 (74)	80 (82)	NS
Loose housing system	50 (51)	55 (59)	NS
Ventilation	44 (44)	37 (38)	NS
Milking parlour	54 (53)	59 (60)	NS
Barn drying system	18 (18)	21 (21)	NS
Size of the farm during the past 10 years of work			
Total size, hectares	95±56	97±58	NS
Size of fodder lands, hectares	52±34	57±34	NS
Size of cereal production, hectares	24±35	29±42	NS
Number of cattle	109±69	107±72	NS
Number of cows	43±21	44±23	NS

Data are presented as n, n (%) or mean ± SD, unless otherwise stated.

COPD: chronic obstructive pulmonary disease; DF-COPD: dairy farmers with COPD; DF-Control: dairy farmers in control group.

**Additional Table 3.** Adjusted odds ratios for markers of atopy in the whole population considering three tested indicators

	<b>COPD</b>		
	OR	95% CI	p-value
<b>Model 1 (Food allergens)</b>			
Farmers; ref: non-farmer	1.41	0.59 - 3.51	0.4383
PAL; ref: no	<b>2.90</b>	<b>1.33 - 6.85</b>	<b>0.0099</b>
Age; continuous	0.98	0.94 - 1.02	0.3367
Sex; ref:female	2.11	0.69 - 9.91	0.2406
Pack-years; ref<1	1		
1-15	0.73	0.22 - 2.13	0.5846
>15	0.77	0.30 - 1.94	0.5769
<b>Model 2 (Seasonal inhalant allergens)</b>			
Farmers; ref: non-farmer	<b>0.40</b>	<b>0.18 - 0.85</b>	<b>0.0201</b>
PAL; ref: no	<b>2.64</b>	<b>1.34 - 5.47</b>	<b>0.0066</b>
Age; continuous	1.00	0.96 - 1.05	0.8178
Sex; ref:female	2.13	0.85 - 6.53	0.1376
Pack-years; ref<1	1		
1-15	1.40	0.48 - 3.99	0.5258
>15	1.11	0.45 - 2.90	0.8179
<b>Model 3 (Polysensitized (at least 3 IgE))</b>			
Farmers; ref: non-farmer	0.99	0.39 - 2.52	0.9926
PAL; ref: no	<b>3.47</b>	<b>1.48 - 9.10</b>	<b>0.0064</b>
Age; continuous	0.96	0.92 - 1.01	0.1325
Sex; ref:female	0.59	0.24 - 1.62	0.2761
Pack-years; ref<1	1		
1-15	0.57	0.12 - 2.01	0.4123
>15	0.94	0.35 - 2.55	0.8979

## 5. Article 8: Never-smokers with COPD have better exercise capacities and ventilatory efficiency than matched ever-smokers with COPD

### *a. Contexte et objectif*

La BPCO du non-fumeur, dont fait partie la BPCO secondaire aux poussières organiques, est encore assez mal caractérisée. De ce fait, sa prise en charge reste à l'heure actuelle calquée sur celle de la BPCO tabagique. Il a cependant été suggéré que la présentation clinique et radiologique de la BPCO du non-fumeur était différente de celle de la BPCO tabagique. En effet, la BPCO du non-fumeur semble associée avec une moindre prévalence de l'emphysème pulmonaire que la BPCO tabagique.

Bien qu'ils aient par définition une fonction ventilatoire peu altérée, les patients présentant une BPCO tabagique légère à modérée ont une tolérance à l'exercice qui est moins bonne que celle des sujets sains. La principale anomalie constatée à l'exercice chez ces patients consiste en une distension dynamique. Il a par ailleurs été montré que l'administration d'un traitement bronchodilatateur dans la BPCO permettait d'améliorer la dyspnée et la tolérance à l'exercice. La tolérance à l'exercice et la mécanique ventilatoire de la BPCO du non fumeur reste à ce jour inconnue. Par ailleurs, la précédente étude (article 4) n'a pas permis de comparer correctement du fait de nombreux biais de confusion (VEMS, tabagisme).

L'objectif de cette étude était de comparer l'adaptation à l'exercice de non-fumeurs présentant une BPCO avec celle de fumeurs présentant une BPCO (appariés sur la sévérité de l'obstruction bronchique) et avec celle de sujets sains fumeurs et non-fumeurs.

### *b. Manuscrit*

Article "Never-smokers with COPD have better exercise capacities and ventilatory efficiency than matched ever-smokers with COPD". **Soumagne T**, Guillien A, Roche R, Annesi-Maesano I, Andujar P, Laurent L, Jouneau S, Botbol M, Laplante JJ, Dalphin JC, Degano B.  
*En cours de soumission.*



**Never-smokers with COPD have better exercise capacities and ventilatory efficiency  
than matched ever-smokers with COPD**

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Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide (1). Although tobacco smoking is known to be the main risk factor for COPD, it is now admitted that around 30% of patients with COPD have never smoked (2, 3). Risk factors associated with COPD in never-smokers include occupational or environmental dust exposure.

COPD in never-smokers is still poorly characterized, and follow-up and treatment of these patients are based on recommendations made for patients with COPD related to tobacco smoking. Nevertheless, there is increasing evidence for differences in clinical, radiological and physiological presentations between smokers and non-smokers with COPD (3-6). As an example, never-smokers with COPD are less likely to have emphysema, and they have higher lung transfer capacity for carbon monoxide (TLCO) compared to smokers with COPD (3, 7, 8).

Smokers with COPD have a higher ventilatory requirement than normal during exercise even at mild stages of the disease, resulting in poorer perceived health status, higher chronic activity-related dyspnoea and reduced activity levels (9-11). Inhaled long acting bronchodilator improve exertional dyspnoea and exercise endurance in these patients (12, 13). As it is unknown whether or not never-smokers with COPD have similar impairment at exercise as their smoking counterparts, it is also unknown whether or not bronchodilators should be tested in never-smokers with COPD with the aim to improve exercise tolerance and ventilatory mechanics.

The objective of the current study was therefore to compare several physiological parameters measured at exercise between never-smokers with mild-to-moderate COPD, ever-smokers with COPD (matched for the severity of airway obstruction) and healthy subjects (either never-smokers or ever-smokers), all matched for age and sex.

## Methods

### *Subjects and study design*

COPD patients and control subjects were recruited through a regional COPD screening programme (BALISTIC study; ClinicalTrials.gov Identifier: NCT02540408), as previously described. For the current analysis, we identified 4 groups: ever-smokers with COPD; never-smokers with COPD; ever-smokers with normal spirometry; never-smokers with normal spirometry. Never-smokers were individuals who had never smoked in their lifetime (in practice, all have been exposed to organic dusts for at least 20 years) and ever-smokers (either current or former smokers) were individuals who had smoked more than 15 pack-years. The four groups were matched in terms of age and sex. The 2 groups with COPD were pair-matched in terms of FEV<sub>1</sub> and FEV<sub>1</sub>/FVC.

Patients with COPD had (i) a medical history compatible with the disease; (ii) persistent airflow limitation defined as a FEV<sub>1</sub>/FVC ratio < 5<sup>th</sup> centile lower limit of normal according to the last GLI-2012 equations (14) and (iii) a FEV<sub>1</sub> > 50% of predicted value according to the GLI-2012 equations (15, 16). Control subjects had normal spirometry. Exclusion criteria for COPD and controls were (i) a history of any medical conditions that could cause or contribute to breathlessness (*i.e.*, a respiratory disease other than COPD (including asthma, either self-reported or confirmed by a doctor) and/or a cardiovascular disease) and/or (ii) any other disorder that could interfere with exercise testing (10, 11).

Subjects were in stable condition (for COPD patients, no exacerbation during the previous 6 weeks) and were not taking any oral or inhaled anti-inflammatory drugs, including corticosteroids. COPD patients were asked to interrupt short and long-acting bronchodilators 72 hours prior to the visit, if required.

Ethical approval was received from the local Ethics Committee (CPP Est; P-2011-119) and

written consent was obtained from all subjects.

### *Procedures*

Subjects attended a single visit. If required, COPD subjects were asked to interrupt any respiratory medication such as short and long-acting bronchodilators 72h prior to the visit. Subjects were asked to arrive early in the morning. They then underwent pulmonary function testing prebronchodilator and 30 min post- bronchodilator administration, as previously described. A symptom-limited incremental cycle ergometer cardiopulmonary exercise testing (CPET) was performed on the same day, at least 6 h after the bronchodilator test and at least 4 h after the meal.

Routine spirometry, constant-volume body plethysmography and single breath lung transfer for carbon monoxide (TLCO) were performed in accordance with recommended techniques (Platinum Elite; MGC Diagnostics Corporation, Saint Paul, Minnesota, USA) (17, 18). Symptom-limited incremental CPET was performed on an electronically braked cycle ergometer (Ergometrics 900; Ergoline; Bitz, Germany), as previously described (11). Briefly, after a steady-state resting period, a 3 min warm-up was conducted at about 20% of individually estimated maximal work load, and the load was increased every minute such that the CPET duration was between 8 and 12 min (19). Tests were terminated at the point of symptom limitation (peak exercise). Physiological data were obtained breath by breath (MGC-CPX System; MGC Diagnostics Corporation) and were expressed as 30 s averages, according to recommended guidelines (19). Exercise variables were compared with predicted values (20). Blood samples were drawn from the arterialised earlobe. Subjects rated the magnitude of their perceived breathing and leg discomfort by pointing to a number on the 10-point Borg scale (21). Changes in end-expiratory lung volume were estimated from inspiratory capacity (IC) measurements at rest,

at the end of each 1min increment of exercise and at peak exercise. Dynamic hyperinflation was defined as a decrease of >150 mL in IC compared with resting levels at any time point during exercise (9).

Ventilatory equivalent for carbon dioxide ( $V'E/V'CO_2$  ratio) plotted against WR and its nadir were used as surrogate markers of ventilatory efficiency. Dead space (VD)/tidal volume (VT) ratio was calculated by standard equations using  $P_{cap}CO_2$  at maximal exercise (22). In the relationship between VT and minute ventilation (VE), there is an inflection point beyond which almost no further change in VT occurs despite a continued increase in VE (23). This inflection in the VT response marks the point where dyspnoea sharply increases because of mechanical constraints on VT expansion (23). This inflection point was determined by two trained observers (BD and TS) for each patient by analysing individual plots of VE versus VT (24).

### *Statistical analysis*

A sample size of 22 subjects in each group was estimated to provide 90% power to detect a significant difference between never-smokers with COPD and ever-smokers with COPD in dyspnoea intensity (Borg scale) measured at a standardised WR during incremental cycle exercise based on a relevant difference in Borg ratings of  $\pm 1$ , an SD=1 unit,  $\alpha=0.05$  and a two-tailed test of significance (10, 11).

Between-group comparisons of subjects' characteristics were performed using paired t tests. Comparisons of categorical data between groups were performed using Fisher's exact test. Repeated measures analysis of variance (ANOVA) was performed to evaluate differences between groups for measurements made at different time points and/or intensities during exercise. Scheffe's tests were applied to evaluate pairwise comparisons when a significant difference was found by ANOVA. Pearson's  $r$  assessed linear association between continuous

variables.

All reported p values were two-sided, with a significance level set at  $p < 0.05$ . Statistical analysis was performed with R version 3.5.0 and RStudio version 1.1.453 (R Foundation for Statistical Computing, Vienna, Austria).

## **Results**

### *Subject characteristics*

A total of 88 subjects were included (22 in each group). By design, all groups had similar sex distribution and age (Table 1). Comorbidities in the groups were similar unless for dyslipidaemia which was more frequent in smokers (supplemental Table 1). Among the 88 studied subjects, only one patient with COPD was taking long-term bronchodilators at the time of screening, and none were taking inhaled corticosteroids (supplemental Table 2).

### *Health status and pulmonary function*

The 2 groups of patients with COPD had similar chronic activity-related dyspnoea ( $mMRC \geq 1$ ), chronic cough and/or chronic sputum production (Table 1). Among never-smokers, COPD and controls had similar baseline dyspnoea index (BDI); BDI and COPD assessment test (CAT) was poorer in ever-smokers with COPD compared with never-smokers with COPD (table 1).

By design, post-BD  $FEV_1$  and  $FEV_1/FVC$  was similar in the 2 groups with COPD (Table 1). Small airways dysfunction, judged by a reduction in maximal mid-expiratory flows ( $FEF_{25-75}$ ), was absent in controls and was significant (mean z-score  $< -1.64$ ) in the two groups with COPD. Of note, never-smokers with COPD had significantly higher TLCO and KCO compared to ever-smokers with COPD.

### *Symptom-limited incremental cycle exercise*

All controls and COPD performed a maximal exercise (respiratory exchange ratio >1.1 at peak exercise). Among never-smokers, COPD and controls had similar peak work rate (WR) and peak oxygen consumption ( $\text{VO}_2$ ). By contrast, among ever-smokers, COPD had significantly lower peak WR and  $\text{VO}_2$  than controls. In addition, ever-smokers with COPD had significantly lower peak WR and  $\text{VO}_2$  than never-smokers with COPD (Table 2).

Dyspnoea/WR slope was similar in never-smokers with COPD and in controls but was significantly higher in ever-smokers with COPD compared to the three other groups (ANOVA repeated measures). Borg scale rating was lower by more than 1 unit in never-smokers with COPD than in ever-smokers with COPD at 80 W (Figure 2 and Table 3) and was also similar to controls. Similarly, Borg scale ratings as a function of VE were similar in never-smokers with COPD and in controls, and was higher in ever-smokers with COPD at 50W (Figure 2 and Table 4).

Never-smokers with COPD and ever-smokers with COPD had a greater reduction in IC during exercise than controls (ANOVA repeated measures; Figure 3). A significant dynamic hyperinflation was found in 14 never-smokers with COPD (64%) and 13 ever-smokers with COPD (59%).

Among never-smokers, the  $V_T/\text{VE}$  inflection point occurred at similar VE and  $V_T$  in COPD and in controls; among ever-smokers, and this inflection point occurred at lower  $V_T$  and VE in COPD than in controls (Figure 4). In addition, nadir  $\text{VE}/\text{VCO}_2$  was modestly but significantly lower (*i.e.*, better) in never-smokers with COPD than in ever-smokers with COPD ( $30.3 \pm 4.1$  vs.  $32.8 \pm 3.9$ , respectively;  $p < 0.05$ ), and  $\text{VD}/\text{VT}$  ratio tended to be lower (*i.e.*, better) in never-smokers with COPD than in ever-smokers with COPD ( $p = 0.06$ ; Table 2). Of note, ventilatory efficiency (*i.e.* nadir  $\text{V}'\text{E}/\text{V}'\text{CO}_2$ ) was different between COPD and control only in ever-

smokers; VD/VT did not differ between COPD and matched control in both ever and never-smokers.

#### *Functional correlates of ventilatory inefficiency*

Patients with low peak  $\text{VO}_2$  presented with greater ventilatory inefficiency (i.e. high  $\text{V}'\text{E}/\text{V}'\text{CO}_2$ ) ( $r = 0.83$ ,  $p < 0.0001$ ). Low TLCO was associated with higher  $\text{V}'\text{E}/\text{V}'\text{CO}_2$  nadir ( $r = -0.62$ ,  $p < 0.001$ ), higher VD/VT at maximal exercise ( $r = -0.36$ ,  $p < 0.05$ ) and higher dyspnoea intensity at 80W ( $r = -0.35$ ,  $p < 0.05$ ). Dyspnoea intensity did not correlate with IC (%baseline) at 80W.

#### **Discussion**

In the present study, we confirmed that never-smokers with mild to moderate COPD had preserved TLCO compared to ever-smokers with mild to moderate COPD. In addition, we demonstrated that never-smokers with COPD had lower dyspnoea intensity during incremental exercise and better peak exercise capacity even with similar dynamic hyperinflation than matched ever-smokers with COPD. These never-smokers with COPD tend to have closer ventilatory efficiency to those of healthy controls than those of ever-smokers with COPD.

It is now currently admitted that 20 to 30% of people diagnosed with COPD have never-smoked (3, 25, 26). As a matter of fact, a growing number of published studies in the past decade have suggested that indoor and outdoor air pollutants as well as occupational hazards including exposure to organic dusts have been associated with accelerated impairment in lung function and with increased risk of COPD (27). In addition, it has been shown that COPD was associated with a reduction in life expectancy for never smokers but somewhat in a lower extent than ever-smokers (5, 28). However there is a huge lack of knowledge in this field. Further researches to

precise the phenotype of COPD in never smokers have therefore become a priority. For these reasons, findings from our study are of particular interest.

One key finding is that mild to moderate COPD in never-smokers is associated with relatively preserved exercise capacities and dyspnoea intensity similarly to healthy controls on the contrary of COPD in ever-smokers. This difference might not be driven by airway obstruction as never-smokers and ever-smokers with COPD have been carefully paired-matched on FEV1 and that subsequent dynamic hyperinflation is similar among ever and never-smokers with COPD. One potential explanation is that never-smokers with COPD have relatively preserved TLCO, KCO and TLCO/FVC which might indicate lower emphysema burden in contrast to ever-smokers with COPD (29-31). These results are consistent with previously reported data. Indeed, in a large cross sectional study, Tan et al. reported that never-smokers with COPD were less likely to have reduced diffusing capacity and emphysema on CT scan than ever-smokers with COPD and therefore could be labelled as a “airway predominant phenotype” (3). Lower prevalence of emphysema was also found in COPD related to biomass smoke exposure (7, 32).

Emphysema is known to induce marked alveolar ventilation/perfusion ( $VA'/Q'$ ) mismatching and therefore might increase arterial–alveolar  $CO_2$  difference (22). The high dead space (VD)/tidal volume (VT) ratio reflecting increased “wasted” ventilation is a sensitive tool to estimate this increased arterial–alveolar  $CO_2$  difference (22, 33). Underlying mechanisms of lower dyspnoea intensity in never-smokers with COPD might result of a better ventilatory efficiency stemming from a lower physiological dead space due to lower  $VA'/Q'$  heterogeneity compared to ever-smoker with COPD. Jones et al. found similar findings by comparing mild COPD patients to healthy controls (29). Our data provide evidence that lower exertional dyspnoea in never-smoker with COPD might be related to lower wasted ventilation in



emphysematous areas compared to ever-smokers.

Because large COPD trials have systematically excluded patients who have never smoked, the role and the benefit of bronchodilation in never-smoking COPD remains a mystery. Our findings may therefore have consequences for the management of never-smokers with COPD. Some physiological studies have suggested a rationale for the utility of long-acting bronchodilators in patients with mild COPD, with a view to decreasing dynamic gas trapping, improving resultant restrictive mechanical constraints on tidal volume expansion and thus improving exercise capacity (10, 34, 35). Our approach provides evidence that up to 60% of never-smokers with COPD have significant dynamic hyperinflation: the benefits of inhaled bronchodilators in these subjects should therefore be tested.

#### Limitations

Our study population was carefully selected in order to avoid as far as possible confounders that could interfere with our results. Our 3 groups were carefully matched for age, sex and FEV<sub>1</sub>, as these factors can impact on dyspnoea evaluation (36). We also paid attention to exclude from our study subjects with cardiac comorbidities. This is important, because an association between chronic heart failure and COPD frequently coexists (37), and dynamic hyperinflation during exercise has been reported in patients with chronic heart failure and normal spirometry (38). In our study, significant cardiac impairment was unlikely to have contributed to dyspnoea and/or to dynamic hyperinflation since heart rate responses, O<sub>2</sub> pulse at peak exercise and blood pressure measurements were similar in both COPD groups. Although we did not record any information on usual physical activity in the subjects in our study, it is unlikely that never-smokers with COPD minimised dyspnoea compared with matched ever-smokers with COPD through activity avoidance because WR, V'O<sub>2</sub>, heart rate and breath frequency at ventilatory threshold were

similar in these two groups (online supplementary table 2), suggesting that deconditioning, if any, was also similar.

Organic dust exposure was the sole cause of COPD in never-smoker. However, findings were consistent with those found in COPD induced by biomass exposure (7, 32). Finally, as HRCT was not available we were unable to evaluate the extent of emphysema among never-smokers. However, low TLCO has been clearly linked to structural markers of early emphysema and could therefore represent a valuable surrogate (3).

In summary, our findings suggest that never-smokers with COPD have relatively preserved exercise capacities and lower exertional dyspnea than ever-smokers. These differences may be in part driven by a better ventilatory efficiency stemming from a preserved diffusing capacity. The effect of bronchodilators on physiological and perceptual responses to exercise in never-smokers with COPD who have dynamic hyperinflation remains to be determined.

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### **Author Contributions:**

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

JCD and BD made substantial contributions to the conception and design of the work.

TS and AG performed all analyses.

JCD and BD obtained study funding.

All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work. TS wrote the first draft of the manuscript. BD, TS, AG, NR, IAM, PA, LL, SJ, JLL and JCD revised the manuscript for important intellectual content. All authors approved the manuscript.

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**Table 1.** Characteristics, respiratory symptoms and pulmonary function in subjects with chronic obstructive pulmonary disease (COPD), and healthy controls.

	Never-smokers			Ever-smokers			Never vs ever-smokers with COPD p value
	COPD (n=22)	Controls (n=22)	p value	COPD (n=22)	Controls (n=22)	p value	
<b>Demographics</b>							
Male, %	20 (91)	20 (91)	NS	20 (91)	20 (91)	NS	
Age, yr	56.4 ± 8.3	58.5 ± 7.8	NS	58.8 ± 7.3	59.0 ± 7.8	NS	NS
Body mass index, kg/m <sup>2</sup>	26.1 ± 4.2	26.6 ± 2.9	NS	25.3 ± 3.8	27.1 ± 3.7	NS	NS
Current smokers, %	-	-		36 %	64%	<0.05	<0.0001
Tobacco, pack-years	-	-		36.0 ± 18.8	28.7 ± 13.7	NS	<0.0001
<b>Symptoms</b>							
Chronic cough, n (%)	10 (45)	4 (18)	NS	8 (36)	4 (18)	NS	NS
Chronic sputum, n (%)	10 (45)	4 (18)	NS	9 (41)	4 (18)	NS	NS
mMRC dyspnoea scale (0-4)	0.24 ± 0.44	0.14 ± 0.35	NS	0.55 ± 0.80	0.36 ± 0.90	NS	NS
BDI dyspnoea scale (0-12)	11.3 ± 1.04	11.6 ± 1.0	NS	10.1 ± 2.1	11.3 ± 1.5	<0.05	<0.05
SGRQ	14.6 ± 10.9	6.1 ± 5.4	<0.01	18.1 ± 15.4	11.7 ± 10.2	NS	NS
CAT score (0-40)	8.3 ± 4.8			11.9 ± 6.1			<0.05
<b>Pulmonary function tests</b>							
Post-BD FEV <sub>1</sub> , L (z-score)	2.78 ± 0.68 (-1.45 ± 0.78)	3.66 ± 0.65 (0.55 ± 0.79)	<0.0001	2.65 ± 0.54 (-1.43 ± 0.69)	3.67 ± 0.65 (0.59 ± 0.83)	<0.0001	NS
Post-BD FEV <sub>1</sub> /FVC, % (z-score)	59.8 ± 7.1 (-2.44 ± 0.72)	78.7 ± 4.5 (0.11 ± 0.68)	<0.0001	59.2 ± 5.2 (-2.45 ± 0.61)	79.1 ± 3.4 (0.16 ± 0.51)	<0.0001	NS
Pre-BD pulmonary function							
FEV <sub>1</sub> , L (z-score)	2.56 ± 0.63 (-1.87 ± 0.72)	3.55 ± 0.62 (0.31 ± 0.65)	<0.0001	2.46 ± 0.48 (-1.79 ± 0.68)	3.56 ± 0.62 (0.37 ± 0.79)	<0.0001	NS
FVC, L (z-score)	4.48 ± 0.93 (0.01 ± 0.91)	4.72 ± 0.74 (0.57 ± 0.57)	NS	4.27 ± 0.81 (-0.06 ± 0.79)	4.65 ± 0.87 (0.47 ± 0.89)	NS	NS
FRC, L (z-score)	4.13 ± 0.70 (1.19 ± 1.16)	3.80 ± 0.56 (0.63 ± 0.81)	NS	4.20 ± 0.90 (1.32 ± 1.39)	3.72 ± 0.90 (0.45 ± 1.35)	NS	NS
IC, L	3.32 ± 0.56	3.54 ± 0.76		2.98 ± 0.77	3.55 ± 0.44	<0.01	NS
FEF25-75, % (z-score)	1.17 ± 0.48 (-2.28 ± 0.49)	2.83 ± 0.95 (-0.16 ± 0.73)	<0.0001	1.09 ± 0.32 (-2.20 ± 0.49)	3.07 ± 0.83 (0.11 ± 0.73)	<0.0001	NS
RV, L (z-score)	2.82 ± 0.54 (1.42 ± 1.60)	2.49 ± 0.44 (0.48 ± 0.96)	<0.05	2.79 ± 0.73 (1.22 ± 1.82)	2.48 ± 0.47 (0.42 ± 1.17)	NS	NS
TLC, L (z-score)	7.44 ± 0.84 (1.08 ± 0.89)	7.35 ± 1.02 (0.97 ± 0.82)	NS	7.19 ± 1.19 (0.85 ± 1.27)	7.27 ± 1.05 (0.86 ± 1.09)	NS	NS
VA/TLC, %	90.3 ± 7.7	91.5 ± 7.6	NS	93.1 ± 10.8	92.8 ± 10.4	NS	NS
TLCO, mmol/min/kPa (z-score)	8.45 ± 2.24 (-0.43 ± 1.22)	9.96 ± 2.36 (0.72 ± 1.08)	<0.05	6.64 ± 1.67 (-1.69 ± 1.22)	8.66 ± 2.00 (-0.15 ± 1.33)	<0.001	<0.01
TLCO/FVC	1.92 ± 0.46	2.11 ± 0.33	NS	1.57 ± 0.35	1.90 ± 0.46	<0.01	<0.01
KCO, mmol/min/kPa/L (z-score)	1.31 ± 0.27 (-0.73 ± 1.14)	1.47 ± 0.24 (0.07 ± 1.00)	<0.05	1.09 ± 0.23 (-1.75 ± 1.15)	1.33 ± 0.22 (-0.54 ± 1.07)	<0.001	<0.01

COPD = chronic obstructive pulmonary disease; mMRC = modified medical research council; BDI = baseline dyspnoea index; SGRQ = St. George's Respiratory Questionnaire; CAT = COPD Assessment Test; BD = bronchodilator; FEV<sub>1</sub> = forced expiratory volume in 1 second; z-score = standardized residual; FVC = forced vital capacity; FRC = functional residual capacity; IC = inspiratory capacity (z-score was omitted because standard deviation of theoretical values were not available); FEF25-75 = maximal mid-expiratory flows; RV = residual volume; TLC = total lung capacity; TLCO = lung transfer for carbon monoxide; KCO = carbon monoxide transfer coefficient. Values are means ± SD.

**Table 2** Measurements at peak symptom-limited incremental cycle exercise

	Never-smokers		Ever-smokers		Never vs ever-smokers with COPD p value
	COPD (n=22)	Controls (n=22)	COPD (n=22)	Controls (n=22)	
Dyspnoea, Borg scale	7.8 ± 2.2	8.4 ± 2.3	7.9 ± 2.1	9.0 ± 1.5	NS
Leg discomfort, Borg scale	8.0 ± 2.1	8.2 ± 2.3	9.0 ± 1.5	8.5 ± 2.2	NS
<i>Reason for stopping</i>					NS
Leg discomfort	9 (41)	16 (73)	15 (68)	14 (64)	
Breathing discomfort	7 (32)	4 (18)	4 (18)	8 (36)	
Work rate, Watts (% pred)	173 ± 36 (89 ± 14)	185 ± 51 (97 ± 20)	145 ± 44 (78 ± 20)	174 ± 38 (92 ± 18)	< 0.05
VO <sub>2</sub> , L/min (% predicted)	2.22 ± 0.46 (97 ± 15)	2.47 ± 0.62 (110 ± 21)	1.91 ± 0.48 (87 ± 19)	2.30 ± 0.53 (104 ± 23)	< 0.05
RER	1.12 ± 0.09	1.13 ± 0.09	1.14 ± 0.09	1.16 ± 0.07	NS
HR, % predicted maximum	98.6 ± 7.9	99.5 ± 9.0	95.3 ± 9.8	99.0 ± 9.0	NS
O <sub>2</sub> pulse, mL O <sub>2</sub> /beat	13.6 ± 2.6	15.3 ± 3.3	12.3 ± 2.4	14.5 ± 3.4	< 0.05
PeapO <sub>2</sub> , kPa	11.09 ± 1.13	11.71 ± 0.91	10.92 ± 1.30	11.93 ± 1.01	< 0.01
PeapCO <sub>2</sub> , kPa	4.59 ± 0.56	4.66 ± 0.52	4.84 ± 0.54	4.63 ± 0.59	NS
V <sub>T</sub> E, L/min (% estimated MVV)	85.9 ± 19.2 (99.3 ± 22.1)	93.7 ± 26.0 (75.3 ± 15.9)	76.8 ± 18.2 (89.5 ± 15.0)	93.1 ± 21.1 (75.5 ± 16.1)	< 0.01
f, breaths/min	35.8 ± 7.1	36.0 ± 8.1	36.9 ± 6.3	35.6 ± 5.9	NS
VT, L	2.44 ± 0.46	2.60 ± 0.46	2.11 ± 0.46	2.63 ± 0.49	< 0.01
IC, L	3.35 ± 0.61	3.48 ± 0.55	3.08 ± 0.68	3.57 ± 0.65	< 0.05
IRV, L	0.89 ± 0.37	0.88 ± 0.32	0.96 ± 0.45	0.95 ± 0.38	NS
VE/VO <sub>2</sub>	39.2 ± 7.3	37.9 ± 5.3	41.1 ± 6.9	41.1 ± 7.0	NS
VE/VC0 <sub>2</sub>	34.6 ± 4.7	33.4 ± 3.7	36.1 ± 5.0	35.3 ± 5.1	NS
VD/VT max	0.23 ± 0.11	0.23 ± 0.09	0.28 ± 0.08	0.27 ± 0.07	NS

Values are means ± SD

f, respiratory rate; HR, heart rate; IC, inspiratory capacity; IRV, inspiratory reserve volume; MVV, maximal voluntary ventilation; PeapCO<sub>2</sub>, arterialised pCO<sub>2</sub>; PeapO<sub>2</sub>, arterialised pO<sub>2</sub>; RER, respiratory exchange ratio; VE, minute ventilation; VO<sub>2</sub>, oxygen uptake; VE/VO<sub>2</sub> and VE/VC0<sub>2</sub>, ventilatory equivalents for oxygen and carbon dioxide, respectively; VT, tidal volume.



**Table 3.** Measurements at highest common work rate (80 Watts)

	Never-smokers		Ever-smokers		p value	Never vs ever-smokers with COPD p value
	COPD (n=22)	Controls (n=22)	COPD (n=22)	Controls (n=22)		
Dyspnoea, Borg scale	1.9 ± 1.4	2.2 ± 1.5	4.1 ± 1.8	2.6 ± 1.8	NS	<0.01
Leg discomfort, Borg scale	2.6 ± 1.7	2.6 ± 1.4	5.1 ± 2.9	3.0 ± 1.8	NS	<0.05
V'O <sub>2</sub> , L/min (% predicted)	1.17 ± 0.09 (52 ± 9)	1.21 ± 0.11 (55 ± 10)	1.20 ± 0.13 (56 ± 10)	1.18 ± 0.13 (54 ± 9)	NS	NS
RER	0.93 ± 0.08	0.89 ± 0.08	0.96 ± 0.10	0.93 ± 0.09	NS	NS
HR, % predicted maximum	69.3 ± 9.5	68.1 ± 9.6	74.0 ± 9.1	72.2 ± 12.0	NS	NS
O <sub>2</sub> pulse, mL O <sub>2</sub> /beat	10.4 ± 1.4	11.2 ± 1.7	10.2 ± 1.3	10.4 ± 1.8	NS	NS
SpO <sub>2</sub> , %	97.0 ± 1.5	97.8 ± 0.9	97.1 ± 1.4	97.8 ± 1.1	NS	NS
V'E, L/min (% estimated MVV)	34.5 ± 6.0 (42.8 ± 19.5)	33.5 ± 5.7 (28.2 ± 8.9)	39.7 ± 7.5 (49.0 ± 18.3)	35.1 ± 5.8 (29.2 ± 7.9)	NS (<0.01)	<0.05 (<0.001)
f, breaths/min	21.0 ± 4.5	20.2 ± 3.8	25.9 ± 6.6	20.1 ± 4.2	NS	<0.01
VT, L	1.67 ± 0.22	1.68 ± 0.22	1.58 ± 0.28	1.78 ± 0.24	NS	<0.05
IC, L	3.53 ± 0.64	3.51 ± 0.63	3.28 ± 0.85	3.53 ± 0.57	NS	NS
IRV, L	1.86 ± 0.60	1.83 ± 0.63	1.70 ± 0.84	1.75 ± 0.56	NS	NS
V'E/V'O <sub>2</sub>	29.7 ± 5.6	27.6 ± 3.2	33.3 ± 6.7	29.8 ± 5.0	NS	<0.05
V'E/V'CO <sub>2</sub>	31.8 ± 4.3	31.0 ± 2.8	34.5 ± 4.7	31.9 ± 3.4	NS	<0.05

Values are means±SD.

f, respiratory rate; HR, heart rate; IC, inspiratory capacity; IRV, inspiratory reserve volume; V'O<sub>2</sub>, oxygen uptake; V'E, minute ventilation; V'E/V'CO<sub>2</sub>, ventilatory equivalents for carbon dioxide; VT, tidal volume.

**Table 4.** Measurements at highest common ventilation rate (50 L/min)

	Never-smokers		p value	Ever-smokers		Never vs ever-smokers with COPD p value
	COPD (n=22)	Controls (n=22)		COPD (n=22)	Controls (n=22)	
Dyspnoea, Borg scale	3.7 ± 1.9	4.3 ± 2.1	NS	5.1 ± 1.5	4.4 ± 1.8	NS
Leg discomfort, Borg scale	4.4 ± 2.3	4.9 ± 1.9	NS	5.8 ± 2.7	4.9 ± 2.1	NS
V'O <sub>2</sub> , L/min (% predicted)	1.59 ± 0.25 (70 ± 11)	1.72 ± 0.23 (77 ± 6)	NS	1.49 ± 0.25 (69 ± 13)	1.62 ± 0.22 (74 ± 13)	0.06
RER	1.03 ± 0.06	0.99 ± 0.09	NS	1.03 ± 0.08	1.01 ± 0.08	NS
HR, % predicted maximum	80.9 ± 8.2	80.5 ± 10.3	NS	82.7 ± 8.0	82.6 ± 9.7	NS
O <sub>2</sub> pulse, mL O <sub>2</sub> /beat	12.1 ± 2.1	13.4 ± 2.2	NS	11.2 ± 1.8	12.4 ± 2.4	NS
f <sub>i</sub> breaths/min	23.8 ± 4.8	23.4 ± 3.9	NS	27.9 ± 5.4	23.7 ± 3.5	< 0.01
IRV, L	1.20 ± 0.48	1.32 ± 0.44	NS	1.33 ± 0.64	1.41 ± 0.49	NS
IC, L	3.40 ± 0.62	3.53 ± 0.57	NS	3.20 ± 0.78	3.54 ± 0.59	NS
VT, L	2.18 ± 0.42	2.19 ± 0.35	NS	1.86 ± 0.33	2.16 ± 0.33	< 0.01

Values are means ± SD.

f<sub>i</sub>, respiratory rate; HR, heart rate; IC, inspiratory capacity; IRV, inspiratory reserve volume; V'O<sub>2</sub>, oxygen uptake; V'E, minute ventilation; V'E/V'CO<sub>2</sub>, ventilatory equivalents for carbon dioxide; VT, tidal volume.

**Supplemental Table 1.** Cardiovascular Comorbidities and Use of Medication among Subjects with COPD

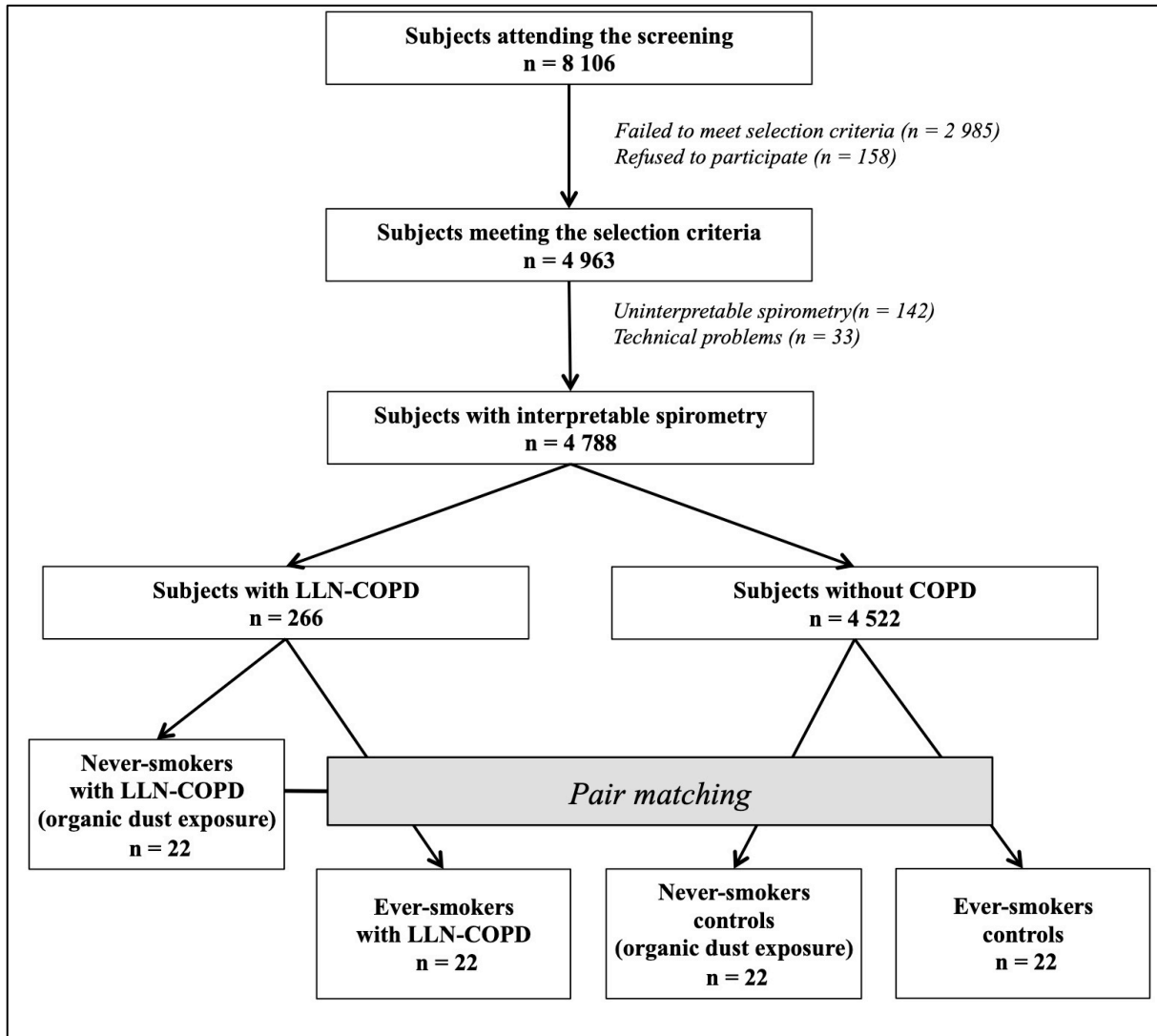
	Never-smokers		Exposure to tobacco smoking		Organic dust vs tobacco smoking COPD p value	
	COPD (n=22)	Controls (n=22)	p value	COPD (n=22)		Controls (n=22)
<b>Comorbidities</b>						
High blood pressure, %	2 (9)	3 (14)	NS	7 (32)	5 (23)	NS
Diabetes, %	0	0	-	3 (14)	1 (5)	NS
Dyslipidemia, %	2 (9)	3 (14)	NS	10 (45)	7 (32)	NS
Osteoporosis, %	0	0	-	2 (9)	0	NS
Obstructive sleep apnea syndrome, %	2 (9)	1 (5)	NS	4 (18)	1 (5)	NS
Gastroesophageal reflux, %	0	0	-	2 (9)	0	NS
<b>Use of pulmonary medication*, %</b>						
Short-acting b2 agonists, %	1 (5)	0	NS	1 (5)	0	NS
Long-acting b2 agonists (LABA), %	0	0	-	0	0	-
Combined LABA and inhaled corticosteroids, %	0	0	-	1 (5)	0	NS
Short-acting anticholinergics, %	1 (5)	0	NS	0	0	-
Long-acting anticholinergics, %	0	0	-	1 (5)	0	NS
Theophylline, %	0	0	-	0	0	-
Inhaled corticosteroids, %	0	0	-	0	0	-

**Supplemental Table 2.** Measurement at ventilatory threshold

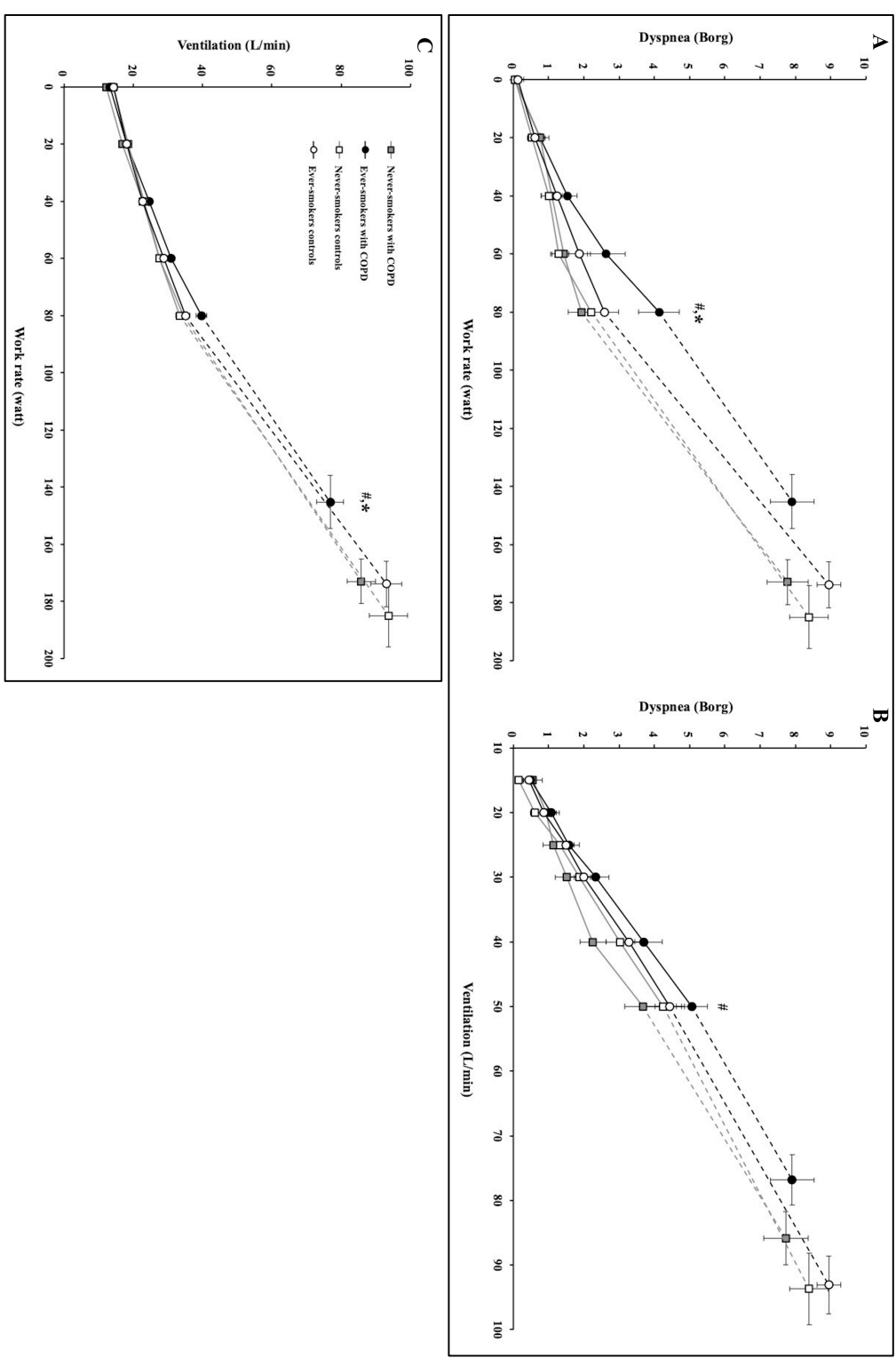
	Never-smokers			Ever-smokers			Never vs ever-smokers with COPD p value
	COPD (n=22)	Controls (n=22)	p value	COPD (n=22)	Controls (n=22)	p value	
Work rate, W (% predicted)	103.4 ± 23.6	118.9 ± 28.4	0.06	82.5 ± 23.1	102.3 ± 26.4	< 0.05	< 0.01
HR, beat/min (% predicted maximum)	53 ± 12	62 ± 10	< 0.01	44 ± 11	55 ± 14	< 0.05	< 0.05
V̇O <sub>2</sub> , L/min (% predicted)	122.6 ± 12.1	126.2 ± 13.0	NS	119.6 ± 12.1	125.3 ± 13.4	NS	NS
RER	75 ± 9	78 ± 8	NS	74 ± 8	78 ± 9	NS	NS
O <sub>2</sub> pulse, mL O <sub>2</sub> /beat	1.43 ± 0.34	1.66 ± 0.35	< 0.05	1.22 ± 0.26	1.46 ± 0.37	< 0.05	< 0.05
f <sub>i</sub> , breaths/min	63 ± 14	74 ± 12	< 0.01	56 ± 12	66 ± 17	< 0.05	NS
V̇E, L/min (% estimated MVV)	0.97 ± 0.05	0.97 ± 0.06	NS	0.96 ± 0.08	0.96 ± 0.06	NS	NS
V <sub>T</sub> , L	11.6 ± 2.3	13.2 ± 2.7	< 0.05	10.2 ± 1.9	11.7 ± 2.9	< 0.05	< 0.05
Dyspnoea, Borg scale	22.8 ± 5.4	22.7 ± 3.9	NS	23.8 ± 2.9	21.2 ± 3.7	< 0.05	NS
Leg discomfort, Borg scale	42.8 ± 10.6	47.1 ± 9.2	NS	38.2 ± 6.0	42.7 ± 10.7	NS	NS
	50.9 ± 18.1	38.6 ± 8.0	< 0.01	45.3 ± 8.8	35.1 ± 9.8	< 0.001	NS
	31.1 ± 4.5	29.5 ± 3.3	NS	33.5 ± 4.4	30.7 ± 3.4	< 0.05	NS
	1.91 ± 0.42	2.11 ± 0.38	NS	1.64 ± 0.34	2.03 ± 0.36	< 0.001	< 0.05
	2.6 ± 2.0	3.9 ± 2.0	NS	3.2 ± 0.9	3.5 ± 1.2	NS	NS
	3.3 ± 1.8	4.5 ± 1.9	NS	3.5 ± 1.4	4.1 ± 1.8	NS	NS

**Figure 1.** Flow chart of participants included in the study. Subjects were recruited through a screening program in two branches of the social security. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma, hypersensitivity pneumonitis.

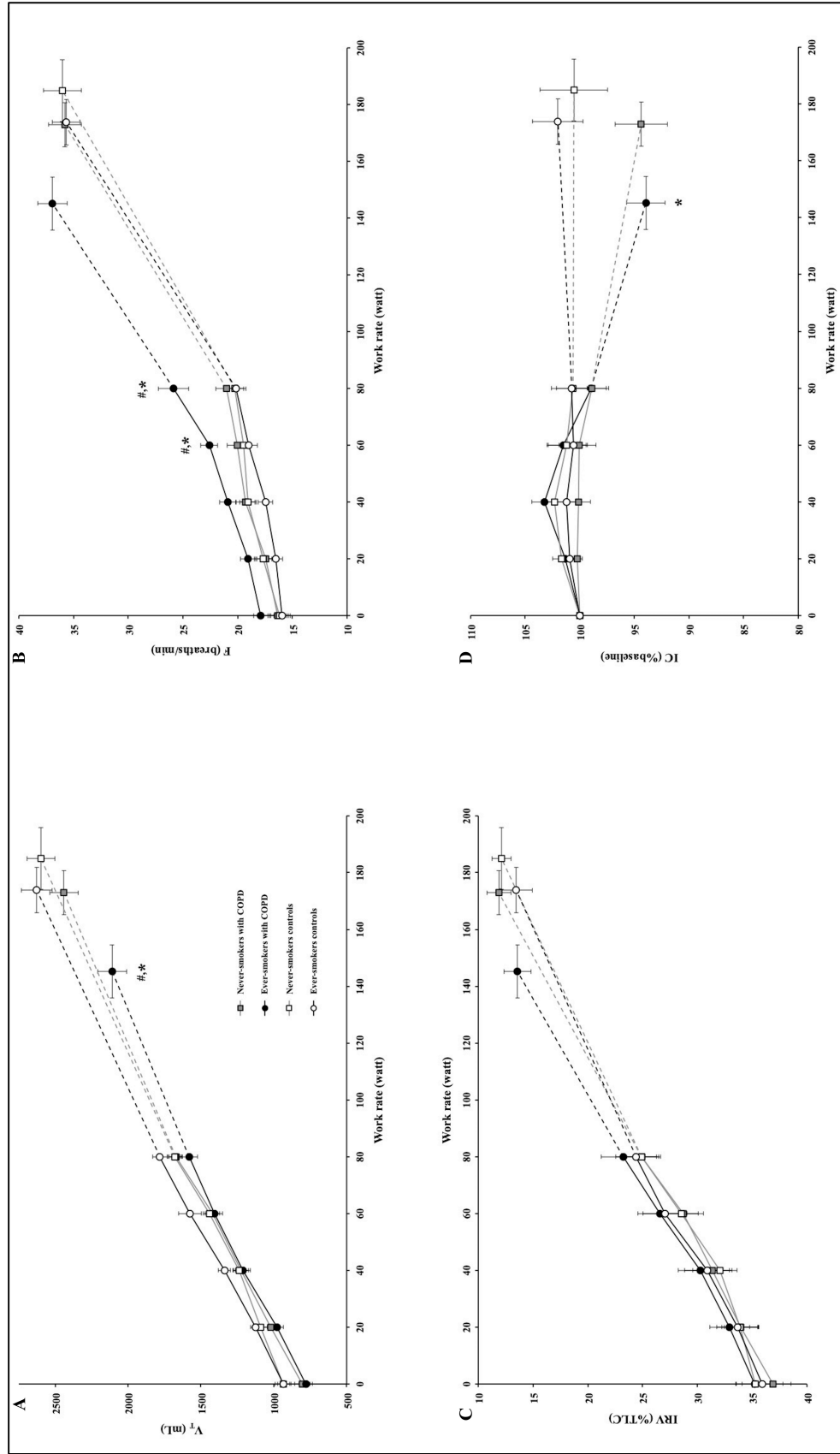
Abbreviations: COPD: chronic obstructive lung disease, LLN: lower limit of normal.



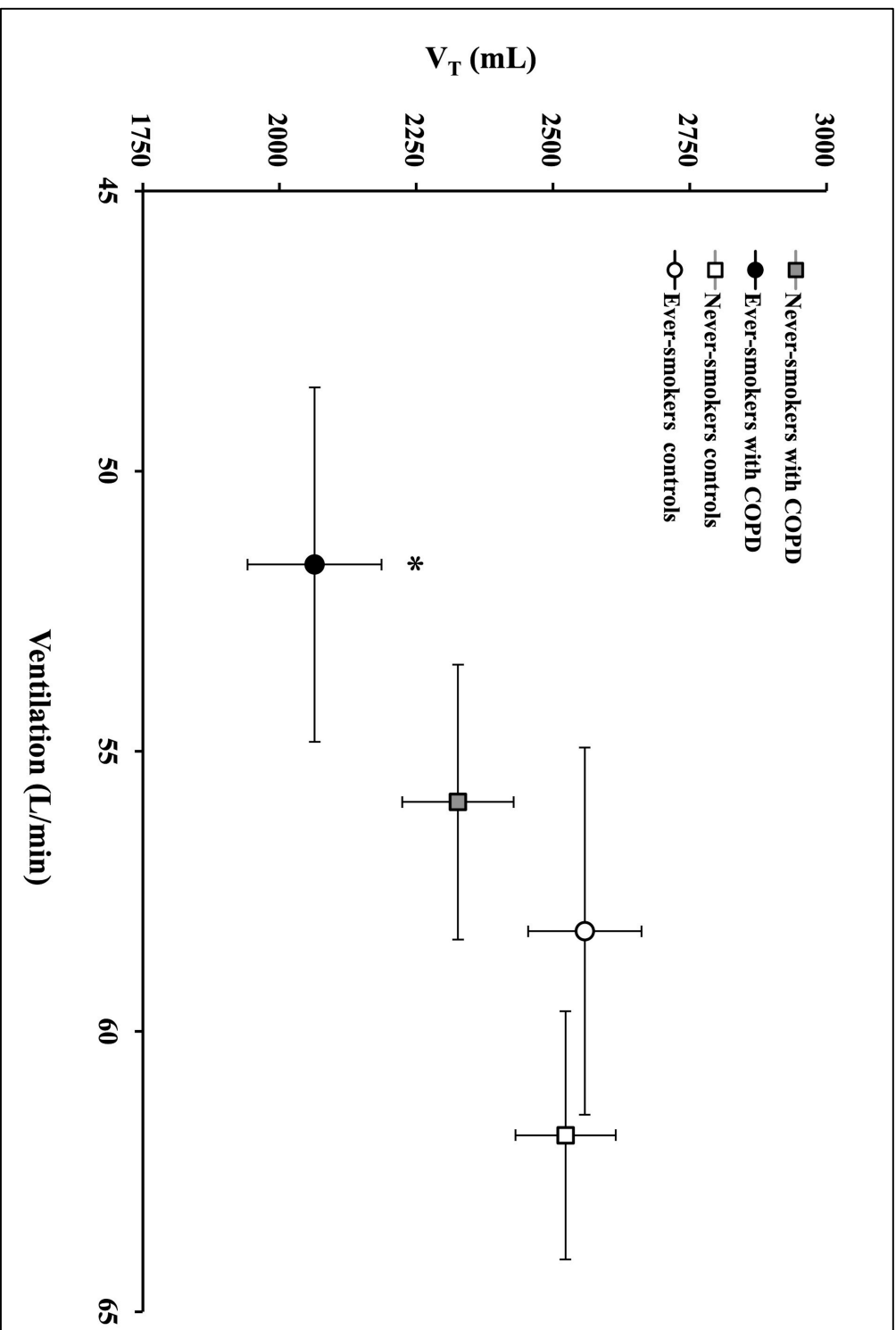
**Figure 2.** Exertional dyspnoea intensity during incremental cycle exercise testing is shown relative to work rate (A) and minute ventilation (B). Dyspnoea to work rate and dyspnoea to minute ventilation slopes were significantly different between ever-smokers with COPD and never-smokers with COPD (analysis of variance repeated measures). Of note, the relationship between work rate and minute ventilation was superimposed in all the groups (C). Values are means±SEM. #*p*<0.05 versus never-smokers with COPD and \**p*< 0.05 versus controls at a standardised work rate (Scheffe's tests).



**Figure 3.** Tidal volume (VT; A), breathing frequency (F; B), inspiratory reserve volume (IRV, expressed as a percentage of total lung capacity (TLC); C) and inspiratory capacity (IC, expressed as a percentage of the value measured at rest during the exercise test; D) are plotted in relation to work rate. Values are means±SEM. #p<0.05 versus never-smokers with COPD and \*p<0.05 versus controls at a standardised work rate (Scheffe's tests).



**Figure 4.** On evaluation of individual plots of minute ventilation ( $V^{\prime}E$ ) versus tidal volume ( $V_T$ ) (Hey plots), the group of asymptomatic subjects with airway obstruction had an inflection point of the relationship between  $V_T$  and  $V^{\prime}E$  between those of controls and those of never-smokers with COPD. By contrast, the inflection point occurred at a lower  $V_T$  for a lower  $V^{\prime}E$  in ever-smokers with COPD compared to controls ( $p < 0.05$ ). Graphs represent mean  $\pm$  SE values. \*  $p < 0.05$  versus controls







## C. BPCO secondaire à l'exposition aux poussières organiques: conséquences systémiques

### 1. Article 9 : In patients with mild-to-moderate COPD, tobacco smoking, and not COPD, is associated with a higher risk of comorbidity.

#### *a. Contexte et objectif*

Les comorbidités sont fréquentes dans la BPCO et contribuent à la sévérité et à la mortalité globale de la maladie. La prévalence de ces différentes comorbidités est variable suivant le sexe, la sévérité de l'obstruction bronchique et probablement le type d'exposition. Néanmoins, la prévalence des comorbidités a été peu étudiée dans la BPCO légère à modérée. Par ailleurs, il est maintenant communément admis que près de la moitié des cas de BPCO est associée à une autre exposition que le tabagisme, notamment les expositions professionnelles. L'existence de comorbidités dans BPCO non-tabagique a également été rarement évaluée.

L'objectif de cette étude était d'évaluer la prévalence des comorbidités et d'évaluer ses déterminants chez des sujets présentant une BPCO ou des sujets sains, exposés aux poussières organiques, au tabac ou aux deux.

#### *b. Manuscrit*

Article "In patients with mild-to-moderate COPD, tobacco smoking, and not COPD, is associated with a higher risk of comorbidity". **Soumagne T**, Guillien A, Roche N, Annesi-Maesano I, Andujar P, Laurent L, Jouneau S, Botbol M, Laplante JJ, Dalphin JC, Degano B. *En cours de finalisation.*



## **IN PATIENTS WITH MILD-TO-MODERATE COPD, TOBACCO SMOKING, AND NOT COPD, IS ASSOCIATED WITH A HIGHER RISK OF COMORBIDITY**

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## **Abstract**

**Background:** Comorbidities including cardiovascular disease (CVD) are very common in chronic obstructive pulmonary disease (COPD) secondary to tobacco smoking and contribute to the overall severity of the disease. In non-smoking COPD, which accounts for up to 50% of COPD cases worldwide, the current knowledge on the prevalence of comorbidities remains scarce.

**Objectives:** To assess the prevalence of major comorbidities and to evaluate their determinants in a group of non-selected patients with mild-to-moderate COPD and in controls (without COPD) exposed to organic dust exposure (i.e. dairy farmers), tobacco smoking or both.

**Methods:** 4665 subjects (2323 dairy farmers and 2342 non-farmers) including 355 patients with COPD and 4310 controls without pre-bronchodilator airflow limitation were recruited through a large COPD screening program. Self-reported presence of physician-diagnosed diseases with plausible links to COPD and tobacco or organic dust exposure were prospectively recorded.

**Results:** COPD secondary to organic dust was associated with a lower prevalence of comorbidities including CVD and diabetes mellitus compared to tobacco smoking COPD. Multivariate analysis demonstrated that exposure to organic dust was associated with a lower prevalence of CVD and diabetes mellitus, independently to potential confounder such as age, sex and tobacco smoking.

**Conclusions:** Analysis of unselected, consecutively diagnosed patients with COPD derived from a large a screening program suggests that organic dust exposure is a potential protective factor for CVD in patients with or without COPD.

Chronic obstructive pulmonary disease (COPD) is an important cause of morbidity and mortality worldwide (1). Progressive respiratory failure accounts for only one third of all causes of mortality in patients with COPD, especially in those with severe disease, suggesting that many patients die from other causes (2). Comorbidities are very common in COPD, and contribute to the overall severity of the disease, impairing quality of life and increasing mortality (3, 4). The prevalence of the different comorbidities in COPD varies according to several conditions such as gender, severity of lung function impairment and possibly type of exposure (3, 5).

To diagnose COPD at mild-to-moderate stages (i.e., in patients with preserved or moderately altered pulmonary function) and, if possible, at early stages (i.e., at the beginning of the pulmonary function decline) has been identified as a priority for secondary prevention (6). In addition, an appropriate diagnosis of COPD is a prerequisite to modify exposure to risk factors and initiate appropriate therapy among those who have the disease, and is essential to help avoid unnecessary costs and potential side effects of treatments and to search for an appropriate diagnosis among those who do not have the disease (6, 7). Making a diagnosis of COPD at early and/or mild-to-moderate stages can also allow the earlier introduction of preventative medication for conditions associated with COPD, such as coronary artery disease and depression (8). There is therefore a room for COPD case-finding and for identification of associated comorbidities in at-risk populations (7).

Cigarette smoking is the main risk factor for COPD in developed countries, and a large majority of the current knowledge on comorbidities in COPD comes from analyses made in smoking-related COPD. Nevertheless, up to 50% of COPD cases worldwide can involve other factors, including indoor and outdoor air pollutants as well as occupational hazards (9). Among occupational causes, exposure to organic dusts has been associated with accelerated impairment in lung function and with increased risk of COPD (10-12).

The prevalence of the major comorbidities reported to be associated with COPD has been rarely studied in patients at early and/or mild-to-moderate stages, and there are few data regarding the prevalence of comorbidities in COPD secondary to causes other than tobacco smoking. We therefore sought to assess the prevalence of comorbidities and to evaluate their determinants in a group of non-selected patients with mild-to-moderate COPD and in controls (without COPD) exposed to either organic dust, tobacco smoking or both, all detected by screening.

## **METHODS**

### **Study design and subjects**

Patients with COPD and control subjects without COPD were consecutively recruited between January 2012 and November 2015 through a regional COPD screening program involving general practitioners and occupational physicians (BALISTIC study; ClinicalTrials.gov Identifier: NCT02540408). This program was conducted at the University Hospital of Besançon in collaboration with the French national social security system for all agricultural workers (Mutualité Sociale Agricole, MSA) and with the care homes federation of the Franche-Comté region (Fédération des Maisons de Santé Comtoises, FéMaSaC) (13). The study was set up primarily to obtain data on the prevalence of COPD and on characteristics of the disease occurring in dairy farmers (i.e. COPD secondary to organic dust exposure) by comparison with COPD patients who were not exposed to organic dusts (14). Every five years, all persons affiliated to the MSA are invited to attend a free health check-up, and around 20% of those invited usually attend (15). Among all persons aged 40-74 years who attended the health check-up between January 2012 and November 2015, participation in the screening phase of the BalistiC study was offered to those who had been exposed either to organic dusts (dairy farmers) and/or to tobacco smoking and also to those with no identified

exposure. Exclusion criteria were an established diagnosis of asthma, bronchiectasis and/or hypersensitivity pneumonitis. Patients with a documented diagnosis of severe COPD were also excluded from the screening program. In order to include subjects with an exposure as homogeneous as possible, subjects having/having had a history of occupational exposure other than dairy farming (including farmers with any other professional activity than dairy farming) were also excluded.

In parallel with the screening by the MSA, GPs working with the FéMaSaC proposed the participation in the screening phase of the BalistiC study to their patients fulfilling the above inclusion and exclusion criteria. These patients were invited to attend free screening sessions during which spirometry was performed by their GPs.

The criteria for retaining a diagnosis of mild-to-moderate COPD were (i) to have a history of exposure to risk factors (tobacco smoking and/or daily exposure to organic dusts) and (ii) to have a evidence of persistent airflow limitation defined as a post-bronchodilator forced expiratory volume in 1 second ( $FEV_1$ ) on forced vital capacity (FVC) ratio < lower limit of normal (LLN) together with a  $FEV_1 > 50\%$  of predicted value according to the GLI-2012 equations (1, 16). COPD patients were classified into 3 groups: (i) COPD secondary organic dust exposure, i.e., COPD patients with a history of at least 20 years of exposure to organic dust together with a smoking history of less 1 pack-year in; (ii) COPD secondary to tobacco smoking; and (iii) COPD secondary to both organic dusts and tobacco smoking exposures.

Subjects without airflow limitation participating in the screening program with otherwise identical selection criteria as COPD patients were also included in the current analysis.

Ethics committee approval was received from the local Ethics Committee (CPP Est; P-2011-119), and written consent was obtained from all subjects.

## **Spirometry**



Spirometry tests were performed using a pneumotachograph (MedGraphics; MSE Medical, Strasbourg, France). All spirometers were calibrated at least once daily using a 3-L syringe. Bronchodilation was performed by administering 400 µg of the short-acting β<sub>2</sub>-agonist salbutamol (Ventoline, GlaxoSmithKline, Marly-le-Roi, France) and spirometry was repeated after a 10- to 15-min interval, as recommended. Only subjects with valid spirometry at screening were further included in the study.

### **Questionnaire**

The study examined the self-reported presence of physician-diagnosed diseases with plausible links to COPD and tobacco or organic dust exposure asking participants whether they had been diagnosed or treated for selected medical condition. The following conditions (considered as “comorbidities”) were selected and recorded for all patients at the time of the first visit: hypertension, heart disease (including coronary artery disease (CAD), chronic heart failure, atrial fibrillation and valvulopathy), peripheral artery disease (PAD), cerebrovascular disease, thromboembolic disease, obstructive sleep apnea syndrome (OSAS), diabetes mellitus, dyslipidemia, thyroid disorders, gastroesophageal reflux, osteoporosis, solid neoplasms (cancer), leukemia, lymphoma, chronic renal failure, liver disease, neurodegenerative disorder, epilepsy, anxious depressive syndrome and collagen disease. Responses were dichotomous. The non-age-adjusted Charlson index and the COTE (COPD specific comorbidity test) index were calculated for every patient, as described elsewhere (3, 17).

### **Statistical Analysis**

Bivariate logistic regression analysis and chi-square tests were performed for categorical data and t-test for quantitative data. Data were expressed as mean (SD); number (percentage); or z

scores, as appropriate. Continuous variables used for logistic regression were all categorized to obtain categorical variables. The variables of age and tobacco habits were divided into three categories each (namely, aged < 55 years, 55–64 years,  $\geq$  65 years; and never smokers, former smokers, current smokers, respectively). The other variables were binary: sex (male versus female); BMI (< 25 versus  $\geq$ 25); and presence of comorbidity (yes versus no).

Multivariate logistic regression, with diagnosis of the comorbidity as a dependent variable, included variables with a level of significance  $\leq$  0.20 in the bivariate analysis. In order to obtain the final model, a stepwise backward selection of variables was performed.

All analyses were performed with R version 3.5.0 and RStudio version 1.1.453 (R Foundation for Statistical Computing, Vienna, Austria).

## **Results**

### **Subject characteristics**

The flowchart of the participating population is displayed in Figure 1. Of the 4963 subjects who underwent COPD screening, 4665 (94%) had a spirometry test of acceptable quality together with a complete report of comorbidities and were therefore included in the current analysis. Of these 4665 subjects, 266 patients (5.70%) had mild-to-moderate COPD and 4399 had normal spirometry (controls subjects).

On average, dairy farmers were slightly older, were more frequently men and were much less smoker than non-dairy farmers (Supplemental Table 1). The main characteristics of the 3 subgroups of COPD patients and their counterparts without airway obstruction separated according to exposure (i.e., tobacco smoking, organic dusts and both exposures), as well as characteristics of control subjects without airway obstruction and without any exposure, are given in Table 1. All subgroups (patients with COPD and control subjects) had the same age. COPD patients were more frequently men than their counterparts with normal spirometry,

except in the subgroup of subjects exposed only to tobacco smoking. In subgroups exposed to tobacco smoking (either exclusively or in association with organic dust exposure), smoking intensity was higher in COPD than in controls, and patients with COPD were more frequently active smokers (instead of former smokers) than controls. The subgroup of COPD patients with an exclusive exposure to tobacco smoking had a significantly lower FEV1 compared with the two other subgroups of COPD. Of note, the mean  $\pm$  standard deviation z-scores for FEV1 and FVC for all control subjects without COPD were very close to  $0 \pm 1$ , indicating a very good fit of our “control” population with the healthy population of the Global Lung Initiative for spirometry (18).

### **Comorbidities**

Compared with non-dairy farmers, dairy farmers had a lower prevalence of comorbidities including cardiovascular diseases (CVD), diabetes mellitus, anxious depressive syndrome and cancer (Supplemental Table 1). By contrast, there were very few differences for each of the reported comorbidities between COPD patients and their counterparts with normal spirometry (Table 2). Similarly, in each of the 3 subgroups separated according to exposure, COPD patients had values of the non-age-adjusted Charlson comorbidity index that differed of less than 1 compared with their counterparts without COPD. As the item “COPD” counts 1 point in the Charlson comorbidity index, a difference of less than 1 between COPD and non-COPD indicates that values are in fact similar.

The non-age-adjusted Charlson comorbidity index and the COTE index were poorer for COPD patients exposed tobacco smoking compared with COPD patients exposed to organic dust alone – those exposed to both organic dusts and tobacco smoking having intermediate values.

### **Factors Associated with comorbidities**

Tables 3-5 show the results of the univariate and multivariate analyses for the association between three selected comorbidities (i.e., coronary artery disease, diabetes mellitus and hypertension) and several selected variables. This analysis shows that in our group of patients with mild-to-moderate COPD, COPD was not associated with an increased risk of these three major comorbidities. By contrast, we found that exposure to organic dusts was associated with a lower risk of diabetes mellitus and of coronary artery disease. Conversely, a smoking intensity of more than 15 packs-years was associated with an increased risk of each of the three selected major comorbidities.

### **DISCUSSION**

The analysis of a large population comprising 5.70% of patients with mild-to-moderate COPD of various etiologies (i.e., exposure to tobacco smoking and/or to organic dusts), all identified by screening, reveals that (1) whatever the exposure, patients with COPD did not have a higher prevalence of comorbidities index than their counterparts without airflow limitation; (2) after adjustment on airflow limitation severity (i.e., on FEV1), comorbidity indexes were poorer in COPD secondary to tobacco smoking than in COPD secondary to organic dusts exposure; (3) a higher risk of major comorbidities was associated with tobacco smoking (but not with COPD) and (4) a lower risk of major comorbidities was associated with exposure to organic dusts (here, to have been a dairy farmer for more than 20 years).

Few studies have investigated the prevalence of comorbidities among the farming population. Regarding CVD, in a study conducted in Ireland, Van Doorne et al. suggested that livestock farmers were considered at high-risk for cardiovascular disease as about one of two had hypertension and elevated total cholesterol (19). By contrast, in our cross sectional study, we found lower CVD prevalence (including CAD and PAD) in dairy farmers. This could possibly

be explained by difference in lifestyle behaviors as prevalence of obesity (ie BMI  $\geq 25$ ) was only of 63% in our study compared to 86% in van Doorn et al.

Several large scale studies have showed that organic dust exposure, occurring in dairy farming, is independently associated with higher prevalence of COPD even farmers tend to smoke less (15, 21, 22). COPD is a complex respiratory disease and associated with systemic manifestations (23). Indeed, there is accumulating evidence that individuals with COPD are more likely to suffer from several comorbidities that account for a significant part of overall mortality in COPD (3). Tobacco smoking COPD and comorbidities such as cardiovascular diseases (CVD) share common risk factors, such as smoking or advanced age, but the mechanisms linking COPD to these comorbidities are complex, multifactorial and not entirely understood (24, 25). In addition, low-grade systemic inflammation is thought play a role in the pathophysiology of COPD and its comorbidities.

In the current study, we showed that individuals COPD secondary to organic dusts have a lower prevalence of CVD disease (ie CAD and PAD). Two possible reasons could explain this lower prevalence. First, tobacco smoking is a well-known common risk factor for both COPD and CVD (24), so does not organic dust exposure. It is plausible that the nature of the noxious agents (tobacco or organic dust exposure) could have a different impact on peripheral or coronary arteries. Second, the systemic inflammatory response to exposure to organic dust may differ from that of tobacco smoking. Indeed, it is thought to be associated with a predominantly Th2-type lymphocyte production profile in organic dust exposure (26-28). On the contrary, COPD due to tobacco smoking is dominated by Th1-type lymphocyte response and is associated with Th17 cytokine production (26).

The prevalence of the different comorbidities among patients with tobacco smoking COPD were lower than those previously reported (3, 29, 30). This could in part be explained by the fact that comorbidities were self-reported and that a large proportion of patient in our study

had mild COPD.

Current knowledge of comorbidities in non-smoking COPD remains scarce, as only one retrospective study has focused on this purpose. Regarding patients with COPD exposed to organic dust, our results were in line with this previous study including non-smoking COPD patients (5). Indeed, Golpe et al found biomass exposure was independently associated with a lower prevalence of CVD in patients with COPD.

### **Limitations**

Some limitations must be pointed out. First, as this is a cross-sectional study of disease prevalence rather than incidence, there is uncertainty in the degree to which exposure preceded the outcomes observed. Second, we did not quantify the level of organic dust exposure of all subjects included in the current analysis. However, exposure was studied in a subset of our population and showed that dairy farmers with and without COPD was exposed to higher mold and actinomycete concentrations (14). Third, as the study was based on self-reported assessment of comorbidities and health-related variables, there may be misclassification in the outcome and covariate data. Undiagnosed and untreated cases may not have been reported, and there may have been misreporting of diagnoses or sensitive health-related characteristics such as weight, level of physical activity and alcohol use.

### **Conclusion**

In conclusion, we found a different distribution of comorbidities for patients with COPD secondary to organic dust and tobacco smoking COPD. Exposure to organic dust was associated with a significant lower risk for CVD. These results suggest that the systemic effects of organic dust exposure might be different to those of tobacco and that organic dust exposure could have a potential protective factor for CVD in patients with or without COPD.

However, these findings must be taken with caution as other factor like lifestyle behavior could be potential confounders. Further studies are needed to confirm these results.

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### **Author Contributions:**

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

BD, JCD and TS made substantial contributions to the conception and design of the work.

TS and AG performed all analyses.

JCD and BD obtained study funding.

All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work. TS wrote the first draft of the manuscript. BD, TS, AG, NR, IAM, PA, LL, SJ, MB, JIL and JCD revised the manuscript for important intellectual content. All authors approved the manuscript.

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**Table 1.** Main characteristics of patients with COPD and control subjects, separated into 3 subgroups according to exposure to tobacco smoking, to organic dusts or both.

	Exposure to organic dusts			Exposure to both tobacco and organic dusts			Exposure to tobacco smoking			Controls without any exposure (n = 1077)
	COPD (n = 72)	Controls (n = 1555)	p	COPD (n = 75)	Controls (n = 521)	p	COPD (n = 119)	Controls (n = 1246)	p	
<b>Age, years</b>	57.0 ± 9.7	58.4 ± 9.9	NS	58.1 ± 9.3	56.5 ± 9.9	NS	55.7 ± 8.9	56.0 ± 8.8	NS	57.3 ± 9.2
<b>Males</b>	52 (72)	877 (56)	< 0.01	69 (92) <sup>a</sup>	425 (82)	< 0.05	67 (56) <sup>ab</sup>	673 (54)	NS	361 (34)
<b>BMI, kg·m<sup>-2</sup></b>	26.0 ± 5.0	27.2 ± 4.5	NS	27.4 ± 4.7	27.7 ± 4.4	NS	24.9 ± 4.4 <sup>b</sup>	26.4 ± 4.9	< 0.001	26.4 ± 5.1
<b>Smoking status</b>										
Never	72 (100)	1555 (100)		-	-		-	-		1077 (100)
Former	-	-		31 (41)	351 (67)		50 (42)	690 (55)		-
Current	-	-		44 (59)	170 (33)		69 (58)	556 (45)		-
<b>Smoking intensity</b>										
< 1 pack-year	72 (100)	1555 (100)		-	-	< 0.001	-	-	< 0.001	1077 (100)
1-15 pack-years	-	-		25 (33)	287 (55)		34 (29)	588 (47)		-
> 15 pack-years	-	-		50 (67)	234 (45)		85 (71)	657 (53)		-
<b>Pre-bronchodilator spirometry</b>										
FVC L	4.34 ± 1.24	3.90 ± 1.09	< 0.01	4.50 ± 1.37	4.27 ± 0.93	NS	3.84 ± 1.13 <sup>ab</sup>	3.92 ± 0.91	NS	3.66 ± 0.99
z-score	0.24 ± 1.10	0.10 ± 1.05	NS	0.06 ± 1.43	0.05 ± 1.05	NS	-0.40 ± 1.29 <sup>ab</sup>	-0.12 ± 1.04	< 0.0001	0.00 ± 1.01
FEV1 L	2.72 ± 0.87	3.07 ± 0.87	< 0.0001	2.77 ± 0.93	3.32 ± 0.74	< 0.0001	2.39 ± 0.81 <sup>ab</sup>	3.08 ± 0.71	< 0.0001	2.93 ± 0.77
z-score	-1.20 ± 1.02	0.15 ± 1.07	< 0.0001	-1.36 ± 1.21	0.01 ± 1.07	< 0.0001	-1.75 ± 1.17 <sup>ab</sup>	-0.10 ± 1.05	< 0.0001	0.16 ± 1.02
FEV1/FVC %	62.0 ± 5.9	78.9 ± 5.7	< 0.0001	61.0 ± 5.5	77.8 ± 5.7	< 0.0001	61.6 ± 6.7	78.7 ± 5.8	< 0.0001	80.6 ± 5.3
z-score	-2.20 ± 0.52	0.04 ± 0.82	< 0.0001	-2.26 ± 0.49	-0.10 ± 0.80	< 0.0001	-2.30 ± 0.64	-0.01 ± 0.86	< 0.0001	0.23 ± 0.79
<b>Post-bronchodilator spirometry</b>										
FVC L	4.36 ± 1.22	-		4.59 ± 1.340	-		3.94 ± 1.12 <sup>ab</sup>	-		-
z-score	0.29 ± 1.06	-		0.20 ± 1.45	-		-0.24 ± 1.21 <sup>ab</sup>	-		-
FEV1 L	2.75 ± 0.87	-		2.88 ± 0.94	-		2.45 ± 0.80 <sup>ab</sup>	-		-
z-score	-1.14 ± 1.02	-		-1.16 ± 1.17	-		-1.61 ± 1.12 <sup>ab</sup>	-		-
FEV1/FVC %	62.4 ± 6.4	-		62.1 ± 5.6	-		61.9 ± 7.1	-		-
z-score	-2.16 ± 0.55	-		-2.12 ± 0.45	-		-2.26 ± 0.68	-		-

<sup>a</sup>: p < 0.05 vs. organic dust COPD; <sup>b</sup>: p < 0.05 vs. COPD with both exposures (Bonferroni's correction).

**Table 2.** Prevalence of main comorbidities in the different subgroups

	Exposure to organic dusts			Exposure to both tobacco and organic dusts			Exposure to tobacco smoking			Controls without exposure	
	COPD (n = 72)	Controls (n = 1555)	p	COPD (n = 75)	Controls (n = 521)	p	COPD (n = 119)	Controls (n = 1246)	p	p	Controls without exposure (n = 1077)
Charlson comorbidity index*	1.13 ± 0.44	0.17 ± 0.48	< 0.0001	1.15 ± 0.42	0.23 ± 0.56	< 0.0001	1.35 ± 0.63 <sup>a</sup>	0.31 ± 0.65	< 0.0001	< 0.0001	0.22 ± 0.56
COTE index	0.15 ± 0.78			0.28 ± 1.08			0.41 ± 1.15 <sup>a</sup>				
Hypertension	6 (8)	293 (19)	< 0.05	16 (21) <sup>a</sup>	99 (19)	NS	18 (15)	245 (20)	NS	NS	194 (18)
Diabetes mellitus	1 (1)	54 (4)	NS	4 (4)	21 (4)	NS	10 (8) <sup>a</sup>	95 (8)	NS	NS	52 (5)
Dyslipidemia	4 (6)	157 (10)	NS	7 (9)	59 (11)	NS	9 (8)	134 (11)	NS	NS	109 (10)
Coronary artery disease	0	28 (2)	NS	3 (4)	21 (4)	NS	7 (6) <sup>a</sup>	64 (5)	NS	NS	31 (3)
Chronic heart failure	0	2 (0.1)	NS	0	2 (0.4)	NS	0	2 (0.2)	NS	NS	2 (0.2)
Valvulopathy	0	14 (1)	NS	1 (1)	1 (0.2)	NS	2 (2)	14 (1)	NS	NS	13 (1)
Atrial fibrillation	0	31 (2)	NS	3 (4)	13 (3)	NS	1 (1)	23 (2)	NS	NS	17 (2)
Pacemaker	0	5 (0.3)	NS	0	0	NS	1 (1)	2 (0.2)	NS	NS	1 (0.1)
Cerebrovascular disease	1 (1)	20 (1)	NS	3 (4)	5 (1)	< 0.05	3 (3)	17 (1.4)	NS	NS	11 (1)
Peripheral artery disease	0	2 (0.1)	NS	0	3 (0.6)	NS	3 (3)	18 (1)	NS	NS	1 (0.1)
Thromboembolic disease	0	17 (1)	NS	1 (1)	5 (1)	NS	3 (3)	19 (2)	NS	NS	17 (2)
Gastroesophageal reflux	1 (1)	58 (4)	NS	1	17 (3)	NS	7 (6)	72 (6)	NS	NS	58 (5)
Thyroid disorder	2 (3)	61 (4)	NS	2 (3)	21 (4)	NS	9 (8)	86 (7)	NS	NS	97 (9)
Osteoporosis	2 (3)	15 (1)	NS	0	0	NS	2 (2)	7 (1)	NS	NS	17 (2)
Chronic kidney disease	0	3 (0.2)	NS	0	1 (0.2)	NS	0	2 (0.2)	NS	NS	1 (0.1)
Liver disease	0	1 (0.1)	NS	0	2 (0.4)	NS	0	4 (0.3)	NS	NS	1 (0.1)
Cancer, leukemia or lymphoma	3 (4)	32 (2)	NS	0	17 (3)	NS	5 (4)	47 (4)	NS	NS	28 (3)
Connective tissue disease	0	26 (2)	NS	0	15 (3)	NS	1 (1)	10 (1)	NS	NS	18 (2)
Anxious depressive syndrome	0	46 (3)	NS	1 (1)	9 (2)	NS	7 (6) <sup>a</sup>	69 (6)	NS	NS	66 (6)
Neurodegenerative disorder	0	5 (0.3)	NS	0	0	NS	0	3 (0.2)	NS	NS	9 (1)
Epilepsia	1 (1)	6 (0.4)	NS	1 (1)	2 (0.4)	NS	2 (2)	3 (0.2)	< 0.05	< 0.05	4 (0.4)
OSAS	0	2 (0.1)	NS	0	1 (0.2)	NS	0	1 (0.1)	NS	NS	1 (0.1)

\*Non-age-adjusted Charlson comorbidity index; <sup>a</sup>: p < 0.05 vs. organic dust COPD (Bonferroni's correction).

**Table 3.** Univariate and multivariate logistic regression analysis for coronary artery disease

Variable	Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p
<b>Male</b>	3.35	2.29 - 5.04	<b>&lt; 0.0001</b>	3.07	2.05 - 4.72	<b>&lt; 0.0001</b>
<b>Age, years</b>						
40-54	ref					
55-64	2.59	1.62 - 4.27	<b>&lt; 0.0001</b>	2.42	1.48 - 4.08	<b>&lt; 0.0001</b>
65-75	4.88	3.14 - 7.85	<b>&lt; 0.0001</b>	4.88	3.05 - 8.08	<b>&lt; 0.0001</b>
<b>BMI</b>						
< 25	ref					
≥ 25	1.86	1.30 - 2.71	<b>&lt; 0.0001</b>			
<b>COPD</b>	1.15	0.56 - 2.11	0.67			
<b>Organic dust exposure</b>	0.55	0.39 - 0.77	<b>&lt; 0.0001</b>	0.58	0.40 - 0.84	<b>&lt; 0.0001</b>
<b>Smoking status</b>						
Never	ref					
Former	2.66	1.87 - 3.80	<b>&lt; 0.0001</b>			
Current	1.38	0.85 - 2.18	0.18			
<b>Smoking intensity</b>						
< 1 pack-year	ref			ref		
1-15 pack-years	1.11	0.66 - 1.82	0.67	0.92	0.54 - 1.54	0.77
> 15 pack-years	3.20	2.25 - 4.58	<b>&lt; 0.0001</b>	2.13	1.44 - 3.17	<b>&lt; 0.0001</b>
<b>zscore FEV1 &lt; 0</b>	0.39	1.06 - 2.05	<b>&lt; 0.05</b>			
<b>zscore FEV1 &lt; -1.64</b>	0.48	1.61 - 2.63	0.07			
<b>Hypertension</b>	1.84	1.28 - 2.61	<b>&lt; 0.0001</b>			
<b>Dyslipidemia</b>	1.40	0.85 - 2.19	0.16			
<b>Diabetes mellitus</b>	5.03	3.25 - 7.58	<b>&lt; 0.0001</b>	2.71	1.67 - 4.26	<b>&lt; 0.0001</b>

*Abbreviations:* BMI: body mass index; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in 1 second;

OR: odds ratio.

**Table 4.** Univariate and multivariate logistic regression analysis for diabetes mellitus

Variable	Univariate			Multivariate		
	OR	95 % CI	p	OR	95 % CI	p
Male	1.19	0.92 - 1.56	0.19			
Age, years						
40-54						
55-64	3.68	2.44 - 5.69	< 0.0001	2.51	1.60 - 4.03	< 0.0001
65-75	6.58	4.45 - 10.05	< 0.0001	3.40	2.16 - 5.50	< 0.0001
BMI, kg.m <sup>-2</sup>						
< 25						
≥ 25	6.72	4.42 - 10.77	< 0.0001	5.56	3.46 - 9.50	< 0.0001
COPD	1.12	0.63 - 1.86	0.66			
Organic dust exposure	0.54	0.41 - 0.71	< 0.0001	0.51	0.37 - 0.70	< 0.0001
Smoking status						
Never						
Former	1.94	1.44 - 2.60	< 0.0001	1.24	0.88 - 1.72	0.22
Current	1.42	0.99 - 2.02	0.053	1.84	1.20 - 2.77	< 0.01
Smoking intensity						
< 1 pack-year						
1-15 pack-years	1.09	0.74 - 1.59	0.64			
> 15 pack-years	2.14	1.59 - 2.87	< 0.0001			
zscore FEV1 < 0	2.21	1.68 - 2.94	< 0.0001			
zscore FEV1 < -1.64	2.34	1.58 - 3.38	< 0.0001			
Dyslipidemia	4.82	3.59 - 6.42	< 0.0001	2.00	1.29 - 3.04	< 0.01
Hypertension	6.52	4.99 - 8.54	< 0.0001	2.48	1.77 - 3.44	< 0.0001
Ischemic heart disease	5.03	3.25 - 7.58	< 0.0001	3.60	2.67 - 4.86	< 0.0001
Peripheral vascular disease	4.32	1.44 - 10.64	< 0.01	3.12	1.88 - 5.04	< 0.0001
OSAS	4.69	0.24 - 31.83	0.17			

*Abbreviations:* BMI: body mass index; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in 1 second; OR: odds ratio; OSAS: obstructive sleep apnea syndrome.

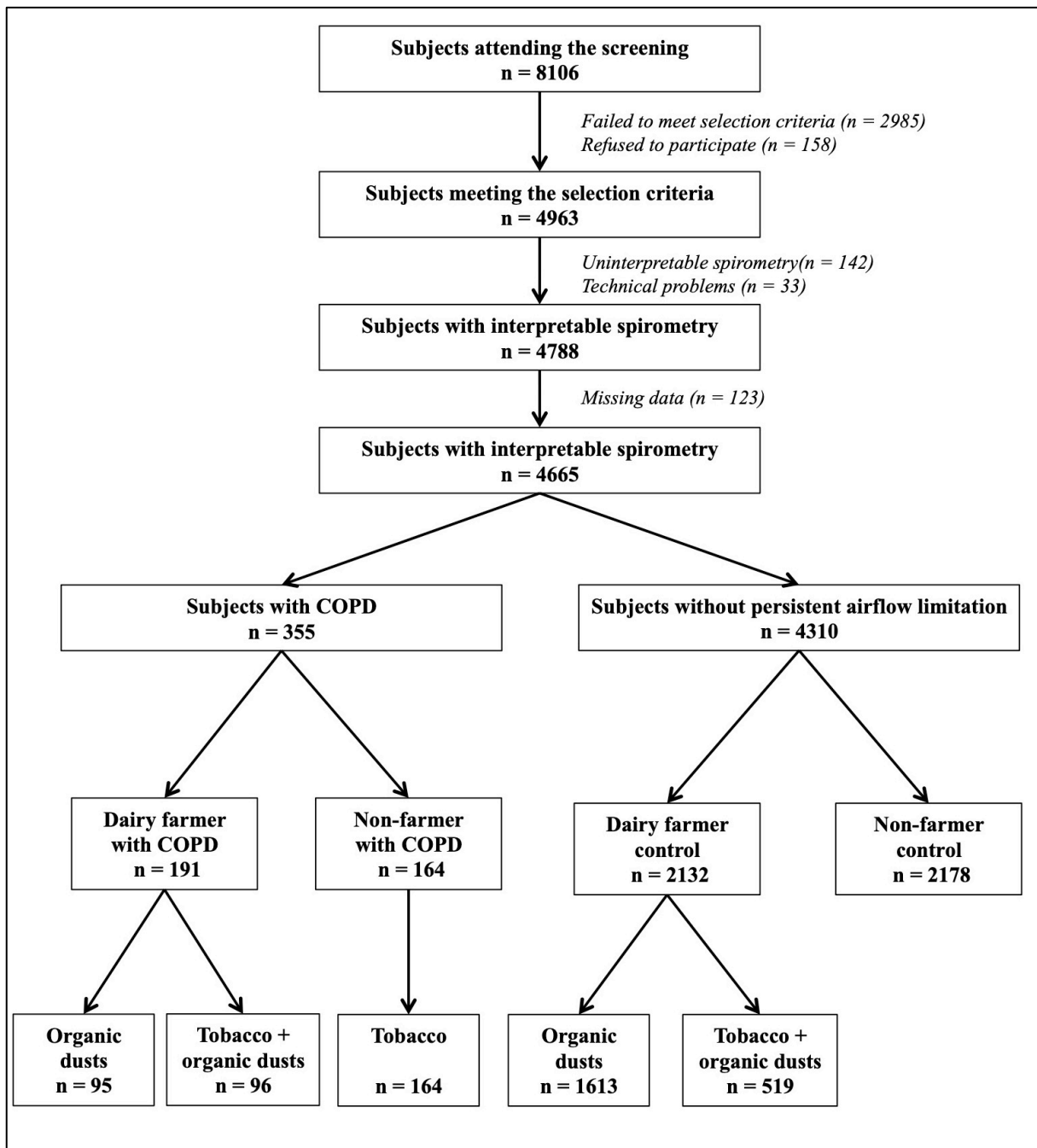
**Table 5.** Univariate and multivariate logistic regression analysis for hypertension

Variable	Univariate				Multivariate			
	OR	95 % CI	p	OR	95 % CI	p		
<b>Male</b>	0.79	0.68	0.92	0.65	0.55	0.77	< 0.0001	
<b>Age, years</b>								
40-54	1			1				
55-64	3.69	2.96	4.61	3.04	2.42	3.83	< 0.0001	
65-75	7.56	6.11	9.42	5.49	4.38	6.93	< 0.0001	
<b>BMI, kg.m<sup>-2</sup></b>								
< 25	1			1				
≥ 25	3.16	2.65	3.79	2.36	1.95	2.87	< 0.0001	
<b>COPD</b>	0.76	0.53	1.06				0.12	
<b>Organic dust exposure</b>	0.99	0.86	1.15				0.94	
<b>Smoking status</b>								
Never	1							
Former	1.24	1.05	1.47				< 0.05	
Current	0.85	0.69	1.05				0.14	
<b>Smoking intensity</b>								
< 1 pack-year	1			1				
1-15 pack-years	0.87	0.71	1.07	1.13	0.90	1.42	0.28	
> 15 pack-years	1.23	1.03	1.47	1.39	1.13	1.71	< 0.01	
<b>zscore FEV1 &lt; 0</b>	1.42	1.22	1.64	1.29	1.09	1.53	< 0.001	
<b>zscore FEV1 &lt; -1.64</b>	1.27	0.97	1.65				0.08	
<b>Dyslipidemia</b>	4.12	3.38	5.02	2.40	1.92	2.99	< 0.0001	
<b>Diabetes mellitus</b>	6.52	4.99	8.54	3.34	2.47	4.52	< 0.0001	
<b>Ischemic heart disease</b>	1.84	1.28	2.61				< 0.001	
<b>Peripheral vascular disease</b>	3.02	1.36	6.47				< 0.01	
<b>OSAS</b>	2.91	0.38	17.58				0.24	

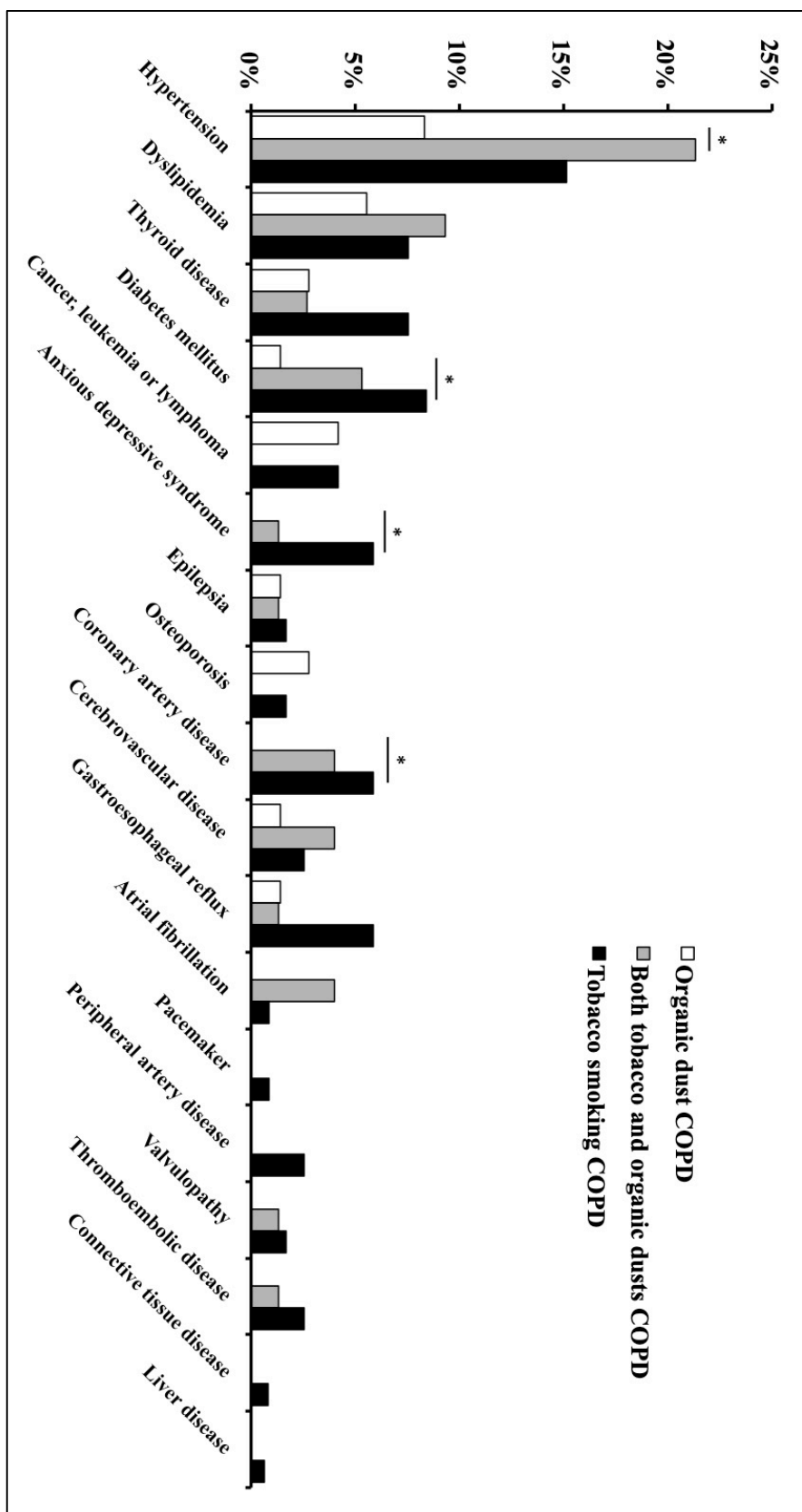
*Abbreviations:* BMI: body mass index; CI: confidence interval; COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in 1 second; OR: odds ratio; OSAS: obstructive sleep apnea syndrome.

**Figure 1. Flow chart of participants included in the study.** Subjects were recruited through a screening program set up by two national health insurance organizations. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma and hypersensitivity pneumonitis.

Abbreviations: COPD = chronic obstructive lung diseases.



**Figure 2. Prevalence of comorbidities in patients with COPD separated into 3 subgroups according to exposure to tobacco smoking, to organic dusts or both. \*  $p < 0.05$ .**





**Online table 1.** Main characteristics and comorbidities in dairy farmers and non dairy farmers

	<b>Dairy farmers</b> <b>n = 2323</b>	<b>Non farmers</b> <b>n = 2342</b>	<b>p</b>
<b>Age, years</b>	57.9 ± 9.9	56.6 ± 9.0	<b>&lt; 0.0001</b>
<b>Males</b>	1423 (61)	1101 (47)	<b>&lt; 0.01</b>
<b>BMI, kg.m<sup>-2</sup></b>	26.8 ± 4.5	26.3 ± 5.0	<b>&lt; 0.0001</b>
<b>Smoking status</b>			<b>&lt; 0.0001</b>
Never	1708 (74)	1026 (44)	
Former	394 (17)	705 (30)	
Current	221 (10)	611 (26)	
<b>Smoking intensity</b>			<b>&lt; 0.0001</b>
< 1 pack-year	1708 (74)	1026 (44)	
1-15 pack-years	309 (13)	541 (23)	
> 15 pack-years	306 (13)	775 (33)	
<b>Charlson comorbidity index*</b>	0.27 ± 0.56	0.34 ± 0.68	<b>&lt; 0.001</b>
<b>Hypertension</b>	414 (18)	457 (20)	NS
<b>Diabetes mellitus</b>	80 (3)	157 (7)	<b>&lt; 0.0001</b>
<b>Dyslipidemia</b>	227 (10)	252 (11)	NS
<b>Coronary artery disease</b>	52 (2)	102 (4)	<b>&lt; 0.001</b>
<b>Chronic heart failure</b>	4 (0.2)	4 (0.2)	NS
<b>Valvulopathy</b>	16 (1)	29 (1)	NS
<b>Atrial fibrillation</b>	47 (2)	41 (2)	NS
<b>Pacemaker</b>	5 (0.2)	4 (0.2)	NS
<b>Cerebrovascular disease</b>	29 (1)	32 (1)	NS
<b>Peripheral artery disease</b>	5 (0.2)	22 (1)	<b>&lt; 0.01</b>
<b>Thromboembolic disease</b>	23 (1)	39 (2)	NS
<b>Gastroesophageal reflux</b>	77 (3)	137 (6)	<b>&lt; 0.001</b>
<b>Thyroid disorder</b>	86 (4)	192 (8)	<b>&lt; 0.0001</b>
<b>Osteoporosis</b>	17 (1)	26 (1)	NS
<b>Chronic kidney disease</b>	4 (0.2)	3 (0.1)	NS
<b>Liver disease</b>	3 (0.1)	5 (0.2)	NS
<b>Cancer, leukemia or lymphoma</b>	52 (2)	80 (3)	<b>&lt; 0.05</b>
<b>Connective tissue disease</b>	41 (2)	29 (1)	NS
<b>Anxious depressive syndrome</b>	56 (2)	142 (6)	<b>&lt; 0.0001</b>
<b>Neurodegenerative disorder</b>	5 (0.2)	12 (1)	NS
<b>Epilepsia</b>	10 (0.4)	9 (0.4)	NS
<b>OSAS</b>	3 (0.1)	2 (0.1)	NS

\*Non-age-adjusted; BMI = body mass index; OSAS = obstructive sleep apnea syndrome.

## 2. Article 10 : Cardiovascular risk in COPD: deciphering the contribution of tobacco smoking

### *a. Contexte et objectif*

Les pathologies cardiovasculaires sont fréquentes chez les patients présentant une BPCO tabagique et contribuent de manière importante à la mortalité notamment dans les BPCO légère à modérée. L'athérosclérose est un des éléments clé dans la genèse de ces pathologies cardiovasculaires.

Chez des sujets indemnes de pathologies cardiovasculaires, la rigidité artérielle évaluée par la mesure de l'onde de pouls est un facteur prédictif indépendant d'évènements et de mortalité cardiovasculaires. Il a par ailleurs été mis en évidence que la rigidité artérielle était plus importante chez des BPCO tabagiques que chez des sujets témoins appariés sur le tabagisme. Néanmoins, cette approche ne permet pas d'évaluer la contribution respective du tabagisme et de la BPCO dans la genèse du risque cardiovasculaire. Par ailleurs, l'importance de ce risque cardiovasculaire n'a jamais été étudiée dans la BPCO du non-fumeur.

L'objectif de cette étude était d'évaluer la rigidité artérielle et d'évaluer ses déterminants dans un groupe de patients présentant une BPCO légère à modérée et de sujets témoins exposés aux poussières organiques, au tabac ou aux deux et indemne de pathologie cardiovasculaire.

### *b. Manuscrit*

Article “ Cardiovascular risk in COPD: deciphering the contribution of tobacco smoking”. <b>Soumagne T</b> , Roche N, Guillien A, Bouhaddi M, Rocchi S, Hue S, Claudé F, Bizard L, Andujar P, Dalphin JC, Degano B. <i>En cours de soumission</i> .
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**Title: Cardiovascular risk in COPD: deciphering the contribution of tobacco smoking**

**Running head: Cardiovascular risk in COPD and tobacco smoking**

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**Summary conflict of interest statements**

Pr. Roche reports grants and personal fees from Boehringer Ingelheim, Pfizer and Novartis, personal fees from Teva, GSK, AstraZeneca, Chiesi, Mundipharma, Cipla, Sano , Sandoz, 3M, Zambon, outside the submitted work. Dr. Dalphin reports grants, personal fees and non-financial support from Novartis Pharma, GSK, Chiesi, Intermune, AstraZeneca, Boehringer Ingelheim and non-financial support from Stallergenes, outside the submitted work.

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The other authors declare that they have no conflict of interest for the submitted work.

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## **ABBREVIATIONS LIST**

AIx = central augmentation index; aPWV = aortic pulse wave velocity; BRS = baroreflex sensitivity; COPD = chronic obstructive pulmonary disease; CVD = cardiovascular disease; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; GOLD = Global lung initiative for Obstructive Lung Disease; HRCT = high-resolution computed tomography; hsCRP = high-sensitivity C-reactive protein; IL = interleukin; LLN = lower limit of normal; OSA = obstructive sleep apnea; PaO<sub>2</sub> = arterial oxygen partial pressure; TLCO = lung transfer for carbon monoxide; TNFR1 = tumor necrosis factor receptor 1.

## ABSTRACT

**Background:** The observation that chronic obstructive pulmonary disease (COPD) is an independent risk factor for cardiovascular disease (CVD) comes from comparisons between smokers with COPD and smokers without COPD. The mechanisms that explain increased risk of CVD in patients with COPD are still unclear.

**Objectives:** To compare systemic arterial stiffness (a predictor of CVD mortality) and some inflammatory markers between three groups of patients with mild-to-moderate COPD secondary to either tobacco smoking, organic dust exposure, or both.

**Methods:** Systemic arterial stiffness was assessed by aortic pulse wave velocity (aPWV). Measurements were made in 142 COPD patients (consecutively diagnosed through a screening program) and 155 healthy controls matched for age, sex, body mass index and tobacco smoking, exposed to tobacco smoking ( $n=56/70$  for COPD/controls, respectively), organic dusts ( $n=44/48$ ), or both ( $n=42/37$ ).

**Main Results:** aPWV was higher in COPD than in healthy controls in subjects exposed to tobacco smoking and to both dusts and tobacco smoking. By contrast, among never-smokers exposed to organic dusts, COPD patients and matched controls had identical aPWV. Multivariate analysis of the 142 COPD patients (exposed to tobacco smoking and/or to organic dusts) demonstrated that tobacco smoking was independently associated with high aPWV. Moreover, soluble ST2, a marker of major cardiovascular events, was correlated with aPWV in these patients.

**Conclusions:** Analysis of an unselected group of COPD patients with different causes suggests that 1) COPD by itself is not sufficient to explain increased cardiovascular risk and 2) tobacco smoking is an independent risk factor for CVD in COPD.

**Keywords:** pulmonary disease, chronic obstructive; cardiovascular diseases; vascular stiffness.

Chronic obstructive pulmonary disease (COPD) is a common disease with high global morbidity and mortality.<sup>1</sup> Tobacco smoking is the most frequent cause of COPD in developed countries.<sup>1</sup> Nevertheless, up to 50% of COPD cases worldwide can involve other factors, including indoor and outdoor air pollutants as well as occupational hazards.<sup>2</sup> Among occupational causes, exposure to organic dusts has been associated with accelerated impairment in lung function and with increased risk of COPD.<sup>3-5</sup>

The prevalence of cardiovascular diseases (CVD) is high in patients with smoking-related COPD, and most of these patients die from CVD rather than from respiratory causes.<sup>6-8</sup> COPD and CVD share common risk factors such as tobacco smoking, advanced age and sedentary lifestyle.<sup>9,10</sup> Chronic systemic inflammation could play a role in the pathophysiology of CVD in smoking-related COPD,<sup>11</sup> but clear evidence for this relationship is lacking.<sup>12,13</sup> Emphysema has also been associated with CVD in smoking-related COPD, a supposed link between the two diseases being increased elastin degradation.<sup>14</sup> Mechanical interactions may also be involved, including deleterious effects of lung hyperinflation on left ventricular filling and diastolic function.<sup>15,16</sup> In non-smoking COPD, including disease secondary to organic dust exposure, the prevalence and the putative mechanisms of cardiovascular diseases remain to be studied.

Abnormalities of systemic vessels are centrally involved in the genesis of CVD, and measurements of systemic vascular function are therefore used to estimate the risk of CVD.<sup>17</sup> In individuals who are free of clinically apparent heart disease, arterial stiffness, measured non-invasively by aortic pulse wave velocity (aPWV), is an independent predictor of cardiovascular events and mortality that has been positively associated with several systemic inflammatory markers.<sup>18,19</sup> aPWV is higher in patients with COPD than in disease-free controls.<sup>17</sup> A large majority of studies in this area have been based on comparisons between patients with smoking-



related COPD and matched smokers without COPD.<sup>20</sup> It is therefore impossible to determine whether tobacco smoking remains an independent factor associated with abnormal systemic vascular function in this specific population of patients. In addition, despite the burden of COPD secondary to exposures other than tobacco smoking, systemic vascular function in patients with non-smoking COPD has not yet been extensively studied.<sup>21</sup>

We therefore sought to assess systemic arterial stiffness and to evaluate its determinants in a group of patients with mild-to-moderate COPD secondary to organic dust exposure, tobacco smoking or both.

## **METHODS**

### **Study Design and Subjects**

Patients and control subjects were consecutively recruited between September 2012 and November 2015 through a regional COPD screening program involving general practitioners and occupational physicians and set up by two national health insurance organizations (BALISTIC study; ClinicalTrials.gov Identifier: NCT02540408), as described elsewhere.<sup>22</sup> All patients had a medical history compatible with COPD, a history of risk factors (tobacco smoking and/or daily exposure to organic dusts) and evidence of persistent mild-to-moderate airflow limitation (defined either as a post-bronchodilator forced expiratory volume in 1 second ( $FEV_1$ ) on forced vital capacity (FVC) ratio  $< 0.70$  or as  $FEV_1/FVC < 5^{\text{th}}$  centile lower limit of normal)<sup>23</sup> together with  $FEV_1 > 50\%$  of predicted value according to the GLI-2012 equations.<sup>1,24</sup> Patients were in stable condition (no exacerbation of COPD during the previous 6 weeks) and were not taking any oral or inhaled anti-inflammatory drugs, including corticosteroids. COPD subjects were asked to interrupt short and long-acting bronchodilators 72 hours prior to the visit, if required. Exclusion

criteria were the following: long-term oxygen therapy; a history of asthma, pulmonary fibrosis, tuberculosis, bronchiectasis, lung cancer, or lung resection; conditions known to affect vascular function, including obstructive sleep apnea, cardiovascular, cerebrovascular, and peripheral vascular disease; uncontrolled hypertension; diabetes; inflammatory conditions such as rheumatoid arthritis or psoriasis.

Healthy subjects participating in the screening program (subjects without airflow limitation or respiratory symptoms) with otherwise identical selection criteria as the COPD patients were also included in the current analysis. These subjects were frequency matched with the COPD patients in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and sex.

Ethical approval was received from the local Ethics Committee (CPP Est; P-2011-119) and written consent was obtained from all subjects.

## **Procedures**

*Lung function.* Routine spirometry, constant-volume body plethysmography and single breath lung transfer for carbon monoxide (TLCO) were performed in accordance with recommended techniques (Platinum Elite; MGC Diagnostics Corporation, Saint Paul, Minnesota, USA), as described elsewhere.<sup>25</sup>

*Vascular function.* Studies were performed in the morning, after 30 minutes of supine rest in a quiet, dimly lit, temperature-controlled room (22-25°C). Subjects had fasted overnight and abstained from coffee, tea, and alcohol for the 24 hours before the study. All medications were withheld on the morning of the study.

Aortic pulse wave velocity (aPWV) was estimated by the noninvasive measurement of carotid-

femoral pulse wave velocity using the SphygmoCor™ system (AtCorMedical, Sydney, Australia). Briefly, ECG-gated carotid and femoral artery waveforms were recorded and the distance between the two recording sites was divided by the wave transit time, as described in detail elsewhere.<sup>26,27</sup> A measurement was accepted when it was reproducible three times with minimal variation, and the retained aPWV measurement was the mean of the three measurements.

Baroreflex sensitivity (BRS), a critical physiological feedback mechanism that regulates short-term (beat-to-beat) arterial blood pressure and that demonstrates a negative relationship with aPWV, was assessed as described elsewhere.<sup>28</sup> Briefly, beat-to-beat finger blood pressure and heart rate were measured and recorded over 5 min in the supine position (Finometer-Midi; Finapres Medical Systems, Amsterdam, the Netherlands). Sequences of three or more beats in which progressive increases/decreases in systolic blood pressure are followed by progressive lengthening/shortening in R-R interval were considered as spontaneous baroreflex sequences. A linear regression was calculated for each chosen recording epoch; this slope is considered to depict the cardiac BRS.

*Laboratory analysis.* For each patient, blood gases were measured from samples drawn from the arterialized earlobe, and measurements were corrected in order to estimate arterial oxygen partial pressure (PaO<sub>2</sub>).<sup>29</sup> In addition, a venous blood sample was collected in the fasting state, and serum samples were stored at -80°C for further analyses. Measurement of several inflammatory markers was carried out using a bead-based cytometric immunoassay (R&D Systems Europe, Abingdon, UK). Serum high-sensitivity C-reactive protein (hsCRP) concentrations were measured using a highly sensitive immunonephelometric assay.

## **Statistical Analysis**

Data are presented as number (percent) and mean  $\pm$  standard deviation for qualitative and quantitative variables, respectively. Quantitative variables were compared with the Student t test or the Wilcoxon test, as appropriate. Qualitative variables were compared with the Chi-square ( $\chi^2$ ) test. Bonferroni's correction for multiple comparisons was used as appropriate.

Comparisons were made between six groups defined by their exposures: non-smoking dairy farmers, smokers with no occupational exposure and smoking dairy farmers (with smokers defined as those having smoked on average more than one cigarette, one cigar, or one pipe a day for a year), and by the presence or absence of COPD. To identify factors associated with aPWV among COPD patients, bivariate linear regressions for all candidate factors were performed. The same analyses were repeated twice, once with persistent airflow limitation defined according to the Global lung initiative for Obstructive Lung Disease (GOLD; FEV<sub>1</sub>/FVC <0.70) and once with persistent airflow limitation defined according to the Global Lung function Initiative (GLI; FEV<sub>1</sub>/FVC <LLN).

A *P* value < 0.05 was considered statistically significant. Analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

## **RESULTS**

### **Subject Characteristics**

The screening program identified 186 patients with COPD according to the GOLD criterion. Thirty-five patients (19%) were ineligible for the current analysis because of the presence of at

least one exclusion criterion (Fig 1). Of the 151 eligible patients, 9 were excluded because of unsuccessful aPWV measurements. The characteristics of these 9 patients were similar to those of the 142 remaining patients (data not shown).

The main characteristics of the 3 subgroups of COPD patients and their healthy counterparts separated according to exposure (tobacco smoking, organic dusts and both exposures) are given in Table 1. Demographic characteristics of all subgroups were similar. Smoking habits were not significantly different between the subgroups exposed to tobacco smoking. All subgroups exposed to organic dusts also had a similar exposure type and duration (Table 1 and e-Fig 1). The 3 subgroups of COPD patients had similar airflow limitation and similar arterial blood gases. Conversely, the subgroup of patients with COPD due only to exposure to tobacco smoking had a significantly lower TLCO than the other two subgroups. Very few COPD patients received long-acting bronchodilators, and the proportion of patients receiving this medication was similar in the 3 subgroups (e-Table 1). About one third had at least one self-reported cardiovascular comorbidity, that is, either high blood pressure, diabetes mellitus and/or dyslipidaemia (e-Table 1).

### **Vascular Function**

The group of 142 patients with COPD had a significantly higher aPWV than their healthy matched controls ( $8.7 \pm 2.7$  vs.  $8.0 \pm 1.9$  m/s, respectively,  $P < 0.01$ ). When analysing subgroups, aPWV was higher in COPD than in healthy controls only in the 2 sub-groups exposed to tobacco smoking, while COPD patients exposed only to organic dusts had similar aPWV to their matched controls (Table 2 and Fig 2). Central augmentation index (AIx) was similar in all subgroups (Table 2). Among COPD patients, BRS was significantly lower in the subgroup exposed only to tobacco smoking than in the other two subgroups (Table 2). Of note, COPD patients exposed

only to tobacco smoking also had lower diastolic blood pressure than the other two subgroups of COPD patients.

### **Factors Associated with aPWV in COPD**

The univariate analysis performed in the group of 142 COPD patients demonstrated that factors classically associated with poorer systemic vascular function (higher age, higher mean systemic artery pressure and higher body mass index) were significantly correlated with a higher aPWV (Table 3). In these 142 patients, there was also a significant association between aPWV and the magnitude of tobacco consumption (Table 3). In addition, the presence of at least one cardiovascular co-morbidity, greater gas exchange impairment (lower estimated PaO<sub>2</sub> and higher alveolar-arterial oxygen difference), lower baroreflex sensitivity and several markers of systemic inflammation and/or of vascular dysfunction were also associated with increased aPWV (Table 3). By contrast, in these patients with mild-to-moderate COPD, the magnitude of organic dust exposure, the severity of COPD (assessed by a lower FEV<sub>1</sub>) and the severity of emphysema (assessed by a lower TLCO) did not correlate with aPWV.

After adjustment for age, mean blood pressure and BMI, there was still a significant association between aPWV and the magnitude of tobacco consumption (Table 3). The presence of at least one cardiovascular co-morbidity, a higher alveolar-arterial oxygen difference and higher sST2, a biomarker that provides prognostic information on cardiovascular risk, were also significantly correlated with aPWV.

Similar results were found when persistent airflow limitation was defined by an FEV<sub>1</sub>/FVC ratio below the lower limit of normal (e-Table 2).<sup>23</sup>

## DISCUSSION

Analysis of a group of unselected, consecutively diagnosed patients with mild-to-moderate COPD confirmed that aPWV, a predictor of cardiovascular morbidity and mortality, is higher in COPD than in healthy matched subjects even at mild stages of the disease. We found, however, that aPWV was higher in COPD than in controls only in subjects with COPD attributed to tobacco smoking. Among never-smokers exposed to organic dusts, COPD patients and matched controls had identical aPWV. In addition, analysis of our group of patients with COPD of different causes (exposure to tobacco smoking and/or to organic dusts) demonstrated for the first time that tobacco smoking is an independent factor associated with aPWV in patients with an established diagnosis of COPD.

Increased arterial stiffness has been widely reported in patients with COPD, mostly in those with severe forms of the disease.<sup>17,20,27,30,31</sup> Although patients with mild COPD are currently considered at low risk for poor cardiovascular outcomes, systemic vascular dysfunction has been very recently reported in such patients.<sup>14,32</sup> Arterial stiffness is a sign of early cardiovascular damage.<sup>17</sup> By measuring carotid-femoral pulse wave velocity, a surrogate for aPWV that is considered the “gold standard” for assessment of arterial stiffness, our study corroborates in a large number of unselected consecutive patients the finding of impaired systemic vascular function in the less severe forms of COPD.

In patients with severe COPD, arterial stiffness is independently associated with the severity of emphysema<sup>30</sup> and of airflow limitation.<sup>31</sup> Systemic elastin degradation (elastin fragmentation and collagen replacement in the extracellular matrix) has been suggested as a common mechanism for arterial stiffness and pulmonary emphysema.<sup>33</sup> Even in mild COPD, a positive correlation

between arterial stiffness and emphysema burden, as quantified by high-resolution computed tomography (HRCT), has recently been reported in 12 patients with a history of tobacco smoking.<sup>14</sup> Low single breath lung transfer for carbon monoxide (TLCO) has been clearly linked to structural markers of early emphysema.<sup>34</sup> HRCT was not available for emphysema quantification, but we were unable to find any correlation between TLCO and aPWV in our large group of unselected COPD patients. This finding suggests that the hypotheses of 1) a causal pathophysiological relationship between emphysema burden and systemic arterial stiffness and 2) a common mechanism for emphysema and arterial stiffness might be reconsidered.

An exaggerated activation of the sympathetic nervous system may explain how COPD leads to the development of increased arterial stiffness.<sup>35</sup> In line with this hypothesis, we found that decreased baroreflex sensitivity, indicating sympathetic/parasympathetic imbalance in favour of the sympathetic system, was associated on univariate analysis with aPWV in our series of patients. Nevertheless, this association was no longer found after adjustment for age, systemic blood pressure and BMI, suggesting that one or more of these factors contributed significantly to BRS impairment.

A pathophysiological link between persistent low-grade systemic inflammation and cardiovascular diseases in COPD has been suggested, but clear evidence for this relationship is still lacking.<sup>12,13</sup> One study has shown a weak positive correlation between arterial stiffness and two inflammatory biomarkers, (interleukin (IL)-6 and soluble tumor necrosis factor receptor 1 (sTNFR1)), in COPD,<sup>31</sup> but this finding has not been confirmed by others.<sup>27</sup> We found an association between aPWV and sTNFR1 only on univariate analysis, and we were unable to find any association between aPWV and IL-6. We were also unable to find any correlation between aPWV and hsCRP, an inflammatory marker that was found to be elevated only in a small subset



of our patients. Our findings therefore go against the hypothesis of a low-grade systemic inflammation that may determine vascular dysfunction, at least in the mild stages of COPD.

In recent years, novel biomarkers of CVD have emerged including soluble ST2, a member of the interleukin-1 receptor family that is believed to reflect cardiovascular stress and to have a prognostic value.<sup>36</sup> In population-based studies, higher sST2 levels have been associated with increased risk of heart failure, death and major cardiovascular events.<sup>36</sup> Here, we report for the first time an association between aPWV and sST2 in COPD patients without any overt cardiovascular disease. Further studies are needed to determine whether sST2 has some value in routine clinical practice for the estimation of cardiovascular risk in COPD patients.

The attempt to dissect the intrinsic contribution of COPD to augmented risk of CVD is complicated by the presence of common risk factors for both, notably smoking. To answer that question, a large majority of studies have compared patients with smoking-related COPD with controls without COPD, matched for tobacco consumption.<sup>20</sup> With such designs, it is impossible to determine whether smoking is 1) solely a risk factor shared by COPD and cardiovascular disease, or 2) a factor that remains independently associated with increased cardiovascular risk in patients with COPD.<sup>10</sup> Using an innovative approach (analysing a group of patients with COPD of different causes, that is, exposure to tobacco smoking and/or to organic dusts), we provide for the first time evidence that tobacco smoking remains independently associated with a marker of cardiovascular risk in COPD. In addition, we found that the magnitude of tobacco smoking, rather than current smoking status, was the significant determinant of a higher aPWV.

The systemic consequences of COPD caused by exposures other than tobacco smoking have not been fully investigated. In particular, the cardiovascular consequences of COPD secondary to

organic dust exposure are unknown.<sup>2</sup> We provide the first evidence that organic dust exposure is not associated with increased risk of CVD in patients with mild-to-moderate COPD. Only one recent study has assessed cardiovascular variables in patients with COPD related to an exposure other than tobacco smoking: Golpe *et al.* compared a group of patients with COPD caused by biomass smoke exposure with a group of patients with tobacco-related COPD matched for sex, age, and pulmonary function.<sup>21</sup> These authors found similar carotid intima-media thickness, number of carotid plaques and endothelial function variables in both patient groups.<sup>21</sup> There could be several explanations for the discrepancies with our findings. First, Golpe *et al.* studied COPD patients with a mean post-bronchodilator FEV<sub>1</sub> that was ~ 30% lower than in the current study, and it is plausible that cardiovascular alterations depend at least in part on COPD severity (due to severity of lung hyperinflation, inflammation and hypoxemia).<sup>17</sup> Second, exposures to biomass smoke and to organic dust are not equivalent, as biomass smoke is composed of both gaseous pollutants (including carbon monoxide and nitrogen dioxide) and microparticles (including black carbon),<sup>2</sup> while the organic dusts to which dairy farmers are exposed include mostly bacterial and fungal components.<sup>37</sup>

### **Limitations of the Study**

There are several limitations in our study. First, we acknowledge that arterial stiffness is only one among other surrogate markers of cardiovascular morbidity in COPD, and we did not evaluate cardiovascular function with other measures such as flow-mediated vasodilation and/or carotid intima-media thickness. Nevertheless, among these markers, arterial stiffness has the strongest predictive value for cardiovascular events beyond that of classic cardiovascular risk factors and is

the most suited for use in routine clinical practice.<sup>17,38</sup> Second, some other factors linked to the severity of airway obstruction that may contribute to arterial stiffness, including low level of physical activity, were not specifically assessed. Third, the cross-sectional nature of this study did not permit any assessment of causality.

## **Conclusions**

In addition to confirming that increased arterial stiffness may be found in patients with COPD even at mild-to-moderate severity stages, our study indicates for the first time that tobacco smoking is an independent factor associated with increased arterial stiffness in patients with an established diagnosis of COPD. As cardiovascular diseases are a major cause of death in COPD, our finding reinforces the need for patients with COPD to stop smoking. We also report that patients with mild-to-moderate COPD secondary to organic dust exposure are not at high risk for cardiovascular diseases. This suggests that COPD by itself is not sufficient to explain increased cardiovascular risk, and that causal factors of the disease are also of importance. Finally, soluble ST2, a marker associated with increased risk of heart failure, death and major cardiovascular events, is correlated with arterial stiffness in COPD and could be of interest for prospective evaluations of cardiovascular risk in COPD patients.

### **Guarantor statement**

TS is the guarantor of the content of the manuscript, including the data and analysis.

### **Author Contributions:**

All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Authors made the following contributions:

- BD, FC and TS made substantial contributions to the conception and design of the work.
- AG, LB, SH, MB, BD and TS performed all analyses.
- JCD and BD obtained study funding.
- All authors made substantial contributions to the acquisition, analysis, or interpretation of data for the work.
- TS wrote the first draft of the manuscript. BD, TS, MB, SH, PA, NR and JCD revised the manuscript for important intellectual content.
- All authors approved the manuscript.

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**Table 1.** Main Characteristics of Patients with COPD and Control Subjects, Separated Into 3 Subgroups According to Exposure to Tobacco Smoking, to Organic Dusts, or Both

Demographics	Exposure to tobacco smoking			Exposure to both tobacco and organic dusts			Exposure to organic dusts		
	COPD (n = 56)	Controls (n = 70)	P	COPD (n = 42)	Controls (n = 37)	P	COPD (n = 44)	Controls (n = 48)	P
Age	60.7 ± 8.0	60.4 ± 6.7	0.83	58.2 ± 8.3	57.2 ± 8.9	0.58	59.6 ± 9.1	58.8 ± 8.7	0.67
Men, %	82	74	0.29	86	92	0.49	82	89	0.29
Body mass index, kg.m <sup>-2</sup>	25.7 ± 3.7	26.8 ± 3.7	0.09	25.8 ± 3.3	26.9 ± 4.2	0.19	25.9 ± 3.8	26.1 ± 3.0	0.74
<b>Exposure</b>									
Former/current smokers, %	43 <sup>a</sup> /57 <sup>a</sup>	51/49	0.34	55/45	70/30	0.16	-	-	
Tobacco, pack-years	34.1 ± 23.2 <sup>a</sup>	28.8 ± 15.9	0.15	24.4 ± 16.9	17.6 ± 11.5	0.04	-	-	
Exposure to organic dust, years	-	-		37.7 ± 5.9	36.4 ± 5.8	0.35	38.2 ± 6.3	37.7 ± 5.9	0.73
<b>Pulmonary function tests</b>									
Post-bronchodilator FVC, % predicted	103 ± 13	107 ± 12	0.14	105 ± 17	105 ± 14	0.91	107 ± 14	106 ± 10	0.70
Post-bronchodilator FEV <sub>1</sub> , % predicted	81 ± 14	110 ± 12	<0.001	85 ± 15	105 ± 11	<0.001	87 ± 14	107 ± 11	<0.001
Post-bronchodilator FEV <sub>1</sub> /FVC, %	61 ± 7	80 ± 4	<0.001	63 ± 6	79 ± 4	<0.001	63 ± 7	78 ± 4	<0.001
TLCO, z-score	-1.43 ± 1.33 <sup>a,b</sup>	0.03 ± 1.19	<0.001	0.09 ± 1.36	0.54 ± 1.08	0.12	-0.36 ± 1.20	0.61 ± 1.16	<0.001
PaCO <sub>2</sub> at rest, mmHg	37.5 ± 3.2	37.7 ± 3.2	0.68	37.9 ± 4.1	37.0 ± 3.1	0.32	36.8 ± 3.8	37.1 ± 2.9	0.68
PaO <sub>2</sub> at rest*, mmHg	79.1 ± 10.4	84.6 ± 10.3	0.003	83.6 ± 9.9	85.6 ± 10.2	0.38	80.0 ± 7.9	86.7 ± 9.6	<0.001
Alveolar-arterial difference, mmHg	23.8 ± 10.1	18.1 ± 10.1	0.002	19.0 ± 9.4	17.8 ± 11.6	0.62	23.7 ± 10.0	16.7 ± 9.9	0.001

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; FEV<sub>1</sub> = forced expiratory volume in 1 s; FVC = forced vital capacity; PaCO<sub>2</sub> = arterial carbon dioxide tension; PaO<sub>2</sub> = estimated arterial oxygen tension according to the correction proposed by Aguilaniu *et al.*<sup>29</sup>; TLCO = lung transfer for carbon monoxide. Data are presented as mean ± SD or as percentages.

<sup>a</sup>. *P* < 0.01 vs. organic dust COPD; <sup>b</sup>. *P* < 0.01 vs. COPD with both exposures (Bonferroni's correction).



**Table 2.** Vascular Function and Systemic Inflammation in Subjects with COPD and in Matched Controls

	Exposure to tobacco smoking		Exposure to both tobacco and organic dusts		Exposure to organic dusts				
	COPD (n = 56)	Controls (n = 70)	P	COPD (n = 42)	Controls (n = 37)	P	COPD (n = 44)	Controls (n = 48)	P
<b>Vascular function</b>									
Aortic pulse wave velocity, m.s <sup>-1</sup>	9.0 ± 2.8	8.0 ± 1.9	0.02	8.8 ± 3.0	7.7 ± 1.6	0.04	8.4 ± 2.1	8.3 ± 2.1	0.85
Central augmentation index, %	25.6 ± 9.9	25.2 ± 8.1	0.79	23.7 ± 9.9	20.0 ± 10.2	0.12	21.0 ± 12.0	19.6 ± 10.7	0.56
Systolic blood pressure, mmHg	125 ± 15	132 ± 18	0.02	126 ± 18	126 ± 15	0.96	131 ± 19	129 ± 15	0.55
Diastolic blood pressure, mmHg	70 ± 9 <sup>b</sup>	75 ± 9	0.006	75 ± 10	75 ± 9	0.93	74 ± 11	75 ± 10	0.68
Mean blood pressure, mmHg	89 ± 10	94 ± 12	0.02	93 ± 12	93 ± 11	0.96	94 ± 14	93 ± 11	0.96
Cardiac frequency, beats per min	69 ± 11	64 ± 8	0.003	68 ± 9	66 ± 11	0.42	65 ± 7	66 ± 7	0.43
Baroreflex sensitivity, ms.mmHg <sup>-1</sup>	7.3 ± 4.2 <sup>a</sup>	8.5 ± 3.7	0.09	9.4 ± 4.5	9.5 ± 4.3	0.97	9.8 ± 4.5	9.7 ± 4.5	0.85
<b>Systemic inflammatory markers</b>									
hsCRP, mg.L <sup>-1</sup>	3.27 ± 2.78	2.78 ± 2.68	0.32	2.15 ± 1.94	2.99 ± 3.50	0.21	3.12 ± 4.05	2.06 ± 1.89	0.12
sTNFR1, pg.mL <sup>-1</sup>	4391 ± 1278 <sup>b</sup>	4251 ± 912	0.51	3747 ± 708	4523 ± 2463	0.07	4244 ± 955 <sup>b</sup>	4243 ± 1165	1.00
Interleukin-8, pg.mL <sup>-1</sup>	15.1 ± 11.0 <sup>a</sup>	13.1 ± 5.7	0.22	12.1 ± 5.4	11.7 ± 5.3	0.72	10.8 ± 4.1	12.7 ± 5.7	0.08
sST2, ng.mL <sup>-1</sup>	13.1 ± 5.5	12.5 ± 5.0	0.55	13.9 ± 8.7	14.0 ± 4.9	0.98	12.9 ± 5.9	15.2 ± 6.1	0.09
sICAM-1, ng.mL <sup>-1</sup>	1031 ± 425	966 ± 481	0.43	922 ± 405	895 ± 304	0.74	845 ± 325	1004 ± 437	0.05
sVCAM-1, ng.mL <sup>-1</sup>	409 ± 108	381 ± 136	0.22	364 ± 106	398 ± 137	0.22	386 ± 135	420 ± 170	0.30

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; hsCRP = high-sensitivity C-reactive protein; sICAM-1 = intercellular adhesion molecule-1; sST2 = soluble ST2; sTNFR1 = soluble tumor necrosis factor receptor 1; sVCAM-1 = vascular cell adhesion molecule-1.

Data are presented as mean ± SD or as percentages.

<sup>a</sup>:  $P < 0.017$  vs. organic dust COPD; <sup>b</sup>:  $P < 0.017$  vs. COPD with both exposures (Bonferroni's correction).

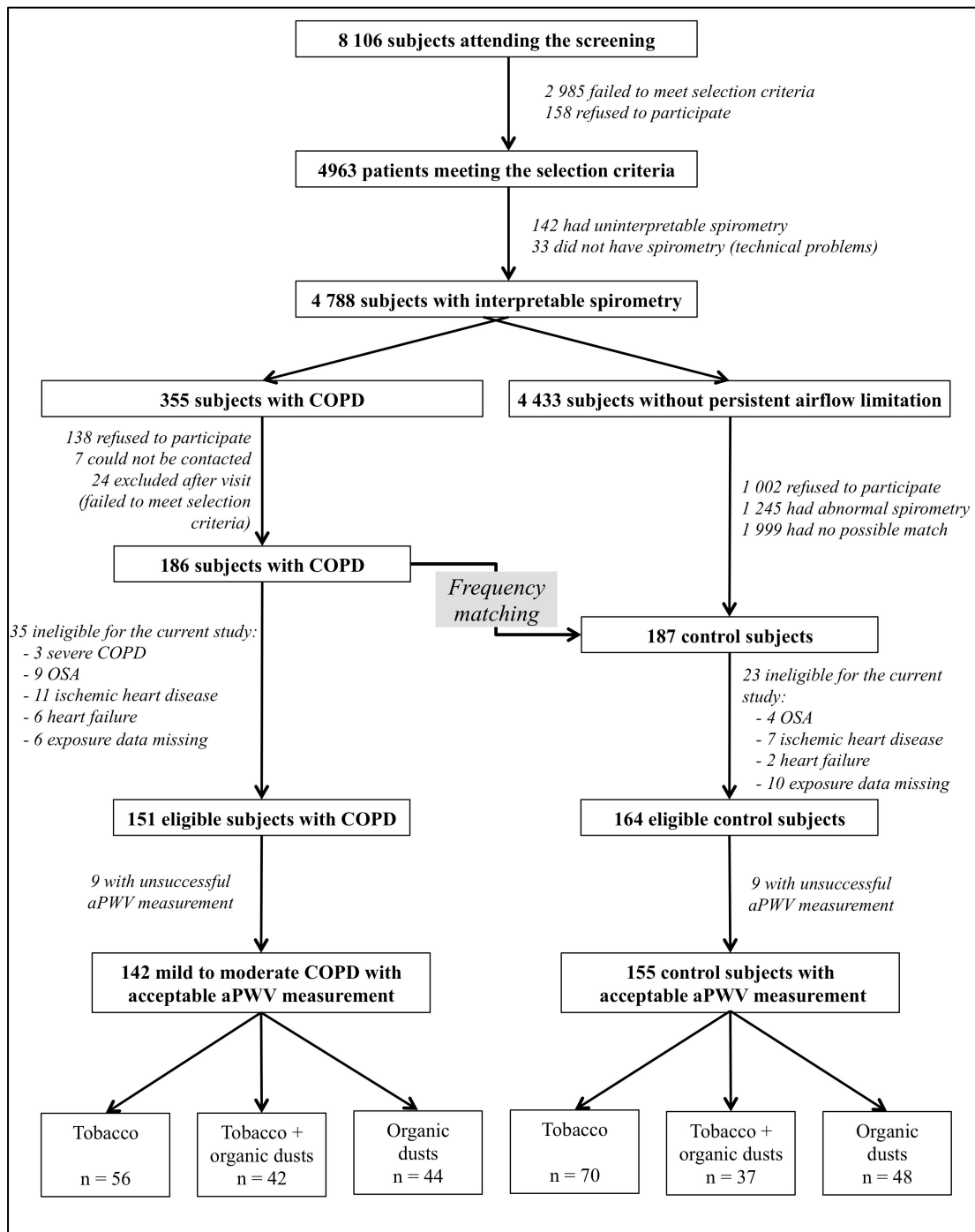
**Table 3.** Simple and Multivariate Linear Regression with Aortic Pulse Wave Velocity as the Dependent Variable in Patients with COPD ( $n = 142$ )

	Simple linear regression		Multiple linear regression <sup>#</sup>	
	$\beta$	p	$\beta$	p
Age, years	0.11	< 0.001	-	-
Mean systemic blood pressure, mmHg	0.06	<b>0.002</b>	-	-
Body mass index, kg.m <sup>-2</sup>	0.17	<b>0.005</b>	-	-
Tobacco smoking, pack-years	0.03	<b>0.001</b>	0.03	<b>0.001</b>
Smoking status (vs. never-smoker)				
Former smoker	1.00	0.07	0.84	0.10
Current smoker	0.04	0.95	0.64	0.20
Organic dust exposure (vs. no exposure)	-0.43	0.35	-0.47	0.26
Duration of exposure to organic dust, years	-0.005	0.66	-0.02	0.15
Post-bronchodilator FEV <sub>1</sub> , % predicted	0.01	0.44	0.003	0.86
Post-bronchodilator FEV <sub>1</sub> /FVC, %	0.005	0.88	0.001	0.97
TLCO, % predicted	0.002	0.82	0.002	0.85
At least one cardiovascular comorbidity, %	1.84	< 0.001	1.28	<b>0.002</b>
Baroreflex sensitivity, ms/mmHg	-0.14	<b>0.005</b>	-0.06	0.16
PaO <sub>2</sub> at rest, mmHg	-0.07	<b>0.004</b>	-0.04	0.09
Alveolar-arterial difference, mmHg	0.08	<b>0.0004</b>	0.05	<b>0.03</b>
Inflammatory markers				
hsCRP, mg.L <sup>-1</sup>	0.04	0.63	-0.06	0.34
sTNFR1, pg.mL <sup>-1</sup>	0.05	<b>0.07</b>	0.03	0.26
Interleukin-8, pg.mL <sup>-1</sup>	0.0005	<b>0.01</b>	0	0.25
sST2, ng.mL <sup>-1</sup>	0.097	<b>0.002</b>	0.06	<b>0.04</b>
sICAM-1, ng.mL <sup>-1</sup>	0	0.95	0	0.72
sVCAM-1, ng.mL <sup>-1</sup>	0.004	<b>0.02</b>	0	0.21

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; hsCRP = high-sensitivity C-reactive protein; FEV<sub>1</sub> = forced expiratory volume in 1 s; FVC = forced vital capacity; sICAM-1 = intercellular adhesion molecule-1; PaO<sub>2</sub> = estimated arterial oxygen tension according to the correction proposed by Aguilaniu *et al.*<sup>29</sup>; sST2 = soluble ST2; TLCO = lung transfer for carbon monoxide; sTNFR1 = soluble tumor necrosis factor receptor 1; sVCAM-1 = vascular cell adhesion molecule-1.

<sup>#</sup>: *after adjustment for age, mean blood pressure and body mass index (BMI)*

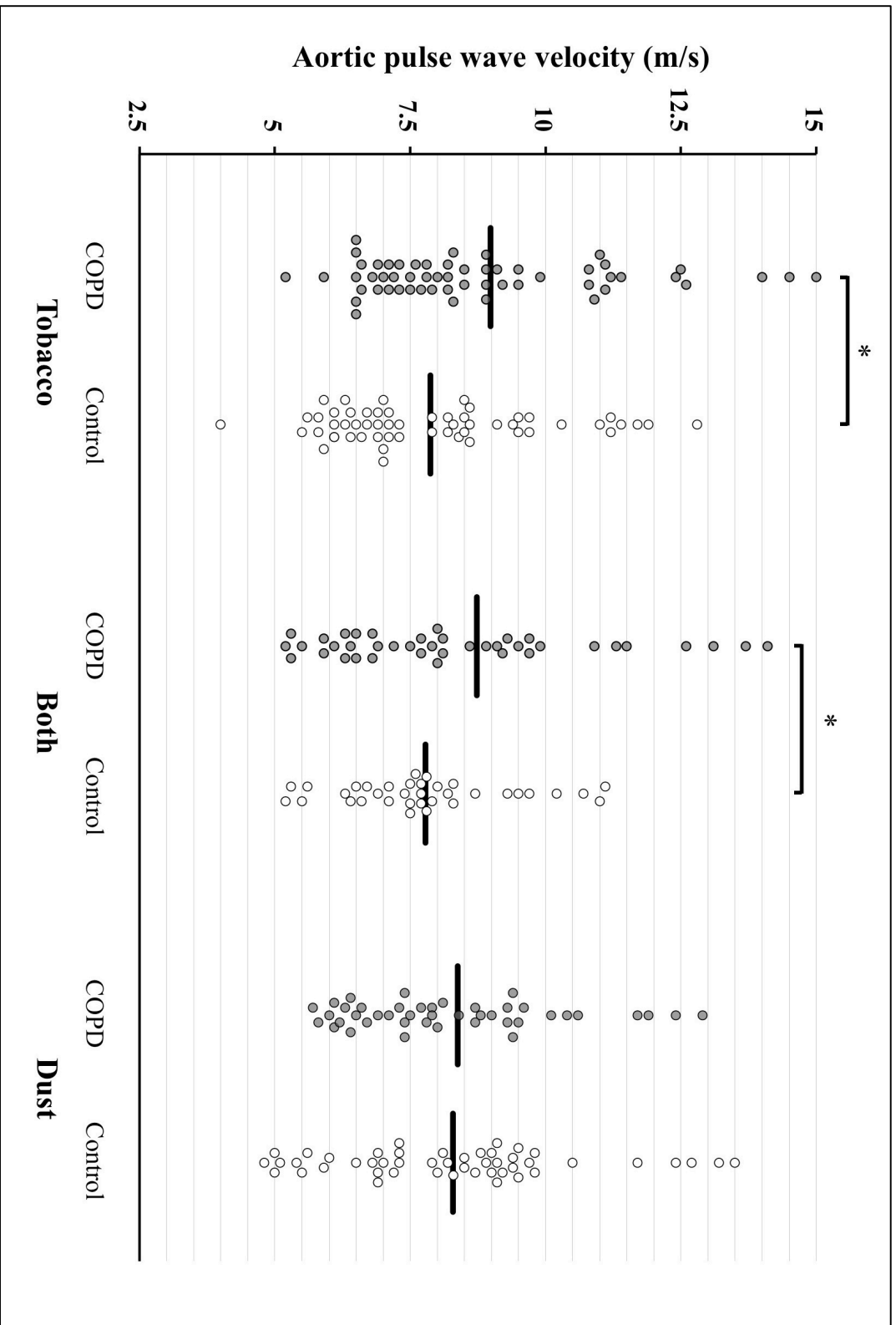
**Figure 1. Flow chart of participants included in the study.**



Subjects were recruited through a screening program set up by two national health insurance organizations. Inclusion criteria in the screening programs were: men or women aged 40 to 74 years, with no history of chronic respiratory disease including asthma and hypersensitivity pneumonitis.

Abbreviations: aPWV = aortic pulse wave velocity; COPD = chronic obstructive lung disease; OSA = obstructive sleep apnea.

Figure 2. Aortic pulse wave velocity in patients with COPD and controls.



\*  $P < 0.05$  versus controls.

## **E-appendix 1**

### **Methods**

#### *Microbial quantification by qPCR*

Exposure was quantified through the analysis of dust collected passively by sterilized electrostatic wipes placed for 10 weeks in the bedroom of the dwelling of each subject (dwelling airborne dust being considered to be representative of both indoor and outdoor occupational activities). Dust was analysed by real-time quantitative PCR (qPCR) as described elsewhere.<sup>1</sup>

### **Reference**

1. Barrera C, Rocchi S, Degano B, et al. Microbial exposure to dairy farmers' dwellings and COPD occurrence. *International journal of environmental health research* 2018:1-13.

**e-Table 1.** Cardiovascular Comorbidities and Use of Medication among Subjects with COPD

	Exposure to tobacco smoking <i>n</i> = 56	Exposure to both tobacco and organic dusts <i>n</i> = 42	Exposure to organic dust <i>n</i> = 44	<i>P</i>
<b>Cardiovascular comorbidities</b>				
High blood pressure, %	23	31	20	0.50
Diabetes, %	9	0	0	<b>0.01</b>
Dyslipidemia, %	29	19	27	0.53
None, %	61	60	70	0.50
<b>Other comorbidities</b>				
Osteoporosis, %	5	0	0	0.11
Gastroesophageal reflux, %	13	17	5	0.19
<b>Use of pulmonary medication*, %</b>				
Short-acting $\beta$ 2 agonists, %	5	12	7	0.45
Long-acting $\beta$ 2 agonists (LABA), %	0	0	0	1.00
Combined LABA and inhaled corticosteroids, %	4	7	0	0.23
Short-acting anticholinergics, %	2	2	5	0.82
Long-acting anticholinergics, %	0	0	2	0.61
Theophylline, %	4	2	0	0.63
Inhaled corticosteroids, %	0	0	0	1.00
Oral corticoids, %	0	0	0	1.00
Antileukotrienes, %	0	0	0	1.00
Influenza vaccine, %	19 <sup>b</sup>	2	9	<b>0.02</b>
Pneumococcal vaccine, %	2	0	2	1.00
Long-term oxygen therapy, %	0	0	0	1.00
<b>Use of cardiovascular and other medication, %</b>				
Blood pressure lowering medication, %	63 <sup>a,b</sup>	38	30	<b>0.003</b>
ACE, %	23	18	26	
$\beta$ -blockers, %	11	14	9	0.80
ARB, %	5	12	0 <sup>b</sup>	<b>0.04</b>
Diuretics, %	13	7	9	0.73
Calcium antagonists, %	2	5	5	0.62
Antiaggregants, %	2	2	0	
Antiarrhythmics, %	11 <sup>a</sup>	2	0	<b>0.03</b>
Coumarins, %	2	2	0	0.75
Oral antidiabetics, %	2	5	0	0.38
Statins, %	4	0	0	
Anxiolytics, %	18	14	16	0.89
Antidepressants, %	5	5	0	0.33
Sleep medication, %	5	2	2	0.73
	4	0	2	0.78

\* Pulmonary medications were interrupted 72 h prior to the visit. <sup>a</sup>:  $P < 0.017$  vs. organic dust COPD; <sup>b</sup>:  $P < 0.017$  vs. with both exposures (Bonferroni's correction).

*Definition of abbreviations:* ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blockers; COPD = chronic obstructive pulmonary disease.

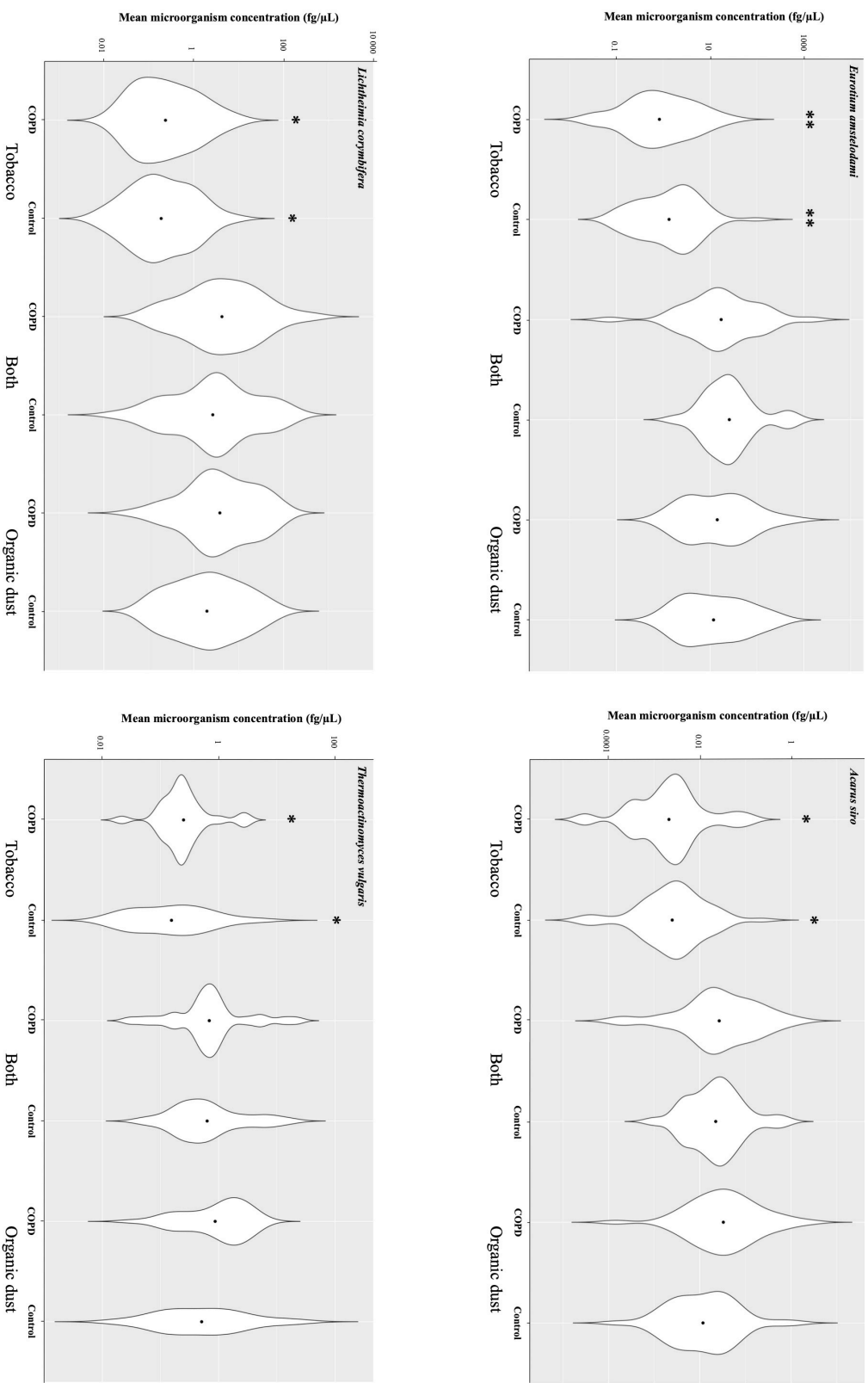
**e-Table 2.** Simple and Multivariate Linear Regression with Aortic Pulse Wave Velocity as the Dependent Variable in Subjects with LLN COPD ( $n = 90$ )

	Simple linear regression		Multiple linear regression <sup>#</sup>	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>
Age, years	0.11	< <b>0.001</b>	-	-
Mean systemic blood pressure, mmHg	0.05	<b>0.04</b>	-	-
Body mass index, kg.m <sup>-2</sup>	0.20	<b>0.01</b>	-	-
Tobacco smoking, pack-years	0.04	<b>0.001</b>	0.19	<b>0.008</b>
Smoking status (vs. never-smoker)				
Former smoker	1.51	<b>0.04</b>	0.87	0.20
Current smoker	0.001	1.00	0.61	0.64
Organic dust exposure (vs. no exposure)	-0.43	0.46	-0.31	0.56
Duration exposure to organic dust, years	-0.004	0.81	-0.01	0.42
Post-bronchodilator FEV <sub>1</sub> , % predicted	0.02	0.37	0.02	0.25
Post-bronchodilator FEV <sub>1</sub> /FVC, %	0.00	1.00	0.04	0.35
TLCO, % predicted	0.003	0.79	0.004	0.68
At least one cardiovascular comorbidity, %	1.87	<b>0.001</b>	1.27	<b>0.02</b>
Baroreflex sensitivity, ms/mmHg	-0.16	<b>0.02</b>	-0.05	0.46
PaO <sub>2</sub> at rest, mmHg	-0.10	<b>0.001</b>	-0.07	<b>0.01</b>
Alveolar-arterial difference, mmHg	0.10	< <b>0.001</b>	0.07	<b>0.01</b>
Inflammatory markers				
hsCRP, mg.L <sup>-1</sup>	0.009	0.92	-0.11	0.17
sTNFR1, pg.mL <sup>-1</sup>	0.09	<b>0.02</b>	0.06	0.06
Interleukin-8, pg.mL <sup>-1</sup>	0.0004	0.09	0	0.37
sST2, ng.mL <sup>-1</sup>	0	0.31	0	0.74
sICAM-1, ng.mL <sup>-1</sup>	0.0001	<b>0.04</b>	0	0.25
sVCAM-1, ng.mL <sup>-1</sup>	0.03	0.44	0.01	0.78

<sup>#</sup>: after adjustment for age, mean blood pressure and body mass index (BMI)

*Definition of abbreviations:* COPD = chronic obstructive pulmonary disease; hsCRP = high-sensitivity C-reactive protein; FVC = forced vital capacity; FEV<sub>1</sub> = forced expiratory volume in 1 s; sICAM-1 = intercellular adhesion molecule-1; LLN = lower limit of normal; PaO<sub>2</sub> = estimated arterial oxygen tension according to the correction proposed by Aguilaniu *et al.*; sTNFR1 = soluble tumor necrosis factor receptor 1; sST2 = soluble ST2; TLCO = lung transfer for carbon monoxide; sVCAM-1 = vascular cell adhesion molecule-1.

**e-figure 1.** Violin plots showing microorganism concentrations of four species, quantified by qPCR from Electrostatic Dust Collector (EDC) among the six subgroups. Microorganism concentrations are expressed in DNA equivalents (fg/ $\mu$ L). The point represents the mean value. \*  $p < 0.05$  and \*\*  $p < 0.01$  vs. each group of subjects with exposure to organic dusts (Bonferroni's correction)







### 3. Article 11 : Anxiety and depression among dairy farmers: the impact of COPD

#### *a. Contexte et objectif*

L'anxiété et la dépression sont deux comorbidités fréquentes de la BPCO. La présence de ces comorbidités chez les patients BPCO a une incidence sur la survie, la durée des hospitalisations et l'intensité des symptômes respiratoires. Par ailleurs, la littérature montre que la prévalence de l'anxiété et celle de la dépression sont plus importantes chez les agriculteurs que dans le reste de la population. Toutefois, la littérature ne traite pas de la prévalence de l'anxiété et de la dépression chez des sujets étant à la fois agriculteurs et atteints BPCO.

L'article qui suit a donc utilisé les données de l'étude BalistiC pour évaluer l'anxiété et la dépression chez des sujets étant à la fois agriculteurs (producteurs laitiers) et porteurs de BPCO, ces sujets étant comparés à des agriculteurs (producteurs laitiers) indemnes de BPCO, des non agriculteurs porteurs de BPCO et des non agriculteurs indemnes de BPCO. L'anxiété et la dépression étaient évaluées à l'aide de l'échelle Hospital Anxiety and Depression scale. Pour cet article, les 4 groupes de l'étude BalistiC étaient analysés. Cependant un sujet « BA » n'avait pas rempli l'échelle HAD et a donc été exclu des analyses. Ainsi, cet article s'appuyait sur les données de 100 BA (BPCO producteur laitier), 85 BN (BPCO Non Agricole), 98 TA (Témoin producteur laitier) et 89 TN (Témoin Non Agricole).

#### *b. Manuscrit*

Article "Anxiety and depression among dairy farmers: the impact of COPD". Guillien A, Laurent L, **Soumagne T**, Puyraveau M, Laplante JJ, Andujar P, Annesi-Maesano I, Degano B, Dalphin JC. *International Journal of COPD* 2017.



# Anxiety and depression among dairy farmers: the impact of COPD

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**Background:** Chronic obstructive pulmonary disease (COPD) and farming are two conditions that have been associated with an increased risk of anxiety and depression. Dairy farming is an independent risk factor for COPD.

**Objective:** To test the hypotheses that the prevalence of anxiety and/or depression is higher in dairy farmers with COPD than in farmers without COPD, and higher in dairy farmers with COPD than in non-farmers with COPD.

**Methods:** Anxiety and depression were evaluated using the Hospital Anxiety and Depression Scale in 100 dairy farmers with COPD (DF-COPD), 98 dairy farmers without COPD (DF-controls), 85 non-farming patients with COPD (NF-COPD) and 89 non-farming subjects without COPD (NF-controls), all identified by screening in the Franche-Comté region of France. Anxiety and depression were considered present when the Hospital Anxiety and Depression Scale score was  $\geq 8$ . COPD was defined by a post-bronchodilator forced expiratory volume in 1 second/forced vital capacity ratio  $< 0.7$ .

**Results:** The crude prevalence of anxiety did not differ between the four groups, ranging from 36% in NF-controls to 47% in NF-COPD ( $p=0.15$  between groups). Similarly, the prevalence of depression did not differ significantly between the four groups ( $p=0.16$  between groups). In dairy farmers ( $n=198$ ), the only factors associated with anxiety were quality of life and current smoking. Depression in dairy farmers was associated with airflow limitation (lower forced expiratory volume in 1 second and COPD grade 2 or more) as well as with some COPD-related features (dyspnea severity, current smoking, and poorer quality of life). In non-farmers, both anxiety and depression were associated with airflow limitation and COPD-related features.

**Conclusion:** In our population, the prevalence of anxiety and/or depression was similar in dairy farmers with and without COPD and in non-farmers with COPD. Nevertheless, the degree of airway obstruction and some COPD-related features were associated with depression among dairy farmers, whereas these factors were not associated with anxiety.

**Keywords:** COPD, farmers, forced expiratory volume in 1 second, Hospital Anxiety and Depression Scale, St George's Respiratory Questionnaire

## Introduction

Chronic obstructive pulmonary disease (COPD) is currently the most common respiratory disease, affecting between 5% and 10% of subjects over 40 years of age.<sup>1</sup> This disease leads to a deterioration of the patient's quality of life<sup>2,3</sup> and involves considerable costs due to health care, professional absenteeism and morbidity–mortality.<sup>4</sup> COPD primarily affects the lungs, but also has consequences on other organs and functions.<sup>5</sup> Anxiety and depression are among the most frequent comorbidities identified in COPD patients.<sup>6–8</sup> The causal relationships between COPD and anxiety and/or depression are multifaceted.<sup>9,10</sup> Patients with COPD are

more likely to develop anxiety and/or depression than control subjects, while COPD patients with anxiety and/or depression have a higher risk of COPD-related mortality than COPD patients without anxiety and/or depression.<sup>10,11</sup>

Although the main causal risk factor for COPD in developed countries is tobacco smoking, COPD is also attributable to environmental and/or professional exposures.<sup>12,13</sup> COPD in dairy farmers is, in this regard, a typical example of COPD attributable to exposure to organic dusts at work.<sup>14</sup> A recent study showed that COPD prevalence among farmers is approximately twice as high as in subjects with no occupational exposure.<sup>15</sup> Many studies have shown that farming may be associated with an increased risk of anxiety and depression.<sup>16-19</sup> This could partly be explained by the fact that in some cases, farmers are daily subjected to multiple stress factors such as economic circumstances, weather conditions, labor problems and social conditions.<sup>16,19,20</sup> Nevertheless, the prevalence of anxiety and depression among farmers with COPD is currently unknown.

Given that COPD and dairy farming may both be risk factors for anxiety and/or depression, we aimed to test the hypothesis that a higher prevalence of anxiety and/or depression exists in farmers with COPD than in farmers without COPD, together with a higher prevalence of anxiety and/or depression in farmers with COPD than in non-farmers with COPD. We also aimed to study the relationship between anxiety and/or depression and selected COPD-related features in farmers and in non-farmers. To achieve this aim, we assessed anxiety and depression by the self-administered Hospital Anxiety and Depression Scale (HADS) in four groups of subjects: dairy farmers with and without COPD, as well as non-farmers with and without COPD, all identified by screening.

## Subjects and methods

### Screening programs

Data for this study were collected as part of the BALISTIC project (COPD in dairy farmers: screening, characterization and constitution of a cohort; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02540408) Identifier: NCT02540408) which was conducted from 2011 to 2015 at the University Hospital of Besançon in collaboration with the French national social security system for agricultural workers (Mutualité Sociale Agricole [MSA]) and the federation of community health practices of Franche-Comté (Fédération des Maisons de Santé Comtoises). This study was set up primarily to assess the prevalence and some specific characteristics of COPD in dairy farmers (COPD secondary to organic dust exposure) in comparison with COPD in patients without any occupational exposure and with matched controls without COPD.<sup>21</sup>

COPD patients and matched controls were recruited through two parallel screening programs (screening phase of the BALISTIC study). Inclusion criteria in the screening programs were: men or women aged between 40 and 74 years, with no history of chronic respiratory disease including asthma and COPD or related conditions (chronic bronchitis, emphysema), and either being a dairy farmer (dairy farmer subgroups) or not being exposed to any occupational hazard associated with COPD (non-farmer subgroups). One of these two programs was conducted during free health checkups organized by the MSA for their affiliated members; COPD screening was proposed to all invited subjects who attended the health checkup and who fulfilled the inclusion criteria. In parallel, general practitioners of the Fédération des Maisons de Santé Comtoises conducted a second program whereby they invited all their patients who met the inclusion criteria to take part in the COPD screening program.

For screening, spirometry was performed as previously described.<sup>15</sup> Spirometry outcomes included forced expiratory volume in 1 second ( $FEV_1$ ) and forced vital capacity (FVC). A bronchodilation test was performed when the  $FEV_1$ /FVC ratio was  $<0.70$ .<sup>22</sup> Predicted values were based on the European Coal and Steel Community equations.<sup>23</sup>

### Study groups

A diagnosis of COPD was retained when the  $FEV_1$ /FVC ratio post-bronchodilator was  $<0.70$ . COPD patients were rated as either grade 1 ( $FEV_1 >80\%$  of the predicted value) or grade 2 or more ( $FEV_1 \leq 80\%$  of the predicted value). Spirometry was considered normal when the  $FEV_1$ /FVC ratio was  $>0.70$  and  $FEV_1$  was  $>80\%$  of the predicted value before bronchodilator administration.

During the study period, all subjects with COPD detected during the screening phase of the BALISTIC study were invited to participate in the characterization phase of the study. Those who agreed to participate in the characterization phase were included in two subgroups, namely, dairy farmers with COPD (DF-COPD) and non-farmers with COPD (NF-COPD). An equivalent number of subjects with normal spirometry (as defined above) who had participated in the screening phase of the study were frequency matched with the COPD subjects in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and gender. These subjects with normal spirometry made up two additional subgroups, namely, dairy farmers without COPD (DF-controls) and non-dairy farmers without COPD (NF-controls).

Subjects from these four subgroups underwent a characterization visit at the University Hospital of Besançon, during

which examinations were performed and questionnaires were administered, as described in detail elsewhere.<sup>21</sup> The distribution of the study population is detailed in Figure 1.

Ethical approval was received from the local Ethics Committee (Comité de Protection des Personnes Est; authorization 11/617). All participants were informed of the study procedures, and written consent was obtained from all included subjects.

## Questionnaires

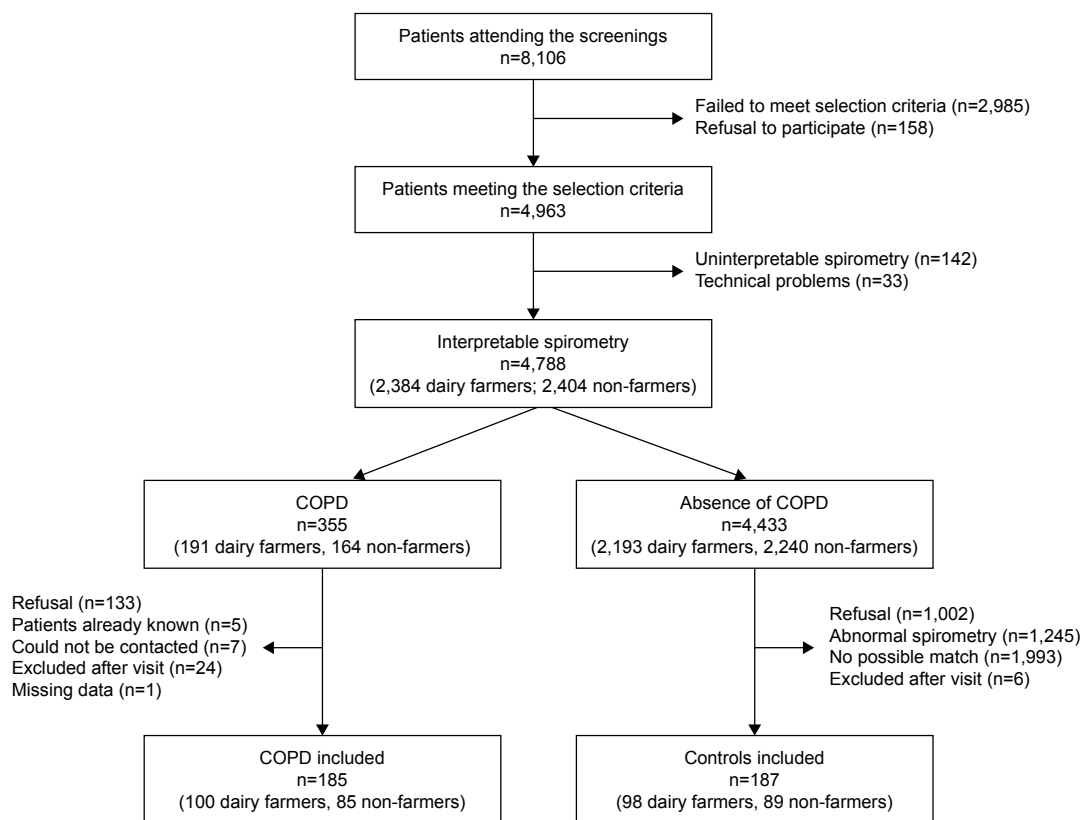
During the characterization visit, several standardized questionnaires were administered to the four participating subgroups.

A medical questionnaire (completed by the physician) collected data on anthropometric and demographic characteristics, symptoms and smoking history. Each patient was asked about their history of exacerbation, sputum and allergies. Dyspnea was assessed using two scales, namely, the Baseline Dyspnea Index (BDI) and the modified Medical Research Council scale (mMRC). A 6-minute walk test was performed to calculate the BMI, airflow Obstruction, Dyspnea and Exercise capacity (BODE) index. This score

combines BMI, FEV<sub>1</sub>, mMRC and 6-minute walk test.<sup>24</sup> Smoking status was recorded and the number of pack-years was calculated. Subjects who smoked on average less than one cigarette (or cigar or pipe) a day for a year were categorized as never-smokers. If the amount smoked was higher than this value, patients were considered as current smokers. Subjects who had stopped smoking for at least 1 month were considered as former smokers.

Patients completed the self-administered HADS to assess anxiety and depression. The HADS is composed of 14 questions, 7 for the anxiety subscale and 7 for the depression subscale. Each question has four responses rated on a scale of 0–3, with a maximum score of 21 for each subscale (indicating a high level of distress). For each subscale, a score of 0–7 corresponds to an absence of symptoms of anxiety and/or depression, a score between 8 and 11 corresponds to borderline symptoms of anxiety and/or depression and a score >11 corresponds to the certain presence of symptoms of anxiety and/or depression.<sup>17,25,26</sup>

Quality of life was assessed by the St George's Respiratory Questionnaire (SGRQ). The SGRQ is a questionnaire of 50 items designed to measure the quality of life in patients



**Figure 1** Flowchart of the population.

**Abbreviation:** COPD, chronic obstructive pulmonary disease.

with obstructive lung disease. A high score indicates a significant impairment of quality of life.<sup>27</sup> It is assumed that a difference of 4 points in the SGRQ is clinically relevant to differentiate groups of subjects.<sup>28</sup>

## Statistical analysis

Data are presented as number (percent) and mean  $\pm$  standard deviation for qualitative and quantitative variables, respectively.

Anxiety and depression variables were dichotomized with a cutoff at 8 for absence of anxiety or depression symptoms vs possible/certain presence of anxiety or depression symptoms.<sup>17,25,26</sup> Hereinafter, these two conditions are designated as absence and presence of anxiety and depression, for simplification.

Characteristics of COPD and control subjects, stratified by professional status (dairy farmers vs non-farmers), were compared using Student's *t*-test and chi-square test for quantitative and qualitative variables, respectively. Characteristics of DF-COPD and NF-COPD subjects were compared using the same methodology.

The prevalence of anxiety and depression was compared in the four groups using the chi-square test. Logistic regressions adjusted for post-bronchodilator FEV<sub>1</sub> (% predicted value) were performed to compare the prevalence of anxiety between dairy farmers and non-farmers. The same

methodology was used to compare the prevalence of depression between dairy farmers and non-farmers.

To identify factors associated with anxiety among dairy farmers and non-farmers, bivariate logistic regressions for all candidate factors were performed. Odds ratios (ORs) were presented using forest plots. The same methodology was used to identify factors associated with depression.

A *p*-value <0.05 was considered statistically significant. Analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

## Results

### Population characteristics

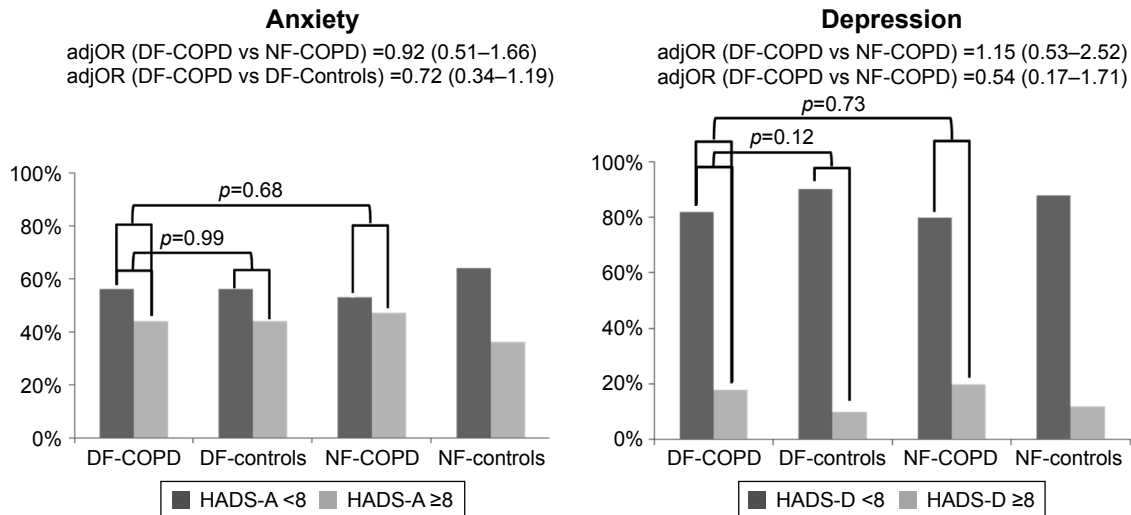
Among the 8,106 subjects who underwent screening, 4,963 met the inclusion criteria for the study (61.2%). In total, 355 patients with COPD (191 dairy farmers and 164 non-farmers) were identified (7.4% of all subjects with interpretable spirometry) and 4,433 had normal spirometry. A total of 210 COPD patients and 193 non-COPD matched controls agreed to participate in the characterization phase of the study. Thirty patients were excluded, mainly owing to a history of asthma or occupational exposure other than dairy farming (Figure 1). In addition, one DF-COPD patient was not included in this analysis because of missing HADS data. Finally, the DF-COPD, DF-controls, NF-COPD and NF-controls groups comprised 100, 98, 85 and 89 patients, respectively (Figure 1 and Table 1).

**Table 1** Population characteristics

Characteristics	DF-COPD (n=100)		DF-controls (n=98)		<i>p</i> -value <sup>a</sup>	NF-COPD (n=85)		NF-controls (n=89)		<i>p</i> -value <sup>b</sup>	<i>p</i> -value <sup>c</sup>
	Mean $\pm$ SD	%	Mean $\pm$ SD	%		Mean $\pm$ SD	%	Mean $\pm$ SD	%		
Age (years)	60.3 $\pm$ 9.1	–	59.3 $\pm$ 8.9	–	0.448	61.4 $\pm$ 7.6	–	60.2 $\pm$ 6.5	–	0.240	0.342
Men	–	86	–	88	0.715	–	78	–	73	0.481	0.139
Smoking status					0.289					0.313	<0.001
Non-smoker	–	47	–	55		–	8	–	14		
Former smoker	–	33	–	33		–	42	–	47		
Active smoker	–	20	–	12		–	50	–	39		
Number of pack-years	12.7 $\pm$ 16.8	–	8.1 $\pm$ 12.1	–	0.028	31.4 $\pm$ 23.1	–	24.4 $\pm$ 17.8	–	0.026	<0.001
FEV <sub>1</sub> (% predicted)	90 $\pm$ 16	–	112 $\pm$ 12	–	<0.001	84 $\pm$ 17	–	115 $\pm$ 13	–	<0.001	0.020
GOLD stage											0.029
I	–	75	–	–	–	–	60	–	–	–	–
$\geq$ 2	–	25	–	–	–	–	40	–	–	–	–
mMRC	0.40 $\pm$ 0.55	–	0.15 $\pm$ 0.36	–	<0.001	0.61 $\pm$ 0.77	–	0.25 $\pm$ 0.57	–	<0.001	0.041
BDI	10.9 $\pm$ 1.4	–	11.5 $\pm$ 1.0	–	<0.001	10.1 $\pm$ 2.1	–	11.3 $\pm$ 1.4	–	<0.001	0.006
Symptoms <sup>d</sup>	–	74	–	42	<0.001	–	79	–	42	<0.001	0.420
SGRQ	14.8 $\pm$ 12.6	–	7.5 $\pm$ 7.3	–	<0.001	19.2 $\pm$ 14.8	–	9.4 $\pm$ 9.1	–	<0.001	0.028
BODE	0.20 $\pm$ 0.55	–	0.04 $\pm$ 0.21	–	0.012	0.42 $\pm$ 0.81	–	0.11 $\pm$ 0.41	–	0.002	0.034

**Notes:** <sup>a</sup>Comparison between DF-COPD and DF-controls. <sup>b</sup>Comparison between NF-COPD and NF-controls. <sup>c</sup>Comparison between DF-COPD and NF-COPD. <sup>d</sup>Symptoms: presence of at least one symptom among dyspnea, chronic cough and chronic sputum. Dairy farmers with COPD (DF-COPD), dairy farmers without COPD (DF-controls), non-farmers with COPD (NF-COPD), and non-farmers without COPD (NF-controls).

**Abbreviations:** BDI, Baseline Dyspnea Index; BODE, Body mass index, airflow Obstruction, Dyspnea and Exercise capacity index; COPD, chronic obstructive pulmonary disease; DF, dairy farmer; FEV<sub>1</sub>, forced expiratory volume in 1 second; GOLD, Global Initiative for Chronic Obstructive Lung Disease; mMRC, modified Medical Research Council scale; NF, non-farmer; SD, standard deviation; SGRQ, St George's Respiratory Questionnaire.



**Figure 2** Prevalence of anxiety and depression among dairy farmers with COPD (DF-COPD), dairy farmers without COPD (DF-controls), non-farmers with COPD (NF-COPD) and non-farmers without COPD (NF-controls). ORs are adjusted (adjOR) on FEV<sub>1</sub> post-bronchodilator.

**Abbreviations:** COPD, chronic obstructive pulmonary disease; FEV<sub>1</sub>, forced expiratory volume in 1 second; HADS-A; Hospital Anxiety and Depression Scale – anxiety subscale; HADS-D; Hospital Anxiety and Depression Scale – depression subscale; OR, odds ratio.

The DF-COPD and NF-COPD subgroups were composed mainly of mild COPD with preserved FEV<sub>1</sub> (Table 1). The number of current smokers and former smokers was lower among farmers than among non-farmers (Table 1). The proportion of men was higher among farmers than among non-farmers. Dyspnea was more frequent and quality of life scores were lower among COPD patients than among control subjects without COPD (Table 1).

## Prevalence of anxiety and depression

The crude prevalence of anxiety (HADS-anxiety subscale score  $\geq 8$ , indicating possible/probable symptoms of anxiety) was similar in the four subgroups. Neither COPD nor dairy farming was associated with an increased frequency of anxiety (Figure 2).

Similarly, the prevalence of depression (HADS-depression subscale score  $\geq 8$ , indicating possible/probable depression) was similar in the four subgroups (Figure 2).

## Factors associated with anxiety and depression

Among farmers, anxiety was associated with current smoking (OR: 2.53 [1.12–5.70],  $p=0.03$ ) and with poorer quality of life as measured by the SGRQ score (OR: 1.05 [1.02–1.08],  $p=0.003$ ), which is shown in Figure 3. Among non-farmers, anxiety was associated with lower FEV<sub>1</sub> (OR: 0.98 [0.97–0.99],  $p=0.03$ ), more severe dyspnea (higher mMRC score [OR: 2.76 {1.62–4.69}]) and lower BDI [OR: 0.73 {0.61–0.89}],  $p<0.001$  for both), female gender (OR: 3.23

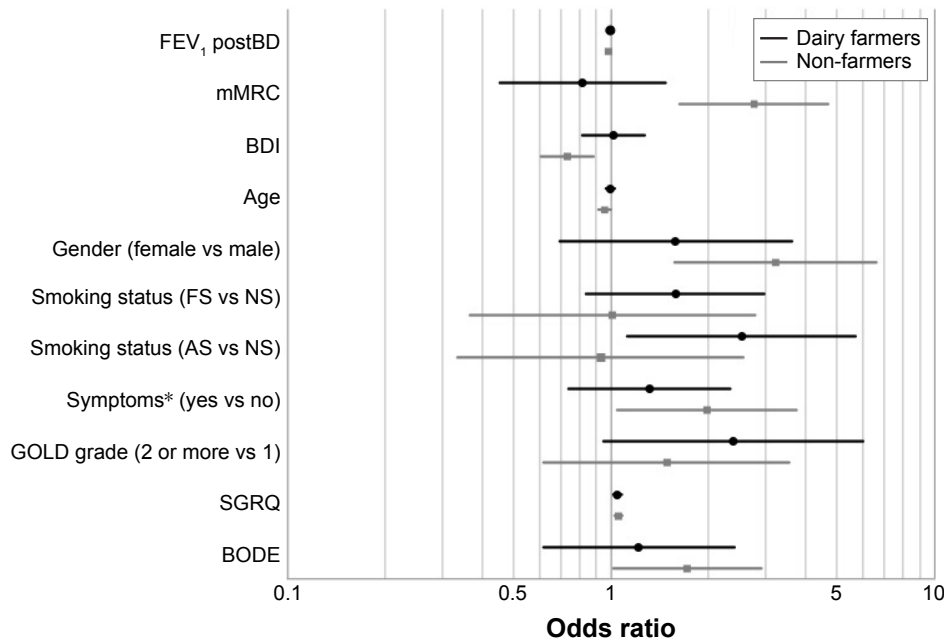
[1.58–6.60],  $p=0.001$ ), younger age (OR: 0.95 [0.91–0.99],  $p=0.03$ ), presence of COPD-related symptoms (OR: 1.98 [1.05–3.74],  $p=0.04$ ), lower quality of life as evaluated by the SGRQ (OR: 1.06 [1.03–1.08],  $p<0.001$ ) and higher BODE score (OR: 1.72 [1.02–2.91],  $p=0.04$ ).

Depression among dairy farmers was associated with lower FEV<sub>1</sub> (OR: 0.96 [0.94–0.98],  $p<0.001$ ), more severe COPD grades, that is, grade 2 or more (OR: 4.13 [1.41–12.07],  $p=0.003$ ), more severe dyspnea on the mMRC scale (OR: 2.23 [1.07–4.65],  $p=0.03$ ), lower quality of life as measured by the SGRQ score (OR: 1.08 [1.04–1.12],  $p<0.001$ ) and current smoking (OR: 3.37 [1.29–8.81],  $p<0.01$ ), as shown in Figure 4. Among non-farmers, depression was associated with lower FEV<sub>1</sub> (OR: 0.98 [0.96–0.99],  $p=0.02$ ), grade 2 or more COPD (OR: 3.59 [1.18–10.93],  $p=0.02$ ), more severe dyspnea using either the mMRC scale (OR: 2.94 [1.69–5.12],  $p<0.001$ ) or the BDI score (OR: 0.68 [0.56–0.84],  $p=0.006$ ), presence of symptoms (OR: 3.63 [1.31–10.09],  $p=0.009$ ), female gender (OR: 3.35 [1.44–7.80],  $p=0.004$ ), poorer quality of life (OR: 1.06 [1.03–1.09],  $p=0.001$ ) and a higher BODE score (OR: 2.00 [1.19–3.39],  $p=0.01$ ).

## Discussion

This study performed in dairy farmers and in subjects with no occupational exposure in the French Franche-Comté region shows that 1) the prevalence of anxiety and depression as assessed by the HADS was similar in dairy farmers and non-farmers, whether with or without COPD; 2) some COPD-associated features, such as COPD severity,





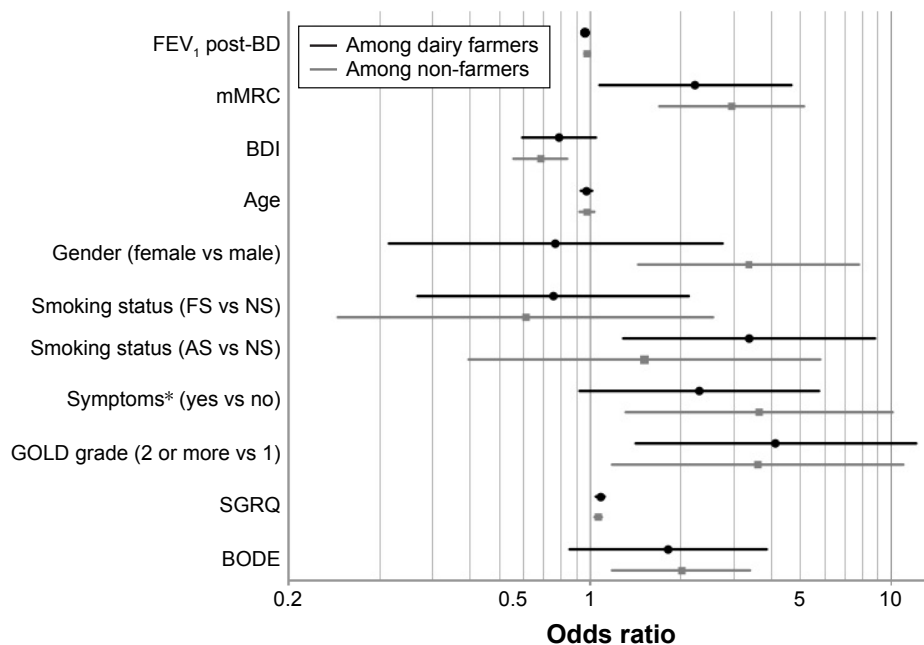
**Figure 3** Factors associated with anxiety among dairy farmers and non-farmers.

**Note:** \*Symptoms: presence of at least one symptom among dyspnea, chronic cough and chronic sputum.

**Abbreviations:** AS, active smoker; BDI, Baseline Dyspnea Index; BODE, Body mass index, airflow Obstruction, Dyspnea and Exercise capacity index; FEV<sub>1</sub> post-BD, forced expiratory volume in 1 second post-bronchodilator; FS, former smoker; GOLD, Global Initiative for Chronic Obstructive Lung Disease; mMRC, modified Medical Research Council scale; NS, non-smoker; SGRQ, St George's Respiratory Questionnaire.

dyspnea and quality of life, were associated with depression in dairy farmers as well as in subjects with no occupational exposure; and 3) there was no relationship between anxiety and COPD-associated features among dairy farmers.

Several studies have shown that the farming population is at higher risk of anxiety, depression and even suicide than the general population. In a study conducted in the USA, male farmers, fishermen and foresters had the highest age-adjusted



**Figure 4** Factors associated with depression among dairy farmers and non-farmers.

**Note:** \*Symptoms: presence of at least one symptom among dyspnea, chronic cough and chronic sputum.

**Abbreviations:** AS, active smoker; BDI, Baseline Dyspnea Index; BODE, Body mass index, airflow Obstruction, Dyspnea and Exercise capacity index; FEV<sub>1</sub> post-BD, forced expiratory volume in 1 second post-bronchodilator; FS, former smoker; GOLD, Global Initiative for Chronic Obstructive Lung Disease; mMRC, modified Medical Research Council scale; NS, non-smoker; SGRQ, St George's Respiratory Questionnaire.

suicide rates (476 per 100,000), well above those of non-farmers (113 per 100,000,  $p < 0.05$ ).<sup>29</sup> In another study using the HADS, male farmers ( $n=1,100$ ) had a higher prevalence of depression than non-farming male workers ( $n=10,026$ ), with an age-adjusted OR of 1.49 (95% CI: 1.22–1.83).<sup>17</sup> Nevertheless, in this latter study, the prevalence of anxiety was similar in farmers and non-farmers.<sup>17</sup> Although our study was not designed to compare anxiety and/or depression between healthy farmers and healthy non-farmers, we did not observe any difference between these two groups. A point of note is that similar prevalences of anxiety and depression were found in our study in dairy farmers with COPD and in non-farmers with COPD ( $p=0.68$  and  $p=0.73$  for anxiety and depression, respectively). This may be due to the particular situation of dairy farmers in the Franche-Comté region. They sell their milk to the cheese manufacturing sector, in particular, for the production of the highly reputed Comté cheese, which is safeguarded by a protected designation of origin, guaranteeing a higher selling price for the milk. It may also be related to the design of our study, in which 1) only subjects who agreed to undergo a health checkup were included in the COPD screening program and 2) only half of the subjects who had COPD detected by screening agreed to attend the hospital visit for characterization. It has previously been reported that among all subjects who are invited for the health checkups organized by the French agricultural health insurance system (MSA), those who attend these checkups and those who do not have different health characteristics.<sup>30</sup> It is, therefore, possible that our population was not representative of the whole population of dairy farmers.

Some data have previously highlighted the association between COPD and anxiety and/or depression. More than 15 years ago, a study reported that among 79 patients with grade 2 or more COPD, 50% had anxiety and 28% had depression as assessed by a HADS score  $\geq 8$ .<sup>25</sup> More recently, a study of 302 COPD patients (106 in grade 1 and 196 in grade 2 or more) showed that 53% had anxiety and 45% had depression, also assessed by a HADS score  $\geq 8$ .<sup>26</sup> In this study, anxiety and depression were not limited to the most severe forms of COPD, since 34% of patients with anxiety and 28% of patients with depression had grade 1 COPD.<sup>26</sup> Nevertheless, large differences in the prevalence of anxiety and depression in COPD have been observed from one study to another, ranging from 10% to 55% for anxiety and from 15% to 36% for depression.<sup>7,8</sup> In our study, we failed to find any difference in anxiety and/or depression prevalence between COPD patients detected by screening – the majority being Global Initiative for Chronic Obstructive Lung Disease grade 1 – and selected controls matched for

age, gender and tobacco habits. Again, this finding might be due to a selection bias secondary to the study design. The discrepancy with the literature could also be due to the choice of the threshold value of 8 for the HADS. In our population, most subjects with a score  $> 8$  had a score  $< 11$ , placing them in the borderline category, and only a minority had a score  $> 11$  (probable anxiety and/or depression). Nonetheless, a robust literature review revealed an optimal balance between sensitivity and specificity for a threshold of 8, for both anxiety and depression.<sup>31</sup>

In our population, anxiety and depression were associated with higher SGRQ scores, reflecting poorer quality of life. The mean difference in SGRQ between patients with vs without anxiety was 5–8 points, and the average difference between depressed and non-depressed patients was more than 10 points. These differences are well above the 4-point threshold defining the minimum clinically important difference. For a similar level of anxiety and depression, the impact on the quality of life measured by the SGRQ tended to be greater among non-farmers than among farmers, despite similar mean FEV<sub>1</sub> values in both groups. In addition, anxiety and depression were associated with more severe dyspnea scores among non-farmers only. These results are consistent with studies conducted in the general population, which observed a link between intensity of depression and severity of dyspnea.<sup>32,33</sup> A point of note is that this association suggests that there is an overlap between symptoms of anxiety and depression on the one hand, and symptoms of COPD on the other hand; this overlap may explain at least in part the association observed in the study. Nevertheless, this result also suggests that among farmers, anxiety and depression are associated with factors other than dyspnea, which may not have been explored in our study.

It seems difficult to characterize anxiety and depression by a single score. It might have been appropriate to use other scores in addition to the HADS, such as the Beck Depression Inventory, Beck Anxiety Inventory and State-Trait Anxiety Inventory.<sup>34,35</sup> A diagnostic questionnaire, such as the Mini-International Neuropsychiatric Interview or the Composite International Diagnostic Interview questionnaire, could have been administered to our positive cases to confirm the presence of anxiety and/or depression.<sup>36,37</sup> Finally, a clinical evaluation of anxiety and depression could also have been performed because it can lead to results differing from those of the HADS. For example, in our population, being a woman was associated with the level of anxiety and depression only among non-farmers. In general, the prevalence, incidence and morbidity of depression are higher among women than among men, as early as in their

teenage years.<sup>38</sup> Nevertheless, several studies have reported that the depression component of the HADS score was higher in men than in women.<sup>39</sup> This finding raises questions about the gender-specific validity of the HADS questionnaire. However, it also reinforces the results observed in our population of non-farmers regarding the link between anxiety and/or depression and female gender.

In conclusion, we have shown that dairy farmers with COPD have prevalences of anxiety and depression that are similar to those of dairy farmers without COPD. In accordance with previous reports, we also found that depression was associated with some COPD-related features, such as COPD severity, dyspnea and quality of life, and that this association was similar in dairy farmers and in subjects with no occupational exposure. In contrast, no association between anxiety and COPD-related features was found in our population of dairy farmers. In view of the particular situation of dairy farmers from Franche-Comté in terms of milk selling price, the observation that dairy farming is not associated with increased risk of anxiety and/or depression needs additional studies with other populations.

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## Availability of data and material

The data that support the findings of this study are available from the corresponding author.

## Disclosure

The authors report no conflicts of interest in this work.

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#### **4. Article 12 : Dietary Patterns and Prevalence of Post-Bronchodilator Airway Obstruction in Dairy Farmers Exposed to Organic Dusts**

##### ***a. Contexte et objectif***

On considère que seuls 10 à 20 % des sujets exposés à des substances à risque de BPCO (tabagisme, poussières organiques, ...), développent effectivement une BPCO. Ceci suggère que d'autres facteurs sont impliqués dans le développement de la pathologie. De nombreuses données indiquent que ces facteurs ne sont pas uniquement liés à l'hôte mais incluent également des facteurs modifiables.

Les habitudes alimentaires et notamment la consommation de produits transformés à base de viande font partie de ces facteurs de risque modifiables de BPCO. Ce facteur de risque n'a cependant pas été étudié dans la BPCO du non-fumeur.

L'objectif de cette étude était d'évaluer et de comparer les habitudes alimentaires de sujets présentant une BPCO et celles de témoins sains, exposés aux poussières organiques ou au tabac.

##### ***b. Manuscrit***

<p>Article " Dietary Patterns and Prevalence of Post-Bronchodilator Airway Obstruction in Dairy Farmers Exposed to Organic Dusts." Saussereau J, Guillien A, <b>Soumagne T</b>, Laplante JJ, Laurent L, Bouhaddi M, Rocchi S, Annesi-Maesano I, Roche R, Dalphin JC, Degano B. <i>Journal of COPD - Accepté.</i></p>
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## **Dietary Patterns and Prevalence of Post-Bronchodilator Airway Obstruction in Dairy Farmers Exposed to Organic Dusts**

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## **Abstract**

Exposure to organic dusts is an independent causative factor of chronic obstructive pulmonary disease (COPD). Unhealthy dietary patterns have been associated with poor lung function in smokers. This study investigated whether dietary patterns were associated with post-bronchodilator airway obstruction, a hallmark of COPD, in dairy farmers exposed to organic dusts.

All subjects were identified by screening programs and patients with airflow obstruction were matched with subjects with normal spirometry. Six groups were compared, defined by their exposures (non-smoking dairy farmers, smokers  $\geq 10$  pack-years with no occupational exposure, and smoking dairy farmers) and the presence or absence of post-bronchodilator airflow obstruction, resulting in 321 study subjects (ranging from 27 in the group of dairy-farmers  $\geq 10$  pack-years with normal spirometry to 68 non-dairy farmers  $\geq 10$  pack-years with post-bronchodilator airway obstruction). The Alternative Healthy Eating Index (AHEI) score was calculated based on an adapted food frequency questionnaire.

Mean total AHEI scores were similar in all groups. Comparison between smokers with post-bronchodilator airway obstruction and subjects with post-bronchodilator airway obstruction related to occupational exposure found minimal differences in dietary patterns: dairy farmers had lower scores for the ratio of white to red meat and higher scores for cereal fiber consumption. As in previous studies, smokers with post-bronchodilator airway obstruction exhibited higher lipid intakes and lower carbohydrate intakes than their counterparts with normal spirometry.

No evidence of any meaningful difference in dietary patterns was found between subjects with post-bronchodilator airway obstruction detected by screening and healthy controls, either in dairy farmers or in smokers with no occupational exposure.

**Key words:** COPD, occupational exposure, dairy farmers, dietary patterns, organic dusts, tobacco smoking

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease characterized by the association of post-bronchodilator airway obstruction with chronic respiratory symptoms (dyspnea and/or cough and/or sputum production) (1). COPD is one of the leading causes of morbidity and mortality worldwide and results in an increasing economic and social burden (2). The most important etiological factor for COPD is tobacco smoking (3), but many other factors can trigger lung inflammation, leading to COPD. Occupational exposure to biological dusts ranks high among these factors (4), especially in dairy farmers (5, 6). Nevertheless, COPD develops only in a subset of subjects exposed to these noxious particles (7), indicating that other factors leading to disease development are involved in these patients. There is increasing evidence to suggest that these factors include not only genetic factors but also many modifiable conditions (8).

Diet counts among these modifiable conditions. Using the Alternative Healthy Eating Index (AHEI), a score based on the results of food frequency questionnaires that quantifies diet quality (9), dietary patterns have been associated with the risk of COPD development, with a lower risk being associated with high intake of antioxidants (10-12) and a greater risk with high intake of processed meats (13-15). Nevertheless, most studies have essentially focused on smoking-related COPD, and very little is known regarding the effects of diet on the development of COPD in non-smokers exposed to noxious particles.

The main objective of the present study was to investigate whether dietary patterns differed between subjects with post-bronchodilator airway obstruction exposed to tobacco smoking and those exposed to organic dusts. We also aimed to study body composition in these subjects. For this purpose, we compared AHEI scores and body composition between 6 groups of patients: 3 groups with mild-to-moderate post-bronchodilator airway obstruction

(exposed either to tobacco smoking, to organic dusts or to both) and 3 groups of matched counterparts with normal spirometry.

## **METHODS**

### **Screening programs**

Data for this study were collected as part of the BALISTIC project (COPD in dairy farmers: screening, characterization and constitution of a cohort; ClinicalTrials.gov Identifier: NCT02540408) which was conducted from 2011 to 2015 at the University Hospital of Besançon in collaboration with the French national social security system for agricultural workers (Mutualité Sociale Agricole, MSA) and the federation of community health practices of Franche-Comté (Fédération des Maisons de Santé Comtoises, FeMaSaC). This study was set up primarily to assess the prevalence and some specific characteristics associated with post-bronchodilator airway obstruction in dairy farmers (subjects exposed to organic dusts) as compared with subjects with smoking-related post-bronchodilator airway obstruction without occupational exposure and matched controls without airway obstruction (16).

Subjects with post-bronchodilator airway obstruction and matched controls were recruited through 2 parallel screening programs (the screening phase of the BALISTIC study). Inclusion criteria in the screening programs were: men or women aged 40-74 years, with no previous diagnosis of asthma or hypersensitivity pneumonitis and who were either dairy farmers (“dairy farmers” subgroups) or who were unexposed to any occupational hazard associated with COPD (“non-farmers” subgroups). One of these 2 screening programs was conducted during free health check-ups organized by the MSA for their affiliated members, where COPD screening was proposed to all subjects who attended the health check-up and fulfilled the inclusion criteria. In parallel, general practitioners of the FeMaSaC conducted a

second COPD screening program targeting all their patients who met the same inclusion criteria.

For COPD screening, both programs used the same material, that is, a pneumotachograph (MedGraphics; MSE Medical, Strasbourg, France) calibrated at least once daily using a 3-L syringe, and the same protocol. Spirometry was performed according to the ATS/ERS recommendations by nurses or technicians who had followed the same training course in the same pulmonary function laboratory, as previously described (17). Spirometry outcomes included forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC). A bronchodilation test was applied when the FEV<sub>1</sub>/FVC ratio was less than 0.70. Predicted values were based on the European Coal and Steel Community (ECSC) equations (18).

### **Study groups**

A diagnosis of post-bronchodilator airway obstruction was retained when the post-bronchodilator FEV<sub>1</sub>/FVC ratio was less than 0.70. Subjects with post-bronchodilator airway obstruction were rated either as stage 1 (FEV<sub>1</sub> > 80% of the predicted value) or stage 2+ (FEV<sub>1</sub> ≤ 80% of the predicted value). Spirometry was considered normal when the FEV<sub>1</sub>/FVC ratio was > 0.70 and FEV<sub>1</sub> was > 80% of the predicted value before bronchodilator administration.

During the study period, all subjects with post-bronchodilator airway obstruction detected during the screening phase of the BALISTIC study were invited to participate in the characterization phase of the study. Those who accepted were matched 1:1 with subjects with normal spirometry (as defined above) in terms of age, body mass index (BMI), tobacco smoking (in pack-years) and sex.

All subjects who participated in the characterization phase attended a visit at the University Hospital of Besançon, during which examinations were performed and questionnaires were

administered as described in detail elsewhere (16). The distribution of the study population is shown in Table 1.

Ethical approval was received from the local Ethics Committee (CPP Est; 11/617), and written consent was obtained from all subjects.

## **Questionnaires**

A medical questionnaire (completed by the physician) collected data on demographic characteristics, medical history, treatments, symptoms and smoking history. Dyspnea was assessed using the modified Medical Research Council (mMRC) scale. Smoking status was recorded and the number of pack-years was calculated. Subjects who smoked on average less than one cigarette (cigarette, cigar or pipe) a day for a year were categorized as never-smokers. If the amount smoked was higher than this value, patients were considered as smokers, former smokers being those who had abstained from smoking for the last 30 days before the characterization visit.

The dietary questionnaire was an adaptation of a French Food Frequency Questionnaire (FFQ) that includes 24 items regarding food and drinks (19). The FFQ was qualitative, without information on portion size. Its objective was to assess retrospectively the usual intake during the past year for foods or food groups. The questionnaire was self-administered in a single interview. The questionnaire included semi-quantitative questions about the frequency of consumption (with a “never” option as well as options ranging from very infrequent to several times a day), and portion size information (with portion size images to enhance reporting accuracy).

## **Alternate Healthy Eating Index (AHEI)**

An adapted Alternate Healthy Eating Index (AHEI) (9) was calculated for each completed FFQ by converting each food item to a daily intake. The original components of the index include vegetables, fruit, nuts and soy, the ratio of white (seafood and poultry) to red meat, cereal fiber, *trans* fat, the ratio of polyunsaturated fatty acids to saturated fatty acids, long-term multivitamin use (< 5 or ≥ 5 years), and alcohol consumption. Because multivitamin use was not available in our nutrient data set, we adapted the score using the eight other components, with a potential contribution of 0-10 points to the total score for each. Intermediate intakes were scored proportionately between 0 and 10, with scoring decisions made a priori, based on food nutrient composition. All the component scores were summed to obtain a total AHEI score, which ranged from 0 to 80, with higher scores corresponding to healthier diets.

### **Bioelectrical impedance analysis**

A nurse measured height and weight. Body mass index (BMI) was calculated as weight/height<sup>2</sup> (kg/m<sup>2</sup>). Body composition was assessed by multiple frequency bioelectrical impedance analysis (BIA) (Z-Metrix, Bioparhom, France) under standardized conditions, i.e., ambient temperature between 23°C and 25°C, fasting during at least 3 hours, empty bladder, clean skin surface. For body composition assessment, participants were asked to remain in the supine position for at least 10 minutes before starting the measurement, with legs and arms slightly abducted at 30° so there was no contact between the extremities and the trunk.

A standard tetrapolar technique was used, with the measuring electrodes placed on the anterior surface of the wrist and ankle, and the injecting electrodes placed on the dorsal surface of the hand and the foot. Impedance (Z) was determined at 2 frequencies (5 and 250 kHz) with an imperceptible electrical current of 800 mA. Z at 5 kHz and Z at 250 kHz were considered as inversely related to extracellular water (ECW) and total body water (TBW),

respectively, TBW being the sum of ECW and intracellular water (ICW). Fat-free mass (20) and FFM index (fat-free mass index [FFMI] equal to FFM/height<sup>2</sup>) were calculated by estimating that FFM is composed of 73% of water, and thus FFM=TBW/0.73 (21).

### **Statistical analysis**

Data are presented as number (percent) and mean  $\pm$  standard deviation for qualitative and quantitative variables, respectively. Quantitative variables were compared with the Student t test or the Wilcoxon test, as appropriate. Qualitative variables were compared with the Chi-square ( $\chi^2$ ) test.

Comparisons were made between 6 groups defined by their exposures (non-smoking dairy farmers, smokers > 10 pack-years with no occupational exposure and smoking dairy farmers) and the presence or absence of post-bronchodilator airflow obstruction. To identify factors associated with post-bronchodilator airway obstruction among dairy farmers and non-farmers, bivariate logistic regressions for all candidate factors were performed.

Sensitivity analysis was conducted using the lower limit of normal to define airflow obstruction (22) (results shown as Supplemental data).

A *p*-value < 0.05 was considered statistically significant. Analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

## **RESULTS**

### **Population characteristics**

The flowchart of subjects is shown in Figure 1. A total of 186 subjects with post-bronchodilator airway obstruction and 187 matched controls with normal spirometry were included in the characterization phase of the study. Thirty patients were excluded after their visit, mainly owing to a history of asthma or occupational exposure other than dairy farming.

In addition, 8 subjects with post-bronchodilator airway obstruction (4 dairy farmers and 4 non-farmers) and 13 control subjects (8 dairy farmers and 5 non-farmers) were not included in the current analysis because of missing data for the dietary evaluation. Finally, 31 non-farmers (13 with post-bronchodilator airway obstruction and 18 with normal spirometry) who had smoked less than 10 pack-years were not analyzed because group sizes were too small.

The main characteristics of the population analyzed are displayed in Table 1 and supplemental Figure 1. The number of current smokers and former smokers was much lower among farmers than among non-farmers. The proportion of men was higher among farmers than among non-farmers. A large majority of subjects with post-bronchodilator airway obstruction had preserved FEV<sub>1</sub>. Nevertheless, dyspnea scores were poorer among subjects with post-bronchodilator airway obstruction than among subjects with normal spirometry. Comorbidities and treatments were similar in all subgroups (supplemental Table 1).

Comparisons made between subjects recruited through the MSA and those recruited through the FeMaSaC demonstrated that the main characteristics of the recruited subjects were similar in both conditions of recruitment (supplemental Table 2).

### **Dietary patterns**

Total AHEI score and each dietary component of the score are shown in Table 2. The mean total AHEI score was very similar in all groups. Each available component of the AHEI score was compared between groups. The only significant difference was for the ratio of white to red meat and for cereal fiber between dairy farmers with post-bronchodilator airway obstruction and the other groups of subjects with post-bronchodilator airway obstruction (Table 2). In addition, there was no relationship between FEV<sub>1</sub> and AHEI in patients with post-BD airway obstruction, the coefficient of correlation ( $r$ ) being equal to [95% confidence interval] 0.13 [-0.20;0.43],  $p=0.44$ ; -0.08 [-0.34;0.18],  $p=0.53$ ; and 0.07 [-0.17;0.31],  $p=0.55$



in dairy farmers  $\geq 10$  pack-years, dairy farmers  $< 10$  pack-years and non-dairy farmers  $\geq 10$  pack-years, respectively.

Table 2 also lists the participants' nutritional intake ratios of lipids, carbohydrates and proteins according to the FFQ, showing significantly higher intakes of lipids and lower intakes of carbohydrates in smokers with post-bronchodilator airway obstruction than in their counterparts with normal spirometry. The same results were found when the LLN criterion was used to define airflow obstruction (supplemental Table 3).

### **Bioelectrical impedance analysis**

In the entire population, there was a positive correlation between AHEI score and fat mass expressed as a percentage of body weight ( $p = 0.04$ ). Fat mass (% body weight) was also positively correlated with the ratio of white to red meat ( $p < 0.001$ ) and negatively correlated with cereal fiber ( $p = 0.002$ ).

The body composition in each group is shown in Table 3. Patients were overweight in all groups, with mean BMI ranging from 25.9 to 27.0 kg.m<sup>-2</sup>. FFMI was preserved in all groups, with mean values between 19.7 and 20.9 kg.m<sup>-2</sup>. There was no significant difference in terms of body composition between groups. All these results were similar using FEV1/FVC  $<$  LLN to define airflow obstruction (supplemental Table 4).

## **DISCUSSION**

In this study performed in dairy farmers and in subjects with no occupational exposure in the Franche-Comté region of France, we were unable to find any difference in terms of dietary patterns or body composition between patients with post-bronchodilator airway obstruction and their healthy counterparts. In addition, comparisons between smokers with post-bronchodilator airway obstruction and subjects with post-bronchodilator airway

obstruction related to occupational exposure revealed only minimal differences in terms of dietary patterns. Conversely, smokers with post-bronchodilator airway obstruction exhibited slightly higher intakes of lipids and lower intakes of carbohydrates than their counterparts with normal spirometry.

Several associations between dietary patterns on the one hand and COPD prevalence and severity on the other have been previously reported. In a large prospective cohort of US men followed up for 12 years, the risk of newly diagnosed COPD increased with a greater consumption of cured meats (15). Consumption of processed meats has also been significantly associated with lower FEV1 and lower FEV1/FVC (14). In addition, the negative association between higher processed meat consumption and poorer lung function has been shown to be greater in subjects who also have lower fruit and vegetable consumption and low dietary total antioxidant capacity (23). In line with these findings, it has been reported that higher current cured meat consumption increases the risk of readmission to hospital in COPD patients (24). Other studies have reported a negative association between fish intake and COPD-related outcomes (25-28), potentially linked to the beneficial effects of omega-3 polyunsaturated fatty acid (PUFA) on the production of anti-inflammatory and pro-resolving mediators (29). Fish and omega-3 PUFA are an important component of the prudent dietary pattern recommended by the AHEI-2010. A prospective cohort analysis of more than 120,000 US women and men has demonstrated that a higher AHEI-2010 diet score, reflecting high intakes of whole grains, polyunsaturated fatty acids, nuts, and long-chain omega-3 fats and low intakes of red and processed meats, refined grains, and sugar sweetened drinks, was associated with a lower risk of newly diagnosed COPD (30). Some specific nutrients such as black tea have been shown to be protective against developing COPD in male smokers, possibly through an increase in the level of cellular antioxidant defences (31). Here, with the AHEI score adapted to our FFQ data, we found similar global AHEI scores in subjects with

post-bronchodilator airway obstruction and in healthy controls, even after stratification for occupational exposure and tobacco smoking. Detailed items of the AHEI score showed no difference in consumption of each food category between subjects with post-bronchodilator airway obstruction and controls. We only found that farmers with post-bronchodilator airway obstruction who had smoked  $\geq 10$  pack-years had a lower ratio of white to red meat consumption and a higher consumption of cereal fiber than non-farming smokers with post-bronchodilator airway obstruction. This could be related to better access of dairy farmers to these food categories (cereal fiber and red meat). Of note, dairy farmers with and without post-bronchodilator airway obstruction had strictly similar dietary patterns. In accordance with previous findings, the proportion of total energy from carbohydrates was low in comparison with recommendations while lipid proportions were high (32).

Body weight and body composition are important discriminants in classifying disease heterogeneity. Body weight loss in patients with COPD is at least in part related to disturbance of hormonal appetite stimulation and activation of inflammation by hypoxia (33, 34). Fat-free mass index (FFMI) depletion, calculated by bioelectrical impedance analysis (BIA), has been shown to be a better predictor of mortality than BMI in COPD patients (35), and could be a predictor of COPD severity as it is strongly correlated with exercise capacity, dyspnea, respiratory muscle function and FEV<sub>1</sub> (36). Lung function has an impact on nutritional status, as both BMI and FFMI values are poorer in the more severe COPD stages than in patients with mild disease (37). Analysis of dietary intakes in patients with moderate to severe COPD showed that patients with low FFMI had lower mean energy intakes than patients with normal FFMI, related to lower daily consumption of dairy products and red meat (38). In this study, patients with post-bronchodilator airway obstruction (a hallmark of COPD) had few symptoms, suggesting a low impact on overall nutritional status, as confirmed by BIA and FFMI comparisons between subjects.

## **Limitations of the study**

Our study has some limitations. Firstly, there was a small number of patients in each group, leading to some lack of power. Statistical power had been calculated for the primary endpoint of the BalistiC study (16). For the study presented here, 52 subjects had to be excluded (therefore, only 321 subjects of the 373 included in the “original” BalistiC study were analyzed). The initial power calculation was therefore no longer appropriate for the current study, resulting in a lower effective statistical power that could explain the lack of significance of our results. Secondly, the FFQ used in our study was not as effective as that used by others (12). It seems difficult to characterize a diet with a single score based on a 24-item FFQ, leading to more approximate data in the AHEI-2010 scoring. This could explain at least in part the lack of significant difference between the groups. Nevertheless, the FFQ used in this study was validated to cover all lipid, carbohydrate and protein intakes, and this dietary score had been validated as a referential score adapted from the Healthy Eating Index (HEI) developed by the US Department of Agriculture. The HEI measures adherence to the Dietary Guidelines for Americans (39) and is designed to target food choices and macronutrient sources associated with reduced chronic disease risk (9). Thirdly, physical activity, which may differ between farmers and non-farmers, is a confounding factor in terms of body composition that was not taken into account in our study. Fourthly, there is a time-dependent confounding factor, as the diet questioning was cross-sectional, with no longitudinal evaluation. It has now been demonstrated that standard methods of analysis may be biased in the presence of such a confounder (40). Fifthly, only one half of subjects detected with post-bronchodilator airway obstruction agreed to attend the hospital for characterization. It has been previously reported that among all subjects who are invited to health check-ups organized by the French agricultural health insurance (Mutualité Sociale Agricole), those who

attend such check-ups and those who do not have different health characteristics (41). Our study population may therefore not be representative of the whole population of dairy farmers.

## **Conclusion**

In conclusion, no evidence of any meaningful difference in dietary patterns was found between subjects with post-bronchodilator airway obstruction detected by screening in the Franche-Comté region and healthy controls, whether in dairy farmers or in smokers with no occupational exposure. Further studies, including more patients and with analysis of micronutrient intakes, would improve our understanding of diet as a risk factor for the onset of COPD, especially in non-smokers exposed to organic dusts.

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**Table 1.** Main characteristics and pulmonary function of the study participants according to exposure to dairy-farming, number of smoked pack-years and presence of post-bronchodilator airway obstruction (defined by FEV1/FVC<0.70)

	Dairy Farmers				Non-Dairy Farmers			
	≥ 10 Pack-Years		< 10 Pack-Years		≥ 10 Pack-Years		< 10 Pack-Years	
	Post-BD airway obstruction (n=38)	Normal spirometry (n=27)	Post-BD airway obstruction (n=59)	Normal spirometry (n=63)	Post-BD airway obstruction (n=68)	Normal spirometry (n=66)	p-value	p-value
Age (years)	61 ± 9	56 ± 10	60 ± 9	60 ± 9	61 ± 8	61 ± 6	0.95	0.87
Men, n (%)	34 (89)	26 (96)	48 (81)	53 (84)	54 (79)	48 (73)	0.69	0.36
Smoking status, n (%)								
Non-smoker	-	-	48 (81)	49 (78)	-	-		0.30
Former smoker	21 (55)	16 (59)	9 (15)	13 (21)	29 (43)	34 (52)		
Active smoker	17 (45)	11 (41)	2 (3)	1 (1)	39 (57)	32 (48)		
Number of pack-years	29 ± 14	24 ± 10	0.9 ± 2.3	1.0 ± 2.2	37.7 ± 22.1 <sup>#</sup>	31.1 ± 14.2	0.88	0.04
mMRC dyspnea > 0	19 (50)	5 (19)	18 (31)	8 (13)	33 (49)	15 (23)	0.01	0.002
Post-bronchodilator spirometry								
FEV1, L	2.76 ± 0.64	3.58 ± 0.58	2.86 ± 0.78	3.41 ± 0.74	2.56 ± 0.76	3.37 ± 0.67	< 0.001	< 0.001
z-score	-1.06 ± 0.96	0.45 ± 0.86	-0.95 ± 1.03	0.39 ± 0.72	-1.43 ± 0.99	0.48 ± 0.81	< 0.001	< 0.001
FVC, L	4.42 ± 0.89	4.63 ± 0.83	4.51 ± 1.18	4.32 ± 0.92	4.21 ± 1.08	4.22 ± 0.90	0.34	0.92
z-score	0.29 ± 1.01	0.51 ± 0.98	0.37 ± 1.15	0.30 ± 0.69	0.02 ± 1.03	0.28 ± 0.78	0.69	0.11
FEV1/FVC, %	62.5 ± 6.5	77.6 ± 3.7	68.5 ± 7.1	78.8 ± 4.5	60.4 ± 7.8	80.0 ± 4.2	< 0.001	< 0.001
z-score	-1.98 ± 0.73	-0.10 ± 0.51	-1.92 ± 0.83	0.12 ± 0.68	-2.22 ± 0.82	0.28 ± 0.60	< 0.001	< 0.001
Severity of airway obstruction, n (%)								
Stage I	22 (58)	-	41 (69)	-	30 (44)*	-		
Stage II+	16 (42)	-	18 (31)	-	38 (56)*	-		

*Definition of abbreviations:* post-BD: post-bronchodilator; mMRC: modified medical research council; FEV<sub>1</sub>: forced expiratory volume in 1 second; z-score: standardized residual; FVC: forced vital capacity. Stage I: FEV1 > 80% of the predicted value; Stage II+: FEV1 > 80% of the predicted value. Values are means ± SD.

<sup>#</sup>: p < 0.05 vs. dairy farmers with post-BD airway obstruction who had smoked ≥ 10 pack-years; \*: p < 0.05 vs. dairy farmers with post-BD airway obstruction < 10 pack-years

**Table 2.** Adapted Alternate Healthy Eating Index scores and subscores and nutritional intake ratios with the results of food-frequency questionnaires according to exposure to dairy farming, number of pack-years smoked and presence of post-bronchodilator airway obstruction (defined by FEV1/FVC <0.70)

	Dairy Farmers						Non-Dairy Farmers			
	≥ 10 Pack-Years			< 10 Pack-Years			≥ 10 Pack-Years			
	Post-BD airway obstruction (n=38)	Normal spirometry (n=27)	<i>p-value</i>	Post-BD airway obstruction (n=59)	Normal spirometry (n=63)	<i>p-value</i>	Post-BD airway obstruction (n=68)	Normal spirometry (n=66)	<i>p-value</i>	
Total AHEI score	37 ± 9	36 ± 7	0.85	37 ± 4	38 ± 7	0.90	36 ± 7	36 ± 8	0.73	
AHEI subscores										
Vegetables	5.6 ± 2.0	5.1 ± 1.7	0.24	5.5 ± 1.5	5.6 ± 1.7	0.72	5.8 ± 1.8	5.5 ± 1.7	0.19	
Fruits	4.4 ± 3.6	3.8 ± 3.2	0.62	4.8 ± 3.2	5.3 ± 3.2	0.36	4.8 ± 3.4	5.0 ± 3.4	0.70	
Nuts and soy proteins	3.2 ± 1.2	3.3 ± 1.1	0.93	3.0 ± 1.2	3.0 ± 1.0	0.66	2.9 ± 1.0	2.9 ± 1.4	0.96	
Ratio of white to red meat	3.5 ± 1.2	3.8 ± 1.3	0.49	3.6 ± 1.3	3.6 ± 1.2	0.75	4.2 ± 1.2 <sup>#</sup>	4.2 ± 1.3	0.89	
Cereal fiber	5.0 ± 1.3	4.8 ± 1.6	0.57	4.9 ± 1.3	4.9 ± 1.3	0.81	4.3 ± 1.7 <sup>#</sup>	4.5 ± 1.8	0.49	
Long-chain omega 3 fatty acids	3.9 ± 2.1	3.8 ± 2.0	0.81	3.2 ± 1.8	3.2 ± 1.9	0.97	3.4 ± 2.8	2.8 ± 2.7	0.18	
Polysaturated fatty acids	4.6 ± 1.4	4.2 ± 1.6	0.17	4.6 ± 1.6	4.5 ± 1.8	0.82	4.2 ± 1.7	4.1 ± 1.4	0.62	
Alcohol	6.7 ± 3.4	7.6 ± 2.9	0.17	7.9 ± 2.5	7.4 ± 2.7	0.33	6.7 ± 2.8	7.0 ± 2.5	0.46	
Nutritional intake ratios										
Lipids (%)	19.8 ± 2.4	19.8 ± 2.3	0.92	19.5 ± 2.4	19.5 ± 2.8	0.85	21.0 ± 3.7	19.0 ± 2.6	<b>0.0003</b>	
Proteins (%)	21.0 ± 1.8	20.9 ± 2.0	0.86	20.9 ± 1.9	21.0 ± 1.8	0.74	21.0 ± 2.2	21.1 ± 2.1	0.63	
Carbohydrates (%)	59.1 ± 3.1	59.4 ± 3.4	0.88	59.5 ± 3.4	59.5 ± 3.8	0.97	58.1 ± 4.2	59.7 ± 4.0	<b>0.03</b>	

*Definition of abbreviations:* post-BD: post-bronchodilator; AHEI: Alternate Healthy Eating Index. Values are means ± SD.

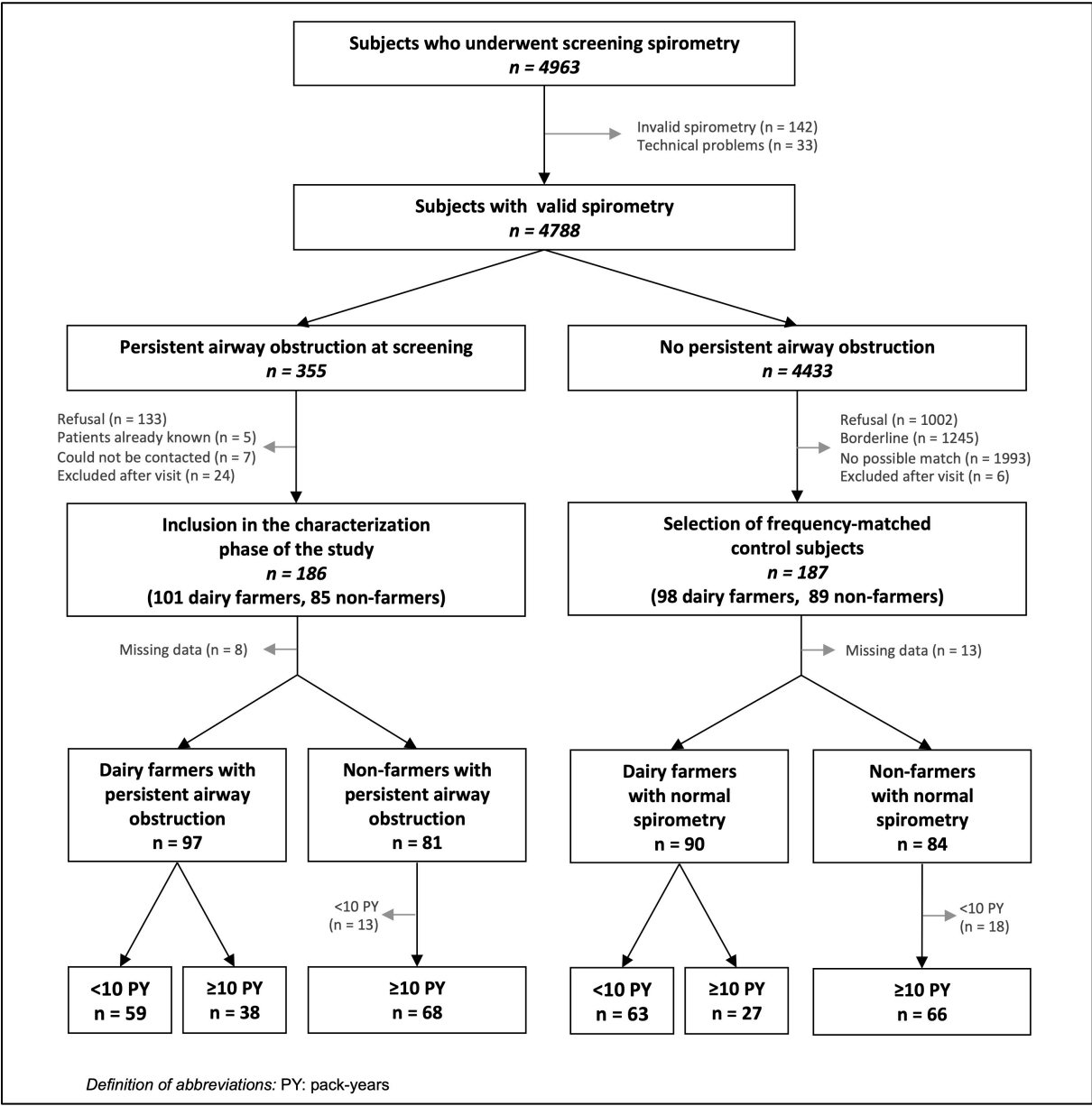
<sup>#</sup>: *p* < 0.05 vs. dairy farmers with post-bronchodilator airway obstruction who had smoked ≥ 10 pack-years

**Table 3.** Morphometric characteristics and body composition evaluated by bioelectrical impedance analysis according to exposure to dairy-farming, number of smoked pack-years and presence of post-bronchodilator airway obstruction (defined by FEV1/FVC <0.70)

	Dairy Farmers				Non-Dairy Farmers			
	≥ 10 Pack-Years		< 10 Pack-Years		≥ 10 Pack-Years		< 10 Pack-Years	
	Post-BD airway obstruction (n=38)	Normal spirometry (n=27)	Post-BD airway obstruction (n=59)	Normal spirometry (n=63)	Post-BD airway obstruction (n=68)	Normal spirometry (n=66)	<i>p</i> - value	<i>p</i> - value
Weight (kg)	78 ± 14	81 ± 14	79 ± 15	78 ± 13	79 ± 15	79 ± 13	0.60	0.99
Height (cm)	172 ± 8	173 ± 8	173 ± 9	170 ± 8	173 ± 8	170 ± 8	0.06	0.11
BMI (kg/m <sup>2</sup> )	27.0 ± 3.8	25.9 ± 3.7	26.6 ± 4.8	27.2 ± 3.7	25.9 ± 4.2	26.7 ± 3.9	0.41	0.23
FFMI (kg/m <sup>2</sup> )	20.9 ± 3.6	20.4 ± 3.1	20.1 ± 4.3	20.9 ± 3.3	19.7 ± 3.9	20.2 ± 3.6	0.29	0.46
Fat mass (% body weight)	22.6 ± 9.0	21.0 ± 5.6	23.6 ± 9.6	22.8 ± 7.3	24.0 ± 8.2	24.6 ± 7.6	0.61	0.69
Total body water (L)	43.0 ± 7.9	44.8 ± 8.0	43.6 ± 9.3	44.4 ± 7.3	42.1 ± 7.5	41.2 ± 6.7	0.60	0.47
Extra-cellular water (%)	37.9 ± 2.3	37.0 ± 2.3	38.3 ± 4.4	38.1 ± 3.1	39.2 ± 4.4	38.4 ± 2.7	0.86	0.24
Intra-cellular water (%)	72.1 ± 6.1	74.7 ± 7.3	69.7 ± 9.3	72.5 ± 7.8	69.6 ± 7.2	70.8 ± 7.3	0.08	0.33

*Definition of abbreviations:* post-BD: post-bronchodilator; BMI: body mass index; FFMI: fat-free mass index. Values are means ± SD.

**Figure 1.** Flowchart of the population



## **Online Data Supplement**

### **METHODS**

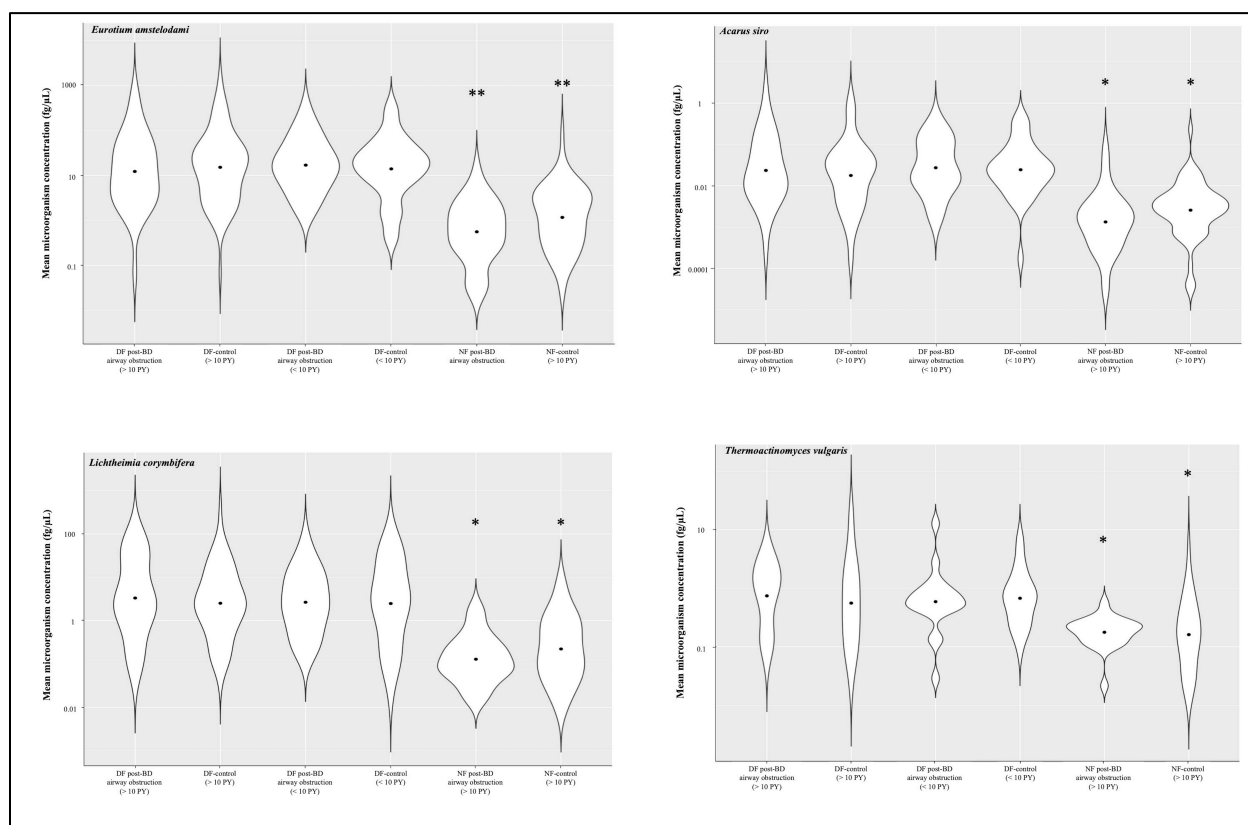
#### *Microbial quantification by qPCR*

Exposure was quantified through the analysis of dust collected passively by sterilized electrostatic wipes placed for 10 weeks in the bedroom of the dwelling of each subject (dwelling airborne dust being considered to be representative of both indoor and outdoor occupational activities). Dust was analyzed by real-time quantitative PCR (qPCR) as described elsewhere (1).

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**Supplemental Figure 1.** Violin plots showing microorganism concentrations of 4 species, quantified by qPCR from electrostatic dust collector (EDC) samples from the 6 subgroups. Microorganism concentrations are expressed in DNA equivalents (fg/ $\mu$ l). The point represents the mean value.



\*  $p < 0.05$  and \*\*  $p < 0.01$  vs each group of dairy farmers.

Abbreviations: post-BD: post-bronchodilator; DF: dairy farmers; NF: non-farmers; PY: pack-years.

**Supplemental table 1.** Comorbidities and treatments taken according to exposure to dairy-farming, number of smoked pack-years and presence of post-bronchodilator airway obstruction (defined by a FEV1/FVC ratio <0.70)

	Dairy Farmers				Non-Dairy Farmers			
	≥ 10 Pack-Years		< 10 Pack-Years		≥ 10 Pack-Years		< 10 Pack-Years	
	Post-bronchodilator airway obstruction (n=38)	Normal spirometry (n=27)	Post-bronchodilator airway obstruction (n=59)	Normal spirometry (n=63)	Post-bronchodilator airway obstruction (n=68)	Normal spirometry (n=66)	<i>p</i> -value	<i>p</i> -value
<b>Comorbidities</b>								
Hypertension	11 (29)	3 (11)	14 (24)	12 (19)	16 (24)	15 (23)	0.68	1
Coronary disease	2 (5)	1 (4)	1 (2)	3 (5)	7 (10)	2 (3)	0.62	0.16
Chronic heart failure	1 (3)	0 (0)	2 (3)	0 (0)	5 (7)	2 (3)	0.23	0.44
Diabetes mellitus	1 (3)	2 (7)	0 (0)	4 (6)	9 (13)	2 (3)	0.12	0.06
Dyslipidemia	13 (34)	5 (19)	13 (22)	9 (14)	20 (29)	13 (20)	0.38	0.27
<b>Treatments</b>								
ACE inhibitors	5 (13)	1 (4)	9 (15)	7 (11)	10 (15)	5 (8)	0.68	0.30
β-blockers	7 (18)	1 (4)	5 (8)	2 (3)	11 (16)	5 (8)	0.26	0.18
ARBs	3 (8)	2 (7)	6 (10)	6 (10)	9 (13)	4 (6)	1	0.27
Diuretics	3 (8)	3 (11)	5 (8)	0 (0)	2 (3)	5 (8)	<b>0.02</b>	0.27
Antiplatelet drugs	5 (13)	4 (15)	3 (5)	4 (7)	19 (28)	9 (14)	1	0.19
Statins	10 (26)	3 (11)	8 (14)	9 (14)	19 (28)	12 (18)	1	0.26
Oral anticoagulants	4 (11)	1 (4)	2 (3)	0 (0)	2 (3)	2 (3)	0.23	1

ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blocker

**Supplemental table 2.** Characteristics of subjects screened through the MSA and FeMaSaC

	<b>MSA (n=223)</b>	<b>FeMaSaC (n=98)</b>	<b>p-value</b>
Post-bronchodilator airway obstruction, n (%)	113 (51)	52 (53)	0.78
Dairy farmers, n (%)	187 (84)	0 (0)	< 0.001
≥ 10 pack-years, n (%)	101 (45)	98 (100)	< 0.001
Age (years)	59.8 ± 8.8	61.3 ± 7.3	0.12
Men, n (%)	192 (86)	71 (72)	0.006
Smoking status, n (%)			< 0.001
Non-smoker	97 (43)	-	
Former smoker	81 (36)	41 (42)	
Active smoker	45 (20)	57 (58)	
Number of pack-years	13.2 ± 16.5	36.1 ± 19.7	< 0.001
mMRC dyspnea > 0	64 (29)	34 (35)	0.36
Post-bronchodilator spirometry			
FEV <sub>1</sub> , L	3.16 ± 0.79	2.80 ± 0.78	< 0.001
z-score	-0.28 ± 1.15	-0.69 ± 1.32	0.009
FVC, L	4.48 ± 0.99	4.05 ± 0.95	< 0.001
z-score	0.38 ± 0.94	0.01 ± 0.94	0.001
FEV <sub>1</sub> /FVC, %	70 ± 10	69 ± 12	0.33
z-score	-0.97 ± 1.25	-1.11 ± 1.48	0.20

*Definition of abbreviations:* MSA: Mutualité Sociale Agricole; FeMaSaC: Fédération des Maisons de Santé Comtoises; mMRC: modified Medical Research Council; FEV<sub>1</sub>: forced expiratory volume in 1 second; z-score: standardized residual; FVC: forced vital capacity. Values are means ± SD.



**Supplemental table 3.** Dietary patterns according to exposure to dairy farming, number of pack-years smoked and presence of post-bronchodilator airway obstruction (defined by the LLN criterion)

	Dairy Farmers				Non-Dairy Farmers			
	≥ 10 Pack-Years		< 10 Pack-Years		≥ 10 Pack-Years			
	Post-bronchodilator airway obstruction (n=23)	Normal spirometry (n=42)	Post-bronchodilator airway obstruction (n=34)	Normal spirometry (n=88)	Post-bronchodilator airway obstruction (n=47)	Normal spirometry (n=87)	<i>p</i> -value	
Total AHEI score	37 ± 7	36 ± 8	38 ± 7	37 ± 7	36 ± 7	36 ± 7	0.90	0.87
AHEI subscores								
Vegetables	5.9 ± 2.0	5.1 ± 1.7	5.5 ± 1.5	5.6 ± 1.7	5.7 ± 1.7	5.6 ± 1.8	0.91	0.86
Fruits	4.1 ± 3.4	4.1 ± 3.5	4.9 ± 3.2	5.1 ± 3.2	4.6 ± 3.4	5.0 ± 3.5	0.68	0.45
Nuts and soy proteins	3.3 ± 1.1	3.2 ± 1.2	3.1 ± 1.1	3.0 ± 1.1	2.9 ± 1.0	2.9 ± 1.3	0.42	0.96
Ratio of white to red meat	3.3 ± 1.1	3.8 ± 1.3	3.6 ± 1.4	3.6 ± 1.2	4.2 ± 1.3 <sup>#</sup>	4.2 ± 1.3	0.84	0.78
Cereal fiber	5.4 ± 1.1	4.7 ± 1.5	4.9 ± 1.2	4.9 ± 1.3	4.2 ± 1.7 <sup>#</sup>	4.5 ± 1.7	0.88	0.37
Long-chain omega 3 fatty acids	3.9 ± 1.9	3.8 ± 2.2	3.3 ± 1.98	3.2 ± 1.9	3.8 ± 2.8	2.8 ± 2.7	0.73	0.05
Polyunsaturated fatty acids	4.6 ± 1.4	4.3 ± 1.6	4.6 ± 1.6	4.5 ± 1.7	4.4 ± 1.97	4.0 ± 1.4	0.71	0.27
Alcohol	6.6 ± 3.4	7.3 ± 3.1	7.8 ± 2.8	7.6 ± 2.5	6.5 ± 2.9	7.0 ± 2.5	0.80	0.34
Nutritional intake ratios								
Lipids (%)	19.7 ± 2.5	19.9 ± 2.2	19.5 ± 2.4	19.5 ± 2.7	21.3 ± 3.7	19.3 ± 2.9	0.93	<b>0.001</b>
Proteins (%)	21.0 ± 1.6	20.9 ± 2.0	21.2 ± 1.8	20.8 ± 1.9	21.2 ± 2.3	21.0 ± 2.1	0.24	0.63
Carbohydrates (%)	59.3 ± 3.4	59.2 ± 3.1	59.4 ± 3.4	59.6 ± 3.6	57.5 ± 4.6	59.6 ± 3.7	0.73	<b>0.009</b>

*Definition of abbreviations:* AHEI: Alternate Healthy Eating Index. Values are means ± SD.

<sup>#</sup>: *p* < 0.05 vs. dairy farmers with post-bronchodilator airway obstruction (defined by the LLN criterion) who had smoked ≥ 10 pack-years

**Supplemental table 4.** Morphometric characteristics and body composition evaluation by bioelectrical impedance analysis according to exposure to dairy farming, number of pack-years smoked and presence of post-bronchodilator airway obstruction (defined by the LLN criterion)

	Dairy Farmers				Non-Dairy Farmers			
	≥ 10 Pack-Years		< 10 Pack-Years		≥ 10 Pack-Years			
	Post-bronchodilator airway obstruction (n=23)	Normal spirometry (n=42)	Post-bronchodilator airway obstruction (n=34)	Normal spirometry (n=88)	Post-bronchodilator airway obstruction (n=47)	Normal spirometry (n=87)		
		<i>p</i> -value		<i>p</i> -value		<i>p</i> -value		
Weight (kg)	78 ± 15	0.88	80 ± 16	79 ± 14	76 ± 16	78 ± 13	0.54	
Height (cm)	170 ± 7	<b>0.04</b>	173 ± 11	171 ± 7	172 ± 8	171 ± 8	0.85	
BMI (kg/m <sup>2</sup> )	25.6 ± 3.3	0.32	26.1 ± 4.1	26.9 ± 3.9	25.7 ± 3.9	27.3 ± 4.0	<b>0.02</b>	
FFMI (kg/m <sup>2</sup> )	21.4 ± 4.1	0.06	20.6 ± 4.2	20.5 ± 3.6	19.8 ± 3.8 <sup>#</sup>	20.0 ± 3.7	0.82	
Fat mass (% body weight)	22.5 ± 10.9	0.53	22.2 ± 8.5	23.5 ± 8.5	23.2 ± 6.7	24.9 ± 8.4	0.21	
Total body water (L)	43.3 ± 7.9	0.68	42.7 ± 9.1	44.6 ± 8.0	41.1 ± 7.3	41.9 ± 7.0	0.57	0.82
Extra-cellular water (%)	37.6 ± 2.3	0.81	37.9 ± 5.4	38.3 ± 2.9	39.4 ± 5.1 <sup>#</sup>	38.5 ± 2.6	0.24	0.82
Intra-cellular water (%)	72.9 ± 6.0	0.92	69.7 ± 11.3	71.7 ± 7.4	69.6 ± 8.0	70.6 ± 6.8	0.49	0.82

*Definition of abbreviations:* BMI: body mass index; FFMI: fat-free mass index. Values are means ± SD.



## V. SYNTHÈSE ET PERSPECTIVES

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### Synthèse

Les pathologies respiratoires associées à l'agriculture ont été l'une des premières maladies professionnelles mises en évidence. Dès le moyen-âge, les dangers de l'inhalation de poussières de céréales ont été décrits. Malgré cette reconnaissance précoce, ce n'est qu'au 20<sup>ème</sup> siècle que ce problème a été étudié et documenté avec soin.

Parce que l'agriculture est intimement liée à la terre, elle a généré de nombreux mythes sur la santé des agriculteurs (171). Malheureusement, le mythe de l'agriculteur robuste et en bonne santé est en réalité un mythe qui ne correspond pas aux réalités de la vie agricole. De nombreuses données montrent l'ampleur et la gravité des risques respiratoires en agriculture (172). Pour autant, les agriculteurs ont une espérance de vie plus élevée et une prévalence de maladies notamment cancéreuses plus faible que la population générale.

Les maladies respiratoires constituent un problème clinique important pour les travailleurs agricoles du fait de leur exposition à des quantités généralement importantes de poussières organiques. De nombreuses études ont clairement démontré le risque de morbidité et de mortalité respiratoires chez les agriculteurs et les ouvriers agricoles. Dans le département du Doubs, on considère qu'environ 10 % des actifs agricoles régulièrement exposés présentent une pathologie respiratoire. Celles-ci incluent principalement la pneumopathie d'hypersensibilité (maladie du poumon de fermier) et la bronchite chronique agricole (173, 174). A l'inverse, certaines pathologies respiratoires sont moins fréquentes en secteur de production laitière que dans le reste de la population. C'est le cas du cancer du poumon et de l'asthme et des allergies (101, 143, 175).

Notre équipe effectue des recherches sur les maladies respiratoires chez les producteurs laitiers depuis 30 ans. Deux études longitudinales contrôlées ont été menées dans deux zones géographiques différentes de notre région (143, 173). Nous avons observé un excès de symptômes respiratoires, en particulier une bronchite chronique et, dans une moindre mesure, une obstruction bronchique chez les agriculteurs par rapport à des témoins non agricoles (143, 173). Ces deux cohortes ont été régulièrement mises à jour et les analyses les plus récentes ont révélé un déclin légèrement accéléré, mais significatif, de la fonction respiratoire chez les producteurs laitiers (103, 176, 177). Le fait de travailler dans une ferme traditionnelle, en particulier dans les petites exploitations fortement exposées à la poussière organique et aux

micro-organismes, était associé à une détérioration de la fonction pulmonaire (98, 105, 144, 178, 179).

Nous nous intéressons maintenant à la BPCO en milieu agricole. Les connaissances concernant la BPCO liée aux poussières organiques sont en effet assez limitées comme pour la plupart des autres étiologies de la BPCO du non-fumeur. Dans une étude transversale incluant près de 5000 sujets, nous avons confirmé l'excès de risque de BPCO selon les définitions de GOLD et de LLN dans plusieurs catégories d'élevage en France, y compris l'élevage de bétail et ce indépendamment du tabagisme (93).

Afin de faire un état des lieux des connaissances sur le sujet, nous avons réalisé une revue générale sur les principales caractéristiques des BPCO professionnelles (article 1). Pour estimer la prévalence de la BPCO chez les agriculteurs, nous avons réalisé une revue systématique de la littérature suivie d'une méta-analyse (article 2). Nous avons mis en évidence que les éleveurs bovins, les éleveurs de porcs et les éleveurs de volailles présentaient un sur-risque de BPCO. Néanmoins, les critères définissant la BPCO étaient très variable d'une étude à l'autre (existence d'une bronchite chronique, existence d'un TVO). Parmi les 22 études incluses dans la méta-analyse, une seule étude évaluait la prévalence de la BPCO selon sa définition actuelle. Les résultats de la méta-analyse sur la prévalence de la BPCO doivent donc être interprétés avec vigilance.

Par ailleurs, la cohorte AGRICOH qui regroupe les données de 18 cohortes issus de 11 pays et incluant plus de 200 000 patients, nous a permis d'évaluer la prévalence de la BPCO à l'échelle mondiale (étude 3). Bien que les critères définissant la BPCO étaient également variables d'une étude à l'autre, la cohorte AGRICOH a permis de mettre en évidence une grande disparité de la prévalence de la BPCO à travers le monde variant de 0,5 à 15% en milieu agricole et était similaire entre les hommes et les femmes. Les facteurs expliquant cette variation n'ont pas pu être étudiés du fait de l'absence d'évaluation du type et de la diversité des pratiques agricoles dans les différentes cohortes. Par ailleurs, la disparité des définitions de BPCO représente également une limite importante de cette étude. Ce travail collaboratif a néanmoins le mérite de donner un aperçu de l'impact global de la BPCO dans le milieu agricole dans de nombreuses régions et environnements géographiques et dans diverses populations d'étude.

De multiples questions demeurent quant à la caractérisation pulmonaire et systémique ainsi que la prise en charge de la BPCO secondaire aux poussières organiques. C'est dans cette optique que l'étude BALISTIC-1 a été initiée en 2012 (141).

La première partie de l'étude a consisté en un dépistage au sein de 2 populations incluant des sujets exposés aux poussières organiques (agriculteurs) et des sujets non exposés (population générale). Parmi les 4 788 sujets inclus, la moitié était des agriculteurs en milieu de production laitière et l'autre moitié n'avait pas d'exposition aux poussières organiques.

Les principaux résultats de cette première partie sont:

- la prévalence de la BPCO secondaire aux poussières organiques est similaire à celle de la BPCO tabagique mais avec une prévalence du tabagisme beaucoup plus faible. La prévalence est estimée à 8.0% selon les critères GOLD et de 6.2% selon les critères LIN (article 5). Il s'agit d'une cause importante de BPCO chez le non-fumeur.

- la BPCO secondaire aux poussières organiques est associée avec une moindre prévalence de comorbidités tel que le diabète ou la cardiopathie ischémique par rapport au BPCO tabagique (article 9). Le tabagisme, et non pas l'existence d'une BPCO, apparaît comme le principal déterminant de l'existence de comorbidités chez les patients présentant une BPCO légère à modérée.

Parmi ces sujets dépistés, 400 patients ont été sélectionnés afin de constituer 2 groupes de sujets exposés aux poussières organiques (BPCO et témoins) et 2 groupes de sujets non exposés (BPCO et témoins). Ces 4 groupes ont permis de caractériser la BPCO secondaire à l'exposition aux poussières organiques sur le plan pulmonaire et systémique (2<sup>nd</sup>e partie).

Cette seconde partie de l'étude a permis de montrer que par rapport aux sujets présentant une BPCO tabagique, les sujets présentant une BPCO secondaire à l'exposition aux poussières organiques :

- ont une dyspnée d'effort moins importante et une meilleure qualité de vie (article 5);
- se présentent avec une capacité de diffusion moins altérée suggérant une moindre destruction parenchymateuse (article 5 et 8) ;

- semblent avoir une inflammation systémique moins importante et être associé à un phénotype Th2 (article 5) ;

- ont de meilleures capacités et une meilleure adaptation ventilatoire à l'exercice, indépendamment de la sévérité de l'obstruction bronchique (article 8);

- ont un risque cardiovasculaire, évaluée par la rigidité vasculaire, plus faible. Le tabagisme, et non pas l'existence d'une BPCO, semble être le facteur déterminant du risque cardiovasculaire (article 10) ;

- présentent une prévalence comparable d'anxiété et dépression. La dépression est associée à la sévérité de l'obstruction bronchique (article 11) ;

- ont des habitudes alimentaires semblables (article 12).

Par ailleurs, l'exposition aux poussières organiques, évaluée soit sous forme de questionnaire (caractéristiques de la ferme et de l'exposition), soit de manière quantitative, n'est pas suffisante pour expliquer à elle seule la survenue de la BPCO (article 5 et 6). Enfin, les facteurs liés à l'hôte semblent être un des déterminants de la BPCO liée aux poussières organiques. En effet, nous avons mis en évidence que les patients présentant une BPCO secondaire aux poussières organiques avaient des marqueurs d'atopie plus élevés que les témoins exposés (article 7).

### **Perspectives : conséquences des résultats obtenus**

L'absence de données phénotypiques de la BPCO du non-fumeur a longtemps semé le doute quant à son appartenance à la classique BPCO du fumeur. A cet égard, la caractérisation de la BPCO secondaire aux poussières organiques qui est une cause de BPCO chez le non-fumeur est intéressante. Au même titre que les autres BPCO du non-fumeur, il est intéressant de constater que la fréquence des symptômes respiratoires tels que la toux et les expectorations est similaire à celle de la BPCO tabagique. La fréquence et la gravité des exacerbations respiratoires est également comparable à celle de la BPCO tabagique (138).

Par ailleurs, bien que les patients présentant une BPCO liées aux poussières organiques et ceux présentant une BPCO tabagique aient une obstruction bronchique et une distension dynamique à l'effort, les non-fumeurs présentant une BPCO ont une capacité de diffusion moins altérée (et donc une moindre destruction parenchymateuse pulmonaire) que les BPCO tabagique. La BPCO liée aux poussières organiques semble donc induire principalement un remodelage des voies aériennes plutôt qu'un emphysème à la différence de la BPCO liée au tabagisme et peut être assimilé à un phénotype d'atteinte prédominante des bronches (180).

Cette atteinte bronchique prédominante pourrait en partie expliquer la moindre prévalence des comorbidités cardiaques ou extra-pulmonaires dans la BPCO secondaires aux poussières organique. Une autre explication pourrait être une implication différente des voies de signalisation de l'inflammatoires. Le tabagisme est en effet associé à une augmentation de l'inflammation systémique qui est un élément important de la physiopathologie des maladies cardiovasculaires (181, 182). Conformément à cette hypothèse, les niveaux de biomarqueurs inflammatoires chez les patients atteints de BPCO tabagique dans notre étude étaient légèrement plus élevés que chez les patients présentant une BPCO liée aux poussières

organiques. L'augmentation des niveaux de biomarqueurs inflammatoires pourrait également être expliquée par des événements inflammatoires survenant dans les poumons et reflétant une activité accrue de la maladie et se propageant dans la circulation systémique (183).

L'étude actuelle pourrait également avoir des implications importantes. En effet, les recommandations en vigueur quant à la prise en charge de la BPCO ont été établies à partir des données de la BPCO tabagique.

Le premier point clé pour la prise en charge optimale dans la BPCO réside dans le diagnostic précoce qui, associé à une prise en charge adaptée, est indispensable pour diminuer l'impact humain et financier de cette pathologie. Le travail actuel est important à cet égard car il renforce l'importance de l'exposition aux poussières organiques dans la genèse de la BPCO. Chez les patients exposés à des poussières organiques et présentant des symptômes respiratoires, il paraît important de rechercher une BPCO.

Le second point clé est la réduction de l'exposition des sujets (prévention primaire) et des patients (prévention secondaire) aux aérocontaminants responsables du développement de la BPCO à l'instar du sevrage tabagique (184). La prévention de l'exposition aux poussières organiques doit être précoce et bien conduite. Elle doit comporter d'une part une prévention technique collective (modernisation du lieu de travail), qui permet la réduction des émissions de poussières organiques, en favorisant leur évacuation et en développant l'automatisation des tâches, ce qui permet de limiter le contact avec l'ambiance polluée. D'autre part, une prévention technique individuelle, est nécessaire et consiste essentiellement à utiliser des appareils de protection respiratoire (port de masque) et à conseiller l'agriculteur sur ses pratiques professionnelles. Cela est d'autant plus important chez les patients fumeurs, car l'association du tabagisme à l'exposition professionnelle en milieu agricole a un effet additif, voire synergique, sur le déclin de la fonction respiratoire (98, 184). La lutte contre le tabagisme est donc un objectif important dans cette population doublement exposée.

Le troisième point clé concerne la recherche des comorbidités associée à la BPCO. De multiples comorbidités coexistent avec la BPCO tabagique, indépendamment de sa sévérité, et ont un impact important dans l'évolution de la maladie. La BPCO tabagique doit donc faire l'objet d'une prise en charge globale. A l'inverse, la BPCO secondaire aux poussières organiques n'est pas associée avec un sur-risque de comorbidités. Le tabagisme, plus que la BPCO, semble jouer un rôle important dans la genèse de ces comorbidités. La recherche systématique de comorbidités dans la BPCO secondaire aux poussières organiques n'est donc probablement pas utile.



Le dernier point clé concerne la prise en charge thérapeutique de cette BPCO. L'étude actuelle pourrait avoir d'importantes implications sur la prise en charge médicamenteuses. En effet, l'ensemble des essais thérapeutiques dans la BPCO publiés à ce jour ont systématiquement exclus les patients non fumeurs. De telles études ont principalement montré les avantages des traitements bronchodilatateurs et les effets néfastes potentiels d'une monothérapie avec des corticostéroïdes inhalés dans la BPCO, par opposition à l'asthme pour lequel l'inverse est vrai. Le rôle de ces médicaments dans la BPCO agricole et plus largement dans la BPCO du non-fumeur est inconnu. En l'absence de données fiables, l'incertitude quant à la prise en charge thérapeutique est importante, puisque certains sont vraisemblablement traités comme des patients atteints de BPCO et d'autres comme des patients asthmatique. A l'aune des résultats de notre étude, un traitement bronchodilatateur semble être une option thérapeutique adaptée compte tenu d'une distension dynamique à l'effort, en complément des mesures de prévention (185). Par ailleurs, nous avons montré que le phénotype de la BPCO secondaire aux poussières organiques était différent de la BPCO tabagique. Ce phénotype semble être proche de celui de la BPCO secondaire à l'exposition à la biomasse dans lequel le profil inflammatoire serait plus de type Th2 (comme dans l'asthme) (186-190). L'intérêt d'une corticothérapie inhalée dans la BPCO secondaire aux poussières organiques reste à définir.

Notre attention a été focalisée depuis longtemps sur les dangers du tabagisme ; il apparaît aujourd'hui important de cesser de perpétuer le mythe selon lequel la BPCO ne concerne que les fumeurs. Les futurs travaux de recherche doivent donc se focaliser sur la prise en charge thérapeutique de la BPCO des non-fumeurs. Bien que son pronostic soit meilleur que celui de la BPCO tabagique, il reste moins bon par rapport à celui de sujets ne présentant pas de BPCO et est associé à un risque accru d'hospitalisations pour cause respiratoire (138). Le défi des années à venir est de trouver de nouvelles solutions pour les quelques 100 millions de non-fumeurs présentant une BPCO dans le monde.

### **Perspectives : recherches en cours et à venir**

Ces premiers résultats donnent un aperçu des différences phénotypiques potentielles, mais doivent être interprétés avec prudence et doivent être validés à partir des données longitudinales.

Un suivi avec une réévaluation clinique et fonctionnelle est en cours (étude BALISTIC-2 - NCT03468101) depuis septembre 2018. Tous les sujets inclus dans l'étude BALISTIC-1 et

présentant une BPCO sont invités à venir au CHU de Besançon pour réaliser différents examens environ six ans après leur première visite. Les examens réalisés sont identiques à ceux réalisés lors de la première visite avec, en plus, la réalisation d'un scanner thoracique. L'objectif principal est d'analyser la relation entre l'étiologie de la BPCO (exposition à des poussières organiques vs. liée à une exposition tabagique) et le déclin de la fonction respiratoire (correspondant au déclin annuel du VEMS, exprimé en mL/an). Les objectifs secondaires seront d'analyser la relation entre l'étiologie de la BPCO et l'évolutivité de la BPCO (dyspnée, qualité de vie, mesures physiologiques respiratoires au repos et à l'effort), l'épigénétique (méthylation et acétylation des histones et méthylation de l'ADN) et l'existence d'un emphysème. Cette étude permettra des données supplémentaires sur la physiopathologie ainsi qu'un certain nombre de caractéristiques de la BPCO secondaire à l'exposition à des poussières organiques.

### **Conclusion**

La BPCO secondaire à l'exposition aux poussières organiques est une cause de BPCO chez le non-fumeur. Elle est caractérisée par une atteinte prédominante des voies aériennes et la moindre présence d'une inflammation systémique et de comorbidités notamment cardiovasculaire. Un suivi longitudinal de ces sujets est nécessaire pour évaluer leur pronostic à long terme. La prise en charge pharmacologique de cette BPCO du non-fumeur reste à définir mais un diagnostic précoce et une prévention primaire et secondaire vis à vis des poussières organiques sont essentiels.



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## VII. ANNEXES

### Annexe 1.

Code inclusion\* |\_|\_|



Patient n° |\_|\_| |\_|\_|\_| (code inclusion suivi du numéro d'inclusion par ordre chronologique)

Date de la visite de screening : |\_|\_| |\_|\_| 201|\_|

Lieu de la visite de screening : .....

# BALISTIC

**BPCO EN MILIEU RURAL : DEPISTAGE,  
CARACTERISATION ET CONSTITUTION D'UNE  
COHORTE**

**Questionnaire médical de screening - MSA**

*Cette première page est réservée aux enquêteurs et vous n'avez pas à la remplir.  
Commencez le remplissage du questionnaire page suivante.*

\* BA : BPCO Agricole  
BN : BPCO Non agricole

TA : Témoin Agricole  
TN : Témoin Non agricole

*Cadre réservé aux IDE :*  
*Poids : |\_|\_|\_| ; |\_| kg*  
*Taille : |\_|\_|\_| cm*





## 4. FACTEURS DE RISQUE

### 4.1. ANTECEDENTS MEDICAUX

4.1.1. Avez-vous fait (ou avez-vous) une maladie respiratoire ? NON  OUI

**Si OUI**, laquelle ? En quelle année ? Depuis combien d'années ?

Pneumopathie d'hypersensibilité (PHS) (poumon de fermier, maladie des éleveurs d'oiseaux, autres PHS) <input type="checkbox"/>	_ _ _	_
Asthme <input type="checkbox"/>	_ _ _	_
Dilatation des bronches <input type="checkbox"/>	_ _ _	_
Autre(s) ; précisez <input type="checkbox"/>	_ _ _	_


.....  
.....

4.1.2. Avez-vous fait (ou avez-vous) une maladie cardiaque ou vasculaire ? NON  OUI

**Si OUI**, laquelle ? .....  
En quelle année ? |\_|\_|\_| Depuis combien d'années ? |\_|

4.1.3. Etes-vous actuellement atteint d'une maladie grave ? NON  OUI

**Si OUI**, laquelle ? .....  
Depuis combien d'années ? |\_|

 **Si vous avez une pneumopathie d'hypersensibilité (maladie du poumon de fermier, maladie des éleveurs d'oiseaux, ...), vous n'êtes pas obligé(e) de remplir la suite de ce questionnaire.**

### 4.2. TRAITEMENTS MEDICAMENTEUX

4.2.1. Prenez-vous actuellement des médicaments (gélules, comprimés, injections, sprays, aérosols...)? NON  OUI


**Si OUI**, lequel (lesquels) ?  
.....  
.....  
.....  
.....

4.2.2. Prenez-vous actuellement un médicament par la bouche ou en injection (ne comptez pas les sprays ou les aérosols) qui contient de la cortisone ? NON  OUI

**Si OUI**, lequel ?  
.....  
.....

4.2.3. Prenez-vous actuellement un médicament immuno-suppresseur ? NON  OUI


**Si OUI**, lequel ?  
.....

 **Si vous prenez un médicament contenant de la cortisone ou un immuno-suppresseur, vous n'êtes pas obligé(e) de remplir la suite de ce questionnaire.**

### 4.3. HABITUDES TABAGIQUES

4.3.1. Fumez-vous ou avez-vous fumé antérieurement ?

NON  OUI

 Si vous répondez "Non" à la question, passez directement à la question 4.3.3.

**Si OUI,**

A quel âge avez-vous commencé ?

|\_|\_|

Actuellement ou avant d'arrêter de fumer, combien fumez ou fumiez-vous en moyenne de :

- cigarettes par jour |\_|\_|
- cigarillos par jour |\_|\_|
- cigares par semaine |\_|\_|
- tabac à pipe par semaine |\_|\_|

4.3.2. Si vous avez arrêté de fumer, depuis combien de temps ?

|\_|\_| an(s)

Si moins d'1 an, |\_|\_| mois

4.3.3. Si vous n'avez jamais fumé, y a-t-il, dans votre entourage familial ou professionnel proche, des personnes fumant régulièrement (tous les jours) en votre présence ?

NON  OUI

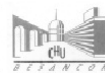
**Si OUI,** depuis combien d'années ?

|\_|\_|

**A remplir par l'enquêteur** 4.3.4. Nombre de paquets/année |\_|\_|

**Merci d'avoir répondu à ce questionnaire !**

**Nom de l'enquêteur :** .....



## Synthèse screening

A remplir par l'enquêteur

Patient d'accord pour l'étude BALISTIC :  oui  non

1. Patient exclu

**Motifs :**

- Profession   
Age   
Grossesse   
Maladie   
Médicaments   
Autres

Précisez.....

2. Patient incluable

A   
N

3. Spirométrie

- BA      Sexe       Age       Tabac\* : NF – EF – FA      PA
- BN      Sexe       Age       Tabac\* : NF – EF – FA      PA
- TA      Sexe       Age       Tabac\* : NF – EF – FA      PA
- TN      Sexe       Age       Tabac\* : NF – EF – FA      PA

**\* Enquêteur : Entourez la bonne réponse et insérez le nombre de PA chez les fumeurs et ex-fumeurs.**

## Annexe 2. Questionnaire médical (étude BalistiC)

Code inclusion\* |\_|\_|

Patient n° |\_|\_| |\_|\_|\_|

Date de la visite : |\_|\_| |\_|\_| 201|\_|

**BALISTIC**

**BPCO EN MILIEU RURAL :**

**DEPISSTAGE, CARACTERISATION ET**

**CONSTITUTION D'UNE COHORTE**

**Visite initiale – Questionnaire médical**

\* BA : BPCO Agricole  
BN : BPCO Non agricole

TA : Témoin Agricole  
TN : Témoin Non agricole



## 4. SYMPTOMES EN ETAT STABLE

### 4.1. TOUX ET / OU EXPECTORATION CORRESPONDANT A UNE BRONCHITE CHRONIQUE\* :

OUI  NON

**Si oui : Crises de sifflements thoraciques ? :**

OUI  NON

### 4.2. DYSPNEE

• Echelle MRC : Score :

- 0 : Essoufflé seulement pour des efforts intenses
- 1 : Essoufflé en hâtant le pas ou en montant une légère côte
- 2 : Marche sur terrain plat plus lentement que les sujets de son âge  
**ou** doit s'arrêter en marchant à son rythme sur terrain plat
- 3 : Doit s'arrêter après 100 m ou quelques minutes de marche
- 4 : Trop essoufflé pour sortir de la maison

• Baseline Dyspnea Index : (merci de compléter l'annexe n°2 puis de reporter le score ici)

Handicap fonctionnel :

Amplitude de l'activité :

Amplitude de l'effort :

**Total** :

## 5. EXACERBATION

### 5.1. ANTECEDENTS D'EXACERBATION

oui  non

Nombre d'exacerbations (caractérisées par au moins 1 des items listés au paragraphe 5.2)  
dans l'année précédente :

exacerbations

Dont :  Traités exclusivement en ambulatoire avec consultation en urgence.

et / ou AB   
et / ou corticoïdes

Traités en hospitalisation, sans assistance ventilatoire.

Traités en hospitalisation, avec assistance ventilatoire.

### 5.2. SYMPTOMES APPARUS OU MAJORES (pendant au moins 48h) :

- Toux
- Expectoration (volume)
- Purulence de l'expectoration
- Sibilants
- Dyspnée

## 6. NEZ

- Existence de symptômes rhino-sinusiens : oui  non   
Si oui, précisez :
  - Obstruction chronique
  - Rhinorrhée chronique
  - Anosmie
- Symptômes : - Saisonniers   
- Per annuels

## 7. CO-MORBIDITES CONNUES

### Définition :

Maladie chronique (en dehors de la BPCO) diagnostiquée et nécessitant un traitement au long cours.

Oui  Non

### Précisez :

- HTA oui  non
- Cardiopathie ischémique oui  non
- Insuffisance cardiaque oui  non   
Si oui (origine):
  - rythmique
  - valvulaire
  - ischémique
  - non connue
- Diabète oui  non
- Dyslipidémie oui  non
- Ostéoporose traitée oui  non   
Si oui,
  - fracturaire
  - non fracturaire
- Polyarthrite rhumatoïde oui  non
- Impotence fonctionnelle d'origine articulaire oui  non
- Reflux gastro-oesophagien oui  non
- Syndrome d'apnées obstructives du sommeil oui  non   
Si oui, Diagnostiquée avec indication appareillage oui  non
- Pathologie relevant de la psychiatrie oui  non
- Cancer bronchique oui  non   
Si oui, date du premier diagnostic | | | | | | | | | |
- Activités physiques : questionnaire rempli oui  non

•Autres maladies chroniques (précisez): .....

.....



## 8. TRAITEMENT USUEL

**Enquêteur : Joignez une copie de l'ordonnance du patient ou notez en Annexe n°1 tous les médicaments pris par le sujet et remplissez secondairement les paragraphes 8.1 et 8.2.**

### 8.1. TRAITEMENT(S) RESPIRATOIRE(S) AU LONG COURS oui non

#### Inhalés

- Bêta<sub>2</sub> courte durée
- Bêta<sub>2</sub> longue durée
- Bêta<sub>2</sub> longue durée + corticoïde
- Anticholinergique courte durée
- Anticholinergique longue durée
- Théophylline
- Corticostéroïde inhalé

#### Per os

- Corticostéroïde oral au long cours\*   
*si oui, posologie moyenne ..... en mg*
- Antileucotriènes
- Roflumina

#### Vaccins

- Vaccin anti-grippal
- Vaccin anti-pneumococcique

#### Autres

- Oxygénothérapie de longue durée
- VI
- VNI
- Kinésithérapie
- Réhabilitation antérieure
- Programme d'éducation

### 8.2. TRAITEMENT(S) EXTRA-RESPIRATOIRE(S) EN COURS oui non

- IEC
- Bêta bloquants
- Sartans/ARA2
- Diurétiques
- Antiagrégants (aspirine)
- Antiagrégants (autres)
- Statines
- Anti-arythmiques
- Anticoagulants
- Anxiolytiques
- Antidépresseurs
- Hypnotiques
- Traitement de biothérapie

• Autres traitements pertinents

Précisez : .....



## ANNEXE N° 2

### BASELINE DYSPNEA INDEX

Cochez une réponse décrivant le mieux la manière dont l'affection respiratoire influe sur la vie quotidienne du patient / de la patiente.

Les activités habituelles correspondent aux tâches de la vie quotidienne, à l'entretien et au nettoyage du logement, aux travaux d'entretien de la cour et de jardinage, aux courses, etc.

#### 1. Handicap fonctionnel :

Degré 4	<input type="checkbox"/>	<i>Aucun handicap.</i> Capable d'effectuer les activités et les occupations habituelles sans manquer de souffle.
Degré 3	<input type="checkbox"/>	<i>Léger handicap.</i> Handicap marqué lors d'au moins une activité, mais aucune des activités n'étant complètement abandonnée. Réduction des activités professionnelles ou de la vie courante, que le manque de souffle provoque légèrement ou de façon non évidente.
Degré 2	<input type="checkbox"/>	<i>Handicap modéré.</i> Le patient a dû changer d'emploi <i>et/ou</i> abandonner au moins une de ses activités habituelles suite à ses problèmes de manque de souffle.
Degré 1	<input type="checkbox"/>	<i>Grave handicap.</i> Le patient est incapable de travailler ou a dû abandonner la plupart ou l'intégralité de ses activités habituelles suite à ses problèmes de manque de souffle.
Degré 0	<input type="checkbox"/>	<i>Très grave handicap.</i> Le patient est incapable de travailler et a dû abandonner la plupart ou l'intégralité de ses activités habituelles suite à ses problèmes de manque de souffle.
W	<input type="checkbox"/>	<i>Niveau indéterminé.</i> Le manque de souffle handicape le patient, mais son niveau ne peut être spécifié. Les informations ne permettent pas de catégoriser le handicap.
X	<input type="checkbox"/>	<i>Inconnu.</i> Pas d'information disponible sur le handicap.
Y	<input type="checkbox"/>	<i>Handicap provoqué par des causes autres que le manque de souffle.</i> Par exemple, problème musculo-squelettique ou douleur dans la poitrine.

Exemples d'activités limitées : .....

.....

.....

.....

Cochez une réponse

**2. Amplitude de l'activité :**

Degré 4	<input type="checkbox"/>	<i>Extraordinaire.</i> Devient essoufflé lors d'activités extraordinaires telles que le fait de porter des charges lourdes sur une surface plane, de monter une côte avec des charges plus légères ou de courir. Pas d'essoufflement lors de tâches ordinaires
Degré 3	<input type="checkbox"/>	<i>Importante.</i> Devient essoufflé lors d'activités importantes telles que le fait de monter une forte côte, plus de trois étages par l'escalier ou de porter une charge modérée sur une surface plane.
Degré 2	<input type="checkbox"/>	<i>Moyenne.</i> Devient essoufflé lors d'activités modérées ou d'intensité moyenne telles que le fait de monter une côte de déclivité moyenne, moins de trois étages par l'escalier ou de porter une charge légère sur une surface plane.
Degré 1	<input type="checkbox"/>	<i>Légère.</i> Devient essoufflé lors d'activités légères telles que le fait de marcher sur une surface plane, de se laver ou de rester en position debout.
Degré 0	<input type="checkbox"/>	<i>Aucune activité.</i> Est au repos, en position assise ou couchée.
W	<input type="checkbox"/>	<i>Niveau indéterminé.</i> L'essoufflement handicape le patient lors de ses activités, mais son niveau ne peut être spécifié. Les informations ne permettent pas de catégoriser le handicap.
X	<input type="checkbox"/>	<i>Inconnu.</i> Pas d'information disponible sur les limites de l'amplitude des activités.
Y	<input type="checkbox"/>	<i>Handicap provoqué par des causes autres que l'essoufflement.</i> Par exemple, problème musculo-squelettique ou douleur dans la poitrine.

Cochez une réponse décrivant le mieux la manière dont l'affection respiratoire influe sur la vie quotidienne du patient / de la patiente.

**3. Amplitude de l'effort :**

Degré 4	<input type="checkbox"/>	<i>Extraordinaire.</i> Devient essoufflé uniquement au cours du plus grand effort possible. Pas d'essoufflement au cours d'un effort normal.
Degré 3	<input type="checkbox"/>	<i>Importante.</i> Devient essoufflé au cours d'efforts importants mais non maximaux. Les tâches sont réalisées sans pause sauf pour les tâches demandant un effort extraordinaire, réalisées alors avec pauses.
Degré 2	<input type="checkbox"/>	<i>Moyenne.</i> Devient essoufflé au cours d'efforts moyens. Les tâches sont réalisées avec des pauses intermittentes et demandent plus de temps que pour une personne normale.
Degré 1	<input type="checkbox"/>	<i>Faible.</i> Devient essoufflé au cours d'un effort léger. Les tâches requérant peu d'efforts ou les tâches plus difficiles sont exécutées avec des pauses intermittentes et demandent plus de temps (50 à 100%) que pour une personne normale.
Degré 0	<input type="checkbox"/>	<i>Aucun effort.</i> Est essoufflé au repos, en position assise ou couchée.
W	<input type="checkbox"/>	<i>Niveau indéterminé.</i> L'essoufflement restreint la capacité du patient à fournir des efforts, mais son niveau ne peut être spécifié. Les informations ne permettent pas de catégoriser le handicap.
X	<input type="checkbox"/>	<i>Inconnu.</i> Pas d'information disponible sur la limitation des efforts.
Y	<input type="checkbox"/>	<i>Handicap provoqué par des causes autres que l'essoufflement.</i> Par exemple, problème musculo-squelettique ou douleur dans la poitrine.

Exemples d'efforts concernés : .....

.....

.....

.....

**Baseline Dyspnea Index : Total : | | | / 12**

**Merci d'avoir rempli ce questionnaire.**

**Annexe 3. Auto-questionnaire sur les symptômes, les allergies, les antécédents médicaux, et la qualité de vie (étude BalistiC)**

Code inclusion\* |\_|\_|

Patient n° |\_|\_| |\_|\_|

Date de la visite : |\_|\_| |\_|\_| 201|\_|

**BALISTIC**

**BPCO EN MILIEU RURAL :**

**DEPISSTAGE, CARACTERISATION ET**

**CONSTITUTION D'UNE COHORTE**

**Visite initiale – Auto-questionnaire**

\* BA : BPCO Agricole  
BN : BPCO Non agricole

TA : Témoin Agricole  
TN : Témoin Non agricole

## 1. SIFFLEMENTS – ASTHME - ALLERGIES

### 1.1. SIFFLEMENTS

1.1.1. Avez-vous eu des sifflements dans la poitrine, à un moment quelconque, dans les 12 derniers mois ? NON  OUI

**Si OUI :**

1.1.1.1. Avez-vous eu été essoufflé(e), même légèrement, quand vous aviez ces sifflements ? NON  OUI

1.1.1.2. Avez-vous eu ces sifflements quand vous n'étiez pas enrhumé(e) ? NON  OUI

1.1.2. Avez-vous déjà eu des sifflements dans la poitrine à un moment quelconque de votre vie ? NON  OUI

1.1.3. Vous êtes-vous réveillé(e) avec une sensation de gêne respiratoire, à un moment quelconque, dans les 12 derniers mois ? NON  OUI

### 1.2. ASTHME


1.2.1. Avez-vous eu une crise d'essoufflement, au repos, pendant la journée, à un moment quelconque, dans les 12 derniers mois ? NON  OUI

1.2.2. Avez-vous eu une crise d'essoufflement après un effort intense, à un moment quelconque, dans les 12 derniers mois ? NON  OUI

1.2.3. Avez-vous été réveillé(e) par une crise d'essoufflement, à un moment quelconque, dans les 12 derniers mois ? NON  OUI

1.2.4. Avez-vous déjà eu des crises d'essoufflement au repos avec des sifflements dans la poitrine ? NON  OUI

1.2.5. Avez-vous déjà eu de l'asthme ? NON  OUI

 **Si Non, passez à la partie 1.3 : Allergies Personnelles**

1.2.5.1. Cela a-t-il été confirmé par un médecin ? NON  OUI

1.2.5.2. A quel âge avez-vous eu votre dernière crise d'asthme ?   ans

1.2.5.3. Pendant quels mois de l'année avez-vous habituellement des crises d'asthme ?  
Printemps  Été  Automne  Hiver

1.2.5.4. Avez-vous eu une crise d'asthme dans les 12 derniers mois ? NON  OUI

**Si OUI :** Combien de crises avez-vous eues dans les 12 derniers mois  
  crises

1.2.5.5. Prenez-vous actuellement des médicaments pour l'asthme (y compris produits inhalés, aérosols, comprimés) ? NON  OUI

### 1.3. ALLERGIES PERSONNELLES

1.3.1. Avez-vous déjà eu des allergies nasales, y compris le « rhume des foins » ?

NON  OUI

**Si OUI :**

1.3.1.1. Le diagnostic a-t-il été confirmé par un médecin ?

NON  OUI

1.3.1.2. Dans les 12 derniers mois, avez-vous eu le nez bouché ou le nez qui coulait en dehors de tout rhume ou état grippal ?

NON  OUI

1.3.2. Avez-vous déjà eu de l'eczéma ou d'autres allergies de la peau ?

NON  OUI

**Si OUI :**

1.3.2.1. Le diagnostic a-t-il été confirmé par un médecin ?

NON  OUI

1.3.2.2. Dans les 12 derniers mois, avez-vous eu de l'eczéma ou d'autres allergies de la peau ?

NON  OUI

1.3.3. Etes-vous allergique aux piqûres d'insectes ?

NON  OUI

1.3.4. Avez-vous déjà eu une conjonctivite ?

NON  OUI

**Si OUI :**

1.3.4.1. Le diagnostic a-t-il été confirmé par un médecin ?

NON  OUI

1.3.4.2. Dans les 12 derniers mois, vous est-il arrivé d'avoir les yeux larmoyants, qui piquaient ou qui brûlaient, en dehors de tout état grippal ?

NON  OUI

1.3.5. Avez-vous déjà eu des difficultés pour respirer, un œdème et/ou une éruption après avoir pris des médicaments ?

NON  OUI

### 1.4. ALLERGIES DANS LA FAMILLE

1.4.1. Combien de frères avez-vous (ou aviez-vous) ?

□□□



**Si vous n'avez aucun frère, passez à la question 1.4.2.**

1.4.1.1. Combien de frères plus âgés que vous ?

□□□

1.4.1.2. Combien de frères plus jeunes que vous ?

□□□

1.4.1.3. Combien de frères ont déjà eu de l'asthme ?

□□□

1.4.1.1. Combien d'autres frères ont eu de l'eczéma, une allergie de la peau, une allergie nasale ou le rhume des foins ?

□□□



1.4.2. Combien de soeurs avez-vous (ou aviez-vous) ?

□□□



**Si vous n'avez aucune soeur, passez à la question 1.4.3.**

1.4.1.1. Combien de soeurs plus âgées que vous ?

□□□

1.4.1.2. Combien de soeurs plus jeunes que vous ?

□□□

1.4.1.3. Combien de soeurs ont déjà eu de l'asthme ?

□□□

1.4.1.1. Combien d'autres soeurs ont eu de l'eczéma, une allergie de la peau, une allergie nasale ou le rhume des foins ?

□□□

1.4.3. Est-ce que votre mère a déjà eu de l'asthme ? NON  OUI  Ne sait pas

1.4.4. Est-ce que votre mère a déjà eu de l'eczéma, une allergie de la peau, une allergie nasale ou le rhume des foins ?

NON  OUI  Ne sait pas

1.4.5. Est-ce que votre père a déjà eu de l'asthme ? NON  OUI  Ne sait pas

1.4.6. Est-ce que votre père a déjà eu de l'eczéma, une allergie de la peau, une allergie nasale ou le rhume des foins ?

NON  OUI  Ne sait pas

1.4.7. Avez-vous partagé régulièrement votre chambre à coucher avec un ou des enfant(s) plus âgé(s) que vous avant l'âge de 5 ans ?

NON  OUI  Ne sait pas

1.4.8. Etes-vous allé(e) à l'école, dans une garderie, ou à la crèche, avec d'autres enfants avant l'âge de 5 ans ?

NON  OUI  Ne sait pas

## 2. TOUX / EXPECTORATION

2.1 Avez-vous été réveillé(e) par une quinte de toux, à un moment quelconque, dans les 12 derniers mois ?

NON  OUI

2.2 Toussez-vous habituellement en vous levant, en hiver ?

NON  OUI

2.3 Toussez-vous habituellement pendant la journée ou la nuit, en hiver ?

NON  OUI

☞ Si Non, passez à la question 2.4

2.3.1 Si oui, tousez-vous comme cela presque tous les jours pendant 3 mois de suite chaque année ?

NON  OUI

2.3.2 Si oui, depuis combien d'années ?

|\_|\_| années

2.4 Avez-vous habituellement des crachats provenant de la poitrine en vous levant, en hiver ?

NON  OUI

2.5 Avez-vous habituellement des crachats provenant de la poitrine pendant la journée ou la nuit, en hiver ?

NON  OUI

☞ Si Non, passez à la partie 3 : « Dyspnée »

2.5.1 Si oui, crachez-vous comme cela presque tous les jours pendant 3 mois de suite chaque année ?

NON  OUI

2.5.2 Si oui, depuis combien d'années ?

|\_|\_| années

## 3. DYSPNEE

3.1 Vous sentez-vous essoufflé(e) ?

NON  OUI

Si oui, pour quelle raison ? .....

3.2 Etes-vous essoufflé(e) en marchant vite en terrain plat, ou en montant une côte légère à un pas normal ?

NON  OUI

3.3 Etes-vous essoufflé(e) quand vous marchez avec d'autres gens de votre âge en terrain plat ?

NON  OUI

3.4 Vous arrive-t-il de vous arrêter pour reprendre votre souffle quand vous marchez à votre propre allure en terrain plat ?

NON  OUI

## ANNEXE N° 1

### ECHELLE D'EPWORTH POUR L'EVALUATION DE LA SOMNOLENCE

Dans les 8 circonstances suivantes, avez-vous un risque de vous endormir dans la journée ?

- Si ce risque est inexistant : cochez 0
- Si ce risque est minime : cochez 1
- Si ce risque est modéré : cochez 2
- Si ce risque est important : cochez 3

**Exemple :**

*Si le risque de vous endormir « assis en lisant un livre ou le journal » est modéré : cochez 2*

- |   |                            |                            |                            |                            |
|---|----------------------------|----------------------------|----------------------------|----------------------------|
| 1) Assis en lisant un livre ou le journal   | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 2) En regardant la télévision   | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 3) Assis, inactif, dans un lieu public<br>(cinéma, théâtre, salle d'attente)                      | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 4) Si vous êtes passager d'une voiture<br>pour un trajet d'une heure                              | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 5) En étant allongé après le repas de midi<br>lorsque les circonstances le permettent             | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 6) En étant assis, en parlant avec quelqu'un  | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 7) En étant assis, après un repas sans boisson alcoolisée   | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |
| 8) En étant au volant de la voiture, au cours d'un arrêt<br>de la circulation de quelques minutes | 0 <input type="checkbox"/> | 1 <input type="checkbox"/> | 2 <input type="checkbox"/> | 3 <input type="checkbox"/> |

TOTAL :

## ANNEXE N° 2 - ECHELLE HAD

### Pourquoi ces questions ?

Les médecins savent que les émotions jouent un rôle important dans la plupart des maladies. Si votre médecin est au courant des émotions que vous éprouvez, il pourra mieux vous aider. Ce questionnaire a été conçu de façon à permettre à votre médecin de se familiariser avec ce que vous éprouvez vous-même sur le plan émotif.

Ne faites pas attention aux chiffres et aux lettres imprimés à gauche du questionnaire. Lisez chaque série de questions et **soulignez la réponse qui exprime le mieux ce que vous avez éprouvé au cours de la semaine qui vient de s'écouler**. Ne vous attardez pas sur la réponse à faire, votre réaction immédiate à chaque question fournira probablement une meilleure indication de ce que vous éprouvez, qu'une réponse longue et méditée.

<b>A</b>	<b>Je me sens tendu(e) ou énervé(e)</b>	<b>D</b>	<b>J'ai l'impression de fonctionner au ralenti</b>
3	La plupart du temps	3	Presque toujours
2	Souvent	2	Très souvent
1	De temps en temps	1	Parfois
0	Jamais	0	Jamais
<b>D</b>	<b>Je prends plaisir aux mêmes choses qu'autrefois</b>	<b>A</b>	<b>J'éprouve des sensations de peur et j'ai l'estomac noué</b>
0	Oui, tout autant	0	Jamais
1	Pas autant	1	Parfois
2	Un peu seulement	2	Assez souvent
3	Presque plus	3	Très souvent
<b>A</b>	<b>J'ai une sensation de peur comme si quelque chose d'horrible allait m'arriver</b>	<b>D</b>	<b>Je ne m'intéresse plus à mon apparence</b>
3	Oui, très nettement	3	Plus du tout
2	Oui, mais ce n'est pas trop grave	2	Je n'y accorde pas autant d'attention que je le devrais
1	Un peu, mais cela ne m'inquiète pas	1	Il se peut que je n'y fasse plus autant attention
0	Pas du tout	0	J'y prête autant d'attention que par le passé
<b>D</b>	<b>Je ris facilement et vois le bon côté des choses</b>	<b>A</b>	<b>J'ai la bougeotte et n'arrive plus à tenir en place</b>
0	Autant que par le passé	3	Oui, c'est tout à fait le cas
1	Plus autant qu'avant	2	Un peu
2	Vraiment moins qu'avant	1	Pas tellement
3	Plus du tout	0	Pas du tout
<b>A</b>	<b>Je me fais du souci</b>	<b>D</b>	<b>Je me réjouis d'avance à l'idée de faire certaines choses</b>
3	Très souvent	0	Autant qu'avant
2	Assez souvent	1	Un peu moins qu'avant
1	Occasionnellement	2	Bien moins qu'avant
0	Très occasionnellement	3	Presque jamais
<b>D</b>	<b>Je suis de bonne humeur</b>	<b>A</b>	<b>J'éprouve des sensations soudaines de panique</b>
3	Jamais	3	Vraiment très souvent
2	Rarement	2	Assez souvent
1	Assez souvent	1	Pas très souvent
0	La plupart du temps	0	Jamais
<b>A</b>	<b>Je peux rester tranquillement assis(e) à ne rien faire et me sentir décontracté(e)</b>	<b>D</b>	<b>Je peux prendre plaisir à un bon livre ou à une bonne émission radio ou de télévision</b>
0	Oui, quoi qu'il arrive	0	Souvent
1	Oui, en général	1	Parfois
2	Rarement	2	Rarement
3	Jamais	3	Très rarement

Score HAD\* :          Score Anxiété\* :          Score Dépression\* :

\* Enquêteur : Reportez le score en vous servant de la grille spécifique de cotation HAD.

## ANNEXE N° 3

*Cette page est à remplir par l'enquêteur.*

*Enquêteur : Reportez le score en utilisant la grille spécifique de cotation QSG*

### **QUESTIONNAIRE DU SAINT GEORGE'S HOSPITAL**

*(5 pages suivantes à remplir)*

Questionnaire complété :    Oui     Non

• **Symptômes :** .....

• **Activité :** .....

• **Impact :** .....

• **Total :** .....

## 1ère PARTIE

Les questions qui suivent cherchent à déterminer l'importance des problèmes respiratoires que vous avez pu ressentir **AU COURS DES 12 DERNIERS MOIS**  
(Mettez une croix dans la case correspondant à votre réponse à chaque question)

	Presque tous les jours de la semaine (5-7 jours)	Plusieurs jours par semaine (2-4 jours)	Quelques jours par mois	Seulement pendant une infection respiratoire	Pas du tout										
1) Au cours des 12 derniers mois, avez-vous toussé ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>										
2) Au cours des 12 derniers mois, avez-vous craché ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>										
3) Au cours des 12 derniers mois, avez-vous été essoufflé(e) ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>										
4) Au cours des 12 derniers mois, avez-vous eu des crises de sifflement dans la poitrine ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>										
5) Au cours des 12 derniers mois, combien de fois avez-vous eu de crises graves ?	<table border="1"> <tr> <td>Plus de 3 crises</td> <td><input type="checkbox"/></td> </tr> <tr> <td>3 crises.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>2 crises.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>1 crise.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>Aucune crise.....</td> <td><input type="checkbox"/></td> </tr> </table>					Plus de 3 crises	<input type="checkbox"/>	3 crises.....	<input type="checkbox"/>	2 crises.....	<input type="checkbox"/>	1 crise.....	<input type="checkbox"/>	Aucune crise.....	<input type="checkbox"/>
Plus de 3 crises	<input type="checkbox"/>														
3 crises.....	<input type="checkbox"/>														
2 crises.....	<input type="checkbox"/>														
1 crise.....	<input type="checkbox"/>														
Aucune crise.....	<input type="checkbox"/>														
<i>(passez à la question 7 si vous n'avez pas eu de crise grave)</i>															
6) Au cours des 12 derniers mois, combien de temps a duré la crise la plus pénible ?	<table border="1"> <tr> <td>Une semaine ou plus ....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>3 jours ou plus.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>1 ou 2 jours.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>Moins d'une journée.....</td> <td><input type="checkbox"/></td> </tr> </table>					Une semaine ou plus ....	<input type="checkbox"/>	3 jours ou plus.....	<input type="checkbox"/>	1 ou 2 jours.....	<input type="checkbox"/>	Moins d'une journée.....	<input type="checkbox"/>		
Une semaine ou plus ....	<input type="checkbox"/>														
3 jours ou plus.....	<input type="checkbox"/>														
1 ou 2 jours.....	<input type="checkbox"/>														
Moins d'une journée.....	<input type="checkbox"/>														
7) Au cours des 12 derniers mois, dans une semaine ordinaire, combien avez-vous eu de journées sans grand problème respiratoire ?	<table border="1"> <tr> <td>Aucune journée.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>1 ou 2 jours.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>3 ou 4 jours.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>Presque tous les jours..</td> <td><input type="checkbox"/></td> </tr> <tr> <td>Tous les jours.....</td> <td><input type="checkbox"/></td> </tr> </table>					Aucune journée.....	<input type="checkbox"/>	1 ou 2 jours.....	<input type="checkbox"/>	3 ou 4 jours.....	<input type="checkbox"/>	Presque tous les jours..	<input type="checkbox"/>	Tous les jours.....	<input type="checkbox"/>
Aucune journée.....	<input type="checkbox"/>														
1 ou 2 jours.....	<input type="checkbox"/>														
3 ou 4 jours.....	<input type="checkbox"/>														
Presque tous les jours..	<input type="checkbox"/>														
Tous les jours.....	<input type="checkbox"/>														
8) Quand vous avez des sifflements, est-ce pire le matin ?	<table border="1"> <tr> <td>oui.....</td> <td><input type="checkbox"/></td> </tr> <tr> <td>non.....</td> <td><input type="checkbox"/></td> </tr> </table>					oui.....	<input type="checkbox"/>	non.....	<input type="checkbox"/>						
oui.....	<input type="checkbox"/>														
non.....	<input type="checkbox"/>														

---

## 2ème PARTIE

---

**SECTION 1** Mettez une croix dans la case correspondant à votre réponse à chaque question.

### QUE PENSEZ-VOUS DE VOTRE ETAT RESPIRATOIRE ?

- C'est mon plus gros problème.....
- Cela me pose pas mal de problèmes.....
- Cela me pose quelques problèmes.....
- Cela ne me pose aucun problème.....

### SI VOUS AVEZ OU SI VOUS AVEZ EU UNE ACTIVITE PROFESSIONNELLE

- Mes problèmes respiratoires m'ont obligé(e) à ne plus travailler.....
- Mes problèmes respiratoires m'empêchent parfois de travailler.....
- Mes problèmes respiratoires ne m'empêchent pas de travailler.....

---

### SECTION 2 VOICI QUELQUES SITUATIONS QUI, HABITUELLEMENT, VOUS ESSOUFFLENT.

Répondez en mettant une croix dans la case correspondant à à votre situation ces jours-ci

- |  | VRAI                     | FAUX                     |
|--|--------------------------|--------------------------|
| Etre assis au repos.....                         | <input type="checkbox"/> | <input type="checkbox"/> |
| Faire sa toilette ou s'habiller.....             | <input type="checkbox"/> | <input type="checkbox"/> |
| Marcher dans la maison.....                      | <input type="checkbox"/> | <input type="checkbox"/> |
| Marcher à l'extérieur sur terrain plat.....      | <input type="checkbox"/> | <input type="checkbox"/> |
| Monter un étage.....                             | <input type="checkbox"/> | <input type="checkbox"/> |
| Monter une côte.....                             | <input type="checkbox"/> | <input type="checkbox"/> |
| Pratiquer une activité physique ou sportive..... | <input type="checkbox"/> | <input type="checkbox"/> |

**SECTION 3 VOICI ENCORE QUELQUES SITUATIONS CONCERNANT VOTRE TOUX ET VOTRE ESSOUFFLEMENT.**

*Voulez-vous signaler celles qui correspondent à votre état ces jours-ci ?*

	VRAI	FAUX
Ca me fait mal quand je tousse.....	<input type="checkbox"/>	<input type="checkbox"/>
Ca me fatigue quand je tousse.....	<input type="checkbox"/>	<input type="checkbox"/>
Je suis essoufflé quand je parle.....	<input type="checkbox"/>	<input type="checkbox"/>
Je suis essoufflé quand je me penche.....	<input type="checkbox"/>	<input type="checkbox"/>
Ma toux ou ma respiration perturbe mon sommeil.....	<input type="checkbox"/>	<input type="checkbox"/>
Je m'épuise vite en faisant une activité quotidienne (par exemple : toilette, habillage, ménage).....	<input type="checkbox"/>	<input type="checkbox"/>

**SECTION 4 VOICI D'AUTRES EFFETS QUE VOS PROBLÈMES RESPIRATOIRES PEUVENT ENTRAÎNER CHEZ VOUS.**

*Voulez-vous signaler celles qui s'appliquent à vous ces jours-ci ?*

	VRAI	FAUX
Devant les autres je me sens gêné de tousser ou d'être essoufflé.....	<input type="checkbox"/>	<input type="checkbox"/>
Mes problèmes respiratoires gênent ma famille, mes amis ou mon voisinage.....	<input type="checkbox"/>	<input type="checkbox"/>
J'ai peur ou je panique quand je n'arrive plus à respirer .....	<input type="checkbox"/>	<input type="checkbox"/>
Je sens que je ne peux pas contrôler ma respiration .....	<input type="checkbox"/>	<input type="checkbox"/>
Je pense que mon état respiratoire ne va pas s'améliorer .....	<input type="checkbox"/>	<input type="checkbox"/>
Je suis devenu un grand malade en raison de mon état respiratoire .....	<input type="checkbox"/>	<input type="checkbox"/>
L'exercice physique est dangereux pour moi.....	<input type="checkbox"/>	<input type="checkbox"/>
Tout me demande un effort.....	<input type="checkbox"/>	<input type="checkbox"/>

**SECTION 5 CETTE SECTION CONCERNE VOTRE TRAITEMENT (Médicaments, aérosols, oxygène, kinésithérapie...)**

	VRAI	FAUX
Mon traitement ne m'aide pas beaucoup.....	<input type="checkbox"/>	<input type="checkbox"/>
Devant les autres, je me sens gêné de suivre mon traitement.....	<input type="checkbox"/>	<input type="checkbox"/>
Mon traitement a des effets désagréables chez moi.....	<input type="checkbox"/>	<input type="checkbox"/>
Mon traitement me gêne beaucoup dans ma vie de tous les jours.....	<input type="checkbox"/>	<input type="checkbox"/>



**SECTION 6 CETTE SECTION CONCERNE LES ACTIVITES QUOTIDIENNES QUI POURRAIENT ETRE GENEES PAR VOTRE RESPIRATION**

	VRAI	FAUX
J'ai besoin de beaucoup de temps pour faire ma toilette ou pour m'habiller.....	<input type="checkbox"/>	<input type="checkbox"/>
Je ne peux pas prendre un bain ou une douche, ou alors j'ai besoin de beaucoup de temps pour le faire.....	<input type="checkbox"/>	<input type="checkbox"/>
Je marche plus lentement que les autres ou je m'arrête pour me reposer.....	<input type="checkbox"/>	<input type="checkbox"/>
Des travaux comme le ménage me prennent beaucoup de temps ou je dois m'arrêter pour me reposer.....	<input type="checkbox"/>	<input type="checkbox"/>
Si je monte un étage, je dois aller lentement ou m'arrêter.....	<input type="checkbox"/>	<input type="checkbox"/>
Si je me dépêche ou si je marche vite, je dois m'arrêter ou ralentir.....	<input type="checkbox"/>	<input type="checkbox"/>
Ma respiration rend pénible les activités telles que monter une côte, porter des objets en montant un étage, effectuer des travaux légers de jardinage, danser, jouer aux boules.....	<input type="checkbox"/>	<input type="checkbox"/>
Ma respiration rend pénible les activités telles que porter des charges lourdes, bêcher le jardin, déblayer la neige, faire du jogging ou marcher rapidement, jouer au tennis, nager.....	<input type="checkbox"/>	<input type="checkbox"/>
Ma respiration rend pénible les activités telles que les travaux manuels lourds, la course à pied, le vélo, la natation rapide ou les sports de compétition.....	<input type="checkbox"/>	<input type="checkbox"/>

**SECTION 7 DECRIVEZ AVEC QUELLE INTENSITE VOTRE ETAT RESPIRATOIRE RETENTIT D'ORDINAIRE SUR VOTRE VIE QUOTIDIENNE ?**

	VRAI	FAUX
Je ne peux pratiquer aucun sport.....	<input type="checkbox"/>	<input type="checkbox"/>
Je ne peux pas sortir pour me distraire ou me détendre.....	<input type="checkbox"/>	<input type="checkbox"/>
Je ne peux pas sortir faire les courses.....	<input type="checkbox"/>	<input type="checkbox"/>
Je ne peux pas faire le ménage ou bricoler.....	<input type="checkbox"/>	<input type="checkbox"/>
Je ne peux pas m'éloigner beaucoup de mon lit ou de mon fauteuil.....	<input type="checkbox"/>	<input type="checkbox"/>

**MAINTENANT, POURRIEZ-VOUS COCHER LA CASE CORRESPONDANT À CE QUI DÉCRIT LE MIEUX, SELON VOUS, LA MANIÈRE DONT VOTRE ETAT RESPIRATOIRE VOUS GÈNE**

*(Ne cochez qu'une seule case)*

- Cela ne m'empêche pas de faire ce que je veux.....
- Cela m'empêche de faire une ou deux choses que j'aurais envie de faire.....
- Cela m'empêche de faire la plupart des choses que j'aurais envie de faire.....
- Cela m'empêche de faire tout ce que j'aurais envie de faire.....

**Avant de terminer, veuillez vérifier que vous avez bien répondu à toutes les questions. Merci d'avoir rempli ce questionnaire.**

## Annexe 4. Questionnaire professionnel pour les non-agriculteurs (étude Balistic)

Code inclusion | | | |

Numéro : | | | | / | | | |

Date : | | | | | | | | | |

### BALISTIC

#### Questionnaire Professionnel Non agricole



Nom : \_\_\_\_\_ Prénom : \_\_\_\_\_ Sexe : \_\_\_\_\_  
 Adresse : \_\_\_\_\_  
 Code postal : \_\_\_\_\_ Commune : \_\_\_\_\_  
 Date de naissance : \_\_\_\_\_ Téléphone : \_\_\_\_\_

#### I - Coursus professionnel :

Ce questionnaire a pour but d'évaluer votre exposition professionnelle présente et passée à d'éventuels polluants autres que le tabac  
 1-Pouvez-vous préciser succinctement, pour chacun de vos emplois successifs (éventuels) : date de début, date de fin, secteur d'activité et poste de travail ?

	Date de début (mois/année)	Date de fin (mois/année)	Secteur d'activité Exemples :	Poste de travail	Décrivez vos principales tâches à ce poste	A ce poste étiez vous exposé à des poussières, gaz ou fumées ? Si oui précisez
<i>Exemple 1</i>	12/1974	05/1985	Industrie automobile	contrôle ébavurage	Nettoyage des pièces avec un solvant, contrôle visuel à la recherche de défaut puis ébavurage	
<i>Exemple 2</i>	06/1985	-	BTP	maçon	Construction de murs, quelques travaux de démolition	
1 <sup>er</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
2 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
3 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
4 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
5 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
6 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
7 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
8 <sup>e</sup> emploi	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>
<b>Emploi actuel</b>	--/--	--/--				oui <input type="checkbox"/> non <input type="checkbox"/> <u>Si oui précisez</u>

*Ne pas remplir les cases grisées réservées au codage par les enquêteurs*

1/2

Code inclusion | | |

Numéro : | | | / | | | | |

Date : | | | | | | | | | |

**2-Travaillez-vous actuellement ?** : oui  (Passer à la question 3)

non  (Passer à la question 2.1)

**2.1 Si vous vous ne travaillez plus actuellement ? :**

2.1.1 : êtes-vous officiellement en retraite ? oui  non

2.1.2 : êtes-vous en pré-retraite? oui  non

2.1.3 : autre motif d'inactivité (précisez) \_\_\_\_\_ oui  non

2.2 : Quand avez vous arrêté de travailler ? mois | | | année | | | | |

2.3: Quelle est la raison principale de la fin de votre activité professionnelle ?:

age

raison médicale si oui :  problème respiratoire

autre maladie

raison économique

autre motif : précisez .....

## **II Exposition non-professionnelle**

**3-Etes-vous issu d'une famille d'agriculteur ?**

oui  non

si oui : 3.1 pourquoi n'exercez vous pas le métier d'agriculteur ?

par manque d'intérêt pour la profession

pour des raisons économiques

pour des raisons médicales

**4-Vivez-vous ou avez-vous vécu dans une exploitation agricole ?**

oui  non

si oui : 5.1 Année de début | | | | | Année de fin | | | | |

**5-Participez-vous ou avez-vous déjà participé aux travaux dans une exploitation agricole ?**

oui  non

si oui : 6.1 Année de début | | | | | Année de fin | | | | |

6.2 Décrivez succinctement vos principales tâches lors de ces travaux \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

*Ne pas remplir les cases grisées réservées au codage par les enquêteurs*

2/2

## Annexe 5. Questionnaire professionnel pour les producteurs laitiers (étude BalistiC)

Code inclusion [ ][ ]

Numéro : [ ][ ]/[ ][ ][ ][ ]

Date : [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

### BALISTIC

#### Questionnaire Professionnel Agricole



Nom : \_\_\_\_\_ Prénom : \_\_\_\_\_ Sexe : \_\_\_\_\_  
Adresse : \_\_\_\_\_  
Code postal : \_\_\_\_\_ Commune : \_\_\_\_\_  
Date de naissance : \_\_\_\_\_ Téléphone : \_\_\_\_\_

#### I. ACTIVITE ACTUELLE

**1. Travaillez vous actuellement:** oui  (Passer à la question 2)  
non  (Passer à la question 1.1)

##### 1.1 Si vous ne travaillez plus:

1.1.1 : êtes-vous officiellement en retraite ? oui  non   
1.1.2 : êtes-vous en pré-retraite? oui  non   
1.1.3 : autre motif d'inactivité ) \_\_\_\_\_ oui  non

1.2 : Quand avez vous arrêté de travailler? mois [ ][ ] année [ ][ ][ ][ ]

1.3 : Quelle est la raison principale de la fin de votre activité professionnelle agricole ?:

- Age  
 Raison médicale si oui :  problème respiratoire  
 autre maladie  
 raison économique  
 autre motif : précisez .....

1.4 : Vivez-vous toujours dans l'exploitation ? oui  non

1.5 : Participez vous aux activités agricoles à titre bénévole ? oui  non

1.5.1 si oui combien de temps chaque jour en moyenne  
 moins d'une heure/jour  
 plus d'une heure/jour

Quelles sont alors vos principales tâches \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

#### II. CARACTERISTIQUES DE LA FERME

**2 Bâtiments d'habitation séparés**  non  oui depuis quand :

si oui : 3.1 quelle est actuellement la distance qui sépare les bâtiments : [ ][ ][ ][ ] en mètres

**3 Stabulation libre**  non  
 oui mais seulement dans une partie des bâtiments, depuis quand : \_\_\_\_\_  
 oui partout , depuis quand : \_\_\_\_\_

**4 Couloir central**  non  
 oui mais seulement dans une partie des bâtiments, depuis quand : \_\_\_\_\_

*Ne pas remplir les cases grisées réservées au codage par les enquêteurs*

1/4

Code inclusion [ ][ ]

Numéro : [ ][ ] / [ ][ ] [ ][ ]

Date : [ ][ ] [ ][ ] [ ][ ] [ ][ ] [ ][ ] [ ][ ]

oui partout , depuis quand : \_\_\_\_\_

**5 Griffe de chargement** non  oui  depuis quand :

**6 Griffe d'alimentation** oui  non  depuis quand :

**7 Pailleuse** oui  non  depuis quand :

**8 Salle de traite**  non  
 oui mais seulement dans une partie des bâtiments, depuis quand : \_\_\_\_\_  
 oui partout , depuis quand : \_\_\_\_\_

**9 Ventilation de l'étable**  
 non  
 oui mais seulement dans une partie des bâtiments, depuis quand : \_\_\_\_\_  
 oui partout, depuis quand : \_\_\_\_\_

Si oui : 9.1 système électrique ? oui  non

### III. TAILLE DE LA FERME

	Actuellement	<u>En moyenne au cours</u> <u>de vos 10 dernières années</u> <u>d'activité</u>
<b>10 Nombre d'hectares exploités</b>	[ ][ ][ ][ ]	[ ][ ][ ][ ]
<b>11 Nombre d'hectares de fourrage</b>	[ ][ ][ ][ ]	[ ][ ][ ][ ]
<b>12 Nombre d'hectares de céréales</b>	[ ][ ][ ][ ]	[ ][ ][ ][ ]
<b>13 Nombre de têtes de bétail</b>	[ ][ ][ ][ ]	[ ][ ][ ][ ]
13.1 (nombre de vaches)	[ ][ ][ ][ ]	[ ][ ][ ][ ]

### IV. MODE DE CONDITIONNEMENT DES FOURRAGES

	Date de début	Date de fin
<b>14 Vrac traditionnel</b> (sans séchage artificiel en grange)	[ ][ ][ ][ ]	[ ][ ][ ][ ]
<b>15 Moyenne densité</b>	[ ][ ][ ][ ]	[ ][ ][ ][ ]
<b>16 Grosses balles</b> (rondes ou carrés)	[ ][ ][ ][ ]	[ ][ ][ ][ ]
<b>17 Vrac avec séchage en grange</b>	[ ][ ][ ][ ]	[ ][ ][ ][ ]

**18 Si plusieurs conditionnements, préciser le mode majoritaire :** .....

**19 Si séchage artificiel en grange**

**19.1 depuis quand ?** .....

**19.2 réchauffage ?** oui  non

Si oui : 19.2.1  fuel ;  gaz ;  solaire  électrique

Ne pas remplir les cases grisées réservées au codage par les enquêteurs

2/4

Code inclusion

Numéro :  /

Date :

**20 Quelle est la qualité de séchage des fourrages actuellement utilisés ?**

(coter de 1 : mauvais à 4 : très bon)

**V. COMPLEMENTS D'ALIMENTATION**

**21 Céréales - farines :**

21.1  non donneur

21.2  ex-donneur : de quand : ..... (année) à quand : ..... (année)

21.3  donneur actuel : depuis quand .....

21.4 Si donneur :  Tous les jours

> 1 fois par semaine

< 1 fois par semaine

**22 Ensilage :** oui  non

Si oui : 22.1 - quel type : .....

22.2 - pourcentage de la ration alimentaire quotidienne : ..... %

**VI. DUREE D'EXPOSITION AUX PARTICULES ORGANIQUES**

**23 Nombre d'heures par jour dans l'étable durant la période de stabulation :** (en heures par jours)

23.1 Actuel : .....

23.2 Moyenne sur vos 10 dernières années d'activité : .....

**24 Durée annuelle de la période de stabulation : (en mois)**

24.1 Au cours des 12 derniers mois : .....

24.2 Moyenne sur vos 10 dernières années d'activité : .....

**25 Travaux effectués personnellement :**

25.1 Traite en étable :  actuellement  interrompue  jamais

Date de début..... Date de fin.....

Si actuel, temps passé quotidiennement : ..... minutes par jour

25.2 Traite en salle de traite :  actuellement  interrompue  jamais

Date de début..... Date de fin.....

Si actuel, temps passé quotidiennement : ..... minutes par jour

25.3 Affouragement manuel :  actuellement  interrompue  jamais

Date de début..... Date de fin.....

Si actuel, temps passé quotidiennement : ..... minutes par jour

25.4 Affouragement mécanisé :  actuellement  interrompue  jamais

Date de début..... Date de fin.....

Si actuel, temps passé quotidiennement : ..... minutes par jour

*Ne pas remplir les cases grisées réservées au codage par les enquêteurs*

3/4



## Annexe 6. Questionnaire CAT pour les sujets BPCO (étude BalistiC)

Nom:

Date:



### Quel est l'état de votre BPCO? Répondez au questionnaire CAT (COPD Assessment Test™) pour évaluer votre BPCO

Ce questionnaire vous aidera, ainsi que votre médecin, à mesurer l'impact de la BPCO (BronchoPneumopathie Chronique Obstructive) sur votre bien-être et votre vie au quotidien. Vous pourrez, ainsi que votre médecin, utiliser les réponses et les scores du questionnaire pour mieux prendre en charge votre BPCO et obtenir le meilleur bénéfice de votre traitement.

Pour chaque élément ci-dessous, veuillez indiquer d'une croix (x) la case qui correspond le mieux à votre état actuel. Prenez soin de ne sélectionner qu'une seule réponse par question.

**Exemple:** Je suis très heureux  0  1  2  3  4  5 Je suis très triste  
(heureuse)

	POINTS
Je ne tousse jamais <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 Je tousse tout le temps	<input type="text"/>
Je n'ai pas du tout de glaires (mucus) dans les poumons <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 J'ai les poumons entièrement encombrés de glaires (mucus)	<input type="text"/>
Je n'ai pas du tout la poitrine oppressée <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 J'ai la poitrine très oppressée	<input type="text"/>
Quand je monte une côte ou une volée de marches, je ne suis pas essoufflé(e) <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 Quand je monte une côte ou une volée de marches, je suis très essoufflé(e)	<input type="text"/>
Je ne suis pas limité(e) dans mes activités chez moi <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 Je suis très limité(e) dans mes activités chez moi	<input type="text"/>
Je ne suis pas inquiet(e) quand je quitte la maison, en dépit de mes problèmes pulmonaires <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 Je suis très inquiet(e) quand je quitte la maison, en raison de mes problèmes pulmonaires	<input type="text"/>
Je dors bien <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 Je dors mal à cause de mes problèmes pulmonaires	<input type="text"/>
Je suis plein(e) d'énergie <input type="radio"/> 0 <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/> 4 <input type="radio"/> 5 Je n'ai pas d'énergie du tout	<input type="text"/>
	<b>SCORE TOTAL</b> <input type="text"/>

COPD Assessment Test et le logo CAT est une marque commerciale du groupe de sociétés GlaxoSmithKline.  
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Last Updated: February 24, 2012



## Annexe 7. Questionnaire sur les habitudes alimentaires (étude BalistiC)



### **CENTRE HOSPITALIER UNIVERSITAIRE DE BESANÇON** **Pôle Cœur Poumon** **PHYSIOLOGIE – EXPLORATIONS FONCTIONNELLES**

Etiquette du patient

Date de l'examen=

#### **ENQUETE ALIMENTAIRE**

*Cocher les quantites consommées*

1)-quelle quantite de pain blanc ou baguette / **jour**



0    30    60    90    120    150    200    250+

2)-quelle quantite de pains speciaux ,pates, riz pomme de terre vapeur / **jour**



0    15    30    45    60    75    90    120    150+

3)- combien de tasse de lait / **jour** ( 1tasse=70ml, 1bol=4tasses)



0    ½    1    2    3    4    5+

4)-combien de tasse de cafe/**jour**



0    1    2    3    4    5    6    7    8    9+

5)-combien de portion de fromage/ **jour** (1 portion = 30gr)



0    ¼    ½    1    2    3    4    5+

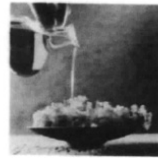
6)-quantite de fruit /**jour** (1pomme 1poire 1banane=200gr)

0 50 100 150 200 250 300 350 400 500 600+



7)-combien de cuillere à soupe d'huile / **jour** ( pour la cuisson, assaisonnement....)

0 ¼ ½ 1 1.5 2 3 4 5+



8)-combien de portion de beurre/**jour**  
( pour vos tartines, la cuisson...portion individuelle=10g)

0 ¼ ½ 1 1.5 2 3 4 5+



9)-combien de salade/ **semaine**

0 1 2 3 4 5 6 7 8 9+



10)- combien de portion de salade à chaque fois (1portion=60gr)

0 ½ 1 1.5 2

11)-combien de portion de haricots verts,legumes verts autres/ **semaine** ( 1portion=100gr)

0 ½ 1 1.5 2 2.5 3+



12)-combien de portion de carottes, petit pois, betterave / **semaine** (1portion=100gr)

0 ½ 1 1.5 2 2.5 3+



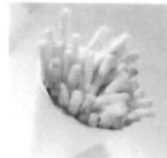
13)-combien de portion de legumes secs / **semaine**  
(lentilles, haricots...1portion=100gr)

0 ¼ ½ 1 2 3 4 5+



14)-combien de portion de frites ou de pomme de terre rissolees / **semaine** (1portion=100gr)

0 ¼ ½ 1 2 3 4 5+



15)-combien d'oeuf / **semaine**

0 1 2 3 4 5 6+



16)- quelle quantite de poisson /**semaine**

( poisson pane 1portion=50gr)  
0 50 100 150 200 300 400 500+



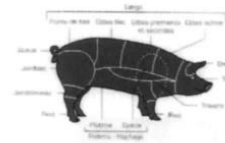
7)-quelle quantite de volaille / **semaine** (1 cuisse =100gr)

0 50 100 150 200 300 400 500+



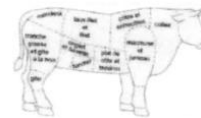
18)-quelle quantite de porc / **semaine**  
(1cotelette=2tranches jambon=2saucisses=100gr)

0 50 100 150 200 300 400 500+



19)-quelle quantite de boeuf / **semaine** (1portion=100gr)

0 50 100 150 200 300 400 500+



20)-quelle quantite de vin / **semaine**

0 1verre 2verres 3verres 1bouteille 2bouteilles  
3bouteilles 4bouteilles+



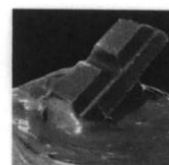
21)-combien de yaourt / **semaine**

0 1 2 3 4 5 6 7 8 9 10 11+



22)-quelle quantite de chocolat / **semaine**

0 2carres 1barre 2barres 3barres 1tablette  
2tablettes+



23)-combien de paquet de cereales( petit dej) / **mois** (1paquet375gr)

0 1 2 3 4 5+

24)-combien de fruit oleagineux / mois (1portion=60gr)

0   ½   1   2   3   4   5   6+



**Titre:** BPCO secondaire à l'exposition aux poussières organiques: caractérisation pulmonaire et systémique

**Mots clés :** BPCO, agriculteurs, poussières organiques, comorbidités

**Résumé :** La broncho-pneumopathie chronique obstructive (BPCO) est caractérisée par l'existence de symptômes respiratoires et d'une obstruction permanente des voies aériennes. Le tabagisme reste le principal facteur de risque bien que des causes environnementales et professionnelles comme l'exposition aux poussières organiques aient été identifiées. La prévalence de la BPCO secondaire à l'exposition aux poussières organiques est de 8% et est similaire à celle de la BPCO tabagique malgré une prévalence du tabagisme beaucoup plus faible. Les facteurs liés à l'hôte tel que l'atopie semblent être des déterminants de son développement plus que l'importance de l'exposition aux poussières organiques. Par rapport à la BPCO

tabagique, la BPCO secondaire aux poussières organiques est caractérisée par une moindre dyspnée d'effort, une meilleure qualité de vie, une capacité de diffusion alvéolo-capillaire moins altérée suggérant une moindre destruction parenchymateuse et une meilleure tolérance à l'exercice. Les comorbidités notamment cardiovasculaires sont moins fréquentes et sont liés au tabagisme plus qu'à l'existence d'une BPCO. L'inflammation systémique est également moins importante avec un profil de type Th2.

Un suivi longitudinal de ces patients est actuellement en cours (NCT03468101). Par ailleurs, la prise en charge thérapeutique et le pronostic de cette BPCO reste à déterminer.

**Title:** COPD secondary to organic dust exposure: pulmonary and systemic characterization.

**Keywords:** COPD, farmers, organic dust, comorbidities

**Abstract:** Chronic obstructive pulmonary disease (COPD) is characterized by persistent respiratory symptoms and airflow limitation. The main risk factor for COPD is tobacco smoking but other environmental exposures such as organic dust exposure may contribute.

The prevalence of COPD secondary to organic dust exposure is similar (8%) to those of COPD although prevalence of tobacco smoking is lower. Host factors like atopy appear to be important determinants for its development rather than the magnitude of organic dust exposure. Compared to COPD related to tobacco smoking, COPD

secondary to organic dust exposure is associated with less dyspnoea, better quality of life, less impaired exercise capacity and diffusing capacity suggesting a lower emphysema burden. In addition, comorbidities including cardio-vascular diseases are less frequent and are related to tobacco smoking rather than COPD. Systemic inflammation is at a lower level and has a predominantly Th2 profile.

Longitudinal follow-up is currently underway (NCT03468101). Therapeutic management and prognosis of this COPD remain to be determined.