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TEMPORAL CHANGES IN RESPIRATORY SYMPTOMS/DISEASES AND ASSOCIATED RISK FACTORS IN REAL LIFE

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ABSTRACT

Background: The prevalence of respiratory diseases increased during last centuries and the rapid rise in the prevalence of such diseases cannot be explained by genetic factors. Analyses on general population samples investigated at interval of some years can permit to better comprehend these increases and the associated risk factors, adding new evidences on this important item taking into account the *real life* setting.

Aims: to assess *real life* temporal changes in respiratory diseases/symptoms (asthma, allergic rhinitis - AR - and chronic obstructive pulmonary disease - COPD) and associated risk factors in general population samples investigated during a period of 30 years.

Methods: a general population sample from 4 studies (PISA1: 1985-1988; PISA2: 1991-1993; PISA3: 2009-2011; AGAVE: 2011-2014), completing a questionnaire on respiratory symptoms/diseases, risk factors exposure and performing spirometry and skin prick test, was analyzed.

Respiratory symptoms/diseases cumulative incidence, remission, persistence, multimorbidity and phenotypes were computed.

Cross-sectional risk factors exposure (smoking habits, occupational exposure, vehicular traffic exposure) and longitudinal changes in risk factors exposure (incidence, persistence, remission) were considered.

Multiple logistic regression, multinomial logistic regression and latent transition analyses were run to assess the relationship between respiratory disease outcomes and risk factors exposure.

Results: substantial temporal changes in respiratory symptoms/diseases in general population samples were found: respiratory symptoms/diseases new onset ranging from 3.4% for asthma to 33.1% for AR; 52.5% of asthma remission and 39.6% of COPD

remission; 17.2% of asthma underdiagnosis and 30.4% of COPD underdiagnosis (defined as symptoms persistence without lifetime physician diagnosis); 18.6% of respiratory multimorbidity new onset. Moreover, the following longitudinal trajectories of respiratory symptoms/diseases phenotypes were computed over 18 years: 2% improving health status, 52.2% persistent health status, 22.9% persistent AR, 9% persistent usual cough/phlegm, 13.8% worsening health status.

Temporal changes (i.e. persistence and incidence) in lifetime habits and in exposure to risk factors were differently associated with the temporal changes in respiratory diseases occurrence in a *real life* setting.

Smoking habits were related to: persistent disease condition, in particular persistent usual cough/phlegm phenotype; worsening health status due to new onset of usual cough/phlegm, COPD and respiratory mutimorbidity. *Occupational exposure* was related to: persistent medical condition, in particular persistent usual cough/phlegm phenotype; new onset of respiratory multimorbidity and morbidity, in particular of allergic diseases and bronchitic symptoms/diseases. *Vehicular traffic exposure* was related to: new onset of respiratory multimorbidity and morbidity, in particular of allergic diseases and of asthma symptoms.

Moreover, passive smoke exposure and occupational exposure reduced the probability of asthma remission; living in an urban area reduced the probability of asthma and COPD remission; smoking habits seemed to facilitate COPD diagnosis, while masking asthma diagnosis.

Comorbidities reduced the probability of asthma and COPD remission and facilitated COPD diagnosis.

Conclusions: few longitudinal studies on general population are available in literature; the Pisa survey permitted to assess an important topic, like the increase of respiratory

symptoms/diseases, addressing different and specific aspects, with different methodological approaches, and adding new scientific evidences.

The obtained results should be considered for primary prevention strategies in order to reduce the burden of chronic diseases in the general population and for prevention and management strategies of respiratory diseases in health care setting.

ABBREVIATIONS

AO: Airway Obstruction

AR: Allergic Rhinitis

ATS: American Thoracic Society

BIC: Bayesian Information Criterion

BMI: Body Mass Index

95% CI: 95% Confidence Interval

COPD: Chronic Obstructive Pulmonary Disease

ERS: European Respiratory Society

LLN: Lower Limit of Normal

LTA: Latent Transition Analysis

OR: Odds Ratio

PI1: Pisa 1 survey

PI2: Pisa 2 survey

PI3: Pisa 3 survey

SPT: Skin Prick Test

WHO: World Health Organization

ORIGINAL PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. *Sara Maio*, Sandra Baldacci, Laura Carrozzi, Francesco Pistelli, Marzia Simoni, Anna Angino, Stefania La Grutta, Vito Muggeo, Giovanni Viegi. **Incidence of respiratory symptoms and diseases is still elevated: the 18-yr follow-up of the Pisa epidemiological study.** (*Submitted).*

II. *Sara Maio*, Sandra Baldacci, Marzia Simoni, Anna Angino, Stefania La Grutta, Vito Muggeo, Nicola Murgia, Giovanni Viegi, on behalf of the AGAVE group. **Asthma remission and underdiagnosis and associated risk factors in Italian general population samples.** (*Submitted).*

III. *Sara Maio*, Sandra Baldacci, Marzia Simoni, Anna Angino, Stefania La Grutta, Vito Muggeo, Giovanni Viegi. **COPD remission/persistence and associated risk factors in Italian general population samples.** (*In preparation).*

IV. *Sara Maio*, Sandra Baldacci, Salvatore Fasola, Marzia Simoni, Anna Angino, Stefania La Grutta, Vito Muggeo, Giovanni Viegi. **Temporal changes in multimorbidity and associated risk factors in Italian general population samples.** *(In preparation).*

V. *Sara Maio*, Sandra Baldacci, Salvatore Fasola, Stefania La Grutta, Vito Muggeo, Giovanni Viegi. **Trajectories of respiratory disease phenotypes and associated risk factors in the 18-yr Pisa epidemiological study.** (*In preparation).*

I. INTRODUCTION

Recent cross-sectional studies showed an increasing prevalence of respiratory symptom/diseases in the last decades.

Epidemiological surveys on general population samples investigated during different periods of their life can be an important source of data to better comprehend the risk factors associated with the increase of respiratory diseases in the *real life* setting; longitudinal studies can add further information about the increasing trend of respiratory symptom/diseases, permitting to assess their natural history and long term outcomes. In particular, incidence, persistence and remission rates can be assessed.

Nevertheless, few longitudinal studies on general population are available in literature.

Moreover, a current challenge in chronic respiratory diseases assessment is the evaluation of multimorbidity and disease phenotypes for a better diseases management and identification of specific risk factors.

This work is an epidemiological study on respiratory health status in general population samples. It was conducted in Tuscany (Pisa), in a cohort of subjects living in a suburban/urban area. The study started in 1985 and the last follow-up was performed in 2011. Data coming from this study were already analyzed in previous papers using statistical approaches for cross-sectional and longitudinal assessments (i.e. generalized estimating equation – GEE).

The aim of this thesis was to assess *real life* temporal changes in respiratory diseases/symptoms (asthma, allergic rhinitis and chronic obstructive pulmonary disease) and associated risk factors in Pisa general population samples investigated during a period of 30 years, applying simple and more complex statistical approaches.

II. BACKGROUND

Strong scientific evidences showed that the prevalence of respiratory symptoms/diseases increased worldwide.

Table 1. Respiratory symptoms/diseases prevalence trend

	Reference	Country	Population	Follow-up period	Prevalence (%)
Asthma diagnosis	Ekerljung 2010 [1]	Sweden	n=~5600 20-69 yrs	1996/2007 11 yrs	From 7.6% to 9.3%
	Fukutomi 2011 [2]	Japan	n=12152 and n=3829 ≥ 15 yrs	1985/1999 14 yrs	From 2.1% to 3.9%
	De Marco 2012 [3]	Italy	n=6031 and n=10494 20-44 yrs	1991/2010 10 yrs	From 4.1% to 6.6%
AR	Bjerg 2011 [4]	Sweden	n=8982 and n=9156 20-44 yrs	1990/2008 18 yrs	From 21.6% to 30.9%
	De Marco 2012 [3]	Italy	n=6031 and n=10494 20-44 yrs	1991/2010 10 yrs	From 16.8% to 25.8%
	Maio 2016 [5]	Italy	n=3865 and n=1620 4-97 yrs	1985/2010 25 yrs	From 16.2% to 37.4%
COPD	Gershon 2010 [6]	Ontario	More than 5 millions* ≥ 35 yrs	1991/2007 16 yrs	From 7.8% to 9.5%
	Waatevik 2013 [7]	Norway	n=1664 35-90 yrs	1996/2003 8 yrs	From 7.0% to 14.0%
	Maio 2016 [5]	Italy	n=3865 and n=1620 4-97 yrs	1985/2010 25 yrs	From 2.1% to 6.8%

* health administrative data.
AR: allergic rhinitis; COPD: chronic obstructive pulmonary disease.

To better understand this increasing trend of respiratory symptoms/diseases, data coming from longitudinal studies, focusing on diseases natural history and long term outcomes, are necessary; in particular, disease incidence, persistence and remission rates should be assessed. Moreover, an accurate evaluation of multimorbidity and disease phenotypes is needed for a better diseases management and identification of specific risk factors.

The following sections address the current knowledge on respiratory health, focusing on incidence, remission, underdiagnosis, multimorbidity and disease phenotypes.

II.1 INCIDENCE OF RESPIRATORY SYMPTOMS/DISEASES

Recent studies showed significant values of respiratory symptoms/diseases incidence in general population (table 2).

Asthma incidence rates ranged from 2% to 6% in adult subjects at 10-20 yr follow-up [8-11]; among adolescents an asthma cumulative incidence of 7% emerged at 6.5-yr follow-up [12].

A 9-yr follow-up of European young adults showed a 12% allergic rhinitis (AR) cumulative incidence [13]; a more recent follow-up of German young adults showed a cumulative incidence value of 8.0% at 20-yr follow-up [14].

Finnish investigators found a chronic bronchitis cumulative incidence of 14% at 30-yr follow-up in adult subjects [15]. Cough and phlegm showed lower incidence (about 3%) in a US adult cohort at 3-yr follow-up [16]. Airway obstruction (AO) incidence values ranging from 3% to 7% at about 10-yr follow-up were found [17-18].

Table 2. Respiratory symptoms/diseases cumulative incidence rates

	Reference	Country	Population	Follow-up period	Cum. incid. (%)	Yearly incid. (‰)
Asthma diagnosis	Torén 2011 [8]	Western Sweden	n=15761 16-75 yrs	1990-2008 18 yrs	2.3	1.3
	Pallasaho 2011 [9]	Finland	n=4302 20-69 yrs	1996-2007 11 yrs	4.0	3.6
	Hansen 2015 [10]	Switzerland	n=5128 18-60 yrs	1991-2001 20 yr	6.4	3.2
	Verlato 2016 [11]	Italy	n=3187 20-54 yrs	1999-2008 9 yrs	4.6	5.1
	Hedman 2015 [12]	Sweden	n=3151 11-12 yrs	2000 6.5 yrs	7.2	11.1
AR	Radon 2008 [13]	27 European countries	n=4994 20-44 yrs	1992-2001 9 yrs	12.0	13.3
	Gallmeier 2014 [14]	Germany	n=754 20-44 yrs	1990-1992 20 yrs	7.9	4.0
COPD or	Johannesse	Norway	n=908	1987-1996	6.1	6.7

CB	n 2005 [19]		18-74 yrs	9 yrs		
	Pelkonnen 2006 [15]	Finland	n=345 men 40-59 yrs	1959-1989 30 yrs	14.0	4.7
Usual cough	Mirabelli 2012 [16]	US	n=8967 45-64 yrs	'80s 3 yrs	3.5	11.7
Usual phlegm					3.4	11.3
LLN AO	Mehta 2012 [17]	Switzerland	n=4023 18-62 yrs	1991-2002 11 yrs	7.4	6.7
	Marcon 2018 [18]	Europe	n=4205 20-44 yrs	1991-1993 9 yrs	2.6	2.9

Cum.: cumulative; incid.: incidence; AR: allergic rhinitis; COPD: chronic obstructive pulmonary disease; CB: chronic bronchitis; LLN AO: airway obstruction according to lower limit of normal.

The rapid rise of such diseases cannot be explained by genetic factors alone [20]. Main environmental risk factors associated with lifetime increase of respiratory symptoms/diseases prevalence and incidence are tobacco use, air pollutants and occupational exposure, as reported in World Health Organization (WHO) official statements [21-23]. In particular, rapid urbanization and industrialization throughout the world have increased air pollution and population exposures, so that most epidemiologic studies are focusing on possible links between air pollution and respiratory diseases [22, 24-26].

II.2 REMISSION AND UNDERDIAGNOSIS OF RESPIRATORY SYMPTOMS/DISEASES

In the last decades, nevertheless effective pharmacological treatments and management strategies have been developed, chronic respiratory diseases remain treatable but not curable diseases. Thus, much effort was put into studying the risk factors associated with their inception and progression, since understanding these factors represents the first necessary step for developing effective prevention strategies [27-28].

From a clinical standpoint, to elucidate the asthma and chronic obstructive pulmonary disease (COPD) natural history and long term outcomes is a issue of primary importance.

Studies on asthmatic cohorts can help [27]. Asthma may onset at any age (even if manly in children) and is known to clinically persist, possibly resolve and/or present remission over time. For these reasons, asthma progression is difficult to be characterized and predicted [29] and little is known about the asthma activity and inactivity and its correlated risk factors [29-30]. Indeed, few epidemiological studies have focused on factors associated with asthma remission [30-34].

COPD has been described by the WHO GOLD guidelines as a disease characterized by airflow limitation that is not fully reversible [35], but remission of COPD symptoms (chronic cough and chronic phlegm) is possible; nevertheless, few studies have examined the remission of respiratory symptoms in general population cohorts and the associated factors [36].

Another problem related to asthma and COPD is their underdiagnosis even if the first international guidelines were published since ‘90s [35,37]. Most patients, in particular elderly, don't report their symptoms to the general practitioner (GP) because they don't recognize them or are suffering from other comorbidities with similar clinical manifestations like heart failure; thus, they remain unknown and undiagnosed. On the other side, patients who do present with respiratory problems and who have reduced lung function are not always recognized as such or they are underestimated [38-40].

II.3 MULTIMORBIDITY

Multimorbidity denotes multiple medical conditions within a single patient. Multimorbidity is distinct from comorbidity because there is no primary or index

condition. Nowadays, there is no a standard definition and this fact has led to wide discrepancies in prevalence estimates (ranging from 13% to 72% in general population) [41]. A study of 28 countries, using the World Health Survey, showed that the increasing prevalence of multiple chronic conditions is now a global phenomenon [42] and it is expected to continue rising [43]. Indeed, the lifespan of population is increasing because of improvements in public health measures and the significant success of modern medicine and technology. However, although populations are living longer, they are not necessarily living disease-free for a longer period [44].

Evidence from high-income countries suggested that multimorbidity is highly prevalent in older populations (over 65 years of age), but it also affects younger people. As such, multimorbidity from chronic conditions is now widespread, with at least 50 million people affected in the European Union alone [43,45].

Most of the evidence on multimorbidity came from cross-sectional studies and longitudinal studies are limited [43], even if there are some findings highlighting that the prevalence of some types of multimorbidity increased over time [46].

The main risk factor for multimorbidity increase is the ageing population but also other factors such as high body-mass index, urbanisation and the growing burden of non communicable diseases play a role. Few data are available about modifiable factors that predict the risk of different types of multimorbidity [41]: it remains unknown whether there are biological, environmental or behavioural factors. The identification of any such factors, and the assessment of the likelihood of causality, requires data from prospective observational studies [43].

II.4 DISEASES PHENOTYPES

Chronic respiratory diseases are complex diseases characterized by a strong clinical heterogeneity and possible phenotypic variability over time [47]. An accurate assessment of phenotypes is needed for better diseases management and for better identification of phenotype-specific risk factors. Disentangling disease phenotypes is a current challenge [47-48].

Beside the "candidate" approach that identifies a priori phenotypes on the basis of one or few disease characteristics, unsupervised or data driven approaches have been proposed to unravel the heterogeneity of chronic diseases by means of a clustering approach integrating multiple disease features, possibly identifying and defining objective, novel or previously unrecognised phenotypes [47,49]. The application of data-driven approaches has yielded phenotypic classifications that are clinically meaningful and interpretable and that are relevant to prognosis [48].

To date, clustering analyses have been performed in a cross-sectional manner (Latent Class Analysis - LCA), by integrating several domains of the disease measured at one point in time, or longitudinally, by using a single disease characteristic assessed at several time points to define trajectories [47,49]. On the contrary, the application of data-driven approaches to define longitudinal phenotypes of chronic diseases remains largely unexplored. Latent transition analysis (LTA) permits to incorporate the longitudinal pattern of several disease manifestations into one statistical model to simultaneously define phenotypes and to examine transitions over time [47-49]. If disease prevalence changes, there is a need to understand what dynamics contributed to the change; transition probabilities better explain the nature of such changes [49].

III. AIMS

The overall aim of this thesis was to assess *real life* temporal changes in respiratory diseases/symptoms (asthma, AR and COPD) and associated risk factors in general population samples investigated during a period of 30 years.

III.1 SPECIFIC AIMS

1. To assess incidence of respiratory symptoms/diseases and associated risk factors (PAPER I);

2. To assess remission and persistence of respiratory symptoms/diseases and associated risk factors (PAPERS II, III);

3. To assess respiratory multimorbidity and associated risk factors (PAPER IV);

4. To assess trajectories of respiratory disease phenotypes over time and associated risk factors (PAPER V).

IV. METHODS

IV.1 STUDY POPULATION

This study takes into account 4 population-based samples living in the urban/suburban area of Pisa (Central Italy) and investigated from 1985 to 2014: Pisa 1 survey (PI1) (1985–1988, n=3865), Pisa 2 survey (PI2) (1991-1993, n=2841), Pisa 3 survey (PI3) (2009-2011, n=1620), AGAVE survey (2011-2014, n=480).

PI1: in 1985–1988, a general population sample of 3865 subjects (84% of the invited subjects) was investigated with the aim to assess the COPD natural history and the related risk factors. The sample was enrolled through a randomized, stratified by socio-economic status, family-cluster design [50] (figure 1).

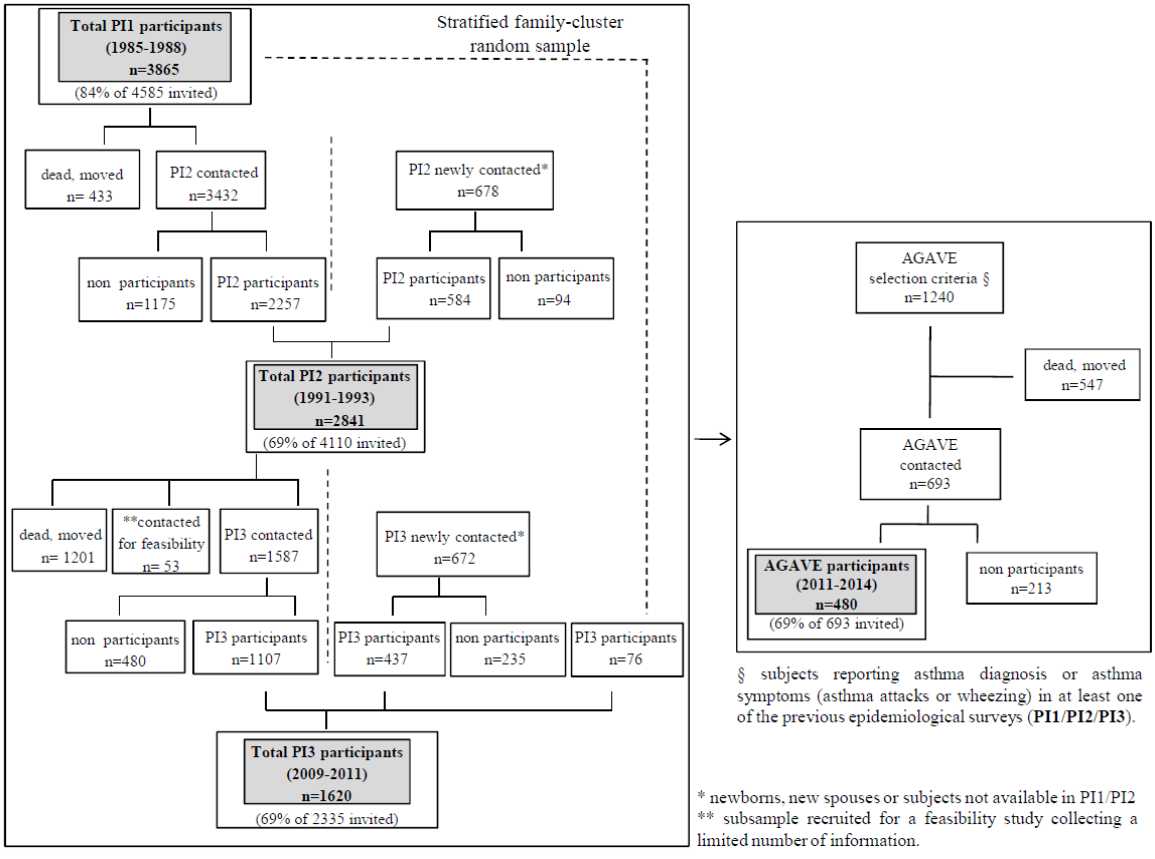
PI2: a second cross-sectional survey was carried out in 1991–1993. Beside those participating in PI1, new volunteer subjects belonging to the family unit were recruited: newborns, new spouses and subjects not available to participate in PI1. Overall, 2841 subjects (69% of the invited subjects) were investigated. 2257 subjects participated in both PI1 and PI2 surveys, corresponding to a longitudinal participation rate of 58% (66% if those dead or moved were excluded from the computation) with a mean follow-up of 6 years (figure 1).

PI3: a third cross-sectional survey was carried out in 2009-2011 within the European IMCA2 (Indicators for Monitoring COPD and Asthma in the EU) project. Beside those participating in PI1 and/or PI2, new volunteer subjects belonging to the family unit were recruited: newborns, new spouses and subjects not available to participate in PI1 and/or PI2. Overall, 1620 subjects (69% of invited) were investigated. 1107 subjects participated in both PI2 and PI3 surveys, corresponding to a longitudinal participation rate of 39% (68% excluding those dead or moved) with a mean follow-up of 18 years. 849

subjects participated in all the 3 surveys PI1, PI2 and PI3, corresponding to a longitudinal participation rate of 22% (38% excluding those dead or moved) with a mean follow-up of 25 years (figure 1).

AGAVE: in a more recent survey (the AGAVE project, 2011-2014), subjects reporting asthma diagnosis or asthma symptoms (asthma attacks or wheezing) in at least one of the previous epidemiological surveys (PI1, PI2, PI3) were newly investigated. Overall, 480 subjects (69% of invited) were investigated, with a mean follow-up of 20 years (figure 1).

Figure 1. Flow chart of the participation



For this thesis, the following longitudinal sub-samples were analyzed:

1) subjects participating in both PI2 and PI3 surveys (n=1107) (PAPERS I, IV, V);

2) subjects participating in AGAVE, that is subjects reporting asthma symptoms/diagnosis in at least one of the previous surveys PI1, PI2, PI3 that accepted to participate in AGAVE survey (n=480) (PAPER II);

3) subjects participating in PI3 and reporting COPD symptoms/diagnosis in at least one of the previous surveys PI1, PI2 (n=257) (PAPER III);

4) subjects participating in PI1, PI2 and PI3 surveys (n=849) (PAPER IV).

IV.2 ETHICAL COMMITTEE

Italian law didn't request the approval of Ethical Committee at the time of PI1/PI2. The protocol was approved by an Internal Revision Board within the Preventive Medicine Targeted Project of the Italian CNR.

PI3 study protocol, patient information sheet and consent form were approved by the ethics committee of the Pisa University Hospital (Azienda Ospedaliero-Universitaria Pisana, Prot. no. 23887, April 16, 2008).

AGAVE study protocol, patient information sheet and consent form were approved by the ethics committee of the Pisa University Hospital (Azienda Ospedaliero-Universitaria Pisana, Prot. no. 17658, March 21, 2011).

IV.3 DATA COLLECTION TOOLS

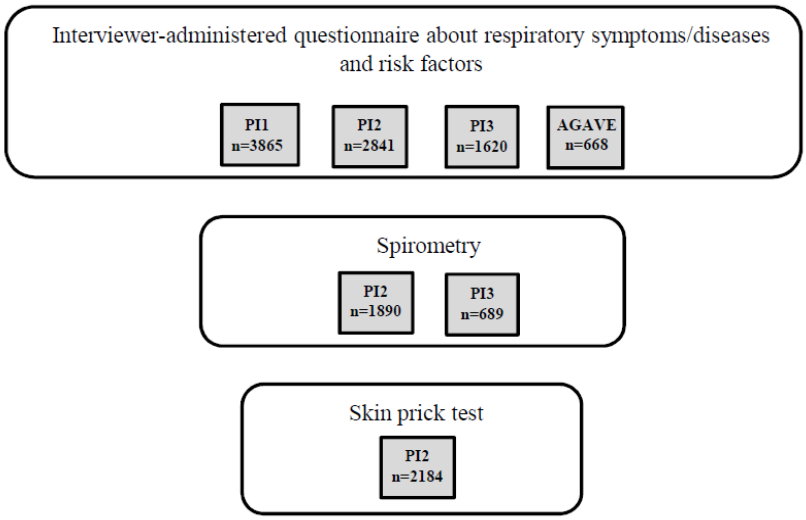
The same study protocol was used in **PI1** and **PI2**. Information on respiratory symptoms/diseases and risk factors were obtained through a standardized interviewer-administered questionnaire developed by the National Research Council [51]. Moreover, in PI2, a subsample performed instrumental measurements to assess respiratory failures and atopy: a) all ≤ 75 year subjects were invited to perform spirometry (forced vital capacity-FVC- manoeuvre) according to the American Thoracic Society (ATS) protocol [52],

through a water-sealed spirometer (Baires, Biomedin) [53] (participation rate 67%); b) the sensitization to 12 local allergens (pollens, house dust mites, animal dander, moulds) was assessed in volunteers by skin-prick test reactivity using a standardized protocol (participation rate 77%) [54].

As regards **PI3**, an interviewer-administered questionnaire on respiratory symptoms/diseases and risk factors was designed using questions from previously validated questionnaires [5,51,55-56]. Moreover, all subjects were invited to perform spirometry (FVC manoeuvre) according to the ATS/European Respiratory Society (ERS) protocol [57], through an hand-held ultrasonic spirometer (EasyOne Model 2001 Spirometer, NDD Medical Technologies) (participation rate 55%) (n=689).

In the **AGAVE** project, subjects were investigated using an epidemiological questionnaire based on the main items of the GINA guidelines [37] regarding asthma symptoms/diagnosis, clinical characteristics, asthma treatment, exacerbations, symptoms' control, comorbidities and risk factors exposure. The questionnaire was reviewed and approved by an interdisciplinary internal board comprised of pneumologists, allergists and epidemiologists.

Figure 2. Tools for data collection



IV.4 DEFINITIONS

The variables of importance to this thesis are below described. All variables, except airway obstruction and allergic sensitization, were based on questionnaire reports.

Procedures of harmonization of the different databases and objectives measurements and procedures of data quality assessment, necessary to compute new comparable variables in the four surveys (PI1-PI2-PI3-AGAVE), were performed (table 3, table 4).

Table 3. Data harmonization: comparison among different questions of the four surveys and computed outcome variables

	PI1 (n=3865)	PI2 (n=2841)	PI3 (n=1620)	AGAVE (n=480)
Asthma diagnosis (yes, no)				
Asthma confirmed by a physician	x	x	x	x
Current asthma attacks (yes, no)				
Current attacks of shortness of breath with wheezing apart from common colds	x	x	---	---
Last 12 months attacks of shortness of breath with wheezing apart from common colds	---	---	x	x
Current wheeze (yes, no)				
Current wheeze apart from common colds	x	x	---	---
Last 12 months wheeze apart from common colds	---	---	x	x
AR symptoms/diagnosis (yes, no)				
Hay fever or other conditions causing runny or blocked nose, apart from common colds	x	x	x	x
Usual phlegm (yes, no)				
Usual phlegm for some part of the year apart from common colds	x	x	x	---
Usual cough (yes, no)				
Usual cough for some part of the year apart from common colds	x	x	x	---

COPD diagnosis (yes, no)				
Chronic bronchitis or emphysema confirmed by a physician	x	x	---	---
Chronic bronchitis, emphysema or COPD confirmed by a physician	---	---	x	x
LLN AO (yes, no)				
FEV_1/FVC percentage predicted < LLN [58]*	---	x (33.0 % mys)	x (58.0% mys)	---
SPT positivity (yes, no)				
Mean wheal diameter ≥ 3 mm than that determined by the negative control [54]**	---	x (23.0% mys)	---	---

AR: allergic rhinitis; COPD: chronic obstructive pulmonary disease; AO: airway obstruction; SPT: skin prick test; mys: missing.

* LLN was derived from population-specific prediction equations [59].

** allergic sensitization at least one of 12 local allergens (pollens, house dust mites, animal dander, moulds).

Table 4. Data harmonization: comparison among different questions of the four surveys and computed demographic characteristics and risk factors variables

	PI1 (n=3865)	PI2 (n=2841)	PI3 (n=1620)	AGAVE (n=480)
Age (continuous variable)				
Difference between date of issue and date of birth	x	x	x	x
Sex (males, females)	x	x	x	x
Body mass index (BMI) (continuous variable)				
Rate between reported weight (Kg)/ reported height^2 (meter)	x (0.5% mys)	x	---	x
Rate between measured weight (Kg)/ measured height^2 (meter)	---	---	x (40% mys)	---
BMI groups (underweight, normal weight, overweight, obese)				
Underweight (BMI<18.50) Normal weight (18.51<BMI<25.00) Overweight (25.01<BMI<30.00) Obese (BMI>30.01)	x (0.5% mys)	x	x (40% mys)	x
Educational level (< 8 yrs, 8-13 yrs, > 13 yrs)				
< 8 yrs of education (elementary school) 8-13 yrs (medium high / high school)	x	x	x	x

> 13 yrs (university and other)				
Area of residence (urban, suburban)				
Urban (living in Pisa) Suburban (living in Cascina)	x	x	x	---
Family history of COPD (yes, no)				
Family history (at least one parent) of emphysema or chronic bronchitis	---	x	---	---
Family history of respiratory diseases (yes, no)				
Family history (at least one parent) of emphysema or chronic bronchitis or asthma	---	x	---	---
Family history of allergic rhinitis (yes, no)				
Family history (at least one parent) of allergic rhinitis	---	x	---	---
Second hand smoke exposure (yes, no)				
Usual passive smoke exposure	---	x	---	x
Current home passive smoke exposure	---	---	x	---
Smoking habits (current smokers, ex smokers, non smokers)				
Life time smoking habits (yes, no) + age of end: current smoker (current smoking habits) ex smoker [smoking habits + (age of end < subject's age)] non smokers (no lifetime smoking habits)	x	x	x	x
Occupational exposure (yes, no)				
Exposure to fumes/gases/dusts at work	x	x	x	x
Vehicular traffic exposure (yes, no)				
Reported exposure to vehicular traffic near home (yes, no)	---	x	---	---
Reported frequency of vehicular traffic near home (continuously+frequently, seldom+never)	---	---	x	x

COPD: chronic obstructive pulmonary disease; mys: missing.

IV.5 OUTCOMES

Different outcomes were computed for achieving the thesis aims:

IV.5.1 Respiratory symptoms/diseases incidence (PAPER I)

Respiratory symptoms/diseases cumulative *incidence* was computed in the longitudinal subsample of the subjects reporting symptoms or diagnoses in PI3 but without symptoms & diagnoses in PI2 ("population at risk"), considering as incident cases the following:

- for asthma diagnosis/asthma attacks: reported asthma diagnosis/asthma attacks in PI3 questionnaire but neither asthma diagnosis nor current asthma attacks in PI2 questionnaire (n=1008);
- for AR symptoms/diagnosis: reported AR diagnosis or AR symptoms in PI3 questionnaire but neither AR diagnosis nor AR symptoms in PI2 questionnaire (n=885);
- for COPD diagnosis/usual phlegm/usual cough: reported COPD diagnosis/usual phlegm/usual cough in PI3 questionnaire but neither COPD diagnosis nor COPD symptoms (usual phlegm and usual cough) in PI2 questionnaire (n=866);
- for LLN AO: LLN AO in PI3 but not in PI2 (n=368).

Cumulative rates for the incidence of symptoms and diseases over the eighteen-year follow-up were calculated as follows: "incidence" = "incident cases" / "population at risk".

IV.5.2 Asthma remission/persistence (PAPER II)

Asthma was classified according to the longitudinal changes in the presence/absence of symptoms/diagnosis from previous surveys (PI1/PI2/PI3) to the AGAVE survey, as (mutually exclusive categories): *Remittent asthma*, asthma symptoms (current asthma

attacks or wheeze) or asthma diagnosis in at least one of the previous surveys and absence of current (last 12 months) asthma symptoms & diagnosis & use of asthma medications in AGAVE survey; *Persistent asthma symptoms*, only current asthma symptoms (without a lifetime physician diagnosis) in both previous surveys and AGAVE survey (a condition interpreted as asthma "underdiagnosis"); *Persistent asthma*, current asthma (with lifetime physician diagnosis) in both previous surveys and AGAVE survey.

IV.5.3 COPD remission/persistence (PAPER III)

COPD was classified according to the longitudinal changes in the presence/absence of symptoms/diagnosis from previous surveys (PI1, PI2) to the PI3 survey, as (mutually exclusive categories): *Remittent COPD*, COPD diagnosis or symptoms (usual cough or usual phlegm) in at least one of the previous surveys and absence of current COPD symptoms & diagnosis in PI3 survey; *Persistent COPD symptoms*, only current usual cough or usual phlegm (without a lifetime physician diagnosis) in both previous surveys and PI3 survey (a condition interpreted as COPD "underdiagnosis"); *Persistent COPD*, current COPD (with lifetime physician diagnosis) in both previous surveys and PI3 survey.

IV.5.4 Longitudinal changes in multimorbidity (PAPER IV)

Respiratory multimorbidity (multiple medical conditions within a single subject) was computed considering the co-presence of asthma, AR and COPD symptoms/diagnosis.

Longitudinal changes in the number of medical conditions within a single subject were computed (mutually exclusive categories): *Never/improving*, never asthma & AR & COPD / reduction in the number of medical conditions (i.e. from 1 to 0 condition; from 2 to 1 condition; from 3 to 2/1 conditions); *Persistent medical condition*; *Incident morbidity*

(i.e. from 0 to 1 condition); *Incident multimorbidity* (i.e. from 0/1 to 2/3 conditions; from 2 to 3 conditions).

IV.5.5 Longitudinal respiratory disease phenotypes (PAPER V)

LTA was used to characterize the respiratory disease phenotypes (see section "Latent transition analysis" for major details). Manifest variables, considered to characterize the phenotypes, were presence/absence of the following symptoms/diseases at PI2 and PI3: asthma, attacks of asthma, AR, COPD, usual cough, usual phlegm, SPT positivity, LLN AO. Estimate of four phenotypes for each time yielded the best Bayesian Information Criterion (BIC).

Based on respiratory symptoms/diseases frequency, cross-sectional mutually exclusive phenotypes were labelled in PI2 and PI3 as: *"Non atopic, healthy", "Atopic, AR", "Non atopic, usual cough/phlegm", "Atopic, asthma & usual cough/phlegm & AR"* (labelled as "*Atopic, asthma & AR*" in PI2). At each time, the subjects were assigned to the phenotype associated with the maximum posterior probabilities of latent class membership [60].

Based on the matrices of transition probabilities, the following longitudinal phenotypes (longitudinal trajectories) were defined: "*Persistent healthy*" ("Non atopic, healthy" phenotype in both PI2 and PI3 surveys); "Persistent AR" ("Atopic, AR" in both PI2 and PI3); "*Persistent usual cough/phlegm*" ("Non atopic, usual cough/phlegm" in both PI2 and PI3); "*Improving health status*" (improvement of health status from PI2 to PI3: "Non atopic, usual cough/phlegm" changing in "Non atopic, healthy"; "Atopic, asthma & AR" changing in "Non atopic, healthy"); "*Worsening health status*" (worsening of health status from PI2 to PI3: "Non atopic, healthy" changing in "Non atopic, usual cough/phlegm"; "Atopic, AR" changing in "Atopic, asthma & usual cough/phlegm &

AR"; "Atopic, asthma & AR" changing in "Atopic, asthma & usual cough/phlegm & AR").

IV.6 LONGITUDINAL RISK FACTORS

Temporal changes in exposure to the main risk factors for respiratory diseases were analyzed in papers I, IV and V:

Smoking habits were coded into 5 mutually exclusive categories: *Never* (non smoker subjects both in PI2 and PI3), *Persistent* (smoker subjects both in PI2 and PI3), *Incident* (subjects starting to smoke in the period between PI2 and PI3), *Remittent for < 18 yrs* (subjects stopping smoking in the period between PI2 and PI3), *Remittent for ≥ 18 yrs* (subjects who had stopped smoking before PI2).

Occupational exposure (self-reported exposure to dusts/fumes/gases at work) was codified into 4 mutually exclusive categories: *Never* (unexposed subjects both in PI2 and PI3), *Persistent* (exposed subjects both in PI2 and PI3), *Incident* (subjects becoming exposed in the period between PI2 and PI3), *Remittent* (subjects quitting exposure between PI2 and PI3).

Vehicular traffic exposure (self-reported exposure to vehicular traffic near home) was codified into 4 mutually exclusive categories: *Never* (unexposed subjects both in PI2 and PI3), *Persistent* (exposed subjects both in PI2 and PI3), *Incident* (subjects becoming exposed in the period between PI2 and PI3), *Remittent* (subjects quitting exposure between PI2 and PI3).

V. STATISTICAL ANALYSES

Overall, statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS version 17.0); R software (version 3.4.2) was used for running the latent transition analysis.

Comparisons between groups were performed by chi-square test for categorical variables and analysis of variance for continuous variables.

A post-hoc analysis was run to assess the source of statistically significant result in case of contingency table larger than a 2 x 2. An adjusted standardized residual having absolute value that exceeded 2, when there was few cells, or 3, when there was many cells (≥12), was used to define the significant contribution of the cell to the magnitude of the resulting value obtained by chi-square test [61-62].

Multivariate analyses by logistic regression models were used to calculate Odds Ratio (OR) and Relative Risk Ratio (RRR) with 95% confidence interval (95% CI).

In particular the following analyses were run:

V.1 MULTIPLE LOGISTIC REGRESSION ANALYSIS

Logistic regression is a statistical tool used to model the probability of success of a binary response variable (e.g. presence/absence of a disease) as a function of continuous and/or categorical (in this case, the analysis is also referred to as "logit" regression) explanatory variables (also referred as covariates or predictors) and parameters to be estimated [63].

In a logistic regression model, the coefficient of the categorical explanatory variables represents log-odds ratios (ORs) and quantifies the covariate effects on the probability of success. However, the results of logistic regression are usually reported as

estimated ORs, i.e. the odd of success given a particular exposure, compared to the odd of success in the absence of that exposure.

Multiple logistic regression is used to estimate ORs in presence of several explanatory variables; its main advantage is to correct for potential confounding effects by including all the predictors at the same time in the model.

Since the success probability ranges between 0 and 1, what is actually modelled is the logarithm of the odds of success, namely:

$$\text{Logit}(\pi) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots \beta_m x_m \quad (1)$$

where π indicates the success probability (e.g. the presence of a disease), β_k are the regression coefficients, and the x_k are *m* explanatory variables. The "reference group" is constituted by individuals having all the covariates equal to 0 (or all categorical variables equal to the reference level), and their log odds of success is represented by β_0. The goal of logistic regression is to estimate the m+1 unknown parameters β in Equation (1). This is usually accomplished by the maximum likelihood method, searching for the set of parameters, which maximizes the probability of the observed data given the hypothesized model [61].

In this thesis, multiple logistic regression models were used to estimate the effect of:

1) longitudinal changes in risk factors exposure (smoking habits, occupational exposure, vehicular traffic exposure) on respiratory symptoms/diseases incidence, controlling for anthropometric parameters, family history of respiratory/allergic diseases and allergic sensitization (PAPER I);

2a) risk factor exposure (smoking habits, second hand smoke exposure, occupational exposure, area of residence) on asthma/COPD remission, controlling for anthropometric parameters (PAPERS II and III);

2b) risk factor exposure (smoking habits, second hand smoke exposure, occupational exposure, area of residence) on asthma/COPD symptoms persistence, controlling for anthropometric parameters (PAPERS II and III).

V.2 MULTINOMIAL LOGISTIC REGRESSION ANALYSIS

Multinomial logistic regression is an extension of binary logistic regression that allows using a dependent variable with more than two categories. In particular, the effect of the predictors on the odd of success for each category, in comparison to the "reference category", is estimated. Multinomial regression simultaneously estimates the parameters in Equation (1) for several outcome categories.

In this thesis, multinomial logistic regression models were used to estimate the effect of:

a) longitudinal changes in risk factors exposure (smoking habits, occupational exposure, vehicular traffic exposure) on longitudinal changes in multimorbidity, controlling for anthropometric parameters, family history of respiratory/allergic diseases and allergic sensitization (PAPER IV);

b) longitudinal changes in risk factors exposure (smoking habits, occupational exposure, vehicular traffic exposure) on longitudinal respiratory disease phenotypes, controlling for anthropometric parameters and family history of respiratory/allergic diseases; all these analyses were performed on the whole sample and stratified by sex (PAPER V).

V.3 LATENT TRANSITION ANALYSIS

LTA was used to define the respiratory disease phenotypes over time (PAPER V). LTA identifies unobservable (latent) subgroups (classes or statuses) of individuals within a

population based on the values of multiple observed variables. Latent statuses (phenotypes) are mutually exclusive and exhaustive and they are not assumed stable over time [60].

LTA uses longitudinal data to estimate the probabilities of transition from one latent class to another and enables to estimate class membership over time. LTA is a methodology that addresses questions like "if an individual belong to a particular latent class at time *t*, what is the probability that he has given characteristics?" and "what is the probability that he will belong to a given that latent class at time t + 1?". Parameters estimated in LTA include [60]:

- probabilities of membership in latent status *s* at time *t* (δ_{st}); since the latent statuses are mutually exclusive and exhaustive at each time, it follows that:

$$\sum_{s_t=1}^{S} \delta_{s_t} = 1.$$

- "item response probabilities" ($\rho_{j,r_{jt}|s_t}$), which are used to characterize the structure of the latent classes, to provide information on how different the latent statuses are, and to label each status accordingly; they represent the probability of response r_{jt} to observed variable *j,* conditional on membership in latent status s_t at time *t.* For each combination of latent status *s,* observed variable *j,* and time *t,* there are R_j item-response probabilities, and

$$\sum_{r_{j,t}=1}^{R_j} \rho_{j,r_{j,t}|s_t} = 1$$

for all *j, t.*

- matrices of transition probabilities ($\tau_{s_{t+1}|s_t}$), i.e. probabilities of belonging to the latent status *s* at time *t* +1, conditional on membership in latent status *s* at time *t*. Again, since the statuses are mutually exclusive and exhaustive, it follows that

$$\sum_{s_{t+1}=1}^{S} \tau_{s_{t+1}|s_t} = 1.$$

Let's define an indicator function $I\ (y_{j,t} = r_{j,t})$ that equals 1 when the response to variable j equals r_J at time t, and 0 otherwise. Then, the likelihood function of the model can be expressed as a function of the probabilities of latent status membership at time 1 (δ_{s1}), the transition probabilities (the τ's), and item response probabilities (the ρ's):

$$P(\mathbf{Y}=\mathbf{y}) = \sum_{s_1=1}^{S} \cdots \sum_{s_T=1}^{S} \delta_{s_1} \tau_{s_2|s_1} \cdots \tau_{s_T|s_{T-1}} \prod_{t=1}^{T} \prod_{j=1}^{J} \prod_{r_{j,t}=1}^{R_j} \rho_{j,r_{j,t}|s_t}^{I(y_{j,t}=r_{j,t})}. \tag{2}$$

If there are only two time points, Equation (2) reduces to:

$$P(\mathbf{Y}=\mathbf{y}) = \sum_{s_1=1}^{S} \sum_{s_2=1}^{S} \delta_{s_1} \tau_{s_2|s_1} \prod_{t=1}^{2} \prod_{j=1}^{J} \prod_{r_{j,t}=1}^{R_j} \rho_{j,r_{j,t}|s_t}^{I(y_{j,t}=r_{j,t})}.$$

The Bayesian Information Criterion (BIC) can be used to choose the optimal number of latent classes in a LTA model. BIC allows comparing competing models in terms of balance between fit and parsimony. A smaller value represents a more optimal balance of model fit and parsimony; thus, a model with the minimum BIC might be selected [60].

VI. RESULTS

This thesis highlighted significant temporal changes in respiratory symptoms/diseases in general population samples and it showed that temporal changes (i.e. persistence and incidence) in lifetime habits and risk factors exposure were differently associated with the temporal changes in respiratory diseases occurrence in a *real life* setting.

The main results of the thesis were reported in the following sections; more exhaustive information can be found in the original papers (see Appendix).

VI.1 INCIDENCE AND ASSOCIATED RISK FACTORS (PAPER I)

In the longitudinal sample participating in both PI2 and PI3 survey, respiratory symptoms/diseases 18-yr cumulative incidence was: 3.4% for asthma, 6.3% for asthma attacks, 33.1% for AR, 4.3% for COPD, 14.8% for usual cough, 15.7% for usual phlegm, 12.5% for AO.

38.5% of subjects reported a new exposure to vehicular traffic in PI3 with respect to PI2, 18.3% to fumes/gases/dusts at work and 6.5% began smoking between PI2 and PI3 surveys. 25.5% of subjects quitted smoking before PI2 survey and 16.1% between PI2 and PI3 surveys; 12.1% was no more exposed to fumes/gases/dusts at work in PI3 with respect to PI2 and 10.8% to vehicular traffic.

The results of the multiple logistic regression analyses showed a significantly higher risk of AR and asthma incidence in subjects with occupational and vehicular traffic exposure (table 5) and of bronchitic symptoms/diseases incidence in smokers and in subjects with occupational exposure (tables 6a-6b).

Table 5. Longitudinal risk factors for asthma/allergic symptoms/diseases incidence*: ORs and 95% CI

	Asthma diagnosis	Asthma attacks	Allergic rhinitis
Smoking habits:			
incident	1.03 (0.24-4.40)	0.97 (0.25-3.85)	0.94 (0.48-1.83)
persistent	0.61 (0.16-2.36)	*2.15 (0.92-5.06)*	0.95 (0.55-1.62)
remittent for <18 yrs	0.92 (0.30-2.84)	1.11 (0.46-2.69)	1.27 (0.79-2.06)
remittent for ≥18 yrs	0.94 (0.36-2.44)	1.17 (0.55-2.48)	1.06 (0.69-1.62)
(ref: never)	1.00	1.00	1.00
Occupational exposure:			
incident	1.92 (0.68-5.45)	0.57 (0.22-1.46)	**2.35 (1.52-3.64)**
persistent	**3.37 (1.28-8.88)**	1.20 (0.59-2.43)	**1.93 (1.27-2.96)**
remittent	0.87 (0.17-4.32)	0.89 (0.35-2.23)	1.14 (0.66-1.97)
(ref: never)	1.00	1.00	1.00
Vehicular traffic exposure:			
incident	*2.31 (0.88-6.07)*	**2.18 (1.09-4.36)**	**1.57 (1.05-2.34)**
persistent	0.87 (0.24-3.21)	0.49 (0.18-1.38)	1.42 (0.91-2.21)
remittent	1.62 (0.43-6.17)	0.53 (0.14-1.94)	0.83 (0.45-1.52)
(ref: never)	1.00	1.00	1.00

ORs: odds ratios.

Reference category: No disease.

In italic: borderline values; in bold: statistically significant values.

Adjusted for age, sex, BMI, second hand smoke exposure, family history of allergic rhinitis, family history of respiratory diseases, SPT positivity.

* the analyses were conducted in the sub-sample of subjects participating in both PI2 and PI3 surveys (n=1107); incidence was computed as presence of symptoms or diagnoses in PI3 without symptoms & diagnoses in PI2; longitudinal risk factors were computed considering changes of exposure between PI2 and PI3 surveys.

Table 6a. Longitudinal risk factors for COPD and LLN AO incidence*: ORs and 95% CI

	COPD	LLN AO
Smoking habits:		
incident	---	---
persistent	**3.91 (1.22-12.56)**	**3.18 (1.15-8.83)**
remittent for <18 yrs	*2.84 (0.95-8.45)*	1.60 (0.55-4.71)
remittent for ≥18 yrs	*2.28 (0.88-5.91)*	1.31 (0.55-3.12)
(ref: never)	1.00	1.00
Occupational exposure:		
incident	0.83 (0.26-2.71)	0.85 (0.30-2.41)
persistent	**2.43 (1.02-5.76)**	1.67 (0.70-3.95)
remittent	0.21 (0.03-1.69)	1.26 (0.42-3.80)
(ref: never)	1.00	1.00
Vehicular traffic exposure:		
incident	2.04 (0.71-5.83)	0.57 (0.25-1.28)
persistent	2.13 (0.70-6.53)	*0.42 (0.16-1.07)*
remittent	2.04 (0.71-5.83)	0.30 (0.06-1.52)
(ref: never)	1.00	1.00

ORs: odds ratios; COPD: Chronic Obstructive Pulmonary Disease; LLN AO: airway obstruction according to lower limit of normal.

Reference category: No disease.

In italic: borderline values; in bold: statistically significant values.

Adjusted for age, sex, BMI, second hand smoke exposure, family history of allergic rhinitis and family history of respiratory diseases.

* the analyses were conducted in the sub-sample of subjects participating in both PI2 and PI3 surveys (n=1107); incidence was computed as presence of symptoms or diagnoses in PI3 without symptoms & diagnoses in PI2; longitudinal risk factors were computed considering changes of exposure between PI2 and PI3 surveys.

Table 6b. Longitudinal risk factors for bronchitic symptoms incidence*: ORs and 95% CI

	Usual phlegm	Usual cough
Smoking habits:		
incident	*2.06 (0.97-4.39)*	**2.71 (1.25-5.89)**
persistent	**2.24 (1.22-4.11)**	1.65 (0.86-3.17)
remittent for <18 yrs	0.94 (0.50-1.77)	0.95 (0.50-1.83)
remittent for ≥18 yrs	1.03 (0.62-1.73)	0.95 (0.56-1.60)
(ref: never)	1.00	1.00
Occupational exposure:		
incident	**1.68 (1.01-2.81)**	**1.71 (1.03-2.85)**
persistent	**1.75 (1.06-2.89)**	1.23 (0.73-2.08)
remittent	0.65 (0.31-1.37)	*0.48 (0.22-1.06)*
(ref: never)	1.00	1.00
Vehicular traffic exposure:		
incident	1.19 (0.73-1.94)	0.93 (0.57-1.52)
persistent	1.23 (0.71-2.13)	0.98 (0.56-1.69)
remittent	1.22 (0.62-2.43)	1.05 (0.52-2.12)
(ref: never)	1.00	1.00

ORs: odds ratios.
Reference category: No disease.
In italic: borderline values; in bold: statistically significant values.
Adjusted for age, sex, BMI, second hand smoke exposure, family history of allergic rhinitis and family history of respiratory diseases.
* the analyses were conducted in the sub-sample of subjects participating in both PI2 and PI3 surveys (n=1107); incidence was computed as presence of symptoms or diagnoses in PI3 without symptoms & diagnoses in PI2; longitudinal risk factors were computed considering changes of exposure between PI2 and PI3 surveys.

VI.2 ASTHMA REMISSION/PERSISTENCE AND ASSOCIATED RISK FACTORS (PAPER II)

In the longitudinal sample participating in AGAVE survey and reporting asthma symptoms/diagnosis in previous surveys (PI1, PI2, PI3), 52.5% of subjects were remittent, 17.2% persistent symptomatic without a lifetime physician diagnosis (a clue for underdiagnosis) and 30.3% persistent asthmatic.

The results of the multiple logistic regression analyses showed a significantly lower risk of asthma remission vs asthma persistence in subjects living in an urban area, with passive smoke exposure and asthma comorbidities (recurrent respiratory infections, AR, COPD and sleep apnoea) (table 7a). A higher risk of underdiagnosis vs asthma diagnosis was found in subjects with current smoking habits, second hand smoke exposure, obstructive sleep apnoea; a lower risk of underdiagnosis was found in subjects with AR (table 7b).

Table 7a. Risk factors for remittent asthma symptoms/diagnosis*: ORs and 95% CI

	Remittent asthma
Urban area of residence	**0.59 (0.38-0.94)**
(ref: rural)	1.00
Smoking habits:	
smokers	0.67 (0.40-1.14)
ex smokers	1.27 (0.81-2.00)
(ref: non smokers)	1.00
Passive smoke exposure	**0.48 (0.28-0.82)**
(ref: no)	1.00
Occupational exposure	0.71 (0.48-1.07)
(ref: no)	1.00
Recurrent respiratory infections	**0.45 (0.24-0.84)**
(ref: no)	1.00
AR	**0.26 (0.17-0.39)**
(ref: no)	1.00
COPD	**0.40 (0.20-0.77)**
(ref: no)	1.00
Sleep apnoea	**0.47 (0.26-0.87)**
(ref: no)	1.00

ORs: odds ratios; AR: allergic rhinitis; COPD: Chronic Obstructive Pulmonary Disease.
Reference category: Persistent asthma symptoms + Persistent asthma.
In bold: statistically significant values.
Adjusted for sex, age, BMI, educational level, diabetes, anxiety/depression.
* the analyses were conducted in the sub-sample of subjects participating in AGAVE survey (n=480). Longitudinal asthma changes were computed as follows: Remittent asthma, asthma symptoms (current asthma attacks or wheeze) or asthma diagnosis in at least one of the previous surveys and absence of current (last 12 months) asthma symptoms

& diagnosis & use of asthma medications in AGAVE survey; Persistent asthma symptoms, only current asthma symptoms (without a lifetime physician diagnosis) in both previous surveys and AGAVE survey; Persistent asthma, current asthma (with lifetime physician diagnosis) in both previous surveys and AGAVE survey. Risk factors pertained to the AGAVE survey.

Table 7b. Risk factors for persistent asthma symptoms ("underdiagnosis")*: ORs and 95% CI

	Persistent asthma symptoms
Urban area of residence	0.57 (0.27-1.19)
(ref: rural)	1.00
Smoking habits:	
smokers	**2.67 (1.25-5.73)**
ex smokers	1.14 (0.56-2.32)
(ref: non smokers)	1.00
Passive smoke exposure	**2.30 (1.13-4.68)**
(ref: no)	1.00
Occupational exposure	0.78 (0.42-1.44)
(ref: no)	1.00
Recurrent respiratory infections	0.74 (0.34-1.62)
(ref: no)	1.00
AR	**0.31 (0.17-0.56)**
(ref: no)	1.00
COPD	0.78 (0.33-1.80)
(ref: no)	1.00
Sleep apnoea	**2.27 (1.08-4.76)**
(ref: no)	1.00

ORs: odds ratios; AR: allergic rhinitis; COPD: Chronic Obstructive Pulmonary Disease.
Reference category: Persistent asthma.
In bold: statistically significant values.
Adjusted for sex, age, BMI, educational level, diabetes, anxiety/depression.
* the analyses were conducted in the sub-sample of subjects participating in AGAVE survey (n=480). Longitudinal asthma changes were computed as follows: Persistent asthma symptoms, only current asthma symptoms (without a lifetime physician diagnosis) in both previous surveys and AGAVE survey; Persistent asthma, current asthma (with lifetime physician diagnosis) in both previous surveys and AGAVE survey. Risk factors pertained to the AGAVE survey.

VI.3 COPD REMISSION/PERSISTENCE AND ASSOCIATED RISK FACTORS (PAPER III)

In the longitudinal sample participating in PI3 survey and reporting COPD symptoms/diagnosis in previous surveys (PI1, PI2), 39.6% of subjects were remittent, 30.4% persistent symptomatic without a lifetime physician diagnosis (a clue for underdiagnosis) and 30.0% persistent COPD.

The results of the multiple logistic regression analyses showed a significantly lower risk of COPD remission vs COPD persistence in subjects living in an urban area with respect to suburban one (table 8a); a lower risk of underdiagnosis vs COPD diagnosis was found in subjects with past smoking habits and comorbidites (heart failures and asthma) (table 8b).

Table 8a. Risk factors for remittent COPD symptoms/diagnosis*: ORs and 95% CI

	Remittent COPD
Smoking habits:	
smokers	0.57 (0.26-1.25)
ex-smokers	1.31 (0.67-2.58)
(ref: non smokers)	1.00
Urban area of residence	**0.52 (0.29-0.90)**
(ref: suburban)	1.00
Occupational exposure	0.63 (0.35-1.13)
(ref: no)	1.00
Heart failure	0.51 (0.17-1.51)
(ref: no)	1.00
Asthma	*0.42 (0.16-1.07)*
(ref: no)	1.00

ORs: odds ratios.
Reference category: Persistent COPD symptoms + Persistent COPD.
In italic: borderline values; in bold: statistically significant values.
Adjusted for sex, age, educational level.
* the analyses were conducted in the sub-sample of subjects participating in PI3 and reporting COPD symptoms/diagnosis in at least one of the previous surveys PI1, PI2

(n=257). Longitudinal COPD changes were computed as follows: Remittent COPD, COPD diagnosis or symptoms (usual cough or usual phlegm) in at least one of the previous surveys and absence of current COPD symptoms & diagnosis in PI3 survey; Persistent COPD symptoms, only current usual cough or usual phlegm (without a lifetime physician diagnosis) in both previous surveys and PI3 survey; Persistent COPD, current COPD (with lifetime physician diagnosis) in both previous surveys and PI3 survey. Risk factors pertained to PI3 survey.

Table 8b. Risk factors for persistent COPD symptoms ("underdiagnosis")*: ORs and 95% CI

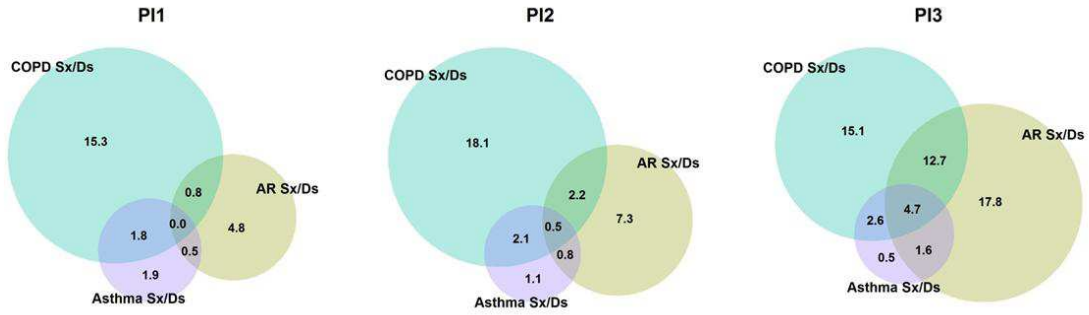
	Persistent COPD symptoms
Smoking habits:	
smokers	*0.36 (0.13-1.02)*
ex-smokers	**0.19 (0.07-0.49)**
(ref: non smokers)	1.00
Urban area of residence	0.83 (0.41-1.72)
(ref: suburban)	1.00
Occupational exposure	1.29 (0.57-2.91)
(ref: no)	1.00
Heart failure	**0.25 (0.07-0.91)**
(ref: no)	1.00
Asthma	**0.23 (0.07-0.71)**
(ref: no)	1.00

ORs: odds ratios.
Reference category: Persistent COPD.
In italic: borderline values; in bold: statistically significant values.
Adjusted for sex, age, educational level.
***** the analyses were conducted in the sub-sample of subjects participating in PI3 and reporting COPD symptoms/diagnosis in at least one of the previous surveys PI1, PI2 (n=257). Longitudinal COPD changes were computed as follows: Persistent COPD symptoms, only current COPD symptoms (without a lifetime physician diagnosis) in both previous surveys and PI3 survey; Persistent COPD, current COPD (with lifetime physician diagnosis) in both previous surveys and PI3 survey. Risk factors pertained to the PI3 survey.

VI.4 TEMPORAL CHANGES IN MULTIMORBIDITY AND ASSOCIATED RISK FACTORS (PAPER IV)

In the longitudinal sample participating in PI1, PI2 and PI3, a significant increase in the co-presence of "COPD&AR" and of "Asthma & AR" from PI1/PI2 to PI3 was found; the same results were found for the co-presence of all the three conditions "Asthma & AR & COPD" (figure 3).

Figure 3. Venn diagram of respiratory morbidity in the longitudinal sample PI1/PI2/PI3



AR: allergic rhinitis; COPD: chronic obstructive pulmonary disease; Sx/Ds: symptoms or diagnosis.

In the longitudinal sample participating in both PI2 and PI3, the following longitudinal changes in respiratory morbidity were found: 48.4% "Never/improving", 11.7% "Persistent medical conditions", 21.3% "Incident morbidity" and 18.6% "Incident multimorbidity".

The results of the multinomial logistic regression analyses showed a significantly higher risk of "Persistent medical condition" vs "Never/improving" in persistent smokers and in subjects with persistent or remittent occupational exposure; a significantly higher risk of "Incident morbidity" emerged in subjects with incident or persistent occupational

exposure and in those with persistent vehicular traffic exposure; at last, a significantly higher risk of "Incident multimorbidity" was found in persistent smokers, in subjects with incident or persistent occupational exposure and in those with incident vehicular traffic exposure (table 9).

Table 9. Longitudinal risk factors for longitudinal changes in respiratory morbidity*: RRRs and 95% CI

	Persistent medical condition	Incident morbidity	Incident multimorbidity
Smoking habits:			
incident	1.84 (0.73-4.64)	0.81 (0.38-1.72)	*1.90 (0.90-4.02)*
persistent	**2.02 (1.02-4.00)**	1.19 (0.69-2.07)	**1.86 (1.03-3.35)**
remittent for < 18 yrs	1.45 (0.75-2.80)	0.87 (0.51-1.46)	1.23 (0.70-2.16)
remittent for ≥ 18 yrs	*1.61 (0.92-2.85)*	1.02 (0.66-1.59)	1.17 (0.72-1.89)
(ref: never)	1.00	1.00	1.00
Occupational exposure:			
incident	*1.87 (0.97-3.59)*	**1.83 (1.15-2.90)**	**2.24 (1.35-3.71)**
persistent	**2.95 (1.68-5.18)**	**1.63 (1.04-2.57)**	**2.49 (1.55-4.01)**
remittent	**1.98 (1.03-3.79)**	1.02 (0.58-1.78)	0.63 (0.31-1.26)
(ref: never)	1.00	1.00	1.00
Vehicular traffic exposure:			
incident	1.22 (0.71-2.11)	1.35 (0.88-2.07)	**2.33 (1.45-3.74)**
persistent	1.30 (0.72-2.37)	**1.59 (1.00-2.54)**	1.46 (0.85-2.51)
remittent	1.50 (0.75-3.02)	1.10 (0.59-2.05)	1.24 (0.62-2.49)
(ref: never)	1.00	1.00	

Reference category: Never/improving.
RRRs: relative risk ratios.
Adjusted for: age, sex, BMI, educational level, family history of respiratory diseases, family history of allergic rhinitis, positivity to skin prick test.
In italic: borderline values; in bold: statistically significant values.
* the analyses were conducted in the sub-sample of subjects participating in both PI2 and PI3 surveys (n=1107). Longitudinal changes in morbidity were computed as follows: Never/improving, never asthma & AR & COPD / reduction in the number of medical conditions (i.e. from 1 to 0 condition; from 2 to 1 condition; from 3 to 2/1 conditions); Persistent medical condition; Incident morbidity (i.e. from 0 to 1 condition); Incident

multimorbidity (i.e. from 0/1 to 2/3 conditions; from 2 to 3 conditions). Longitudinal risk factors were computed considering changes of exposure between PI2 and PI3.

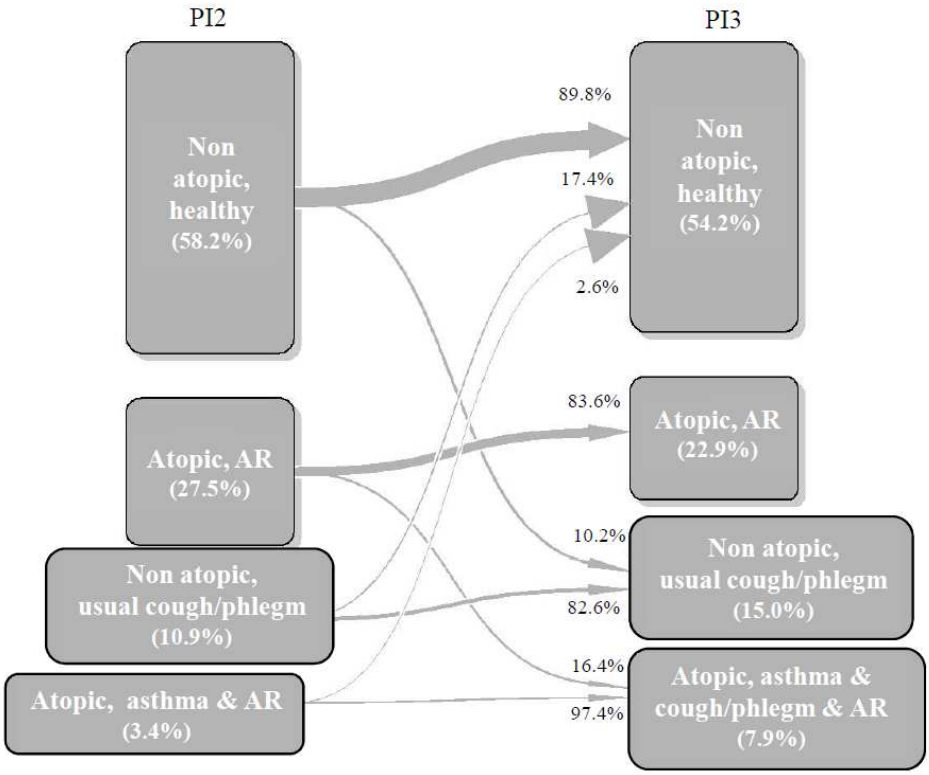
VI.5 TRAJECTORIES OF RESPIRATORY DISEASE PHENOTYPES AND ASSOCIATED RISK FACTORS (PAPER V)

In the longitudinal sample participating in both PI2 and PI3, 4 cross-sectional phenotypes were found: "Non atopic, healthy", "Atopic, AR", "Non atopic, usual cough/phlegm" and "Atopic, asthma & usual cough/phlegm & AR" (labelled "Atopic, asthma & AR" in PI2) (figure 4).

Despite the use of the same labels to identify the cross-sectional phenotypes at different time points, there were some differences in the characteristics of the four phenotypes between PI2 and PI3; in particular, a higher frequency of AR was found in "Non atopic, healthy" (25.0% vs 4.3%), "Atopic, AR" (41.1% vs 22.0%) and "Non atopic, usual cough/phlegm" (56.6% vs 12.4%), comparing PI3 with PI2. As regards the PI3 "Atopic, asthma & usual cough/phlegm & AR", with respect to PI2 "Atopic, asthma & AR", an increase in the frequency of bronchitic and allergic status (39.5% PI2 vs 54.0% PI3 for usual cough, 23.7% PI2 vs 59.8% PI3 for usual phlegm and 42.1% PI2 vs 66.7% PI3 for AR) and a decrease in the frequency of asthma status (84.2% PI2 vs 33.3% PI3 for asthma and 60.5% PI2 vs 35.6% PI3 for asthma attacks) emerged.

Overall, subjects didn't show a strong probability of changing phenotypes across time (about 85% of persistent status) (figure 4); the transition probabilities between phenotypes varied from 2.6% to 17.4%. Atopic phenotypes had the highest probabilities of worsening (16.4% "Atopic, AR" towards "Atopic, asthma & usual cough/phlegm & AR") and non atopic phenotypes the highest probabilities of improving (17.4% "Non atopic, usual cough/phlegm" towards "Non atopic, healthy") (figure 4).

Figure 4. Phenotypes transition plot from PI2 to PI3 survey



According to the transition probabilities matrix, the main longitudinal patterns of disease phenotypes were: "Improving health status" (2.0%), "Persistent healthy" (52.2%), "Persistent AR" (22.9%), "Persistent usual cough/phlegm" (9.0%), "Worsening health status" (13.8%).

Multinomial logistic regression analysis showed a significantly lower risk of "Improving health status" vs "Persistent health status" in subjects with incident and persistent vehicular traffic exposure; a significantly higher risk of "Persistent allergic rhinitis" in subjects with remittent occupational exposure; a significantly higher risk of "Persistent usual cough/phlegm" in persistent smokers and remittent smokers for < 18 yrs and in subjects with incident and persistent occupational exposure; a significantly higher risk of "Worsening health status" in incident and persistent smokers (table 10).

Table 10a. Longitudinal risk factors for longitudinal changes in disease phenotypes*:

RRRs and 95% CI

	Improving health status	Persistent allergic rhinitis	Persistent usual cough/phlegm	Worsening health status
Smoking habits:				
incident	---	0.77 (0.40-1.51)	0.57 (0.07-4.55)	**2.16 (1.07-4.36)**
persistent	2.73 (0.82-9.01)	0.68 (0.39-1.19)	**5.92 (2.86-12.26)**	**1.95 (1.09-3.49)**
remittent for < 18 yrs	1.60 (0.49-5.25)	1.07 (0.69-1.68)	**2.27 (1.07-4.79)**	0.96 (0.53-1.76)
remittent for ≥ 18 yrs	0.46 (0.12-1.85)	0.85 (0.57-1.26)	*1.79 (0.93-3.43)*	1.07 (0.65-1.76)
(ref: never)	1.00	1.00	1.00	1.00
OE:				
incident	2.39 (0.75-7.65)	1.03 (0.66-1.60)	**2.63 (1.27-5.45)**	1.46 (0.89-2.40)
persistent	1.22 (0.36-4.22)	1.21 (0.80-1.82)	**3.51 (1.83-6.74)**	1.43 (0.88-2.33)
remittent	1.28 (0.30-5.47)	**1.60 (1.00-2.56)**	1.73 (0.77-3.87)	0.74 (0.37-1.48)
(ref: never)	1.00	1.00	1.00	1.00
VT:				
incident	**0.33 (0.12-0.94)**	0.85 (0.58-1.25)	1.09 (0.60-1.98)	*1.61 (0.97-2.65)*
persistent	**0.13 (0.03-0.62)**	1.08 (0.71-1.63)	0.87 (0.45-1.69)	*1.59 (0.92-2.75)*
remittent	0.32 (0.07-1.59)	0.78 (0.45-1.36)	1.06 (0.47-2.40)	1.32 (0.67-2.61)
(ref: never)	1.00	1.00	1.00	1.00

RRRs: relative risk ratios; OE: occupational exposure; VT: vehicular traffic exposure.
Reference category: Persistent health status.
Adjusted for: age, sex, educational level, BMI, family history of respiratory diseases, family history of allergic rhinitis.
In italic: borderline values; in bold: statistically significant values.
* the analyses were conducted in the sub-sample of subjects participating in both PI2 and PI3 surveys (n=1107). Longitudinal changes in disease phenotypes were computed as follows: "Persistent healthy" ("Non atopic, healthy" phenotype in both PI2 and PI3); "Persistent AR" ("Atopic, AR" in both PI2 and PI3); "Persistent usual cough/phlegm" ("Non atopic, usual cough/phlegm" in both PI2 and PI3); "Improving health status" (improvement of health status from PI2 to PI3: "Non atopic, usual cough/phlegm" changing in "Non atopic, healthy"; "Atopic, asthma & AR" changing in "Non atopic, healthy"); "Worsening health status" (worsening of health status from PI2 to PI3: "Non atopic, healthy" changing in "Non atopic, usual cough/phlegm"; "Atopic, AR" changing in "Atopic, asthma & usual cough/phlegm & AR"; "Atopic, asthma & AR" changing in "Atopic, asthma & usual cough/phlegm & AR"). Longitudinal risk factors were computed considering changes of exposure between PI2 and PI3.

The same analyses were run stratified by sex: *in males* multinomial logistic regression analysis showed a significantly higher risk of "Persistent disease status" in remittent smokers for < 18 yrs (OR 1.90, 95% CI 1.05-3.47); a significantly higher risk of "Worsening health status" in incident smokers (OR 3.85, 95% CI 1.38-10.74) and a significant inverse association in subjects with remittent vehicular traffic exposure (OR 0.27, 95% CI 0.07-0.99).

In females, a significantly higher risk of "Persistent disease status" was found in subjects with persistent occupational exposure (OR 1.80, 95% CI 1.05-3.11) and with persistent vehicular traffic exposure (OR 1.68, 95% CI 1.00-2.80); a significantly higher risk of "Worsening health status" was found in subjects with remittent vehicular traffic exposure (OR 3.99, 95% CI 1.43-11.16).

VII. DISCUSSION

In this section the main results are put into context and discussed in relation to previous findings. A more detailed discussion is reported in the original papers (see Appendix).

VII.1 INCIDENCE AND ASSOCIATED RISK FACTORS (PAPER I)

Elevated respiratory symptoms/diseases incidence values at 18-yr follow-up were shown. These data were in line with those of other studies, indicating an increasing trend in particular for allergic diseases/symptoms (table 2).

Through multiple logistic regression models the following associations emerged: occupational exposure and vehicular traffic exposure with allergic/asthmatic diseases incidence; smoking habits and occupational exposure with COPD incidence.

As regards occupational exposure, higher risks for asthma, AR, COPD, usual cough and usual phlegm incidence were found in subjects with incident and persistent exposure with respect to unexposed subjects. These results are in line with those of other European cohorts; few studies took into account the effect of incident exposure (table 11).

Table 11. Occupational exposure and respiratory symptoms/diseases incidence

	Reference	Country	Population	Follow-up period	Exposure	Results
Asthma diagnosis	Torén 2011 [8]	Western Sweden	n=15761 16-75 yrs	1990-2008 18 yrs	dusts/fumes exposure	HR 1.8
	Storaas 2015 [65]	Northern Europe	n=16191 25-64 yrs	1990-1999 9 yrs	ever welding	HR 1.4
	PAPER I	Italy	n=1107 8-78 yrs	1991-1993 18 yrs	persistent dusts/fumes/ gases	OR 3.4
AR	Riu 2007 [66]	Germany	n=3785 16-18 yrs	1995-2002 7 yrs	incident chemicals/irritants/fumes and/or ETS	OR 1.5

	Storaas 2015 [65]	Northern Europe	n=16191 25-64 yrs	1990-1999 9 yrs	ever welding	HR 1.4
	PAPER I	Italy	n=1107 8-78 yrs	1991-1993 18 yrs	persistent dusts/fumes/ gases	OR 1.9
	PAPER I	Italy	n=1107 8-78 yrs	1991-1993 18 yrs	incident dusts/fumes/ gases	OR 2.4
COPD	Pallasaho 2014 [67]	Finland	n~6000 20-69 yrs	1996-2007 11 yrs	dusts/gases/f umes	OR 2.1
	PAPER I	Italy	n=1107 8-78 yrs	1991-1993 18 yrs	persistent dusts/fumes/ gases	OR 2.4
LLN AO	Mehta 2012 [16]	Switzerla nd	n=4267 18-62 yrs	1991-2001 10 yrs	vapours/gase s/dusts/fumes high exposure	IRR 2.5
Usual phlegm	Skorge 2009 [68]	Norway	n=2401 15-70 yrs	1985-1996 11 yrs	gases/fumes	OR 1.4 in women
	Mirabelli 2012 [15]	US	n=8967 45-64 yrs	'80s 3 yrs	gases/fumes	OR 1.4
	PAPER I	Italy	n=1107 8-78 yrs	1991-1993 18 yrs	persistent dusts/fumes/ gases	OR 1.8
	PAPER I	Italy	n=1107 8-78 yrs	1991-1993 18 yrs	incident dusts/fumes/ gases	OR 1.7

AR: allergic rhinitis; COPD: chronic obstructive pulmonary disease; LLN AO: airway obstruction according to lower limit of normal; ETS: environmental tobacco smoke; HR: hazard ratio; OR: odds ratio; IRR: incident rate ratio.

In Pisa, as in many other urban areas, vehicular traffic is increased in the last years. Incident vehicular traffic exposure was related to higher risk of having AR and asthma attacks incidence. Several studies supported air pollution role in childhood onset asthma, but few in adult-onset asthma [69]. There is evidence that people living in urban areas more frequently experience allergic respiratory diseases and bronchial asthma than those living in rural areas: one of the main causes is the increased presence of outdoor air pollutants resulting from energy consumption and vehicles exhaust emissions [70-72].

Smoking habits is the main risk factor for COPD occurrence and exacerbation. In these analyses, persistent smokers had an elevated risk of developing COPD, AO and usual phlegm, as well as incident smokers had a higher risk of developing usual cough. These results are in line with other European cohorts [67,73]. The relationship was confirmed also by the results of objective measurements like spirometry [74-75]. On the other hand, smoking habits did not affect the incidence of asthma, in agreement with another Italian study [11].

VII.2 DISEASES REMISSION/PERSISTENCE AND ASSOCIATED RISK FACTORS (PAPER II, PAPER III)

VII.2.1 Asthma/COPD remission and persistence

High values of diseases remission and symptoms persistence without receiving lifetime physician diagnosis (a clue for underdiagnosis) were shown during a mean 22-yr follow-up: 52.5% of asthma remission, 39.6% of COPD remission, 17.2% of asthma symptoms persistence and 30.4% of COPD symptoms persistence.

These results are in line with other scientific evidences [32,36,76-77], even if both higher [31,39,78-79] and lower values were found in literature [18,33,80-82] (figure 5, figure 6).

Figure 5. Asthma/COPD remission

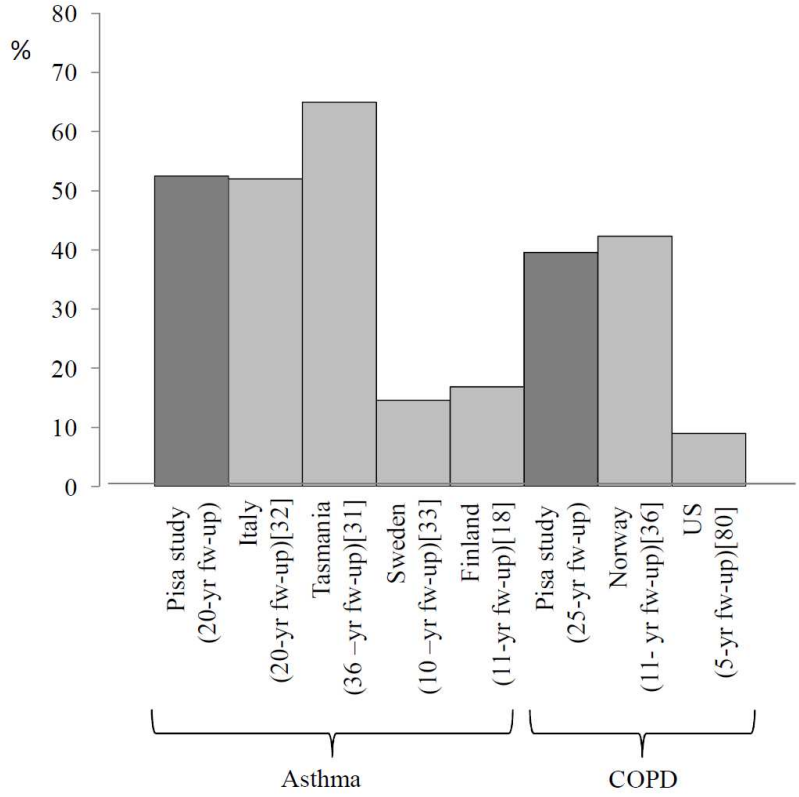
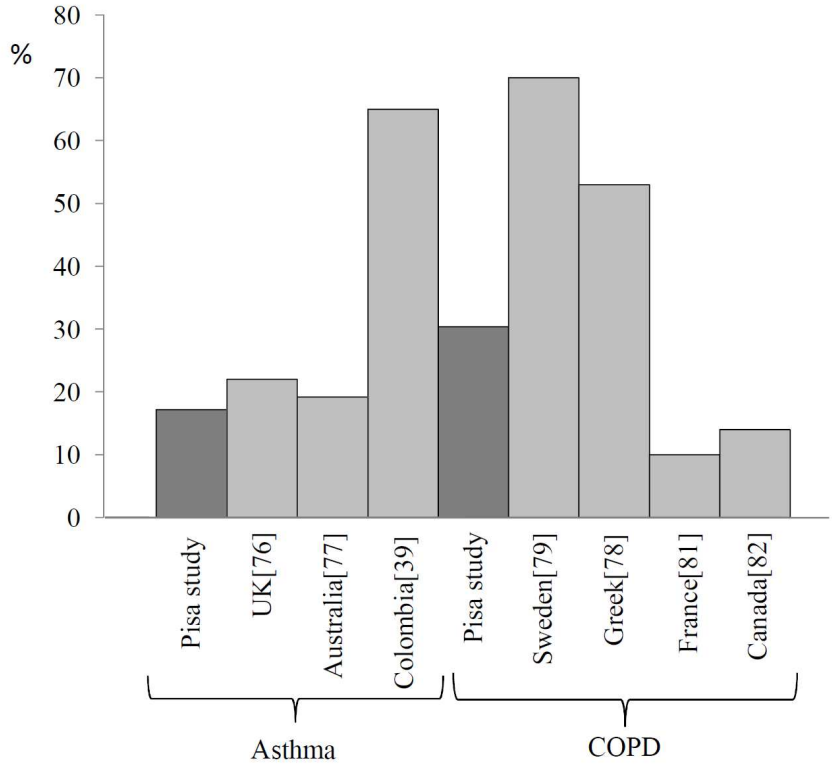


Figure 6. Asthma/COPD underdiagnosis



These variations among studies may partly be explained by different follow-up periods and quite different definitions of remission (i.e. clinical remission or complete remission) and underdiagnosis [27,83].

From a clinical standpoint, to elucidate the asthma and COPD natural history and long term outcomes is an issue of primary importance. Studies on patient cohorts can help [27].

VII.2.2 Risk factors

Few epidemiological studies focused on risk factors associated with disease remission and underdiagnosis; the results of this thesis added new evidences about this topic.

Asthma/COPD remission

Asthma and COPD remitted less in subjects with comorbidities. Some comorbidities arise independently of asthma/COPD, some others have common risk factors, other can increase the risk of asthma/COPD developing or severity worsening. Thus, the coexistence with other diseases may have a significant impact on prognosis.

In line with other international researches, asthma showed a lower risk of remission in subjects with AR [30-34], with COPD [29,84-85], with respiratory infections [28,86] and with sleep obstructive apnoea [87-88].

COPD showed a borderline lower risk of remission in subjects with asthma, in line with past and recent evidences highlighting an association between bronchial hyper-responsiveness (BHR) (an important clinical characteristic of active asthma) and risk of COPD [17,89].

Living in urban area was inversely related to asthma/COPD remission. This result can be explained by the strong scientific evidences showing that urban living is an important risk factor for disease onset and exacerbation [22-23,90-91]; other previous

findings found in Pisa samples showed a significant association between urban living and BHR [92] and between living near traffic roads and risk of COPD in males [93].

Asthma "underdiagnosis"

The results about "underdiagnosis" were separately discussed because different associations were found in asthma and COPD.

Asthma "underdiagnosis" showed a strong relationship with current smoking and second-hand smoke. European and USA studies confirmed that smoking habit and second-hand smoke were significant independent risk factors for undiagnosed wheeze and asthma [76,94-97]. Probably, respiratory symptoms like wheeze or breathlessness could be misinterpreted as due to smoking, rather than to asthmatic condition, thus not reported to the physician. In the other hand, asthma-like symptoms are common also in subjects with COPD, which is often smoke-related, making difficult to disentangle the relationship between smoking and asthma-like symptoms.

These data showed that having AR reduced the risk of asthma underdiagnosis; on the contrary, presence of obstructive sleep apnoea was a risk factor for underdiagnosis. These findings highlighted the possible influence of asthma comorbidities on asthma management. On one side, the co-presence of diseases strictly related to asthma, like allergic rhinitis, can facilitate asthma diagnosis, both by promoting contact with a doctor and by increasing doctor awareness toward diagnosis [94]. On the other side, the co-presence of other diseases with similar clinical manifestations, like the obstructive sleep apnoea, could lead to not recognize or to misinterpret asthma symptoms [98].

COPD "underdiagnosis"

These data showed that being ex smokers and having asthma and heart failures reduced the risk of COPD underdiagnosis.

A possible explanation of these results is that exposure to well know COPD risk factors and the presence of COPD comorbidities could increase the physician awareness of patients' symptoms and, thus, the possibility of a correct disease diagnosis. Indeed, GOLD guidelines for the COPD management highlight the relationship between COPD and smoking habits, advocating active case finding like performing spirometry in patients with symptoms and/or risk factors exposure [35]. On the other hand, an asthma or heart failure diagnosis (COPD comorbidities), in addition to bronchitic symptoms (cough/phlegm), could facilitate the COPD diagnosis by physician and reduce the risk of underdiagnosis [35,98].

VII.3 TEMPORAL CHANGES IN MULTIMORBIDITY AND ASSOCIATED RISK FACTORS (PAPER IV)

VII.3.1 Multimorbidity temporal changes

Most of the evidence on multimorbidity came from cross-sectional studies and longitudinal studies are limited [43]. Our data added new information in a longitudinal general population sample, showing a respiratory multimorbidity incidence of about 19% during a 18-yr follow-up; in particular the highest increases were for asthma/COPD and AR/COPD overlap, maybe due to ageing of the cohort [89] and cumulative long term exposure to risk factors.

21.6% of respiratory multimorbidity was found in PI3, a result within the prevalence range found in other general population samples (13%-72%) [41,99-100].

VII.3.2 Risk factors

Morbidity and multimorbidity incidence was higher in subjects with incident and persistent occupational exposure and with incident and persistent vehicular traffic exposure;

moreover, persistent smoking habit was related to a significantly higher risk of multimorbidity incidence.

Cross-sectional studies explored associations between multimorbidity prevalence and risk factors exposure, but the paucity of longitudinal data determined that the direction of any relationship is not clear. Nonetheless, given the causal relationship between smoking habits, environmental and occupational exposure and many chronic conditions, it is likely that these risk factors directly contribute to the development of multimorbidity [43,101].

Moreover, these results confirmed that persistent smoking habit and persistent occupational exposure were related to persistent respiratory medical condition vs never/improving condition [21,23]. As regards the relationship between persistent medical condition and remittent occupational exposure, it could be interpreted as reverse causation (i.e. subjects change their lifestyle habits after developing a disease).

As above reported, recent reviews and reports highlighted that few data are available about modifiable factors that predict the risk of different types of multimorbidity [41]: it remains unknown whether there are biological, environmental or behavioural factors. The identification of any such factors, and the assessment of the likelihood of causality, requires data from prospective observational studies [41,43].

The results obtained in the Pisa longitudinal study added new information about modifiable risk factors and onset/increase of multimorbidity.

VII.4 TRAJECTORIES OF RESPIRATORY DISEASE PHENOTYPES AND ASSOCIATED RISK FACTORS (PAPER V)

VII.4.1 Respiratory phenotypes temporal changes

Using an unsupervised method (LTA), four distinct classes of respiratory phenotypes were identified in general population. This thesis identified classes determined by clinical characteristics and allergy profiles focusing on respiratory and allergic multimorbidities. Moreover, the longitudinal design permitted to address latent class stability over time.

Four main phenotypes of respiratory diseases in a general population sample were identified: "Non atopic, healthy", "Atopic, AR", "Non atopic, usual cough/phlegm", "Atopic, asthma & AR".

The phenotypes prevalence found in Pisa sample, using LTA, was very similar to previously published prevalence values of respiratory symptoms/diseases found in general population samples [3,5,102-104], suggesting plausibility and meaningfulness of computed phenotypes (table 12).

Table 12. Comparison among respiratory symptoms/diseases phenotypes and respiratory symptoms/diseases prevalence in general population samples

	Reference	Country	Population	Period	Prevalence (%)
"Atopic, AR"	PAPER IV	Pisa, Italy	n=1107 8-78 yrs	1991-1993 2009-2011	27.5% 22.9%
	Maio 2016 [5]	Pisa, Italy	n=2841 and n=1620 4-97 yrs	1991-1993 2009-2011	20.2% 37.4%
	De Marco 2012 [3]	Italy	n= 6031 and n=10494 20-44 yrs	1991-1993 2007-2010	16.8% 25.8%
"Non atopic, usual cough/phlegm"	PAPER IV	Pisa, Italy	n=1107 8-78 yrs	1991-1993 2009-2011	10.9% 15.0%
	Maio 2016 [5]	Pisa, Italy	n=2841 and n=1620 4-97 yrs	1991-1993 2009-2011	14.4% cough 12.0% phlegm 16.5% cough

					19.5% phlegm
	Backman 2014 [102]	Sweden	n=8333 and n= 7997 20-69 yrs	1996 2006	12.4% cough 19.0% phlegm 10.1% cough 15.0% phlegm
"Atopic, asthma & AR"	PAPER IV	Pisa, Italy	n=1107 8-78 yrs	1991-1993 2009-2011	3.4% 7.9%
	Bugiani 2005 [103]	Italy	n=17666 20-44 yrs	1999-2000	5.2%
	Gough 2015 [104]	Germany	n=942 20 yrs	2010	6.0%

AR: allergic rhinitis.

Moreover, the reduction of frequency of "Non atopic, healthy" phenotype and the increase of frequency of respiratory disease phenotypes, found from PI2 to PI3, are in line with the increasing prevalence of respiratory symptoms/diseases highlighted in recent scientific papers [3,5,7,98].

Overall, 13.8% of subjects had a "worsening health status", probably due to the ageing of this population but also to the long-term exposure to environmental risk factors and lifetime habits, as confirmed by results of the multinomial logistic regression analysis.

According to the transition matrix, 97.4% of subjects remained stable in "atopic, asthma & AR" phenotype (labelled "atopic, asthma & usual cough/phlegm & AR" in PI3); nevertheless, this condition was considered as "worsening health status" longitudinal phenotype, due to the fact that a significant increase of usual cough/phlegm and a significant reduction of asthma and asthma attacks emerged from PI2 "atopic, asthma & AR" to PI3 "atopic, asthma & usual cough/phlegm & AR". This change can be suggestive of asthma evolution towards COPD [35] or of the coexistence between asthma and COPD in the same subject, a condition described as Asthma and COPD Overlap Syndrome [98].

Finally, results showed stability in atopic/non atopic condition from PI2 to PI3 and highlighted as atopic subjects had a worst course of disease status with respect to non

atopic subjects; in particular "atopic allergic" subjects and "atopic allergic asthmatic" subjects tended to move towards PI3 "atopic, asthma & cough/phlegm & AR", suggesting allergy as risk factor for development of asthma [105] and, directly or indirectly, of COPD [35,98,106].

VII.4.2 Risk factors

“Improving health status” was significantly lower in subjects living exposed to vehicular traffic; “Persistent cough/phlegm” was higher in smokers and in subjects with occupational exposure; “Persistent AR” was higher in subjects with remittent occupational exposure; “Worsening health status” was higher in smokers.

These findings confirm the strong relationship between tobacco use, air pollutants and occupational exposure and respiratory symptoms/diseases prevalence and incidence, as reported in WHO official statements [21,23] and already above discussed.

As regards the relationship between persistent AR and remittent occupational exposure and between persistent usual cough/phlegm and remittent smoking, it could be interpreted as reverse causation (i.e. subjects change their lifestyle habits after developing a disease).

Additional results were found comparing longitudinal respiratory symptoms/diseases phenotypes between sexes, suggesting a different susceptibility to risk factors exposure: smoking habits were related to "Persistent disease status" and "Worsening health status" in males; occupational and vehicular traffic exposure was related to "Persistent disease status" in females.

A higher frequency of smokers was found in males with respect to females, a result in line with the 2010 DOXA Italian data [107]; this lifetime habit may have determined a

significant relationship between respiratory disease phenotypes and smoke in males but not in females.

Vehicular traffic and occupational exposure was related to females and not to males. Women are more susceptible to inflammatory lung disease induced by air pollution and show worse adverse pulmonary health outcomes than men. A possible mechanism underlying these differences could be a sex difference in the expression of lung inflammatory mediators that affect sex-specific immune responses to environmental toxicants [108]. Moreover as regards the occupational exposure, women, with respect to men, complain more frequently about major threats to respiratory health tract coming from cleaning and sterilizing agents used in health care facilities, as well as from dust in the textile and clothing industry [109].

It is to mention that, nowadays, LTA or cluster analyses were used to assess phenotypes focusing on single diseases (i.e COPD or asthma) [47-48,110]. These results put a new insight into the possibility of performing LTA to assess phenotypes using population-based data, integrating at the same time different disease domains (diagnoses, symptoms, objective measurements, multiple diseases) and time repeated measurements.

VII.5 LIMITS AND STRENGTHS

VII.5.1 Limits

Use of a questionnaire for collecting data about respiratory symptoms/diseases and about environmental exposure might be a limitation because it is potentially affected by a reporting bias, as it relies upon individual memory and subjects' answers about exposure could be influenced by their current health status; nevertheless, the standardized questionnaire is one of the main investigation tool in respiratory epidemiology [111-112]. Moreover, Sanchez et al, comparing the answers to simple questions about asthma (i.e.

‘‘ever asthma’’) through repeated measurements with data coming from a comprehensive drugs reimbursement database, found a good correlation, highlighting the possibility of conducting studies on asthma variability using questionnaire as a proxy of clinical assessments [113].

In addition, objective outcomes (lung function), not affected by such potential bias, were also applied.

It is to point out that spirometry was performed using different instruments in PI2 and PI3; a correction factor was derived to overcome this limit and to permit the comparison between studies, as reported in a previous paper [5].

Except for smoking habits, no information about the period of change (i.e. age, year) in exposure status was available in the database; thus, it was not possible to analyze a precise temporal association between changes in disease status and changes in risk factors exposure.

As regards the subjects lost to follow-up, a comparison of the baseline characteristics and health status of subjects who were followed-up (PI2&PI3 participants) with those of subjects who weren’t (only PI2 participants) was performed; some differences regarding age and prevalence of COPD symptoms/disease were found, with the highest values in only PI2 participants than in PI2&PI3 participants. Thus, the incidence value was computed in a sample of younger healthy subjects, with a possible conservative estimate of the reported incidence values. Such occurrence was confirmed by a recent article showing that, among long-term participants in population surveys, disease prevalence rates tend to be slightly lower than for the total baseline population, suggesting that subjects who continue to participate in a study are healthier than those who quit [114].

Other specific limits related to the outcomes computation were reported in the original papers (see Appendix).

VII.5.2 Strengths

A strength of this population-based study is to have applied, over a 25-yr follow-up, the same study design, sampling frame and study protocol in repeated cross-sectional surveys on general population random samples living in the same area. In all the surveys, questions were derived from validated international questionnaires, which already had passed the scrutiny of independent reviewers.

Moreover, a wide general population sample spanning from childhood to the elderly was analyzed, with a vast amount of individual qualitative and quantitative data.

Another strength is the consistency of the results with those from other international studies, adding new evidences about longitudinal risk factors exposure associated with longitudinal changes in respiratory symptoms/diseases occurrence in general population samples using simple methodological approaches (longitudinal changes in presence/absence of 1 disease outcome) and more complex ones (LTA).

LTA allowed to simultaneously accounts for several disease domains repeatedly measured over time. If disease prevalence changes, there is a need to understand what dynamics contributed to the change; transition probabilities better explain the nature of such changes [49]. This approach applied to a longitudinal dataset is exploratory and hypothesis generating. However, the phenotypes observed were plausible and meaningful.

VIII. CONCLUSIONS

Few longitudinal studies on general population are available in literature; the Pisa study permitted to assess an important topic, like the increase of respiratory symptoms/diseases, addressing different and specific aspects, with different methodological approaches, and adding new scientific evidences.

These results highlighted substantial temporal changes in respiratory symptoms/diseases in general population samples, living in an urban/suburban area: high values of incidence, remission and persistence and increase in respiratory multimorbidity.

Temporal changes in lifetime habits and in risk factors exposure were differently associated with the natural history of respiratory diseases in a *real life* setting:

Smoke:

- persistent smoking habits were related to: persistent disease condition (PAPER IV), in particular persistent usual cough/phlegm phenotype (PAPER V); worsening health status (PAPER V) due to new onset of usual cough/phlegm (PAPER I), bronchitic diseases (COPD, AO) (PAPER I) and respiratory mutimorbidity (PAPER IV);
- incident smoking habits were related to: worsening health status (PAPER V), in particular due to new onset of usual cough/phlegm (PAPER I);
- passive smoke exposure reduced the probability of asthma remission (PAPER II);
- smoking habits seemed to facilitate COPD diagnosis (PAPER III), while masking asthma diagnosis (PAPER II).

Occupational exposure:

- incident and persistent occupational exposure was related to: persistent disease condition (PAPER IV), in particular persistent usual cough/phlegm phenotype (PAPER V); new

onset of respiratory morbidity and multimorbidity (PAPER IV), in particular of allergic diseases (PAPER I) and bronchitic symptoms/diseases (PAPER I);

- persistent and current occupational exposure was related to: asthma onset (PAPER I) and lower probability of asthma remission (PAPER II), respectively.

Vehicular traffic exposure:

- incident and persistent vehicular traffic exposure was related to: new onset of respiratory morbidity and multimorbidity (PAPER IV), in particular of allergic diseases (PAPER I) and of asthma symptoms (PAPER I), reducing the probability of improving health status (PAPER V);

- living in an urban area reduced the probability of asthma remission (PAPER II) and COPD remission (PAPER III).

Moreover, *comorbidities* reduced the probability of asthma (PAPER II) and COPD remission (PAPER III) and facilitated COPD diagnosis (PAPER III).

All these information should be considered for primary prevention strategies in order to reduce the burden of chronic diseases in the general population and for prevention and management strategies of respiratory diseases in health care setting.

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X. REFERENCES

1. Ekerljung L, Andersson Å, Sundblad B-M, Rönmark E, Larsson K, Ahlstedt S, et al. Has the increase in the prevalence of asthma and respiratory symptoms reached a plateau in Stockholm, Sweden?. Int J Tuberc Lung Dis 2010;14:764–771.
2. Fukutomi Y, Taniguchi M, Watanabe J, Nakamura H, Komase Y, Ohta K, et al. Time Trend in the Prevalence of Adult Asthma in Japan: Findings from Population-Based Surveys in Fujieda City in 1985, 1999, and 2006. Allergology International 2011;60:443-448.
3. De Marco R, Cappa V, Accordini S, Rava M, Antonicelli L, Bortolami O, et al; for the GEIRD study group. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. Eur Respir J 2012;39:883–892.
4. Bjerg A, Ekerljung L, Middelveld R, Dahlén SE, Forsberg B, Franklin K, et al. Increased prevalence of symptoms of rhinitis but not of asthma between 1990 and 2008 in Swedish adults: comparisons of the ECRHS and GA2LEN Surveys. PLoS ONE 2011;6:e16082.
5. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, et al. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Resp Med 2016;110:58-68.
6. Gershon AS, Wang C, Wilton AS, Raut R, To T. Trends in Chronic Obstructive Pulmonary Disease Prevalence, Incidence, and Mortality in Ontario, Canada, 1996 to 2007. A Population-Based Study. Arch Intern Med 2010;170:560-565.
7. Waatevik M, Skorge TD, Omenaas E, Bakke PS, Gulsvik A, Johannessen A. Increased prevalence of chronic obstructive pulmonary disease in a general population. Respir Med 2013;107:1037–1045.

8. Torén K, Ekerljung L, Kim J-L, Hillström J, Wennergren G, Rönmark E, et al. Adult-onset asthma in west Sweden e Incidence, sex differences and impact of occupational exposures. Respir Med 2011;105:1622-1628.

9. Pallasaho P, Juusela M, Lindqvist A, Sovijärvi A, Lundbäck B, Rönmark E. Allergic rhinoconjunctivitis doubles the risk for incident asthma e Results from a population study in Helsinki, Finland. Respir Med 2011;105:1449-1456.

10. Hansen S, Probst-Hensch N, Keidel D, Dratva J, Bettschart R, Pons M, et al. Gender differences in adult-onset asthma: results from the Swiss SAPALDIA cohort study. Eur Respir J 2015;46:1011–1020.

11. Verlato G, Nguyen G, Marchetti P, Accordini S, Marcon A, Marconcini R, et al. Smoking and New-Onset Asthma in a Prospective Study on Italian Adults. Int Arch Allergy Immunol 2016;170:149–157.

12. Hedman L, Andersson M, Bjerg A, Forsberg B, Lundbäck B, Rönmark E. Environmental risk factors related to the incidence of wheeze and asthma in adolescence. Clin Exp Allergy 2015;45:184-191.

13. Radon K, Gerhardinger U, Schulze A, Zock JP, Norback D, Toren K, et al; Occupational Group of the ECRHS study. Occupation and adult onset of rhinitis in the general population. Occup Environ Med 2008;65:38-43.

14. Gallmeier K, Becker E, Kirsten A, Wölke G, Manuwald O, Meyer H, et al. Prediction of new-onset asthma and nasal allergy by skin prick test and RAST in a cohort of adults. Eur Respir J 2014; 43: 92-102

15. Pelkonen M, Notkola IL, Nissinen A, Tukiainen H, Koskela H. Thirty-year cumulative incidence of chronic bronchitis and COPD in relation to 30-year pulmonary function and 40-year mortality: a follow-up in middle-aged rural men. Chest 2006;130:1129-1137.

16. Mirabelli MC, London SJ, Charles LE, Pompeii LA, Wagenknecht LE. Occupation and three-year incidence of respiratory symptoms and lung function decline: the ARIC Study. Respir Res 2012;13:24.

17. Mehta AJ, Miedinger D, Keidel D, Bettschart R, Bircher A, Bridevaux PO, et al; the SAPALDIA Team. Occupational Exposure to Dusts, Gases, and Fumes and Incidence of Chronic Obstructive Pulmonary Disease in the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults. Am J Respir Crit Care Med 2012;185:1292–1300.

18. Marcon A, Locatelli F, Keidel D, Beckmeyer-Borowko AB, Cerveri I, Dharmage SC, et al; Ageing Lungs in European Cohorts (ALEC) study. Airway responsiveness to methacholine and incidence of COPD: an international prospective cohort study. Thorax 2018;73:825-832.

19. Johannessen A, Omenaas E, Bakke P, Gulsvik A. Incidence of GOLD-defined chronic obstructive pulmonary disease in a general adult population. Int J Tuberc Lung Dis 2005;9:926–932.

20. Dijk FN, de Jongste JC, Postma DS, Koppelman GH. Genetics of onset of asthma. Curr Opin Allergy Clin. Immunol 2013;13:193–202.

21. World Health Organization. Report Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. 2007. http://www.who.int/gard/publications/GARD_Manual/en/. Accessed May 2018.

22. World Health Organization 2013, Review of evidence on health aspects of air pollution - REVIHAAP Project: final technical report. http://www.euro.who.int/data/assets/pdf_file/0004/193108/REVIHAAP-Final-technical-report-final-version.pdf. Accessed May 2018.

23. Prüss-Ustün A, Wolf J, Corvalán C, R Bos and M Neira. Preventing disease through healthy environments. A global assessment of the burden of disease from environmental risks. WHO 2016. http://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/. Accessed May 2018.

24. Maio S, Cerrai S, Simoni M, Sarno G, Baldacci S, Viegi G, Environmental risk factors: indoor and outdoor pollution. In: Pawankar R, Canonica GW, Holgate ST , Lockey RF, Blaiss MS (Eds.), World Allergy Organization (WAO). White Book on Allergy: Update, 2013, pp. 91-98.

25. Jenerowicz D, Silny W, Dańczak-Pazdrowska A, Polańska A, Osmola-Mańkowska A, Olek-Hrab K. Environmental factors and allergic diseases. Ann Agric Environ Med 2012;19:475-81.

26. Anderson H, Favarato G, Atkinson RW. Long-term exposure to air pollution and the incidence of asthma: meta-analysis of cohort studies. Air Qual Atmos Health 2013;1:47–56.

27. Guerra S. Clinical remission of asthma: what lies beyond? Thorax 2005;60:5–6.

28. Anderson HM, Jackson DJ. Microbes, allergic sensitization, and the natural history of asthma. Curr Opin Allergy Clin Immunol 2017;17:116–122.

29. Gershon A, Guan J, Victor JC, Wang C, To T. The course of asthma activity: a population study. J Allergy Clin Immunol 2012;129:679-86.

30. Fuchs O, Bahmer T, Rabe KF, von Mutius E. Asthma transition from childhood into adulthood. Lancet Respir Med 2017;5:224-234.

31. Burgess JA, Matheson MC, Gurrin LC, Byrnes GB, Adams KS, Wharton CL, et al. Factors influencing asthma remission:longitudinal study from childhood to middle ag e. Thorax 2011;66:508-13.

32. Pesce G, Locatelli F, Cerveri I, Bugiani M, Pirina P, Johannessen A, et al. Seventy Years of Asthma in Italy: Age, Period and Cohort Effects on Incidence and Remission of Self-Reported Asthma from 1940 to 2010. PLoS ONE 2015;10:e0138570.

33. Ekerljung L, Rönmark E, Larsson K, Sundblad B-M, Bjerg A, Ahlstedt S, et al. No further increase of incidence of asthma: Incidence, remission and relapse of adult asthma in Sweden. Respir Med 2008;102:1730-1736.

34. Yavuz ST, Civelek E, Comert S, Sahiner UM, Buyuktiryaki B, Tuncer A, et al. Development of rhinitis may be an indicator for the persistence of childhood asthma. International Journal of Pediatric Otorhinolaryngology 2014;78:843–849.

35. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. http://www.goldcopd.org/guidelines-global-strategy-for-diagnosis-management.htm. Update 2018. Accessed July 2018.

36. Eagan TML, Gulsvik A, Eide GE, Bakke PS. Remission of respiratory symptoms by smoking and occupational exposure in a cohort study. Eur Respir J 2004; 23: 589–594.

37. Global Initiative for Asthma. Global strategy for asthma management and Prevention. Update 2015. http://ginasthma.org/archived-reports/. Accessed date June 2016.

38. van Schayck OC. Global strategies for reducing the burden from asthma. Prim Care Respir J 2013;22:239-43.

39. Gonzalez-Garcia M, Caballero A, Jaramillo C, Maldonado D, Torres-Duque CA. Prevalence, risk factors and underdiagnosis of asthma and wheezing in adults 40 year s and older: A population-based study. Asthma 2015;52:823-30.

40. Maio S, Baldacci S, Carrozzi L, Pistelli F, Viegi G. The global burden of chronic respiratory diseases. Breathe 2006;3:20-29.

41. The Lancet. Making more of multimorbidity: an emerging priority. www.thelancet.com 2018;391. Accessed October 2018.

42. Afshar S, Roderick PJ, Kowal P, Dimitrov BD, Hill AG. Multimorbidity and the inequalities of global ageing: a cross-sectional study of 28 countries using the World Health Surveys. BMC Public Health 2015;15:776-786.

43. The Academy of Medical Sciences. Multimorbidity: a priority for global health research. April 2018. https://acmedsci.ac.uk/policy/policy-projects/multimorbidity. Accessed October 2018.

44. Nicholson K, Makovski TT, Griffith LE, Raina P, Stranges S, van den Akker M. Multimorbidity and comorbidity revisited: Refining the concepts for international health research. Journal of Clinical Epidemiology 2019;105:142-146.

45. Rijken M, Struckmann V, Dyakova M, Melchiorre MG, Rissanen ST, Van Ginneken E. ICARE4EU: improving care for people with multiple chronic conditions in Europe. Eurohealth 2013;19:29–31.

46. Ryan A, Murphy C, Boland F, Galvin R, Smith SM. What Is the Impact of Physical Activity and Physical Function on the Development of Multimorbidity in Older Adults Over Time? A Population-Based Cohort Study. J Gerontol A Biol Sci Med Sci 2018;73:1538–1544.

47. Boudier A, Curjuric I, Basagan˜a X, Hazgui H, Anto JM, Bousquet J, et al. Ten-Year Follow-up of Cluster-based Asthma Phenotypes in Adults A Pooled Analysis of Three Cohorts. Am J Respir Crit Care Med 2013;188:550–560.

48. Garden FL, Simpson JM, Mellis CM, Marks GB for the CAPS Investigator. Change in the manifestations of asthma and asthma-related traits in childhood: a latent transition analysis. Eur Respir J 2016;47: 499–509.

49. Soto-Ramírez N, Ziyab AH, Karmaus W, Zhang H, Kurukulaaratchy RJ, Ewart S, et al. Epidemiologic Methods of Assessing Asthma and Wheezing Episodes in Longitudinal Studies: Measures of Change and Stability. J Epidemiol 2013;23:399-410.

50. Carrozzi L, Giuliano G, Viegi, G, Paoletti P, Di Pede F, Mammini U, et al. The Po river delta epidemiological study of obstructive lung disease: sampling methods, environmental and population characteristics. Eur J Epidemiol 1990;6:191-200.

51. Viegi G, Paoletti P, Carrozzi L, Vellutini M, Diviggiano E, Di Pede C, et al. Prevalence rates of respiratory symptoms in Italian general population samples, exposed to different levels of air pollution. Environ Health Perspect 1991;94:95-99.

52. American Thoracic Society. Statement of the American Thoracic Society. Standardization of spirometry: 1987 update. Am Rev Respir Dis 1987;136:1285–1298.

53. Viegi G, Matteelli G, Angino A, Scognamiglio A, Baldacci S, Soriano JB, et al. The Proportional Venn Diagram of Obstructive Lung Disease in the Italian General Population. Chest 2004;126:1093–1101.

54. Baldacci S, Modena P, Carrozzi L, Pedreschi M, Vellutini M, Biavati P, et al. Skin prick test reactivity to common aeroallergens in relation to total IgE, respiratory symptoms, and smoking in a general population sample of northern Italy. Allergy 1996;51:149–156.

55. European Community Respiratory Health Survey. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Health Survey (ECRHS). Eur Respir J 1996;9:687–695.

56. Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, et al; on behalf of the BOLD Collaborative Research Group. International variation in the prevalence of COPD (the BOLD study): a population-based prevalence study. Lancet 2007;370:741–750.

57. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J 2005;26:319–38.

58. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948–968.

59. Pistelli F, Bottai M, Carrozzi L, Baldacci S, Simoni M, Di Pede F, et al. Reference equations for spirometry from a general population sample in central Italy. Respir Med 2007;101:814-25.

60. Collins LM and Lanza ST. Latent Class and Latent Transition Analysis with applications in the social, behavioral, and health sciences. Wiley series in probability and statistics 2010. A John Wiley & sons, inc., publication; Editors: David 1 Balding, Noel A. C. Cressie, Garrett M. Fitzmaurice, Iain M. Johnstone, Geert Molenberghs, Llavid Scott, Adrian F M. Smith, Ruey S. Tsay, Sanford Weisberg. Pages 1-283.

61. Agresti A. An Introduction to Categorical Data Analysis. Second Edition 2007. Wiley-Interscience. A John Wailey & Sons, Inc., Publication.

62. Sharpe D. Your Chi-Square Test is Statistically Significant: Now What?. Practical Assessment, Research & Evaluation 2015;20:1-10.

63. Sperandei S. Understanding logistic regression analysis. Biochemia Medica 2014;24:12-18.

64. McCullagh P. Regression Models for Ordinal Data. Journal of the Royal Statistical Society. Series B (Methodological) 1980;42:109-142.

65. Storaas T, Zock J-P, Morano AE, Holm M, Bjørnsson E, Forsberg B, et al. Incidence of rhinitis and asthma related to welding in Northern Europe. Eur Respir J 2015;46:1290–1297.

66. Riu E, Dressel H, Windstetter D, Weinmayr G, Weiland S, Vogelberg C, et al. First months of employment and new onset of rhinitis in adolescents. Eur Respir J 2007;30:549–555.

67. Pallasaho P, Kainu A, Sovijärvi A, Lindqvist A, Piirilä PL. Combined effect of smoking and occupational exposure to dusts, gases or fumes on the incidence of COPD. COPD 2014;11:88-895.

68. Skorge TD, Eagan TML, Eide GE, Gulsvik A, Bakke PS. Occupational exposure and incidence of respiratory disorders in a general population. Scand J Work Environ Health 2009;35:454-461.

69. Jacquemin B, Siroux V, Sanchez M, Carsin AE, Schikowski T, Adam M, et al. Ambient Air Pollution and Adult Asthma Incidence in Six European Cohorts (ESCAPE). Environ Health Perspect 2015;123:613-621.

70. D'Amato G, Cecchi L, D'Amato M, Liccardi G. Urban Air Pollution and Climate Change as Environmental Risk Factors of Respiratory Allergy: An Update. J Investig Allergol Clin Immunol 2010;20:95-102.

71. D'Amato M, Cecchi L, Annesi-Maesano I, D'Amato G. News on climate change, air pollution, and allergic triggers of asthma. J Investig Allergol Clin Immunol 2018; 28:91-7.

72. Baldacci S, Maio S, Cerrai S, Sarno G, Baïz N, Simoni Met al; HEALS Study. Allergy and asthma: Effects of the exposure to particulate matter and biological allergens. Respir Med 2015;109:1089-1104.

73. Holm M, Torén K, Andersson E. Incidence of chronic bronchitis: a prospective study in a large general population. Int J Tuberc Lung Dis 2014;18:870–75.

74. Probst-Hensch NM, Curjuric I, Pierre-Olivier B, Ackermann-Liebrich U, Bettschart RW, Brändli O, et al. Longitudinal change of prebronchodilator spirometric obstruction and health outcomes: results from the SAPALDIA cohort. Thorax 2010;65:150-156.

75. Luoto JA, Elmståhl S, Wollmer P, Pihlsgård M. Incidence of airflow limitation in subjects 65–100 years of age. Eur Respir J 2016;47:379–81.

76. Raza A, Kurukulaaratchy RJ, Grundy JD, Clayton C, Mitchell FA, Roberts G, et al. What does adolescent undiagnosed wheeze represent? Findings from the Isle of Wight Cohort. Eur Respir J 2012;40:580–588.

77. Adams RJ, Wilson DH, Appleton S, Taylor A, Dal Grande E, Chittleborough CR, et al. Underdiagnosed asthma in South Australia. Thorax 2003;58:846–850.

78. Spyratos D, Chloros D, Michalopoulou D, Sichletidis L. Estimating the extent and economic impact of under and overdiagnosis of chronic obstructive pulmonary disease in primary care. Chronic Respi Dis 2016;13:240–246.

79. Lindström M, Jönsson E, Larsson K, Lundbäck B. Underdiagnosis of chronic obstructive pulmonary disease in Northern Sweden. Int J Tuberc Lung Dis 2001;6:76–84.

80. Kim V, Zhao H, Boriek AM, Anzueto A, Soler X, Bhatt SP, et al, and the COPDGene Investigators. Persistent and Newly Developed Chronic Bronchitis Are

Associated with Worse Outcomes in Chronic Obstructive Pulmonary Disease. Ann Am Thorac Soc 2016;13:1016–1025.

81. Quach A, Giovannelli J, Chérot-Kornobis N, Ciuchete A, Clément G, Matran R, et al. Prevalence and underdiagnosis of airway obstruction among middle-aged adults in northern France: The ELISABET study 2011-2013. Respir Med 2015;109:1553-1561.

82. Gershon AS, Thiruchelvam D, Chapman KR, Aaron SD, Stanbrook MB, Bourbeau J, et al; Canadian Respiratory Research Network. Health Services Burden of Undiagnosed and Overdiagnosed COPD. Chest 2018;153:1336-1346.

83. Holm M, Omenaas E, Gíslason T, Svanes C, Jögi R, Norrman E, et al, on behalf of the RHINE study group. Remission of asthma: a prospective longitudinal study from northern Europe (RHINE study). Eur Respir J 2007;30:62–65.

84. Mirabelli MC, Beavers SF, Chatterjee AB. Active Asthma and the Prevalence of Physician-Diagnosed COPD. Lung 2014;192:693–700.

85. Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for COPD in a longitudinal study. Chest 2004;126:59-65.

86. Iikura M, Hojo M, Koketsu R, Watanabe S, Sato A, Chino H, et al. The importance of bacterial and viral infections associated with adult asthma exacerbations in clinical practice. PLoS One 2015 22;10:e0123584.

87. Teodorescu M, Polomis DA, Teodorescu MC, Gangnon RE, Peterson AG, Consens FB, et al. Association of obstructive sleep apnea risk or diagnosis with daytime asthma in adults. J Asthma 2012;49:620-628.

88. Qiao Yi-X, Xiao Y. Asthma and Obstructive Sleep Apnea. Chin Med J 2015;128:2798-2804.

89. Xu X, Rijcken B, Schouten JP, Weiss ST. Airways responsiveness and development and remission of chronic respiratory symptoms in adults. Lancet 1997;350:1431–1434.

90. Thurston GD, Kipen H, Annesi-Maesano I, Balmes J, Brook RD, Cromar K, et al. A joint ERS/ATS policy statement: what constitutes an adverse health effect of air pollution? An analytical framework. Eur Respir J 2017;49:1600419.

91. De Marco R, Pesce G, Marcon A, Accordini S, Antonicelli L, Bugiani M, et al. The Coexistence of Asthma and Chronic Obstructive Pulmonary Disease (COPD). Prevalence and Risk Factors in Young, Middle-aged and Elderly People from the General Population. Plos One 2013;8:e62985.

92. Maio S, Baldacci S, Carrozzi L, Polverino E, Angino A, Pistelli F, et al. Urban residence is associated with bronchial hyperresponsiveness in Italian general population samples. Chest 2009;135:434-441.

93. Nuvolone D, della Maggiore R, Maio S, Fresco R, Baldacci S, Carrozzi L, et al. Geographical information system and environmental epidemiology: a cross-sectional spatial analysis of the effects of traffic-related air pollution on population respiratory health. Environ Health 2011;10:12.

94. Yeatts K, Davis KJ, Sotir M, Herget C, Shy C. Who gets diagnosed with asthma? Frequent wheeze among adolescents with and without a diagnosis of asthma. Pediatrics 2003;111:1046-1054.

95. Siersted HC, Boldsen J, Hansen HS, Mostgaard G, Hyldebrandt N. Population based study of risk factors for underdiagnosis of asthma in adolescence: Odense schoolchild study. BMJ 1998;316:651–657.

96. Annesi-Maesano I, Sterlin C, Caillaud D, de Blay F, Lavaud F, Charpin D, et al. Factors related to under-diagnosis and undertreatment of childhood asthma in metropolitan France. Multidisciplinary Respir Med 2012;7:24.

97. Kurukulaaratchy RJ, Zhang H, Raza A, Patil V, Karmaus W, Ewart S, et al. The Diversity of Young Adult Wheeze; a Cluster Analysis in a Longitudinal Birth Cohort. Clin Exp Allergy 2014;44:724–735.

98. Alshabanat A, Zafari Z, Albanyan O, Dairi M, FitzGerald JM. Asthma and COPD Overlap Syndrome (ACOS): A Systematic Review and Meta Analysis. PLoS ONE 2015;10:e0136065.

99. Rzewuska M, de Azevedo-Marques JM, Coxon D, Zanetti ML, Zanetti ACG, Franco LJ, et al. Epidemiology of multimorbidity within the Brazilian adult general population: Evidence from the 2013 National Health Survey (PNS 2013). PLoS ONE 2017;12: e0171813.

100. Puth MT, Weckbecker K, Schmid M, Münster E. Prevalence of multimorbidity in Germany: impact of age and educational level in a cross-sectional study on 19,294 adults. BMC Public Health 2017;17:826.

101. Wikström K, Lindström J, Harald K, Peltonen M, Laatikainen T. Clinical and lifestyle-related risk factors for incident multimorbidity: 10-year follow-up of Finnish population-based cohorts 1982-2012. Eur J Intern Med 2015;26:211-216.

102. Backman H, Hedman L, Jansson S-A, Lindberg A, Lundbäck B, Rönmark E. Prevalence trends in respiratory symptoms and asthma in relation to smoking - two cross-sectional studies ten years apart among adults in northern Sweden, WAO Journal 2014;7:1.

103. Bugiani M, Carosso A, Migliore E, Piccioni P, Corsico A, Olivieri M, et al; ISAYA (ECRHS Italy) Study Group. Allergic rhinitis and asthma comorbidity in a survey of young adults in Italy. Allergy 2005;60:165-170.

104. Gough H, Grabenhenrich L, Reich A, Eckers N, Nitsche O, Schramm D, et al; MAS study group. Allergic multimorbidity of asthma, rhinitis and eczema over 20 years in the German birth cohort MAS. Pediatr Allergy Immunol 2015;26:431-437.

105. Egan M, Bunyavanich S. Allergic rhinitis: the "Ghost Diagnosis" in patients with asthma. Asthma ResPract 2015;1:8.

106. Jin J-M, Sun Y-C. Allergy and Chronic Obstructive Pulmonary Disease 2017; 130: 2017–2020.

107. Gallus S, Muttarak R, Martínez-Sánchez JM, Zuccaro P, Colombo P, La Vecchia C. Smoking prevalence and smoking attributable mortality in Italy, 2010. Prev Med 2011;52:434-438.

108. Amaral AFS, Strachan DP, Burney PGJ, Jarvis DL. Female Smokers Are at Greater Risk of Airflow Obstruction Than Male Smokers. UK Biobank. Am J Respir Crit Care Med 2017;195:1226-1235.

109. Cabello N, Mishra V, Sinha U, DiAngelo SL, Chroneos ZC, Ekpa NA, et al. Sex differences in the expression of lung inflammatory mediators in response to ozone. Am J Physiol Lung Cell Mol Physiol 2015;309:L1150-63.

110. Pinto LM, Alghamdi M, Benedetti A, Zaihra T, Landry T, Bourbeau J. Derivation and validation of clinical phenotypes for COPD: a systematic review. Respir Res 2015;16:50.

111. Bakke PS, Rønmark E, Eagan T, Pistelli F, Annesi-Maesano I, Maly M, et al; European Respiratory Society Task Force. Recommendations for epidemiological studies on COPD. Eur Respir J 2011;38:1261-1277.

112. Pistelli F, Maio S. Questionnaires and lung function. In: Annesi-Maesano I, Lundback B, Viegi G eds. Respiratory Epidemiology. Sheffield (UK): Eur Respir Monograph 2014;65:257-272.

113. Sanchez M, Bousquet J, Le Moual N, Jacquemin B, Clavel-Chapelon F, Humbert M, et al. Temporal Asthma Patterns Using Repeated Questionnaires over 13 Years in a Large French Cohort of Women. PLoS ONE 2013;8:e65090.

114. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Long term follow-up in European respiratory health studies e patterns and implications. BMC Pulm Med 2014;14:63.

XI. APPENDIX

XI.1 PAPER I

Incidence of respiratory symptoms and diseases is still elevated: the 18-yr follow-up of the Pisa epidemiological study.

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ABSTRACT

Background: Few data on respiratory symptoms/diseases (RSD) incidence and on effects of risk factors exposure changes are available.

Aim: assessing RSD incidence and its association with changes in risk factors exposure.

Methods: a longitudinal general population sample from two studies (PISA2:1991-1993; PISA3:2009-2011; n=1107), completing a questionnaire on RSD, risk factors exposure and performing spirometry.

18-yr cumulative incidence of RSD and longitudinal changes (persistence, incidence, remittance) in risk factors exposure (smoking habits-SH, occupational exposure-OE, vehicular traffic exposure-VT) were computed.

Results: Cumulative incidence values were: 3.4%, asthma; 6.3%, asthma attacks; 33.1%, allergic rhinitis-AR; 4.3%, chronic obstructive pulmonary disease-COPD; 14.8%, usual cough; 15.7%, usual phlegm; 12.5%, airway obstruction.

Multiple logistic regression models showed significant associations among changes of risk factors and RSD incidence values: OE persistence and asthma (OR 3.4), AR (OR 1.9), phlegm (OR 1.8), COPD (OR 2.4); OE incidence and AR (OR 2.4), phlegm (OR 1.7), cough (OR 1.7); VT incidence and asthma attacks (OR 2.2), AR (OR 1.6); SH persistence and COPD (OR 3.9), phlegm (OR 2.2), airway obstruction (OR 3.2); SH incidence and cough (OR 2.7).

Conclusions: Our study showed elevated RSD incidence values during an 18-yr follow-up in a general population sample living in Central Italy and it indicated that changes in life-style and in environmental exposures can influence the RSD onset: changes in OE and VT were related to allergy/asthma incidence and changes in SH and OE were related to COPD incidence. Such information should be considered for primary prevention strategies.

INTRODUCTION

Respiratory symptoms/diseases (RSD) prevalence rates are still on the increase in the last decade [1,4], but few data on incidence values and the effects of changes in risk factors exposure are available.

Swedish [5] and Swiss [6] 20-yr follow-up studies reported an asthma cumulative incidence of 2.3% and 6.4% in adult subjects. The Gene-Environment Interactions in Respiratory Diseases (GEIRD) study in Italian young adults found an asthma incidence value of 4.6% at 9-yr follow-up [7]. Among adolescents participating in the Obstructive Lung Disease In Northern Sweden (OLIN) studies, an asthma cumulative incidence of 7.2% emerged at 6.5-yr follow-up [8].

In a 9-yr follow up of the European Community Respiratory Health Survey (ECRHS) the cumulative incidence of allergic rhinitis (AR) was 12% [9]; a more recent follow-up of the ECRHS German sample showed a cumulative incidence value of 7.9% at 20-yr follow-up [10].

A chronic bronchitis (CB) cumulative incidence ranging from 22% in smokers to about 10% in ex smokers and non smokers was found in Finnish adult subjects at 30-yr follow-up [11]. Incidence values slightly over 3% of cough and phlegm were found in a US adult subjects cohort at 3-yr follow-up [12]. Different airway obstruction (AO) incidence values according to spirometric criteria were shown in a Swiss study at 11-yr follow-up: 15.5% according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criterion [13] and 7.4% according to the lower limit of normal (LLN) criterion [14]. A more recent study showed a cumulative incidence value of LLN AO of about 3% in European young adults at 9-yr follow-up [15].

According to the World Health Organization (WHO), main environmental risk factors associated with lifetime increase of RSD prevalence/incidence are tobacco use, air pollutants, occupational exposure and climate changes [16-19]. Chronic Obstructive Pulmonary Disease (COPD) attributable fractions are: 35% to smoking habit [20], 36% to indoor/outdoor air pollution, 12% to occupational exposure [19]. Furthermore, 44% of asthma is attributable to total occupational risks, indoor and ambient air pollutants [19].

The purpose of our population-based study was to evaluate 18-yr cumulative incidence of RSD and the relationship with changes in risk factors exposure.

MATERIALS AND METHODS

Sample

Detailed information on population characteristics and methods are available elsewhere [1,21].

Briefly, a multistage stratified family-cluster random sample of general population, living in Pisa, was investigated in three subsequent cross-sectional surveys: first survey (PI1) (1985–1988); second survey (PI2) (1991–1993); third survey (PI3) (2009-2011).

Since spirometry data were unavailable in PI1, only subjects participating in both PI2 and PI3 (n=1107) were taken into account herein: the mean follow-up was 18-yr.

Information on RSD and risk factors were obtained through standardized interviewer-administered questionnaires developed by CNR [1,22-23].

In PI2, all ≤ 75 year subjects were invited to perform spirometry (forced vital capacity-FVC- manoeuvre) according to the American Thoracic Society protocol [24], through a water-sealed spirometer (Baires, Biomedin): the participation rate was 67% [25]. In PI3, all subjects were invited to perform spirometry (FVC manoeuvre) according to the American Thoracic Society/European Respiratory Society protocol [26], through a hand-held ultrasonic spirometer (EasyOne Model 2001 Spirometer, NDD Medical Technologies): the participation rate was 55%.

At the time of PI2, Italian law didn't request an Ethical Committee approval; thus, the protocol was approved by an Internal Revision Board within the CNR Preventive Medicine Targeted Project. PI3 study protocol, patient information sheet, and consent form were approved by the Pisa University Hospital Ethics Committee (Prot. no. 23887, April 16, 2008).

RSD incidence and longitudinal risk factors

Detailed information on investigated respiratory outcomes and risk factors are reported in the Online Resource.

Briefly, 18-yr cumulative incidence values of AR, asthma, asthma attacks, COPD, usual cough, usual phlegm, and AO were computed as the proportion of subjects without symptoms & diagnoses in PI2 ("population at risk") who reported symptoms or diagnoses in PI3 ("incidence"= "incident cases"/"population at risk").

For the longitudinal changes of smoking habits-SH, dusts/fumes/gases occupational exposure-OE, and self-reported vehicular traffic exposure near home-VT, the following

mutually exclusive categories were computed: “Never” (unexposed subjects in both PI2 and PI3), “Persistent” (exposed subjects in both PI2 and PI3), “Incident” (subjects who started to be exposed between PI2 and PI3), “Remittent” (subjects who quit to be exposed between PI2 and PI3).

Statistical analyses

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS version 16.0). Comparisons among groups were performed by chi-square test for categorical variables and analysis of variance for continuous variables.

Logistic regression models were used to estimate the effect of longitudinal changes in risk factors exposure on RSD incidence. Possible PI2 confounding factors were accounted for:age, sex, body mass index -BMI, second hand smoke exposure, family history (at least one parent) of allergic rhinitis and/or respiratory diseases (asthma, CB or emphysema). For AR and asthma, also skin prick test (SPT) positivity to house dust mites, pets, moulds or pollens was taken into account. Odds ratio (OR) and 95% confidence interval (CI) were calculated.

The significance level was set at 0.05.

RESULTS

Descriptive characteristics of PI2 and PI3 participants are shown in Table 1: 54.2%were females, 32.2% had SPT positivity, 47.3% had family history of allergic disorders and 49.0% of respiratory diseases, 55.6% reported current second hand smoke exposure. Significantly higher percentages of obesity (26.7% vs 11.6%), OE (45.8% vs 39.6%) and VT (63.1% vs 35.5%) and significantly lower percentage of smokers (18.8% vs 24.7%) were found in PI3 with respect to PI2.

18-yr cumulative incidence values of RSD were: 3.4%, asthma; 6.3%, asthma attacks; 33.1%, AR; 4.3%, COPD; 14.8%, usual cough; 15.7%, usual phlegm; 12.5%, AO (Fig.1a-1b).

The highest incidence value in risk factor exposure was shown by VT (38.5%), followed by OE (18.3%) and SH (6.5%). The highest remittance value was exhibited by SH (25.5% for ≥ 18 yrs, 16.1% for < 18 yrs), followed by OE (12.1%), VT (10.8%) (figure 2).

Asthma symptoms/diseases and AR showed no significant associations with SH. COPD incidence was significantly higher in persistent (7.1%) and remittent smokers

(about 6.0%) with respect to the other categories; usual phlegm was significantly higher in persistent (27.1%) and incident smokers (21.9%) (table 2).

RSD incidence was significantly higher in persistent and incident OE: 38.5% and 43.0% for AR, 21.4% and 20.1% usual phlegm, 16.0% and 20.1% usual cough. COPD incidence was higher in persistent exposed (8.7%) with respect to the other categories (table 3).

Asthma attacks incidence showed the significantly highest value in incident VT (10.0%), whilst AR in persistent and incident VT (about 36% for both). Bronchitic symptoms/diseases showed no significant associations with VT (table 3).

Tables 4a-4b report the results of logistic regression models.

OE and VT were significantly associated with the cumulative incidence values of AR and asthma: persistent OE with asthma (OR 3.4) and AR (OR 1.9); incident OE with AR (OR 2.4); incident VT with asthma attacks (OR 2.2) and AR (OR 1.6) (table 4a).

SH and OE were significantly associated with the cumulative incidence of bronchitic symptoms/diseases: persistent SH with COPD (OR 3.9), usual phlegm (OR 2.2) and LLN AO (OR 3.2); incident SH with usual cough (OR 2.7); persistent OE with COPD (OR 2.4) and usual phlegm (OR 1.8); incident SH with usual phlegm (OR 1.7) and usual cough (OR 1.7) (table 4b).

DISCUSSION

We found elevated 18-yr cumulative incidence values for RSD in a general population sample, living in an urban/suburban area in Central Italy.

Occupational exposure and vehicular traffic exposure mainly influenced AR/asthma incidence, as well as smoking habits and occupational exposure did for COPD incidence.

Allergic/asthmatic diseases

Significantly higher risks of asthma and AR incidence were found in subjects with persistent OE: three-fold for asthma, two-fold for AR. A recent Northern European study showed a significantly higher risk of having rhinitis and asthma incidence among ever welders (hazard ratio (HR) 1.5 for both diseases), with respect to never welders [27]. A higher risk of developing asthma in subjects exposed to dusts/fumes was confirmed by a Swedish general population study showing an HR of 1.8 and 9.4% of OE attributable new-onset asthma [5].

A two-fold higher incidence risk of AR in subjects with incident OE with respect to unexposed subjects was found. This relationship was confirmed by a 7-yr follow-up study performed on German adolescents: those working in high-risk occupations (i.e. high exposure levels to chemicals/irritants/fumes and/or environmental tobacco smoke) had an increased risk for rhinitis onset (OR 1.5), with the highest incidence shown by those who had been working for 2-9 months [28], highlighting the need of strong and early preventive measures in workplaces also in the 21st century.

In Pisa, as in many other urban areas, vehicular traffic is increased in the last years. VT incidence was related to 1.6-2.2 fold higher risk of AR and asthma attacks incidence values, with respect to non exposure. These results are in line with data about six European cohorts suggesting a deleterious effect of pollutants emitted by vehicular traffic on adult-onset asthma incidence [29]. Furthermore, there is evidence that people living in urban areas more frequently experience AR and bronchial asthma than those living in rural areas: one of the main determinants is the increased presence of outdoor air pollutants from energy consumption and vehicles exhaust emissions [17,30-31]. Indeed, damage to airway mucous membranes and impaired muco-ciliary clearance caused by air pollution may facilitate access of inhaled allergens to the immune system cells, promoting airways sensitization [30-31].

COPD symptoms/diagnosis

SH is the main risk factor for COPD occurrence and exacerbations. In our study, persistent smokers had an elevated risk of developing COPD, AO and usual phlegm, as well as incident smokers had a higher risk of developing usual cough.

Similar results have been shown in other European cohorts [11,32-33]. In two rural Finnish cohorts, the 30-yr CB cumulative incidence was 22% in smokers and about 10% in ex smokers and non smokers [11]. In another Finnish cohort, current smoking emerged as an independent risk factor for incident COPD (OR 4.40) [32]. A Swedish cohort showed a 1.5/1000 person-year productive cough incidence rate in smokers [33].

The relationship between SH and bronchitic conditions was confirmed also by the results of objective measurements (spirometry), showing a 3-fold higher risk of developing LLN AO in persistent smokers. In a Swiss cohort, heavy smoking was related to higher risk for AO incidence based on fixed ratio criterion (RR 1.51) [13]. More recently, in a Swedish elderly cohort, a significant effect of current smoking for both fixed ratio (RR 1.75) and LLN criteria (RR 2.16) was observed [34].

OE was significantly associated with the cumulative incidence of COPD symptoms/diagnosis. In particular, incident OE was related to usual phlegm and usual cough and persistent OE was related to COPD and usual phlegm, confirming results of other general population cohorts. A Norwegian 11-year community cohort study showed that exposure to workplace gases/fumes was significantly related to incident phlegm in women (OR 1.42) [35]. North-American authors reported that, during an approximately 3-yr follow-up, subjects working in specific occupations, including mechanic and repair occupations and cleaning and building services, had significantly higher risks of developing chronic phlegm and chronic cough [12]. A recent Swiss Cohort Study indicated a fourfold higher risk of COPD incidence in subjects exposed to high levels of vapours/gases/dusts/fumes with respect to low exposure [14]. These findings were confirmed in a Finnish cohort where occupational exposure to dusts/gases/fumes, assessed by both self-reported exposure and job exposure matrix, was an independent risk factor for incident COPD (OR 2.14 for self-reported OE) [32].

Cumulative and yearly incidence values

In order to compare our findings with those of other European/US studies, considering the different follow-up periods, we computed the mean yearly incidence values, after dividing cumulative incidence rates by years of follow-up (table 5).

Consistently with other studies, there was a general increasing trend, in particular for AR (table 5). Indeed, our AR incidence values were the highest (18.4‰), indicating that the worldwide increase of allergic respiratory diseases during the last 3 decades in industrialized countries is not over [9-10,30]. Further, there were elevated yearly incidence values for bronchitic symptoms (cough and phlegm), ranging from 8.2‰ to 11.7‰ (table 5). Not surprisingly, the incidence of diagnosed disease was lower than the symptoms incidence, but values were not negligible (from 1.3‰ to 5.1‰ for asthma and from 2.4‰ to 4.7‰ for COPD) (table 5). The slight differences among the findings of the reported studies are likely due to the different periods of data collection and definitions of symptoms/disease diagnoses.

Limits and strengths

Use of questionnaires for collecting RSD data might be a limitation because it is potentially affected by a reporting bias, as it relies upon individual memory; nevertheless, the standardized questionnaire is one of the main investigation tool in respiratory

epidemiology [36-37]. Moreover, in our study an objective respiratory outcome (lung function), not affected by such potential bias, was also applied.

It is to point out that in PI3 some differences in the used questionnaire exist, but only comparable or identical questions to PI2 ones were chosen.

Spirometry was performed using different instruments in PI2 and PI3; a correction factor was derived to overcome this limit and to permit the comparison between studies, as reported in our previous paper [1].

Sensitivity analyses were performed to assess some potential critic aspects of our study.

First, considering that less than 50% of the original sample performed spirometry in both surveys (n=417), a comparison of incidence rates and frequencies of longitudinal risk factors between the subsample of subject performing spirometry and the whole sample was performed: no difference was found letting us to be confident in the generalizability of results about AO incidence to the whole Pisa sample.

Second, a comparison of the baseline characteristics and health status of subjects who were followed-up (PI2 and PI3 participants) with those of subjects who weren't (only PI2 participants) was performed: differences regarding age and prevalence of COPD symptoms/disease were found, with the highest values in only PI2 participants than in PI2 and PI3 participants. Thus, the incidence value computation was made in a sample of younger healthy subjects with respect to the total PI2sample, with a possible conservative estimate of the reported incidence values. This aspect was confirmed by a recent article showing that, among long-term participants in population surveys, disease prevalence rates tend to be slightly lower than for the total baseline population, suggesting that subjects who continue to participate in a study are healthier than those who quit [38].

A strength of our population-based study is to have applied, over an 18-yr follow-up, the same study design, sampling frame and study protocol in repeated cross-sectional surveys on general population samples living in the same area. Moreover, a wide general population sample spanning from childhood to the elderly was analyzed, with a vast amount of individual qualitative and quantitative data.

At last, another strength is the consistency of our results with those of other international studies.

CONCLUSION

In conclusion, our study showed elevated18-yr cumulative incidence values of RSD in a general population sample living in Central Italy and it indicated that changes in lifestyle and in environmental exposure can influence the RSD onset: in particular, changes in OE and VT were related to AR/asthma incidence and changes in SH and OE were related to COPD incidence.

Such information should be taken into account for primary prevention strategies in order to reduce the burden of chronic diseases in the general population.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

REFERENCES

1. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, et al. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Respir Med. 2016;110:58-68.
2. De Marco R, Cappa V, Accordini S, Rava M, Antonicelli L, Bortolami O, et al.; GEIRD Study Group. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. EurRespir J. 2012;39:883–92.
3. Gershon AS, Wang C, Wilton AS, Raut R, To T. Trends in Chronic Obstructive Pulmonary Disease Prevalence, Incidence, and Mortality in Ontario, Canada, 1996 to 2007. A Population-Based Study. Arch Intern Med. 2010;170:560-5.
4. Ekerljung L, Andersson A, Sundblad BM, Rönmark E, Larsson K, Ahlstedt S, et al. Has the increase in the prevalence of asthma and respiratory symptoms reached a plateau in Stockholm, Sweden? Int J Tuberc Lung Dis. 2010;14:764-71.
5. Torén K, Ekerljung L, Kim J-L, Hillström J, Wennergren G, Rönmark E, et al. Adult-onset asthma in west Sweden e Incidence, sex differences and impact of occupational exposures. Respir Med. 2011;105:1622-8.
6. Hansen S, Probst-Hensch N, Keidel D, Dratva J, Bettschart R, Pons M, et al. Gender differences in adult-onset asthma: results from the Swiss SAPALDIA cohort study. Eur Respir J. 2015;46:1011–20.

7. Verlato G, Nguyen G, Marchetti P, Accordini S, Marcon A, Marconcini R, et al. Smoking and New-Onset Asthma in a Prospective Study on Italian Adults. Int Arch Allergy Immunol. 2016;170:149–57.
8. Hedman L, Andersson M, Bjerg A, Forsberg B, Lundbäck B, Rönmark E. Environmental risk factors related to the incidence of wheeze and asthma in adolescence. Clin Exp Allergy 2015;45:184-91.
9. Radon K, Gerhardinger U, Schulze A, Zock JP, Norback D, Toren K, et al; Occupational Group of the ECRHS study. Occupation and adult onset of rhinitis in the general population. Occup Environ Med. 2008;65:38-43.
10. Gallmeier K, Becker E, Kirsten A, Wölke G, Manuwald O, Meyer H, et al. Prediction of new-onset asthma and nasal allergy by skin prick test and RAST in a cohort of adults. Eur Respir J. 2014;43:92-102.
11. Pelkonen M, Notkola IL, Nissinen A, Tukiainen H, Koskela H. Thirty-year cumulative incidence of chronic bronchitis and COPD in relation to 30-year pulmonary function and 40-year mortality: a follow-up in middle-aged rural men. Chest. 2006;130:1129-37.
12. Mirabelli MC, London SJ, Charles LE, Pompeii LA, Wagenknecht LE. Occupation and three-year incidence of respiratory symptoms and lung function decline: the ARIC Study. Respir Res. 2012;13:24.
13. Probst-Hensch NM, Curjuric I, Pierre-Olivier B, Ackermann-Liebrich U, Bettschart RW, Brändli O, et al. Longitudinal change of prebronchodilator spirometric obstruction and health outcomes: results from the SAPALDIA cohort. Thorax. 2010;65:150-6.
14. Mehta AJ, Miedinger D, Keidel D, Bettschart R, Bircher A, Bridevaux PO, et al.; the SAPALDIA Team. Occupational Exposure to Dusts, Gases, and Fumes and Incidence of Chronic Obstructive Pulmonary Disease in the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults. Am J RespirCrit Care Med. 2012;185:1292–300.
15. Marcon A, Locatelli F, Keidel D, Beckmeyer-Borowko AB, Cerveri I, DharmageSH, et al, on behalf of the Ageing Lungs in European Cohorts (ALEC) study. Airway responsiveness to methacholine and incidence of COPD: an international prospective cohort study. Thorax. 2018;73:825–32.

16. Pesce G, Bugiani M, Marcon A,Marchetti P, Carosso A, Accordini S, et al. Geo-climatic heterogeneity in self-reported asthma, allergic rhinitis and chronic bronchitis in Italy. Sci Total Environ. 2016; 544: 645–52.
17. D'Amato M, Cecchi L, Annesi-Maesano I, D'Amato G. News on climate change, air pollution, and allergic triggers of asthma. J Investig Allergol Clin Immunol. 2018; 28:91-7.
18. World Health Organization. Report Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. 2007. http://www.who.int/gard/publications/GARD_Manual/en/. Accessed October 2018.
19. Prüss-Ustün A, Wolf J, Corvalán C, Bos R and Neira M. Preventing disease through healthy environments. A global assessment of the burden of disease from environmental risks. WHO 2016. http://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/. Accessed May 2018.
20. Institute for Health Metrics and Evaluation (IHME). GBD Compare Data Visualization. Seattle, WA: IHME, University of Washington, 2017. Available from http://vizhub.healthdata.org/gbd-compare. Accessed May 2018.
21. Viegi G, Pedreschi M, Baldacci S. Prevalence rates of respiratory symptoms and diseases in general population samples of North and Central Italy. Int J Tuberc Lung Dis. 1999;3:1034–42.
22. Viegi G, Paoletti P, Carrozzi L. Prevalence rates of respiratory symptoms in Italian general population samples, exposed to different levels of air pollution. Environ Health Perspect. 1991;94:95-9.
23. US Department of Health Education and Welfare. Proceedings of the first NHLBI epidemiology workshop. Washington DC, US Government Printing Office, 1971.
24. Statement of the American Thoracic Society. Standardization of spirometry: 1987 update. Am Rev Respir Dis. 1987;136:1285–98.
25. Viegi G, Matteelli G, Angino A, Scognamiglio A, Baldacci S, Soriano JB, etal.The Proportional Venn Diagram of Obstructive Lung Disease in the Italian General Population. Chest. 2004;126:1093–101.
26. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al.; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J. 2005;26:319–38.

27. Storaas T, Zock J-P, Morano AE, Holm M, Bjørnsson E, Forsberg B, et al. Incidence of rhinitis and asthma related to welding in Northern Europe. Eur Respir J. 2015;46:1290–7.
28. Riu E, Dressel H, Windstetter D, Weinmayr G, Weiland S, Vogelberg C, et al. First months of employment and new onset of rhinitis in adolescents. Eur Respir J. 2007;30:549–55.
29. Jacquemin B, Siroux V, Sanchez M, Carsin AE, Schikowski T, Adam M, et al. Ambient Air Pollution and Adult Asthma Incidence in Six European Cohorts (ESCAPE). Environ Health Perspect. 2015;123:613-21.
30. D'Amato G, Cecchi L, D'Amato M, Liccardi G. Urban Air Pollution and Climate Change as Environmental Risk Factors of Respiratory Allergy: An Update. J Investig Allergol Clin Immunol. 2010;20:95-102.
31. Baldacci S, Maio S, Cerrai S, Sarno G, Baïz N, Simoni M, et al.; HEALS Study. Allergy and asthma: Effects of the exposure to particulate matter and biological allergens. Respir Med. 2015;109:1089-104.
32. Pallasaho P, Kainu A, Sovijärvi A, Lindqvist A, Piirilä PL. Combined effect of smoking and occupational exposure to dusts, gases or fumes on the incidence of COPD. COPD. 2014;11:88-95.
33. Holm M, Torén K, Andersson E. Incidence of chronic bronchitis: a prospective study in a large general population. Int J Tuberc Lung Dis. 2014;18:870–75.
34. Luoto JA, Elmståhl S, Wollmer P, Pihlsgård M. Incidence of airflow limitation in subjects 65–100 years of age. Eur Respir J. 2016;47:379–81.
35. Skorge TD, Eagan TML, Eide GE, Gulsvik A, Bakke PS. Occupational exposure and incidence of respiratory disorders in a general population. Scand J Work Environ Health. 2009;35:454-61.
36. Bakke PS, Rønmark E, Eagan T, Pistelli F, Annesi-Maesano I, Maly M, et al.; European Respiratory Society Task Force. Recommendations for epidemiological studies on COPD. Eur Respir J. 2011;38:1261-77.
37. Pistelli F, Maio S. Questionnaires and lung function. In: Annesi-Maesano I, Lundback B, Viegi G eds. Respiratory Epidemiology. Eur Respir Monograph. Sheffield (UK); 2014;65:257-72.

38. Johannessen A, Verlato G, Benediktsdottir B, Forsberg B, Franklin K, Gislason T, et al. Long term follow-up in European respiratory health studies e patterns and implications. BMC Pulm Med. 2014;14:63.
39. Pallasaho P, Juusela M, Lindqvist A, Sovija¨rvi A, Lundba¨ck B, Ro¨nmark E. Allergic rhinoconjunctivitis doubles the risk for incident asthma e Results from a population study in Helsinki, Finland. Respir Med. 2011;105:1449-56.
40. Johannessen A, Omenaas E, Bakke P, Gulsvik A. Incidence of GOLD-defined chronic obstructive pulmonary disease in a general adult population. Int J Tuberc Lung Dis. 2005;9:926–32.

Table 1 Descriptive characteristics of PI2-PI3 longitudinal subjects

(n=1107; males=507; females=600)

	PI2 (1991-1993)	PI3 (2009-2011)	p-value
Sex (%):			
males	45.8	45.8	
females	54.2	54.2	
Age (mean±SD)	**42.3±16.3**	**60.1±16.2**	**0.000**
Age range	8-78	25-96	
BMI (n=688)* (mean±SD)	**25.4±4.2**	**27.5±4.6**	**0.000**
BMI (n=688) (%):			**0.000**
Underweight/normal weight	**48.0**	**29.8**	
Overweight	**40.4**	**43.5**	
Obese	**11.6**	**26.7**	
missing values (n=419)	*37.8*	*37.8*	
Smoking habits (%):			**0.000**
Smokers	**24.7**	**18.8**	
Ex smokers	**28.5**	**35.4**	
Non smokers	**46.8**	**45.8**	
Occupational exposure (%)	**39.6**	**45.8**	**0.003**
Vehicular traffic exposure (%)	**35.5**	**63.1**	**0.000**
Second hand smoke exposure	55.6	n.a.	
Positivity to SPT** (n=987) (%)	32.2	n.a.	
missing values (n=120)	*10.8*		
Family history (at least one parent) of respiratory diseases*** (%)	49.0	n.a.	
Family history (at least one parent)	47.3	n.a.	

of allergic rhinitis (%)			

SD: standard deviation; BMI: body mass index; SPT: skin prick test

* BMI was available only in 688 subjects in PI3; thus, the comparison between PI2 and PI3 values was performed on this subsample

**positivity (mean wheal diameter ≥ 3 mm than that of the negative control) to at least one allergen among house dust mites, pets, moulds, pollens

***asthma or chronic bronchitis or emphysema

n.a. not available

Information about second hand smoke exposure were not comparable in PI2 and PI3, thus only baseline value was taken into account.

In bold: statistically significant values.

Table 2 Symptoms/diseases incidence by longitudinal changes in smoking habits (%)

	Persistent**	Remittent for <18 yrs**	Remittent for ≥ 18 yrs**	Incident**	Never	p-value
N*	126	164	257	63	398	
Asthma diagnosis	3.2	3.0	3.5	4.8	3.3	0.977
Asthma attacks	9.5	5.5	7.0	4.8	5.3	0.463
N	111	150	234	61	329	
Allergic rhinitis	27.9	35.3	36.3	36.1	31.0	0.452
N	85	129	217	64	371	
COPD	**7.1**	**6.2**	**6.0**	**0.0**	**2.7**	**0.050**
Usual phlegm	**27.1**	**13.2**	**15.2**	**21.9**	**13.2**	**0.014**
Usual cough	20.0	11.6	13.0	21.9	14.3	0.212
N	51	56	102	25	134	
LLN AO	*19.6*	*12.5*	*15.7*	*0.0*	*9.7*	*0.098*

COPD: Chronic Obstructive Pulmonary Disease; LLN AO: Airway obstruction computed according to the lower limit of normal.

*N=population at risk; **Persistent: smokers at PI2 and PI3; Remittent for < 18 yrs: quitters between PI2 and PI3; Remittent for ≥ 18 yrs: quitters before PI2; Incident: beginners between PI2 and PI3.

In italic: borderline values; in bold: statistically significant values.

Table 3 Symptoms/diseases incidence by longitudinal changes in occupational exposure and vehicular traffic exposure (%)

	Persistent	Remittent	Incident	Never	p-value
Occupational exposure					
N	275	125	181	427	
Asthma diagnosis	*5.1*	*1.6*	*5.0*	*2.1*	*0.065*
Asthma attacks	8.7	6.4	3.9	5.6	0.177
N	244	105	158	378	
Allergic rhinitis	**38.5**	**25.7**	**43.0**	**27.5**	**0.001**
N	206	103	159	398	
COPD	**8.7**	**1.0**	**2.5**	**3.5**	**0.002**
Usual phlegm	**21.4**	**9.7**	**20.1**	**12.6**	**0.004**
Usual cough	**16.0**	**7.8**	**20.1**	**13.8**	**0.043**
N	109	40	74	145	
LLN AO	17.4	15.0	10.8	9.0	0.212
Vehicular traffic exposure					
N	245	106	391	265	
Asthma diagnosis	*1.6*	*3.8*	*5.1*	*2.3*	*0.073*
Asthma attacks	**3.3**	**3.8**	**10.0**	**4.5**	**0.001**
N	216	95	343	230	
Allergic rhinitis	**37.0**	**24.2**	**36.2**	**28.7**	**0.039**
N	206	93	340	227	
COPD	5.8	4.3	4.7	2.2	0.290
Usual phlegm	17.0	17.2	15.9	13.7	0.767
Usual cough	15.5	15.1	14.4	14.5	0.986
N	95	33	142	97	
LLN AO	10.5	6.1	12.0	17.5	0.277

COPD: Chronic Obstructive Pulmonary Disease; LLN AO: Airway obstruction computed according to the lower limit of normal.

In italic: borderline values; in bold: statistically significant values.

Table 4a. Longitudinal risk factors for asthma/allergic symptoms/diseases incidence: OR and 95% CI

	Asthma diagnosis	Asthma attacks	Allergic rhinitis
Smoking habits:			
never	1.0	1.0	1.0
persistent	0.6(0.2-2.4)	*2.2(0.9-5.1)*	0.9(0.6-1.6)
remittent for <18 yrs	0.9(0.3-2.8)	1.1(0.5-2.7)	1.3(0.8-2.1)
remittent for ≥18 yrs	0.9(0.4-2.4)	1.2(0.6-2.5)	1.1(0.7-1.6)
incident	1.0(0.2-4.4)	1.0(0.2-3.9)	0.9(0.5-1.8)
Occupational exposure:			
never	1.0	1.0	1.0
persistent	**3.4(1.3-8.9)**	1.2(0.6-2.4)	**1.9(1.3-3.0)**
remittent	0.9(0.2-4.3)	0.9(0.4-2.2)	1.1(0.7-2.0)
incident	1.9(0.7-5.5)	0.6(0.2-1.5)	**2.4(1.5-3.6)**
Vehicular traffic exposure:			
never	1.0	1.0	1.0
persistent	0.9(0.2-3.2)	0.5(0.2-1.4)	1.4(0.9-2.2)
remittent	1.6(0.4-6.2)	0.5(0.1-1.9)	0.8(0.5-1.5)
incident	*2.3(0.9-6.1)*	**2.2(1.1-4.4)**	**1.6(1.0-2.3)**

A logistic regression model for each considered outcome was used to estimate the effect of longitudinal changes in risk factors exposure (smoking habits, occupational exposure and vehicular traffic exposure) on respiratory symptoms/diseases incidence, controlling for baseline factors closely related to the onset of respiratory symptoms/diseases (age, sex, body mass index -BMI, second hand smoke exposure, positivity to skin prick test, family history of allergic rhinitis and family history of respiratory diseases (asthma, chronic bronchitis or emphysema)).

In italic: borderline values; in bold: statistically significant values.

Table 4b. Longitudinal risk factors for bronchitic symptoms/diseases incidence: OR and 95% CI

	COPD	Usual phlegm	Usual cough	LLN AO
Smoking habits:				
never	1.0	1.0	1.0	1.0
persistent	**3.9(1.2-12.6)**	**2.2(1.2-4.1)**	1.7(0.9-3.2)	**3.2(1.2-8.3)**
remittent for <18 yrs	*2.8 (0.9-8.5)*	0.9(0.5-1.8)	1.0(0.5-1.8)	1.6(0.6-4.7)
remittent for ≥18 yrs	*2.3(0.9-5.9)*	1.0(0.6-1.7)	1.0(0.6-1.6)	1.3(0.6-3.1)
incident	---	*2.1(0.9-4.4)*	**2.7(1.3-5.9)**	---
Occupational exposure:				
never	1.0	1.0	1.0	1.0
persistent	**2.4(1.0-5.8)**	**1.8(1.1-2.9)**	1.2(0.7-2.1)	1.7(0.7-4.0)
remittent	0.2(0.0-1.7)	0.7(0.3-1.4)	*0.5(0.2-1.1)*	1.3(0.4-3.8)
incident	0.8(0.3-2.7)	**1.7(1.0-2.8)**	**1.7(1.0-2.8)**	0.9(0.3-2.4)
Vehicular traffic exposure:				
never	1.0	1.0	1.0	1.0
persistent	2.1(0.7-6.5)	1.2(0.7-2.1)	1.0(0.6-1.7)	*0.4(0.2-1.1)*
remittent	2.0(0.7-5.8)	1.3(0.6-2.4)	1.1(0.5-2.1)	0.3(0.1-1.5)
incident	2.0(0.7-5.8)	1.2(0.7-1.9)	0.9(0.6-1.5)	0.6(0.3-1.3)

A logistic regression model for each considered outcome was used to estimate the effect of longitudinal changes in risk factors exposure (smoking habits, occupational exposure and vehicular traffic exposure) on respiratory symptoms/diseases incidence, controlling for baseline factors closely related to the onset of respiratory symptoms/diseases (age, sex, body mass index -BMI, second hand smoke exposure, positivity to skin prick test, family history of allergic rhinitis and family history of respiratory diseases (asthma, chronic bronchitis or emphysema)).

COPD: Chronic Obstructive Pulmonary Disease; LLN AO: Airway obstruction computed according to the lower limit of normal

In italic: borderline values; in bold: statistically significant values.

Table 5. Comparison of respiratory symptoms/diseases incidence values among different studies

	Reference	Country	Population	Follow-up period	Cumulative incidence rate (%)	Yearly incidence rate (‰)
Asthma diagnosis	Torén et al 2011[5]	Western Sweden	n=15761 16-75 yrs	1990-2008 18 yrs	2.3	1.3
	Pallasaho et al 2011[39]	Finland	n=4302 20-69 yrs	1996-2007 11 yrs	4.0	3.6
	Hansen et al 2015[6]	Switzerland	n=5128 18-60 yrs	1991-2001 20 yrs	6.4	3.2
	Verlato et al 2016[7]	Italy	n=3187 20-54 yrs	1999-2008 9 yrs	4.6	5.1
	Hedman et al, 2015[8]	Sweden	n=3151 11-12 yrs	2000 6.5 yrs	7.2	11.1
	Maio et al	Italy	n=1008 8-78 yrs	1992-2010 18 yrs	3.4	1.8
Allergic rhinitis	Gallmeieret al 2014[10]	Germany	n=754 20-44 yrs	1990-1992 20 yrs	7.9	4.0
	Radon et al 2008[9]	27 European countries	n=4994 20-44 yrs	1992-2001 9 yrs	12.0	13.3
	Maio et al	Italy	n=885 8-78 yrs	1992-2010 18 yrs	33.1	18.4
COPD or CB	Johannessen et al 2005[40]	Norway	n=908 18-74 yrs	1987-1996 9 yrs	6.1	6.7
	Pelkonnen et al 2006[11]	Finland	only men n=345 40-59 yrs	1959-1989 30 yrs	14.0	4.7
	Maio et al	Italy	n=866 8-78 yrs	1992-2010 18 yrs	4.3 *(4.7)***	2.4 *(2.6)*
Usual cough	Mirabelli et al 2012[12]	US	n=8967 45-64 yrs	‘80s 3 yrs	3.5	11.7
	Maio et al	Italy	n=866 8-78 yrs	1992-2010 18 yrs	14.8 *(15.1)***	8.2 *(8.4)*
Usual phlegm	Mirabelli et al 2012[12]	US	n=8967 45-64 yrs	‘80s 3 yrs	3.4	11.3
	Maio et al	Italy	n=866 8-78 yrs	1992-2010 18 yrs	15.7 *(15.5)***	8.7 *(8.6)*
LLN AO	Mehta et al	Switzerla	n=4023	1991-2002	7.4	6.7

	2012[14]	nd	18-62 yrs	11 yrs		
	Marcon et al, 2018[15]	Europe	n=4205 20-44 yrs	1991-1993 9 yrs	2.6	2.9
	Maio et al	Italy	n=368 8-70 yrs	1992-2010 18 yrs	12.5 *(13.7)***	6.9 *(7.6)*

COPD: Chronic Obstructive Pulmonary Disease; CB: chronic bronchitis; LLN AO: Airway obstruction computed according to the lower limit of normal.

* general health insurance beneficiaries (health care utilization)

** the incidence rate computed only in adult subjects was reported in italic

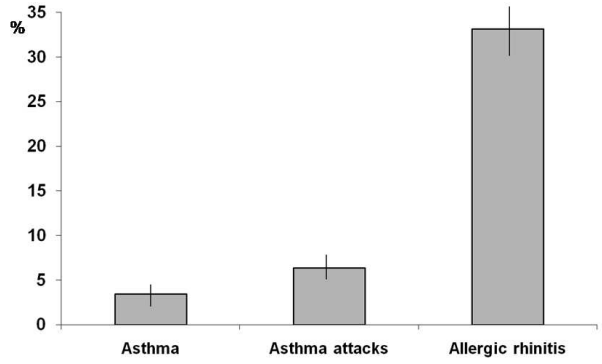
Fig 1. Symptoms/diseases cumulative incidence* and 95% confidence intervals from PI2 to PI3

a) asthma and allergic rhinitis symptoms/disease b) COPD symptoms/disease

* Incidence in PI3 subjects not reporting symptoms and diagnoses in PI2.

COPD: Chronic Obstructive Pulmonary Disease; LLN AO: Airway obstruction computed according to the lower limit of normal.

a)



b)

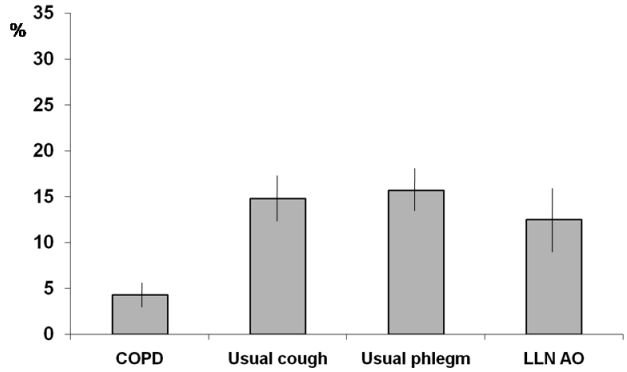
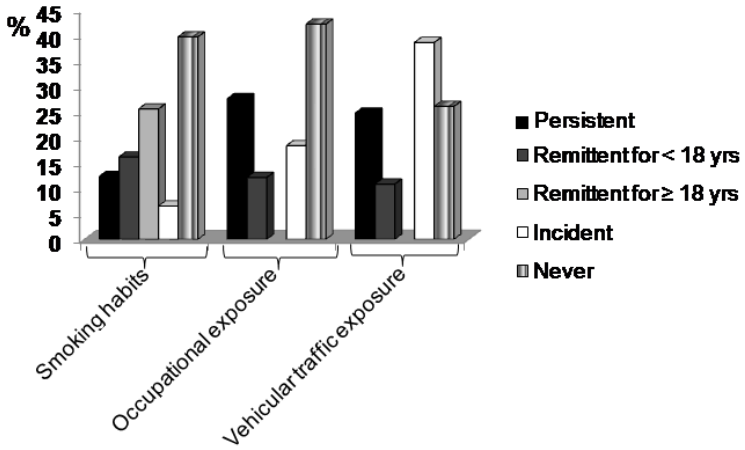


Fig 2. Changes in risk factors exposure from PI2 to PI3 (n=1107) (%)

Remittent for < 18 yrs: subjects stopping smoking between PI2 and PI3; Remittent for ≥ 18 yrs: subjects stopping smoking before PI2.



Supplemental material

Respiratory symptoms/diseases incidence and risk factors in the 18-yr Pisa epidemiological study

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Investigated respiratory symptoms/diseases

Since in PI2 and PI3 different questionnaires were used, only comparable questions were used for these analyses:

- Asthma diagnosis, if the subjects reported asthma confirmed by a physician (PI2, PI3);
- Current asthma attacks, if the subjects reported attacks of shortness of breath with wheezing, apart from common colds, currently (PI2) or in the last 12 months (PI3);
- Allergic rhinitis (AR) symptoms/diagnosis, if the subjects reported hay fever or other conditions causing runny or blocked nose, apart from common colds (PI2) or if the subjects reported problems with sneezing or a runny or blocked nose, apart from common colds (PI3);
- Usual cough (or phlegm), if the subjects reported usual cough (or phlegm) apart from common colds (PI2, PI3);
- COPD diagnosis, if the subjects reported chronic bronchitis or emphysema confirmed by a physician (PI2) or if the subjects reported chronic bronchitis, emphysema or COPD confirmed by a physician (PI3);
- Airway obstruction (AO) was detected using the ERS/ATS criterion: FEV_1/FVC percentage predicted < lower limit of normal (LLN) (Pellegrino 2005); the LLN was derived from population-specific prediction equations (Pistelli 2007) (PI2,PI3). Considering that two different spirometers were used in PI2 and PI3, adjusted value of FEV_1 and FVC were used in PI3, as detailed in an our previous paper (Maio 2016).

Incidence was computed in the subsample of the subjects reporting symptoms or diagnoses in PI3 but without symptoms & diagnoses in PI2, as follows:

- for asthma diagnosis: subjects reporting asthma diagnosis in PI3 but reporting neither asthma diagnosis nor asthma attacks in PI2 (n=1008);
- for asthma attacks: subjects reporting asthma attacks in PI3 but reporting neither

asthma diagnosis nor asthma attacks in PI2 (n=1008);

- for AR symptoms/diagnosis: subjects reporting AR diagnosis or AR symptoms in PI3 but reporting neither AR diagnosis nor AR symptoms in PI2 (n=885);
- for COPD diagnosis: subjects reporting COPD in PI3 but reporting neither COPD diagnosis nor COPD symptoms (phlegm and cough) in PI2 (n=866);
- for usual phlegm: subjects reporting usual phlegm in PI3 but reporting neither COPD diagnosis nor COPD symptoms (phlegm and cough) in PI2 (n=866);
- for usual cough: subjects reporting usual cough in PI3 but reporting neither COPD diagnosis nor COPD symptoms (phlegm and cough) in PI2 (n=866);
- for AO: subjects having AO in PI3 but not in PI2 (n=368).

Cumulative rates for the incidence of the symptoms and diseases over the eighteen-year follow-up were calculated as follows: "incidence" = "incident cases" / ("incident" + "never").

Risk factors

Longitudinal changes of the main risk factors for respiratory diseases were computed.

Smoking habits were coded into 5 groups: “Never” (non smoker subjects both in PI2 and PI3), “Persistent” (smoker subjects both in PI2 and PI3), “Incident” (subjects starting to smoke in the period between PI2 and PI3), “Remittent for < 18 yrs” (subjects stopping smoking in the period between PI2 and PI3), “Remittent for ≥ 18 yrs” (subjects who had stopped smoking before PI2).

Occupational exposure (exposure to dust/fume/gas at work) was codified into 4 groups: “Never” (unexposed subjects both in PI2 and PI3), “Persistent” (exposed subjects both in PI2 and PI3), “Incident” (subjects becoming exposed in the period between PI2 and PI3), “Remittent” (subjects quitting exposure between PI2 and PI3).

Vehicular traffic exposure (self-reported exposure to vehicular traffic near home) was codified into 4 groups: “Never” (unexposed subjects both in PI2 and PI3), “Persistent” (exposed subjects both in PI2 and PI3), “Incident” (subjects becoming exposed in the period between PI2 and PI3), “Remittent” (subjects quitting exposure between PI2 and PI3).

References

1. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948–968.
2. Pistelli F, Bottai M, Carrozzi L, et al. Reference equations for spirometry from a general population sample in central Italy. Respir Med 2007;101:814-25.
3. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, Sarno G, Cerrai S, Martini F, Fresta M, Silvi P, Di Pede F, Guerriero M, Viegi G. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Resp Med 2016; 110:58-68.

XI.2 PAPER II

Asthma remission and underdiagnosis and associated risk factors in Italian general population samples

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Keywords: asthma status longitudinal changes, general population, risk factors

Abstract

Background: asthma underdiagnosis and remission data from longitudinal epidemiological studies are scanty.

Aim: assessing risk factors for asthma underdiagnosis and remission.

Methods: two general population samples living in Po Delta (North Italy) and Pisa (Central Italy) were investigated, through health status and risk factors questionnaire, in cross-sectional studies between 1980-2011.

Individuals reporting asthma symptoms/diagnosis in the previous surveys were enrolled in a survey about asthma (AGAVE project, 2011-2014).

Subjects were classified as: remittent (not reporting current asthma symptoms and treatment in AGAVE survey) (REMIT); persistent symptomatic (reporting current asthma symptoms but not diagnosis in both PD/PI previous and AGAVE surveys) (Sx-PERS); persistent asthmatic (reporting current asthma diagnosis in both PD/PI previous and AGAVE surveys) (DSx-PERS). Statistical analyses were performed to assess risk factors associated with Sx-PERS, a condition interpreted as underdiagnosis, and REMITIT.

Results: 668 subjects participated: 52.5% REMITIT, 17.2% Sx-PERS, 30.3% DSx-PERS. The multinomial logistic regression analysis (DSx-PERS, reference category) showed a significant inverse association between REMITIT and living in urban area (OR 0.46) and asthma comorbidities (OR 0.40 recurrent respiratory infections; OR 0.18 allergic rhinitis; OR 0.35 COPD). Sx-PERS was significantly related to smoking habits (OR 2.33), second hand smoke exposure (OR 2.16), obstructive sleep apnoea (OR 2.02) and inversely related to allergic rhinitis (OR 0.34).

Conclusions: 52.5% of asthma remission and 17.2% of underdiagnosis was found in general population at mean 20-yr follow-up.

Asthma remission and underdiagnosis showed associations with different risk factors; such information could help affected patients and health care providers in prevention and management strategies.

Introduction

Asthma is a chronic inflammatory airway disease whose prevalence is still on the increase. The prevalence reached epidemic proportions (1-18%) due to risk factors such as atopic predisposition, exposure to allergens or indoor and outdoor pollutants (including occupational exposure) and viral infections. Patients suffering from this disease have a reduced quality of life, lower productivity and increasing medical costs[1]. In the last decades, nevertheless effective pharmacological treatments and management strategies have been developed, asthma remains a treatable but not a curable disease. Thus, much effort was put into studying the risk factors associated with the inception and progression of asthma, since understanding these factors represents the first necessary step for developing effective prevention strategies[2,3].

From a clinical standpoint, to elucidate the asthma natural history and long term outcomes is not an issue of secondary importance; studies on asthmatic cohorts can help[2]. Asthma may onset at any age (even if manly in children) and is known to clinically persist, possibly resolve and/or present remission over time. For these reasons, asthma progression is difficult to be characterized and predicted[4] and little is known about the asthma activity and inactivity and its correlated risk factors[4,5]. Indeed, few epidemiological studies have focused on asthma remission associated factors[5-10].

Another problem related to asthma is its underdiagnosis even if the first international guidelines were published in 1991[11]. Most patients, in particular elderly, don't report their symptoms to the general practitioner (GP) because they don't recognize them and/or are suffering from other comorbidities with similar clinical manifestations like COPD or heart failure; thus, they remain unknown and undiagnosed. On the other side, patients who do present with respiratory problems and who have reduced lung function are not always recognized as such or they are underestimated[12,13].

In this framework, data of the *AGAVE project (*Severe Asthma: epidemiological and clinical cohorts follow up by registry and questionnaires; therapeutic appropriateness and outcome assessment, according to GINA guidelines*)* were analyzed.

The AGAVE project, performed in 2011-2014, was funded by the Italian Medicines Agency (AIFA) and it was aimed at assessing the effectiveness of therapeutic strategies for asthma patients, in epidemiological and clinical samples, through the implementation of an on-line Registry (http://www.iss.it/site/asmagrave/)[14].

This manuscript focuses on the AGAVE epidemiological arm with the aim to assess the risk factors associated with asthma underdiagnosis and remission in two general population samples assessed during a mean 20-yr follow-up.

Materials and methods

Background

A random general population sample, living in the rural Po Delta area (PD, North Italy), was investigated in two subsequent cross-sectional surveys with the aim to assess respiratory symptoms/diseases and the related risk factors: the first survey (PD1) was performed in 1980–1982 on 3284 subjects; the second survey (PD2) in 1988–1991 on 2841 subjects[15].

The same protocol and selection method were used to enrol a random general population sample, living in the urban and suburban area of Pisa (PI, Central Italy), investigated in three subsequent cross-sectional surveys: the first survey (PI1) was performed in 1985–1988 on 3865 subjects; the second survey (PI2) in 1991–1993 on 2841 subjects; the third survey (PI3) in 2009-2011 on 1620 subjects. Data were collected through standardized interviewer-administered questionnaires[16].

Detailed information on population characteristics and methods of the previous studies were already published[15,16].

AGAVE study population

In the AGAVE project (2011-2014), subjects reporting asthma diagnosis or asthma symptoms (asthma attacks or wheezing) in at least one of the previous epidemiological surveys (PD and PI) were enrolled (figure 1): n=480 from Pisa survey and n=188 from PD survey.

Methods

In the AGAVE project, subjects were investigated using an epidemiological questionnaire based on the main items of the GINA guidelines[11] regarding asthma symptoms/diseases, clinical characteristics, asthma treatment, exacerbations, symptoms' control, comorbidities and risk factors exposure. The questionnaire was reviewed and

approved by an interdisciplinary internal board comprised of pneumologists, allergists and epidemiologists.

The AGAVE study protocol, patient information sheet and consent form were approved by the ethics committee of the Pisa University Hospital (Azienda Ospedaliero-Universitaria Pisana, Prot. no. 17658, March 21, 2011).

Statistical analyses

Subjects were classified according to the temporal changes in the presence/absence of asthma symptoms/diagnosis from previous surveys (PD and PI) to the start of the AGAVE survey, as: remittent (REMIT), subjects reporting asthma symptoms or diagnosis in at least one of the PD and PI previous surveys and not reporting current (last 12 months) asthma symptoms & diagnosis & use of asthma medications in AGAVE project; persistent symptomatic (Sx-PERS), subjects reporting only current asthma symptoms (without lifetime physician diagnosis, i.e. "underdiagnosis") in both PD and PI previous surveys and AGAVE survey; persistent asthmatic (DSx-PERS), subjects reporting current asthma (with lifetime physician diagnosis) in both PD and PI previous surveys and AGAVE survey (figure 2).

One-way analysis of variance was used for comparing continuous variables among different groups of patients, while categorical variables were evaluated by chi-square test.

A post-hoc analysis was run to assess the source of statistically significant result in case of contingency table larger than a 2 x 2, using adjusted standardized residual.

A multinomial logistic regression analysis, taking as dependent variable the longitudinal changes in asthma symptoms/diagnosis (reference category: DSx-PERS), and as independent variables: age (≤ 46 yrs, 47-65 yrs, > 65 yrs), sex (males, females), BMI groups (obese, overweight, normal/under weight), educational level (0-8 yrs, 8-13 yrs, > 13 yrs), area of residence (urban, rural), occupational exposure to gases/fumes/dusts (yes, no), smoking habits (smokers, ex-smokers, non smokers), second hand smoke exposure (yes, no), obstructive sleep apnoea (yes, no), AR (yes, no), COPD (yes, no), recurrent (> 3 annual episodes) lower respiratory infections (yes, no), anxiety/depression (yes, no), diabetes (yes, no).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), rel 17.0. A p-value ≤0.05 was considered statistically significant, a 0.05<p-value<0.10 was considered as borderline value.

Results

General characteristics

Six hundred and sixty eight subjects reporting asthma diagnosis or asthma symptoms in the PD and PI previous surveys were analyzed (participation rate 68%) (table 1, figure 1).

The majority were females (54.3%) and the mean age was 57.8 years. Educational level was middle-low with only 16.1% with an academic degree. 71.9% of the sample lived in urban PI (table 1). Body mass index (BMI) was slightly high with 15.5% of subjects having a BMI indicating obesity. 37.2% were ex-smokers, while 19.3% current smokers. 16.7% were exposed to second hand smoke, 51.8% to vehicular traffic near home and 39.1% to fumes/gases/dusts at work (table 1).

Longitudinal asthmatic status

52.5% of subjects were remittent (REMIT), 17.2% persistent symptomatic (Sx-PERS) and 30.3% persistent asthmatic (DSx-PERS) (figure 3).

A significantly higher frequency of REMIT was found in: males with respect to females (56.1% vs 49.6%), subjects living in PD rural area with respect to PI urban area (64.4% vs 47.9%), medium educational level (high school) vs low (elementary/medium high school) and high (university) educational level (57.3% vs 49.7% and 53.3%), age 47-65 yrs vs age ≤ 46 yrs and > 65 yrs (54.6% vs 49.7% and 52.9%). A significantly higher frequency of Sx-PERS was found in males with respect to females (19.0% vs 15.7%), in subjects with low educational level (21.8% vs 14.1% (medium) and 9.3% (high)) and in subjects with age > 65 yrs (22.5% vs 9.7% (≤ 46 yrs) and 17.9% (47-65 yrs)). A significantly higher frequency of DSx-PERS was found in females with respect to males (34.7% vs 24.9%), in subjects living in PI urban area with respect to PD rural area (35.0% vs 18.0%), in subjects with high educational level (37.4% vs 28.5% (low) and 28.6% (medium)) and in subjects with age ≤ 46 yrs (40.6% vs 27.5% (47-65 yrs) and 24.6% (> 65 yrs)) (table 2).

A higher frequency of REMIT was found in subjects with asthma diagnosis and asthma symptoms onset at younger age (< 12 yrs) with respect to older age (12-40 yrs, > 40 yrs) (39.8% vs 25.6% and 11.1%; 39.2% vs 23.9% and 12.2%, respectively). A higher frequency of DSx-PERS was found in subjects with asthma diagnosis and asthma symptoms onset at older age (> 40 yrs) with respect to younger age (< 12 yrs, 12-40 yrs) (88.9% vs 60.2% and 74.4%; 87.8% vs 60.8% and 76.1%, respectively) (table 2).

Table 3 shows the relationship between risk factors exposure, current asthma comorbidities and longitudinal asthmatic status.

REMIT was significantly lower in current smokers with respect to ex smokers and no smokers (44.2% vs 55.2% and 53.8%) and in subjects with occupational exposure (43.1% vs 57.7%) and second hand smoke exposure (35.5% vs 55.6%) with respect to those without them; Sx-PERS was significantly higher in current smokers (28.7% vs 17.0% ex smokers and 12.4% non smokers) and in subjects with second hand smoke exposure (29.0% vs 15.0%); ADs-PERS frequency was significantly higher in subjects with occupational exposure (36.2% vs 26.6%) (table 3).

As regards current comorbidities, REMIT was significantly lower in subjects with asthma comorbidities with respect to those without them: recurrent respiratory infections (24.4% vs 56.4%), obstructive sleep apnoea (30.9% vs 55.7%), AR (36.5% vs 62.4%), COPD (28.2% vs 55.5%) and depression/anxiety (42.0% vs 56.7%); Sx-PERS was significantly higher in subjects with obstructive sleep apnoea (34.6% vs 14.8%) and diabetes (31.8% vs 16.0%); ADs-PERS was significantly higher in subjects with the other comorbidities: AR (49.2% vs 18.8%), COPD (47.9% vs 28.4%) and depression/anxiety (40.3% vs 26.4%) (table 3).

The results of the multinomial logistic regression analysis, taking into account DSx-PERS as reference category, were shown in figure 3. An inverse significant association was found between REMIT and living in PI urban area (OR 0.46, 95% CI 0.26-0.80) and asthma comorbidities (OR 0.40, 95% CI 0.20-0.78 for recurrent respiratory infections; OR 0.18, 95% CI 0.11-0.28 for allergic rhinitis; OR 0.35, 95% CI 0.17-0.74 for COPD) (figure 3); a borderline inverse association was found in subjects exposed to fumes/gases/dusts at work (OR 0.66, 95% CI 0.41-1.06) (data not shown).

Sx-PERS was significantly related to current smoking habits (OR 2.33, 95% CI 1.13-4.83), second hand smoke exposure (OR 2.16, 95% CI 1.09-4.28), obstructive apnoea (OR 2.02, 95% CI 1.00-4.09) and inversely related to allergic rhinitis (OR 0.34, 95% CI 0.19-0.60) (figure 4).

Discussion

Our results showed 52.5% of asthma remission and 17.2% of asthma symptoms persistence without receiving asthma diagnosis in general population samples assessed during a 30-yr follow-up. The latter may be considered a clue for underdiagnosis.

Asthma remitted less in subjects living in an urban area and in those with asthma comorbidities. Asthma underdiagnosis was higher in current smokers, in those exposed to second hand smoke and in subjects with sleep apnoea, but lower in those with allergic rhinitis.

Asthma remission

Asthma can persist, completely remit, or show possible combinations of remissions and relapses over time. Few patients show lifespan symptoms. Most children with asthma remit symptoms during adolescence and early adulthood[5,10].

Our study showed about 50% of asthma remission, a value in line with other findings[5,7]. Some studies showed higher values (65-75% at 30/40-yr follow-up)[5,6], others lower values (15-20% at 10-yr follow-up)[8,17,18]. These variations among studies may partly be explained by different follow-up periods and quite different definitions of remission (i.e. clinical remission or complete remission)[2,17]. Only a minority of patients has complete remission. The remaining patients no longer report symptoms but retain airway hyper-responsiveness or airway inflammation. It suggests that symptoms remission does not reflect remission of underlying airway pathology[5]. However, the clinical asthma remission (absence of symptoms and medication use) is the most commonly used criterion in epidemiology and it is also mostly used in clinical algorithms for disease management[2].

In the few epidemiological studies focusing on asthma remission, male sex, early-onset asthma and absence of allergic rhinitis were clearly associated with symptoms remission[5-7,10].

We found relationship between REMIT and asthma comorbidities, which was in line with other international researches; in particular, the association with allergic rhinitis is evident[5,9]. In Swedish and Italian general population samples, presence of rhinitis was inversely associated with 10-yr (OR 0.33, 95% CI 0.16-0.67)[8] and 20-yr remission (OR 0.48, 0.43–0.53)[7], respectively. In a Tasmanian Longitudinal Health Study, childhood (OR 0.38, 0.25-0.58) and later-onset allergic rhinitis (OR 0.42, 0.29-0.63) were inversely associated with remission at 40-yr follow up[6].

REMIT was lower in subjects with COPD (OR 0.35). A US study on a general adult population sample showed that COPD was higher in subjects with active asthma with respect to inactive asthma (34.6% vs 14.7%)[19]; the same result was found in a Canadian study, using health administrative databases, showing that rates of asthma claims were higher among patients with COPD with respect to those without COPD[4]. Moreover, in a

US study, subjects with active asthma showed a higher hazard ratios than inactive or non asthmatic subjects for acquiring COPD[20].

REMIT was lower in subjects with recurrent respiratory infections (OR 0.40). This association could be explained by the strong evidences demonstrating the association between asthma exacerbations and viral infections in the community. Several viral infections are an "adjuvant" to the inflammatory response and promote the development of airway injury by enhancing airway inflammation and possibly inducing asthma exacerbation[11]. Respiratory syncytial virus and human rhinovirus are known to be major causes of asthma exacerbations in children[3]. 41–78% of adult asthmatic exacerbation was associated with virus presence[21]. Influenza virus infection also can induce asthma attacks in adults, with higher frequency than in children[21].

Living in urban area (PI), with respect to living in a rural area (PD), showed an inverse significant association with REMIT (OR 0.46). This result can be explained by the strong scientific evidences showing that urban living, characterized by more elevated levels of air pollutants concentration than rural environment, is an important risk factor for asthma onset and exacerbations[22,23]. Moreover, the current data are in line with our previous observation showing that urban living (PI), with respect to rural living (PD), was associated with higher bronchial hyper-responsiveness (an important clinical characteristic of active asthma)[24].

Even if with borderline values, exposure to gas/dusts/fumes at work seems to influence REMIT, confirming that occupational exposures could be an important risk factors for a more uncontrolled and symptomatic asthma[25].

Asthma symptoms persistence ("underdiagnosis")

We classified subjects with underdiagnosed asthma if they reported persistence of asthma symptoms at follow-up without a reported physician diagnosis of asthma across the surveys (Sx-PERS). Underdiagnosis of asthma is common in adults and it has been related to reduced perception or under reported symptoms and to the difficulty in differentiating this condition from other comorbidities like COPD, angina pectoris and heart trouble[13,26].

Our study showed about 17% of asthma underdiagnosis, a value in line with a UK study (22%)[27], but lower than that of other studies: a Colombian study on adult subjects showed that about 65% of subjects with wheezing had not physician-diagnosed asthma[13]; a Dutch study showed that 66% of patients did not present bronchial symptoms to their GP, even if in presence of decreased lung function, so they remained unknown and

undiagnosed and 23% of patients with respiratory problems and reduced lung function were not always recognised as such[12].

In our study a strong relationship was found with current smoking and second-hand smoke (OR 2.33 and OR 2.16, respectively). Published evidences about the relationship between asthma underdiagnosis and second-hand smoke exposure regard children samples: a French study reported that undiagnosed asthmatic children were more exposed to maternal smoking with respect to diagnosed ones (36.0% vs 30.0%)[28]; furthermore, Danish and US studies showed that second-hand smoking was a risk factor for asthma underdiagnosis (OR 2.39, 1.16-4.92 and OR 1.59, 95% CI 1.50-1.70, respectively)[29,30].

UK and USA studies confirmed that smoking habit was as significant independent risk factor for undiagnosed wheeze (OR 2.54, 1.19–5.41 and OR 2.60, 95% CI 2.43-2.79)[27,30]. In a recent paper about the same UK sample, a cluster analysis was used to define clinically relevant young adult wheeze clusters and 19.4% of subjects were identified as "undiagnosed-wheezers"; personal smoking habits showed the highest prevalence (74.6%) in this cluster with respect to the other ones[31]. A possible explanation of the relationship between Sx-PERS and smoke is that respiratory symptoms like wheeze or breathlessness could be miss-interpreted as due to smoking, rather than to asthmatic condition, thus not reported to the physician. In the other hand, asthma-like symptoms are common also in subjects with COPD, which is often smoke-related, making difficult to disentangle the relationship between smoking and asthma-like symptoms. However, the result of the multinomial regression showed an influence of current smoking on symptoms persistence and not of COPD.

With regard to comorbidities, our study showed that allergic rhinitis was inversely associated with asthma underdiagnosis (OR 0.34), as found in the quoted French study (undiagnosed asthmatic children had a higher prevalence of AR with respect to diagnosed ones 66.9% vs 54.4%)[28]; on the contrary, presence of obstructive sleep apnoea was a risk factor for underdiagnosis (OR 2.02). These findings highlighted the possible influence of asthma comorbidities on asthma management. On one side, the co-presence of diseases strictly related to asthma, like allergic rhinitis, can facilitate the asthma diagnosis, both by promoting contact with a doctor and by increasing doctor awareness toward diagnosis[30]. Indeed, the international guidelines for allergic rhinitis management (the Allergic Rhinitis and its Impact on Asthma initiative) recommend to consider the presence of asthma in all patients with rhinitis. On the other side, the co-presence of other diseases with similar

clinical manifestations, like the obstructive sleep apnoea, could lead to not recognizing or to mis-interpreting asthma symptoms[32].

Limitations and strengths

A limitation of this study is the use of questionnaire for collecting data on respiratory symptoms/diseases, potentially affected by a reporting bias, as it relies upon individual memory; nevertheless, the standardized questionnaire is one of the main investigation tool in respiratory epidemiology[33].

As regards the definition of asthma remission, it was based on absence of symptoms and of drugs consumption in the last 12 months. This period might seem too short to identify the occurrence of asthma remission, determining an overestimation of its proportion. However, other investigators used the same time frame[9,34] and a longer period could raise the problem of recall bias. One year without symptoms has been accepted as a minimum period, given the frequent seasonal variability of asthma[34].

A strength of our study is to have applied, over a 30-year follow-up, the same study design, sampling frame and study protocol in subjects living in two different Italian Regions. In all the surveys, questions were derived from validated international questionnaires, which already had passed the scrutiny of independent reviewers.

At last, the added value of our study is to have analyzed two general population samples with a large age range from childhood to the elderly, adding new evidences about risk factors associated with asthma remission and underdiagnosis in general population.

Conclusion

Asthma remission and underdiagnosis showed associations with different risk factors: REMIT was lower in subjects living in an urban area and in those with asthma comorbidities; Sx-PERS was higher in subjects exposed to active and second hand smoke, with sleep apnoea and lower in those with allergic rhinitis. Such information could be useful to affected patients and health care providers for prevention and management strategies.

Conflict of interest

Conflicts of interest: none.

Acknowledgments

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References

1. Maio S, Baldacci S, Simoni M, Angino A, Martini F, Cerrai S, et al, on behalf of ARGA Study Group. Impact of Asthma and Comorbid Allergic Rhinitis on Quality of Life and Control in Patients of Italian General Practitioners. Journal of Asthma 2012;49:854–861.
2. Guerra S. Clinical remission of asthma: what lies beyond? Thorax 2005;60:5–6.
3. Anderson HM, Jackson DJ. Microbes, allergic sensitization, and the natural history of asthma. Curr Opin Allergy Clin Immunol 2017;17:116–122.
4. Gershon A, Guan J, Victor JC, Wang C, To T. The course of asthma activity: a population study. J Allergy Clin Immunol 2012;129:679-86.
5. Fuchs O, Bahmer T, Rabe KF, von Mutius E. Asthma transition from childhood into adulthood. Lancet Respir Med 2017;5:224-234.
6. Burgess JA, Matheson MC, Gurrin LC, Byrnes GB, Adams KS, Wharton CL, et al. Factors influencing asthma remission:
   a longitudinal study from childhood to middle age. Thorax 2011;66:508-513.
7. Pesce G, Locatelli F, Cerveri I, Bugiani M, Pirina P, Johannessen A, et al. Seventy Years of Asthma in Italy: Age, Period and Cohort Effects on Incidence and Remission of Self-Reported Asthma from 1940 to 2010. PLoS ONE 2015; 10:e0138570.

8. Ekerljung L, Rönmark E, Larsson K, Sundblad B-M, Bjerg A, Ahlstedt S, et al. No further increase of incidence of asthma: Incidence, remission and relapse of adult asthma in Sweden. Respir Med 2008;102:1730-1736.
9. Yavuz ST, Civelek E, Comert S, Sahiner UM, Buyuktiryaki B, Tuncer A, et al. Development of rhinitis may be an indicator for the persistence of childhood asthma. International Journal of Pediatric Otorhinolaryngology 2014;78:843–849.
10. Andersson M, Hedman L, Bjerg A, Forsberg B, Lundbäck B, Rönmark E. Remission and Persistence of Asthma Followed From 7 to 19 Years of Age. Pediatrics 2013;132:e435–e442.
11. Global Initiative for Asthma. Global strategy for asthma management and Prevention. Update 2015. http://ginasthma.org/archived-reports/. Accessed date June 2016.
12. van Schayck OC. Global strategies for reducing the burden from asthma. Prim Care Respir J 2013;22:239-43.
13. Gonzalez-Garcia M, Caballero A, Jaramillo C, Maldonado D, Torres-Duque CA. Prevalence, risk factors and underdiagnosis of asthma and wheezing in adults 40 yea rs and older: A population-based study. Journal of Asthma 2015;52:823-830.
14. Maio S, Baldacci S, Bresciani M, Simoni M, Latorre M, Murgia N, et al. AGAVE group. RItA: The Italian severe/uncontrolled asthma registry. Allergy 2018:73:683-695.
15. Viegi G, Pedreschi M, Baldacci S. Prevalence rates of respiratory symptoms and diseases in general population samples of North and Central Italy. Int J Tuberc Lung Dis 1999;3:1034–1042.
16. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, et al. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Respir Med 2016;110:58-68.
17. Holm M, Omenaas E, Gíslason T, Svanes C, Jögi R, Norrman E, et al, on behalf of the RHINE study group. Remission of asthma: a prospective longitudinal study from northern Europe (RHINE study). Eur Respir J 2007;30:62–65.
18. Pallasaho P, Juusela M, Lindqvist A, Sovijärvi A, Lundbäck B, Rönmark E. Allergic rhinoconjunctivitis doubles the risk for incident asthma e Results from a population study in Helsinki, Finland. Respir Med 2011;105:1449-1456.

19. Mirabelli MC, Beavers SF, Chatterjee AB. Active Asthma and the Prevalence of Physician-Diagnosed COPD. Lung 2014;192:693–700.
20. Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for COPD in a longitudinal study. Chest 2004;126:59-65.
21. Iikura M, Hojo M, Koketsu R, Watanabe S, Sato A, Chino H, et al. The importance of bacterial and viral infections associated with adult asthma exacerbations in clinical practice. PLoS One 2015;10:e0123584.
22. Prüss-Ustün A, Wolf J, Corvalán C, Bos R, Neira M. Preventing disease through healthy environments. A global assessment of the burden of disease from environmental risks. WHO 2016. http://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/ (accessed February 2018).
23. Thurston GD, Kipen H, Annesi-Maesano I, Balmes J, Brook RD, Cromar K, et al. A joint ERS/ATS policy statement: what constitutes an adverse health effect of air pollution? An analytical framework. Eur Respir J 2017;49:1600419.
24. Maio S, Baldacci S, Carrozzi L, Polverino E, Angino A, Pistelli F, et al. Urban residence is associated with bronchial hyperresponsiveness in Italian general population samples. Chest 2009;135:434-441.
25. Le Moual N, Carsin AE, Siroux V, Radon K, Norback D, Torén K, et al. Occupational exposures and uncontrolled adult-onset asthma in the European Community Respiratory Health Survey II. Eur Respir J 2014;43:374-786.
26. Yáñez A, Cho S-H, Soriano JB, Rosenwasser LJ, Rodrigo GJ, Rabe KF, et al and the WAO Special Committee on Asthma. Asthma in the elderly: what we know and what we have yet to know. World Allergy Organization Journal 2014;7:8.
27. Raza A, Kurukulaaratchy RJ, Grundy JD, Clayton C, Mitchell FA, Roberts G, et al. What does adolescent undiagnosed wheeze represent? Findings from the Isle of Wight Cohort. Eur Respir J 2012;40:580–588.
28. Annesi-Maesano I, Sterlin C, Caillaud D, de Blay F, Lavaud F, Charpin D, Raherisson C. Factors related to under-diagnosis and undertreatment of childhood asthma in metropolitan France. Multidisciplinary Respir Med 2012;7:24.
29. Siersted HC, Boldsen J, Hansen HS, Mostgaard G, Hyldebrandt N. Population based study of risk factors for underdiagnosis of asthma in adolescence: Odense schoolchild study. BMJ 1998;316:651–657.

30. Yeatts K, Davis KJ, Sotir M, Herget C, Shy C. Who gets diagnosed with asthma? Frequent wheeze among adolescents with and without a diagnosis of asthma. Pediatrics 2003;111:1046-1054.
31. Kurukulaaratchy RJ, Zhang H, Raza A, Patil V, Karmaus W, Ewart S, Arshad SH. The Diversity of Young Adult Wheeze; a Cluster Analysis in a Longitudinal Birth Cohort. Clin Exp Allergy 2014;44:724–735.
32. Qiao Yi-X, Xiao Y. Asthma and Obstructive Sleep Apnea. Chin Med J 2015;128:2798-2804.
33. Pistelli F, Maio S. Questionnaires and lung function. In: Annesi-Maesano I, Lundback B, Viegi G eds. Respiratory Epidemiology. Sheffield (UK): Eur Respir Monograph 2014;65:257-72.
34. Cazzoletti L, Corsico AG, Albicini F, Di Vincenzo EMG, Gini E, Grosso A, et al. The Course of Asthma in Young Adults: A Population-Based Nine-Year Follow-Up on Asthma Remission and Control. PLoS ONE 2014;9:e86956.

Table 1. Descriptive characteristics of the subjects (n=668)

Gender (%):	
males	45.7
females	54.3
Age (mean±SD) (yrs)	57.8±16.3
Age range (yrs)	20-91
Age groups (%):	
≤46 yrs	29.2
47-65 yrs	34.3
>65 yrs	36.5
Educational level (%):	
elementary/medium high school	51.8
high school	32.1
university	16.1
Area of residence (%):	
PI	71.9
PD	28.1
BMI (Kg/m^2) groups (%):	
underweight/normal weight	49.2
overweight	35.3
obese	15.5
Smoking habits (%):	
smokers	19.3
ex-smokers	37.2
non smokers	43.5
Second hand smoke exposure (%)	16.7
Traffic exposure at home (%)	51.8
Occupational exposure (%)	39.1

PI: Pisa urban area; PD: Po Delta rural area

Table 2. Longitudinal changes in asthma symptoms/diagnosis from PD and PI previous surveys to AGAVE survey by descriptive characteristics

	Remittent (REMIT) (n=351)	Persistent symptomatic (Sx-PERS) (n=115)	Persistent asthmatic (DSx-PERS) (n=202)	p-value
Sex:				**0.022**
males (n=305)	**56.1**	**19.0**	**24.9**	
females (n=363)	**49.6**	**15.7**	**34.7**	
Residence:				**0.000**
PI (n=480)	**47.9**	**17.1**	**35.0**	
PD (n=188)	**64.4**	**17.6**	**18.0**	
Educational level:				**0.011**
elementary/medium high school (n=344)	**49.7**	**21.8**	**28.5**	
high school (n=213)	**57.3**	**14.1**	**28.6**	
university (n=107)	**53.3**	**9.3**	**37.4**	

Age (mean±SD) (n=688)	**57.9±15.8**	**63.0±15.4**	**54.6±16.8**	**0.000**
Age groups: <=46 yrs (n=195) 47-65 yrs (n=229) >65 yrs (n=244)	 **49.7** **54.6** **52.9**	 **9.7** **17.9** **22.5**	 **40.6** **27.5** **24.6**	**0.000**
BMI (Kg/m^2) groups: under/normal weight (n=328) over weight (n=235) obese (n=103)	 54.6 53.2 44.7	 14.0 18.7 24.3	 31.4 28.1 31.0	0.124
Age at asthma diagnosis* (mean±SD) (n=216)	**13.5±13.9**	**---**	**26.1±21.3**	**0.000**
Age groups at asthma diagnosis *: <12 yrs (n=88) 12-40 yrs (n=82) >40 yrs (n=46)	 **39.8** **25.6** **11.1**	**---**	 **60.2** **74.4** **88.9**	**0.002**
Age at first asthma symptoms* (mean±SD) (n=227)	**12.8±13.8**	**---**	**24.5±20.9**	**0.000**
Age groups at first asthma symptoms *: <12 yrs (n=97) 12-40 yrs (n=88) >40 yrs (n=42)	 **39.2** **23.9** **12.2**	**---**	 **60.8** **76.1** **87.8**	**0.003**

* performed only on subjects reporting lifetime asthma diagnosis

PI: Pisa urban area; PD: Po Delta rural area

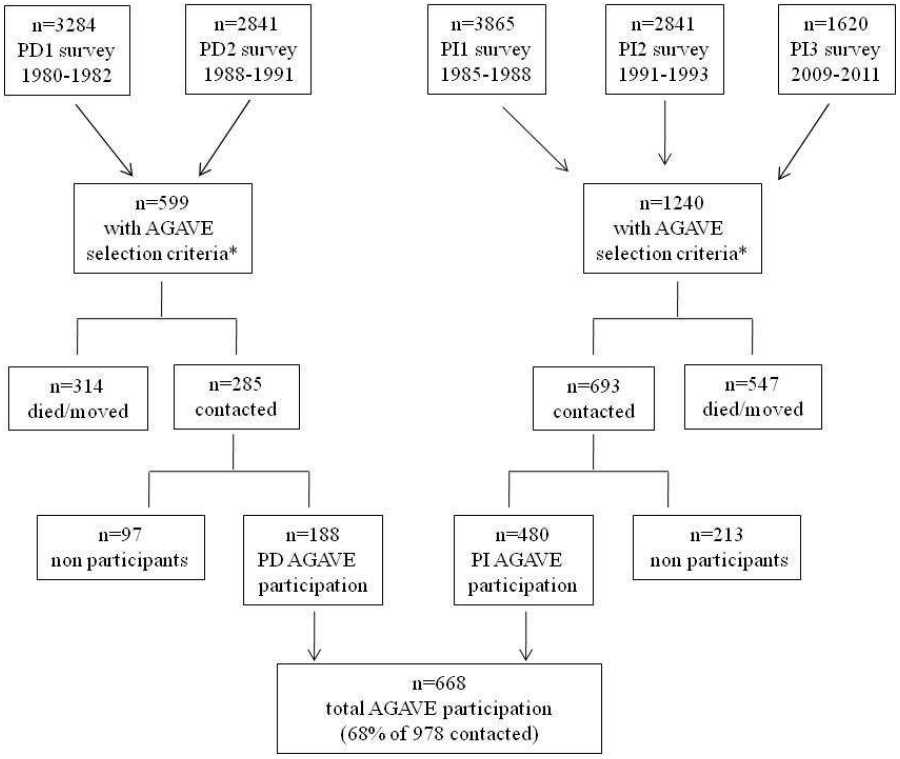
Table 3. Longitudinal changes in asthma symptoms/diagnosis from PD and PI previous surveys to AGAVE survey by risk factors exposure and current comorbidities

	Remittent (REMIT) (n=351)	Persistent symptomatic (Sx-PERS) (n=115)	Persistent asthmatic (DSx-PERS) (n=202)	p-value
Smoking habits: Smokers (n=129) ex smokers (n=248) no smokers (n=290)	 **44.2** **55.2** **53.8**	 **28.7** **17.0** **12.4**	 **27.1** **27.8** **33.8**	**0.001**
Occupational exposure: yes (n=246) no (n=383)	 **43.1** **57.7**	 **20.7** **15.7**	 **36.2** **26.6**	**0.002**
Vehicular traffic exposure: yes (n=345) no (n=321)	 *48.7* *56.7*	 *17.1* *17.1*	 *34.2* *26.2*	*0.062*
Second hand smoke				**0.000**

exposure: yes (n=110) no (n=547)	 **35.5** **55.6**	 **29.0** **15.0**	 **35.5** **29.4**	
Recurrent respiratory infections: yes (n=82) no (n=569)	 **24.4** **56.4**	 **19.5** **17.0**	 **56.1** **26.6**	**0.000**
Chronic sinusitis: yes (n=75) no (n=589)	 *40.0* *54.0*	 *22.7* *16.5*	 *37.3* *29.5*	*0.071*
Obstructive sleep apnoea: yes (n=81) no (n=582)	 **30.9** **55.7**	 **34.6** **14.8**	 **34.5** **29.5**	**0.000**
Allergic rhinitis: yes (n=252) no (n=415)	 **36.5** **62.4**	 **14.3** **18.8**	 **49.2** **18.8**	**0.000**
COPD: yes (n=71) no (n=591)	 **28.2** **55.5**	 **23.9** **16.1**	 **47.9** **28.4**	**0.000**
Depression/anxiety: yes (n=181) no (n=480)	 **42.0** **56.7**	 **17.7** **16.9**	 **40.3** **26.4**	**0.001**
Diabetes: yes (n=44) no (n=616)	 **45.5** **53.2**	 **31.8** **16.0**	 **22.7** **30.8**	**0.024**

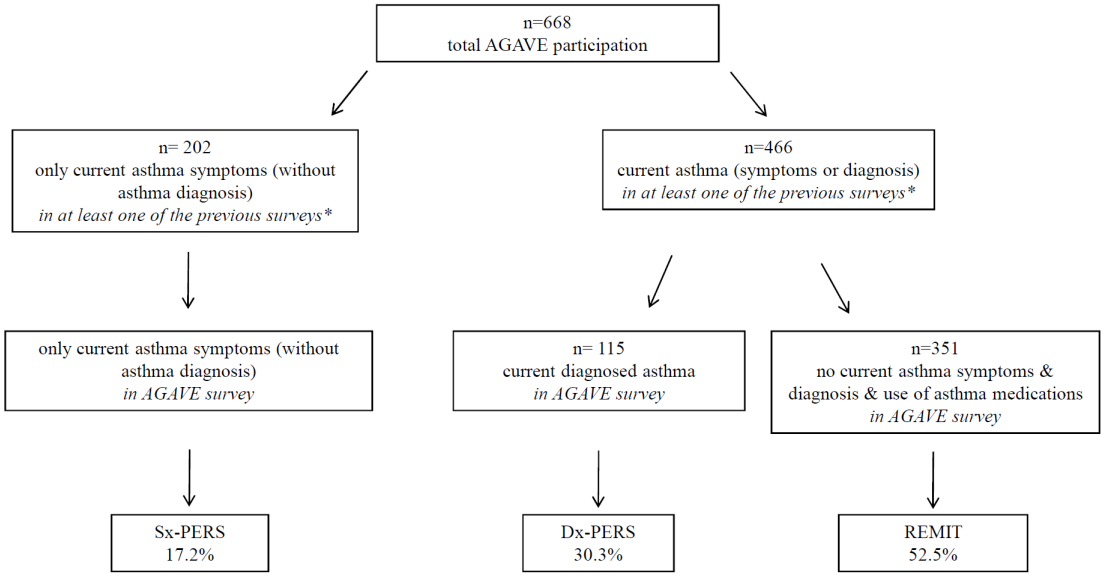
COPD: chronic obstructive pulmonary disease

Figure 1. Study population selection flow chart



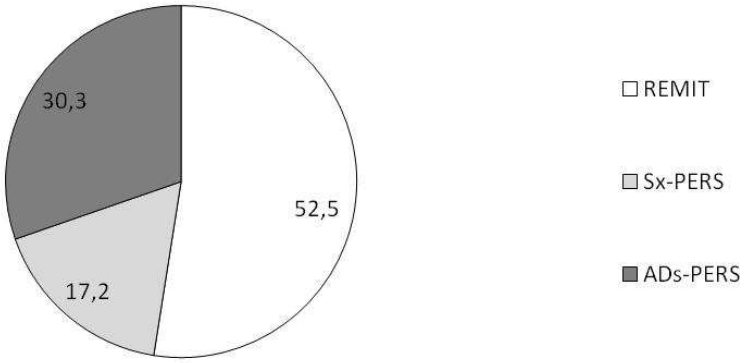
PD: Po Delta survey; PI: Pisa survey

Figure 2. Longitudinal changes in asthma symptoms/diagnosis from previous surveys to AGAVE survey



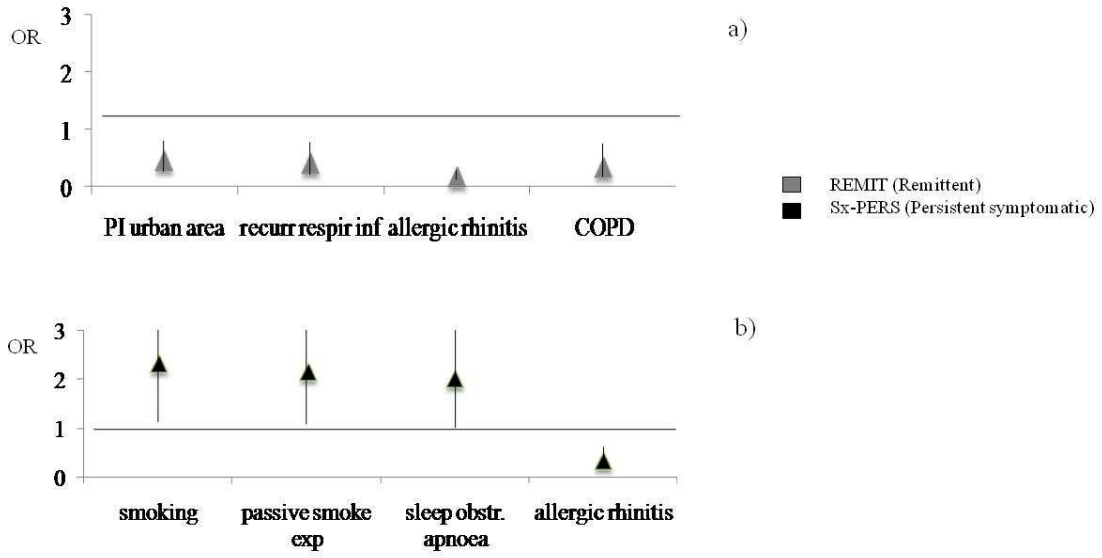
REMIT: remittent; Sx-PERS: persistent symptomatic; DSx-PERS: persistent asthmatic.
*AGAVE selection criteria: subjects reporting asthma diagnosis or asthma symptoms (asthma attacks or wheezing) in at least one of the previous epidemiological surveys (PD and PI).

Figure 3. Longitudinal changes in asthma symptoms/diagnosis from PD/PI previous surveys to AGAVE survey (%)



REMITIT: remittent, subjects reporting no more current asthma symptoms & diagnosis and no current use of asthma drugs in AGAVE survey; Sx-PERS: persistent symptomatic, subjects reporting only current asthma symptoms in both PD and PI previous and AGAVE surveys; ADs-PERS: persistent asthmatic, subjects reporting current asthma diagnosis in both PD and PI previous and AGAVE surveys.

Figure 4. Risk factors for longitudinal changes in asthma symptoms/diagnosis: significant results of multinomial regression analysis (OR, 95%CI) for REMIT (a) and Sx-PERS (b)



Reference category: DSx-PERS
Adjusted for age, sex, BMI, educational level, occupational exposure, diabetes, depression/anxiety

XI.3 PAPER III

COPD remission/persistence and associated risk factors in Italian general population samples

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Keywords: COPD status longitudinal changes, general population, risk factors

Abstract

Background: COPD underdiagnosis and remission data from longitudinal epidemiological studies are scanty.

Aim: assessing risk factors for COPD underdiagnosis and remission.

Methods: a general population sample participated in 3 cross-sectional surveys carried out in Central Italy (Pisa) in 1985-88 (n=3865), 1991-93 (n=2841), 2009-11 (n=1620).

All subjects filled in a standardized questionnaire about health status and risk factors.

The subsample of subjects reporting COPD diagnosis or symptoms (usual cough or usual phlegm) in the previous PI1/PI2 surveys were selected.

Subjects were classified according to the presence/absence of COPD symptoms/diagnosis in PI3 survey as: *remittent* (REMIT), subjects reporting COPD symptoms (usual cough or usual phlegm) or diagnosis in at least one of the previous surveys and not reporting current COPD symptoms & diagnosis in PI3 survey; *persistent symptomatic* (Sx-PERS), subjects reporting only current COPD symptoms (without physician diagnosis) in both previous surveys and PI3 survey (a condition interpreted as COPD "underdiagnosis"); *persistent COPD* (CDs-PERS), subjects reporting current COPD in both previous surveys and PI3 survey.

Results: 257 subjects were analyzed: 39.6% REMIT, 30.4% Sx-PERS, 30.0% CDs-PERS. The multinomial logistic regression analysis (Sx-PERS, reference category) showed a significant inverse association between REMIT and smokers (OR 0.25), living within 75m from main roads (OR 0.39) and asthma (OR 0.18). Sx-PERS showed a significant inverse association with current smoking habits (OR 0.324), ex-smokers (OR 0.21), living within 75m from main roads (OR 0.39) and comorbidites (OR 0.28 for heart failure and OR 0.16 for asthma).

Conclusions: COPD remission and underdiagnosis showed associations with different risk factors; such information could help affected patients and health care providers in prevention and management strategies.

Introduction

In the last decades, nevertheless effective pharmacological treatments and management strategies have been developed, chronic respiratory diseases remain treatable but not curable diseases. Thus, much effort was put into studying the risk factors associated with their inception and progression, since understanding these factors represents the first necessary step for developing effective prevention strategies [1].

From a clinical standpoint, to elucidate the COPD natural history and long term outcomes is not an issue of secondary importance.

COPD has been described by the WHO GOLD guidelines as a disease characterized by airflow limitation that is not fully reversible [2], but remission of COPD symptoms (chronic cough and chronic phlegm) is possible; nevertheless, few studies have examined the remission of respiratory symptoms in general population cohorts and the associated factors [3].

Another problem related to COPD is its underdiagnosis even if the first international guidelines were published since '90s [2]. Most patients, in particular elderly, don't report their symptoms to the general practitioner (GP) because they don't recognize them or are suffering from other comorbidities with similar clinical manifestations like heart failure; thus, they remain unknown and undiagnosed. On the other side, patients who do present with respiratory problems and who have reduced lung function are not always recognized as such or they are underestimated [4-6].

This manuscript has the aim to assess the risk factors associated with COPD underdiagnosis and remission in a general population sample assessed during a 25-yr follow-up.

Materials and methods

Detailed information on population characteristics and methods were previously published [7-9].

In 1985–1988, a general population sample of 3865 subjects (84% of the invited subjects) living in the urban and suburban area of Pisa, Central Italy, was investigated within the first Pisa survey (**PI1**) with the aim to assess the COPD natural history and the related risk factors. The sample was enrolled through a randomized, stratified, family cluster design, similar to the one previously used in the Po Delta Survey [8].

A second cross-sectional survey (**PI2**) was carried out in 1991–1993. Beside those participating in PI1, new subjects were recruited: newborns, new spouses and subjects not available in PI1. There were 433 subjects lost to follow-up (dead or moved). Overall, 2841 subjects (69% of the invited subjects) were investigated. 2257 subjects participated in both PI1 and PI2 surveys, corresponding to a longitudinal participation rate of 58% (66% if those lost to follow-up were excluded from the computation) with a mean follow-up of 6 years.

A third cross-sectional survey (**PI3**) was carried out in 2009-2011 within the European IMCA2 (Indicators for Monitoring COPD and Asthma in the EU) project. Beside those participating in PI1 and/or PI2, new subjects were recruited: newborns, new spouses and subjects not available in PI1 and/or PI2. There were 1201 subjects lost to follow-up (dead or moved). Overall, 1620 subjects (69% of invited) were investigated. 1107 subjects participated in both PI2 and PI3 surveys, corresponding to a longitudinal participation rate of 39% (68% excluding lost to follow-up) with a mean follow-up of 18 years.

The same study protocol was used in PI1 and PI2. Information on respiratory symptoms/diseases and risk factors were obtained through a standardized interviewer-administered questionnaire developed by the National Research Council [10]. As regards PI3, an interviewer-administered questionnaire on respiratory symptoms/diseases and risk factors was designed using questions from previously validated questionnaires [9].

Italian law didn't request the approval of Ethical Committee at the time of PI1/PI2. The protocol was approved by an Internal Revision Board within the Preventive Medicine Targeted Project of the Italian CNR. PI3 study protocol, patient information sheet and consent form were approved by the ethics committee of the Pisa University Hospital (Azienda Ospedaliero-Universitaria Pisana, Prot. no. 23887, April 16, 2008).

Statistical analyses

The subsample of subjects reporting COPD diagnosis or symptoms (usual cough or usual phlegm) in the previous PI1/PI2 surveys were selected.

Subjects were classified according to the presence/absence of COPD symptoms/diagnosis in PI3 survey, as: *remittent* (REMIT), subjects reporting COPD symptoms (usual cough or usual phlegm) or diagnosis in at least one of the previous surveys and not reporting current COPD symptoms & diagnosis in PI3 survey; *persistent symptomatic* (Sx-PERS), subjects reporting only current COPD symptoms (without

lifetime physician diagnosis) in both previous surveys and PI3 survey (a condition interpreted as COPD "underdiagnosis"); *persistent COPD* (CDs-PERS), subjects reporting current COPD (with lifetime physician diagnosis) in both previous surveys and PI3 survey.

One-way analysis of variance was used for comparing continuous variables among different groups of patients, while categorical variables were evaluated by chi-square test.

A post-hoc analysis was run to assess the source of statistically significant result in case of contingency table larger than a 2 x 2, using adjusted standardized residual [11-12].

A multinomial logistic regression analysis, taking as dependent variable the longitudinal changes in COPD symptoms/diagnosis (reference category: CDs-PERS), and as explanatory variables age (< 65 yrs, ≥ 65 yrs), sex (males, females), educational level (0-8 yrs, 8-13 yrs, > 13 yrs), area of residence (urban, suburban), occupational exposure to gases/fumes/dusts (yes, no), smoking habits (smokers, ex-smokers, non smokers), vehicular traffic exposure according to home distance from roads (highly exposed, moderately exposed, non exposed) and COPD comorbidities (heart failure (yes, no) and asthma (yes, no)) assessed during PI3 survey, was run.

As regards home distance from roads, it was computed using the GIS (Geographical Information System) technology (ArcMap 8.2). GIS permits to identify the exact position of the home residence addresses on the ground using the geographical coordinates (geocoding) and to compute the home distance from the roads. For the subjects geocoding, cartographic data provided by the GIS Service of Pisa and Cascina municipalities was used: buildings, streets, topography, population addresses, and house numbers [13]. In particular, subjects were considered as: highly exposed, if living within <75 m from roads; moderately exposed, if living within 75-150 from main roads OR 0-100m from secondary roads; non exposed, if living > 150m from main roads OR > 100m from secondary roads.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), rel 17.0. A p-value ≤0.05 was considered statistically significant, a 0.05<p-value<0.10 was considered as borderline value.

Results

General characteristics

Two hundred and fifty seven subjects reporting COPD diagnosis or COPD symptoms (usual cough or usual phlegm) in the PI previous surveys were analyzed.

The majority were males (52.9%) and the mean age was 66.3 years. Educational level was middle-low with only 8.2% with an academic degree. 54.9% of the sample lived in suburban area and 43.2% had a COPD family history (table 1).

42.4% were ex-smokers, while 22.2% current smokers. 58.6% lived within 75m from roads (i.e. highly exposed to traffic) and 56.0% were exposed to fumes/gases/dusts at work (table 2). About 30% reported cardiovascular risk factors (37% hypertension and 30.0% hypercholesterolemia), 20.6% anxiety and depression, 11.7% asthma, 8.9% heart failure and 8.6% diabetes (table 2).

Longitudinal COPD status

39.6% of subjects were remittent (REMIT), 30.4% persistent symptomatic (Sx-PERS) and 30.0% persistent COPD (CDs-PERS) (figure 1).

REMIT frequency was significantly lower in older age (≥ 65 yrs) with respect to younger age (< 65 yrs) (28.8% vs 57.7%) and in subjects living in a urban area with respect to suburban one (31.0% vs 46.8%); CDs-PERS was significantly higher in males with respect to females (36.8% vs 22.3%) and in older age with respect to younger age (36.9% vs 18.6%) (table 3).

Table 4 shows the relationship between longitudinal COPD status and risk factors exposure, current comorbidities.

REMIT was significantly lower in subjects with occupational exposure (32.6% vs 48.7%); Sx-PERS was significantly lower in ex-smokers with respect to smokers and non smokers (18.3% vs 33.3% and 42.9%); on the contrary, CDs-PERS was significantly more elevated in ex smokers with respect to smokers and non smokers (39.4% vs 29.8% and 18.7%) (table 4).

As regards current comorbidities, only heart failure showed significant relationship with longitudinal COPD status: a higher frequency of CDs-PERS was found in subjects with heart failure (60.9% vs 26.9%) (table 4).

The results of the multinomial logistic regression analysis, taking into account CDs-PERS as reference category, were shown in figure 2. An inverse significant association was found between REMIT and smokers (OR 0.25, 95% CI 0.08-0.72), living within 75m from main roads (OR 0.39, 95% CI 0.16-0.95) and asthma (OR 0.18, 95% CI 0.06-0.57) (figure 2).

Sx-PERS showed a significant inverse association with current smoking habits (OR 0.32, 95% CI 0.12-0.89), ex-smokers (OR 0.21, 95% CI 0.09-0.52), living within 75m

from main roads (OR 0.39, 95% CI 0.16-0.96) and comorbidites (OR 0.28, 95% CI 0.08-0.99 for heart failure; OR 0.16, 95% CI 0.05-0.55 for asthma) (figure 2).

Discussion

These results showed 39.6% of COPD symptoms/diagnosis remission and 30.4% of COPD symptoms (usual cough or usual phlegm) persistence without receiving COPD diagnosis (a clue for underdiagnosis) in general population samples assessed during a 25-yr follow-up.

COPD remitted less in current smokers and ex-smokers, in subjects living exposed to vehicular traffic and in those with COPD comorbidities. COPD "underdiagnosis" was lower in current smokers, in those living exposed to vehicular traffic and in subjects with asthma.

COPD remission

COPD has been described by the WHO GOLD guidelines as a disease characterized by airflow limitation that is not fully reversible [2], but remission of COPD symptoms (chronic cough and chronic phlegm) is possible; nevertheless, few past studies have examined the remission of respiratory symptoms in general population cohorts and the associated risk factors [3].

These data showed about 40% of COPD symptoms/diagnosis remission (of which 85% symptoms remission), a value in line with a Norwegian 11-yr community cohort (1985–1996/1997) showing a cumulative remission that varied from 42.3% of morning cough to 58.4% of chronic cough [3]. A more recent US multicenter study showed 9% of CB remission at 5-yr follow-up [14].

Smoking habits reduced significantly the probability of REMIT (OR 0.25). This result can be explained by the strong scientific evidences showing that smoking habits is the main risk factor associated to COPD onset and exacerbations [15-16]. On the other hand, scientific evidences showed a beneficial effect of smoking cessation on the remission of cough and phlegm [3] and of CB diagnosis [14].

Living near main road (<75 m), with respect to living far, reduced the probability of REMIT (OR 0.39). This result can be explained by the strong scientific evidences showing that exposure to vehicular traffic is an important risk factor for COPD onset and exacerbations [17-18] and living near traffic roads (<100m) is associated with an increased risk of having COPD in males (OR 1.80) [13] and in general population (OR 1.64) [19].

REMIT was lower in subjects with COPD comorbidities (OR 0.18 for asthma). COPD often coexists with other diseases that may have a significant impact on prognosis. Some comorbidities arise independently of COPD, some others have common risk factors, other can increase the risk of COPD developing or worsening the COPD severity. Past and recent evidences highlighted an association between BHR (an important clinical characteristic of active asthma) and risk of COPD: in a 24-yr follow-up Dutch cohort was found a significantly lower risk of asthma/COPD symptoms remission (OR 0.42) in subjects with BHR [20]; on the other side, European cohorts showed a higher risk of developing COPD in subjects with BHR ranging from incident rate ratios (IRR) of 3.41 to IRR of 8.91 at a mean 9-yr follow-up [21].

COPD "underdiagnosis"

Subjects were considered as having underdiagnosed COPD if they reported persistence of COPD symptoms at follow-up without a reported physician diagnosis of COPD across the surveys. COPD symptoms may precede the development of airflow limitation by many years. GPs play a crucial role in detecting and managing COPD, since they manage the vast majority of patients in the early stages. However, the diagnosis of COPD by GPs is not always consistent to GOLD guidelines in daily clinical practice, mainly because of underuse of spirometry. As a result, COPD is a disease that is largely underestimated and underdiagnosed [4-5].

These results showed about 30% of COPD underdiagnosis, a value in line with a Sweden study on adult general population (about 25%) [22], but lower than that of a Greek study (53%) [23] and higher than that of a French study (ranging from 10% to 16%) [24] and a Canadian study (14%) [25] on adult general population. These differences can be due to different criteria used to define COPD and different period of follow-up.

These data showed that being smokers, living exposed to vehicular traffic and having asthma reduced the risk of COPD underdiagnosis.

A possible explanation of these results is that exposure to well know COPD risk factors and the presence of COPD comorbidities could increase the physician awareness of patients' symptoms and, thus, the possibility of a correct disease diagnosis.

Smoking habits and living near main roads (<75 m) showed an inverse significant association with Sx-PERS (OR 0.32 for current smokers, OR 0.21 for ex-smokers and OR 0.39 living near main roads). Strong scientific evidences showing that smoking habits and exposure to air pollution are the main risk factors associated to COPD onset and

exacerbations [2,15-18]. GOLD guidelines for the management of COPD highlight the relationship between COPD and these two risk factors and advocate active case finding like performing spirometry in patients with symptoms and/or risk factors exposure [2]. Thus, the exposure to well-know COPD risk factors can reduce the possibility of underdiagnosis.

As above reported, COPD often coexists with other diseases that may have a significant impact on prognosis. In patients with COPD and heart failure, an exacerbation of heart failure may be accompanied by worsening of COPD and *vice versa* [2].

Asthma and COPD were recognized as distinct disease entities. However, this concept was re-evaluated as many epidemiological studies showed that asthma and COPD may coexist, or at least one condition may evolve into the other. This state is described as Asthma and COPD Overlap Syndrome (ACOS). It is a syndrome in which older adults with a significant smoking history have simultaneously features of asthma and COPD [26]. Therefore, a diagnosis of asthma, in addition to bronchitic symptoms (usual cough/phlegm), could facilitate the COPD diagnosis by physician and reduce the risk of underdiagnosis.

Limitations and strengths

A limitation of this study is the use of questionnaire for collecting data on respiratory symptoms/diseases, potentially affected by a reporting bias, as it relies upon individual memory; nevertheless, the standardized questionnaire is one of the main investigation tool in respiratory epidemiology [27].

The definition of COPD remission was based on absence of current symptoms and diagnosis after a period of at least 18 years from baseline; this timeframe is sufficiently long to be confident in an actual disease/symptoms remission. On the other side, under-reporting is more likely than over-reporting in a general population, determining a possible overestimation of cumulative remission rates [3].

A strength of our study is to have applied, over a 25-year follow-up, the same study design, sampling frame and study protocol. In all the surveys, questions were derived from validated international questionnaires, which already had passed the scrutiny of independent reviewers.

At last, the added value of our study is to have analyzed two general population samples with a large age range from childhood to the elderly, adding new evidences about risk factors associated with COPD remission and underdiagnosis in general population.

Conclusion

COPD remission and underdiagnosis showed associations with different risk factors: REMIT was lower in smokers, in subjects living near main roads and in those with asthma); Sx-PERS was lower in smokers and ex-smokers, in subjects living near main roads and in those with asthma and heart failures. Such information could be useful to affected patients and health care providers for prevention and management strategies.

Conflict of interest

Conflicts of interest: none.

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References

1. Anderson HM, Jackson DJ. Microbes, allergic sensitization, and the natural history of asthma. Curr Opin Allergy Clin Immunol 2017;17:116–122.
2. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. http://www.goldcopd.org/guidelines-global-strategy-for-diagnosis-management.htm. Update 2018 (accessed July 2018).
3. Eagan TML, Gulsvik A, Eide GE, Bakke PS. Remission of respiratory symptoms by smoking and occupational exposure in a cohort study. Eur Respir J 2004; 23: 589–594.
4. López-Campos JL, Tan W, Soriano JB. Global burden of COPD. Respirology 2016;21:14-23.

5. Stafyla E, Kotsiou OS, Deskata K, Gourgoulianis KI. Missed diagnosis and overtreatment of COPD among smoking primary care population in Central Greece: old problems persist. Int J Chron Obstruct Pulmon Dis 2018;13:487-498.
6. Maio S, Baldacci S, Carrozzi L, Pistelli F, Viegi G. The global burden of chronic respiratory diseases. Breathe 2006;3:20-29.
7. Viegi G, Matteelli G, Angino A, Scognamiglio A, Baldacci S, Soriano JB, Carrozzi L. The Proportional Venn Diagram of Obstructive Lung Disease in the Italian General Population. Chest 2004;126:1093–1101.
8. Carrozzi L, Giuliano G, Viegi, G, Paoletti P, Di Pede F, Mammini U, Carmignani G, Saracci R, Giuntini C, Lebowitz MD. The Po river delta epidemiological study of obstructive lung disease: sampling methods, environmental and population characteristics. Eur J Epidemiol 1990;6:191-200.
9. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, Sarno G, Cerrai S, Martini F, Fresta M, Silvi P, Di Pede F, Guerriero M, Viegi G. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Resp Med 2016;110:58-68.
10. Viegi G, Paoletti P, Carrozzi L, Vellutini M, Diviggiano E, Di Pede C, Pistelli G, Giuntini C, Lebowitz M.D. Prevalence rates of respiratory symptoms in Italian general population samples, exposed to different levels of air pollution. Environ Health Perspect 1991;94:95-99.
11. Agresti A. An Introduction to Categorical Data Analysis. Second Edition 2007. Wiley-Interscience. A John Wailey & Sons, Inc., Publication.
12. Sharpe D. Your Chi-Square Test is Statistically Significant: Now What?. Practical Assessment, Research & Evaluation 2015;20:1-10.
13. Nuvolone D, della Maggiore R, Maio S, Fresco R, Baldacci S, Carrozzi L, Pistelli F, Giovanni Viegi. Geographical information system and environmental epidemiology: a cross-sectional spatial analysis of the effects of traffic-related air pollution on population respiratory health. Environ Health 2011;10:12.
14. Kim V, Zhao H, Boriek AM, Anzueto A, Soler X, Bhatt SP, Rennard SI, Wise R, Comellas A, Ramsdell JW, Kinney GL, Han MK, Martinez CH, Yen A, Black-Shinn J, Porszasz J, Criner GJ, Hanania NA, Sharafkhaneh A, Crapo JD, Make B, Silverman EK, Curtis JL and the COPDGene Investigators. Persistent and Newly Developed

Chronic Bronchitis Are Associated with Worse Outcomes in Chronic Obstructive Pulmonary Disease. Ann Am Thorac Soc 2016;13(7):1016–1025.

15. World Health Organization. Report Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. 2007. http://www.who.int/gard/publications/GARD_Manual/en/ (accessed May 2018).
16. Institute for Health Metrics and Evaluation (IHME). GBD Compare Data Visualization. Seattle, WA: IHME, University of Washington, 2017. Available from http://vizhub.healthdata.org/gbd-compare. (Accessed May 2018).
17. Prüss-Ustün A, Wolf J, Corvalán C, R Bos and M Neira. Preventing disease through healthy environments. A global assessment of the burden of disease from environmental risks. WHO 2016. http://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/ (accessed May 2018).
18. World Health Organization 2013, Review of evidence on health aspects of air pollution - REVIHAAP Project: final technical report.
19. Lindgren A, Stroh E, Montnémery P, Nihlén U, Jakobsson K, Axmon A. Traffic-related air pollution associated with prevalence of asthma and COPD/chronic bronchitis. A cross-sectional study in Southern Sweden. Int J Health Geographics 2009, 8:2.
20. Xu X, Rijcken B, Schouten JP, Weiss ST. Airways responsiveness and development and remission of chronic respiratory symptoms in adults. Lancet 1997;350:1431–34.
21. Marcon A, Locatelli F, Keidel D, Beckmeyer-Borowko AB, Cerveri I, Dharmage SC, Fuertes E, Garcia-Aymerich J, Heinrich J, Imboden M, Janson C, Johannessen A, Leynaert B, Pascual Erquicia S, Pesce G, Schaffner E, Svanes C, Urrutia I, Jarvis D, Probst-Hensch NM, Accordini S; Ageing Lungs in European Cohorts (ALEC) study. Airway responsiveness to methacholine and incidence of COPD: an international prospective cohort study. Thorax 2018;73:825-832.
22. Lindström M, Jönsson E, Larsson K, Lundbäck B. Underdiagnosis of chronic obstructive pulmonary disease in Northern Sweden. Int J Tuberc Lung Dis 2001;6:76–84.
23. Spyratos D, Chloros D, Michalopoulou D, Sichletidis L. Estimating the extent and economic impact of under and overdiagnosis of chronic obstructive pulmonary disease in primary care. Chronic Respi Dis 2016;13:240–246.

24. Quach A, Giovannelli J, Chérot-Kornobis N, Ciuchete A, Clément G, Matran R, Amouyel P, Edmé JL, Dauchet L. Prevalence and underdiagnosis of airway obstruction among middle-aged adults in northern France: The ELISABET study 2011-2013. Respir Med 2015;109:1553-1561.
25. Gershon AS, Thiruchelvam D, Chapman KR, Aaron SD, Stanbrook MB, Bourbeau J, Tan W, To T; Canadian Respiratory Research Network. Health Services Burden of Undiagnosed and Overdiagnosed COPD. Chest 2018;153(6):1336-1346.
26. Alshabanat A, Zafari Z, Albanyan O, Dairi M, FitzGerald JM. Asthma and COPD Overlap Syndrome (ACOS): A Systematic Review and Meta Analysis. PLoS ONE 2015;10(9):e0136065.
27. Pistelli F, Maio S. Questionnaires and lung function. In: Annesi-Maesano I, Lundback B, Viegi G eds. Respiratory Epidemiology. Sheffield (UK): Eur Respir Monograph 2014;65:257-272.

Table 1. **PI3 descriptive characteristics of subjects with previous bronchitic symptom/diagnosis (n=257) (%)**

Gender:	
males	52.9
females	47.1
Age (mean±SD) (yrs)	66.3±14.4
Age range (yrs)	29-96
Age groups:	
< 65 yrs	37.7
≥ 65 yrs	62.3
BMI (Kg/m^2) groups:	
obese	33.5
overweight	42.5
underweight/normal weight	24.0
Educational level:	
0-8 yrs	70.4
8-13 yrs	21.4
> 13 yrs	8.2
Area of residence:	
urban	45.1
suburban	54.9
Family history of COPD	43.2

COPD: chronic obstructive pulmonary disease.

Table 2. PI3 risk factors exposure and comorbidities in subjects with previous bronchitic symptom/diagnosis (%)

Smoking habits:	
smokers	22.2
ex-smokers	42.4
non smokers	35.4
Occupational exposure	56.0
Vehicular traffic exposure	63.0
Distance from roads:	
<75 m	35.6
75-150 from main roads OR 0-100m from secondary roads	23.0
> 150m from main roads OR > 100m from secondary roads	41.4
Heart failure	8.9
Diabetes	8.6
Hypertension	37.0
Hypercholesterolemia	30.0
Depression/anxiety	20.6
Asthma	11.7

Table 3. Longitudinal changes in COPD symptoms/diagnosis from PI1/PI2 surveys to PI3 survey by PI3 descriptive characteristics

	Remittent (REMIT) (n=102)	Persistent symptomatic (Sx-PERS) (n=78)	Persistent COPD (CDs-PERS) (n=77)	p-value
Gender:				**0.038**
males	**36.8**	**26.5**	**36.8**	
females	**43.0**	**34.7**	**22.3**	
Age groups:				**0.000**
≥ 65 yrs	**28.8**	**34.4**	**36.9**	
< 65 yrs	**57.7**	**23.7**	**18.6**	
BMI (Kg/m^2) groups:				*0.069*
obese	*30.0*	*30.0*	*40.0*	
overweight	*25.0*	*39.5*	*35.5*	
underweight/normal weight	*46.5*	*34.9*	*18.6*	
Educational level:				*0.093*
0-8 yrs	*34.3*	*33.7*	*32.0*	
8-13 yrs	*50.9*	*23.6*	*25.5*	
> 13 yrs	*57.1*	*19.0*	*23.8*	
Area of residence:				**0.036**

urban	**31.0**	**34.5**	**34.5**	
suburban	**46.8**	**27.0**	**26.2**	
Family history of COPD:				0.179
yes	36.0	27.9	36.0	
no	42.5	32.2	25.3	

SD: standard deviation; COPD: chronic obstructive pulmonary disease.
in italic: borderline values; in bold: statistically significant values.

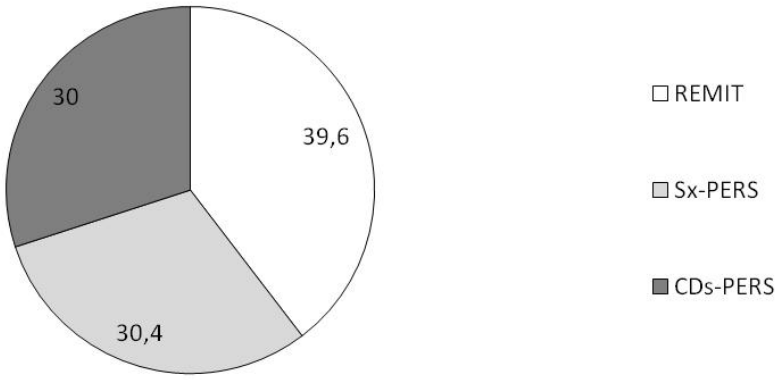
Table 4. Longitudinal changes in COPD symptoms/diagnosis from PI1/PI2 surveys to PI3 survey by PI3 risk factors exposure and comorbidities (%)

	Remittent (REMIT) (n=102)	Persistent symptomatic (Sx-PERS) (n=78)	Persistent COPD (CDs-PERS) (n=77)	p-value
Smoking habits:				**0.002**
smokers	**36.8**	**33.3**	**29.8**	
ex-smokers	**42.2**	**18.3**	**39.4**	
non smokers	**38.5**	**42.9**	**18.7**	
Occupational exposure:				**0.032**
yes	**32.6**	**33.3**	**34.0**	
no	**48.7**	**26.5**	**24.8**	
Vehicular traffic exposure:				0.873
yes	38.9	31.5	29.6	
no	41.1	28.4	30.5	
Distance from road:				*0.060*
<75 m	*32.9*	*29.4*	*37.6*	
75-150 from main roads OR 0-100m from secondary roads	*29.1*	*30.9*	*40.0*	
> 150m from main roads OR > 100m from secondary roads	*45.5*	*33.3*	*21.2*	
Heart failure:				**0.003**
yes	**21.7**	**17.4**	**60.9**	
no	**41.5**	**31.6**	**26.9**	
Diabetes:				0.251
yes	31.8	22.7	45.5	
no	40.4	31.1	28.5	
Hypertension:				0.208
yes	32.6	33.7	33.7	
no	43.8	28.4	27.8	
Hypercholesterolemia:				0.100

yes no	29.9 43.9	33.8 28.9	36.4 27.2	
Depression/anxiety: yes no	 34.0 41.2	 37.7 28.4	 28.3 30.4	0.404
Asthma: yes no	 30.0 41.0	 23.3 31.3	 46.7 27.8	0.104

in italic: borderline values; in bold: statistically significant values.

Figure 1. Longitudinal changes in COPD symptoms/diagnosis from PI1/PI2 surveys to PI3 survey (%)

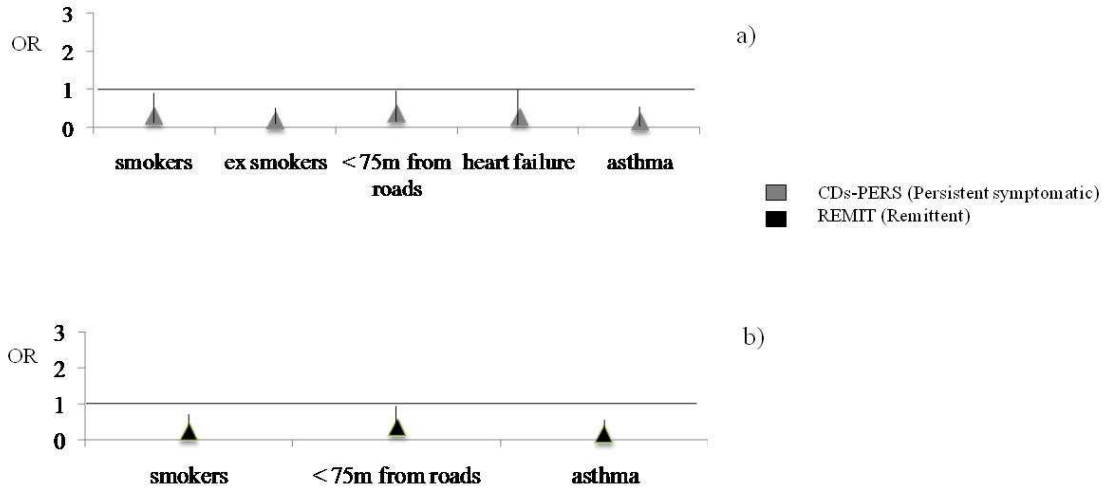


REMIT: remittent, subjects reporting no more COPD symptoms or diagnosis; Sx-PERS: persistent symptomatic, subjects reporting only current COPD symptoms (without physician diagnosis) in both previous surveys and PI3 survey; CDs-PERS: persistent COPD, subjects reporting current COPD in both previous surveys and PI3 survey.

Figure 2. Risk factors for longitudinal changes in COPD symptoms/diagnosis: significant results of multinomial regression analysis (OR, 95%CI) for REM (a) and CPERS (b)

Reference category: CPERS (Persistent COPD)

Adjusted for sex, age, educational level, occupational exposure, area of residence



XI.4 PAPER IV

Temporal changes in multimorbidity and associated risk factors in Italian general population samples

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Abstract

Background: increasing prevalence of multimorbidity (multiple medical conditions within a single subject) is now a global phenomenon and it is expected to continue rising; most of the evidence on multimorbidity came from cross-sectional studies and longitudinal studies are limited.

Aim: assessing temporal changes in respiratory multimorbidity and related risk factors.

Methods: a general population sample participated in 3 cross-sectional surveys carried out in Central Italy (Pisa) in 1985-88 (n=3865), 1991-93 (n=2841), 2009-11 (n=1620).

All subjects filled in a standardized questionnaire about health status and risk factors.

Multimorbidity of asthma, allergic rhinitis and COPD symptoms/diagnosis was assessed in the longitudinal sample participating in PI1, PI2 and PI3 survey (n=849).

Longitudinal changes in mutimorbidity were computed only in subjects participating in both PI2 and PI3 for sample size reasons (n=1107): "Never/reduced number of conditions", "Persistent number of conditions", "Increased number of conditions from 0 to 1 or more", "Increased number of conditions from 1/2 to more".

Results: the following results were found: 48.4% "Never/reduced number of conditions", 11.7% "Persistent number of conditions", 29.4% "Increased number of conditions from 0 to 1 or more" and 10.5% "Increased number of conditions from 1/2 to more".

The multinomial logistic regression analysis showed a significantly higher risk of persistent multimorbidity in persistent smokers and in subjects with persistent and remittent occupational exposure. A significantly higher risk of multimorbidity increase was found in subjects with incident and persistent occupational exposure and in subjects living exposed to incident and persistent vehicular traffic exposure; moreover, persistent smokers were related to a significantly higher risk of multimorbidity increase if already suffering from respiratory diseases.

Conclusions: This study showed a significant increase in the prevalence of multimorbidity in a longitudinal general population sample and it added new scientific evidences about associated longitudinal risk factors.

Introduction

Multimorbidity denotes multiple medical conditions within a single patient. Multimorbidity is distinct from comorbidity because there is no primary or index condition. Nowadays, there is no a standard definition and this fact has led to wide discrepancies in prevalence estimate (ranging from 13% to 72% in the general population) [1].

A study of 28 countries using the World Health Survey showed that the increasing prevalence of multiple chronic conditions is now a global phenomenon [2] and it is expected to continue rising [3]. Indeed, the lifespan of population is increasing because of improvements in public health measures and the significant success of modern medicine and technology. However, although populations are living longer, they are not necessarily living disease-free for a longer period [4].

Evidence from high-income countries suggested that multimorbidity is highly prevalent in older populations (over 65 years of age), but it also affects younger people. As such, multimorbidity from chronic conditions is now widespread, with at least 50 million people affected in the European Union alone [3,5].

Most of the evidence on multimorbidity came from cross-sectional studies and longitudinal studies are limited [3], even if there are some findings highlighting that the prevalence of some types of multimorbidity increased over time [6].

The main risk factors for multimorbidity increase is the ageing population but also by factors such as high body-mass index, urbanization and the growing burden of non communicable diseases. Few data are available about modifiable factors that predict the risk of different types of multimorbidity [1]: it remains unknown whether there are biological, environmental or behavioral factors. The identification of any such factors, and the assessment of the likelihood of causality, requires data from prospective observational studies [3].

The aim of this article is to assess the temporal changes in respiratory multimorbidity and related risk factors in a longitudinal general population sample.

Materials and methods

Materials

Detailed information on population characteristics and methods were previously published [7-9].

In 1985–1988, a general population sample of 3865 subjects (84% of the invited subjects) living in the urban and suburban area of Pisa, Central Italy, was investigated within the first Pisa survey (**PI1**) with the aim to assess the COPD natural history and the related risk factors. The sample was enrolled through a randomized, stratified, family cluster design, similar to the one previously used in the Po Delta Survey [8].

A second cross-sectional survey (**PI2**) was carried out in 1991–1993. Beside those participating in PI1, new subjects were recruited: newborns, new spouses and subjects not available in PI1. There were 433 subjects lost to follow-up (dead or moved). Overall, 2841 subjects (69% of the invited subjects) were investigated. 2257 subjects participated in both PI1 and PI2 surveys, corresponding to a longitudinal participation rate of 58% (66% if those lost to follow-up were excluded from the computation) with a mean follow-up of 6 years.

A third cross-sectional survey (**PI3**) was carried out in 2009-2011 within the European IMCA2 (Indicators for Monitoring COPD and Asthma in the EU) project. Beside those participating in PI1 and/or PI2, new subjects were recruited: newborns, new spouses and subjects not available in PI1 and/or PI2. There were 1201 subjects lost to follow-up (dead or moved). Overall, 1620 subjects (69% of invited) were investigated. 1107 subjects participated in both PI2 and PI3 surveys, corresponding to a longitudinal participation rate of 39% (68% excluding lost to follow-up) with a mean follow-up of 18 years.

The same study protocol was used in PI1 and PI2. Information on respiratory symptoms/diseases and risk factors were obtained through a standardized interviewer-administered questionnaire developed by the National Research Council [10]. As regards PI3, an interviewer-administered questionnaire on respiratory symptoms/diseases and risk factors was designed using questions from previously validated questionnaires [9].

Italian law didn't request the approval of Ethical Committee at the time of PI1/PI2. The protocol was approved by an Internal Revision Board within the Preventive Medicine Targeted Project of the Italian CNR. PI3 study protocol, patient information sheet and consent form were approved by the ethics committee of the Pisa University Hospital (Azienda Ospedaliero-Universitaria Pisana, Prot. no. 23887, April 16, 2008).

Outcome computation

Multimorbidity (multiple medical conditions within a single subject) of asthma, allergic rhinitis (AR) and COPD symptoms/diagnosis was assessed in the longitudinal sample participating in PI1, PI2 and PI3 survey (n=849) (Figure 1).

Longitudinal changes were assessed only in subjects participating in both PI2 and PI3 for sample size reasons (n=1107). In particular, changes in the number of medical conditions within a single subject were computed (mutually exclusive categories): "*Never/reduced number of conditions*", never asthma or AR or COPD or reduction in the number of medical conditions (i.e. from 1 to 0 condition; from 2 to 1 condition; from 3 to 2/1 conditions); "*Persistent number of conditions*"; "*Increased number of conditions from 0 to 1 or more*" (i.e. from 0 to 1/2/3 conditions); "*Increased number of conditions from 1/2 to more*" (i.e. from 1 to 2/3 conditions; from 2 to 3 conditions).

Longitudinal risk factors computation

Longitudinal changes of the main risk factors for respiratory diseases were computed.

Smoking habits were coded into 5 groups: "Never" (non smoker subjects both in PI2 and PI3), "Persistent" (smoker subjects both in PI2 and PI3), "Incident" (subjects starting to smoke in the period between PI2 and PI3), "Remittent for < 18 yrs" (subjects stopping smoking in the period between PI2 and PI3), "Remittent for ≥ 18 yrs" (subjects who had stopped smoking before PI2).

Occupational exposure (exposure to dust/fume/gas at work) was codified into 4 groups: "Never" (unexposed subjects both in PI2 and PI3), "Persistent" (exposed subjects both in PI2 and PI3), "Incident" (subjects becoming exposed in the period between PI2 and PI3), "Remittent" (subjects quitting exposure between PI2 and PI3).

Vehicular traffic exposure (self-reported exposure to vehicular traffic near home) was codified into 4 groups: "Never" (unexposed subjects both in PI2 and PI3), "Persistent" (exposed subjects both in PI2 and PI3), "Incident" (subjects becoming exposed in the period between PI2 and PI3), "Remittent" (subjects quitting exposure between PI2 and PI3).

Statistical analyses

One-way analysis of variance was used for comparing continuous variables among different groups of patients, while categorical variables were evaluated by chi-square test.

A post-hoc analysis was run to assess the source of statistically significant result in case of contingency table larger than a 2 x 2, using adjusted standardized residual [11-12].

An multinomial logistic regression model, taking as dependent variable the longitudinal changes in multimorbidity and as explanatory variables group of age (≤ 44 yrs, > 44 yrs), sex (males, females), educational level (0-8 yrs, 8-13 yrs, > 13 yrs), body mass index – BMI - groups (obese, overweight, normal/under weight), respiratory diseases familiarity, allergic rhinitis familiarity, skin prick test (SPT) results and exposure to longitudinal risk factors (occupational exposure, smoking habits, vehicular traffic exposure), was run.

As regards SPT, the sensitization to 12 local allergens (pollens, house dust mites, animal dander, moulds) was assessed by SPT reactivity using a standardized protocol; the result was considered positive if it yielded a mean wheal diameter ≥ 3 mm than that determined by the negative control [14].

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), rel 17.0. A p-value ≤0.05 was considered statistically significant, a 0.05<p-value<0.10 was considered as borderline value.

Results

General characteristics

Table 1 shows the descriptive characteristics and risk factors exposure of the PI1&PI2&PI3 participants (n=849). 55.5% were females and about 39.0% lived in the urban area; subjects had a mean age of 38.4±15.8 in PI1, 44.2±15.8 in PI2 and 62.0±15.7 in PI3. A significant increase in the educational level was found among the surveys, with high educational level ranging from 2.9% in PI1 to 10.5% in PI3. An increase in percentages of obesity and overweight was found among the surveys (5.7% PI1 vs 28.0% PI3 for obesity and 29.1% PI1 vs 43.9% PI3 for overweight); the same results for occupational exposure (39.8% PI2 vs 45.8% PI3) and vehicular traffic exposure (35.4% PI2 vs 62.8% PI3). A decrease of current smoking habit emerged among the surveys (29.8% PI1 vs 18.0% PI3).

As regards the current respiratory symptoms/diseases prevalence, a significantly higher value of COPD symptoms/diagnosis was found in PI2 with respect to PI1: 15.5% vs 11.8% for usual cough and 6.2% vs 3.7% for COPD (Figure 1). A significantly higher value of asthma attacks and COPD symptoms/diagnosis was found in PI3 with respect to PI2: 8.0% vs 2.7% for asthma attacks, 23.2% vs 11.5% for usual phlegm, 21.3% vs 15.5% for usual cough and 9.7% vs 6.2% for COPD (Figure 1).

Comparing the prevalence of multimorbidity in the three surveys, a significant increase in the co-presence of "COPD&AR" and of "Asthma&AR" from PI1/PI2 to PI3 was found (from 0.8% PI1 and 2.2% PI2 to 12.7% PI3; from 0.5% PI1 and 0.8% PI2 to 1.6% PI3, respectively); the same results were found for the co-presence of all the three conditions "Asthma&AR&COPD" (from 0.0% PI1 and 0.5% PI2 to 4.7% PI3) (Figure 2).

As regards the longitudinal changes in multimorbidity, the following results were found: 48.4% "Never/reduced number of conditions", 11.7% "Persistent number of conditions", 29.4% "Increased number of conditions from 0 to 1 or more" and 10.5% "Increased number of conditions from 1/2 to more" (Figure 3).

"Never/reduced number of conditions" frequency was significantly higher in younger age (≤ 44 yrs) with respect to older age (> 44 yrs) (53.2% vs 43.5%) and in subjects with negative SPT with respect to those with positive results (50.5% vs 43.7%); "Increased number of conditions from 0 to 1 or more" in subjects living in urban area with respect to living in a suburban one (25.4% vs 32.2%); "Increased number of conditions from 1/2 to more" was significantly higher in older subjects with respect to younger (14.9% vs 6.1%), in subjects living in urban area (13.4% vs 8.5%) and with positive SPT (13.8% vs 8.7%) (Table 2).

As regards the relationship with longitudinal risk factors, a significant association was found only with occupational exposure: "Never/reduced number of conditions" frequency was significantly higher in never exposed with respect to the other longitudinal categories (56.2% vs 42.4 incident, 37.5% persistent and 55.2% remittent); "Persistent number of conditions" and "Increased number of conditions from 1/2 to more" were significantly higher in persistent exposure (16.4% vs 10.3% incident, 15.7% remittent and 7.9% never; 15.8% vs 11.8% incident, 8.2% remittent and 7.1% never, respectively); "Increased number of conditions from 0 to 1 or more" was significantly higher in incident exposure (35.5% vs 30.3 persistent, 20.9% remittent and 28.8% never) (Table 3).

The results of the multinomial logistic regression analysis were shown in table 4. A significantly higher risk of persistent multimorbidity was found in persistent smokers (OR 2.16, 95% CI 1.09-4.27) and in subjects with persistent and remittent occupational exposure (OR 2.69, 95% CI 1.55-4.68 and OR 1.91, 95% CI 1.00-3.64); a significantly higher risk of multimorbidity increase (from 0 to 1 or more conditions) was found in subjects with incident and persistent occupational exposure (OR 2.01, 95% CI 1.33-3.04 and OR 1.85, 95% CI 1.24-2.76) and with incident and persistent vehicular traffic exposure

(OR 1.62, 95% CI 1.10-2.37 and OR 1.55, 95% CI 1.01-2.38); a significantly higher risk of multimorbidity increase (from 1/2 to more conditions) was found in persistent smokers (OR 2.71, 95% CI 1.36-5.39), in subjects with incident and persistent occupational exposure (OR 2.31, 95% CI 1.18-4.50 and OR 2.93, 95% CI 1.62-5.28) and with incident vehicular traffic exposure (OR 2.00, 95% CI 1.09-3.69) (table 4).

Discussion

These results showed 11.7% of persistent number of medical conditions within a single subject, 29.4% of increased number of conditions from 0 to 1 or more and 10.5% of increased number of conditions from 1/2 to more in a general population sample assessed during a 18-yr follow-up.

Worsening of health status due to multimorbidity was higher in persistent smokers, in subjects with incident and persistent occupational and vehicular traffic exposure.

Multimorbidity temporal changes

Taking into account the prevalence of multimorbidity found in the 3 Pisa surveys, an increase from 4-6% in PI1/PI2 to 22% in PI3 was shown. These data are within the prevalence range found in general population (13%-72%) [1]. Moreover, a Portuguese study performed on a general population sample in the same period of PI3 showed similar results (24% of multimorbidity prevalence) [15]; a higher value was found in a German general population (40%) [16].

In particular, data showed an elevated increase of co-presence of COPD & AR (from 0.8% in PI1 to 12.7% in PI3) and co-presence of all the 3 conditions (Asthma & COPD & AR) (from 0.0% in PI1 to 4.7% in PI3). As regards the close link between COPD and AR found in PI3, other studies showed that COPD patients had high percentages of SPT positivity and symptoms of allergic rhinitis [17-18]. A review presented the evidence that COPD is associated with significant sinonasal symptoms. Upper airway symptoms in COPD cause impairment to quality of life. The severity of upper airway involvement relates to that present in the lower airway, suggesting that the nose may be used to model the lung in COPD [18].

Most of the evidence on multimorbidity came from cross-sectional studies and longitudinal studies are limited [3]; our data added new information about the multimorbidity in a longitudinal general population sample, showing an increase of about 40% during a 18-yrs follow-up.

Multimorbidity risk factors

Baseline risk factors associated to the multimorbidity increase were age, AR family history and SPT positivity.

Multimorbidity increase is driven by the ageing population, as found in many other studies [3, 15-16].

As regards the role of allergy in multimorbidity, in the German MAS a higher prevalence of multimorbidity was found in participants study (subjects of 20-yrs of age) with allergic parents as compared to those without AR family history (18.5% vs 6.3%) [19] and in a French birth cohort a higher prevalence of multimorbidity was found in infants with allergens sensitization with respect to those without it (20% vs 6%) [20].

Analyzing the effect of longitudinal changes in risk factors exposure, important associations were found between worsening of health status due to multimorbidity and persistent smoking habits, incident and persistent occupational and vehicular traffic exposure.

Cross-sectional studies explored associations between multimorbidity prevalence and tobacco, but the paucity of longitudinal data determined that the direction of any relationship is not clear. Nonetheless, given the causal relationship between smoking and many chronic conditions, it is likely that smoking directly contributes to the development of multimorbidity [3]. One longitudinal Finland study has found that smoking is a predisposing factor for incident multimorbidity during 10-yrs follow-up with an OR of 2.7 in males and 2.6 in females [21].

As for smoking habits, environmental and occupational exposures were linked to the increased risk of several chronic conditions. Thus, these exposures could be associated with multimorbidity. However, to date, there is an evident lack of studies that have specifically investigated an association between multimorbidity and environmental and occupational exposure [3].

Indeed, recent reviews and reports about multimorbidity highlighted that few data are available about modifiable factors that predict the risk of different types of multimorbidity [1]: it remains unknown whether there are biological, environmental or behavioural factors. The identification of any such factors, and the assessment of the likelihood of causality, requires data from prospective observational studies [1,3].

Limitations and strengths

A limitation of this study is the use of questionnaire for collecting data on respiratory symptoms/diseases, potentially affected by a reporting bias, as it relies upon individual memory; nevertheless, the standardized questionnaire is one of the main investigation tool in respiratory epidemiology [22].

In these analyses, multimorbidity was defined according to the number of respiratory/allergic diseases/symptoms within a single subject. Nowadays, there is no a standard definition and in other studies multimorbidity was defined as co-presence of different kind of diseases (i.e cardiovascular, respiratory and metabolic). The heterogeneity in the measurement and reporting of multimorbidity across these studies limited comparability of our results with those of other scientific researches. However, these data add new evidences to this emerging topic, in particular regarding modifiable risk factors associated with multimorbidity longitudinal changes.

A strength of our study is to have applied, over a 25-year follow-up, the same study design, sampling frame and study protocol. In all the surveys, questions were derived from validated international questionnaires, which already had passed the scrutiny of independent reviewers.

At last, the added value of our study is to have analyzed two general population samples with a large age range from childhood to the elderly.

Conclusion

The results obtained in the Pisa longitudinal survey added new information about modifiable risk factors and onset/increase of multimorbidity; in particular, worsening of health status due to multimorbidity was higher in persistent smokers, in subjects with incident and persistent occupational exposure and in those with incident and persistent vehicular traffic exposure. Such information could be useful to affected patients and health care providers for prevention and management strategies.

Conflict of interest

Conflicts of interest: none.

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References

1. The Lancet. Making more of multimorbidity: an emerging priority. www.thelancet.com 2018;391. Access October 2018.
2. Afshar S, Roderick PJ, Kowal P, Dimitrov BD, Hill AG. Multimorbidity and the inequalities of global ageing: a cross-sectional study of 28 countries using the World Health Surveys. BMC Public Health 2015;15:776-786.
3. The Academy of Medical Sciences. Multimorbidity: a priority for global health research. April 2018. https://acmedsci.ac.uk/policy/policy-projects/multimorbidity. Access October 2018.
4. Nicholson K, Makovski TT, Griffith LE, Raina P, Stranges S, van den Akker M. Multimorbidity and comorbidity revisited: Refining the concepts for international health research. Journal of Clinical Epidemiology 2019;105:142-146.
5. Rijken M, Struckmann V, Dyakova M, Melchiorre MG, Rissanen ST, Van Ginneken E. ICARE4EU: improving care for people with multiple chronic conditions in Europe. Eurohealth 2013;19:29–31.
6. Ryan A, Murphy C, Boland F, Galvin R, Smith SM. What Is the Impact of Physical Activity and Physical Function on the Development of Multimorbidity in Older Adults Over Time? A Population-Based Cohort Study. J Gerontol A Biol Sci Med Sci 2018;73:1538–1544.
7. Viegi G, Matteelli G, Angino A, Scognamiglio A, Baldacci S, Soriano JB, Carrozzi L. The Proportional Venn Diagram of Obstructive Lung Disease in the Italian General Population. Chest 2004;126:1093–1101.
8. Carrozzi L, Giuliano G, Viegi, G, Paoletti P, Di Pede F, Mammini U, Carmignani G, Saracci R, Giuntini C, Lebowitz MD. The Po river delta epidemiological study

of obstructive lung disease: sampling methods, environmental and population characteristics. Eur J Epidemiol 1990;6:191-200.

9. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, Sarno G, Cerrai S, Martini F, Fresta M, Silvi P, Di Pede F, Guerriero M, Viegi G. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Resp Med 2016;110:58-68.
10. Viegi G, Paoletti P, Carrozzi L, Vellutini M, Diviggiano E, Di Pede C, Pistelli G, Giuntini C, Lebowitz M.D. Prevalence rates of respiratory symptoms in Italian general population samples, exposed to different levels of air pollution. Environ Health Perspect 1991;94:95-99.
11. Agresti A. An Introduction to Categorical Data Analysis. Second Edition 2007. Wiley-Interscience. A John Wailey & Sons, Inc., Publication.
12. Sharpe D. Your Chi-Square Test is Statistically Significant: Now What?. Practical Assessment, Research & Evaluation 2015;20:1-10.
13. Baldacci S, Modena P, Carrozzi L, Pedreschi M, Vellutini M, Biavati P, Simoni M, Sapigni T, Viegi G, Paoletti P, Giuntini C. Skin prick test reactivity to common aeroallergens in relation to total IgE, respiratory symptoms, and smoking in a general population sample of northern Italy. Allergy 1996; 51:149–156.
14. Rzewuska M, de Azevedo-Marques JM, Coxon D, Zanetti ML, Zanetti ACG, Franco LJ, Santos JLF. Epidemiology of multimorbidity within the Brazilian adult general population: Evidence from the 2013 National Health Survey (PNS 2013). PLoS ONE 2017;12(2): e0171813.
15. Puth MT, Weckbecker K, Schmid M, Münster E. Prevalence of multimorbidity in Germany: impact of age and educational level in a cross-sectional study on 19,294 adults. BMC Public Health 2017;17:826.
16. Neves MC, Neves YC, Mendes CM, Bastos MN, Camelier AA, Queiroz CF, Mendoza BF, Lemos AC, D'Oliveira Junior A. Evaluation of atopy in patients with COPD. J Bras Pneumol 2013;39:296-305.
17. Hurst JR. Upper airway. 3: Sinonasal involvement in chronic obstructive pulmonary disease. Thorax 2010;65:85–90.
18. Gough H, Grabenhenrich L, Reich A, Eckers N, Nitsche O, Schramm D, Beschorner J, Hoffmann U, Schuster A, Bauer CP, Forster J, Zepp F, Lee YA, Bergmann RL, Bergmann KE, Wahn U, Lau S, Keil T; MAS study group.

Allergic multimorbidity of asthma, rhinitis and eczema over 20 years in the German birth cohort MAS. Pediatr Allergy Immunol 2015;26:431-437.

19. Gabet S, Just J, Couderc R, Bousquet J, Seta N, Momas I. Early polysensitization is associated with allergic multimorbidity in PARIS birth co hort infants. Pediatr Allergy Immunol 2016;27:831-837.
20. Wikström K, Lindström J, Harald K, Peltonen M, Laatikainen T. Clinical and lifestyle-related risk factors for incident multimorbidity: 10-year follow-up of Finnish population-based cohorts 1982-2012. Eur J Intern Med 2015;26:211-216.
21. Pistelli F, Maio S. Questionnaires and lung function. In: Annesi-Maesano I, Lundback B, Viegi G eds. Respiratory Epidemiology. Sheffield (UK): Eur Respir Monograph 2014;65:257-272.

Table 1. Descriptive characteristics of longitudinal sample PI1/P2/PI3 (n=849) (%)

	PI1	PI2	PI3	p-value PI2vsPI1	p-value PI3vsPI2
Gender:					
males	44.5	44.5	44.5	---	---
females	55.5	55.5	55.5		
Age (mean±SD) (yrs)	**38.4±15.8**	**44.2±15.8**	**62.0±15.7**	**0.002**	**0.000**
Age range (yrs)	5-74	11-78	28-96		
Age groups:					
≤ 44 yrs	**59.8**	**45.0**	**18.8**	**0.000**	**0.000**
> 44 yrs	**40.2**	**55.0**	**81.2**		
BMI (Kg/m^2) groups:				**0.000**	**0.000**
obese	**5.7**	**12.4**	**28.0**		
overweight	**29.1**	**40.3**	**43.9**		
under/normal weight	**65.2**	**47.3**	**28.0**		
Educational level:				**0.000**	**0.000**
0-8 yrs	**79.6**	**73.6**	**64.3**		
8-13 yrs	**17.4**	**22.6**	**25.2**		
> 13 yrs	**2.9**	**3.8**	**10.5**		
Area of residence:					
urban	39.7	39.2	39.2	1.00	1.00
suburban	60.3	60.8	60.8		
Smoking habits:				**0.000**	**0.000**
smokers	**29.8**	**25.7**	**18.0**		
ex-smokers	**20.0**	**29.0**	**35.5**		
non smokers	**50.2**	**45.3**	**46.5**		
Occupational exposure:				0.805	**0.012**
yes	40.4	**39.8**	**45.8**		
no	59.6	**60.2**	**54.2**		
Vehicular traffic exposure:	---	**35.4**	**62.8**	**---**	**0.000**
yes		**64.6**	**37.2**		
no					

SD: standard deviation; BMI: body mass index. In bold: statistically significant values.

Table 2. Longitudinal changes in multimorbidity by PI2 general characteristics (%)

	Never/ reduced (n=536)	Persistent number of conditions (n=129)	Increased from 0 to 1 or more (n=326)	Increased from 1/2 to more (n=116)	p-value
Sex:					*0.097*
males	*47.1*	*13.2*	*27.4*	*12.2*	
females	*49.5*	*10.3*	*31.2*	*9.0*	
Group of age:					**0.000**
≤ 44 yrs	**53.2**	**11.1**	**29.6**	**6.1**	
> 44 yrs	**43.5**	**12.2**	**29.3**	**14.9**	
BMI (Kg/m^2) groups:					0.310
obese	40.5	15.3	30.5	13.7	
overweight	47.6	11.4	29.5	11.4	
normal	50.9	11.0	29.1	9.0	
Educational level:					*0.067*
0-8 yrs	*46.2*	*11.0*	*31.2*	*11.6*	
8-13 yrs	*53.6*	*12.5*	*26.2*	*7.6*	
> 13 yrs	*55.8*	*17.3*	*19.2*	*7.7*	
Area of residence:					**0.014**
urban	**48.8**	**12.5**	**25.4**	**13.4**	
suburban	**48.2**	**11.1**	**32.2**	**8.5**	
Family history of respiratory diseases:					0.213
yes	48.1	12.0	27.6	12.4	
no	47.9	11.4	31.7	9.0	
Family history of allergic rhinitis:					0.447
yes	47.4	12.5	28.4	11.6	
no	49.4	10.8	30.4	9.4	
SPT:					**0.004**
positive	**43.7**	**15.4**	**27.0**	**13.8**	
negative	**50.5**	**10.3**	**30.5**	**8.7**	

BMI: body mass index; SPT: skin prick test.

In italic: borderline values; in bold: statistically significant values.

Table 3. Longitudinal changes in multimorbidity by longitudinal changes in risk factors exposure (%)

	Never/ reduced (n=536)	Persistent number of conditions (n=129)	Increased from 0 to 1 or more (n=326)	Increased from 1/2 to more (n=116)	p-value
Smoking habits:					*0.095*
incident smokers	*47.2*	*12.5*	*36.1*	*4.2*	
persistent smokers	*43.4*	*14.7*	*26.5*	*15.4*	
remittent for < 18 yrs	*50.6*	*12.9*	*25.3*	*11.2*	
remittent for ≥ 18 yrs	*45.0*	*13.8*	*29.4*	*11.7*	
never smokers	*51.5*	*8.7*	*31.0*	*8.9*	
Occupational exposure:					**0.000**
incident	**42.4**	**10.3**	**35.5**	**11.8**	
persistent	**37.5**	**16.4**	**30.3**	**15.8**	
remittent	**55.2**	**15.7**	**20.9**	**8.2**	
never	**56.2**	**7.9**	**28.8**	**7.1**	
Vehicular traffic exposure:					0.127
incident	46.5	10.6	31.2	11.7	
persistent	44.3	12.8	32.6	10.3	
remittent	48.3	15.8	23.3	12.5	
never	55.4	10.1	26.5	8.0	

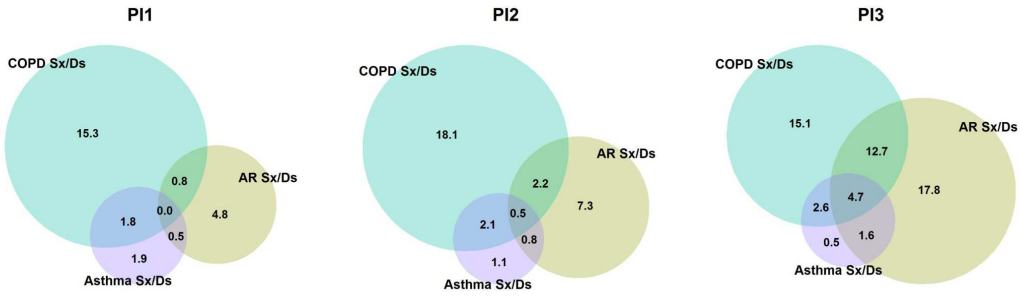
In italic: borderline values; in bold: statistically significant values.

Table 4. Longitudinal risk factors for multimorbidity. Results of the ordinal logistic regression analysis (OR and 95% CI)

	Persistent number of conditions	Increased number of conditions from 0 to 1 or more	Increased number of conditions from 1/2 to more
Smoking habits:			
incident smokers	1.65 (0.66-4.13)	1.35 (0.73-2.50)	0.77 (0.21-2.83)
persistent smokers	**2.16 (1.09-4.27)**	1.10 (0.66-1.84)	**2.71 (1.36-5.39)**
remittent for < 18 yrs	1.50 (0.78-2.89)	0.95 (0.59-1.52)	1.18 (0.58-2.40)
remittent for ≥ 18 yrs	*1.67 (0.95-2.93)*	1.08 (0.72-1.60)	1.00 (0.54-1.84)
(ref: never)	1.00	1.00	1.00
Occupational exposure:			
incident	*1.75 (0.92-3.33)*	**2.01 (1.33-3.04)**	**2.31 (1.18-4.50)**
persistent	**2.69 (1.55-4.68)**	**1.85 (1.24-2.76)**	**2.93 (1.62-5.28)**
remittent	**1.91 (1.00-3.64)**	0.87 (0.52-1.47)	0.85 (0.37-1.94)
(ref: never)	1.00	1.00	1.00
Vehicular traffic exposure:			
incident	1.26 (0.73-2.17)	**1.62 (1.10-2.37)**	**2.00 (1.09-3.69)**
persistent	1.34 (0.74-2.43)	**1.55 (1.01-2.38)**	1.37 (0.69-2.72)
remittent	1.59 (0.79-2.17)	0.90 (0.50-1.62)	*2.03 (0.91-4.51)*
(ref: never)	1.00	1.00	1.00

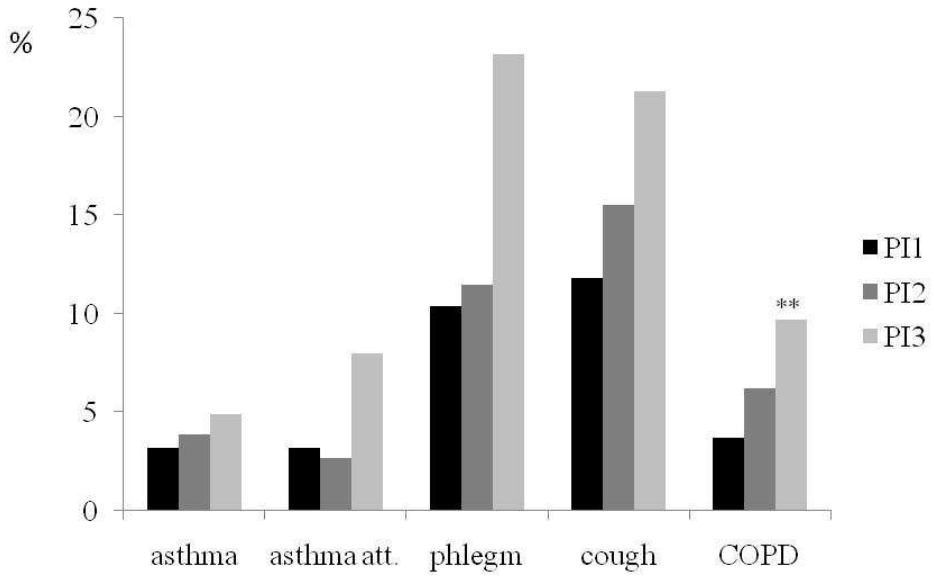
Reference category: never/reduced.
Adjusted for: age, sex, BMI, educational level, family history of respiratory diseases, family history of allergic rhinitis, positivity to skin prick test.
In bold: statistically significant values.

Figure 1. Venn diagram of multimorbidity in longitudinal sample PI1/PI2/PI3



PI1: Pisa 1 survey; PI2: Pisa 2 survey, PI3: Pisa 3 survey; Sx/Ds: symptoms or diagnosis; AR: allergic rhinitis.

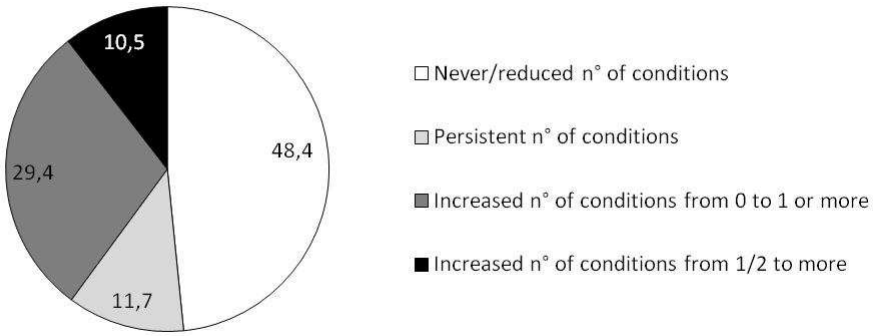
Figure 2. Symptoms/diseases prevalence in longitudinal sample PI1/PI2/PI3 (%)



#: p-value < 0.05 by chi-square test (comparison between PI1 and PI2).

***: p-value < 0.001; **: p-value < 0.01 by chi-square test (comparison between PI2 and PI3).

Figure 3. Longitudinal changes in multimorbidity from PI2 to PI3 survey (%)



XI.5 PAPER V

Trajectories of respiratory disease phenotypes and associated risk factors in the 18-yr Pisa epidemiological study

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ABSTRACT

Background: Few data are available on the temporal pattern of respiratory disease phenotypes in general population.

Aim: assessing trajectories of respiratory disease phenotypes over time and associated risk factors.

Methods: a longitudinal general population sample from two studies (PISA2:1991-1993; PISA3:2009-2011; n=1107), completing a questionnaire on respiratory symptoms/diseases (RSD), risk factors exposure and performing spirometry and skin prick test.

Latent transition analysis (LTA) was performed to assess respiratory disease phenotypes at PI1 and PI2, labelled according to RSD occurrence. Possible longitudinal patterns were persistence, worsening and improvement of the phenotype. Statistical analyses were performed on the whole sample and stratified by sex.

Results: Four different RSD phenotypes (non atopic healthy, atopic AR, non atopic usual cough/phlegm, atopic asthma & AR) and longitudinal trajectories over 18 years (2% improving, 52.2% persistent health status, 22.9% persistent AR, 9% persistent cough/phlegm, 13.8% worsening health status) resulted. "Improving health status" phenotype was lower in subjects exposed to vehicular traffic (OR 0.33 incident, OR 0.13 persistent); "persistent AR" was higher in subjects with remittent occupational exposure (OR 1.60); "persistent cough/phlegm" was higher in smokers (OR 5.92 persistent, OR 2.27 remittent for < 18 yrs) and in subjects with occupational exposure (OR 2.63 incident, OR 3.51 persistent); "worsening health status" was higher in smokers (OR 2.16 incident, OR 1.95 persistent).

Moreover, a higher risk of "Persistent disease status" was found in smoker males (OR 1.91) and in females with occupational and vehicular traffic exposure (OR 1.56).

Conclusions: LTA allowed to identify four different RSD phenotypes and their longitudinal patterns over 18 years and related risk factors in a *real life* setting. Such results brings new perspectives in the analyses of population-based data with the use of a thorough methodological approach, integrating at the same time different disease domains (diagnoses, symptoms, objective measurements and diseases co-presence) and time repeated measurements.

INTRODUCTION

Chronic respiratory diseases are complex diseases characterized by a strong clinical heterogeneity and possible phenotypic variability over time [1]. An accurate assessment of phenotypes is needed for better diseases management and for better identification of phenotype-specific risk factors. Disentangling disease phenotypes is a current challenge [1-2].

Beside the "candidate" approach that identifies a priori phenotypes on the basis of one or few disease characteristics, unsupervised or data driven approaches have been proposed to unravel the heterogeneity of chronic diseases by means of a clustering approach integrating multiple disease features, possibly identifying and defining objective, novel or previously unrecognised phenotypes [1,3]. The application of data-driven approaches has yielded phenotypic classifications that are clinically meaningful and interpretable and that are relevant to prognosis [2].

To date, clustering analyses have been performed in a cross-sectional manner (Latent Class Analysis), by integrating several domains of the disease measured at one point in time, or longitudinally, by using a single disease characteristic assessed at several time points to define trajectories [1,3]. On the contrary, the application of data-driven approaches to define longitudinal phenotypes of chronic diseases remains largely unexplored. Latent transition analysis (LTA) permit to incorporate the longitudinal pattern of several disease manifestations into one statistical model to simultaneously define phenotypes and to examine transitions over time [1-3].

The purpose of our population-based study was to evaluate 18-yr trajectories of respiratory disease phenotypes and associated risk factors in a longitudinal general population sample using LTA.

MATERIALS AND METHODS

Sample

Detailed information on population characteristics and methods are available elsewhere [4-5].

Briefly, a multistage stratified family-cluster random sample of general population, living in Pisa, was investigated in three subsequent cross-sectional surveys: first survey (PI1) (1985–1988); second survey (PI2) (1991–1993); third survey (PI3) (2009-2011).

Since spirometry data were unavailable in PI1, only subjects participating in both PI2 and PI3 (n=1107) were taken into account herein: the mean follow-up was 18-yr.

In PI2, information on respiratory symptoms/diseases and risk factors were obtained through a standardized interviewer-administered questionnaire developed by the National Research Council [6]. A subsample performed instrumental measurements to assess respiratory failures and atopy: a) all ≤ 75 year subjects were invited to perform spirometry (forced vital capacity-FVC- manoeuvre) according to the American Thoracic Society (ATS) protocol [7], through a water-sealed spirometer (Baires, Biomedin) [8]; b) the sensitization to 12 local allergens (pollens, house dust mites, animal dander, moulds) was assessed in volunteers by skin-prick test reactivity using a standardized protocol [9].

As regards PI3, an interviewer-administered questionnaire on respiratory symptoms/diseases and risk factors was designed using questions from previously validated questionnaires [4,6]. Moreover, all subjects were invited to perform spirometry (FVC manoeuvre) according to the ATS/European Respiratory Society (ERS) protocol [10], through an hand-held ultrasonic spirometer (EasyOne Model 2001 Spirometer, NDD Medical Technologies); the participation rate was 55% (n=689).

At the time of PI2, Italian law didn't request an Ethical Committee approval; thus, the protocol was approved by an Internal Revision Board within the CNR Preventive Medicine Targeted Project. PI3 study protocol, patient information sheet, and consent form were approved by the Pisa University Hospital Ethics Committee (Prot. no. 23887, April 16, 2008).

Disease phenotypes characterization

LTA was used to characterize the respiratory disease phenotypes. It identifies unobservable (latent) subgroups (classes or statuses) of individuals within a population based on the values of multiple observed variables. Latent statuses (phenotypes) are mutually exclusive and exhaustive and they are not assumed stable over time.

LTA uses longitudinal data to estimate the probabilities of transitions from one latent class to another and enables to estimate class membership over time.

To choose the optimal LTA model, the Bayesian Information Criterion (BIC) was used. A smaller value represents a more optimal balance of model fit and parsimony; thus, a model with the minimum BIC might be selected [11].

Manifest variables, considered to characterize the phenotypes, were presence/absence of the following symptoms/diseases at PI2 and PI3: asthma, attacks of asthma, allergic rhinitis (AR), COPD, usual cough, usual phlegm, SPT positivity, lower

limit of normal airway obstruction (LLN AO). Estimate of four phenotypes for each time yielded the best BIC.

Based on RSD frequency, "cross-sectional phenotypes" were labelled as:"Non atopic, healthy", "Atopic, AR", "Non atopic, usual cough/phlegm", "Atopic, asthma & AR" in PI2; "Non atopic, healthy", "Atopic, AR", "Non atopic, usual cough/phlegm", "Atopic, asthma & usual cough/phlegm & AR" in PI3 (Figure 1, Figure 2). At each time, the subjects were assigned to the phenotype associated with the maximum posterior probabilities of latent class membership [11].

Longitudinal respiratory disease phenotypes

The following "longitudinal phenotypes" (longitudinal trajectories) were defined: "*Persistent healthy*" ("Non atopic, healthy" phenotype in both PI2 and PI3); "Persistent AR" ("Atopic, AR" in both PI2 and PI3); "*Persistent cough/phlegm*" ("Non atopic, usual cough/phlegm" in both PI2 and PI3); "*Improving health status*" (improvement of health status from PI2 to PI3: "Non atopic, usual cough/phlegm" changing in "Non atopic, healthy"; "Atopic, asthma & AR" changing in "Non atopic, healthy"); "*Worsening health status*" (worsening of health status from PI2 to PI3: "Non atopic, healthy" changing in "Non atopic, usual cough/phlegm"; "Atopic, AR" changing in "Atopic, asthma & usual cough/phlegm & AR"; "Atopic, asthma & AR" changing in "Atopic, asthma & usual cough/phlegm & AR") (Figure 3).

Longitudinal risk factors

Longitudinal changes of the main risk factors for respiratory diseases were computed.

Smoking habits were coded into 5 groups: "Never" (non smoker subjects both in PI2 and PI3), "Persistent" (smoker subjects both in PI2 and PI3), "Incident" (subjects starting to smoke in the period between PI2 and PI3), "Remittent for < 18 yrs" (subjects stopping smoking in the period between PI2 and PI3), "Remittent for ≥ 18 yrs" (subjects who had stopped smoking before PI2).

Occupational exposure (exposure to dust/fume/gas at work) was codified into 4 groups: "Never" (unexposed subjects both in PI2 and PI3), "Persistent" (exposed subjects both in PI2 and PI3), "Incident" (subjects becoming exposed in the period between PI2 and PI3), "Remittent" (subjects quitting exposure between PI2 and PI3).

Vehicular traffic exposure (self-reported exposure to vehicular traffic near home) was codified into 4 groups: "Never" (unexposed subjects both in PI2 and PI3), "Persistent"

(exposed subjects both in PI2 and PI3), “Incident” (subjects becoming exposed in the period between PI2 and PI3), “Remittent” (subjects quitting exposure between PI2 and PI3).

Statistical analyses

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS version 16.0). Comparisons among groups were performed by chi-square test for categorical variables and analysis of variance for continuous variables.

A post-hoc analysis was run to assess the source of statistically significant result in case of contingency table larger than a 2 x 2, using adjusted standardized residual [12-13].

LTA was run using R software (version 3.5.1).

Two different multinomial logistic regression analyses (referred to as a, b) were run, all having the “longitudinal phenotype” as dependent variable (reference category: "Persistent health status"). Explanatory variables were: a) group of age (≤ 44 yrs, > 44 yrs), sex (males, females), BMI groups (obese, overweight, normal/under weight), educational level (0-8 yrs, 8-13 yrs, > 13 yrs), family history of allergic rhinitis, family history of respiratory diseases and exposure to longitudinal risk factors (occupational exposure, smoking habits, vehicular traffic exposure); b) anthropometric parameters (age, sex, educational level), last 12 months use of health services (family physician and specialist visits for respiratory problems) and last 12 months daily activities limitations for respiratory problems.

The significance level was set at 0.05.

Analyses by sex

The same statistical analyses, above reported, were performed separately between males and females to assess possible different longitudinal patterns and associated risk factors between sex.

The same cross-sectional disease phenotypes were found in males and females with respect to the whole sample (figure e1a, figure e1b, figure e2a, figure e2a online supplement).

Longitudinal respiratory disease phenotypes showed a different pattern. The following “longitudinal phenotypes” (longitudinal trajectories) were defined: "*Persistent/improving health status*" ("Non atopic, healthy" phenotype in both PI2 and PI3 in males and females; "Non atopic, usual cough/phlegm" changing in "Non atopic, healthy" in males and in females; "Atopic, asthma & AR" changing in "Atopic, AR" in

males); "*Persistent disease status*" ("Atopic, AR" both in PI2 and PI3 in males and females; "Non atopic, usual cough/phlegm" both in PI2 and PI3 in males and in females); "*Worsening health status*" ("Non atopic, healthy" changing in "Non atopic, usual cough/phlegm" in both males and females; "Atopic, AR" changing in "Atopic, asthma & usual cough/phlegm & AR" in males and in females; "Atopic, asthma & AR" changing in "Atopic, asthma & usual cough/phlegm & AR" in males and in females; "Non atopic, healthy" changing in "Atopic, asthma & usual cough/phlegm & AR" in females; "Non atopic, usual cough/phlegm" changing in "Atopic, asthma & usual cough/phlegm & AR" in females) (Figure 4a, Figure 4b).

RESULTS

Whole sample

Descriptive characteristics of PI2 and PI3 participants are shown in Table 1: 54.2%were females, 32.2% had SPT positivity, 47.3% had family history of allergic disorders and 49.0% of respiratory diseases, 55.6% reported current second hand smoke exposure. Significantly higher percentages of obesity (26.7% vs 11.6%), OE (45.8% vs 39.6%) and VT (63.1% vs 35.5%) and significantly lower percentage of smokers (18.8% vs 24.7%) were found in PI3 with respect to PI2.

As regards the current respiratory symptoms/diseases prevalence, a significantly higher value was found in PI3 with respect to PI2: 7.9% vs 2.7% for asthma attacks, 36.7% vs 11.4% for AR, 22.0% vs 11.5% for usual phlegm, 20.1% vs 14.7% for usual cough, 8.7% vs 5.2% for COPD, 18.5% vs 10.7% for LLN AO (table 2).

According to the item-response probabilities, 4 cross-sectional phenotypes were found in PI2 and in PI3: "Non atopic, healthy" (58.2% and 54.2%), "Atopic, AR" (27.5% and 22.9%), "Non atopic, usual cough/phlegm" (10.9% and 15.0%) and "Atopic, asthma & usual cough/phlegm & AR" (labeled "Atopic, asthma & AR" in PI2) (3.4% and 7.9%) (figure 1, figure 2). Despite the use of the same labels to identify the cross-sectional phenotypes, there were some differences between PI2 and PI3: PI3 "Non atopic, healthy" showed a higher frequency of AR with respect to PI2 (25.0% vs 4.3%); PI3 "Atopic, AR" showed a higher frequency of AR with respect to PI2 (41.1% vs 22.0%); PI3 "Non atopic, usual cough/phlegm" showed a higher frequency of AR and AO with respect to PI2 (56.6% vs 12.4% for AR and 31.4% vs 10.5% for AO). As regards the PI3 "Atopic, asthma & usual cough/phlegm & AR", with respect to PI2 "Atopic, asthma & AR", an increase in the

frequency of bronchitic and allergic status (39.5% vs 54.0% for usual cough, 23.7% vs 59.8% for usual phlegm and 42.1% vs 66.7% for AR) and a decrease in the frequency of asthma status (84.2% vs 33.3% for asthma and 60.5% vs 35.6% for asthma attacks) emerged (figure 1, figure 2).

Overall, subjects didn't show a strong probability of changing phenotypes across time (about 85% of persistent status). The transition probabilities between phenotypes varied from 2.6% to 17.4%. "Atopic, AR" had the highest probabilities of worsening towards "Atopic, asthma & usual cough/phlegm & AR" (16.4%) and the "Non atopic, usual cough/phlegm" had the highest probabilities of improving towards "Non atopic, healthy" (17.4%) (figure 3).

The main longitudinal patterns of disease phenotypes were: "Improving health status" (2.0%), "Persistent healthy" (52.2%), "Persistent AR" (22.9%), "Persistent usual cough/phlegm" (9.0%), "Worsening health status" (13.8%).

Table 3 shows the relationship between PI2 general characteristics, PI2 risk factors exposure and longitudinal patterns of disease phenotypes.

The frequency of "Persistent healthy" was significantly higher in females with respect to males (56.5% and 47.1%); "Persistent AR" was significantly higher in younger age (≤ 44 yrs) with respect to older age (> 44 yrs) (27.1% vs 18.8%); "Persistent usual cough/phlegm" was significantly higher in males with respect to females (12.2% vs 6.3%), in older age with respect to younger age (14.8% vs 3.4%), in obese subjects with respect to overweight and normal weight (17.6% vs 9.8% and 6.5%); finally, "Worsening health status" was significantly more elevated in subjects with a family history of respiratory diseases with respect to those without it (16.8% vs 11.4%) (table 3).

Table 4 shows the relationship between longitudinal risk factors exposure and longitudinal patterns of disease phenotypes.

The frequency of "Improving health status" was significantly lower in subject with never occupational exposure with respect to the other longitudinal categories (1.5% vs 3.0% incident, 2.0% persistent and 2.2% remittent); "Persistent AR" was significantly lower in persistent smokers with respect to the other longitudinal categories (15.4% vs 22.2% incident, 26.4% remittent for < 18 yrs, 21.3% remittent for ≥ 18 yrs and 25.1% never); "Persistent usual cough/phlegm" was significantly higher in persistent smokers (17.6% vs 1.4% incident, 10.1% remittent for < 18 yrs, 12.4% remittent for ≥ 18 yrs and 5.0% never); finally, "Worsening health status" was significantly higher in incident

smokers (23.6% vs 18.4% persistent, 11.2% remittent for < 18 yrs, 13.5% remittent for ≥ 18 yrs and 12.1% never) (table 4).

The results of the 2 different multinomial logistic regression analyses, taking into account "Persistent health" as reference category, were shown in table 5 and table 6.

A significant lower risk of "Improving HS" was found in subjects living exposed to incident and persistent vehicular traffic (OR 0.33, 95% CI 0.12-0.94 and OR 0.13, 95% CI 0.03-0.62); a significant higher risk of "Persistent AR" was found in subjects with remittent occupational exposure (OR 1.60, 95% CI 1.00-2.56); a significantly higher risk of "Persistent usual cough/phlegm" was found in persistent smokers and remittent smokers for < 18 yrs with respect to never (OR 5.92, 95% CI 2.86-12.26 and OR 2.27, 95% CI 1.07-4.79) and in subjects with incident and persistent occupational exposure with respect to never (OR 2.63, 95% CI 1.27-5.45 and OR 3.51, 95% CI 1.83-6.74, respectively); a significant higher risk of "Worsening health status" was found in incident and persistent smokers (OR 2.16, 95% CI 1.07-4.36 and OR 1.95, 95% CI 1.09-3.49) (table 5).

As regards the relationship between longitudinal disease phenotypes an physician visits (family physician and specialist visits for respiratory problems in the last 12 months) and daily activity limitations in the last 12 months, a significantly higher risk of having "Persistent usual cough/phlegm" was found in subjects attending their physician or specialist for respiratory troubles in the last 12 months (OR 5.40, 95% CI 2.87-10.17 and OR 2.08, 95% CI 1.00-4.30, respectively); the same results were found for "Worsening health status" (OR 4.78, 95% CI 2.79-8.20 and OR 4.11, 95% CI 2.32-7.28, respectively) (table 6).

Analyses by sex

The same cross-sectional disease phenotypes were found with respect to the whole sample, but some differences emerged between sex: in both PI2 and PI3 surveys, a significantly higher frequency of "Non atopic, healthy" was found in females with respect to males (62.8% vs 50.7% in PI2 and 58.7% vs 49.3% in PI3); on the contrary, a significantly higher frequency of "Non atopic, usual cough/phlegm" and "Atopic, asthma & usual cough/phlegm & AR" was found in males with respect to females (17.0% vs 8.2% and 6.3% vs 2.5%, respectively, in PI2; 18.3% vs 10.3% and 8.9% vs 4.7%, respectively, in PI3) (figure e1a, figure e1b, figure e2a, figure e2a online supplement).

As in the whole sample, males and females didn't show a strong probability of changing phenotypes across time, with a higher stability in females with respect to males (90% vs 85% of persistent status) (figure 4a, figure 4b).

The main longitudinal patterns of the disease phenotypes were: "Persistent/improving health status" (49.7%), "Persistent disease status" (35.9%), "Worsening health status" (14.4%) *in males*; "Persistent/improving health status" (58.7%), "Persistent disease status" (31.3%), "Worsening health status" (10.0%) *in females*. Males showed a significantly higher frequency of "Persistent disease status" and "Worsening health status" with respect to females.

Results of descriptive and bivariate analyses performed separately in males and in females were reported in the online supplement (table e1, table e2, table e3, table e4).

As regards the results of the multinomial logistic regression analysis, *in males* a significantly higher risk of "Persistent disease status" was found in remittent smokers for < 18 yrs with respect to never (OR 1.90, 95% CI 1.05-3.47). A significantly higher risk of "Worsening health status" was found in incident smokers (OR 3.85, 95% CI 1.38-10.74) and a significant inverse association was found in subjects with remittent vehicular traffic exposure (OR 0.27, 95% CI 0.07-0.99) (Table 7). *In females*, a significantly higher risk of "Persistent disease status" was found in subjects with persistent occupational exposure (OR 1.80, 95% CI 1.05-3.11) and with persistent vehicular traffic exposure (OR 1.68, 95% CI 1.00-2.80). A significantly higher risk of "Worsening health status" was found in subjects with remittent vehicular traffic exposure (OR 3.99, 95% CI 1.43-11.16) (Table 7).

Results of the multinomial logistic regression analysis taking into account the relationship between longitudinal RSD phenotypes and use of health services and daily activity limitation were reported in the online supplement (table e5).

DISCUSSION

Whole sample

Respiratory phenotypes temporal changes

The use of LTA permitted to identify 4 main baseline (PI2) phenotypes of respiratory diseases in this general population sample: “Non atopic, healthy”, “Atopic, AR”, “Non atopic, usual cough/phlegm”, “Atopic, asthma & AR”. The same phenotypes were found in PI3. Despite the use of the same labels to identify the cross-sectional phenotypes, there were some differences between PI2 and PI3: a higher frequency of AR

in PI3 "Non atopic, healthy", "Atopic, AR" and "Non atopic, usual cough/phlegm" with respect to PI2 phenotypes. Moreover, PI3 "Atopic, asthma & usual cough/phlegm & AR", with respect to PI2 "Atopic, asthma & AR", showed an increase in the frequency of usual cough/phlegm and AR and a decrease in the frequency of asthma symptoms/diagnosis. These results confirm the increasing prevalence of respiratory symptoms/diseases found in recent scientific papers [4, 14-15].

Moreover, the frequency values of cross-sectional phenotypes were comparable to the respiratory symptoms/diseases prevalence values found in a previous paper about the Pisa surveys [4]. In particular, "Non atopic, usual cough/phlegm" phenotype (10.9% PI2 and 15.0% PI3) had comparable results with the prevalence of cough/phlegm found in PI2 and in PI3 (14.4% and 16.5% for usual cough; 12.0% and 19.5% for usual phlegm); "Atopic, AR" phenotype had comparable results (27.5% PI2 and 22.9% PI3) with the prevalence of AR found in PI2 and in PI3 (20.2% in PI2 and 37.4% in PI3), even if it was not found the same increasing prevalence as in previous paper [4].

"Atopic, asthma & AR" phenotype showed a prevalence of 3.4% in PI2. The prevalence of asthma & AR was not computed in previous paper about the Pisa surveys [4]; however, in an Italian young adult general population sample comparable results were found (5.2%) [16]. PI2 "Atopic, asthma & AR" phenotype changes in "Atopic, asthma & usual cough/phlegm & AR" at PI3; in particular, a reduction of asthma symptoms/diagnosis frequency and an increase of usual cough/phlegm resulted. This change can be suggestive of asthma evolution towards COPD, a condition described as ACOS [17].

Longitudinal phenotypes were defined as "Improving health status" (2.0%), "Persistent healthy" (52.2%), "Persistent usual cough/phlegm" (9.0%), "Persistent AR" (22.9%) and "Worsening health status" (13.8%). Prevalently, the "Improving health status" was characterized by subjects with asthma and COPD remission and "Worsening health status" by subjects developing usual cough/phlegm and asthma.

Risk factors

"Improving health status" was significantly lower in subjects living exposed to vehicular traffic (persistent and incident exposure). "Persistent cough/phlegm" was higher in smokers (persistent and remittent smokers for < 18 yrs) and in subjects with occupational exposure (persistent and incident exposure). "Worsening health status" was higher in smokers (persistent and incident smokers).

These results confirm the strong relationship between tobacco use, air pollutants and occupational exposure and lifetime increase of respiratory symptoms/diseases prevalence and incidence, as reported in WHO official statements [18-19]. 35% of COPD is attributable to smoking habit [20], 36% to indoor/outdoor air pollution, 12% to occupational exposure [19]; 44% of asthma is attributable to total occupational risks, indoor and ambient air pollutants [19].

No published research about the relationship between longitudinal exposure to risk factors and longitudinal pattern of RSD phenotypes was found by the authors in literature. Thus, these results add new information about this important topic.

Finally, the results of multinomial logistic regression analyses about the use of health services in the last 12 months highlighted that “Persistent cough/phlegm” and “Worsening health status” phenotypes, with respect to “Persistent AR” and “Improving health status”, were characterized by subjects with the worst health status needing of physician and specialist visits at PI3; these results were considered as a possible validation of the goodness of the computed longitudinal phenotypes.

Nowadays, LTA or cluster analyses were used to assess phenotypes focusing on single diseases (i.e COPD or asthma) [1-2,21]. These results put a new insight into the possibility of performing LTA to assess phenotypes using population-based data, integrating at the same time different disease domains (diagnoses, symptoms, objective measurements, multiple diseases) and time repeated measurements. Indeed, using LTA, 4 meaningful and plausible disease phenotypes and longitudinal trajectories were found in *real life* setting.

Analyses by sex

The same longitudinal phenotypes were found in males and females, but with a higher frequency of "Persistent/improving health status” in females with respect to males (58.7% vs 49.7%).

A higher risk of "Persistent disease status" was found in smoker males (OR 1.91) and in females exposed to fumes/dusts/gases at work and to vehicular traffic (ORs 1.56). These results suggest a different susceptibility of males and females to risk factors exposure.

Risk of respiratory diseases persistence was related to smoking habits only in males, probably due to the fact that in the Pisa sample 24% of males were smokers with respect to 16% of females, a result in line with the 2010 DOXA Italian data. However, in

the last decade this sex difference becomes less marked, especially in developed regions where smoking patterns are similar between men and women. This lifetime habits change could influence the relationship between smoking habits and risk of respiratory diseases in females; indeed, it has been suggested that female smokers are more likely to develop COPD than male smokers, and that for the same level of exposure to cigarette smoke women have a higher risk of developing more severe disease at younger ages than men [22].

Risk of respiratory diseases persistence was related to occupational exposure only in females, even if a higher percentage of occupational exposure was found in males (about 60% vs 30%); this could be due different occupational risk, according to the performed working tasks, between males and females: women complain more frequently about upper limb disorders and stress, skin disease and infectious diseases and about major threats to respiratory health tract coming from cleaning and sterilizing agents used in health care facilities, as well as from dust in the textile and clothing industry. Men more frequently have accidents and injuries at work compared to women and they are more likely to suffer from hearing impairment, due to exposure to noisy machines and equipment [23].

As regards the exposure to vehicular traffic, these results showed a significantly higher risk of persistent disease status only in females. Recent scientific evidences reported that women are more susceptible to inflammatory lung disease induced by air pollution and show worse adverse pulmonary health outcomes than men. A possible mechanism underlying these differences could be a sex difference in the expression of lung inflammatory mediators that affect sex-specific immune responses to environmental toxicants [24].

Limits and strengths

Use of questionnaires for collecting RSD data might be a limitation because it is potentially affected by a reporting bias, as it relies upon individual memory; nevertheless, the standardized questionnaire is one of the main investigation tools in respiratory epidemiology [25]. Moreover, in our study an objective respiratory outcome (lung function and SPT), not affected by such potential bias, was also applied.

It is to point out that in PI3 some differences in the used questionnaire exist, but only comparable or identical questions to PI2 ones were chosen.

Spirometry was performed using different instruments in PI2 and PI3; a correction factor was derived to overcome this limit and to permit the comparison between studies, as reported in our previous paper [4].

LTA was used to identify possible longitudinal disease phenotypes. LTA permits to simultaneously account for several domains of the diseases obtaining a more comprehensive characterization of respiratory outcomes. The identified respiratory disease phenotypes can only account for the disease features represented by the variables included in the model; however, the main features of asthma, COPD and AR were taken into account.

A strength of our population-based study is to have applied, over an 18-yr follow-up, the same study design, sampling frame and study protocol in repeated cross-sectional surveys on general population samples living in the same area. Moreover, a wide general population sample spanning from childhood to the elderly was analyzed, with a vast amount of individual qualitative and quantitative data.

LTA allowed to simultaneously accounts for several domains of the diseases repeatedly measured over time. This approach applied to a longitudinal dataset is exploratory and hypothesis generating. However, the phenotypes observed were clinically relevant.

LTA may allow to overcome problems encountered in using incidence to analyze risk factors in follow-up studies. Indeed, as opposed to the analysis of incidence that assumes that chronic diseases are true dichotomous, which is unlikely to be true, LTA provides a novel modelling approach for longitudinal studies that does not make such an assumption. If disease prevalence changes, there is a need to understand what dynamics contributed to the change and transition probabilities better explain the nature of such changes. LTA provides novel information on both stability and changes [3].

CONCLUSIONS

LTA allowed to identify four different phenotypes, their longitudinal patterns and associated risk factors over 18 years. "Improving health status" phenotype was lower in subjects exposed to vehicular traffic; "persistent AR" was higher in subjects with remittent occupational exposure; "persistent cough/phlegm" was higher in smokers and subjects exposed to occupational exposure; "worsening health status" was higher in smokers. Different susceptibility to risk factors exposure was found in males and females.

All these information should be considered for primary prevention strategies in order to reduce the burden of chronic diseases in the general population and for prevention and management strategies of respiratory diseases in health care setting.

Moreover, LTA allowed to identify four plausible different phenotypes based on respiratory symptoms/diseases, their longitudinal patterns over 18 years and related risk factors in a *real life* setting. Such results brings new perspectives in the analyses of population-based data with the use of a thorough methodological approach, integrating at the same time different disease domains (diagnoses, symptoms, objective measurements and diseases co-presence) and time repeated measurements.

REFERENCES

1. Boudier A, Curjuric I, Basagan˜a X, Hazgui H, Anto JM, Bousquet J, Bridevaux PO, Dupuis-Lozeron E, Garcia-Aymerich J, Heinrich J, Janson C, Ku¨nzli N, Leynaert B, de Marco R, Rochat T, Schindler C, Varraso R, Pin I, Probst-Hensch N, Sunyer J, Kauffmann F, Siroux V . Ten-Year Follow-up of Cluster-based Asthma Phenotypes in Adults A Pooled Analysis of Three Cohorts. Am J Respir Crit Care Med 2013;188:550–560.
2. Garden FL, Simpson JM, Mellis CM, Marks GB for the CAPS Investigator. Change in the manifestations of asthma and asthma-related traits in childhood: a latent transition analysis. Eur Respir J 2016; 47: 499–509.
3. Soto-Ramírez N, Ziyab AH, Karmaus W, Zhang H, Kurukulaaratchy RJ, Ewart S, Arshad SH. Epidemiologic Methods of Assessing Asthma and Wheezing Episodes in Longitudinal Studies: Measures of Change and Stability. J Epidemiol 2013;23:399-410.
4. Maio S, Baldacci S, Carrozzi L, Pistelli F, Angino A, Simoni M, Sarno G, Cerrai S, Martini F, Fresta M, Silvi P, Di Pede F, Guerriero M, Viegi G. Respiratory symptoms/diseases prevalence is still increasing: a 25-yr population study. Resp Med 2016;110:58-68.
5. Viegi G, Pedreschi M, Baldacci S. Prevalence rates of respiratory symptoms and diseases in general population samples of North and Central Italy. Int J Tuberc Lung Dis. 1999;3:1034–42.
6. Viegi G, Paoletti P, Carrozzi L, Vellutini M, Diviggiano E, Di Pede C, Pistelli G, Giuntini C, Lebowitz M.D. Prevalence rates of respiratory symptoms in Italian

general population samples, exposed to different levels of air pollution. Environ Health Perspect 1991;94:95-99.

7. American Thoracic Society. Statement of the American Thoracic Society. Standardization of spirometry: 1987 update. Am Rev Respir Dis 1987;136:1285–1298.
8. Viegi G, Matteelli G, Angino A, Scognamiglio A, Baldacci S, Soriano JB, Carrozzi L. The Proportional Venn Diagram of Obstructive Lung Disease in the Italian General Population. Chest 2004;126:1093–1101.
9. Baldacci S, Modena P, Carrozzi L, Pedreschi M, Vellutini M, Biavati P, Simoni M, Sapigni T, Viegi G, Paoletti P, Giuntini C. Skin prick test reactivity to common aeroallergens in relation to total IgE, respiratory symptoms, and smoking in a general population sample of northern Italy. Allergy 1996; 51:149–156.
10. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J 2005;26:319–38.
11. Collins LM and Lanza ST. Latent Class and Latent Transition Analysis with applications in the social, behavioral, and health sciences. Wiley series in probability and statistics 2010. A John Wiley & sons, inc., publication; Editors: David 1 Balding, Noel A. C. Cressie, Garrett M. Fitzmaurice, Iain M. Johnstone, Geert Molenberghs, Llavid Scott, Adrian F M. Smith, Ruey S. Tsay, Sanford Weisberg. Pages 1-283.
12. Agresti A. An Introduction to Categorical Data Analysis. Second Edition 2007. Wiley-Interscience. A John Wailey & Sons, Inc., Publication.
13. Sharpe D. Your Chi-Square Test is Statistically Significant: Now What?. Practical Assessment, Research & Evaluation 2015;20:1-10.
14. De Marco R, Cappa V, Accordini S, Rava M, Antonicelli L, Bortolami O, Braggion M, Bugiani M, Casali L, Cazzoletti L, Cerveri I, Fois AG, Girardi P, Locatelli F, Marcon A, Marinoni A, Panico MG, Pirina P, Villani S, Zanolin ME, Verlato G; for the GEIRD study group. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. Eur Respir J 2012;39:883–892.

15. Waatevik M, Skorge TD, Omenaas E, Bakke PS, Gulsvik A, Johannessen A. Increased prevalence of chronic obstructive pulmonary disease in a general population. Respir Med 2013;107:1037–1045.
16. Bugiani M, Carosso A, Migliore E, Piccioni P, Corsico A, Olivieri M, Ferrari M, Pirina P, de Marco R; ISAYA (ECRHS Italy) Study Group. Allergic rhinitis and asthma comorbidity in a survey of young adults in Italy. Allergy 2005;60:165-170.
17. Alshabanat A, Zafari Z, Albanyan O, Dairi M, FitzGerald JM. Asthma and COPD Overlap Syndrome (ACOS): A Systematic Review and Meta Analysis. PLoS ONE 2015;10(9):e0136065.
18. World Health Organization. Report Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. 2007. http://www.who.int/gard/publications/GARD_Manual/en/(accessed May 2018).
19. Prüss-Ustün A, Wolf J, Corvalán C, R Bos and M Neira. Preventing disease through healthy environments. A global assessment of the burden of disease from environmental risks. WHO 2016. http://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/ (accessed May 2018).
20. Institute for Health Metrics and Evaluation (IHME). GBD Compare Data Visualization. Seattle, WA: IHME, University of Washington, 2017. Available from http://vizhub.healthdata.org/gbd-compare. (Accessed May 2018).
21. Pinto LM, Alghamdi M, Benedetti A, Zaihra T, Landry T, Bourbeau J. Derivation and validation of clinical phenotypes for COPD: a systematic review. Respir Res 2015;16:50.
22. Amaral AFS, Strachan DP, Burney PGJ, Jarvis DL. Female Smokers Are at Greater Risk of Airflow Obstruction Than Male Smokers. UK Biobank. Am J Respir Crit Care Med 2017;195:1226-1235.
23. Raulf M, Brüning T, Jensen-Jarolim E, van Kampen V. Gender-related aspects in occupational allergies – Secondary publication and update. World Allergy Organ J 2017;10: 44.
24. Cabello N, Mishra V, Sinha U, DiAngelo SL, Chroneos ZC, Ekpa NA, Cooper TK, Caruso CR, Silveyra P. Sex differences in the expression of lung inflammatory mediators in response to ozone. Am J Physiol Lung Cell Mol Physiol 2015;309:L1150-63.

25. Pistelli F, Maio S. Questionnaires and lung function. In: Annesi-Maesano I, Lundback B, Viegi G eds. Respiratory Epidemiology. Sheffield (UK): Eur Respir Monograph 2014;65:257-272.

Table 1. Descriptive characteristics of PI2-PI3 longitudinal subjects (n=1107; males=507; females=600)

	PI2 (1991-1993)	PI3 (2009-2011)	p-value
Sex (%):			
males	45.8	45.8	
females	54.2	54.2	
Age (mean±SD)	**42.3±16.3**	**60.1±16.2**	**0.000**
Age range	8-78	25-96	
BMI (n=688)* (mean±SD)	**25.4±4.2**	**27.5±4.6**	**0.000**
BMI (n=688) (%):			**0.000**
Underweight/normal weight	**48.0**	**29.8**	
Overweight	**40.4**	**43.5**	
Obese	**11.6**	**26.7**	
missing values (n=419)	*37.8*	*37.8*	
Smoking habits (%):			**0.000**
Smokers	**24.7**	**18.8**	
Ex smokers	**28.5**	**35.4**	
Non smokers	**46.8**	**45.8**	
Occupational exposure (%)	**39.6**	**45.8**	**0.003**
Vehicular traffic exposure (%)	**35.5**	**63.1**	**0.000**
Second hand smoke exposure	55.6	n.a.	
Positivity to SPT** (n=987) (%)	32.2	n.a.	
missing values (n=120)	*10.8*		
Family history (at least one parent) of respiratory diseases*** (%)	49.0	n.a.	
Family history (at least one parent) of allergic rhinitis (%)	47.3	n.a.	

SD: standard deviation; BMI: body mass index; SPT: skin prick test

* BMI was available only in 688 subjects in PI3; thus, the comparison between PI2 and PI3 values was performed on this subsample

**positivity (mean wheal diameter ≥ 3 mm than that of the negative control) to at least one allergen among house dust mites, pets, moulds, pollens

***asthma or chronic bronchitis or emphysema

n.a. not available

Information about second hand smoke exposure were not comparable in PI2 and PI3, thus only baseline value was taken into account.

In bold: statistically significant values.

Table 2. Current respiratory symptoms/diseases prevalence at PI2 and PI3 (%)

	PI2	PI3	p-value
Asthma	3.6	4.7	0.192
Attacks of asthma	2.7	7.9	<0.001
AR	11.4	36.7	<0.001
Usual phlegm	11.5	22.0	<0.001
Usual cough	14.7	20.1	<0.001
COPD	5.2	8.7	0.001
Atopy (n=987)	32.2	---	
n	889	475	
LLN AO	10.7	18.5	<0.001

AR: allergic rhinitis; COPD: chronic obstructive pulmonary disease; LLN AO: lower limit of normal airway obstruction.

Table 3. Longitudinal changes in disease phenotypes by PI2 general characteristics (%)

	Improving HS (n=22)	Persistent HS (n=578)	Persistent AR (n=254)	Persistent CP (n=100)	Worsening HS (n=153)	p-value
Sex:						**0.003**
males	**2.2**	**47.1**	**24.5**	**12.2**	**14.0**	
females	**1.8**	**56.5**	**21.7**	**6.3**	**13.7**	
Group of age:						**0.000**
≤ 44 yrs	**1.6**	**54.8**	**27.1**	**3.4**	**13.1**	
> 44 yrs	**2.4**	**49.5**	**18.8**	**14.8**	**14.6**	
BMI (Kg/m^2) groups:						**0.002**
obese	**0.8**	**45.8**	**19.1**	**17.6**	**16.8**	
overweight	**3.1**	**50.5**	**22.4**	**9.8**	**14.3**	
normal	**1.4**	**55.0**	**24.3**	**6.5**	**12.8**	
Educational level:						0.172
0-8 yrs	2.3	50.9	22.1	10.2	14.5	
8-13 yrs	1.1	54.4	26.2	5.3	12.9	
> 13 yrs	1.9	61.5	19.2	9.6	7.7	
Area of residence:						0.417
urban	1.6	49.7	23.6	10.5	14.7	
suburban	2.3	54.0	22.5	8.1	13.2	
Family history of respiratory diseases:						**0.005**
yes	**2.5**	**51.9**	**18.9**	**9.8**	**16.8**	
no	**1.6**	**52.1**	**26.6**	**8.3**	**11.4**	
Family history of allergic rhinitis:						*0.087*
yes	*3.9*	*41.2*	*23.5*	*5.9*	*25.5*	
no	*1.9*	*52.7*	*22.9*	*9.2*	*13.3*	

in italic: borderline values; in bold: statistically significant values.

BMI: body mass index; HS: health status; AR: allergic rhinitis; CP: usual cough/phlegm.

Table 4. Longitudinal changes in disease phenotypes by longitudinal changes in risk factors exposure (%)

	Improving HS (n=22)	Persistent HS (n=578)	Persistent AR (n=254)	Persistent CP (n=100)	Worsening HS (n=153)	p-value
Smoking habits:						**0.000**
incident	**0.0**	**52.8**	**22.2**	**1.4**	**23.6**	
persistent	**3.7**	**44.9**	**15.4**	**17.6**	**18.4**	
remittent for < 18 yrs	**2.8**	**49.4**	**26.4**	**10.1**	**11.2**	
remittent for ≥ 18 yrs	**1.1**	**51.8**	**21.3**	**12.4**	**13.5**	
never	**2.1**	**55.8**	**25.1**	**5.0**	**12.1**	
Occupational exposure:						**0.000**
incident	**3.0**	**50.2**	**20.7**	**9.4**	**16.7**	
persistent	**2.0**	**44.1**	**22.0**	**16.1**	**15.8**	
remittent	**2.2**	**50.7**	**29.1**	**9.0**	**9.0**	
never	**1.5**	**58.8**	**22.7**	**4.3**	**12.7**	
Vehicular traffic exposure:						0.123
incident	1.4	53.5	20.9	8.9	15.3	
persistent	0.7	50.2	24.5	9.2	15.4	
remittent	1.7	53.3	20.0	10.0	15.0	
never	4.2	51.9	25.8	8.4	9.8	

in bold: statistically significant values.

HS: health status; AR: allergic rhinitis; CP: usual cough/phlegm.

Table 5. Longitudinal risk factors for longitudinal changes in disease phenotypes. Results of the multinomial logistic regression analysis (OR and 95% CI) (a)

	Improving HS	Persistent AR	Persistent CP	Worsening HS
Smoking habits:				
incident	---	0.77 (0.40-1.51)	0.57 (0.07-4.55)	**2.16 (1.07-4.36)**
persistent	2.73 (0.82-9.01)	0.68 (0.39-1.19)	**5.92 (2.86-12.3)**	**1.95 (1.09-3.49)**
remittent for < 18 yrs	1.60 (0.49-5.25)	1.07 (0.69-1.68)	**2.27 (1.07-4.79)**	0.96 (0.53-1.76)
remittent for ≥ 18 yrs	0.46 (0.12-1.85)	0.85 (0.57-1.26)	*1.79 (0.93-3.43)*	1.07 (0.65-1.76)
(ref: never)	1.00	1.00	1.00	1.00
Occupational exposure:				
incident	2.39 (0.75-7.65)	1.03 (0.66-1.60)	**2.63 (1.27-5.45)**	1.46 (0.89-2.40)
persistent	1.22 (0.36-4.22)	1.21 (0.80-1.82)	**3.51 (1.83-6.74)**	1.43 (0.88-2.33)
remittent	1.28 (0.30-5.47)	**1.60 (1.00-2.56)**	1.73 (0.77-3.87)	0.74 (0.37-1.48)
(ref: never)	1.00	1.00	1.00	1.00
Vehicular traffic exposure:				
incident	**0.33 (0.12-0.94)**	0.85 (0.58-1.25)	1.09 (0.60-1.98)	*1.61 (0.97-2.65)*
persistent	**0.13 (0.03-0.62)**	1.08 (0.71-1.63)	0.87 (0.45-1.69)	*1.59 (0.92-2.75)*
remittent	0.32 (0.07-1.59)	0.78 (0.45-1.36)	1.06 (0.47-2.40)	1.32 (0.67-2.61)
(ref: never)	1.00	1.00	1.00	1.00

Reference category: persistent health status.
Adjusted for: age, sex, educational level, BMI, family history of respiratory diseases, family history of allergic rhinitis at PI2.
in italic: borderline values; in bold: statistically significant values.
HS: health status; AR: allergic rhinitis; CP: usual cough/phlegm.

Table 6. PI3 last 12 months health services use and daily activities limitations in longitudinal disease phenotypes. Results of the multinomial logistic regression analysis (OR and 95% CI) (b)

	Improving HS	Persistent AR	Persistent CP	Worsening HS
Physician visits (ref: no)	---	0.90 (0.47-1.72) 1.00	**5.40 (2.87-10.17)** 1.00	**4.78 (2.79-8.20)** 1.00
Specialist visits (ref: no)	---	0.77 (0.36-1.64) 1.00	**2.08 (1.00-4.30)** 1.00	**4.11 (2.32-7.28)** 1.00
Daily activities limitations (ref: no)	---	1.54 (0.69-3.41) 1.00	0.62 (0.21-1.87) 1.00	0.98 (0.44-2.21) 1.00

Reference category: persistent health status.
Adjusted for: age, sex, educational level at PI3.
In bold: statistically significant values.
HS: health status; AR: allergic rhinitis; CP: usual cough/phlegm.

Table 7. Longitudinal risk factors for longitudinal changes in disease phenotypes by sex. Results of the multinomial logistic regression analysis (OR and 95% CI) (a)

	Males		*Females*	
	DS PERS	HS WORS	DS PERS	HS WORS
Smoking habits:				
incident	1.55 (0.67-3.60)	**3.85 (1.38-10.7)**	0.68 (0.27-1.75)	0.52 (0.11-2.46)
persistent	1.69 (0.86-3.33)	2.05 (0.77-5.48)	1.01 (0.55-1.86)	1.73 (0.76-3.94)
remittent for < 18 yrs	**1.90 (1.05-3.47)**	1.99 (0.82-4.89)	0.84 (0.47-1.53)	0.49 (0.16-1.49)
remittent for ≥ 18 yrs	1.35 (0.77-2.37)	1.87 (0.81-4.32)	0.87 (0.53-1.42)	0.91 (0.43-1.93)
(ref: never)	1.00	1.00	1.00	1.00
Occupational exposure:				
incident	0.89 (0.46-1.72)	1.30 (0.58-2.91)	*1.51 (0.93-2.43)*	1.30 (0.62-2.72)
persistent	1.24 (0.74-2.08)	0.98 (0.48-1.97)	**1.80 (1.05-3.11)**	1.61 (0.72-3.60)
remittent	1.04 (0.52-2.08)	0.63 (0.22-1.79)	*1.71 (0.98-3.01)*	1.18 (0.47-2.94)
(ref: never)	1.00	1.00	1.00	1.00
Vehicular traffic exposure:				
incident	0.79 (0.49-1.30)	1.12 (0.58-2.18)	1.17 (0.72-1.90)	*2.27 (0.97-5.32)*
persistent	0.77 (0.44-1.34)	0.94 (0.44-2.02)	**1.68 (1.00-2.80)**	1.95 (0.77-4.92)
remittent	0.58 (0.29-1.15)	**0.27 (0.07-0.99)**	1.71 (0.86-3.40)	**3.99 (1.43-11.2)**
(ref: never)	1.00	1.00	1.00	1.00

DS PERS: persistent disease status; HS WORS: worsening health status.
Reference category: persistent/improving health status.
Adjusted for: age, educational level, family history of respiratory diseases, family history of allergic diseases at PI2.
in italic: borderline values; in bold: statistically significant values.

Figure 1. PI2 phenotypes: symptom/disease frequency in longitudinal sample PI2/PI3

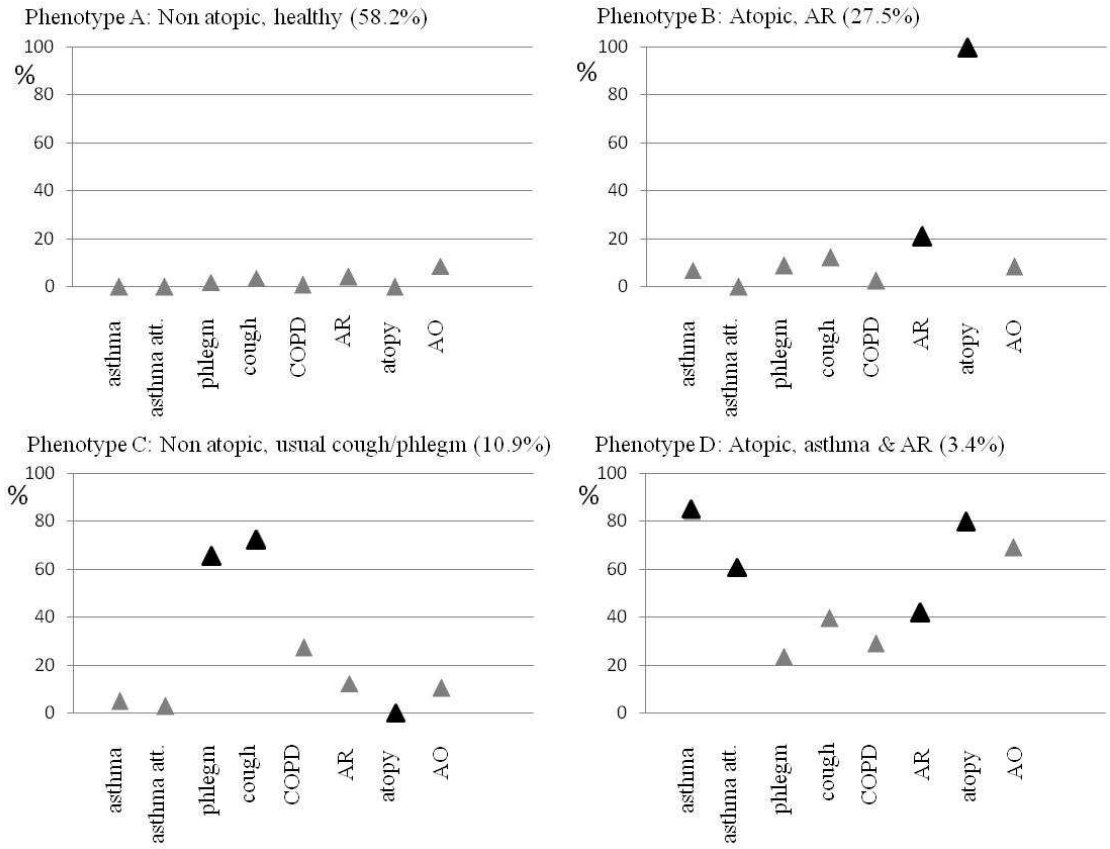


Figure 2. PI3 phenotypes: symptom/disease frequency in longitudinal sample PI2/PI3

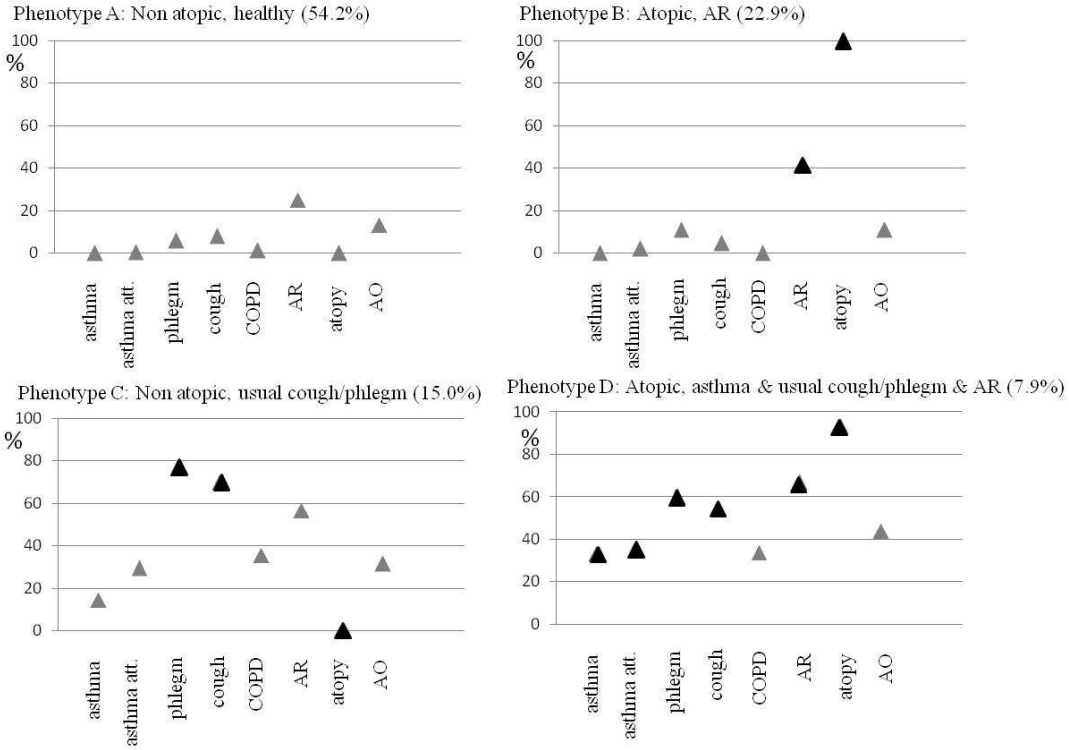


Figure 3. Phenotypes transition plot from PI2 to PI3 survey in longitudinal sample PI2/PI3

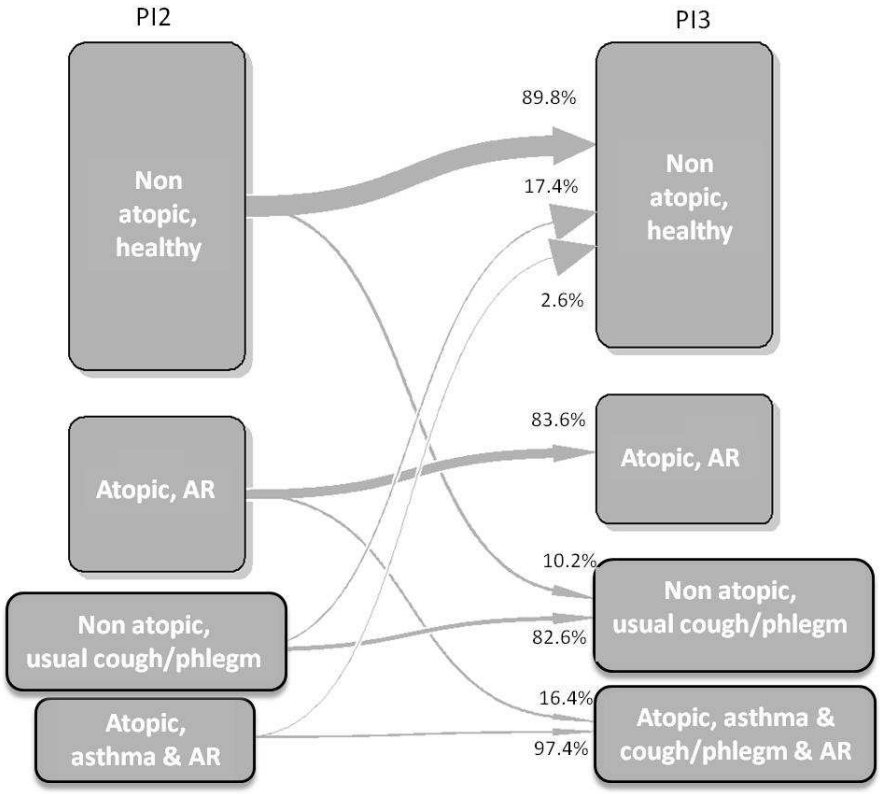


Figure 4a. Phenotypes transition plot *in males* from PI2 to PI3 survey in longitudinal sample PI2/PI3

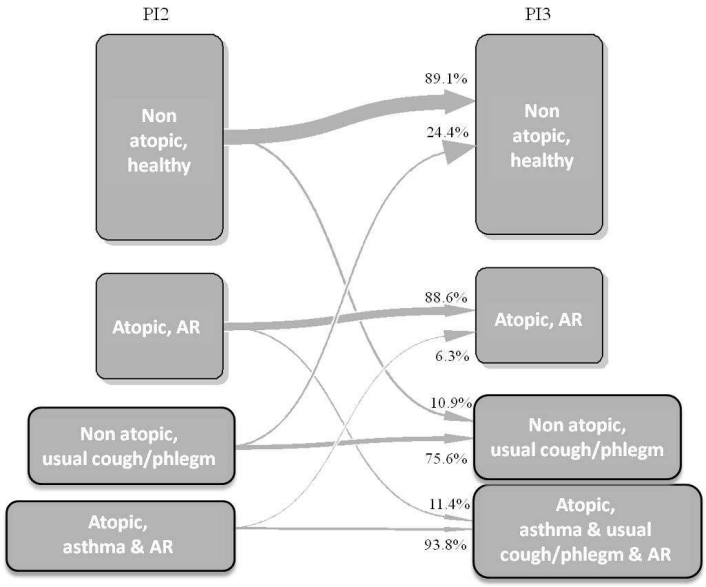
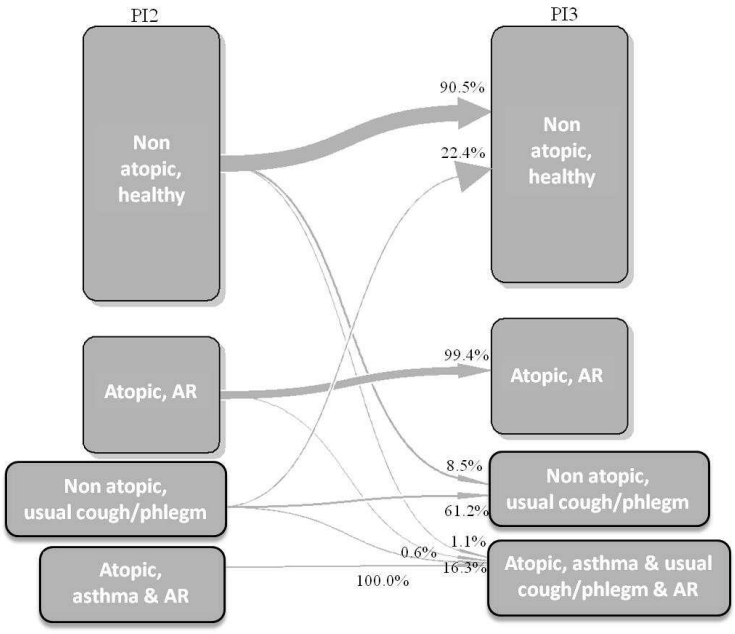


Figure 4b. Phenotypes transition plot *in females* from PI2 to PI3 survey in longitudinal sample PI2/PI3



Supplemental material

Figure e1a. Item-response probabilities *in males* at PI2 survey in longitudinal sample PI2/PI3

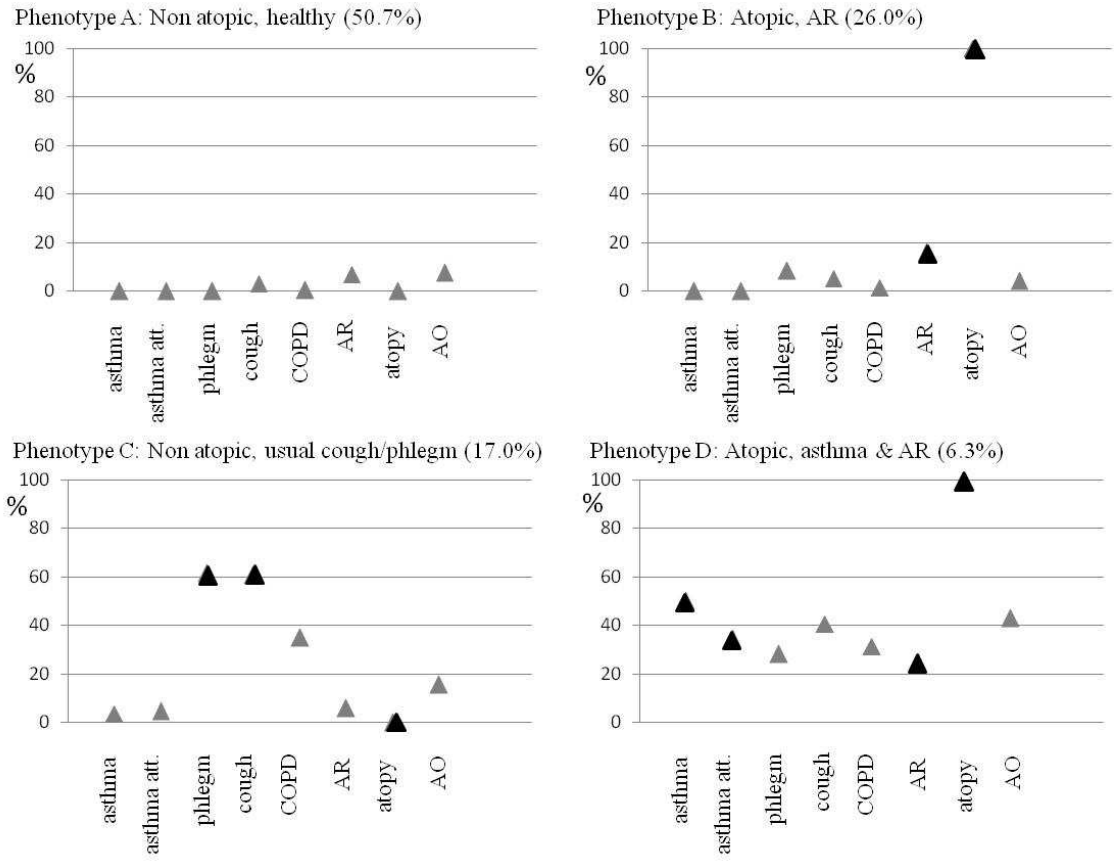


Figure e1b. Item-response probabilities *in females* at PI2 survey in longitudinal sample PI2/PI3

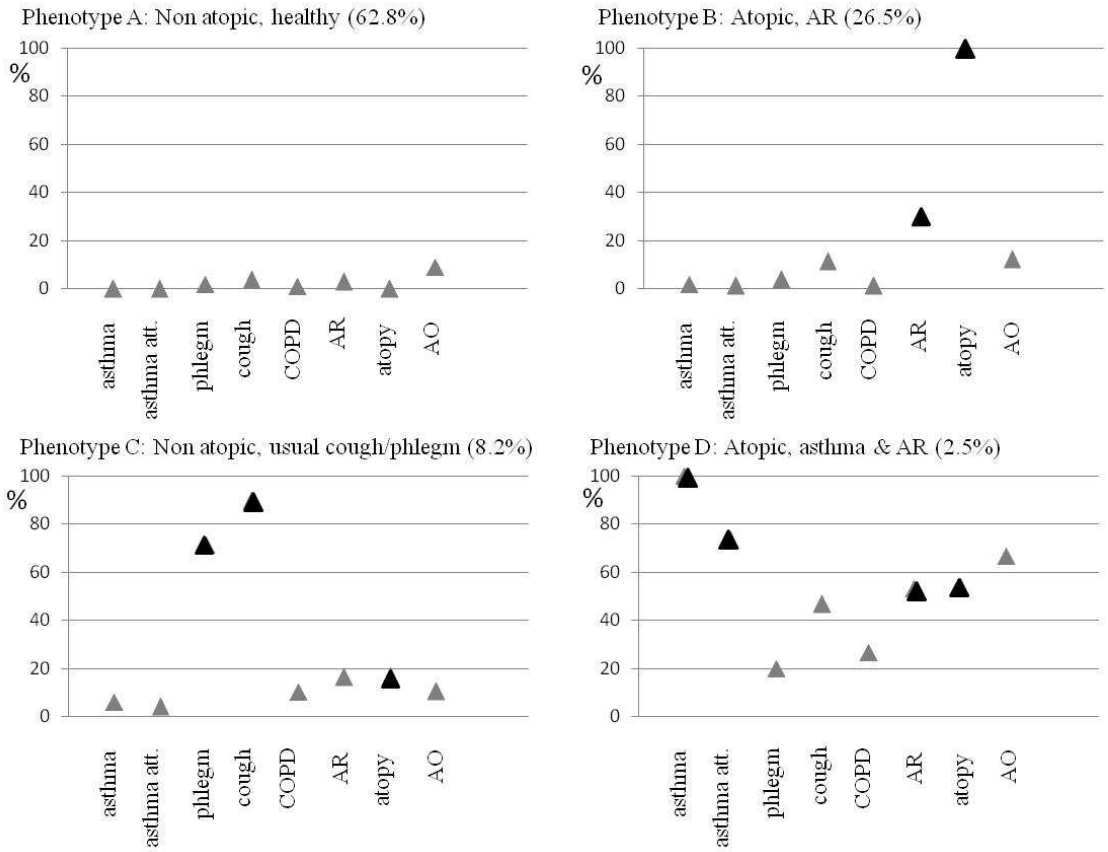


Figure e2a. Item-response probabilities *in males* at PI3 survey in longitudinal sample PI2/PI3

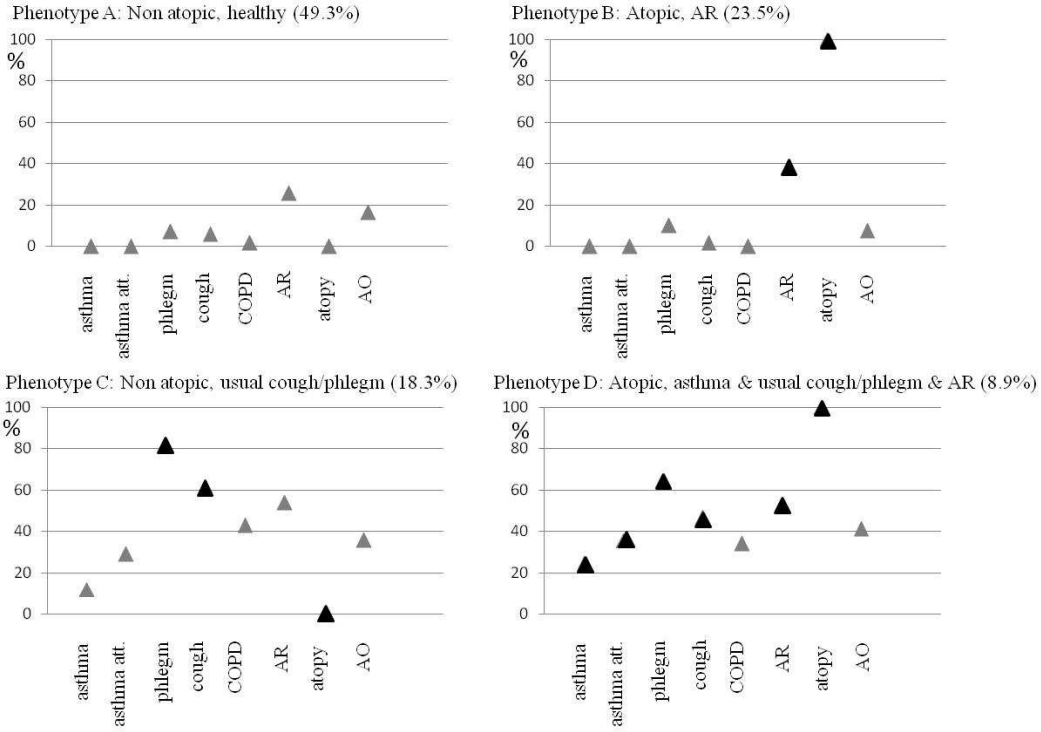


Figure e2b. Item-response probabilities *in females* at PI3 survey in longitudinal sample PI2/PI3

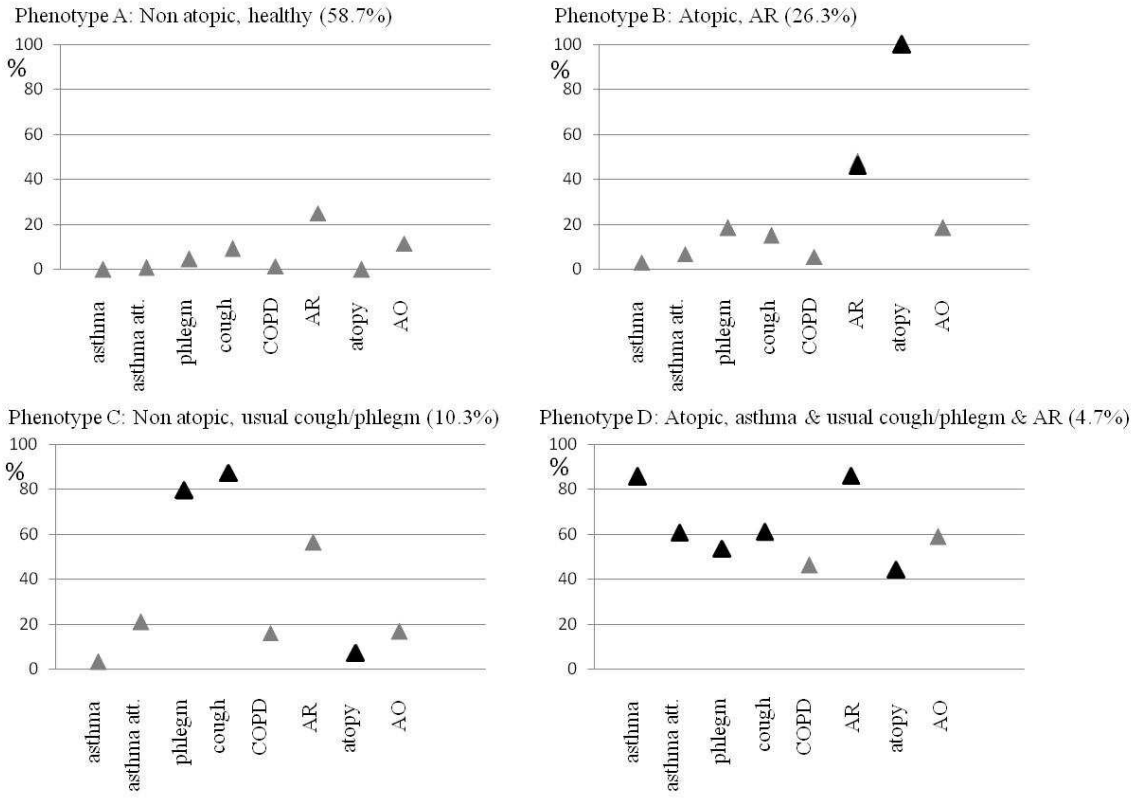


Table e1. Descriptive characteristics and risk factors exposure at PI2 and PI3 in longitudinal sample PI2/PI3 by sex (%)

	PI2			PI3		
	Males	*Females*	p-value	*Males*	*Females*	p-value
N	507	600		507	600	
Age (mean±SD)	**41.1±17.2**	**43.3±15.5**	**0.028**	**58.9±17.1**	**61.1±15.4**	**0.024**
BMI (Kg/m^2) groups:			**0.000**			**0.001**
obese	**9.9**	**13.5**		**25.2**	**28.0**	
overweight	**45.4**	**31.7**		**50.8**	**37.5**	
normal	**44.8**	**54.8**		**23.9**	**34.6**	
Educational level:			0.327			**0.000**
0-8 yrs	69.6	73.2		**54.4**	**65.7**	
8-13 yrs	25.8	22.0		**34.5**	**21.2**	
> 13 yrs	4.5	4.8		**11.0**	**13.2**	
Residence area:			0.840			0.840
urban	40.2	40.8		40.2	40.8	
suburban	59.8	59.2		59.8	59.2	
Smoking habits:			**0.000**			**0.000**
smokers	**28.6**	**21.3**		**21.9**	**16.2**	
ex smokers	**38.5**	**20.2**		**49.7**	**23.3**	
non smokers	**32.9**	**58.5**		**28.4**	**60.5**	
Occupational exposure:			**0.000**			**0.000**
yes	**55.6**	**26.0**		**59.2**	**34.5**	
no	**44.4**	**74.0**		**40.8**	**65.5**	
Vehicular traffic exposure:			0.391			**0.032**
yes	34.2	36.7		**59.8**	**66.0**	
no	65.8	63.3		**40.2**	**34.0**	
Family history of respiratory diseases:			0.106	n.a.	n.a.	
yes	44.6	49.5				
no	55.4	50.5				
Family history of allergic rhinitis:			0.334	n.a.	n.a.	
yes	3.9	5.2				
no	96.1	94.8				

SD: standard deviation; BMI: body mass index; AR: allergic rhinitis; n.a. not available.
in italic: borderline values; in bold: statistically significant values.

Table e2. Respiratory symptoms/diseases at PI2 and PI3 in longitudinal sample PI2/PI3 by sex (%)

	PI2			PI3		
	Males	*Females*	p-value	*Males*	*Females*	p-value
Asthma diagnosis	3.7	3.5	0.826	4.4	5.0	0.607
Asthma attacks	3.0	2.5	0.640	8.5	7.3	0.479
Allergic rhinitis	10.3	12.3	0.278	36.2	37.2	0.731
Usual phlegm	**15.0**	**8.5**	**0.001**	**26.6**	**18.2**	**0.001**
Usual cough	15.8	13.8	0.363	18.7	21.3	0.283
COPD	**8.5**	**2.5**	**0.000**	**11.7**	**6.2**	**0.001**
n	467	520				
Atopy	33.8	30.8	0.304	---	---	---
n	443	446		226	249	
LLN AO	10.2	11.2	0.611	19.9	17.3	0.459

in bold: statistically significant values.

COPD: chronic obstructive pulmonary disease; LLN AO: lower limit of normal airway obstruction.

Table e3. Longitudinal changes in disease phenotypes from PI2 to PI3 by PI2 general characteristics and risk factors exposure by sex (%)

	Males				*Females*			
	HS PERS/ IMPR (n=252)	DS PERS (n=182)	HS WORS (n=73)	p-value	HS PERS/ IMPR (n=352)	DS PERS (n=188)	HS WORS (n=60)	p-value
Age group:				0.474				0.405
≤ 44 yrs	52.2	34.3	13.4		61.4	29.7	9.0	
> 44 yrs	46.9	37.7	15.5		56.1	32.9	11.0	
BMI (Kg/m^2) groups:				0.465				0.138
obese	40.0	42.0	18.0		49.4	37.0	13.6	
over-weight	48.3	37.8	13.9		61.1	26.8	12.1	
normal/ under-weight	53.3	32.6	14.1		59.6	32.5	7.9	
Educational level:				0.194				0.600
0-8 yrs	49.0	36.0	15.0		56.9	32.3	10.7	
8-13 yrs	47.3	38.2	14.5		64.4	27.3	8.3	
> 13 yrs	73.9	21.7	4.3		58.6	34.5	6.9	
Area of residence:				0.421				0.272
urban	46.6	39.2	14.2		56.7	34.7	8.6	
suburban	51.8	33.7	14.5		60.0	29.0	11.0	
Family history of respiratory diseases:				**0.050**				0.276
yes	**48.0**	**33.2**	**18.8**		59.7	28.8	11.5	
no	**50.5**	**38.3**	**11.2**		57.5	33.9	8.6	
Family history of AR:				0.828				*0.066*
yes	50.0	40.0	10.0		*38.7*	*45.2*	*16.1*	
no	49.7	35.7	14.6		*59.8*	*30.6*	*9.7*	

BMI: body mass index; HS PERS/IMPR: persistent/improving health status; DS PERS: persistent disease status; HS WORS: worsening health status; AR: allergi rhinitis.

Table e4. Longitudinal changes in disease phenotypes from PI2 to PI3 by longitudinal changes in risk factors exposure by sex (%)

	Males				*Females*			
	HS PERS/ IMPR	DS PERS	HS WORS	p-value	HS PERS/ IMPR	DS PERS	HS WORS	p-value
Smoking habits:				0.186				0.629
incident	43.2	31.8	25.0		67.9	25.0	7.1	
persistent	46.3	38.8	14.9		52.2	31.9	15.9	
remittent for < 18 yrs	45.3	40.6	14.2		63.9	30.6	5.6	
remittent for ≥ 18 yrs	48.2	36.5	15.3		60.7	29.5	9.8	
never	60.0	30.8	9.2		57.4	32.6	10.0	
Occupational exposure (%):				0.497				0.122
incident	51.3	30.0	18.8		55.3	34.1	10.6	
persistent	45.9	40.0	14.1		48.8	38.1	13.1	
remittent	54.8	35.5	9.7		51.4	38.9	9.7	
never	52.4	33.1	14.5		64.2	26.8	9.0	
Vehicular traffic exposure (%):				0.216				**0.023**
incident	49.2	33.7	17.1		**61.1**	**28.0**	**10.9**	
persistent	49.1	35.3	15.5		**52.2**	**38.2**	**9.6**	
remittent	63.2	31.6	5.3		**47.6**	**34.9**	**17.5**	
never	45.9	40.4	13.7		**66.7**	**27.7**	**5.7**	

HS PERS/IMPR: persistent/improving health status; DS PERS: persistent disease status; HS WORS: worsening health status.
in italic: borderline values; in bold: statistically significant values.

Table e5. PI3 last 12 months health services use and daily activities limitations in longitudinal disease phenotypes by sex. Results of the multinomial logistic regression analysis (OR and 95% CI) (b)

	Males		*Females*	
	DS PERS	HS WORS	DS PERS	HS WORS
Physician visits (ref: no)	**2.41 (1.11-5.21)** 1.00	**3.87 (1.60-9.39)** 1.00	**2.10 (1.14-3.90)** 1.00	**5.06 (2.31-11.06)** 1.00
Specialist visits (ref: no)	1.34 (0.61-2.95) 1.00	**4.60 (2.01-10.5)** 1.00	1.23 (0.51-2.95) 1.00	**5.16 (2.09-12.74)** 1.00
Daily activities limitations (ref: no)	1.46 (0.50-4.26) 1.00	*0.15 (0.02-1.42)* 1.00	0.76 (0.28-2.04) 1.00	*2.66 (0.98-7.19)* 1.00
Hospital (ref: no)	0.47 (0.13-1.68) 1.00	0.41 (0.09-1.92) 1.00	0.81 (0.19-3.39) 1.00	1.54 (0.32-7.53) 1.00

DS PERS: persistent disease status; HS WORS: worsening health status.
Reference category: persistent/improving health status.
Adjusted for: age, educational level at PI3.
in italic: borderline values; in bold: statistically significant values.