Università degli Studi di Torino

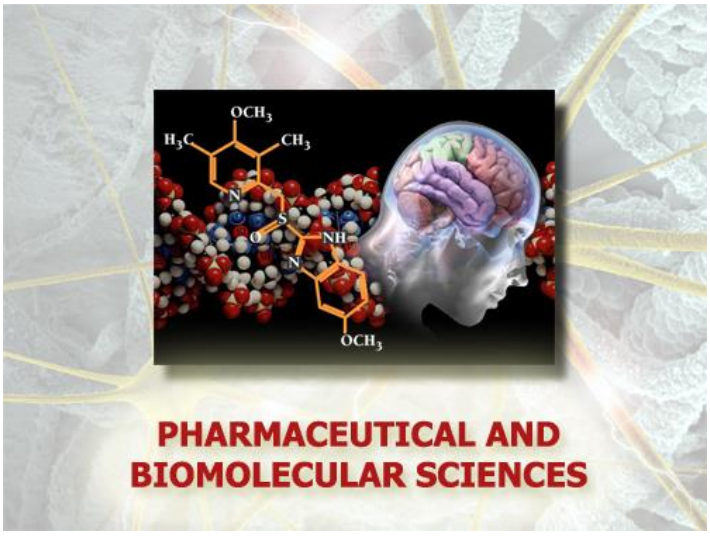
Scuola di Dottorato in

Scienze della Natura e Tecnologie Innovative

Dottorato in

Scienze Farmaceutiche e Biomolecolari

(XXX ciclo)



TITOLO

"Oxidative damage in workers exposed to wood dust. The mechanism of action of this carcinogen according to an innovative approach of the molecular epidemiology".

Candidata: Giulia Trucco **Tutor: Prof. Roberto Bono**

Università degli Studi di Torino

Dottorato in

Scienze Farmaceutiche e Biomolecolari

Tesi svolta presso il

Dipartimento di Scienze della Sanità Pubblica e Pediatriche

CICLO: XXX

"Oxidative damage in workers exposed to wood dust. The mechanism of action of this carcinogen according to an innovative approach of the molecular epidemiology".

TESI PRESENTATA DA: Giulia Trucco

TUTOR(S): Prof. Roberto Bono

COORDINATORE DEL DOTTORATO: Prof. Gianmario Martra

ANNI ACCADEMICI: 2014-2017

SETTORE SCIENTIFICO-DISCIPLINARE DI AFFERENZA: MED/42- Igiene

CONTENTS

TABLE AND FIGURE SUMMARY:

INTRODUCTION: GENERAL CONCEPTS

Physical, chemical and biological risks still account for a substantial proportion of work-related diseases in Europe. Frequently, these risks are associated with chronic illnesses such as musculoskeletal disorders, allergies, respiratory diseases and cancer. Causal multifactorial pathways characterize these work-related diseases but the information are actually inadequate. (Montano, 2014). Dusts represent one of the most common exposure and occupational hazards factors. The main sources of harmful dusts involved in work-related diseases are technological processes. The toxic properties of the dust emitted in the working environment are strongly connected with the properties of the toxic substances from which it is generated.

Wood dust represents one of the most important organic dusts to which humans are exposed. Wood is used and processed primarily for its traditional use of fuel and construction material (Baran, 2009).

1.OCCUPATIONAL EXPOSURE

1.1 Wood dust:

Wood is one of the world's most important renewable resources and grows in forests over the world. These last cover about one-third of the globe's total land area (IARC, 1995).

The Earth has an estimated 12.000 species of tree, each producing a characteristic type of wood. Spermatophytes are subdivided into two classes based on seed type: gymnosperms, which have exposed seeds, and angiosperms, with encapsulated seeds. The terms 'hardwood' and 'softwood' refer to the species of tree and not necessarily to the hardness of the wood. While hardwoods are generally denser than softwoods, the density varies considerably within each family and the hardness of the two groups overlaps somewhat. Gymnosperms collect all trees that yield softwood lumber (**Table 1)** (IARC,1995).

Genus and species	Common name
Softwood	
Abies	Fir
Larix	Larch
Picea	Spruce
Pinus	Pine
Pseudotsuga menziesii	Douglas fir
Sequoia sempervirens	Redwood
Hardwood	
Acer	Maple
Alnus	Alder
Betula	Birch
Carya	Hickory
Carpinus	Hornbeam, white beech
Castanea	Chestnut
Fagus	Beech
Populus	Poplar
Quercus	Oak tree
Junglas	Walnut tree

Table 5: Nomenclature of some hardwood and softwood (IARC,1995).

The essential chemical constituents of wood are cellulose (40-50%), polyoses (hemicelluloses) and lignin, which has a macromolecular structure. Although cellulose is the uniform structural element of all woods, the proportions and chemical composition of lignin and polyoses differ between softwood and hardwood. Wood generally also contains small amounts of polymeric compounds, such as starch, pectic substances and proteins. A heterogeneous mixture of organic and inorganic compounds is detectable in different amounts in different species. The organic matter called “extractives” can be extracted with nonpolar or polar solvents; instead, the inorganic part is reduced mainly to ash in the analysis of wood. Some of these compounds are protective, against injury or fungi attack, and toxic.

Organic extracts can have aliphatic, alicyclic or aromatic structures. Non-polar extractives comprise mainly terpenes, fatty acids, resin acids, waxes, alcohols, sterols, steryl esters and glycerides. The

polar extracts of wood generally restrain aromatic (phenolic) compounds, i.e. tannins, flavonoids, quinones and lignans. The common water-soluble extracts of wood are carbohydrates and their derivatives, alkaloids, proteins and inorganic material. Hardwoods show a higher amount of polar extractives than softwood. Extracts influence wood characteristics as specific weight, hygroscopicity, flammability, permeability, color and odor. "Wood dust" is the name given to the inhalable fraction of dust produced from solid wood, including bark during work activity. In this definition both freshly cut and dried wood dusts are included, but not pulp (cellulose) and paper dusts. Wood dust is frequently described using wood species or as hardwood or as softwood. Wood dust is a complex substance; its composition varies considerably according to the species of tree being processed and reflects the tree chemical compounds, as cellulose, lignin and low-relative-molecular-mass components (IARC, 1995). These last, in particular terpenes and tannins, are useful to distinguish between different species of wood (**Table 2**).

Woody Essence	**Chemical Classes**	**Chemical Biomarkers**
Conifers	Monoterpenes	α-pinene
Exotic wood	Sesquiterpenes	
Decidous trees (chestnut and oak)	Tannins	Ellagic acid
	Tannins	Gallic acid
	Tannins	Total tannins
Cedar	Poliphenols	Plicatic acid

Table 6: Low-relative-molecular-mass components (translate from ISPEL 2008)

Tannins are complex polyphenols present as chemical defenses in most plant species. Wood species differ in their tannin amount, both qualitatively and quantitatively; in particular, hardwoods contain greater amounts of tannins. Tannins can be separated into hydrolysable and condensed types. The hydrolysable are esters of gallic acid (GA) with its dimer (digallic acid), and of ellagic acid with monosaccharides, mainly glucose. Tannins can precipitate proteins and influence cell metabolism. However, although numerous authors are trying to deepen this topic, the cancerogenic mechanism underlying the risk due to exposure to wood dust is not clear (Carrieri, 2016). The amount of GA, a hydrolysable tannin, may be a useful tool to distinguish hardwood from softwood. In this regard, a

higher concentration of GA was found in oak trees than the other species. Its presence could be associated with the carcinogenic activity of the dust. GA is toxic to animals, due to its reactions with macromolecules, such as DNA or proteoglycans (Mamela, 2001).

In addition to composition, wood dusts differ from each other both in their quantity and in their granulometry, according to the operations performed on the raw material. Namely, shattering wood cells during sanding operations produces finer particle size than the ones (e.g.) produced by chipping in sawing and milling industries. In general, harder is the wood more tightly bound are the cells and the finer the dust will be. Similarly, the cells in dry wood are less plastic and more likely to be shattered, leading to more dust formation (Wilbourn, 1995). There is evidence that workplace exposure to wood dust may cause adverse health acute effects such as dermatitis, allergic and non-allergic respiratory effects, and chronic effects as cancer (Bislimovska, 2009; Scarselli, 2007). The International Agency for Research on Cancer (IARC) found sufficient evidence of carcinogenicity of wood dust in humans and consequently, wood dust has been classified as a human carcinogen (Group 1; IARC, 1995). Adenocarcinoma of the nasal cavities and paranasal sinuses is associated with exposure to hardwood dust. The concentration of wood dust and the modalities of exposure may represent important elements in the etiopathogenesis of that disease. Currently, exposure to wood dust has an impact on occupational health, and the occupational prevalence ranges from 10% to 15% (Alonso-Sardón et al., 2015; INAIL, 2016; INAIL, 2012). Odds ratio (OR) for sinonasal tumors caused by moderate levels of dust (≤ 1.0 mg/m^3) is around 3, and concentration ≥5 mg/m^3 cause more dramatic effects (OR ≥ 45) (Wultsch et al., 2015). Directive 2004/37/EC – "Carcinogens or mutagens at work"- aim to protect the workers against health and safety risks including wood dusts exposure. In **Table 3** are shown limits for wood dust exposure.

Agency	**Concentration (mg/m3)**	**Type of limit**
SCOEL	1-1.5 mg/m3	OEL for inhalable fraction
Italy (D.Lgs. 81/08)	5 mg/m3	OEL
NIOSH	1 mg/m3	REL
ACGIH	0.5 mg/m3	TLV-TWA red cedar
ACGIH	1 mg/m3	TLV-TWA other essences without red cedar

Table 7: Regulations and Guidelines Applicable to Wood Dust. (SCOEL: Scientific Committee on Occupational Exposure Limits; OEL: Occupation Exposure Level; NIOSH: National Institute for Occupational Safety and Health; REL: Recommended Exposure Limit; TLV: threshold limit value; TWA: time-weighted average).

Dusts from wooden boards and chemically treated wood are included in the group of “wood dusts”, although these dusts may contain also other chemicals, such as surface coatings (solvents, resins, pigments), glues (formaldehyde and phenols) and engine exhausts (Peteffi, 2015).

1.2 Formaldehyde:

Formaldehyde (FA) (CASRN: 50-00-0) is an important chemical, produced worldwide on a large scale by catalytic, vapor-phase oxidation of methanol. It is also a byproduct of certain natural (e.g., forest fires) and anthropogenic activities (e.g., smoking tobacco and residential wood burning) (IARC, 2012).

FA is one of the component of various types of resin. Phenolic, urea, and melamine resins have wide uses as adhesives and binders in the wood-production, pulp-and-paper and in the production of plastics and coatings. FA is used directly in aqueous solution (known as formalin) as a disinfectant and preservative in many applications. Because of the widespread use of formaldehyde-containing products, it is generally found in higher concentration into indoor environmental than outdoors.

The highest continuous exposures (2–5 ppm; 2.5–6.1 mg/m^3) were measured in the past during varnishing of furniture and wooden floors. Short-term exposures to high levels (3 ppm and higher; ≥ 3.7 mg/m^3) have been reported for embalmers, pathologists, and paper workers. Very wide range of exposure levels has been observed in the production of resins and plastic products. In the last two decades, the development of resins with lower amount of FA and the implementation of the ventilation resulted in lower exposure levels in many industrial settings (IARC,2006).

In 2006, the IARC reclassified FA from Group 2A (probably carcinogenic to humans) to Group 1 (carcinogenic to humans) (IARC,2006) (**Table 4**).

Agency	**Concentration (mg/ m^3)**	**Type of limit**
ACGIH	(0,37 mg/ m^3)	TLV-STEL
NIOSH	(0,02 mg/ m^3)	TLV-TWA
	(0,12 mg/ m^3)	TLV-Ceiling
OSHA	(0,93 mg/ m^3)	TLV-TWA
	(2,48 mg/ m^3)	TLV-STEL
EPA	(0,9 mg/ m^3)	TLV
WHO	(1 mg/ m^3)	IAQC: 30 min at lifelong exposure

Table 8: Regulations and Guidelines Applicable to Formaldehyde (Kim, 2011). (TLV: threshold limit value; TWA: time-weighted average; STEL: short-term exposure limit; Ceiling: the value that should never be exceeded during any length of time; EPA: Environmental Protection Agency.)

Combined exposure to both FA and wood dust is common in the furniture industry, where FA is released from particleboard. Formaldehyde-based glues have been used in the assembly of plywood for over 30 years. Since both compounds are hazardous to the nose, it was considered of interest to investigate whether exposure to a combination of FA and wood dust has an additive adverse effect. Wood dust could act as a carrier of absorbed FA to the lower airways (Holmstrom, 1988; Jafari, 2015).

FA exposure can be via inhalation of its gaseous form and either via ingestion of the substances who contain it but its intake can happen also via dermal absorption. FA is able to induce acute poisoning, cause irritation and other immunotoxic effects. Long-term exposure to elevated level of FA is also reported to be responsible for such serious and chronic health effects as inflammatory and hyperplastic changes of the nasal mucosa. Many transmission routes—inhalation, oral administration, topical application, and subcutaneous injections- has been tested in rodents to study the role of FA as carcinogenic agent. Rodents, exposed to high concentration of airborne FA, showed nasal tumors and it lead to think to FA's carcinogenic effect in occupational exposed workers (Kim, 2011).

There is sufficient evidence for a linkage between FA exposure and nasopharyngeal cancer, nasal and paranasal cancer, and leukemia (Kim, 2011).

2.OXIDATIVE STRESS

Oxidative stress (OS) is considered an important phase of various diseases. OS, characterized by an imbalance between oxidants and antioxidants in favor of oxidants, leads to disruption of redox signaling and physiological function. These redox signaling modifications, performed by reactive oxygen and nitrogen species (ROS and RNS), target protein activities within complex networks of kinases, phosphatases, ion channels, and apoptotic cascades and can cause changes in transcriptional activity (Lushchak,2014). ROS and RNS are known to damage all cellular biomolecules (lipids, sugars, proteins, and polynucleotides). Many defense systems are involved to prevent uncontrolled ROS increase; these systems include from one side nonenzymatic molecules (glutathione, vitamins A, C, and E, and antioxidants present in foods), from the other side enzymatic scavengers of ROS, with superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Marrocco, 2017).

2.1 Biomarkers of oxidative stress

Under physiological conditions, ROS and RNS generated by leukocytes have a role in the immune response to infection. In the presence of oxidative stress, ROS generated in vivo can cause oxidative damage to lipids, proteins and nucleic acids (Wu, 2004). Various markers of oxidative damage have been identified. In the past, some markers were designed and used for lipid peroxidation, such as malondialdehyde (MDA) and F2-isoprostane (Pisoschi, 2015).

ROS can induce lipid peroxidation of polyunsaturated fatty acids (PUFAs), which propagate via peroxyl radicals (ROO•) within the membrane, as well as in the low-density lipoproteins (LDL). In the context of metabolic syndrome and chronic inflammation, the oxidized LDL (oxLDL) activate leukocytes and/or platelets to produce ROS. The direct quantification of ROS is a valuable and promising biomarker that can reflect the disease process. However, given the short half-life of these species, their measurement in biological systems is a complex task (Au, 2007).

- *F_2-isoprostanes*: F_2-isoprostanes are synthesized during non-enzymatic oxidation of arachidonic acid by different types of free radicals, including ROS (Roberts, 2000; Milne, 2015). Based on the position where the oxygen molecule is added to arachidonic acid, four regioisomers are formed, giving each of the four F2-isoprostane series. Furthermore, each series comprises 16 stereoisomers. F_2-isoprostanes can be measured in human biological fluids as human blood and urine (Basu,2008). They have two important characteristics: 1) describe a lipid peroxidation not completely stopped by the antioxidant defenses; this leads to an exponential increase in the isoprostane concentrations; 2) isoprostane quantification represent used as a sensitive expression of systemic oxidative stress

status and inflammatory response (Breintenbach,2015). There are three main techniques used to assay F2-isoprostanes: gas chromatography with mass spectrometry detection (GC-MS), liquid chromatography with tandem mass spectrometry detection (LC-MS/MS), and enzyme-linked immunosorbent assay (ELISA). GC/MS and LC-MS/MS techniques show as many advantages; one is the great sensitivity of the method. The immunoenzymatic ELISA-based tests shows lower performance compared to the chromatography-based techniques due to cross-reactivity. On the other side, ELISA tests allow to measure faster many samples, preserving sensitivity and sensibility. In the epidemiological studies, the 15-F2t-isoprostane is the most used biomarker of oxidative stress. In the last two decades many epidemiological studies investigate, using ELISA technique, 15-F2t-isoprostane as a possible biomarker of oxidative stress in chronic diseases. A positive correlation was found between 15-F2t-isoprostane and asthma, BPCO, and cancer (Antus, 2016; Zinello, 2016). Recent studies shown also a correlation between 15-F2t-isoprostane with tobacco smoking and either exposure to FA (Bellisario, 2016; Romanazzi, 2013).

In addition to damage to lipids and proteins, ROS can interact with DNA and can lead to the modification of the constituent 2-deoxyribonucleosides to yield oxidation products.

- *8-oxo-7,8-dihydro-2-deoxyguanosine* (8-oxodG, also known as 8-OHdG)*:* 8-oxodG is one of the most commonly formed DNA lesions produced in response to oxidative stress and it is considered as a cellular marker for both oxidative stress and oxidative damage to DNA (Brenner, 2014). Guanine is the most easily oxidized nucleotide base, and its oxidation forms 8-oxodG. Measurement of 8-oxodG in DNA is a marker of oxidation induced damage and, possibly, cancer risk. The quantification of 8-oxodG measurement in cells is problematical due to post-sampling changes and artifact. Measurement of 8-oxodG in urine is more straightforward. Extracellular 8-oxodG is excreted in urine without further metabolism. This metabolite is stable in urine, and concentrations are not affected directly by diet or cell death. The origin of 8-oxodG in urine is not clear, but it is believed to be from sanitation of the nucleotide pool. This makes urine 8-oxodG a potentially specific and robust biomarker of “whole body” oxidative stress (Lee, 2009).

In addition to mutagenic role, 8-oxodG may have other negative effects on cell function, including promotion of microsatellite instability and acceleration of telomere shortening (Lam, 2012). The analytical methods applied to measuring urinary 8-oxodG can be divided into two main types: chromatographic and immunoassay. The former includes HPLC in conjunction with tandem mass spectrometric or electro-chemical detection. However, increasingly the strengths of mass spectrometry are being exploited; these include high sensitivity, specificity, and the ability to

measure numerous lesions in a single run. The main disadvantages of massspectrometric techniques are the high cost of equipment and the need for skilled staff for operation and maintenance. As urine is a complex matrix, a cleanup step is often performed to facilitate detection and maximize equipment lifetime. Solid-phase extraction (SPE) has been employed as a relatively fast and simple way to extract and clean up 8-oxodG from urine before massspectrometric detection (Brenner, 2014).

2.2 Sources of oxidative stress

Oxidative stress plays a virtual role in every human disease and environmental exposure can be an important source of contaminants. (Van't Erve, 2017). Oxidative stress is the expression of an imbalance occurring when endogenous or exogenous oxidants overtakes the level of antioxidant defenses indicating a risky condition. The main exogenous sources are the environmental pollution, in particular the atmospherically one, occupation exposure to several toxic chemicals, UV radiation, diet, and cigarette smoke. All these factors may be directly or indirectly responsible of the production of ROS. According to geographical area and socio-economic activities carried out, air pollution may present physical, chemical, mutagenic and toxicological properties, able to induce the production of ROS. The direct exposure to the air pollutants determines for the respiratory system a risky condition for several diseases.

Inhalation of air pollutants and toxic promote the production of ROS, that may result in increased airway inflammation and hyperactivity in response to environmental agents. Among air pollutants, FA is an important chemical widely used in many working activities including construction, wood processing, furniture, textiles, carpeting, hospitals and healthcare. FA represents a ubiquitous pollutant, breathable in every living and working environment (Nielsen, 2017). Furthermore, wood dust is another very important pollutant, present in several working contexts, who represents an important risk factor for several chronic diseases (Baran, 2009). Overall, exposure to FA and wood dust are associated to a wide range of adverse health effects, from mild to severe. Acute exposure can cause irritation (on eyes, nose, throat, and skin), nasal congestion, sore throats, headaches, coughs, conjunctivitis, fatigue, rashes, shortness of breath, nausea and nosebleeds. Both are endowed with genotoxic and oxidant activity and are also known as human carcinogen and as an inducer of chronic toxicity. Long-term exposures to wood dust, formaldehyde and cigarette smoke have all been thought to be etiological factors of paranasal sinuses and nasal cavity cancer. Interestingly, all these etiological factors have been proven capable of induce oxidative stress (Tan,

2106). Therefore, the study of the relationship between exposure to these chemicals, their biological effect and related diseases is crucial in several contexts, but rather complex.

3. OBJECTIVES

The traditional epidemiological techniques have always been the hallmark approach to demonstrate associations between exposure to hazardous substances and development of disease such as cancer. The endpoints of traditional epidemiology for such investigations are usually mortality and disease incidence. The new molecular approach, since several years, has flanked the classical epidemiological techniques with the most current analytical methods of molecular type to elucidate the biochemical or molecular basis of disease etiology.

The aim of the present research has been to investigate the role of occupational exposure to wood dust in the induction of oxidative stress and to contribute to the interpretation about the mechanism involved in diseases-wood dust correlated.

The following points were developed:

1. To evaluate wood dust exposure in different industrial scenario in Piedmont region.
2. To investigate oxidative stress because of occupational exposure of workers to wood dust.
3. To investigate the combined role of FA and wood dust in the induction of oxidative stress in the same population of workers.
4. To quantify the DNA damage due to FA and wood dust occupational exposure.

4.MATERIAL & METHOD

4.1EPIDEMIOLOGICAL SAMPLE:

The four industries who joined the proposal to participate in the study have been contacted thanks to the help of local Trade Associations. At first, manager and responsible for prevention and protection (RSPP) of the chosen Industries have been informed about the study, then workers have been called up for the information meeting. So far 245 volunteers (128 exposed) have joined and participated in the study (165 male, 80 female). All the industries are in Piedmont region; three of them located in province of Cuneo, one in province of Vercelli. In “Cuneo I” the subjects were 68, of which 22 non-exposed to wood dust, in “Cuneo II” 30, all exposed, and in “Cuneo III” 13. Finally, in the Vercelly industry, 39 volunteers were recruited. The sampled industry in Vercelli produces plywood for boat building and for furniture, like “Cuneo II” and “Cuneo III”. “Cuneo I” produces doors.

Concerning types of wood, the industry that produce doors usually processes soft wood. The industry in province of Vercelli works poplar, Okoumé, Cherry, Moabi, Mahogany and Teak. All these types of wood are hardwood. Poplar is also used in Cuneo II.

All subjects signed an informed consent form before starting the study. Afterwards, they filled in a questionnaire to provide personal and lifestyle data (smoking, alcohol and dietary intake), medical status (taking medication), and work history information. Information was also requested on the type of wood material usually used at work.

Each subject received a personal passive air-FA sampler and a personal active air sampler equipped to sampling dust device. These two samplers were both positioned near the respiratory tract. At the end of the working shift, the biological samplers (urine and nasal swab) were collected and then transported to the laboratory, where they were stored at -20° C and the day after moved at -80°C.

4.2 ENVIRONMENTAL ANALYSIS:

Personal dust:

The wood dust was collected on a SKC Button Aerosol sampler equipped with PVC fiber filters (0,5 µm, Ø25 mm, Whatman) operating with a flow rate of 4 L/minute (Gilian 5000, Sensidyne, USA). Wood dust concentrations were determined by gravimetric analysis (HSE, 2014). For the weighing of the filters, an analytical scale with a sensitivity of 0.00001 ug was used. For the transfer of the filters to the pan, tweezers with a flat tip, not knurled, was used to prevent abrasion or damage to the filters. To dehydrate the filters, they were being treated keeping them in a desiccator for 48 hours before carrying out the weighing. This procedure had been conducted every time before and after each sampling. Each group of filters (10 filters + 1 blank) was weighed included the control filter; this allows a constant monitoring of uniformity of the cooling-weighing system. The formula to quantify the wood dust is C=W/V, W = weight of dusty filter – weight prior to sampling filter [mg], V = Air volume sampled [m^3].

Gallic acid:

The determination of GA on the wood dust is obtained through liquid chromatography combined with mass spectrometry, in particular through a system of new generation, UPLC-MS/MS (Acquity UPLC Waters) coupled to a mass detector triple quadrupole (Waters TQD). The wod Briefly, the PVC membranes are extracted with 3 ml of methanol at 20% in water and of internal standard (30 µl PCA 100 µl); the sample is vortexed for 15 min. and centrifuged at 5000 rpm for 5 min; 200 µl of the supernatant are extracted twice with 500 µl of ethyl acetate (liquid-liquid extraction). the sample is vortexed for 15 min. and centrifuged at 5000 rpm for 5 min. The surnatant is dried with dry nitrogen and recovered with 200uL of 1% acetic acid in water in injected into UPLC (3uL). The chromatographic separation is achieved with a column Acquity UPLC HSS T3 1.7µm (2.1 x 50 mm DI) using a gradient characterized by variable percentages of formic acid and 0.1% methanol at a flow rate of 0.5ml/min. The retention time of GA is 0.73 minutes. The method is linear in the range of 5-400 ug/L and is characterized by a LOD equal to 0.5ug/L.

Personal FA:

FA air samples were collected for a working shift (8 hours) using passive, personal air samplers working with radial symmetry (Radiello®). The sampler was clipped near the breathing zone of the subject to quantify as accurately as possible air-FA during the work shift. Each sampler was

equipped with a specific sorbent tube containing florisil 35-50 mesh coated with 2,4-dinitrophenylhydrazine (DNPH). DNPH react with FA and changes by derivatization to the specific 2,4-dinitrophenylhydrazone-FA. This analyte was quantified with a HPLC Perkin-Elmer equipped with an UV detector regulated at 365 nm: NIOSH Method no. 2016. The instrument was set according the following specifications: pump Perkin-Elmer series 200, detector UV Perkin-Elmer LC 295, dilutor model 401 Gilson, automatic sampler Gilson model 231, HPLC column(length 150 mm-4,6 mm diameter), and cartridge 10 m LiChro CART 250-4. The instrumental conditions were as follows: mobile phase, 45% acetonitrile and 55% water; flux in column, 1.9 mL/min; and injection volume, 20 µL. The estimate limit of detection (LOD) was 0.05 µg/mL. The chemical desorption was done as follows: elution with 10 mL of acetonitrile and sonication for 20 min. 200 µL of DNPH solution and a drop of concentrated perchloric acid were added to 1 mL of bubbled sample to promote derivatization to dinitrophenylhydrazone. The reaction proceeded for at least 30 min at room temperature, and then, the samples were transferred in microvials (300 µL) for HPLC analysis. The calibration curve was prepared using a calibration standard (the specific 2,4 dinitrophenilhydrazone formed as above) provided by the sampler manufacturer, Radiello, and had a certificated concentration of 3.83 µg/mL, expressed as FA. The calibration curve was prepared with a range of concentration between 0.05 and 3 µg/mL. The standard solutions and the blank were treated as samples.

4.3 BIOLOGICAL ANALYSIS:

Urinary Isoprostane:

15-F2t-IsoP was measured in urine by ELISA method. A microplate kit specific for urinary 15-F2t-IsoP (Oxford, MI, USA) was used following manufacturers' instructions. This kit is based on a competitive ELISA test and microplates are directly coated with polyclonal antibody specific for F2-IsoPs. Urine sample were mixed with an enhancing reagent to limit interferences due to specific binding. Both samples and standard compete with 15-F2t-IsoP conjugated to horseradish peroxidase (HRP) for binding a polyclonal antibody on the microplate surface. After the addition of the substrate, the HRP activity resulting was measurable in color development. The intensity of the color is inversely proportional to amount of unconjugated 15-F2t-IsoP in the samples or standards. To stop the reaction, sulfuric acid (3M) was then added to each well and the microplate was read at 450 nm using a microplate reader (Tecan). The declared limit of detection is 0.2 ng/ml and the possible

cross-reactivity of this method is fixed below 3%. To achieve better accuracy by the ELISA method, a dilution rate of 1:4 (v/v) was adopted. Finally, 15-F2t-IsoP concentrations were normalized with the CREA levels.

Urinary Cotinine:

Urinary cotinine was measured aiming to consider the role played by tobacco smoke in the onset of an oxidative stress status. Cotinine was measured in urine sample with a solid phase competitive ELISA kit, according to manufacturer's instructions (Abnova, Taiwan). The samples and cotinine enzyme conjugate were added to the wells coated with anti-cotinine antibody. Cotinine in the samples competed with a cotinine enzyme (HRP) conjugate for binding sites. Unbound cotinine and cotinine enzyme conjugate was washed off by washing step. Upon the addition of the substrate, the intensity of color was inversely proportional to the concentration of cotinine in the samples. A standard curve was prepared relating color intensity to the concentration of the cotinine. The assay sensitivity is 1 ng/ml; the kit has been chosen with the purpose of distinguishing the active smokers from non-smokers, avoiding being based on a subjective quantification.

Urinary 8-oxodG:

The determination of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in urine is obtained with Acquity UPLC system coupled with a triple quadrupole Waters TQD mass spectrometer (Waters, Milford, MA, USA). The method is validated according to EMEA bioanalytical method guideline (EMEA 2011). Briefly, after thawing, internal standard ([15N5] 8-oxo-7,8-dihydro-2'-deoxyguanosine) is spiked to urine samples (1.0 mL). After mixing and centrifugation, samples are diluted in 100 mM formic acid solution (1:4), filtered using 0,2 µm Acrodisc Syringe Filter and injected onto UPLC system.

Chromatographic separation is performed on a UPLC BEH equipped with C18 column (2.1 × 100 mm, 1.7 µm) maintained at 40°C and by gradient elution with a mixture containing variable proportions of 20mM formic acid solution and methanol. Flow rate is delivered at 0.5 mL/min, retention time of 8-oxodGuo and its internal standard is 1.8 ± 0.01 minutes. Injection volume is 7.5 µL.

For the mass spectrometer detection, electrospray is operated in positive ion mode and the acquisition is performed in MRM; in particular 8-oxodG: m/z 284.0 → 167.9 for quantification (CV 18, CE 15) and m/z 284.0 → 116.9 for confirmation (CV 18, CE 19); for $^{15}N_5$ 8-OHdG: m/z 289.0 → 173.0 (CV 18, CE 15).

The method is accurate and precise; it provides a broad linear concentration range of 3-100 nM using Sigma synthetic urine LLOQ was 1.5 nM.

4.4 STATISTICAL ANALYSIS:

Statistical analysis were carried out with the statistical package “Stata” (version 12 SE for MS Windows®64 bit) and SPSS (IBM) to test urinary 15-F2t-IsoP, 8-oxodG, cotinine, and environmental samples. According to the Box–Cox regression, the variables had been transformed in logarithmic form; to normalize distribution and reduce heteroscedasticity. A multiple linear regression (MLR) was carried out to assess the effect of covariates on OS markers; using wood dust and FA as independent variables and OS markers (15-F_{2t}-IsoP e 8-oxodG) as dependent variables. For the final regression model, only variables that proved to be significant at 5% level were retained.

5.RESULTS

The characteristics of the population enrolled in the study are described in **Table 5**.

	Subject exposed (n. 128)		**Subjects not exposed (n. 117)**	
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)
Age (years)	43,2 ± 10,4	44 (20; 61)	40,0 ± 10,9	40 (23; 69)
BMI (kg/m2)	23,8 ± 3,1	23,8 (18,4; 34,9)	23,3 ± 0,3	23,0 (15,8; 34,5)
Dust (mg/m3)	0,37 ± 0,33	0,29 (0,03; 2,43)	0,07 ± 0,05	0,04 (0,00; 1,19)
Formaldehyde (µg/m3)	76,0 ± 63,2	57,8 (14,5; 330,2)	19,5 ± 17,3	15,1 (4,0; 106,5)
15-F2t-IsoP (ng/mg crea)	3,04 ± 2,22	2,56 (0,24; 15,96)	3,03 ± 1,96	2,51 (0,19; 12,35)
8-oxodG (µmol/mol)	1,18 ± 0,99	1,03 (0,32; 11,32)	1,17 ± 0,55	1,10 (0,07; 3,21)
Cotinine (ng/mg crea)	47,1 ± 90,1	13,7 (0,4; 616,5)	26,6 ± 60,0	5,8 (0,3; 404,0)

Table 5: Descriptive analysis of the 245 subjects enrolled (BMI: Body Mass Index; S.D.: Standard Deviation)

According to the Box–Cox regression results, the values of 15-F2t-IsoP, 8-oxodG, formaldehyde and exposure to wood were subjected to a logarithmic transformation prior to execute the multiple linear regression (MLR) analysis, to stabilize the variance and normalize the distribution.

BMI has not been shown to be significantly different in the model (p>0.050) and, therefore, were excluded from the computation of the final MLR regression model.

Findings of environmental analysis are shows in **Table 6** as means for each industry and for not exposed. Highest values of dust were found in the Vercelli industry and the lowest in Cuneo I, the door industry. In addition, the dust proves to be significantly higher among the exposed subjects (p<0,001).

Dust	**n.**	**Mean ± S.D. (mg/m3)**	**C.I. (95%)**	**Min-max**
VC	39	0,457 ± 0,327	0.351 - 0.534	0.9 – 1.95
CN1	46	0,182 ± 0,222	0.137 - 0.492	0.00 – 1.07
CN2	30	0,393 ± 0,266	0.293 - 0.492	0.12 – 1.41
CN3	13	0,375 ± 0,54	0.114 - 0.637	0.01 – 2.43
Not exposed	117	0,068 ± 0.05	0.059 – 0.794	0.00 – 0.19
Total	245	0,225 ± 0,282	0.189 – 0.261	0.00 – 2.43

Table 6: Wood dust values sampled in the workers of the four industries and values of subjects non-exposed (S.D. Standard Deviation; C.I. Coefficient Interval)

In **Table 7**, FA concentration are summarized. As for wood dust, in Vercelli industry higher level of FA were recorded, as in Cuneo II. Lowest values of FA were found in Cuneo III. Furthermore, the FA levels recorded among the exposed subjects show a significant higher concentration ($p<0,001$).

Formaldehyde	n.	Mean ± S.D. (mg/m^3)	C.I. (95%)	Min-max
VC	39	**110.3** ± 71.4	87.1 – 133.4	48.5 – 330.2
CN1	46	**35.1** ± 11.8	32.2 – 37.9	11.6 – 62.7
CN2	30	**114.0** ± 63.1	90.4 – 137.5	19.3 – 207.8
CN3	13	**30.1** ± 8.4	26.1 – 34.1	22.2 – 59.1
Not exposed	117	**15.0** ± 15.4	11.7 – 18.2	4.0 – 106.5
Total	245	**49.0** ± 55.0	42.1 – 55.9	4.0 – 330.2

Table 7: Formaldehyde values sampled in the four industries and non-exposed values (SD:Standard Deviation; C.I. Coefficient Interval)

Higher levels of Gallic acid were found in Cuneo III than lower values were found in Cuneo II.

Gallic acid	**n.**	**Mean ± S.D. (mg/m3)**	**C.I. (95%)**	**Min-max**
VC	39	0.006 ± 0.010	0.003 – 0.010	0.000 – 0.061
CN1	46	0.032 ± 0.139	-0.002 – 0.066	0.000 – 1.140
CN2	30	0.000 ± 0.000	0.000 – 0.010	0.000 – 0.002
CN3	13	0.060 ± 0.079	0.022 – 0.099	0.000 – 0.341
Not exposed	117	0.000 ± 0.001	0.000 – 0.001	0.000 – 0.050
Total	245	0.015 ± 0.079	0.005 – 0.025	0.000 – 1.140

Table 8: Gallic acid sampled in industries (SD: Standard Deviation; C.I. Coefficient Interval)

Fondazione Salvatore Maugeri quantified in raw material the GA concentration **(Figure 1).**

Tree	Gallic Acid ng/mg sawdust	Type
Elm	5804,0	hard
National walsnut	388,7	hard
Oak tree	237,7	hard
Mahogany	19,8	hard
Cherry tree	6,0	hard
Padouk	4,2	hard
Fir	1,8	soft
Poplar	1,6	hard
Olive	1,3	hard
Beech	1,2	hard
Bahia walnut	1,1	hard

Figure 1: Gallic acid concentration [ng/mg] in raw material samples conducted by Fondazione Salvatore Maugeri.

The MLR of log GA shows a significant higher level of GA for all the industries if compared to controls, except Cuneo II. This is probably due to the non-use of woods contain this marker in this industry.

Gallic acid	Industries	Mean Log (ng/mg)	p	C.I. (95%)
Non-exposed	VC	-2.37861	< 0.05	-3.0676 – 1.689
	CN1	-2.39804	< 0.05	-2.9759 – 1.820
	CN2	-0.08782	N.S.	-0.8453 – 0.669
	CN3	-4.22454	< 0.05	-5.1313 – 3.317

Table 9. MLR parameters, with 95% C.I. of Log GA.

Subsequently to the environmental analysis, the biological responses was carried out analyzed quantifying them on all the subjects enrolled in the study. The Isoprostane, adjusted by age and gender considered as confounder parameters, shows a positive and significant correlation with wood dust (p = 0,002) (**Table 10**).

Log 15-F2t-IsoP	Coeff.	(95% C.I.)		p
Log wood dust	0.152	0.057	0.246	**0.002**
Wood dust exposed	-0.955	-1.236	-0.673	**0.000**

Table 10. MLR parameters, with 95% C.I., of Log 15-F2t-IsoP.

Robust multiple linear regression analysis shows that 15-F2t-IsoP is positively correlated with the cotinine (p=0,001), thus showing a role of the tobacco smoke exposure in the increase of oxidative stress. This is also confirmed by the robust multiple linear regression analysis showing that the 15-F2t-IsoP is positively correlated with the concentration of urinary cotinine (p = 0.001) (**Figure 2)**. This underlines that cigarette smoking increases the O.S. Conversely, the O.S. does not prove to be influenced by exposure to dusts (p = NS), by the standardized BMI square (p = NS) and by exposure to FA (p = NS). Furthermore, no significant correlation was found with either age (p = NS) or years of work (p = NS).

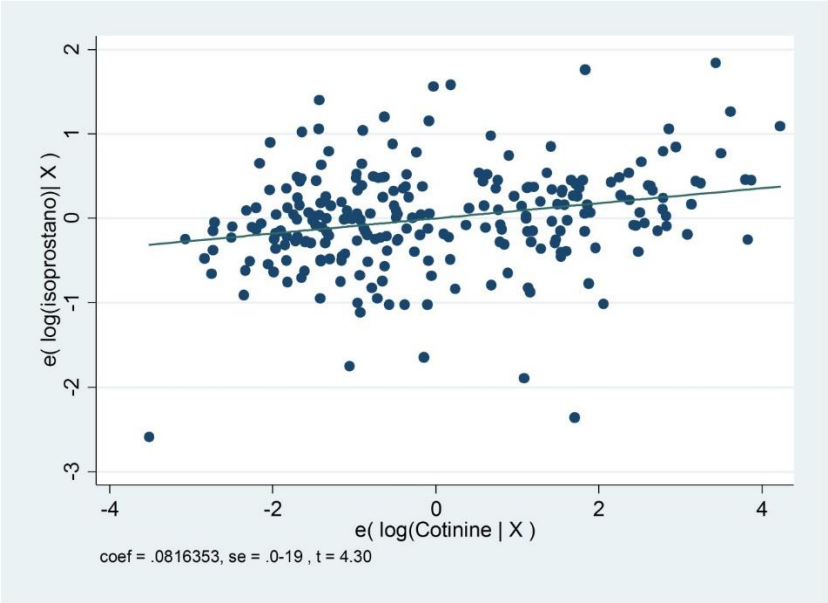


Figure 2. Plot of relationship between 15F2t-IsoP (Log) and cotinine (Log).

Comparison between medians shows that exposure to dust and FA is significantly greater in exposed subjects than controls (p <0.001). On the contrary, the difference between the 15-F2t-IsoP and 8-oxodG levels detected in the two groups is not significant; this shows overlapping levels in the two subgroups. The concentrations of dust and FA are correlated with each other (p=0.041), as well as the concentrations of 15-F2t-IsoP and 8-oxodG (p=0.002) (**Figure 3**).

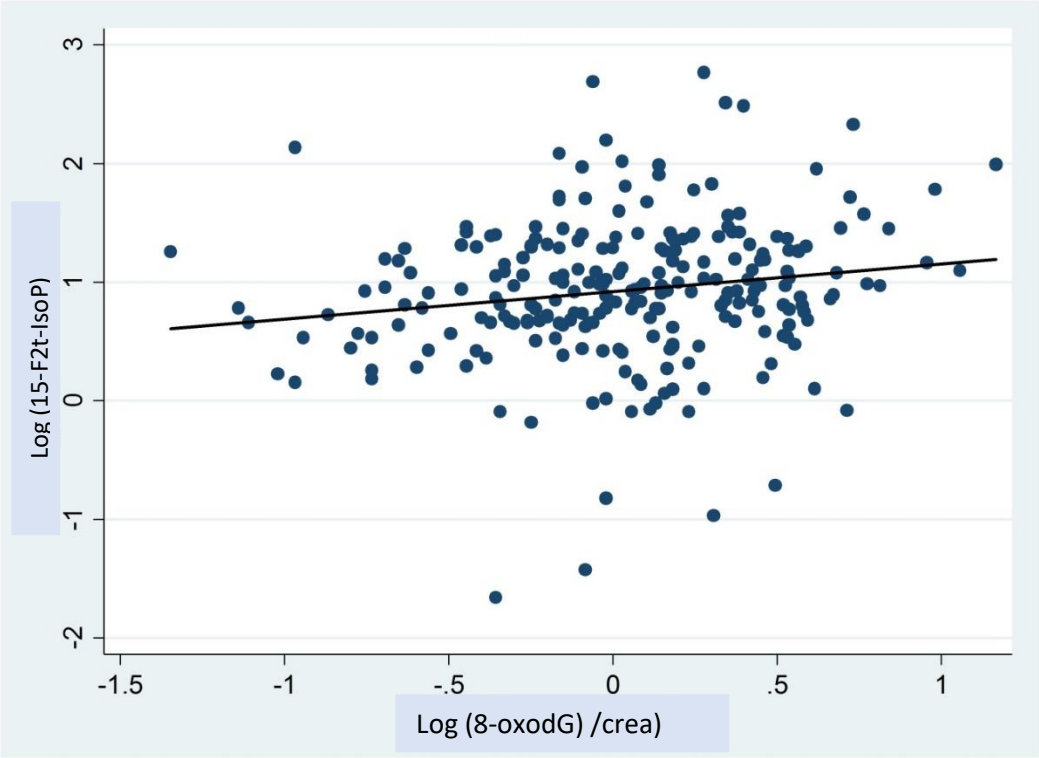


Figure 3. Plot of relationship between 15F2t-IsoP (Log) and 8-oxo-dG (Log).

By adjusting the values of 15-F2t-IsoP for age and years of work, considered as confounding factors, we can observe a positive and significant correlation between 15-F2t-IsoP and dust (p = 0.002). By dividing the measured concentration of dust into tertiles, a significant increase in the levels of 15-F2t-IsoP between the first and second tertiles (p = 0.001) is evident. An increase that was not detected, however, between the second and third (p = NS), as can be seen in **Figure 4** for exposed subjects.

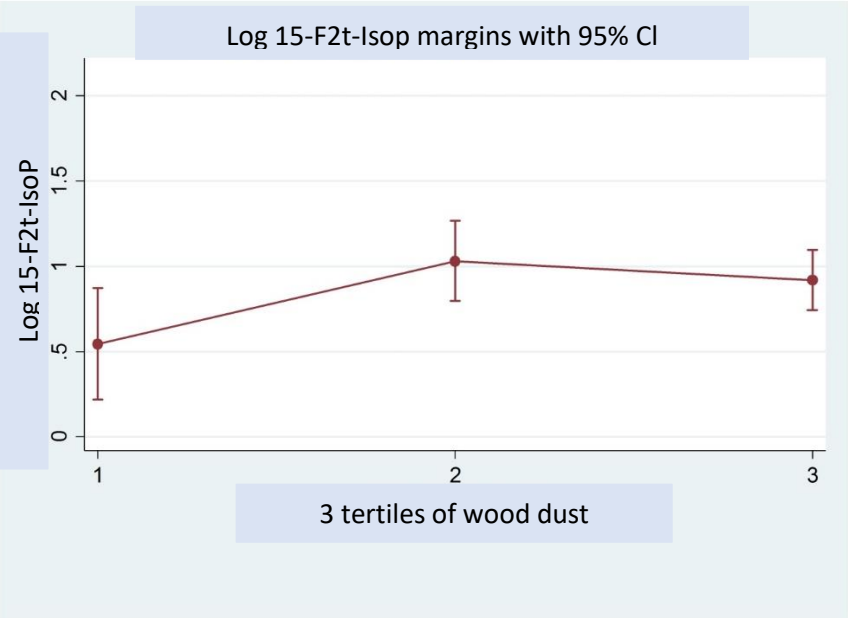


Figure 4. Wood dusts divided in tertiles and 15F2t-IsoP (Log).

The 8-oxo-dG proves positively correlated with the dust (p=0.014). On the contrary, it is not influenced neither by exposure to FA (p = NS), nor by the concentration of urinary cotinine (p = NS). As already for 15-F2t-IsoP, no significant correlation of 8-oxo-dG was found with either age and years of work (p=NS).

The trend between 8-oxo-dG and wood dust is shown in **Figure 5**. After the division in tertiles of 8-oxo-dG is possible to observe an increase of the biomarker passing from the first and the second tertiles and, slightly, passing from second to the third (p=0,035) (**Figure 6**).

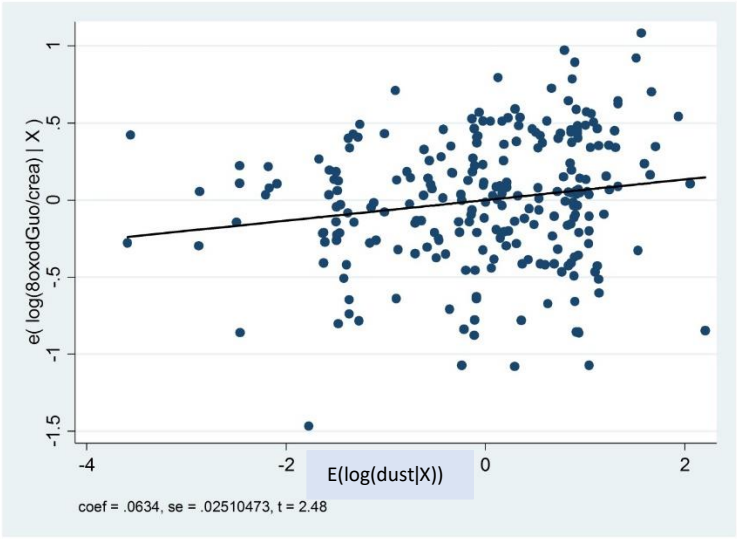


Figure 5: Relationship among 8-oxo-dG and wood dust.

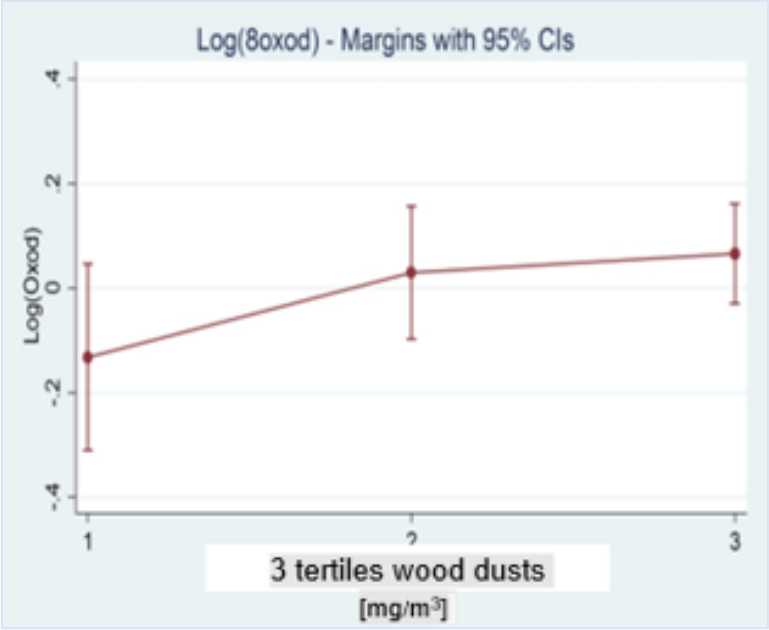


Figure 6. Trend of wood dusts in tertiles and 8-oxo-dG

6. DISCUSSION

The aim of this work was to investigate the wood dust occupational exposure of a group of workers employed in the wood industries in Piedmont region, Italy. This with two main aims: 1) the deepening of the role of environmental wood dust exposure in the development of the oxidative stress and 2) the study of the mechanisms, among which oxidative stress, involved in diseases-wood dust correlated. For the selection of the epidemiological sample, were enrolled a population of exposed to wood dust workers and a population of workers not exposed as controls. The selected exposed population, work in four industries sited in Cuneo and Vercelli area of Piedmont region in Italy. The four industries show different type of use and manufacturing products of wood; from the first step of production as raw material management to produce plywood, to the production of final goods as the doors. The population studied was constituted primarily by men (165 men to 80 women). This is due to the type of manufacture that often sees male workers as protagonists. In addition to exposure to wood dust, exposure to FA was also assessed. The oxidative stress level, as response to these occupational exposures, was monitored through the quantification of urinary 15-F2t-IsoP and 8-oxo-dG.

Occupational exposure to wood dust is known to be associated with a higher risk of many respiratory and allergic diseases, over cancer phenomena (Kaupinnen, 2006). Wood dust has been classified as human carcinogen by IARC, because a marked increase in the rate of sinonasal cancer among workers exposed primarily to hardwood dust were demonstrated. Epidemiological data on the carcinogenic effects of wood dust are weaker for softwoods than for hardwoods, making it very difficult to establish standards for softwood dust exposure (Lovato, 2015).

The wood industry is made up of different stages of production. In the primary wood production stage (sawmills, cutting and upgrading of cut timber), dust generated by mechanical wood working processes is the main environmental hazards. Fine dust may be particularly dangerous when wood particles become airborne and reach the lungs. In the chipboard production process, wood boards are produced by gluing or bonding together laminates or wood particles. The cheapest and most frequently used bonding is FA resin. Therefore, the emission of FA represents one of the main pollutants of this production stage (De Marco, 2009).

Considering the industries studied and their manufactured goods, the main results of this research confirm the different amount and types of dusts in manufacturing steps; Cuneo I shows the lowest levels of wood dust because that industry production are characterized by the final step of productive cycle: the construction of the doors. Instead, At Cuneo II, where raw material (tree

trunks) is worked to produce the plywood sheets, higher amount of dusts, as in Vercelli, were sampled. About the presence of personal airborne FA, the same trend of wood dust is confirmed: the FA concentrations are significantly higher in Cuneo II where, after the use of raw material, rough plywood is packaged and send to furniture industry for the next steps. Plywood are processed in Vercelli I, where a daily use of FA-resin was observed. This different intensity of worker exposure, while respecting the legal levels in all cases, highlights how the different preventive approach to exposure to industrial pollutants may highlight a different intensity of health risk. Environmental agents studied show higher level in the workers exposed than in non-exposed. Wood dusts and FA concentrations, in all the industries, were measured with concentrations under the legislative limits. The medium level of wood exposure is 0.37 mg/m^3, lower than the D.Lgs 81/2008 limit of 5 mg/m^3 and lower than the OEL (1.5 mg/m^3) by SCOEL.

FA shows a medium value of exposure as 76.0 µg/m^3; the TLV-C by ACGIH is 370 µg/m^3. A new FA limit has been introduced in the last few months by ACGIH: TLV-TWA of 120 µg/m^3 and TLV-STEL di 370 µg/m^3. The new limits have been introduced after the sampling period.

To typify the wood dust exposure, in this study the GA amount in each environmental sample was also investigated. Year to day the most serious health effects connected to wood exposure have been observed predominantly among workers exposed to hardwood dusts, such as those from oak and beech. The industries involved in this study used, during the sampling days, both hard and soft woods, dependently on the commercial demand. Define correctly the amount of GA was been important to correlate its presence in air to the “hard wood dust exposure” and the level of oxidative stress investigated.

Carrieri et al. (Carrieri, 2016) considered GA as a good marker for oak dust and thus very useful for estimating the true quantities of dust inhaled. Analyzing the concentration of gallic acid could be useful to quantify the real exposure to oak dust, considered carcinogenic to humans, and to differentiate exposure to oak from that of beech (the other wood considered human carcinogen by ACGIH) or, again, to differentiate it from other types of woods that do not contain GA. Nevertheless, the analysis in this study show a GA amount not only in the industries using oak but also in the ones using other type of wood.

The Fondazione Salvatore Maugeri quantified the GA in raw material (Figure 1) finding his presence not only in oak but also in elm and national walnut, two types of wood used in the industries involved in the present study. Furthermore, GA had been quantified in fir, a soft wood, and use in Cuneo III.

We can hypothesize that carcinogenic potentials are present in the dusts of both hard and soft woods. However, the toxic and carcinogenic potentials of the mixtures of compounds present in wood dusts, as well as the mixtures of natural and synthetic compounds containing additives, paints, glues, etc., remain to be evaluated (IARC, 1995).

In addition, the mechanisms underlying carcinogenesis progression are not yet well understood. Two main hypotheses may been taken into consideration: inhalation of potential carcinogenic agents in the wood dust (e.g. tannins, unsaturated aldehydes), and inhalation of wood dust particles resulting in interference with normal mucociliary action, leading to increasing susceptibility to carcinogens due to the alteration on balance between oxidants and antioxidants (Lushchak, 2014; Naarala, 2003).

Oxidative stress is a complex phenomenon and it can involve many mechanisms. 15F2t-Isoprostane and 8-oxodG are representative to different type of damage by ROS the first is produced by lipid peroxidation; the second is formed because of DNA lesions. So far, no references have been published on this item: these two biomarkers have not been used yet to evaluate oxidative stress in workers employed in the wood industry and in furniture manufacturing. The correlation between these biomarkers from different damage by ROS is positive; in the scientific literature is possible found two conflicting ideas; Wu et al. seems confirm the result of the present study, instead Rossner et al. show different data. (Wu, 2008: Rossner, 2009). The levels of environmental pollutants, however, do not highlight a significant variation in the intensity of oxidative stress markers when compared to controls. This condition can be the result of the coexistence of two factors: the presence in the controls of oxidative stress induced by factors that have not been investigated in my study and, secondly, lower levels of wood dust and FA than I expected.

Both biomarkers of oxidative stress do not show a positive correlation with FA measured on workers exposed to wood dust. Other biomarkers, such as the blood viscosity reduction, better correlate with FA, but only at higher level of exposure in wood workers (Jafari, 2015). In the present study the FA exposure is under the legal limits and this may be a possible explanation of the lack of positive correlation. The low exposure intensity concerning the cumulative exposure to FA, may result from the confounding role of smoking, or from the exposures to other occupational hazards that have not yet been satisfactorily investigated.

Sounding out the exposure detail, the levels of wood dust were divided in tertiles and MLR suggest as the 8-oxodG level increase gradually from the first to the second tertiles (Fig.4) with the rising of wood dust, instead 15F2t-Isoprostane increase only if this biomarker had been adjusted for age and

chronic exposure (Fig.2). To support these results, Rossner et al. in 2008 explained that 8-oxodG is a biomarker of immediate damage instead 15F2t-Isoprostane express a previous exposure (3-4 weeks before). This type of approach is particularly suitable to assess the response to environmental and occupational exposures based on several environmental factors (about 20% in the case of DNA oxidation) and on the genetic component (Broedbaek, 2011). The quantification of 15F2t-Isoprostane could be explain with the interpretation of the lipid damage repair mechanism; this damage will not be immediately after a damage.

The analysis of tertiles also shows as the trend of both biomarkers not change anymore from the second to the third tertiles, lead to think to a threshold limits of wood dust exposure able to induce oxidative stress.

7. CONCLUSION:

The present study provides further evidence that workers in the wood industry are exposed to substances such as dusts and FA, potentially able to induce an alteration of health status. Apart the carcinogenic properties, these risk factors can determine an increase in levels of oxidative stress and, consequently, an increase of an inflammatory harbinger of many chronic diseases. The choice of the two biomarkers I have investigated was determined to obtain more information about wood dust and a better understanding of how these chemical agents act. This is to promote the best preventive strategies to combat oxidative stress and therefore many diseases related to it. The present study also raises the issues of future quantitative individual risk assessment and the need to explore all the potential factors involving in this phenomenon.

Will be useful planning the sampling twice time, to evaluate the seasonality for the two carcinogens compound.

Finally, the results of this research highlight as working in accordance to the precautional principles (D.Lgs 81/2008 and CE normative) can protect the workers from unhealthy situation. Further studies are necessary to explore the different occupational situations (for example higher value of exposure to wood dust and FA) to investigate the environmental exposure and genotoxic and mutagenic effects of these two toxic agents.

BIBLIOGRAPHY:

1. Alonso-Sardón, M., Chamorro A. J., Hernández-García I., Iglesias-de-Sena H., Martín-Rodero H., Herrera C., Marcos M., Mirón-Canelo J. A. 2015. Association between occupational exposure to wood dust and cancer: a systematic review and meta-analysis. *PloS one*, 10(7): e0133024 doi: 10.1371/journal.pone.0133024.
2. Antus, B. 2016. Oxidative stress markers in sputum. *Oxidative Medicine and Cellular Longevity*,vol. 2016,Article ID 2930434, 12 pages doi:10.1155/2016/293043.
3. Au, W. W. 2007. Usefulness of biomarkers in population studies: from exposure to susceptibility and to prediction of cancer. *International journal of hygiene and environmental health*, 210(3): 239-46 doi: 10.1016/j.ijheh.2006.11.001.
4. Basu S. 2008. F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. Antioxid Redox Signal. 10(8):1405-34. doi: 10.1089/ars.2007.1956. Review.
5. Bellisario V., Mengozzi G., Grignani E., Bugiani M, Sapino A., Bussolati G., Bono R. 2016. Towards a formalin-free hospital. Levels of 15-F2t-isoprostane and malondialdehyde to monitor exposure to formaldehyde in nurses from operating theatres. Toxicology ResearchVolume 5, Issue 4, 2016, Pages 1122-1129.
6. Bislimovska, D., Petrovska, S., & Minov J. 2015. Respiratory Symptoms and Lung Function in Never-Smoking Male Workers Exposed to Hardwood Dust. *Open Access Macedonian Journal of Medical Sciences* 3(3):500-5 doi: 10.3889/oamjms.2015.086.
7. Bono, R., Romanazzi V., Bellisario V., Tassinari R., Trucco G., Urbino A., Cassardo C., Siniscalco C., Marchetti P., Marcon, A. 2016. Air pollution, aeroallergens and admissions to pediatric emergency room for respiratory reasons in Turin, northwestern Italy. *BMC public health*, 16(1):722 doi: 10.1186/s12889-016-3376-3.
8. Bono, R., Romanazzi V., Munnia A., Piro S., Allione A., Ricceri F., Guarrera S., Pignata C., Matullo G., Wang P., Giese R. W., Peluso M. 2010. Malondialdehyde− Deoxyguanosine Adduct Formation in Workers of Pathology Wards: The Role of Air Formaldehyde Exposure. *Chemical research in toxicology* 23 (8):1342-48 doi: 10.1021/tx100083x.
9. Bono, R., Bellisario V., Romanazzi V., Pirro V., Piccioni P., Pazzi M., Bugiani M., Vincenti M. 2014. Oxidative stress in adolescent passive smokers living in urban and rural environments. *International journal of hygiene and environmental health*. 217 (2):287-93 doi:10.1016/j.ijheh.2013.06.008.
10. Boysen G., Collins LB, Liao S, Luke AM, Pachkowski BF, Watters JL, Swenberg JA. 2010. Analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine by ultra high pressure liquid chromatography-heat assisted electrospray ionization-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. ;878(3-4):375-80. doi: 10.1016/j.jchromb.
11. Breitenbach M, Eckl P. 2015. Introduction to Oxidative Stress in Biomedical and Biological Research. *Biomolecules.* 5(2):1169-77.
12. Broedbaek, K., Ribel-Madsen R., Henriksen T., Weimann A., Petersen M., Andersen J. T., Afzal S., Hjelvang B., Roberts J., Vaag A., Poulsen, P., Poulsen H. E. 2011. Genetic and environmental influences on oxidative damage assessed in elderly Danish twins. *Free Radical Biology and Medicine.* 50 (11):1488-91 doi:10.1016/ j.freeradbiomed. 2011.02.017
13. Carrieri, M., Scapellato M. L., Salamon F., Gori G., Trevisan A., Bartolucci G. B. 2016. Assessment of exposure to oak wood dust using gallic acid as a chemical marker. *International archives of occupational and environmental health.* 89(1):115-121 doi: 10.1007/s00420-015-1056-8.

14. Darren R. Brenner, Dominique Scherer, Kenneth Muir, Joellen Schildkraut, Paolo Boffetta, Margaret R. Spitz, Loic Le Marchand, Andrew T. Chan, Ellen L. Goode, Cornelia M. Ulrich, and Rayjean J. Hung. A Review of the Application of Inflammatory Biomarkers in Epidemiologic Cancer Research. Cancer Epidemiol Biomarkers Prev. Sep;23(9):1729-51. doi: 10.1158/1055-9965.EPI-14-0064.
15. EMEA .2009. Guideline on bioanalytical method validation. 21 July 2011; Rev. 1 Corr. 2**; Committee for Medicinal Products for Human Use (CHMP).
16. Holmström M, Wilhelmsson B, Hellquist H, Rosén G. 1989. Histological changes in the nasal mucosa in persons occupationally exposed to formaldehyde alone and in combination with wood dust. *Acta Otolaryngol*..107(1-2):120-9.
17. INAIL, 2012. Esposizione lavorativa a polveri di legno.
18. INAIL, 2016. Il Registro Nazionale dei Tumori Naso-Sinusali (ReNaTuNS). Evidenze epidemiologiche, quadro di riferimento, risultati dell'attività di sorveglianza. Primo rapporto.
19. International Agency for Research on Cancer (IARC) 1995. Wood dust and formaldehyde. IARC Monogr Eval Carcinog Risks Hum 62: 1–405.
20. International Agency for Research on Cancer (IARC). 2006. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC Monogr Eval Carcinog Risks Hum, 88: 1–478. PMID:17366697.
21. International Agency for Research on Cancer (IARC) 2012 Formaldehyde. In: IARC Monographs on the *Evaluation of Carcinogenic Risks to Humans,* Volume 100F. IARC, Lyon 2012.
22. Jafari M. J., Rahimi A., Omidi L., Behzadi M. H., Rajabi M. H. 2015. Occupational exposure and health impairments of formaldehyde on employees of a wood industry. *Health promotion perspectives* 5(4):296-303 doi: 10.15171/hpp.2015.035.
23. Jaffe M. 1886. Ueber den Niederschlag welchen Pikrinsäure in normalen Harn erzeugt und über eine neue reaction des Kreatinins. Zeitschrift für physiologische Chemie 10:391–400 doi: 10.1515/bchm1.1886.10.5.391.
24. Kim, K. H., Jahan S. A., Lee J. T. 2011. Exposure to formaldehyde and its potential human health hazards. *Journal of Environmental Science and Health*, Part C 29(4):277-99 doi: 10.1080/10590501.2011.629972.
25. Lam PM, Mistry V, Marczylo TH, Konje JC, Evans MD, Cooke MS. 2012. Rapid measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine in human biological matrices using ultra-high-performance liquid chromatography-tandem mass spectrometry. *Free Radic Biol Med*. 2012 May 15;52(10):2057-63. doi: 10.1016/j.freeradbiomed.2012.03.004. Epub 2012 Apr 15.
26. Lovato A., Staffieri C., G. Ottaviano, Cappelesso R., Giacomelli L., Bartolucci G.B, Scapellato ML., Marioni G..2016. Woodworkers and the inflammatory effects of softwood/hardwood dust: evidence from nasal cytology. *Eur Arch Otorhinolaryngol.* 273:3195–3200 DOI 10.1007/s00405-016-3989-2.
27. Lushchak, V.I. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-Biological Interactions.* 224:164–75 doi: 10.1016/j.cbi.2014.10.016
28. Marrocco I, Altieri F, Peluso I. 2017. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxid Med Cell Longev*. 2017:6501046. doi: 10.1155/2017/6501046.
29. Milne G. L., Dai Q., Roberts, L. J. 2015. The isoprostanes—25 years later. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1851(4):433-45 doi: 10.1016/j.bbalip.2014.10.007.

30. Naarala J., Kasanen J.P., Pasanen P., Pasanen A.L. 2003. The effects of wood dusts on the redox status and cell death in mouse macrophages (RAW 264.7) and human leukocites in vitro. *Journal of Toxicology and Environmental Health*, Part A, 66:1221–1235.
31. NielsenG. D., Larsen S. T., Wolkoff, P. 2017. Re-evaluation of the WHO (2010) formaldehyde indoor air quality guideline for cancer risk assessment. *Archives of toxicology*. 91(1):35 doi: 10.1007/s00204-016-1733-8.
32. Peteffi GP, da Silva LB, Antunes MV, Wilhelm C, Valandro ET, Glaeser J, Kaefer D, Linden R. ; 2015. Evaluation of genotoxicity in workers exposed to low levels of formaldehyde in a furniture manufacturing facility. *Toxicol Ind Health*. 32(10):1763-73. doi: 10.1177/0748233715584250.
33. Pisoschi, A.M., Pop A. 2015. The role of antioxidants in the chemistry of oxidative stress: A review. European Journal of Medicinal Chemistry 97:55-74 doi: 10.1016/j.ejmech.2015.04.040
34. Poulsen HE, Nadal LL, Broedbaek K, Nielsen PE, Weimann A. 2013. Detection and interpretation of 8-oxodG and 8-oxoGua in urine, plasma and cerebrospinal fluid. *Biochim Biophys Acta*. 2014 Feb;1840(2):801-8. doi: 10.1016/j.bbagen.2013.06.009. Epub 2013 Jun 19. Review.
35. Roberts LJ, Morrow JD. 2000. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free *Radic Biol Med*.15;28(4):505-13. Review.
36. Romanazzi V, Pirro V, Bellisario V, Mengozzi G, Peluso M, Pazzi M, Bugiani M, Verlato G, Bono R. 2013. 15-F_2t isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposure to formaldehyde in workers of plastic laminates. *Sci Total Environ.* ;442:20-5. doi: 10.1016/j.scitotenv.2012.10.057.
37. Rossner, P., Gammon M. D., Terry M. B., Agrawal M., Zhang F. F., Teitelbaum S. L., Eng S. M., Gaudet M. M., Neugut A. I., Santella, R. M. 2006. Relationship between urinary 15-F2t-isoprostane and 8-oxodeoxyguanosine levels and breast cancer risk. *Cancer Epidemiology and PreventionBiomarkers* 15 (4):639-44 doi:10.1158/1055-9965. EPI-05-0554.
38. Sie Sie Siew, Jan Ivar Martinsen, Kristina Kjaerheim, Par Sparen, Laufey Tryggvadottir, Elisabete Weiderpass and Eero Pukkala; 2017; Occupational exposure to wood dust and risk of nasal and nasopharyngeal cancer: A case-control study among men in four nordic countries—With an emphasis on nasal adenocarcinoma. *Int. J. Cancer.* 141, 2430–2436.
39. Van der Vaart, H., Postma D.S., Timens W., Hacken N.H.T.T. 2004. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax*. 59:713–21 doi: 10.1136/thx.2003.012468.
40. Zinellu, E., Zinellu A., Fois A.G., Carru C., Pirina P. 2016. Circulating biomarkers of oxidative stress in chronic obstructive pulmonary disease: a systematic review. *Respiratory Research* 17(1):150 doi: 10.1186/s12931-016-0471-z.
41. Wilbourn J, Heseltine E, Møller H. 1995. IARC evaluates wood dust and formaldehyde. International Agency for Research on Cancer. *Scand J Work Environ Health*; 21(3):229-32.
42. Wu, H. C., Wang Q., Yang H. I., Ahsan H., Tsai W. Y., Wang L. Y., Chen S. Y., Chen C. J., Santella, R. M. 2008. Urinary 15-F 2t-isoprostane, aflatoxin B 1 exposure and hepatitis B virus infection and hepatocellular carcinoma in Taiwan. *Carcinogenesis.* 29 (5):971-76 doi:10.1093/carcin/bgn057.
43. Wultsch G, Nersesyan A, Kundi M, Wagner KH, Ferk F, Jakse R, Knasmueller S. 2015. Impact of exposure to wood dust on genotoxicity and cytotoxicity in exfoliated buccal and nasal cells. *Mutagenesis.* 30(5):701-9. doi: 10.1093/mutage/gev034.

Bono et al. BMC Public Health (2016) 16:722
DOI 10.1186/s12889-016-3376-3

BMC Public Health

RESEARCH ARTICLE Open Access

Air pollution, aeroallergens and admissions to pediatric emergency room for respiratory reasons in Turin, northwestern Italy

Roberto Bono[1*], Valeria Romanazzi[1], Valeria Bellisario[1], Roberta Tassinari[1], Giulia Trucco[1], Antonio Urbino[2], Claudio Cassardo[3], Consolata Siniscalco[4], Pierpaolo Marchetti[5] and Alessandro Marcon[5]

Abstract

Background: Air pollution can cause respiratory symptoms or exacerbate pre-existing respiratory diseases, especially in children. This study looked at the short-term association of air pollution concentrations with Emergency Room (ER) admissions for respiratory reasons in pediatric age (0–18 years).

Methods: Daily number of ER admissions in a children's Hospital, concentrations of urban-background $PM_{2.5}$, NO_2, O_3 and total aeroallergens (Corylaceae, Cupressaceae, Gramineae, Urticaceae, Ambrosia, Betula) were collected in Turin, northwestern Italy, for the period 1/08/2008 to 31/12/2010 (883 days). The associations between exposures and ER admissions were estimated, at time lags between 0 and 5 days, using generalized linear Poisson regression models, adjusted for non-meteorological potential confounders.

Results: In the study period, 21,793 ER admissions were observed, mainly (81 %) for upper respiratory tract infections. Median air pollution concentrations were 22.0, 42.5, 34.1 μg/m^3 for urban-background $PM_{2.5}$, NO_2, and O_3, respectively, and 2.9 grains/m^3 for aeroallergens. We found that ER admissions increased by 1.3 % (95 % CI: 0.3-2.2 %) five days after a 10 μg/m^3 increase in NO_2, and by 0.7 % (95 % CI: 0.1-1.2 %) one day after a 10 grains/m^3 increase in aeroallergens, while they were not associated with $PM_{2.5}$ concentrations. ER admissions were negatively associated with O_3 and aeroallergen concentrations at some time lags, but these association shifted to the null when meteorological confounders were adjusted for in the models.

Conclusions: Overall, these findings confirm adverse short-term health effects of air pollution on the risk of ER admission in children and encourage a careful management of the urban environment to health protection.

Keywords: Airborne pollutants, Pollens, Time-series analysis, Pediatric emergency room, Short-term respiratory effects

Background

Over the last decades, the prevalence of respiratory diseases, and in particular of asthma and allergies, has increased considerably, especially in industrialized countries [1, 2]. The etiology of respiratory diseases is multifactorial and includes, among others, interactions between genetic predisposition and environmental factors [3]. The environmental dynamics, characterized by climate change, qualitative and quantitative aspects of chemical air pollution and airborne pollens, may partially explain the increased incidence of respiratory symptoms and respiratory diseases during the last years [4]. The short-term respiratory effects of air pollution include decreases in pulmonary function [5], increases in inflammatory biomarkers [6] and respiratory symptoms [7, 8], exacerbations of chronic obstructive pulmonary disease (COPD), infections [9, 10], school absenteeism [11] and respiratory mortality [12, 13].

The respiratory system is a primary target of air pollution. In children, the small airway caliber allows for a higher chance of being affected by inflammation resulting from air pollution [14, 15]. Due to their respiratory rates, children breathe a proportionately greater volume of air than adults and their oxygen demand is significantly

* Correspondence: robertobono@unito.it
[1]Department of Public Health and Pediatrics, University of Turin, via Santena, 5 bis, 10126 Turin, Italy
Full list of author information is available at the end of the article

higher, as well as their respiration rates. Young people also spend more time engaged in intense activities than adults, often outdoors and during midday when air pollution levels tend to be higher. As a result, children inhale more pollutants per kilogram of body weight. Irritation caused by air pollutants that would produce only a slight response in an adult can result in potentially significant obstruction in the airways of a young child [16].

The environmental risk factors that may have an impact on children's respiratory health, especially in urban areas, include chemical outdoor pollution, aeroallergens, indoor air pollution including environmental tobacco smoke, microorganisms such as virus and bacteria that infect the airways. The latter can exacerbate or re-exacerbate their manifestations in presence of other risk factors.

Several epidemiological studies have documented a positive association between exposure to particulate air pollution and respiratory symptoms of cough and wheeze, especially among children [17, 18]. In this regard, the findings from two Swiss studies showed that the reduction of exposure to particulate matter (PM) <10 μm in aerodynamic diameter (PM_{10}) contributes to improved respiratory health, observed through fewer cases of chronic cough in children [19] and through fewer cough, wheezing and breathlessness in adults [20]. Exposure to ozone (O_3) at environmental concentrations is associated with lung function decrease and respiratory symptoms including cough, shortness of breath and pain on deep inspiration [21]. Nitrogen dioxide (NO_2) concentrations have also been associated with cough, wheeze and breath shortness in children. Residential traffic-related air pollution exposure is associated with reduced expiratory flows in schoolchildren [7, 22]. Variations in lung function that mirror changes in PM exposure have been reported in children who move to areas with different air pollution levels [23].

Pollen is a well know trigger of allergies and asthma aggravation, and actually has a changing profile; in fact new pollen types have emerged following the cultivation and spread of exotic ornamental plants in public and private places [24]. Moreover, global climate change has been linked to an earlier onset and an extended duration of the pollens season, to an increase in pollen production, and a stronger allergenicity for some pollen types [25]. Thunderstorm asthma epidemics may be triggered by pollen grains rupture in the atmosphere and the entrapment of respirable-size particles in the outflows of air masses at ground level [24, 25]. Increasing pollution is responsible for an increase in pollen-induced respiratory allergy, including asthma, because of airway inflammatory reaction and the passage of pollen grains into the lower respiratory tract [24].

The aim of this study was to analyze the short-term relationships between hospital emergency room (ER) admissions for respiratory diseases in children and concentrations of NO_2, $PM_{2.5}$, O_3, and aeroallergens, in Turin, Italy, between 2008 and 2010.

Methods

Turin, the capital of Piedmont region (North-Western Italy) has 900,000 inhabitants, is located at 200 m above sea level and it is one of the most polluted Italian cities [26–29] Additional file 1: Figure S1. Daily data for the period 01/08/2008 to 31/12/2010 (883 days) for the city of Turin were collected or derived as described below. The locations of the data sources are shown in Additional file 1: Figure S1.

ER admissions for respiratory diseases

Daily data on ER admissions (date of admission, primary diagnosis and diagnostic code) for 19 respiratory diseases to "Regina Margherita" Pediatric Hospital of Turin were collected (age range 0–18 years). Diagnoses were coded according to the International Classification of Disease (ICD) 9th edition (Table 1).

Meteorological data

Meteorological data derived from the station placed on the roof of the Department of Physics of the University of Turin, located at about 1 km from the city center. The station is permanently active since 1989 in order to collect and display in real time the weather data in the urban surface layer of the city. The station is equipped with the instruments reported in Additional file 1: Table S1. Data are collected every 5 s by the acquisition system, and subsequently averaged every 5 min and stored in an electronic archive. Data acquired in this way were aggregated in a daily form for the subsequent analysis.

Chemical air pollution data

Daily concentrations of NO_2, $PM_{2.5}$ and O_3 were derived from hourly data collected at the urban background monitoring station "Lingotto" located in Turin (viale Augusto Monti, 21) by the Local Environmental Protection Agency (ARPA Piemonte), coordinated by the regional air pollution service of Piedmont Region, according to the current European legislation (DIR 2008/50/ECX).

Aeroallergen data

Among the pollen taxa usually considered in aerobiological monitoring for being allergenic, Corylaceae, Cupressaceae, Gramineae, Urticaceae, Ambrosia, and Betula were quantified in this study. Daily data were derived from a station located 12 m above the ground, as required by the standard [30], on the flat roof of a building located in a semi-central area of the city of Turin. In this site, atmospheric circulation is local and not affected by surrounding obstacles such as walls or other types of protection. The station is equipped with

Table 1 Distribution of daily ER admissions, for the diagnoses of the respiratory diseases considered in the analysis, during the study period (883 days)

Respiratory disease diagnoses			total n. of admissions	daily n. of admissions		
Group	Description	ICD-IX-CM code	count	mean ± SD	median	min-max
Upper respiratory tract infections	Acute rhino pharyngitis	460	17684	18.1 ± 9.0	17	0-45
	Acute pharyngitis	462				
	Acute tonsillitis	463				
	Acute laryngitis without obstruction	46400				
	Acute laryngitis with obstruction	46401				
	Acute upper respiratory infections	4658				
Lower respiratory tract infections	Acute bronchitis	4660	1989	3.0 ± 3.2	2	0-20
	Acute bronchiolitis other infectious agents	46619				
	Flu with respiratory manifestations	4871				
	Bronchitis	490				
Deep lung infections	Viral pneumonia	4809	839	2.1 ± 2.1	2	0-8
	Bacterial pneumonia	4829				
	Bronchopneumonia	485				
	Pneumonia	486				
Asthma	Asthma, without status asthmatics	49390	1281	1.5 ± 1.6	1	0-7
	Asthma, with status asthmatics	49391				
Total			21793	24.7 ± 11.7	23	0-80

International classification of diseases, 9th edition, clinical modification (ICD-IX-CM) codes

a HIRST sampler, which consists of three main parts: a swivel head, a suction pump and a deposition drum (the sampling part), which rotates at 2 mm / h with 7 days of power reserve. Weekly, a specific adhesive tape is fixed on the drum. This tape captures the aeroallergens avoiding any loss for rebound or natural detachment. The air pump provides a constant airflow of 10 L / min inside the sampler, equivalent to 14.4 m^3 each 24 h. Daily aeroallergen counts were carried out at the Department of Life Science and System Biology, University of Turin, and expressed as concentrations (grains/m^3). For the statistical analysis, daily total aeroallergen concentrations were obtained as the sum of the concentrations of the single aeroallergen types.

Statistical Analysis

Quantitative variables were summarized with means ± SD, medians with interquartile ranges (IQR), and minimum and maximum values. The interquartile ratio (IQR/median ratio) was also computed in order to compare variability across different air pollutants. Linear correlations among exposure variables were evaluated using Pearson's r coefficients.

The association between daily ER admission counts for all diagnoses in Table 1 combined (dependent variable) and air pollution exposure variables were analyzed using Generalized Linear Models (GLMs) fitting a non-stationary Poisson process [30, 31]. We used the following model:

$$\log(\lambda_t) = \alpha + \sum_{i=1}^{k} \beta X_i + NS(Z)$$

Where λ_t denotes the count of daily ER admissions at day t, α is a constant, β is the vector of estimated parameters, X_i is the matrix of k independent variables (exposure and adjustment variables), and $NS(Z_t)$ is a natural spline smoothing function of calendar day Z with 14° of freedom (df), [31] which was included to take the medium/long term trend into account [30, 31]. The number of df of the smoothing function was chosen by minimizing the sum of the absolute values of the partial autocorrelation function (PACF) of the residuals [11, 30, 31]. Day of the week was included when estimating the smoothing function to remove the 7-day positive correlation across PACF residuals. To avoid overfitting, the maximum number of df allowed was 15, which corresponds to about 6 df per calendar year (60-day windows) [32].

The adjustment variables considered were: a) day of the week, b) influenza outbreaks, defined as days when influenza incidence was greater than 2‰ [33], which were

computed by the Regional Reference Service of Epidemiology for the Surveillance, Prevention and Control of Infectious Diseases, ASL Alessandria, Italy Reference Service Regional Epidemiology and Infectious Disease (SeREMI), c) holidays (4-level variable coded as: Christmas and Easter; 3 days around Christmas and Easter; other holidays; other days), d) summer population decrease (from Saturday before Mid-August to the next Sunday for a total of 16 days/year; from 16 July to 31 August, except for the aforementioned period; all other days) [34], e) average daily temperature, f) average relative humidity, and g) cumulative daily precipitations. The following models were fit to the data:

A) One exposure variable + medium/long trend function + non meteorological variables (day of the week, influenza outbreaks, holidays and summer population decrease) (*single-pollutant models*);
B) Model A + meteorological variables (daily temperature, daily relative humidity, cumulative daily precipitations). Temperature and relative humidity were modeled using natural splines with 3 and 2° of freedom, respectively. The number of df was chosen using the PACF criterion as above. Daily precipitations were binary coded (present if ≥1 mm; absent otherwise);
C) One chemical pollutant ($PM_{2.5}$, NO_2 or O_3) + aeroallergens + medium/long trend function + non meteorological variables (*two-pollutant models*). To avoid multicollinearity, two-pollutant models only combined one chemical pollutant per time and aeroallergens, because correlations across chemical pollutants were very strong (Pearson's coefficients of correlation in absolute value $|r| > 0.50$).

Exposure variables were included in the models at single time lags, from the same day when ER admissions were evaluated (Lag 0) to 5 days before (Lag 5). Associations between exposure variables (10 $\mu g/m^3$ increase in $PM_{2.5}$, NO_2, O_3 concentrations; 10 grains/m^3 increase in aeroallergen concentrations) and ER admissions were reported with rate ratios (RR) with 95 % confidence intervals (CI).

Results

In the study period, 21,793 pediatric ER admissions for respiratory diseases were observed, mainly (81 %) for infections of the upper airways (Table 1). Figure 1 shows average daily ER admissions by month of the year. The cold months showed the highest frequency of ER admissions, probably due to the more frequent outbreaks of colds. Table 2 shows a general description of the daily concentrations of airborne pollutants during the study period. NO_2 concentration showed the highest mean absolute levels and, overall, the air pollution concentrations observed underline the poor air condition in Turin as compared to the rest of Europe [26, 35].

Aeroallergen concentrations showed larger variability than chemical air pollution concentrations: the interquartile ratio for aeroallergens was 4 (O_3) to 8-fold (NO_2) the interquartile ratio of the chemical pollutants. Both $PM_{2.5}$ and NO_2 (Fig. 2a and b) showed a prevailing maximum level during the coldest months, which is a typical behavior of primary pollutants. An opposite trend

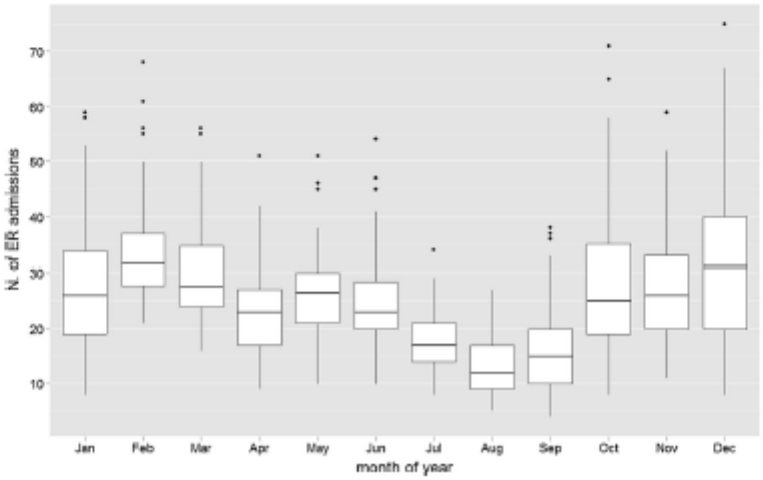


Fig. 1 Monthly distribution of daily ER admissions for respiratory diseases during the study period

Table 2 Distribution of daily concentrations of air pollution and aeroallergens at the Lingotto urban background monitoring station during the study period (883 days)

Exposure variable	available data (days)	median (IQR)	interquartile ratio	min-max	mean ± SD	EU annual reference value[b]
$PM_{2.5}$ (μg/m^3)	833	22.0 (30.0)	1.4	4–157	32.0 ± 26.2	25
NO_2 (μg/m^3)	851	42.5 (32.0)	0.8	7.4–192.9	48.3 ± 25.0	40
O_3 (μg/m^3)	858	34.1 (53.6)	1.6	1.8–123.3	9.6 ± 29.1	
aeroallergens (grains/m^3)[a]	826	2.9 (19.6)	6.7	0–271.9	16.1 ± 29.6	

[a]Includes Corylaceae, Cupressaceae, Gramineae, Urticaceae, Ambrosia, Betula
[b]European Union (EU) Directive 2008/50/CE10 (http://ec.europa.eu/environment/air/quality/legislation/directive.htm)

was shown by O_3, with higher concentrations in summertime (Fig. 2c). The concentrations of aeroallergens were high in the warm season, and virtually absent in winter (Fig. 2d).

There was a strong positive linear correlation between $PM_{2.5}$ and NO_2 ($r = 0.762$, $p < 0.001$). The negative correlations of O_3 with $PM_{2.5}$ and NO_2 were also strong ($r = -0.591$ and -0.695, respectively, all $p < 0.001$) (Fig. 3). The correlations between chemical pollutants and aeroallergens were weaker (|r| between 0.257 and 0.459).

Table 3 shows the mean number of daily ER admissions, air pollution and aeroallergen concentrations according to the potential confounders considered. ER admissions were more frequent during weekends, holidays, and during influenza outbreaks than during the other days. They were also more likely in days with lower temperatures and more extreme (low and high) relative humidity levels. $PM_{2.5}$ and NO_2 were positively associated with temperature, whereas the opposite was true for O_3 and aeroallergens. As expected, airborne pollution was lower during rainy days.

The associations between exposure variables and ER admissions for respiratory diseases, adjusted for non-meteorological potential confounders (model A), is described in Fig. 4. There was no statistically significant association of $PM_{2.5}$ and ER admissions at any time lag (Fig. 4a). Instead, an increase of 10 μg/m^3 of NO_2 concentrations (Fig. 4b) was associated with a significant 1.3 % (95 % CI: 0.3-2.2 %) increase of ER admissions after 5 days (lag 5). O_3 concentrations were significantly negatively associated with ER admissions for respiratory diseases, starting from lag 4 (Fig. 4c). Finally, a 0.7 % (95 % CI: 0.1–1.2 %) increase of ER admissions was observed 1 day after (lag 1) an increase of 10 grains/m^3 of aeroallergens (Fig. 4d). When meteorological variables were also included as adjustment variables in the analyses, the results were consistent, with the exception that the negative associations between O_3 (lags 4–5) and aeroallergen (lag 4) concentrations and ER admissions shifted to the null (Fig. 5). Joint models including individual chemical air pollutants and aeroallergens confirmed the main models results completely (Additional file 1: Figure S2), suggesting that the

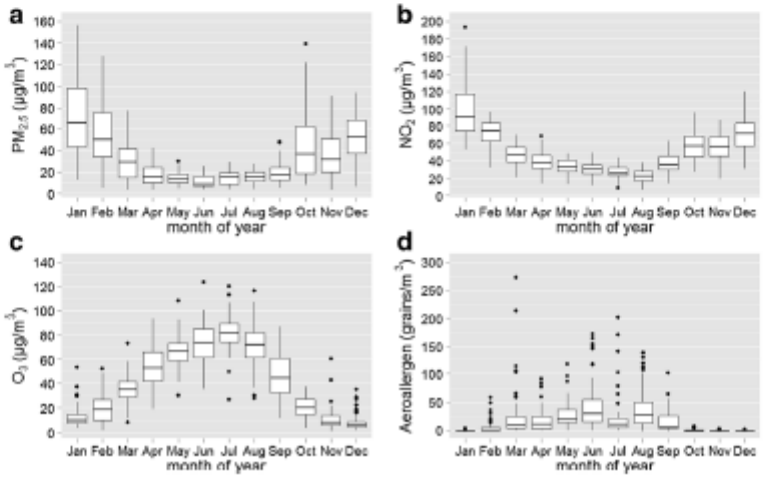


Fig. 2 Monthly distribution of daily $PM_{2.5}$, NO_2, O_3 and aeroallergen concentrations (panels **a** – **d**, respectively) during the study period

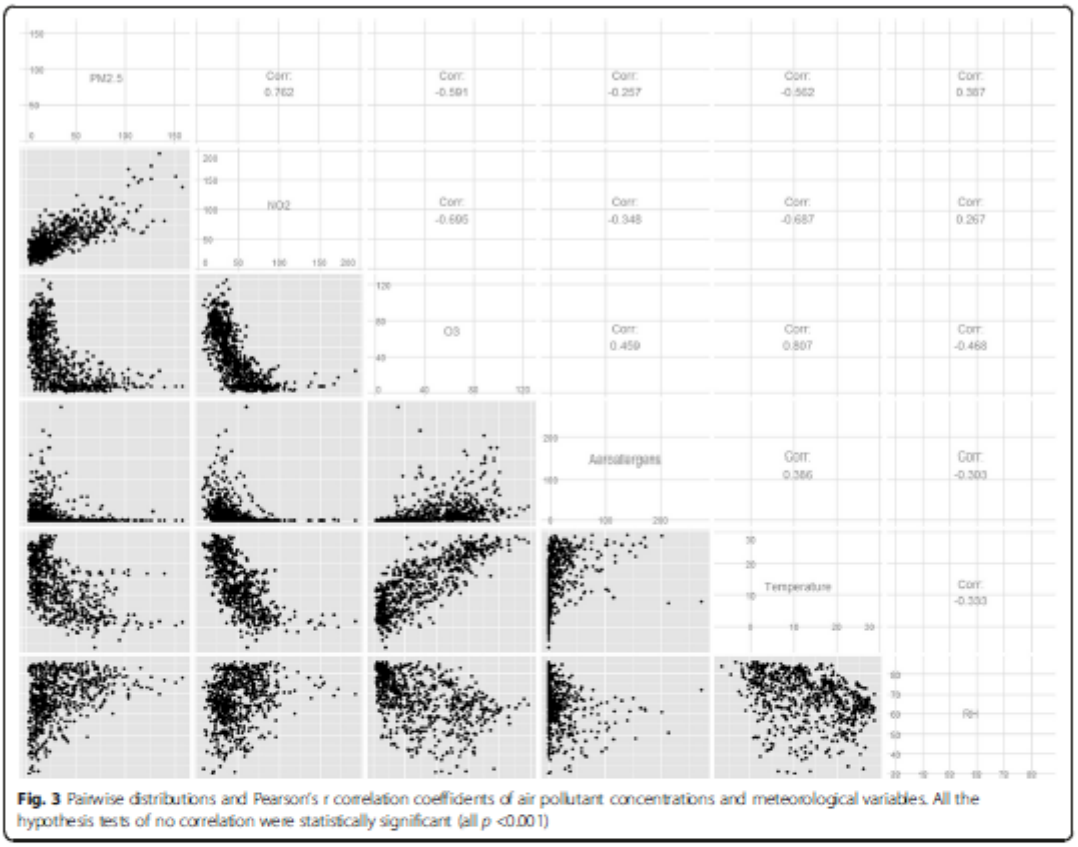


Fig. 3 Pairwise distributions and Pearson's r correlation coefficients of air pollutant concentrations and meteorological variables. All the hypothesis tests of no correlation were statistically significant (all p <0.001)

observed associations of chemical pollutants and aeroallergens with ER admissions were independent.

Discussion and conclusion

The main purpose of this study was to analyze in the selected period the trend of ER admissions for respiratory reasons in a Children's Hospital in Turin and the relationships of ER admissions with urban background chemical air pollutants and aeroallergens.

To achieve this objective, we considered the monitoring site of chemical air pollution where the data were the most complete for the study period and which was the closest to the pediatric emergency room (Additional file 1: Figure S1).

Among the 19 diseases diagnosed at the moment of the access to pediatric emergency room, as expected the upper respiratory tract infections was the most frequent reason for access to the emergency room. These infectious diseases of viral etiology are very common in pediatric age also because children generally attend kindergarten, nurseries and schools and they are therefore more exposed both to the etiological agents and to environmental risk factors such as poor air quality. Air pollution in fact, as well as tobacco smoke, can inhibit defensive mechanisms against oxidative stress [7, 15] and inflammation of the upper respiratory tract, which can favor the development of respiratory virus infections.

Despite the fact that the concentration of $PM_{2.5}$ appears quite high when compared to European levels [29], this pollutant did not reveal any significant short-term association with ER pediatric admission, as documented by other authors [36]. Instead, NO_2 showed a positive significant association with ER admissions, but only after 5 days (lag 5). Also Li et al. have shown a positive association between NO_2 air pollution at lag 5 and ER admission in children of Detroit (MI, U.S.A.) in 2011 [37]. However, in contrast to what we observed, they have also shown a positive and significant association between $PM_{2.5}$ concentrations and ER admissions. This does not seem to depend primarily on the average concentrations of $PM_{2.5}$, which were much lower than in our study area, and it may be due to a different

Table 3 Distribution of the daily number of ER admissions for respiratory diseases and daily concentrations of chemical air pollution and aeroallergens during the study period, according to the potential confounders considered in the analysis[a]

	ER admissions (counts)	$PM_{2.5}$ (μg/m^3)	NO_2 (μg/m^3)	O_3 (μg/m^3)	Aeroallergens (grains/m^3)
Day of the week					
Monday	22.7 ± 10.2	30.4 ± 26.5	47.2 ± 25.1	38.2 ± 27.7	12.7 ± 20.9
Tuesday	21.4 ± 9.8	30.8 ± 25.4	50.3 ± 24.5	37.8 ± 28.6	14.9 ± 28.8
Wednesday	20.4 ± 9.6	32.5 ± 25.4	51.0 ± 23.8	38.9 ± 29.2	18.8 ± 38.1
Thursday	21.6 ± 9.7	35.1 ± 27.8	52.9 ± 25.9	38.9 ± 30.2	15.1 ± 23.0
Friday	23.4 ± 11.1	33.3 ± 25.0	50.9 ± 24.7	39.9 ± 29.7	17.2 ± 32.1
Saturday	31.1 ± 12.4	31.5 ± 26.0	45.0 ± 25.1	42.2 ± 29.8	18.2 ± 33.4
Sunday	32.2 ± 12.8	30.4 ± 27.4	40.9 ± 24.0	41.3 ± 28.5	15.8 ± 27.4
p	<0.001	0.79	0.002	0.87	0.73
Holidays					
Christmas and Easter	47.6 ± 17.5	25.4 ± 15.2	45.1 ± 27.5	32.0 ± 29.9	1.0 ± 1.9
3 days before/after Christmas and Easter	34.9 ± 16.3	37.9 ± 21.7	58.6 ± 25.8	25.7 ± 23.7	6.4 ± 15.8
Other holidays	35.6 ± 15.6	37.4 ± 27.7	49.3 ± 22.7	32.2 ± 30.4	12.6 ± 1.9
Other days	23.8 ± 10.9	31.6 ± 26.4	48.0 ± 25.0	40.4 ± 29.1	16.6 ± 30.2
p	<0.001	0.39	0.23	0.02	0.20
Influenza outbreaks					
No	19.9 ± 9.0	22.8 ± 21.0	36.8 ± 16.9	55.2 ± 27.4	23.8 ± 31.9
Yes	31.0 ± 12.0	43.9 ± 27.5	63.0 ± 25.8	19.9 ± 16.4	6.3 ± 23.0
p	<0.001	<0.001	<0.001	<0.001	<0.001
Summer population decrease					
2 weeks around 15 August	13.4 ± 4.71	15.8 ± 5.6	20.7 ± 6.3	66.1 ± 15.4	34.7 ± 28.5
From 16/7 to 31/8 (except 2 weeks around 15 August)	14.4 ± 5.8	14.5 ± 6.4	26.1 ± 8.5	78.0 ± 19.3	29.1 ± 31.6
Other days	26.4 ± 11.6	34.5 ± 27.1	52.5 ± 24.5	33.7 ± 26.3	13.4 ± 28.7
p	<0.001	<0.001	<0.001	<0.001	<0.001
Temperature[b] (°C)					
−6.4, 6.4	29.1 ± 11.6	57.3 ± 28.3	78.9 ± 27.6	12.1 ± 8.8	0.8 ± 2.5
6.5, 12.8	28.1 ± 11.3	31.7 ± 21.5	51.7 ± 15.2	23.3 ± 17.3	10.1 ± 31.9
12.9, 20.3	24.8 ± 11.5	27.5 ± 23.9	43.4 ± 17.1	41.2 ± 20.2	14.9 ± 21.6
20.4, 28.5	19.5 ± 8.2	14.9 ± 6.8	29.4 ± 9.5	73.7 ± 18.4	32.5 ± 39.7
p	<0.001	<0.001	<0.001	<0.001	<0.001
Cumulative precipitation					
<1 mm	25.4 ± 11.4	35.5 ± 28.0	51.5 ± 26.2	38.0 ± 33.7	16.0 ± 29.9
≥1 mm	25.2 ± 11.5	25.5 ± 19.2	45.0 ± 20.1	33.7 ± 26.0	9.5 ± 27.3
p	0.87	<0.001	0.01	0.10	0.02
Relative humidity [b](%)					
30.4, 60.4	26.4 ± 10.3	17.8 ± 13.1	41.6 ± 17.9	52.1 ± 24.6	25.1 ± 34.3
60.5, 68.6	23.1 ± 11.6	25.5 ± 18.9	43.2 ± 22.2	50.9 ± 31.3	21.8 ± 37.5
68.7, 76.4	25.3 ± 11.3	44.7 ± 30.5	56.9 ± 32.1	28.3 ± 23.0	9.6 ± 25.4
76.5, 85.5	26.7 ± 12.1	42.5 ± 28.8	58.4 ± 22.1	16.7 ± 15.9	1.9 ± 6.0
p	0.01	<0.001	<0.001	<0.001	<0.001

[a]mean ± SD reported; overall *p*-values were calculated using non-parametric Kruskall-Wallis tests, under the null hypothesis that the distribution of a variables is homogeneous among the strata of a potential confounder

[b] coded in groups according to the quantiles of their frequency distribution

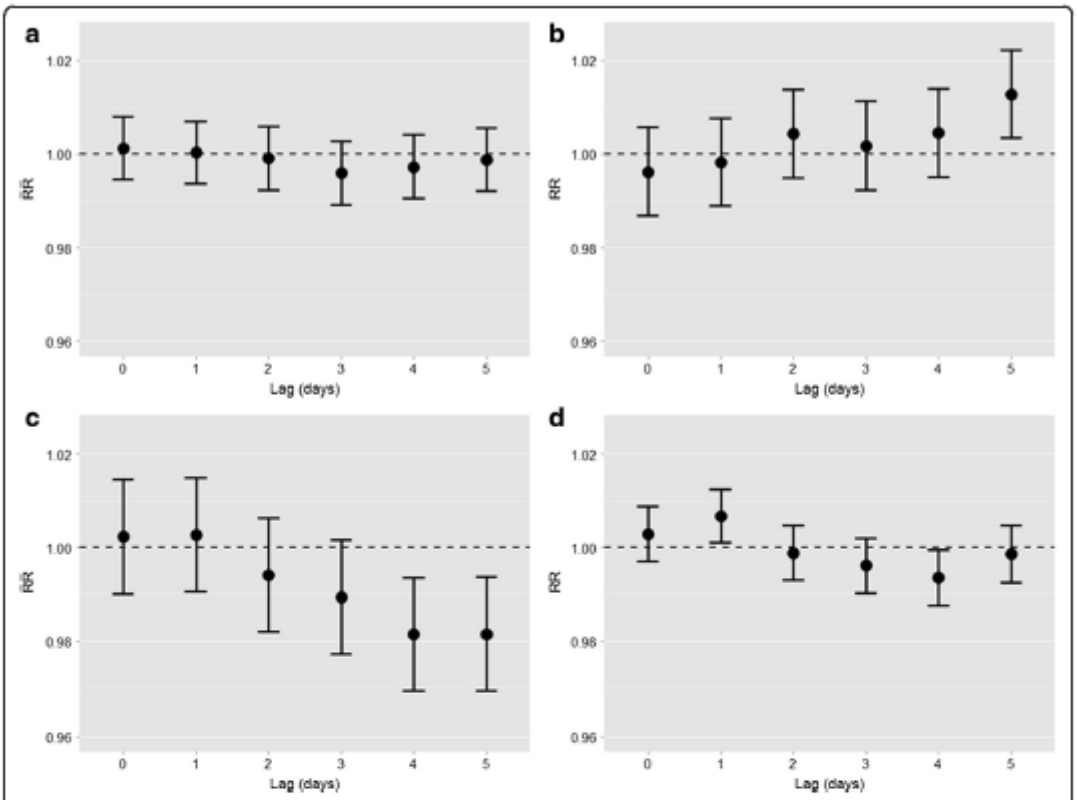


Fig. 4 Estimates of association of daily $PM_{2.5}$, NO_2, O_3, and aeroallergen concentrations (panels **a** – **d**, respectively) with ER admissions for respiratory diseases at different time lags, adjusted for medium/long-term trend function, day of the week, influenza outbreaks, holidays and summer population decrease.* *Single-pollutant models, see "model A" in the Statistical analysis section. Relative risks (RR) with 95%CIs are given for a 10 $\mu g/m^3$ increase in $PM_{2.5}$, NO_2, O_3 concentrations or a 10 grains/m^3 increase in aeroallergen concentrations

composition of particulate, perhaps more toxic in the City of Detroit than in Turin.

The adjusted estimates of relative risk for the effect of O_3 were significantly less than one, seemingly suggesting a little protective effect. In 2009, Jerrett et al. also showed how relative risk for the effect of ozone on the risk of death from cardiovascular causes were significantly less than 1.0 [38].

Such beneficial influence of ozone, however, is currently completely to exclude from toxicological point of view. In experimental studies, O_3 can increase airway inflammation [39] and can worsen pulmonary function and gas exchange [40]. In addition, exposure to elevated concentrations of tropospheric O_3 has been associated with numerous adverse health effects, including the induction [26] and exacerbation [27, 28] of asthma, pulmonary dysfunction [33, 34] and hospitalization for respiratory reasons [31]. In our study, the apparent protective effect of O_3 seems to be due to a confounding by meteorology, or to the fact that O_3 acts as a mediator of the effect of temperature. In fact, when temperature, relative humidity and precipitations were included in the models as adjustment factors, the associations between O_3 and ER admissions shifted to the null. Measurements of $PM_{2.5}$ and NOx obtained using background monitoring stations are probably more representative of population's exposure than measurements of O_3. In fact, O_3 concentrations tend to vary within cities more than $PM_{2.5}$, because of the scavenging of O_3 by NO near roadways and principally, for its photochemical origin [37]. Thus, in the presence of a high density of local traffic, the measurement error is probably higher for exposure to O_3 than for exposure to $PM_{2.5}$. The effects of O_3 could therefore be confounded by the presence of $PM_{2.5}$ because of collinearity between the measurements of the two pollutants and the higher precision of measurements of $PM_{2.5}$ [38].

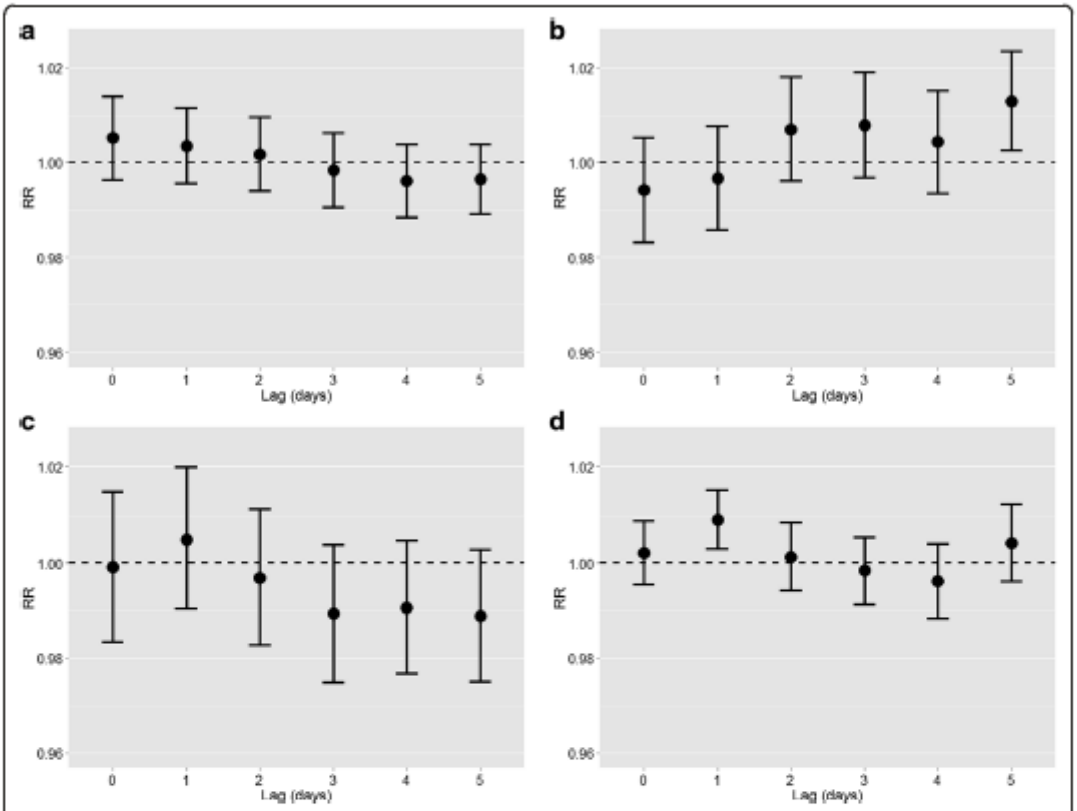


Fig. 5 Estimates of association of daily $PM_{2.5}$, NO_2, O_3, and aeroallergen concentrations (panels **a** – **d**, respectively) with ER admissions for respiratory diseases at different time lags, adjusted for medium/long-term trend function, day of the week, influenza outbreaks, holidays, summer population decrease, and for the meteorological variables (daily temperature, daily relative humidity, cumulative daily precipitations).* * Single-pollutant models, see "model B" in the Statistical analysis section of the article. Relative risks (RR) with 95%CIs are given for a 10 $\mu g/m^3$ increase in $PM_{2.5}$, NO_2, O_3 concentrations or a 10 grains/m^3 increase in aeroallergen concentrations

Finally, a 0.7 % (95 % CI: 0.1–1.2 %) increase of ER admissions for every 10 grains/m^3 increase of aeroallergens was observed at lag 1. This indicates a lower latency between the stimulus and the effect, compared to chemical pollutants. An apparent protective effect of aeroallergens at lag 4 shifted to the null when the models included meteorological adjustment variables. Adverse short-term effects of aeroallergens are supported by other studies [41, 42], although the time lags when excesses of ER admissions are observed vary according to a number of reasons, including differences in study populations, air pollutant mixtures, as well as exposure assessment and statistical methodologies applied.

A limitation in our study is that we used only one monitoring site to estimate air pollution concentrations. However, both chemical pollution and aeroallergen monitoring stations were located close to the children's hospital. It is likely that children are referred to the closest hospital, especially in the case of acute health events that are captured by ER admissions, and we can therefore hypothesize that children lived at relative close distance to the monitoring area. Any measurement error in exposures due to spatial heterogeneity in airborne air pollution concentrations is more likely to bias risk estimates toward the null than in the opposite direction [43].

In conclusion, we observed consistent and positive associations of background NO_2 and aeroallergen concentrations with ER admissions in children in a populated and heavily polluted city in western Italy. Our findings add to the existing evidence and call for urgent public health policies especially in the Po valley in northern Italy, one of the most polluted areas in Europe because

of high emissions but also poor ventilation and precipitation especially in winter. Moreover, replacement of non-allergenic cultivated plant species and their management (for example frequent grassland mowing which limit the production of flowers and consequently of pollens) can reduce the concentrations of allergenic pollens in the air [44]. Air pollution reduction policies are also recommended in the protection and promotion of public health, especially in children.

Additional file

Additional file 1: Table S1. Characteristics of the instruments of the meteorological station. **Figure S1.** Map of the city of Turin (grey line). The dots indicate the locations of the "Regina Margherita" Children's Hospital (red), the aeroallergen sampling station (green), and the chemical air pollution monitoring and weather station (blue) (© 2015 Google maps). **Figure S2.** Estimates of the association of daily PM2.5 + aeroallergens (panel A), NO2 + aeroallergens (panel B), O3 + aeroallergens (panel C) with ER admissions for respiratory diseases at different time lags, adjusted for medium/long trend function, day of the week, influenza outbreaks, holidays and summer population decrease.* (DOCX 931 kb)

Abbreviations

COPD, chronic obstructive pulmonary disease; ER: emergency room; GAMs, generalized additive models; ICD, international classification of diseases; IQR: interquartile ranges, PACF partial autocorrelation function of the residuals; PM, particulate matter

Acknowledgments

The authors are grateful to all the Institutions that have contributed to the construction of the database. In particular: the Pediatrics Emergency of Regina Margherita Children's Hospital, Turin, Italy; the Agency of Environmental Protection of Piedmont (A.R.P.A. Piemonte), S T S.r.L., Botanical Garden of University of Torino, Italy.

Authors' contributions

RB has conceived, designed, and coordinated the study, performed the analysis and wrote the manuscript; VR and VB have made substantial contributions to acquisition of data and to their analysis and interpretation; RT and GT have made substantial intellectual contribution and have contributed to the design of the study and to organization and quality control of database; AU, CC, and CS have made substantial intellectual contribution in the execution of the work and have collected and processed data related to their profession (admission data, aeroallergens, and meteorological data); PM and AM have contributed to the design of the study, revised the manuscript, performed statistical analysis and contributed to the interpretation and discussion of data.
All authors have made substantive intellectual contributions in the study, read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Public Health and Pediatrics, University of Turin, via Santena, 5 bis, 10126 Turin, Italy. [2]Pediatrics Emergency, Regina Margherita Children's Hospital, Piazza Polonia, 94, 10126 Turin, Italy. [3]Department of Physics, University of Turin, Via P. Giuria, 1, 10125 Turin, Italy. [4]Department of Life Sciences and Systems Biology, University of Turin, Viale P. A. Mattioli, 25, 10125 Turin, Italy. [5]Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Strada le Grazie, 8, Verona, Italy.

Received: 24 September 2015 Accepted: 9 June 2016
Published online: 05 August 2016

References

1. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006;368:733–43.
2. 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, GEIRD Study Group. Trends in the prevalence of asthma and allergic rhinitis in Italy between 1991 and 2010. Eur Respir J. 2012;39:883–92.
3. Decramer M, Janssens W, Miravitlles M. Chronic obstructive pulmonary disease. Lancet Lond Engl. 2012;379:1341–51.
4. Huynh BT, Tual S, Turbelin C, Pelat C, Cecchi L, D'Amato G, Blanchon T, Annesi-Maesano I. Short-term effects of airborne pollens on asthma attacks as seen by general practitioners in the Greater Paris area, 2003–2007. Prim Care Respir J J Gen Pract Airw Group. 2010;19:254–9.
5. Lagorio S, Forastiere F, Pistelli R, Iavarone I, Michelozzi P, Fano V, Marconi A, Ziemacki G, Ostro BD. Air pollution and lung function among susceptible adult subjects: a panel study. Environ Health Glob Access Sci Source. 2006;5:11.
6. Zeka A, Zanobetti A, Schwartz J. Individual-level modifiers of the effects of particulate matter on daily mortality. Am J Epidemiol. 2006;163:849–59.
7. Bono R, Tassinari R, Bellisario V, Gilli G, Pazzi M, Pirro V, Mengozzi G, Bugiani M, Piccioni P. Urban air and tobacco smoke as conditions that increase the risk of oxidative stress and respiratory response in youth. Environ Res. 2015;137C:141–6.
8. Delfino RJ, Gong H, Linn WS, Pellizzari ED, Hu Y. Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect. 2003;111:647–56.
9. Dominici F, Peng RD, Bell ML, Pham L, McDermott A, Zeger SL, Samet JM. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA. 2006;295:1127–34.
10. Schwartz J. Air pollution and hospital admissions for respiratory disease. Epidemiol Camb Mass. 1996;7:20–8.
11. Marcon A, Pesce G, Girardi P, Marchetti P, Blengio G, de Zolt SS, Falcone S, Frapporti G, Predicatori F, de Marco R. Association between PM10 concentrations and school absences in proximity of a cement plant in northern Italy. Int J Hyg Environ Health. 2014;217:386–91.
12. Dominici F, McDermott A, Daniels M, Zeger SL, Samet JM. Revised Analyses of the National Morbidity, Mortality, and Air Pollution Study: Mortality Among Residents Of 90 Cities. J Toxicol Environ Health A. 2005;68:1071–92.
13. Ostro B, Feng W-Y, Broadwin R, Green S, Lipsett M. The effects of components of fine particulate air pollution on mortality in california: results from CALFINE. Environ Health Perspect. 2007;115:13–9.
14. Babin SM, Burkom HS, Holtry RS, Tabernero NR, Stokes LD, Davies-Cole JO, Dehaan K, Lee DH. Pediatric patient asthma-related emergency department visits and admissions in Washington, DC, from 2001–2004, and associations with air quality, socio-economic status and age group. Environ Health Glob Access Sci Source. 2007;6:9.
15. Wang I-J, Tung T-H, Tang C-S, Zhao Z-H. Allergens, air pollutants, and childhood allergic diseases. Int J Hyg Environ Health. 2016;219:66–71.
16. Bono R, Bellisario V, Romanazzi V, Pirro V, Piccioni P, Pazzi M, Bugiani M, Vincenti M. Oxidative stress in adolescent passive smokers living in urban and rural environments. Int J Hyg Environ Health. 2014;217:287–93.
17. Pierse N, Rushton L, Harris RS, Kuehni CE, Silverman M, Grigg J. Locally generated particulate pollution and respiratory symptoms in young children. Thorax. 2006;61:216–20.
18. Qian Z, Chapman RS, Hu W, Wei F, Korn LR, Zhang JJ. Using air pollution based community clusters to explore air pollution health effects in children. Environ Int. 2004;30:611–20.
19. Bayer-Oglesby L, Grize L, Gassner M, Takken-Sahli K, Sennhauser FH, Neu U, Schindler C, Braun-Fahrländer C. Decline of ambient air pollution levels and improved respiratory health in Swiss children. Environ Health Perspect. 2005;113:1632–7.
20. Schindler C, Keidel D, Gerbase MW, Zemp E, Bettschart R, Brändli O, Brutsche MH, Burdet L, Karrer W, Knöpfli B, Perruchoud AP, Rapp R, Bayer-Oglesby L, Künzli N, Schwartz J, Liu L-JS, Ackermann-Liebrich U, Rochat T, SAPALDIA Team. Improvements in PM10 exposure and reduced rates of respiratory symptoms in a cohort of Swiss adults (SAPALDIA). Am J Respir Crit Care Med. 2009;179:579–87.
21. Peters JM, Avol E, Gauderman WJ, Linn WS, Navidi W, London SJ, Margolis H, Rappaport E, Vora H, Gong H, Thomas DC. A study of twelve Southern

California communities with differing levels and types of air pollution. II. Effects on pulmonary function. Am J Respir Crit Care Med. 1999;159:768–75.
22. Rosenlund M, Forastiere F, Porta D, De Sario M, Badaloni C, Perucci CA. Traffic-related air pollution in relation to respiratory symptoms, allergic sensitisation and lung function in schoolchildren. Thorax. 2009;64:573–80.
23. Avol EL, Gauderman WJ, Tan SM, London SJ, Peters JM. Respiratory effects of relocating to areas of differing air pollution levels. Am J Respir Crit Care Med. 2001;164:2067–72.
24. D'Amato G, Cecchi L. Effects of climate change on environmental factors in respiratory allergic diseases. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2008;38:1264–74.
25. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62:976–90.
26. Bono R, Degan R, Pazzi M, Romanazzi V, Rovere R. Benzene and formaldehyde in air of two Winter Olympic venues of "Torino 2006.". Environ Int. 2010;36:269–75.
27. Bono R, Bugliosi EH, Schiliro T, Gilli G. The Lagrange Street story: the prevention of aromatics air pollution during the last nine years in a European city. Atmos Environ. 2001;35:107–13.
28. Gilli G, Scursatone E, Bono R. Geographical distribution of benzene in air in northwestern Italy and personal exposure. Environ Health Perspect. 1996; 104 Suppl 6:1137–40.
29. Hazenkamp-Von Arx ME, Gotschi T, Ackermann-Liebrich U, Bono R, Burney P, Cyrys J, Jarvis D, Lillienberg L, Luczynska C, Maldonado JA, Jaen A, de Marco R, Mi YH, Modig L, Bayer-Oglesby L, Payo F, Soon A, Sunyer J, Villani S, Weyler J, Kunzli N. PM2.5 and NO2 assessment in 21 European study centres of ECRHS II: annual means and seasonal differences. London: Research Portal, King's College; 2004.
30. Mandrioli, P. The Italian Aeroallergen Network: report 1990. Aerobiologia, 1990;6(2/1):2–59.
31. Katsouyanni K, Samet JM, Anderson HR, Atkinson R, Le Tertre A, Medina S, Samoli E, Touloumi G, Burnett RT, Krewski D, Ramsay T, Dominici F, Peng RD, Schwartz J, Zanobetti A, HEI Health Review Committee. Air pollution and health: a European and North American approach (APHENA). Res Rep Health Eff Inst. 2009;142:5–90.
32. Peng RD, Dominici F, Louis TA. Model choice in time series studies of air pollution and mortality. J Royal Stat Soc Series A Stat Soc. 2006; 2006;169:179–203.
33. Biggeri A, Baccini M, Bellini P, Terracini B. Meta-analysis of the Italian studies of short-term effects of air pollution (MISA), 1990–1999. Int J Occup Environ Health. 2005;11:107–22.
34. Stafoggia M, Colais P, Serinelli M, Gruppo collaborativo EpiAir. [Methods of statistical analysis to evaluate the short term effects of air pollution in the EpiAir Project]. Epidemiol Prev. 2009;33(6 Suppl 1):53–63.
35. Traversi D, Degan R, De Marco R, Gilli G, Pignata C, Porrazio M, Rava M, Sessarego F, Villani S, Bono R. Mutagenic properties of PM2.5 air pollution in the Padana Plain (Italy) before and in the course of XX Winter Olympic Games of "Torino 2006.". Environ Int. 2008;34:966–70.
36. Linares C, Diaz J. Short-term effect of PM(2.5) on daily hospital admissions in Madrid (2003–2005). Int J Environ Health Res. 2010;20:129–40.
37. Li S, Batterman S, Wasilevich E, Wahl R, Wirth J, Su F-C, Mukherjee B. Association of daily asthma emergency department visits and hospital admissions with ambient air pollutants among the pediatric Medicaid population in Detroit: time-series and time-stratified case-crossover analyses with threshold effects. Environ Res. 2011;111:1137–47.
38. Jerrett M, Burnett RT, Pope CA, Ito K, Thurston G, Krewski D, Shi Y, Calle E, Thun M. Long-term ozone exposure and mortality. N Engl J Med. 2009;360:1085–95.
39. Anderson HR, Ponce de Leon A, Bland JM, Bower JS, Emberlin J, Strachan DP. Air pollution, pollens, and daily admissions for asthma in London 1987–92. Thorax. 1998;53:842–8.
40. Galán I, Tobías A, Banegas JR, Aránguez E. Short-term effects of air pollution on daily asthma emergency room admissions. Eur Respir J. 2003;22:802–8.
41. Gleason JA, Bielory L, Fagliano JA. Associations between ozone, PM2.5, and four pollen types on emergency department pediatric asthma events during the warm season in New Jersey: a case-crossover study. Environ Res. 2014;132:421–9.
42. Allergenicity of the ornamental urban flora: ecological and aerobiological analyses in Cordoba (Spain) and Ascoli Piceno (Italy) [http://www.uco.es/investiga/grupos/rea/publicaciones/andalucia/cordoba/Staffolani_2011.pdf]
43. Darrow LA, Hess J, Rogers CA, Tolbert PE, Klein M, Sarnat SE. Ambient pollen concentrations and emergency department visits for asthma and wheeze. J Allergy Clin Immunol. 2012;130:630–8. e4.
44. Aerobiological and ecological study of the potentially allergenic ornamental plants in south Spain - Springer [http://link.springer.com/article/10.1007/s10453-013-9311-5#/page-1]

Absolute values of lung function explain the sex difference in breathlessness in the general population

Magnus Ekström[1], Linus Schiöler[2], Rune Grønseth[3], Ane Johannessen[4], Cecilie Svanes[5,6], Benedicte Leynaert[7], Deborah Jarvis[8], Thorarinn Gislason[9,10], Pascal Demoly[11], Nicole Probst-Hensch[12], Isabelle Pin[13,14,15], Angelo G. Corsico[16], Bertil Forsberg[17], Joachim Heinrich[18], Dennis Nowak[19], Chantal Raherison-Semjen[20], Shyamali C. Dharmage[21], Giulia Trucco[22], Isabel Urrutia[23], Jesús Martinez-Moratalla Rovira[24], José Luis Sánchez-Ramos[25], Christer Janson[26,27] and Kjell Torén[2,27]

Affiliations: [1]Dept of Respiratory Medicine and Allergology, Institution for Clinical Sciences, Lund University, Lund, Sweden. [2]Section of Occupational and Environmental Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. [3]Dept of Thoracic Medicine, Haukeland University Hospital, Bergen, Norway. [4]Dept of Global Public Health and Primary Care, University of Bergen, Bergen, Norway. [5]Centre for International Health, University of Bergen, Bergen, Norway. [6]Dept of Occupational Medicine, Haukeland University Hospital, Bergen, Norway. [7]Inserm, UMR 1152, Pathophysiology and Epidemiology of Respiratory Diseases, Paris, France. [8]Respiratory Epidemiology and Public Health Group, Imperial College London, National Heart and Lung Institute, London, UK. [9]Dept of Respiratory Medicine and Sleep, Landspitali University Hospital, Reykjavik, Iceland. [10]Faculty of Medicine, University of Iceland, Reykjavik, Iceland. [11]Département de Pneumologie et Addictologie, University Hospital of Montpellier, Montpellier, France. [12]Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland. [13]Pédiatrie, Pôle Couple Enfants, CHU de Grenoble Alpes, Grenoble, France. [14]INSERM U 1209, Grenoble, France. [15]Université Grenoble Alpes, Grenoble, France. [16]Division of Respiratory Diseases, Dept of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy. [17]Division of Occupational and Environmental Medicine, Umeå University, Umeå, Sweden. [18]Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Neuherberg, Germany. [19]Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Inner City Clinic, University Hospital Munich, Ludwig-Maximilians-Universität of Munich, German Center for Lung Research, Munich, Germany. [20]INSERM U897, Institute of Public Health and Epidemiology, Bordeaux University, Bordeaux, France. [21]Allergy and Lung Health Unit, School of Population and Global Health, The University of Melbourne, Melbourne, Australia. [22]Dept of Public Health and Paediatrics, University of Turin, Turin, Italy. [23]Dept of Respiratory Medicine, Galdakao Hospital, Bizkaia, Spain. [24]Unit of Pneumology, University Hospital of Albacete, Albacete, Spain. [25]Dept of Nursing, University of Huelva, Huelva, Spain. [26]Dept of Medical Sciences, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden. [27]Both authors contributed equally.

Correspondence: Magnus Ekström, Dept of Medicine, Blekinge Hospital, SE-37185 Karlskrona, Sweden. E-mail: pmekstrom@gmail.com

@ERSpublications
The sex difference in breathlessness is explained by absolute FEV1 or FVC http://ow.ly/TXoI308DZO3

Cite this article as: Ekström M, Schiöler L, Grønseth R, *et al*. Absolute values of lung function explain the sex difference in breathlessness in the general population. *Eur Respir J* 2017; 49: 1602047 [https://doi.org/10.1183/13993003.02047-2016].

ABSTRACT Activity-related breathlessness is twice as common among females as males in the general population and is associated with adverse health outcomes. We tested whether this sex difference is explained by the lower absolute forced expiratory volume in 1 s (FEV_1) or forced vital capacity (FVC) in females.

This was a cross-sectional analysis of 3250 subjects (51% female) aged 38–67 years across 13 countries in the population-based third European Community Respiratory Health Survey. Activity-related breathlessness was measured using the modified Medical Research Council (mMRC) scale. Associations with mMRC were analysed using ordered logistic regression clustering on centre, adjusting for post-bronchodilator spirometry, body mass index, pack-years smoking, cardiopulmonary diseases, depression and level of exercise.

Activity-related breathlessness (mMRC $\geqslant$1) was twice as common in females (27%) as in males (14%) (odds ratio (OR) 2.21, 95% CI 1.79–2.72). The sex difference was not reduced when controlling for FEV_1 % predicted (OR 2.33), but disappeared when controlling for absolute FEV_1 (OR 0.89, 95% CI 0.69–1.14). Absolute FEV_1 explained 98–100% of the sex difference adjusting for confounders. The effect was similar within males and females, when using FVC instead of FEV_1 and in healthy never-smokers.

The markedly more severe activity-related breathlessness among females in the general population is explained by their smaller spirometric lung volumes.

Introduction

Breathlessness related to daily activities is common, affecting 15–45% of middle-aged and older people in the community [1–3]. More severe disability due to activity-related breathlessness measured on the modified Medical Research Council (mMRC) scale [4] is associated with worse health status [1, 5] and increased mortality [6, 7].

Females report significantly higher prevalence and severity of activity-related breathlessness than males, with odds approximately twice that of males for each level of breathlessness, both in patients with chronic obstructive pulmonary disease (COPD) [8–10] and in the general population [1, 2, 11–13].

The more severe activity-related breathlessness in females is not explained by age, body mass index (BMI), smoking, socioeconomic status, heart disease, chronic airflow limitation (CAL) or lung function impairment [1–3, 9, 11, 13, 14]. However, recent laboratory data indicate that females have a lower maximal ventilatory capacity and more ventilatory constraints during exercise [15, 16]. Furthermore, for a given level of physical activity, females have higher respiratory drive, use more of their maximal ventilatory capacity and are more breathless than males [15–17]. The sex disparity was attenuated when controlling for differences in absolute lung volume in the laboratory [15–17] and in patients with severe COPD and emphysema [10]. Taken together, this suggests revisiting the relationship between dyspnoea, sex and lung function.

No population study has evaluated the association between absolute spirometric lung volumes and the sex difference in breathlessness. Most previous studies have included relative lung function, most often forced expiratory volume in 1 s (FEV_1) or forced vital capacity (FVC) expressed as % predicted when investigating the link between sex and breathlessness [1–3, 9, 11, 13, 14]. Laboratory studies were small, did not evaluate the interplay of multiple factors or the importance of the suggested mechanisms for breathlessness related to activities of daily life [15–17]. As lower spirometric lung volume might be associated with smoking and increased morbidity, analysis in healthy never-smokers would be informative on a possible causal relationship between lung volumes and activity-related breathlessness.

We aimed to test the hypothesis that the sex difference in activity-related breathlessness is mediated through the lower absolute spirometric lung volume (FEV_1 or FVC) in females due to their (on average) smaller lungs, airways and respiratory musculature.

Material and methods

Study design and population

This was a cross-sectional analysis of the third multicentre European Community Respiratory Health Survey (ECRHS III). The ECRHS has been detailed elsewhere [18]. ECRHS III was a population-based study of people aged 38–67 years conducted at 27 centres across 12 European countries and Australia between 2010 and 2014 [19]. The present analysis included people in the random population sample with data on the mMRC breathlessness scale [4, 20]. The exclusion criterion was inability to walk for reasons other than cardiopulmonary disease.

Measurements

All participants had their height, weight and post-bronchodilator spirometry measured at local study centres and completed written questionnaires on smoking habits, respiratory symptoms, exercise habits and comorbidities.

The outcome severity of activity-related breathlessness was measured using a mMRC scale [20] as breathlessness during strenuous exercise (grade 0), when hurrying on the level or up slight hill (grade 1), when walking on the level (grade 2), when walking for a few minutes (grade 3) and at rest or during minimal activity (grade 4). Grades 3 and 4 were merged due to low numbers.

Post-bronchodilator dynamic spirometry was performed using an EasyOne spirometer (NDD, Andover, MA, USA) by certified technicians according to American Thoracic Society/European Respiratory Society

This article has supplementary material available from erj.ersjournals.com

Received: Oct 20 2016 | Accepted after revision: Jan 29 2017

Support statement: The study was supported by unrestricted grants from the Swedish Council for Working Life, Health, and Welfare (FORTE) and Sahlgrenska University Hospital. M. Ekström was supported by unrestricted grants from the Swedish Society of Medicine, the Swedish Respiratory Society, the Swedish Heart–Lung Foundation, the Scientific Committee of Blekinge County Council and the Wera and Emil Cornell Foundation. No funding organisation had a role in the design and conduct of the study, in the analysis and interpretation of data, or in the preparation or approval of the manuscript to be submitted. Funding information for this article has been deposited with the Open Funder Registry.

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

standards [21]. Absolute and relative lung volumes were defined as spirometric FEV_1 and FVC in litres and % pred, respectively. Predicted values were estimated using the Global Lung Function Initiative reference values [22]. CAL was defined as FEV_1/FVC below the 5th percentile (lower limit of normal) of the reference population [21]. Exercise was reported as weekly hours of physical activity that led to sweating. Pack-years of smoking was calculated as (mean number of cigarettes per day) × (years smoking) divided by 20. Occupational exposure was defined as work-related exposure to vapours, gas, dust or fumes. Diagnoses included chronic bronchitis, self-reported asthma, ischaemic heart disease (IHD), hypertension, history of cancer and depression.

Ethical considerations

Informed consent was obtained from each participant prior to inclusion in ECRHS III. Each study centre obtained approval for the study from their regional committee of medical research ethics according to national legislation.

Statistical analyses

Stratified analysis and ordered logistic regression were used to explore associations with the mMRC. The variance in mMRC scores explained by sex was measured using McKelvey and Zavoina's [23] R^2 as the difference in variance explained by the model with sex compared to the model without sex. The measure of primary interest was the reduction in the variance explained by sex by adding the FEV_1 or FVC to the model as absolute volume and % pred, respectively.

Potential confounders of the association between lung function and mMRC score for the final model were selected using a directed acyclical graph (DAG) of the relationships between study variables (online supplementary figure S1) [24]. The DAG was based on the literature and input from co-authors [25]. In addition, we evaluated prespecified models adjusting for diseases (asthma, chronic bronchitis, CAL, IHD, history of cancer and depression), risk factors (pack-years of smoking, exercise, occupational exposure, lung infection before the age of 5 years and hypertension) and physiological variables (age, BMI and CAL). The functional form of continuous covariates was investigated using splines. All models accounted for clustering within countries using robust variance estimation [26]. The analysis included complete cases only. No data were imputed. Associations were expressed as odds ratios (OR) with 95% confidence intervals. For each level of mMRC, an OR of 2.0 for sex is interpreted as the odds of having a higher mMRC score being twice as high in females as in males. The proportional odds assumption of ordinal logistic regression was evaluated by repeating the analyses using a partial proportional odds model, with similar findings. Findings were similar when analysing mMRC dichotomously (⩾1 *versus* 0) using logistic regression.

Analyses were performed in the total study population, females and males separately and in healthy never-smokers, defined as never-smokers without CAL, self-reported chronic bronchitis, IHD, history of cancer or depression. Statistical analyses were performed using Stata (version 12.1; StataCorp, College Station, TX, USA).

Results

Participants

3250 participants (51% female) were included in the analysis. Included and excluded patients had similar characteristics (online supplementary table S1). The mean±SD age of included participants was 54±7.0 years (table 1). Compared with males, females had lower mean absolute FEV_1 (2.7 *versus* 3.7 L), but similar FEV_1 % pred, slightly lower BMI and less smoking exposure, but more asthma and prior depression (table 1).

Sex difference of breathlessness

Activity-related breathlessness (mMRC ⩾1) was twice as common in females (27%) as in males (14%; table 1) (unadjusted OR 2.21, 95% CI 1.79–2.72) for more severe breathlessness. The sex difference was not reduced when controlling for age, BMI, chronic bronchitis, CAL, pack-years of smoking, exercise, IHD and a history of depression (OR 2.63, 95% CI 2.12–3.25) in the final model (table 2).

Predictors of breathlessness

In the final model, independent predictors of increased activity-related breathlessness were lower FEV_1 (absolute or relative value), higher BMI, less exercise, chronic bronchitis (strong association), IHD and history of depression (table 3). Smoking and CAL predicted breathlessness only when not adjusting for absolute FEV_1. The estimates for predictors were similar in males and females, except that females had weaker associations for exercise and IHD, and stronger associations for chronic bronchitis (table 3).

TABLE 1 Characteristics of 3250 subjects from the general population

	Females	Males	All
Subjects	1673 [51]	1577 [49]	3250
Age years	53.7±7.0	54.2±7.0	54.0±7.0
mMRC breathlessness score			
0	1226 [73]	1349 [86]	2575 [79]
1	343 [21]	203 [13]	546 [17]
2	95 [6]	22 [1]	117 [4]
3–4	9 [1]	3 [0]	12 [0]
FEV$_1$ L	2.67±0.46	3.68±0.66	3.16±0.76
FEV$_1$ % predicted	98.8±13.8	98.5±14.5	98.7±14.1
FVC L	3.39±0.56	4.74±0.80	4.04±0.96
FVC % predicted	99.6±12.9	99.2±13.5	99.4±13.2
FEV$_1$/FVC	0.79±0.06	0.78±0.06	0.78±0.06
BMI kg·m^{-2}	26.3±5.0	27.2±4.1	26.7±4.6
<18.5	18 [1]	5 [0]	23 [1]
18.5–<25	770 [46]	480 [30]	1250 [38]
25–30	554 [33]	775 [49]	1329 [41]
>30	331 [20]	317 [20]	648 [20]
Smoking			
Pack-years	1.3 [0–19.2]	7.0 [0–30.5]	3.9 [0–24.3]
Current smoker	283 [17]	398 [19]	581 [18]
Never-smoker	801 [48]	645 [41]	1446 [44]
Occupational exposure	569 [34]	947 [60]	1516 [40]
Exercise h·week^{-1}			
⩾2	688 [41]	763 [48]	1451 [45]
0.5–1	437 [26]	366 [23]	803 [25]
None	548 [33]	448 [28]	996 [31]
Asthma	248 [15]	171 [11]	419 [13]
CAL FEV$_1$/FVC <LLN	73 [4]	87 [6]	160 [5]
Chronic bronchitis	148 [9]	160 [10]	308 [9]
IHD	24 [1]	45 [3]	69 [2]
Severe respiratory infection aged <5 years	168 [10]	146 [9]	314 [10]
History of cancer	95 [6]	82 [5]	177 [5]
History of depression	301 [18]	168 [11]	469 [14]

Data are presented as n [%], mean±SD or median [interquartile range]. Percentages may not sum to 100, due to rounding. mMRC: modified Medical Research Council; FEV$_1$: post-bronchodilatory forced expiratory volume in 1 s; FVC: post-bronchodilatory forced vital capacity; BMI: body mass index; CAL: chronic airflow limitation; LLN: lower limit of normal; IHD: ischaemic heart disease.

Both a lower absolute and relative FEV$_1$ were associated with more severe activity-related breathlessness (online supplementary figure S2). As shown in figure 1, the associations remained when adjusting for possible confounders and were similar in males and females (table 2) for both the absolute FEV$_1$ ($p=0.69$ for interaction) and % pred ($p=0.49$ for interaction).

Spirometric lung volume and sex difference in breathlessness

Adjusting for FEV$_1$ % pred did not reduce the sex difference in activity-related breathlessness (OR 2.66, 95% CI 2.13–3.34). In contrast, the sex difference disappeared when adjusting for absolute FEV$_1$ (OR 0.89, 95% CI 0.69–1.14). This was consistent with stratified analysis (online supplementary table S2). Differences in absolute FEV$_1$ explained 98–100% of the difference (variance) in activity-related breathlessness between males and females (figure 2), which was consistent when controlling for age, BMI, smoking, exercise level, diseases and other risk factors (table 3). Findings were similar when analysing FVC instead of FEV$_1$ (online supplementary figure S3 and table S3), which is also shown in figure 2.

The absolute FEV$_1$ was closely correlated to height ($r=0.72$). In a sensitivity analysis adding height to the final model, the association for FEV$_1$ remained unchanged (OR 0.42 *versus* OR 0.48 in table 3) but the association for height became nonsignificant (OR 1.02, 95% CI 1.00–1.04; $p=0.065$). Compared with height, absolute FEV$_1$ was a stronger predictor and explained more of the sex difference in breathlessness (98% *versus* 55%). Findings were consistent when including weight instead of BMI and when not adjusting for level of exercise in the final model.

Healthy never-smokers

Findings were consistent in healthy never-smokers (n=971 (30%); 455 males and 516 females). The more severe adjusted activity-related breathlessness in females (OR 2.69, 95% CI 1.94–3.73) was not reduced by FEV_1 % pred (OR 2.71, 95% CI 1.96–3.76), but by absolute FEV_1 (OR 1.65, 95% CI 1.07–2.53; p=0.039 for change). The absolute FEV_1 explained 96% of the sex difference in activity-related breathlessness among healthy never-smokers.

Discussion

Main findings

In a middle-aged general population, we found the following. 1) Females reported approximately twice as much activity-related breathlessness as males; 2) the sex difference was eliminated when accounting for the absolute FEV_1 or FVC, whereas it was not reduced when controlling for the level of lung function impairment (% pred); and 3) the association between lower spirometric lung volumes and increased breathlessness was similar across males and females and in healthy never-smokers.

Sex difference in activity-related breathlessness

The finding of increased activity-related breathlessness in females is in line with previous population-based studies [1–3, 14], including one from five Latin American cities [1] and the Burden of Obstructive Lung Disease (BOLD) study of 15 countries, and thus seems to have high validity globally [2, 11].

The sex disparity increased from OR 2.21 to 2.63 when controlling for potential confounders, which was also seen in the BOLD study [2]. This probably reflects that several determinants of more severe breathlessness, such as overweight and IHD were less common in females than males. No factor except absolute spirometric lung volumes was found to decrease the sex difference in the final model, which was unchanged in previous studies adjusting for education level and socioeconomic status [2, 3, 14].

That absolute FEV_1 and FVC explained the sex difference in activity-related breathlessness is consistent with a study of selected patients with severe emphysema [10]. The effect of absolute spirometric lung volume was robust across models controlling for potential confounders.

The impact of absolute spirometric lung volume was not mainly related to sex differences in body size. Although height and absolute FEV_1 were closely correlated, height explained less of the sex difference (55% *versus* 98%) and did not predict breathlessness independent of the absolute FEV_1.

The association between lower spirometric lung volumes and increased breathlessness was not explained by concurrent lung volume impairment, as most participants had normal lung function and findings were similar in healthy never-smokers.

Mechanisms

The present findings are consistent with recent laboratory data that females have smaller absolute lung volumes and experience more dyspnoea for a given absolute work rate, ventilation or metabolic requirement during laboratory-based cardiopulmonary exercise testing in young and older subjects [15–17, 27]. In addition to having smaller lungs, females have narrower airways than males, even when matched on lung size (dysanapsis) [12, 28, 29]. Narrower airways could contribute to the increased exertional breathlessness in females, probably at least partly mediated through reduced ventilatory capacity [30]. In the laboratory, the sex difference in breathlessness disappears when ventilation is expressed as a percentage of maximal voluntary ventilation or when accounting for the reduced exercise capacity in females [15–17, 27]. In other words, females breathe at a higher percentage of their ventilatory capacity, resulting in increased resistive work of breathing and increased neural ventilatory drive [31] for any given work rate or minute ventilation, and therefore experience more breathlessness [17, 32].

Implications

This study extends previous laboratory data and supports that absolute spirometric lung volume has an important impact on the severity of breathlessness related to daily activities, and that it explains the difference in severity between males and females in the general population. This highlights the importance of evaluating both the relative and the absolute lung volume in research and clinical practice. By just focusing on the relative values we may miss associations of which the causal pathway includes the absolute lung volume. Relative lung volume reflects the level of lung volume impairment compared to the predicted normal value, and might reflect an active disease process (such as COPD) that influences the trajectory of absolute lung volume over time, as well as systemic consequences of the disease, health status and mortality [33]. However, the functional impact of a given impairment depends on the person's baseline absolute lung volume, which reflects the remaining ventilatory capacity. Importantly, smaller absolute spirometric lung volume was associated with more severe activity-related breathlessness both overall and

TABLE 2 Absolute forced expiratory volume in 1 s (FEV1) and sex-related difference in breathlessness

Model	Without absolute FEV1		With absolute FEV1		Percentage of sex variance in breathlessness explained by absolute FEV1
	Females *versus* males OR (95% CI)	Variance explained by sex	Females *versus* males OR (95% CI)	Variance explained by sex	
Crude	2.21 (1.79–2.72)	4.8	0.89 (0.69–1.14)	0	100
Risk factors	2.52 (2.01–3.11)	5.2	1.18 (0.88–1.59)	0.1	98
Diseases	2.28 (1.90–2.77)	4.7	0.99 (0.78–1.26)	0	100
Physiology	2.56 (2.07–3.16)	5.2	1.13 (0.79–1.61)	0	100
Final	2.63 (2.12–3.25)	5.1	1.29 (0.89–1.88)	0.1	98

Sex difference in breathlessness expressed as odds ratios (OR) of more severe breathlessness for females compared with males in models with or without the absolute value of FEV1 (n=3250). The sex estimates were crude and adjusted for risk factors (pack-years of smoking, exercise, occupational exposure, lung infection at age <5 years and hypertension); diseases (asthma, chronic bronchitis, chronic airflow limitation (CAL), ischaemic heart disease (IHD) and history of cancer or depression); and physiology (age, body mass index (BMI) and CAL). The final model was controlled for age, BMI, chronic bronchitis, CAL, pack-years smoking, exercise, IHD and history of depression. Analysis performed using ordinal logistic regression clustering on country with 3250 participants in all models. The variance in modified Medical Research Council scores explained by sex was measured as the difference in variance explained by the model with sex compared to the model without sex, using McKelvey and Zavoina's R^2 [23].

within each sex; males with smaller lungs had more severe breathlessness than males with larger lungs, with the same seen among females.

Among females in the present study, a FEV1 of 50% pred corresponds to an average FEV1 of 1.34 L, whereas the mean FEV1 in males with the same level of lung function impairment is 1.84 L, a difference of 500 mL or 37% higher compared with females. Although matched on relative lung volume, males and females can therefore have markedly different absolute lung volume or ventilatory capacity, which may explain the sex disparity in breathlessness seen in previous clinical studies matching on the FEV1 % pred [8–11]. An important implication for future clinical studies is that matching on relative lung volume puts females at a disadvantage in relation to breathlessness due to their average lower absolute lung volume. This sex bias can be overcome by accounting for absolute lung volume.

Clinically and in studies, absolute lung volumes are rarely analysed or reported and its importance in breathlessness has been largely overlooked. We propose that in both research and clinical care, relative and absolute spirometric lung volumes provide complimentary information on the lung volume impairment and remaining ventilatory reserve.

TABLE 3 Final model of absolute forced expiratory volume in 1 s (FEV1) and sex difference in breathlessness in 3250 subjects from the general population

	Overall	Females	Males
Subjects n	3250	1673	1577
Females *versus* males	1.29 (0.89–1.87)		
FEV1 per L	0.48 (0.33–0.69)	0.48 (0.33–0.69)	0.48 (0.30–0.75)
Age per year	0.99 (0.97–1.01)	0.99 (0.98–1.01)	0.98 (0.95–1.02)
BMI per kg·m^{-2}	1.10 (1.07–1.13)	1.10 (1.07–1.13)	1.11 (1.06–1.16)
Exercise ⩾2 h·week^{-1}	Ref.	Ref.	Ref.
Exercise 0.5–1 h·week^{-1}	1.53 (1.20–1.96)	1.24 (0.92–1.69)	2.31 (1.71–3.11)
No exercise	2.17 (1.66–2.84)	1.77 (1.25–2.50)	3.16 (2.20–4.55)
Smoking per pack-year#	1.00 (1.00–1.01)	1.00 (0.99–1.01)	1.01 (1.00–1.01)
CAL FEV1/FVC <LLN	1.40 (0.83–2.37)	1.44 (0.87–2.36)	1.33 (0.63–2.82)
Chronic bronchitis	2.22 (1.71–2.89)	2.68 (1.94–3.70)	1.65 (1.02–2.68)
IHD	1.60 (1.01–2.52)	1.34 (0.67–2.69)	2.01 (1.13–3.56)
History of depression	1.37 (1.10–1.71)	1.53 (1.17–2.00)	1.03 (0.64–1.66)

Data are presented as odds ratio (95% CI), unless otherwise stated. Adjusted associations with the severity of exercise-related breathlessness on the modified Medical Research Council dyspnoea (mMRC) scale overall and in males and females separately, estimated using ordered logistic regression clustering over 24 centres. The model explained 22.8% of the variation in mMRC scores. FEV1: forced expiratory volume in 1 s; BMI: body mass index; CAL: chronic airflow limitation; FVC: forced vital capacity; LLN: lower limit of normal; IHD: ischaemic heart disease; ref.: reference. #: (mean cigarettes per day/20) × (number of years of smoking).

Strengths and limitations

This was an international, multicentre study that included a large general population sample with standardised assessments across 13 countries [21]. The analyses accounted for variability between study centres and potential confounders including BMI, detailed smoking exposure and the presence of cardiopulmonary disease and risk factors. Sensitivity analyses showed consistent results supporting the validity of the findings. Both FEV_1 and FVC were analysed, as they may contribute complimentary information on airway size and respiratory mechanics which influence exertional breathlessness.

Some potential limitations deserve mentioning. First, we excluded subjects with missing study data. However, the representativeness of the sample for the general population is supported in that their characteristics were similar to those of the excluded people and were similar to in previous population-based studies [1, 2]. A previous analysis found no major effect of missing data in ECRHS on analyses of symptoms [34]. The findings pertain to subjects aged 38–67 years. Second, we lacked data on standardised exercise tests. Exercise tests have limited feasibility in large population-based studies. The mMRC is a discriminative and valid measure that performs similar to other instruments of activity-related breathlessness [4, 35, 36]. It is strongly related to health status [1, 4, 5] and mortality [6, 7], and is commonly used, enabling comparisons between studies [1, 2, 4]. Finally, we have not measured static lung volumes, diffusion capacity or cardiopulmonary exercise capacity, which should be evaluated to provide more detailed information concerning both the nature and implication of reduced spirometric lung volumes.

Relationship to prevailing hypotheses

This study does not support the prevailing view that females report more activity-related breathlessness for a given lung function impairment compared to males mainly because of hormonal, affective or sociocultural reasons [11, 12, 37, 38]. Breathlessness is a complex experience that consists of qualitatively distinct sensations that arise through interplay of biochemical, mechanical, neurobiological, affective and sociocultural factors [35]. This is reflected in our final model, where only 22.8% of the mMRC variance was explained in the population. However, accounting for differences in absolute lung volume eliminated the difference in activity-related breathlessness between males and females. Females have been found to select more unpleasant descriptors of breathlessness at peak exercise, which might be due to females breathing closer to their maximum ventilatory capacity [27]. In the same study, the intensity of breathlessness was similar in males and females when expressing ventilation as a percentage of the individual's maximum ventilation [27]. The importance of the ventilatory capacity rather than sex for exertional breathlessness is supported by laboratory findings that males and females experience a similar increase in the intensity and unpleasantness of breathlessness for a similar change in ventilatory motor drive [16].

New hypothesis

The present findings support that the functional impact of a given lung volume impairment depends on the underlying absolute lung volume [11, 39]. We further hypothesise that people with smaller absolute lung volumes are at increased risk of developing significant activity-related breathlessness in relation to different disease processes or noxious exposures such as smoking, which needs to be validated in longitudinal studies.

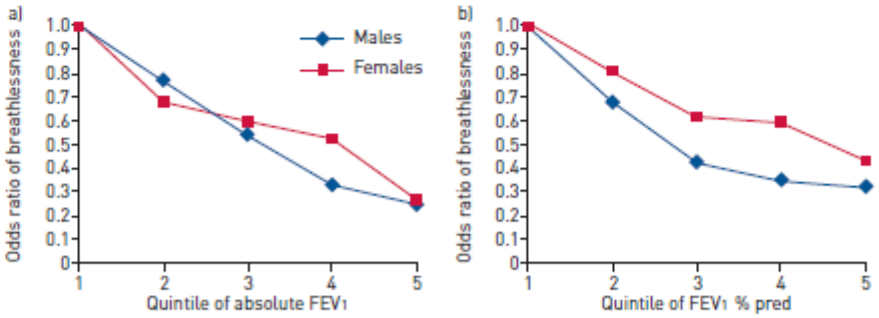


FIGURE 1 Adjusted association between absolute and relative forced expiratory volume in 1 s (FEV_1) and activity-related breathlessness. Lower FEV_1 was associated with more severe activity-related breathlessness in the general population (n=3250) for a) the absolute FEV_1 and b) the relative FEV_1 % predicted. Higher quintiles of FEV_1 imply higher FEV_1 values. Odds ratios are compared with the lowest quintile of FEV_1 (quintile 1), and are adjusted for age, body mass index, hours of exercise per week, pack-years of smoking, chronic bronchitis, chronic airflow limitation, ischaemic heart disease and history of depression. The adjusted association with modified Medical Research Council scores was similar between males and females both for absolute FEV_1 (p=0.69 for interaction) and FEV_1 % pred (p=0.49 for interaction).

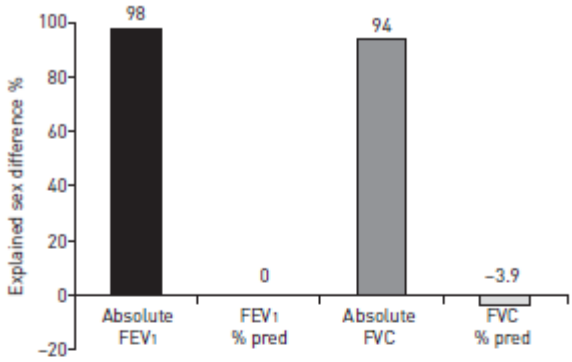


FIGURE 2 Percentage of the sex difference in activity-related breathlessness explained by forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) expressed in absolute volume and % predicted in the general population (n=3250). Sex difference was measured as the variance in the modified Medical Research Council breathlessness score explained by sex in ordinal logistic regression adjusted for age, body mass index, chronic bronchitis, chronic airflow limitation, pack-years smoking, exercise, ischaemic heart disease and a history of depression. The sex disparity disappeared when adjusting for differences in the absolute FEV1 or FVC between males and females. In contrast, the sex difference was not reduced when adjusting for FEV1 or FVC in % pred.

Conclusion

The markedly more severe activity-related breathlessness in females was explained by differences in absolute FEV1 or FVC in the general population. The association between lower lung volumes and increased breathlessness was similar in males and females and among healthy never-smokers. This highlights the importance of both relative and absolute values of lung function to evaluate the level of lung impairment and remaining ventilatory reserve.

Acknowledgements

We thank Thomas Åkesson (Dept of Clinical Physiology, Växjö Hospital, Växjö, Sweden) for valuable discussions and feedback. Authors' contributions: M. Ekström was the guarantor of the study and all authors had full access to all of the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis. Conception: M. Ekström; design: A. Johannessen, L. Schiöler, M. Ekström and R. Grønseth; acquisition of data: all authors; analysis: L. Schiöler and M. Ekström; interpretation of data: A. Johannessen, B. Leynaert, C. Janson, C. Svanes, K. Torén, L. Schiöler, M. Ekström and R. Grønseth; drafting the article: A. Johannessen, L. Schiöler, M. Ekström and R. Grønseth; revision for important intellectual content and approval of the version to be published: all authors.

References

1 Lopez Varela MV, Montes de Oca M, Halbert RJ, *et al.* Sex-related differences in COPD in five Latin American cities: the PLATINO study. *Eur Respir J* 2010; 36: 1034–1041.
2 Grønseth R, Vollmer WM, Hardie JA, *et al.* Predictors of dyspnoea prevalence: results from the BOLD study. *Eur Respir J* 2014; 43: 1610–1620.
3 Bowden JA, To TH, Abernethy AP, *et al.* Predictors of chronic breathlessness: a large population study. *BMC Public Health* 2011; 11: 33.
4 Bestall JC, Paul EA, Garrod R, *et al.* Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. *Thorax* 1999; 54: 581–586.
5 Jones PW, Adamek L, Nadeau G, *et al.* Comparisons of health status scores with MRC grades in COPD: implications for the GOLD 2011 classification. *Eur Respir J* 2013; 42: 647–654.
6 Figarska SM, Boezen HM, Vonk JM. Dyspnea severity, changes in dyspnea status and mortality in the general population: the Vlagtwedde/Vlaardingen study. *Eur J Epidemiol* 2012; 27: 867–876.
7 Nishimura K, Izumi T, Tsukino M, *et al.* Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. *Chest* 2002; 121: 1434–1440.
8 de Torres JP, Casanova C, Hernández C, *et al.* Gender and COPD in patients attending a pulmonary clinic. *Chest* 2005; 128: 2012–2016.
9 de Torres JP, Casanova C, Montejo de Garcini A, *et al.* Gender and respiratory factors associated with dyspnea in chronic obstructive pulmonary disease. *Respir Res* 2007; 8: 18.
10 Martinez FJ, Curtis JL, Sciurba F, *et al.* Sex differences in severe pulmonary emphysema. *Am J Respir Crit Care Med* 2007; 176: 243–252.
11 Lamprecht B, Vanfleteren LE, Studnicka M, *et al.* Sex-related differences in respiratory symptoms: results from the BOLD Study. *Eur Respir J* 2013; 42: 858–860.
12 Becklake MR, Kauffmann F. Gender differences in airway behaviour over the human life span. *Thorax* 1999; 54: 1119–1138.
13 Neuman Å, Gunnbjörnsdottir M, Tunsäter A, *et al.* Dyspnea in relation to symptoms of anxiety and depression: a prospective population study. *Respir Med* 2006; 100: 1843–1849.

14 Lindström M, Kotaniemi J, Jönsson E, *et al.* Smoking, respiratory symptoms, and diseases: a comparative study between northern Sweden and northern Finland: report from the FinEisS study. *Chest* 2001; 119: 852–861.
15 Ofir D, Laveneziana P, Webb KA, *et al.* Sex differences in the perceived intensity of breathlessness during exercise with advancing age. *J Appl Physiol* 2008; 104: 1583–1593.
16 Schaeffer MR, Mendonca CT, Levangie MC, *et al.* Physiological mechanisms of sex differences in exertional dyspnoea: role of neural respiratory motor drive. *Exp Physiol* 2014; 99: 427–441.
17 Guenette JA, Jensen D, Webb KA, *et al.* Sex differences in exertional dyspnea in patients with mild COPD: physiological mechanisms. *Respir Physiol Neurobiol* 2011; 177: 218–227.
18 European Community Respiratory Health Survey II Steering Committee. The European Community Respiratory Health Survey II. *Eur Respir J* 2002; 20: 1071–1079.
19 The European Community Respiratory Health Study. www.ecrhs.org Date last accessed: July 2, 2015.
20 Brooks SM. Surveillance for respiratory hazards. *ATS News* 1982; 8: 12–16.
21 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.
22 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; 40: 1324–1343.
23 McKelvey R, Zavoina W. A statistical model for the analysis of ordinal level dependent variables. *J Math Sociol* 1975; 4: 103–120.
24 Textor J, Hardt J, Knüppel S. DAGitty: a graphical tool for analyzing causal diagrams. *Epidemiology* 2011; 22: 745.
25 Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* 1999; 10: 37–48.
26 Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics* 2000; 56: 645–646.
27 Cory JM, Schaeffer MR, Wilkie SS, *et al.* Sex differences in the intensity and qualitative dimensions of exertional dyspnea in physically active young adults. *J Appl Physiol* 2015; 119: 998–1006.
28 Sheel AW, Guenette JA, Yuan R, *et al.* Evidence for dysanapsis using computed tomographic imaging of the airways in older ex-smokers. *J Appl Physiol* 2009; 107: 1622–1628.
29 Mead J. Dysanapsis in normal lungs assessed by the relationship between maximal flow, static recoil, and vital capacity. *Am Rev Respir Dis* 1980; 121: 339–342.
30 Sheel AW, Guenette JA. Mechanics of breathing during exercise in men and women: sex versus body size differences? *Exerc Sport Sci Rev* 2008; 36: 128–134.
31 Jolley CJ, Moxham J. Dyspnea intensity: a patient-reported measure of respiratory drive and disease severity. *Am J Respir Crit Care Med* 2016; 193: 236–238.
32 Guenette JA, Witt JD, McKenzie DC, *et al.* Respiratory mechanics during exercise in endurance-trained men and women. *J Physiol* 2007; 581: 1309–1322.
33 Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management and Prevention of COPD, 2017. http://goldcopd.org/gold-2017-global-strategy-diagnosis-management-prevention-copd Date last accessed: February 16, 2017.
34 Johannessen A, Verlato G, Benediktsdottir B, *et al.* Longterm follow-up in European respiratory health studies – patterns and implications. *BMC Pulm Med* 2014; 14: 63.
35 Parshall MB, Schwartzstein RM, Adams L, *et al.* An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. *Am J Respir Crit Care Med* 2012; 185: 435–452.
36 Hajiro T, Nishimura K, Tsukino M, *et al.* Analysis of clinical methods used to evaluate dyspnea in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998; 158: 1185–1189.
37 Dales RE, Spitzer WO, Schechter MT, *et al.* The influence of psychological status on respiratory symptom reporting. *Am Rev Respir Dis* 1989; 139: 1459–1463.
38 Camp PG, Goring SM. Gender and the diagnosis, management, and surveillance of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2007; 4: 686–691.
39 Camp PG, O'Donnell DE, Postma DS. Chronic obstructive pulmonary disease in men and women: myths and reality. *Proc Am Thorac Soc* 2009; 6: 535–538.

PLOS ONE

RESEARCH ARTICLE

Assessment of asthma severity in adults with ever asthma: A continuous score

Lucia Calciano[1]*, Angelo Guido Corsico[2], Pietro Pirina[3], Giulia Trucco[4], Deborah Jarvis[5], Christer Janson[6], Simone Accordini[1]

1 Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Verona, Italy, **2** Division of Respiratory Diseases, IRCCS "San Matteo" Hospital Foundation, University of Pavia, Pavia, Italy, **3** Institute of Respiratory Diseases, University of Sassari, Sassari, Italy, **4** Department of Public Health and Pediatrics, University of Turin, Turin, Italy, **5** National Heart & Lung Institute, Imperial College, London, United Kingdom, **6** Department of Medical Sciences, Uppsala University, Uppsala, Sweden

* lucia.calciano@univr.it

Citation: Calciano L, Corsico AG, Pirina P, Trucco G, Jarvis D, Janson C, et al. (2017) Assessment of asthma severity in adults with ever asthma: A continuous score. PLoS ONE 12(5): e0177538. https://doi.org/10.1371/journal.pone.0177538

Editor: Heinz Fehrenbach, Forschungszentrum Borstel Leibniz-Zentrum fur Medizin und Biowissenschaften, GERMANY

Received: June 22, 2016

Accepted: April 29, 2017

Published: May 18, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The GEIRD project was funded by the Cariverona Foundation (Bando 2006), the Italian Ministry of Health (RF-2009-1471235), Chiesi Farmaceutici and the Italian Medicines Agency (AIFA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

In epidemiological studies, continuous measures of asthma severity should be used to catch the heterogeneity of phenotypes. This study aimed at developing and validating continuous measures of asthma severity in adult patients with ever asthma from the general population, to be used in epidemiological studies.

Methods

Respiratory symptoms, anti-asthmatic treatment and lung function were measured on 520 patients with ever asthma aged 20–64 years from the general Italian population (GEIRD study; 2007/2010). The variables that represent the same dimension of asthma severity were identified through an exploratory factor analysis and were summarized through a multiple factor analysis.

Results

Only respiratory symptoms and anti-asthmatic treatment were summarized in a continuous score (STS). STS ranges from 0 (no symptoms/treatment) to 10 (maximum symptom frequency and treatment intensity). STS was positively correlated with the Global Initiative for Asthma classification of asthma severity computed on the 137 cases with a doctor's diagnosis (Spearman's coefficient = 0.61, p-value<0.0001) (concurrent validity). Furthermore, using a cohort of 1,097 European asthmatics (ECRHS II study; 1999/2002), increasing STS levels at baseline (1991/1993) were positively associated with long-term outcomes (hospitalization and lost workdays for breathing problems, asthma attack frequency and use of asthma controllers) (predictive validity). Finally, the STS scores computed from the GEIRD and ECRHS II data were comparable (Lin's coefficient = 0.95, p-value<0.0001) (replication analysis).

Competing interests: AGC and PP have received a grant for the clinical stage of the GEIRD study from Chiesi Farmaceutici. DJ reports grants from EU during the conduct of the study. All remaining authors declare that they have no competing interests. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

Abbreviations: 95%CI, 95% confidence interval; ACQ, Asthma control questionnaire; AHR, Airflow hyperresponsiveness; BMI, Body mass index; COPD, Chronic obstructive pulmonary disease; ECRHS, European Community Respiratory Health Survey; EFA, Exploratory factor analysis; GEIRD, Gene Environment Interactions in Respiratory Diseases; GINA, Global INitiative for Asthma; IQR, Interquartile range; MFA, Multiple factor analysis; RES, Ratio of expected scores; SD, Standard deviation; SoB, Shortness of breath; STS, Symptom frequency and anti-asthmatic Treatment intensity Score; RR, Adjusted rate ratio; RE, Adjusted ratio of expected values.

Conclusions

STS is a valid and replicable measure of asthma severity in adults, which could be used in association studies.

Introduction

Asthma represents a global health problem because of its high morbidity [1] and the heavy socio-economic burden [2–5].

The identification of the level of asthma severity is crucial for treatment decisions in clinical practice and for patients' characterization in epidemiological studies. In fact, asthma is not a single disease and its severity is characterized by different phenotypes that may result from different risk factors [6–8]. However, defining 'asthma severity' is not an easy task, mainly because of its heterogeneity and the lack of a worldwide consensus on its definition [9]. According to the 2002 and 2004 Global Initiative for Asthma (GINA) guidelines [10], a categorical classification of asthma severity, which is based on symptom frequency, lung function and treatment intensity has been used in several epidemiological studies [11–16]. The choice of this composite measure is justified by the fact that no single measures could accurately reflect heterogeneous phenotypes of asthma severity. However, continuous outcomes should be used for epidemiological purposes because any categorical classification of disease severity is biologically unsatisfactory for the majority of chronic diseases [17]. Moreover, individual pathophysiological characteristics of asthma severity are mainly measured on continuous or ordinal scales [9]. In addition, recognizing the nature of asthma as a continuum increases the power of a statistical analysis [18] and has the potential to reduce bias in the evaluation of risk factors for asthma [19].

The present study is aimed at developing and validating continuous measures of asthma severity that summarize the individual information on lung function, symptom frequency and treatment intensity in adult patients with ever asthma from the general population, to be used in epidemiological studies. To fulfill this purpose, the data from the Gene Environment Interactions in Respiratory Diseases (GEIRD) study were used.

Methods

Design of the GEIRD study

GEIRD (www.geird.org) is an ongoing multicentre, (multi)case-control study on respiratory health [20], which includes more than 20,000 subjects who were randomly selected from the general population in seven Italian centres (Ancona, Pavia, Salerno, Sassari, Terni, Turin and Verona). The cases of asthma, chronic obstructive pulmonary disease (COPD), chronic bronchitis and rhinitis, and the subjects without respiratory symptoms (controls) were identified through a two-stage screening process. In the first stage (2007–2010), the participants were administered a screening questionnaire on respiratory health. In the second stage (2008-ongoing), all the responders to the screening questionnaire with symptoms suggestive of asthma, COPD or chronic bronchitis, and a random sample of those with symptoms suggestive of rhinitis or without respiratory symptoms, were invited to undergo a detailed clinical interview, lung function and laboratory tests for accurate phenotyping. Ethics approval was obtained from the appropriate ethics committee ("Comitato Etico per la Sperimentazione dell'Azienda

Ospedaliera Istituti Ospitalieri di Verona"). All participants were fully informed about all the aspects of the research project and they gave written informed consent.

Cases of asthma

The subjects were defined as having asthma if they had reported at least one of the two following conditions at the clinical examination:

1. ever asthma;
2. asthma-like symptoms [wheezing, nocturnal tightness in the chest, shortness of breath (SoB) following strenuous activity, SoB at rest, SoB at night time] or anti-asthmatic treatment in the past 12 months at the clinical interview, and if they had fulfilled at least one of the following spirometric criteria:
   a. being positive to the methacholine challenge test with a <1 mg dose producing a 20% fall in FEV_1;
   b. having a pre-bronchodilator airflow obstruction {FEV_1/FVC <70% or <Lower Limit of Normal for FEV_1/FVC according to Quanjer [21]} and a positive reversibility test (increase in FEV_1 >12% and >200 ml with respect to pre-bronchodilator FEV_1 after 400 mcg of salbutamol);
   c. having pre- but not post-bronchodilator airflow obstruction, and a post-bronchodilator $FEV_1 \geq 80\%$ predicted [21].

At the time of the present analysis, only the data on the patients recruited in the Pavia, Sassari, Turin and Verona centres were available. The screening questionnaire was mailed to 17,084 eligible subjects (GEIRD-stage 1; 2007–2010) in these centres. Among the responders (response rate: 59.4%), 4,792 subjects were invited for further clinical investigations (GEIRD-stage 2; 2008–2010). Among the 1,640 subjects who were phenotyped, 577 subjects were classified as cases of asthma (Fig 1).

Computation of asthma severity scores

Asthma severity scores were devised through a two-step procedure, which was carried out on the 520 cases of asthma (out of the 577 cases identified in the four centres) with complete information on disease severity (respiratory symptoms, lung function and anti-asthmatic treatment).

Step 1. An exploratory factor analysis (EFA) [22] was performed on 11 variables (listed in Table 1) in order to identify the subset of variables representing the same dimension (factor) of disease severity. This model is based on the assumption that the symptom, treatment and lung function variables are correlated through some unobservable factors. The values of each variable were ordered coherently with an increasing level of disease severity. EFA was based on the mixed correlation matrix between each pair of variables [23], i.e. the polychoric correlation between two ordinal variables, the tetrachoric correlation between two dichotomous variables, the Pearson moment correlation between two continuous variables, and the polyserial correlation if one variable is categorical and the other one is continuous.

Factors were retained at EFA if the eigenvalue was greater than the mean of eigenvalues and by means of the break in the scree plot (eigenvalues vs. factors). The uniqueness (i.e. proportion of variance in a given variable that is not due to the extracted common factor) was used to identify the variables that were poorly correlated with the extracted factors. The Kaiser-Meyer-Olkin (KMO) measure was used for assessing the adequacy of the fitted model. The variables

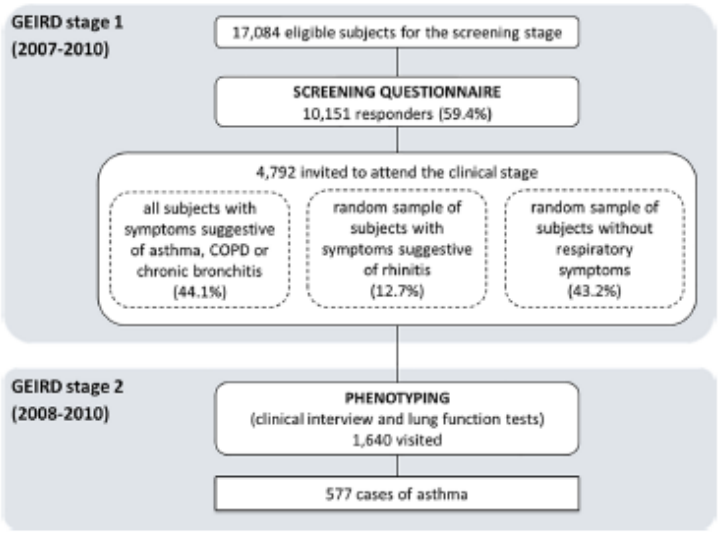


Fig 1. Flow chart of the GEIRD study in the Pavia, Sassari, Turin and Verona centres, and selection of the cases of asthma.

https://doi.org/10.1371/journal.pone.0177538.g001

with a KMO values <0.7 were excluded from the later dimensionality reduction procedure (MFA).

Step 2. A multiple factor analysis (MFA) [24] was performed on the subset of variables representing each dimension of asthma severity (identified at EFA) in order to estimate the weights to be used for computing the corresponding individual score. In MFA, the symptom, treatment and lung function variables were grouped in three different sets. For each dimension of asthma severity, the individual scores were obtained as the weighted linear combination of the variables included in MFA (Methods section in S1 File). All variables were analyzed on the quantitative scale. Any component that had the eigenvalue greater than the mean of the eigenvalues was retained. Moreover, the break in the scree plot was used to retain components. A bootstrap procedure with 50,000 replications was used to obtain a stable solution for eigenvalues, weights and scores.

Concurrent validity

Spearman's coefficients were computed to evaluate the concordance between the identified scores and a categorical classification of asthma severity, which was defined according to the GINA guidelines [25] on the 137 cases who had reported a doctor's diagnosis of asthma and complete information on the GINA classification of asthma severity.

Predictive validity

The data from the European Community Respiratory Health Survey (ECRHS), which is an international, population based cohort study on respiratory health in subjects aged 20–44 years at the time of recruitment (ECRHS I; 1991–1993) [26], were used to evaluate the ability of the identified scores to predict future events. The predictive validity was verified on the subjects with ever asthma from 26 centres, who were identified at the ECRHS I and had

Table 1. Coding and definition of the candidate variables.

Name	Definition	Coding†
Wheezing	"How many times have you had wheezing or whistling in the last 12 months?"	0 (ever)
		1 (sometimes)
		2 (at least once a week)
Asthma attacks	"How many attacks of asthma have you had in the last 12 months?"	0 (none)
		1 (1–11 attacks)
		2 (>12 attacks)
Tightness in chest	"Have you woken up with a feeling of tightness in your chest at any time in the last 12 months?"	0 (no)
		1 (yes)
SOB at rest	"Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months?"	0 (no)
		1 (yes)
SOB after strenuous activity	"Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months?"	0 (no)
		1 (yes)
SOB at night time	"Have you been woken by an attack of shortness of breath at any time in the last 12 months?"	0 (no)
		1 (yes)
Chronic bronchitis	Cough or phlegm on most days for a minimum of three months a year and for at least two successive years.	0 (no)
		1 (yes)
Worsening of respiratory symptoms	"In the last 12 months, have you had any episodes/times when your symptoms (cough, phlegm, shortness of breath) were a lot worse than usual?" or "In the last 12 months have you visited a hospital casualty department or emergency room (for breathing problems)?" or "In the last 12 months, have you spent a night in hospital (for breathing problems)?"	0 (no)
		1 (yes to at least one of the three questions)
Treatment	Intensity of anti-asthmatic treatment in the past 12 months.	0 (no treatment)
		1 (GINA step 1- only relievers)*
		2 (GINA step 1—controllers) **
		3 (GINA steps ≥2)***
FEV_1% predicted	Pre-bronchodilator FEV_1% predicted	(continuous)
FEV_1/FVC	Pre-bronchodilator FEV_1/FVC	(continuous)

† all variables were ordered coherently with an increasing level of asthma severity, i.e. the higher the level of the observed variables, the higher the value of asthma severity.

* GINA step 1—only relievers: treatment with short-acting β2-agonists and/or anticholinergic and/or ketotifen without controllers in the past 12 months.

** GINA step 1—controllers with/without taking relievers: discontinuous treatment with Inhaled glucocorticosteroids (ICS) or Cromones or Oral Methylxanthines or Leukotriene modifiers or ICS & Long-acting β2-agonists or ICS & Methylxanthines or ICS & Leukotriene modifiers with/without taking relievers in the past 12 months.

*** GINA steps ≥2: treatment with ICS (daily) or Cromones (daily) or Oral Methylxanthines (daily) or Leukotriene modifiers (daily) or ICS & Long-acting β2-agonists (daily) or ICS & Methylxanthines (daily) or ICS & Leukotriene modifiers (daily) or Oral glucocorticosteroids or anti-IgE or Injective Corticosteroids with/without taking relievers in the past 12 months.

https://doi.org/10.1371/journal.pone.0177538.t001

participated in the ECRHS II (1999–2002), by evaluating the association between the scores computed at the ECRHS I (using the weights estimated from the GEIRD data) and the following long-term outcomes measured at the ECRHS II: (i) having at least one emergency department visit and/or hospital admission for breathing problems during the follow-up, (ii) number of asthma attacks in the past 12 months, (iii) use of asthma controllers in the past 12 months, and (iv) having lost at least one working day due to breathing problems in the past 12 months. The association between the ECRHS-scores and each outcome was evaluated by using two-level regression models, with subjects (1st level units) nested into centres (2nd level units). The hospitalization Rate Ratios (RR) were computed by using two-level Poisson regression models by setting the person-years equal to half the length of the follow-up in the case of at least one

hospitalization. The adjusted Ratios of Expected number of asthma attacks (RE) were computed by using a two-level negative binomial regression model. We chose a negative binomial model because of the over-dispersed distribution of the "number of asthma attacks" variable (Pearson's moment coefficient of skewness = 8.7) and its better fit as compared to the Poisson model (likelihood ratio test, p-value<0.0001). The association of the ECRHS-scores with the remaining outcomes was measured through the adjusted Ratio of Expected values (RE) obtained by means of a two-level Poisson regression model. All the regression models had a random intercept term at the 2nd level, and the ECRHS-score and potential confounders (gender, age, BMI and smoking habits at baseline) as fixed effects. The 95% confidence intervals (CIs) were obtained by using a robust variance estimator (Huber-White sandwich estimator).

Replicability

The robustness of the identified scores was verified by testing whether the MFA weights change when estimated from another, similar population [27]. The data from the subjects with ever asthma, who were identified at the ECRHS I and had participated in the ECRHS II, were used. The scores obtained by using the weights from the GEIRD data were compared to the scores of the same dimension, which were computed by using the weights from a new MFA on the ECRHS II data. The Lin's concordance correlation coefficient was used (a value $\geq$0.80 indicates a good replicability).

The statistical analyses were carried out using STATA, version 13.0, and R version 3.1.0.

Results

Main characteristics of patients

In our sample, the percentage of females was 51.9%, the mean age was 43.0 years (SD, standard deviation; SD = 9.4) and the median BMI was 24.4 (IQR, interquartile range; IQR = 21.7–27.0) (Table 2). Ever smokers were predominant (51.4%). About two out of three patients reported at least one attack of asthma, the presence of at least one asthma-like symptom, the use of anti-asthmatic treatment, the worsening of symptoms or the use of hospital services in the past 12 months. In addition, 32.5% of these subjects had used anti-asthmatic treatment in the past 12 months, and 15.0% of the 314 asthmatics with available information had reported a not well controlled disease {i.e. 5-item Asthma Control Questionnaire (ACQ) score [28] less than 20}. Finally, pre-bronchodilator FEV_1% predicted and FEV_1/FVC were 102.2 (SD = 14.3) and 78.6 (SD = 7.8) on average, respectively.

Dimensionality reduction procedure

The mixed correlation matrix suggested that there was redundant information in the data and that the two lung function variables were weakly correlated with the variables regarding respiratory symptoms and anti-asthmatic treatment (Table A in S1 File). Moreover, the EFA identified one factor that explained 84% of the total variance, whereas this factor explained 5% and 8% of the variance of pre-bronchodilator FEV_1% predicted and FEV_1/FVC, respectively. In addition, the model adequacy checking indicates that the lung function variables may not belong to the extracted factor (Table B and Figure A in S1 File). Therefore, only the 9 variables regarding symptom frequency and anti-asthmatic treatment intensity represent the same dimension of asthma severity and were considered in the dimensionality reduction procedure.

The MFA extracted two components that accounted for 41% and 19% of the total variance, respectively. Only the first MFA component was considered as a score of asthma severity

Table 2. Main characteristics of the 520 cases of asthma identified in the GEIRD study.

Main characteristic	Sub-characteristic	Summary statistics	Numerical value
Females		%	51.9
Age (years)		mean ± sd	43.0 ± 9.4
Smoking habits	Never smoker	%	48.6
	Past smoker		26.0
	Current smoker		25.4
BMI		median (interquartile range)	24.4 (21.7–27.0)
Wheezing*	Never	%	60.0
	Sometimes		32.7
	At least once a week		7.3
Asthma attacks*	None	%	80.0
	1–11 attacks		15.6
	≥ 12 attacks		4.4
Tightness in chest*		%	21.0
SOB at rest*		%	12.1
SOB after strenuous activity*		%	23.3
SOB at night time*		%	16.0
Chronic bronchitis		%	17.7
Worsening of respiratory symptoms*		%	15.8
Treatment*	None	%	64.4
	GINA step 1—only relievers		14.0
	GINA step 1 –controllers		12.7
	GINA steps ≥ 2		8.9
Pre-bronchodilator FEV_1% predicted		mean ± sd	102.2 ± 14.3
Pre-bronchodilator FEV_1/FVC		mean ± sd	78.6 ± 7.8
ACOS**		%	3.1
ACQ	5–19	%	15.0
	20–24		38.2
	25		46.8

SOB, shortness of breath; ACOS, Asthma-COPD Overlap Syndrome; ACQ, Asthma Control Questionnaire.

* in the past 12 months.

** post-bronchodilator FEV_1/FVC <Lower Limit of Normal according to Quanjer (21).

https://doi.org/10.1371/journal.pone.0177538.t002

because all the weights had the positive sign, as expected for a measure of disease severity (Table C and Figure A in S1 File).

Symptom frequency and anti-asthmatic Treatment intensity Score (STS)

According to the coding of the variables as reported in Table 1, the equation used to compute individual STS is the following:

$$\begin{aligned} STS = {} & 1.03\ (\text{Wheezing}) + 0.85\ (\text{Asthma attacks}) + 0.48\ (\text{Tightness in chest}) \\ & + 0.32\ (\text{SOB at rest}) + 0.46\ (\text{SOB after strenuous activities}) \\ & + 0.38\ (\text{SOB at night time}) + 0.27\ (\text{Chronic bronchitis}) \\ & + 0.34\ (\text{Worsening of respiratory symptoms}) + 1.33\ (\text{Treatment}). \end{aligned}$$

STS ranges from 0 (no respiratory symptoms and no anti-asthmatic treatment) to 10 (maximum level of symptom frequency and treatment intensity) on a continuous scale, and it has a skewed distribution towards low values (Fig 2), which can be approximated to some common

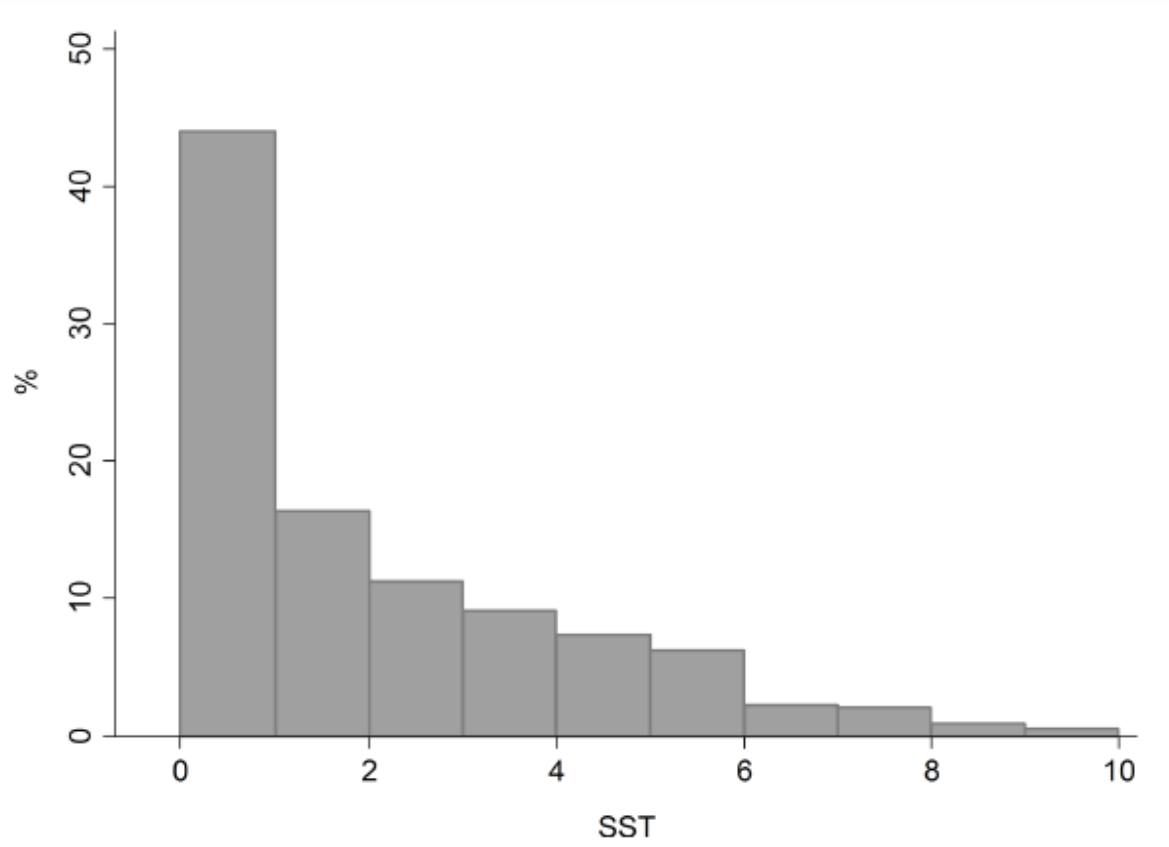


Fig 2. Distribution of the Symptom frequency and anti-asthmatic Treatment intensity Score (STS). Data from the GEIRD study.

https://doi.org/10.1371/journal.pone.0177538.g002

parametric distribution, such as a Zero-inflated Gamma. In our sample, STS ranged from 0 to 9.35 (median = 1.20, IQR = 0–3.53) and 33.5% of the 520 patients had a score equal to 0, because they had reported ever asthma without the presence of respiratory symptoms and without the use of asthma medications in the past 12 months.

Table 3. Median of the Symptom frequency and anti-asthmatic Treatment intensity Score (STS) according to the GINA classification of asthma severity. Data from the GEIRD study.

GINA classification ¯	N	Median (IQR)	p-value
Intermittent	72	3.61 (2.58–4.48)	<0.00001†
mild persistent	17	5.17 (4.07–6.59)	
moderate persistent	17	5.66 (3.99–6.13)	
severe persistent	31	5.99 (5.02–7.70)	

IQR, interquartile range.

¯ GINA classification was calculated on patients with a doctor's diagnosis of asthma.

† Spearman's correlation coefficient, which was computed to evaluate the concordance between STS and the GINA classification of asthma severity.

https://doi.org/10.1371/journal.pone.0177538.t003

Concurrent validity of STS

Among the 137 GEIRD cases with an asthma diagnosis, the individuals who had received daily controller medications had a significantly lower STS (median = 0.46, IQR = 0–1.86) as compared to both the subjects treated with non-daily controller medications (median = 4.47, IQR = 3.48–5.47) and the subjects not treated with controller medications (median = 5.87, IQR = 4.45–7.47) in the past 12 months. Moreover, STS was positively correlated with the GINA classification of asthma severity (Spearman's coefficient = 0.61, p-value<0.0001) (Table 3).

Predictive validity of STS

In the ECRHS cohort, 1,097 subjects with ever asthma had provided complete information on STS at the ECRHS I and on at least one long-term outcome (Table D in S1 File). We found that the long-term outcomes were predicted by increased values of STS (Table 4). In particular, for one-unit increase in STS, the risk of hospitalization for breathing problems during the 9-year follow-up (between the ECRHS I and II) raised by 31% and the expected number of asthma attacks in the past 12 months at ECRHS II raised by 46%. Furthermore, at ECRHS II, the expected number of subjects with at least one working day lost due to breathing problems in the past 12 months increased by 25%; the same figure was observed for the use of controller medications.

Replicability of STS

In the ECRHS cohort, 1,327 subjects with ever asthma had provided complete information on STS at the ECRHS II (Table D in S1 File). The STS scores computed from the GEIRD and ECRHS II data were comparable (Fig 3), the Lin's coefficient being equal to 0.95 (p-value<0.0001), which indicates an excellent replicability.

Discussion

The main results of the present study are the following:

Table 4. Association between the Symptom frequency and anti-asthmatic Treatment intensity Score (STS) at baseline (1991–1993) and long-term outcomes at the end of follow-up (1999–2001). Data from the ECRHS study.

Long-term outcomes (measure of association)	Estimate * [95%CI]	p-value
Hospitalization** (RR)†	1.31 [1.24, 1.39]	<0.001
N. of asthma attacks‡ (RE)§	1.46 [1.30, 1.63]	<0.001
Use of controller drugs‡ (RE)§	1.25 [1.21, 1.29]	<0.001
At least one working day lost‡ (RE)§	1.25 [1.16, 1.35]	<0.001

RR, adjusted rate ratio; RE, adjusted ratio of expected values.

* for one-unit increase in STS.

** at least one emergency department visit and/or hospital admission for breathing problems during the 9-yr follow-up (between the ECRHS I and II).

† adjusted for gender, age, BMI and smoking habits at ECRHS I.

‡ in the past 12 months at ECRHS II.

§ adjusted for gender, age, BMI and smoking habits at baseline, and length of the follow-up.

https://doi.org/10.1371/journal.pone.0177538.t004

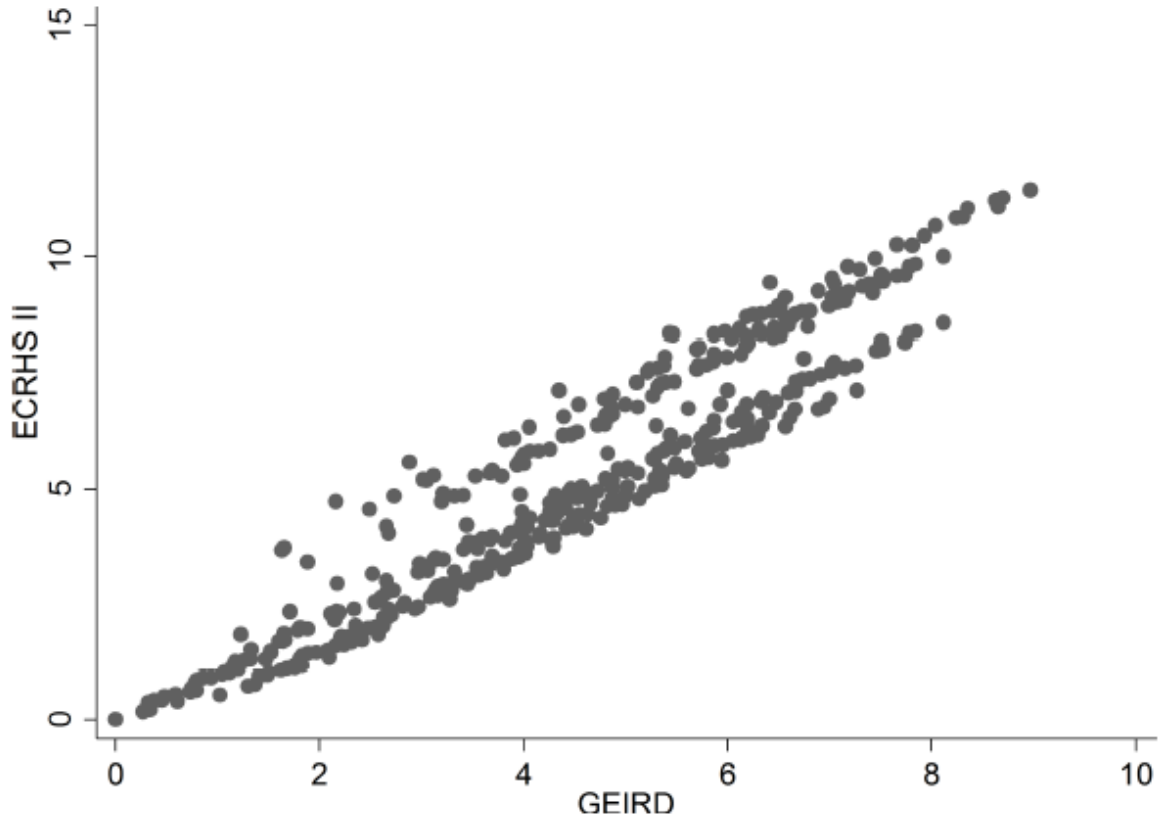


Fig 3. Relationship between the Symptom frequency and anti-asthmatic Treatment intensity Score (STS) computed by using the weights from the GEIRD data (horizontal axis) and STS computed by using the weights from the ECRHS II data (vertical axis).

https://doi.org/10.1371/journal.pone.0177538.g003

1. Lung function variables were weakly correlated with respiratory symptoms and anti-asthmatic treatment in adults. Therefore, lung function, symptom and treatment variables should not be summarized in one dimensional score of asthma severity.
2. A continuous measure of asthma severity has been devised, which summarizes the frequency of respiratory symptoms and the intensity of anti-asthmatic treatment. This score has been proved to be a valid measure of asthma severity in adults and it seems to be replicable in adult populations from different European countries. Moreover, a parametric modelling approach can be adopted in analyzing this score.

Different dimensions of asthma severity

We investigated which scores could summarize the different dimensions of asthma severity in adult patients, i.e. the frequency of respiratory symptoms, the intensity of anti-asthmatic treatment and lung function. Since the list of symptoms described in the GINA guidelines is claimed to be incomplete [29], a larger set of respiratory symptoms was considered in the present analyses.

In order to create summary measures of asthma severity, the redundancy of information in the considered variables is essential. A correlation between respiratory symptoms and lung function is expected because wheezing, tightness in the chest and cough are most likely to be obstructive symptoms. However, other respiratory symptoms, such as shortness of breath, probably reflect non-obstructive dyspnea [29]. We found a weak correlation between our set of symptom and anti-asthmatic treatment variables, and the two lung function measures. This fact is in agreement with the results from other studies, which have shown a modest association between lung function decline and increased respiratory symptoms [30–31]. In addition, only 5% and 8% of the variance of FEV_1% predicted and FEV_1/FVC, respectively, is explained by a common, unobserved factor found in the EFA analysis. When EFA was repeated without the lung function measures, the unobserved factor explains more than 84% of the common variance of the symptom and treatment variables. Therefore, combining respiratory symptoms and lung function variables into a single score is not supported by our data, as found in another study in which lung function had only a small impact on the categorization of asthma severity [32].

MFA helps to remove the redundant information in symptom and treatment variables. We identified two components that explain a high proportion of the total variance (Table C and Figure A in S1 File). The first component can be interpreted as a measure of asthma severity in adult subjects because all weights connected to the frequency of respiratory symptoms and the intensity of anti-asthmatic treatment have a positive sign. The second component separates the patients with the maximum intensity of treatment but no symptoms, from the individuals with no treatment but the maximum frequency of symptoms, because the weights of the treatment and symptom variables have an opposite sign. Therefore, the second component should be a measure of asthma control. In fact, recent guidelines report that asthma control refers to the extent to which the disease manifestations have been reduced or removed by treatment [33]. Despite being different clinical constructs, asthma severity and asthma control are related. In fact, we found a weak negative correlation between STS and the 5-item ACT score (Pearson's coefficient = -0.47, p-value$<$0.0001).

STS and asthma severity

In several epidemiological studies, measures of asthma severity are computed as the sum of symptoms (i.e. equal weights are assigned to each component variable) [34–35]. STS makes it possible to weight the contribution to asthma severity of each symptom and anti-asthmatic treatment in respect to these simple scores. The self-reported intensity of anti-asthmatic treatment has the highest weight in STS. Asthma medications reflect the physician assessment of a patient's underlying severity. In fact, the utilization of a categorical classification of treatment intensity as an approximate index of asthma severity is suggested in population studies where clinical data on disease severity are lacking [36]. Among respiratory symptoms, wheezing has the highest weight in the STS equation, followed by asthma attacks. In fact, different studies have shown that wheezing (alone or in combination with other symptoms) is the most common physical finding in adult asthma [37–39].

The use of continuous scores is recommended in epidemiological studies [18]. In fact, asthma symptoms exist as a continuum in a population [19], and data supporting the existence of one (or more) cut-off points that discriminate subjects into severe (or different levels of severity) and not-severe asthma, are somewhat arbitrary. Furthermore, continuous measures make it possible to increase the power in association analyses, and this fact is particularly important when the sample size is small [19]. Moreover, the power of an association analysis further increases by using a parametric modelling approach for STS, since it is possible to assume a known theoretical distribution of the score [40].

STS is a useful measure of asthma severity in population studies because its validity and replicability, which are two fundamental characteristics of any measurement procedure [41], were demonstrated. Concurrent validity was proved by evaluating the relation between STS and an alternative classification of asthma severity, based on the GINA guidelines. However, the predictive validity is a stronger criterion for validating a measuring instrument [42], as compared to the concurrent validity, because a gold standard for asthma severity does not currently exist. Accordingly, strong positive relationships were observed between an increasing level of STS and the worsening of different long-term outcomes. Moreover, we found a very small variation in the STS weights, when they were computed by using the data from geographically and culturally different European populations (ECRHS). This variation in STS weights (Fig 3) can be attributed to a combination of differences between the clinical questionnaires used in the GEIRD and ECRHS II studies (see "Strengths and weakness of the study" paragraph) and between the characteristics of the GEIRD and ECRHS populations (Table D in S1 File).

Lung function and asthma severity

Pre-bronchodilator FEV_1% predicted and FEV_1/FVC are used as objective measures of respiratory health. The use of FEV_1 is recommended to determine the severity of airflow obstruction, and FEV_1/FVC to confirm an obstructive defect [43]. Lung function measurements are important because patients, especially those who have frequent exacerbations, could have a poor perception of the severity of their symptoms [44]. In addition, people who have a sedentary lifestyle might not experience bothersome symptoms even if they have low lung function [33]. However, lung function tests alone are not sufficient, and the understanding of patient's symptom severity is equally important.

Strengths and weakness of the study

The main strength of the present analysis is that our cases of asthma underwent an accurate phenotyping by means of an extended clinical interview and lung function tests [20]. Moreover, the data were collected in patients who had been identified from the general population, rather than from clinically selected groups, which should guarantee that our sample encompasses a wide spectrum of disease severity.

A few caveats should be taken into account when interpreting our results. Although our patients were identified from large samples of subjects from the general population, the number of asthma cases was relatively small. However, our estimates do not change when computed from a larger number of patients selected from the European population. There are some differences between the clinical questionnaires used in the GEIRD and ECRHS II studies. In particular, in the ECRHS II questionnaire, "wheezing" and "worsening of respiratory symptoms" variables refer to the "presence of wheezing or whistling in the past 12 months" and "having at least one emergency department visit or hospital admission for breathing problems in the past 12 months", respectively. Finally, the age range of the GEIRD (20–64 yrs) and ECRHS (28–57 yrs) cases of asthma are not perfectly overlayable.

Conclusions

Lung function, symptom and treatment variables seem to represent different dimensions of asthma severity in adults with ever asthma. Therefore, we propose STS as a continuous measure of the frequency of respiratory symptoms and the intensity of anti-asthmatic treatment, to be used in epidemiological studies. This score has been proved to be a valid and replicable measure of asthma severity and its distribution can be approximated to some known parametric distribution, such as a Zero-inflated Gamma.

Supporting information

S1 File. Table A—Mixed correlation matrix of the candidate variables. **Table B**—Factor weights, uniqueness and the Kaiser-Meyer-Olkin (KMO) measure for the first factor at EFA, with and without the lung function variables. **Table C**—Mean, 95% confidence interval and coefficient of variation (CV) ** of the maximum eigenvalue, proportion of variance accounted for by each component and weights. **Table D**—Main characteristics of the cases of asthma from the ECRHS study. **Figure A**—Scree plot of eigenvalues from (a) Exploratory Factor Analysis (EFA) and (b) Multiple Factor Analysis (MFA).
(DOCX)

S1 Data. Minimal data set to replicate the reported study findings.
(XLSX)

Author Contributions

Conceptualization: LC SA.

Data curation: LC.

Formal analysis: LC SA.

Investigation: LC AGC PP GT DJ CJ SA.

Methodology: LC SA.

Software: LC.

Supervision: SA.

Visualization: LC.

Writing – original draft: LC SA.

Writing – review & editing: LC AGC PP GT DJ CJ SA.

References

1. Masoli M, Fabian D, Holt S, Beasley R; Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004 May; 59 (5):469–78. https://doi.org/10.1111/j.1398-9995.2004.00526.x PMID: 15080825
2. Weiss KB, Sullivan SD. The economic costs of asthma: a review and conceptual model. Pharmacoeconomics. 1993 Jul; 4(1):14–30. PMID: 10146965
3. Thompson S. On the social cost of asthma. Eur J Respir Dis Suppl. 1984; 136:185–91. PMID: 6428928
4. Accordini S, Corsico A, Cerveri I, Gislason D, Gulsvik A, Janson C, et al. Therapy and Health Economics Working Group of the European Community Respiratory Health Survey II. The socio-economic burden of asthma is substantial in Europe. Allergy. 2008 Jan; 63(1):116–24. https://doi.org/10.1111/j.1398-9995.2007.01523.x PMID: 18053021
5. Accordini S, Corsico AG, Braggion M, Gerbase MW, Gislason D, Gulsvik A, et al. The cost of persistent asthma in Europe: an international population-based study in adults. Int Arch Allergy Immunol. 2013; 160(1):93–101. https://doi.org/10.1159/000338998 PMID: 22948386
6. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet. 2006 Aug 26; 368(9537):804–13. https://doi.org/10.1016/S0140-6736(06)69290-8 PMID: 16935691
7. Lang A, Mowinckel P, Sachs-Olsen C, Riiser A, Lunde J, Carlsen KH, et al. Asthma severity in childhood, untangling clinical phenotypes. Pediatr Allergy Immunol. 2010 Sep; 21(6):945–53. https://doi.org/10.1111/j.1399-3038.2010.01072.x PMID: 20718926
8. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al; National Heart, Lung, and Blood Institute Severe Asthma Research Program. Analyses of asthma severity phenotypes and inflammatory

proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol. 2010 May; 125(5):1028–1036.e13. https://doi.org/10.1016/j.jaci.2010.02.008 PMID: 20398920

9. Blakey JD, Wardlaw AJ. What is severe asthma? Clin Exp Allergy. 2012May; 42(5):617–24. https://doi.org/10.1111/j.1365-2222.2012.03962.x PMID: 22515386
10. From the global strategy for asthma management and prevention, global initiative for asthma (GINA) 2002. Available from: Http://www.ginasthma.org/.
11. Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol. 2004 Jul; 114(1):40–7. https://doi.org/10.1016/j.jaci.2004.04.042 PMID: 15241342
12. Wan YI, Shrine NR, Soler Artigas M, Wain LV, Blakey JD, Moffatt MF, et al. Genome-wide association study to identify genetic determinants of severe asthma. Thorax. 2012 Sep; 67(9):762–8. https://doi.org/10.1136/thoraxjnl-2011-201262 PMID: 22561531
13. Raherison C, Janson C, Jarvis D, Burney P, Cazzoletti L, de Marco R, et al. Evolution of asthma severity in a cohort of young adults: is there any gender difference? PLoS One. 2009 Sep 25; 4(9):e7146. https://doi.org/10.1371/journal.pone.0007146 PMID: 19779616
14. Cazzoletti L, Marcon A, Corsico A, Janson C, Jarvis D, Pin I, et al. Asthma severity according to Global Initiative for Asthma and its determinants: an international study. Int Arch Allergy Immunol. 2010; 151 (1):70–9. https://doi.org/10.1159/000232572 PMID: 19672098
15. Awasthi S, Gupta S, Maurya N, Tripathi P, Dixit P, Sharma N. Environmental risk factors for persistent asthma in Lucknow. Indian J Pediatr. 2012 Oct; 79(10):1311–7. https://doi.org/10.1007/s12098-012-0817-6 PMID: 22696094
16. Bildstrup L, Backer V, Thomsen SF. Increased body mass index predicts severity of asthma symptoms but not objective asthma traits in a large sample of asthmatics. J Asthma. 2015 Sep; 52(7):687–92. https://doi.org/10.3109/02770903.2015.1005840 PMID: 25582044
17. Rose G. The strategy of preventive medicine. The strategy of preventive medicine. 1992.
18. MacCallum RC, Zhang S, Preacher KJ, Rucker DD. On the practice of dichotomization of quantitative variables. Psychol Methods. 2002 Mar; 7(1):19–40. PMID: 11928888
19. Pekkanen J, Sunyer J, Anto JM, Burney P; European Community Respiratory Health Study. Operational definitions of asthma in studies on its aetiology. Eur Respir J. 2005 Jul; 26(1):28–35. https://doi.org/10.1183/09031936.05.00120104 PMID: 15994386
20. de Marco R, Accordini S, Antonicelli L, Bellia V, Bettin MD, Bombieri C, et al. The Gene-Environment Interactions in Respiratory Diseases (GEIRD) Project. Int Arch Allergy Immunol. 2010; 152(3):255–63. https://doi.org/10.1159/000283034 PMID: 20150743
21. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Eur Respir J. 1993 Mar; 6 Suppl 16:5–40.
22. Gorsuch RL. Exploratory factor analysis. Handbook of multivariate experimental psychology. Springer US; 1988. pp 231–258.
23. Drasgow F. Polychoric and polyserial correlations. Encyclopedia of statistical sciences; 1988).
24. Abdi, H, Lynne JW, Domininique V. Multiple factor analysis: principal component analysis for multitable and multiblock data sets. Wiley Interdisciplinary reviews: computational statistics 5.2. 2013; 149–179.
25. de Marco R, Cazzoletti L, Cerveri I, Corsico A, Bugiani M, Accordini S, et al. Are the asthma guideline goals achieved in daily practice? A population-based study on treatment adequacy and the control of asthma. Int Arch Allergy Immunol. 2005 Nov; 138(3):225–34. https://doi.org/10.1159/000088723 PMID: 16210861
26. The European Community Respiratory Health Survey Steering Committee. The European Community Respiratory Health Survey II. Eur Respir J. 2002; 20:1071–9. PMID: 12449157
27. Osborne JW, David CF. Replication analysis in exploratory factor analysis: What it is and why it makes your analysis better. Practical Assessment, Research & Evaluation. 2012; 17(15): 1–8.
28. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol. 2004 Jan; 113(1):59–65. https://doi.org/10.1016/j.jaci.2003.09.008 PMID: 14713908
29. Löwhagen O. Diagnosis of asthma–a new approach. Allergy, 2012, 67.6: 713–717. https://doi.org/10.1111/j.1398-9995.2012.02821.x PMID: 22571439
30. Bacharier LB, Strunk RC, Mauger D, White D, Lemanske RF Jr, Sorkness CA. Classifying asthma severity in children: mismatch between symptoms, medication use, and lung function. Am J Respir Crit Care Med. 2004 Aug 15; 170(4):426–32. https://doi.org/10.1164/rccm.200308-1178OC PMID: 15172893

31. Sunyer J, Basagaña X, Roca J, Urrutia I, Jaen A, Antó JM, et al. Relations between respiratory symptoms and spirometric values in young adults: the European community respiratory health study. Respir Med. 2004 Oct; 98(10):1025–33. PMID: 15481280

32. Birnbaum HG, Ivanova JI, Yu AP, Hsieh M, Seal B, Emani S, et al. Asthma severity categorization using a claims-based algorithm or pulmonary function testing. J Asthma. 2009 Feb; 46(1):67–72. https://doi.org/10.1080/02770900802503099 PMID: 19191141

33. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention 2014. Available from: www.ginasthma.org.

34. Bouzigon E, Siroux V, Dizier MH, Lemainque A, Pison C, Lathrop M, et al. Scores of asthma and asthma severity reveal new regions of linkage in EGEA study families. Eur Respir J. 2007 Aug; 30 (2):253–9. https://doi.org/10.1183/09031936.00162206 PMID: 17459892

35. Rage E, Siroux V, Künzli N, Pin I, Kauffmann F; Epidemiological Study on the Genetics and Environment of Asthma. Air pollution and asthma severity in adults. Occup Environ Med. 2009 Mar; 66(3):182–8. https://doi.org/10.1136/oem.2007.038349 PMID: 19017701

36. Ungar WJ, Chapman KR, Santos MT. Assessment of a medication-based asthma index for population research. Am J Respir Crit Care Med. 2002 Jan 15; 165(2):190–4. https://doi.org/10.1164/ajrccm.165.2.2102012 PMID: 11790653

37. Bai J, Peat JK, Berry G, Marks GB, Woolcock AJ. Questionnaire items that predict asthma and other respiratory conditions in adults. Chest. 1998 Nov; 114(5):1343–8. PMID: 9824012

38. Ringsberg KC, Bjärneman P, Löwhagen O, Odén A, Torén K. Differences in trigger factors and symptoms between patients with asthma-like symptoms and patients with asthma: development of a basis for a questionnaire. Respir Med. 2002 May; 96(5):305–11. PMID: 12113379

39. Sistek D, Tschopp JM, Schindler C, Brutsche M, Ackermann-Liebrich U, Perruchoud AP, et al. Clinical diagnosis of current asthma: predictive value of respiratory symptoms in the SAPALDIA study. Swiss Study on Air Pollution and Lung Diseases in Adults. Eur Respir J. 2001 Feb; 17(2):214–9. PMID: 11334122

40. Accordini S, Calciano L, Bombieri C, Malerba G, Belpinati F, Lo Presti AR, et al. An Interleukin 13 Polymorphism Is Associated with Symptom Severity in Adult Subjects with Ever Asthma. PLoS One. 2016 Mar 17; 11(3):e0151292. https://doi.org/10.1371/journal.pone.0151292 PMID: 26986948

41. Marks GB. Identifying asthma in population studies: from single entity to a multi-component approach. Eur Respir J. 2005 Jul; 26(1):3–5. https://doi.org/10.1183/09031936.05.00055305 PMID: 15994381

42. Sunyer J, Pekkanen J, Garcia-Esteban R, Svanes C, Künzli N, Janson C, et al. Asthma score: predictive ability and risk factors. Allergy. 2007 Feb; 62(2):142–8. https://doi.org/10.1111/j.1398-9995.2006.01184.x PMID: 17298422

43. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis. 1991 Nov; 144(5):1202–18. https://doi.org/10.1164/ajrccm/144.5.1202 PMID: 1952453

44. Killian KJ, Watson R, Otis J, St Amand TA, O'Byrne PM. Symptom perception during acute bronchoconstriction. Am J Respir Crit Care Med. 2000 Aug; 162(2 Pt1):490–6.