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EDITORIAL

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Dampness, indoor mould, fungal DNA and respiratory health – molecular methods in indoor epidemiology

This editorial discusses the findings of the paper in this issue by McSharry et al. [39], pp. 902–907

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Building dampness and indoor mould growth are recognized risk factors for respiratory health, including asthma, rhinitis and asthmatic symptoms [1]. One meta-analysis on the prevalence of dampness and mould in the European housing stock, including published data from 31 European countries, concluded that 12.1% of the homes in Europe had dampness, 10.3% indoor mould and 10.0% water damage [2]. Even higher prevalence of dampness and mould in European homes were found in the European Community Respiratory Health Survey (ECRHS), where 24.8% of the participants reported that they had ever seen mould in their current home and 27.9% reported water damage. Researchers who visited a subset of the homes observed mould in 13.6% and damp spots in 18.2% of the homes [3]. A number of review articles and meta-analysis have been published suggesting associations between dampness and indoor mould and rhinitis [4], bronchitis and airway infections [5] and onset of asthma [6]. These studies are mostly based on population samples and have not specifically studied exacerbation of asthma. One recent review on indoor environmental exposure has focused on exacerbation of asthma [7]. They concluded that there is sufficient evidence of a causal association between *outdoor* culturable fungal exposure and exacerbation in asthmatics sensitized to fungi. They also concluded that there is limited or suggestive evidence of an association between indoor culturable *Penicillium* exposure and exacerbation in asthmatic children with specific sensitization, any fungal sensitization, or unspecific sensitization. Moreover, they concluded that there is limited or suggestive evidence of an association between indoor total culturable fungal exposure and exacerbation of asthma in children with any fungal sensitization. The study has no conclusions concerning exacerbation of asthma in adults in relation to indoor exposure to dampness or mould [7].

Most epidemiological studies on associations between building dampness and indoor mould have investigated respiratory symptoms [1], few have investigated associations for lung function. Two prevalence studies in adults found lower forced expiratory volume in 1 s (FEV1) in damp homes [8] and in a rehabilitation centre with dampness in the floor construction [9]. Another prevalence study found associations between airway obstruction and higher concentration of 1,3-beta-glucan in homes, a marker of fungal exposure [10]. One longitudinal European population study observed increased lung function decline (FEV1) in adults living in homes with dampness and mould, equivalent to smoking 5–10 cigarettes per day [11]. Finally, one study found that asthmatic patients living in homes with confirmed dampness had lower FEV1 than those living in dry homes [12].

Dampness in buildings has been defined broadly and most existing data on building dampness and mould but it is unclear which is exposure that is the causative agent in damp buildings [1]. One consequence of building dampness is an increased growth of bacteria and mould on indoor surfaces and inside the building construction. Lipopolysaccharide (LPS, endotoxin) and peptidoglycan are the two most studied bacterial cell-wall compounds. LPS is a chemical marker for Gram-negative bacteria [13]. Endotoxin is mostly measured by the biological limulus test [13] but 3-hydroxy fatty acids from endotoxin can also be measured by chemical analysis [14]. Peptidoglycan is found in all bacteria but

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in the largest amounts in Gram-positive bacteria [15]. Muramic acid (MuA) is an amino sugar, is found exclusively in peptidoglycan and can be measured by similar chemical analysis [14, 15]. Associations between endotoxin concentration in indoor dust and respiratory health, including asthma and allergies, have been studied in a large number of studies. The effect can depend on exposure timing, dosage, environmental cofactors and genetics [16]. Radon has summarized the effects of endotoxin with respect to different phenotypes of asthma. The risk of atopic asthma, dominated by eosinophilic response, is decreased in those exposed to endotoxin. In contrast, the risk of non-atopic asthma, characterized by a neutrophilic response, is enhanced in subjects with higher endotoxin exposure [13].

Indoor mould is present everywhere and the issue of indoor exposure to mould is complex and most likely the adverse health effects depends on the amount of moulds as well as the species composition. Moreover, there is some evidence of protective effects from fungal exposure on allergies from studies on children in farming environments [17]. Chemical analysis of ergosterol [14] and analysis of beta-1-3 glucan in dust by the limulus method [10] has been used as markers of total fungal load. Detection and quantification of indoor mould is now possible using mould-specific quantitative polymerase chain reaction (real time PCR) [18, 19]. This molecular method can give quantitative data on the occurrence of the most common indoor moulds, irrespectively of viability. EPA scientists have designed and tested primers and probes for over 100 types of mould (http://www.epa.gov/microbes/moldtech.htm). The method is called mould-specific quantitative PCR (MSQPCR) [19, 20].

Mould-specific quantitative PCR has been used to assess mould levels in indoor air and settled dust (surface contamination). The method can detect groups of mould (e.g. *Aspergillus*/*Penicillium*) [18] as well as specific sequences (e.g. *Stachybotrys chartarum*) [21]. In a UK survey of moulds in homes, MSQPCR analysis demonstrated that similar mould species were found in homes in the United States and Great Britain [22]. Researchers as well as commercial laboratories in the United States, Canada and Europe are currently using MSQPCR.

In epidemiological studies, data on fungal DNA in indoor samples can be analysed in different ways. One way is to analyse health association between the concentrations of each fungal DNA sequence in dust or air and the health parameter, mostly asthma or asthmatic symptoms. Two prevalence studies in schools found associations between the concentration of certain fungal DNA sequences in school dust (e.g. from *Aspergillus versicolor* and *Streptomyces*) and respiratory symptoms [23, 24] as well as lower FEV1 [24] in the pupils. One case–control study reported that levels of *Aspergillus versicolor* DNA were higher in asthmatics homes as compared to controls [25]. Another study reported a positive association between levels of *Streptomyces* DNA in home dust and exhaled nitrogen oxide (NO) in asthmatic children [26].

Vesper et al. [27] have developed a concept called Environmental Relative Moldiness Index (ERMI) to quantify the mould burden in homes. The ERMI value is computed by quantifying the concentration of species-specific DNA sequences from 36 indicator mould species in home dust samples. The mould species are divided in two groups. The first group (group 1 mould) consists of 26 mould species that indicate water damage. The second group (group 2 mould) consists of sequences from 10 Group 2 species that can be from outdoor sources and these moulds are commonly found even without water damage [27]. For each home, the mould burden is computed by taking the sum of log-transformed group 1 mould species concentrations minus the sum of log-transformed group 2 mould species concentrations. The ERMI value does not measure the total fungal concentration in the dust or the total fungal exposure. It is used as a way to rank homes with respect to the relative mould burden in homes [28–30].

Environmental Relative Moldiness Index has been used in epidemiological studies, and higher ERMI levels have been found in home dust among children with asthma as compared to controls without asthma [31–33]. One study found no significant association between ERMI in home dust and infant wheeze [34]. One longitudinal study found that early exposure to moulds as measured by ERMI at 1 year of age, but not 7 years of age, increased the risk for asthma at 7 years of age [35]. In addition, one recent study found higher ERMI values in school dust from schools with high prevalence of asthma as compared to schools with low asthma prevalence [36]. Finally, one study found lower lung function (FEV1) among children who lived in homes with higher ERMI values [37].

Few studies have used mould-specific quantitative PCR or the ERMI-index in epidemiological studies on adult respiratory illness. One recent study found an association between ERMI values in home dust and asthma and rhinitis in adults [38]. Moreover, few studies have investigated exacerbation of asthma from mould, assessed by the ERMI-index. Recently, in Clinical and Experimental Allergy, McSharry et al. [39] have extended the use of the ERMI-index and other microbial markers in the home environment to study exacerbation of asthma, measured as decreased FEV1% among non-smoking adult asthmatics in Scotland. They also studied associations between FEV1% and corticosteroid use, asthma control Questionnaire score (ACQ) and St. George's Respiratory questionnaire score. FEV1% were negatively correlated with ACQ and SGRQ scores and weakly with corticosteroid use. Higher ERMI

values in home dust were associated with decreased FEV1% but there was no correlation between FEV1 and other biological contaminants such as concentrations of endotoxin, 1,3-beta-glucan or cat allergen (Fel d 1), dog allergen (Can f 1) or house dust mite allergens (Der p 1 or Der p 2) in home dust [39]. The study adds evidence on the possible role of mould as a cause of exacerbation of asthma in adults and also links the ERMI-index to airway obstruction measured as FEV1. The study supports the view that measurement of fungal DNA in dust in epidemiological studies can be a useful indicator of fungal exposure in indoor environments. Moreover, the study supports the view that the ERMI-index has relevance for respiratory health and can be a useful indicator of relative fungal burden in indoor environments. The ERMI-index may also be useful in patient investigations to identify patients that need to improve their home environment.

However, more prospective studies are needed where ERMI and other types of indoor biological contaminants are measured in parallel in dust samples collected prior to disease development. Moreover, epidemiological studies on respiratory effects of indoor exposure should focus on disease development (e.g. asthma, rhinitis and lung function decline) as well as exacerbation of asthma. Moreover, respiratory effects of different types of indoor biological contaminants, including fungal DNA measured by mould-specific quantitative PCR and calculation of the ERMI-index, should be extended from the home environment to other indoor environments such as day care centres, schools, hospitals and offices. Moreover, as most epidemiological studies on respiratory effects, especially with ERMI-index, are from United States or Europe, similar studies need to be funded and performed in other parts of the world, including Asia, where the current increase of asthma and allergy is high [40].

Conflict of interest: The authors declare no conflict of interest.

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