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DETECTION OF VOLATILE ORGANIC COMPOUNDS IN THE ALVEOLAR AIR OF SUBJECTS WITH STRESS-RELATED PSYCHOPATHOLOGIES

MED/44 OCCUPATIONAL MEDICINE

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INTRODUCTION

In the last years we assisted to an increasing interest from the scientific community towards the work-related psychopathologies which are often a consequence of psychosocial hazards in the workplace. In 2007 the European Agency for Safety and Health at Work has defined the psychosocial hazards "emerging", that is new and in upsurge. It deals with new hazards because they didn't exist before or they were not more probably recognized as such. Besides they are in upsurge because the probability of exposure results great or the effects on the health of the workers seem to worsen (http://osha.europa.eu). The psychosocial hazards follow a trend in growth because tightly in relationship with the recent transformations of the workplaces, contrarily to the chemical, physical and biological hazards that are also reduced in concomitance to the improvements of the hygienic aspects of the workplaces. They are caused by new trials, new technologies, new types of places of job, or by social and organizational transformations

In 1986 the International Labour Organization (ILO) has defined the psychosocial hazards "those hazards springing from the interaction of the job content, the management and organization of the job, environmental and organizational conditions from a side, competences and demands of the dependent workers from the other" (http://ilo.org). Other Authors have defined the psychosocial hazards as "aspects of planning, organization and management of the job, as well as the respective environmental and social contexts that potentially can bring physical or psychological damages" (Cox & Griffiths, 1995) and subsequently as "the whole of organizational, managerial, environmental and report variables that can cause a psychological, social or physical damage to the people with consequent negative effects in the effectiveness and in the image, wide to the organizational, economic, social and environmental levels " (Cox, Griffith & Rial-Gonzalez, 2000). The psychosocial hazards originate from organizational choices that can have a negative relapse on the people both in terms of increase of the accident rate and of uneasiness, up to bring to the development of pathologies. The psychosocial hazards can engrave on the health both physic and psychic of the worker in direct or indirect way through the experience of Stress.

Stress is responsible for various forms of working uneasiness, among which Personal Bullying, Task-related Bullying and Burn-Out that are different expressions of psychosocial Stress. The upsurge of job demand together with more high-level of Stress, have negative effects on the health of workers including mental disorders (Wang and coll., 2006). Manifold studies include depression, anxious and mixed anxious-depressive disorders among the stress-related psychopathologies. The Italian Institute for Insurance against Industrial Accident (INAIL) recognizes and indemnifies the Post-Traumatic Stress Disorder (PTSD) and the Adjustment Disorder as professional illnesses referable to situations of Task-related Bullying (INAIL, Circular n. 71 of December 17th 2003).

In Europe Stress represents the second health problem in the workplace, interesting one in four workers (http://eurofound.europa.eu). The World Health Organization (WHO) esteems that in 2020 depression will represent the second cause of disability and death after the cardiac pathologies (http://who.int). The italian lawmaker has introduced in the D.Lgs 81/08 the mandatory evaluation of work-related stress, comparing this hazard to the physical, chemical and biological ones. medical Few medical centers are able to attend workers with psychic disorders correlated with negative working situations that involve conditions of chronic stress. The evaluation of conditions of stress in the individuals is complex and is based on clinical and biological indicators. The physiopathology of stress has been known for many years and the biomarkers used for the diagnosis of stress such as cortisol in biological matrices like blood, urine and saliva are obtained with invasive methods and nonelementary protocols. The PhD activity focused on the study of stress-related psychopathologies and the alveolar air in subjects that required a clinical assessment to the "Center for the Analysis of Psychosocial Hazards and Pathologies of Working Origin" of the University of Verona. The alveolar air is a biological matrix that hs been studying already for some years as biomarker of neoplastic, inflammatory and metabolic pathologies.

A group of workers with mental and somatoform disorders correlated with situations of chronic stress at work were object of our study. The first aim was to verify if a correspondence exists between the psychiatric disorders complained by the workers for negative conditions at work and those disorders that INAIL recognized and indemnified as professional illnesses. The second aim was to detect one or more molecules in the alveolar air that could be able to correctly identify

subjects with stress-related psychic and psychosomatic disorders and healthy subjects in order to appraise the potential contribution of this biological matrix in the diagnosis of stress-related psychopathologies.

BACKGROUND

WORK-RELATED STRESS

The psychosocial hazards can negatively influence mental health of workers through the experience of a condition of chronic Stress.

The term Stress has been introduced in Medicine by Seyle in 1936 and it has assumed the meaning of physiological reaction of adjustment to any stimulus practiced on the organism by a wide range of factors called “stressors” (Seyle 1936).

In the 1950s Seyles has elaborated the concept of Stress as General Adaptation Syndrome (GAS); it consists in three phases: alarm, resistance and exhaustion (Sayle 1950). In the phase of alarm the individual tries to neutralize the harmful effects of the stressors; the acute activation of the neuro-vegetative system produces noradrenaline and adrenaline and that of the hypothalamic-pituitary-adrenal axis produces ACTH and cortisol, the main mediator of the answer to the Stress. The phase of resistance is characterized from the adaptation to the situation with the chronic neuro-vegetative and hormonal activation and it results nevertheless still reversing. The phase of exhaustion is characterised by the stable iperactivation and not reversing of the compensative neuroendocrine mechanisms that can lead to the onset of psychological, behavioral and somatoform disorders. The clinical disorders that set up as a result of exposure to psychosocial hazards are referable to Stress-related disorders. Emotional, behavioral and psychosomatic symptoms prevail in an early phase; psychiatric and somatoform disorders can subsequently develop if the stressful situation persists. (Mikkalsen 2002).

Work-related Stress is a process that involves the individual in the interaction with the working environment as a result of an imbalance between the applications of the organization and the abilities of an individual. Job is a fundamental value that is able to influence the individual and its personality (ISPESL 2003); it doesn't answer only to the demand of profit, but in our culture it has above all a symbolic value because it is a marker of success and it answers to the need of affiliation and social relationships. Conditions of stress at work can negatively engrave on the quality of life and on the health state of workers. In the last years the awareness of this problem is increased so much that the Italian

Lawmaker has introduced in the D.Lgs 81/08 the mandatory evaluation of work-related stress, so recognizing the injurious potentiality of this hazard to the psycho-physical integrity of the worker.

The National Institute for Occupational Safety and Health (NIOSH) has defined stress at work as "a whole harmful physical and emotional reactions that onset when the work requirements are not commensurate to the abilities, resources or demands of the worker. The work-related stress can negatively influence the health state and it can make easier the event of accidents" (NIOSH, Stress at work, 1999). The European Commission has defined the stress at work as "a reaction to adverse and harmful aspects of the content, of the environment and of the organization of the job and it is characterized by elevated levels of excitement and anxiety, often accompanied by sense of inadequacy."

In 2007 the fourth European Investigation on work conditions has underlined that stress represents the second health problem complained by european workers. The Italian workers complain more meaningfully about sanitary symptoms (lombalgia, headache, general fatigue) likewise to the average of their colleagues in the 25 countries of the European Union; besides 27% of Italian workers mentions the stress as principal sanitary symptom connected with the job in comparison to the 22% of the rest of Europe (22%), (http:/ /eurofound.europa.eu).

The working conditions that can negatively influence the psychic health of the workers through the experience of a condition of chronic Stress are mainly Bullying (Personal Bullying and Task-related Bullying), Work Distress and the Non-specific Work Discomfort.

These conditions have been given various interpretations in literature. Their chief characteristics are reported as follows:

Bullying is defined as negative treatment on the part of one or more persons in the workplace, in a situation in which victims have difficulty in defending themselves; in general it lasts at least six months;

(a) Task-related Bullying refers to hostile actions specifically related to tasks, e.g. the imposition of unreasonable deadlines, unmanageable workloads, the assignment of meaningless tasks or no tasks, excessive monitoring of work, withholding of information or other actions making the work situation hard to bear. In task-related bullying, evident hostile behaviour against one's own image and in relationships are not relevant;

(b) Personal Bullying, on the other hand, is primarily considered to be person-related and arises when victims are subjected to insulting remarks, excessive teasing, gossip and rumours, persistent criticism, practical jokes, intimidation, social isolation and exclusion (Leymann 1993, 1995 and 1996; Ege 1996, 1997, 1998 and 2001);

Work Distress is a negative condition caused by organisational disorder in terms of work context (i.e. organisational abilities, rules in the place of work, career progression, autonomy in decisions, interpersonal relations at work and the balance between work and home life) and work content (workplace environment, task planning, work overload and hours of work) (Hacker 1991);

Non-specific Work Discomfort indicates the presence of significant levels of uneasiness experienced at work which can be of various origins. These may even be linked to personal convictions (for example, unfulfilled job expectations, dissatisfaction at work, task changes etc.). In the case of interpersonal conflict, persecutory intent is not present.

HEALTH EFFECTS OF STRESS

- **Psychosomatic symptoms and somatoform disorders**

The psychosomatic symptoms represent an effect of the body reaction to a condition of Stress extended in the time.

Noradrenaline and adrenaline are responsible for the peripheral vasoconstriction and of the upsurge of heart impairments. Cortisol have effects on the metabolism, on the immune system and the digestive apparatus. Initially the neuroendocrine activation determines functional alterations; organic pathologies can arise in the case of persistence of the condition of Stress. Functional and organic disorders occur to the cardiuvascular apparatus (arrhythmia, arterial hypertension, hearth attack), the gastrointestinal apparatus (dyspepsia, gastro-esophageal reflux disease, peptic ulcer and irritable bowel), the respiratory apparatus (dyspnea, cough and bronchial asthma), the muscle-skeletal apparatus (myalgia, muscular cramps, headache, cervical and low back pain) and the genito-urinary apparatus (menstrual pain, menstrual disorders, impotence, decline in the libido). The endocrine system

can be involved with malfuction of the thyroid gland functionality (hyper - or hypothyroidism) and of the carbohydrate metabolism (diabetes mellitus). The immune system deficiency mediated by the cortisol increases the susceptibility of the individual to contract infectious illnesses and, according to some studies, it also contributes to the onset of cancer. Finally cutaneous pathologies as emotional eritema, dermatitis, itch and psoriasis can occur. The European Heart Network (1998) sustains that the stress at work increases the risk of cardiovascular pathologies, especially in the case of high demand, low control and poor social support and reward. The percentage of cardiovascular illnesses due to the stress at work is about 16% for male workers and 22% for female workers (the percentage climbs to over 50% including the sedentary job) (http://ehnheart.org).

- **Emotional -behavioral disoders and psychiatric pathologies**

Depressive humour, reactions of anxiety, feeling of imminent danger, burst of weeping and insomnia are frequent in the initial phase of the Stress. Sleep disorders take the form of difficulty in falling asleep, recurrent waking with nightmares or negative thoughts and waking early. Difficulty of concentration, memory impairment, anedonia or apathy are other important symptoms (Cassitto, 1999).

The most common behavioral disordes are eating disorders (iporexia or hyperphagia), increase of alcoholic or drug assumption. Behavioral disordes predispose the subject to accidents; work-related stress seems to cause the 60% of the fatal accidents at work (Ispesl 2008).

Psychopathologies in workers who had experienced negative working conditions are represented by various reactive neuroses like mixed anxiety and depressive disorder, adjustment disorder , depression disorder, anxiety disorder and post-traumatic stress disorder (PTSD) (American Psychiatric Association, 2002).

Studies conducted over the last 15-20 years on the subject of bullying at work have provided a major contribution to current knowledge regarding work-related pathologies. Bullying has become such a serious problem since the 1980s due to its severe effects on mental and physical health, as well as on society and industry, that in 2001 the European Parliament decided to focus on the necessity to adapt regulations regarding harassment at work.

Research carried out since the 1980s has revealed that prolonged psychological
oppression in the workplace causes repercussions on health such as somatoform disorders, depression, anxiety, obsessive-compulsive disorders, sense of impotence, anger, despair, social isolation and general maladjustment. It has been demonstrated that these symptoms may lead to serious diseases, suicide or suicidal intent (Leymann 1990 e 1996, Björkqvist et al. 1994; Cassitto, 2002; Einarsen e Mikkelsen, 2003; Leymann, 1996; Mikkelsen e Einarsen 2002; Quine, 1999; Vartia, 2001 and 2003).

Bullying in particular has been considered to be so stressful that it may cause symptoms and reactions in emotional, psychosomatic and behavioural spheres and lead to serious psychiatric diseases (Paoli e Merllié, 2001; Björkqvist et al. 1994; Leymann, 1996; Cassitto, 2003; Punzi et al. 2007; Mikkelsen e Einarsen , 2002; Matthiesen e Einarsen, 2004).

Keashly and Jagatic (2003), in a revision of American literature on the effects of
psychological violence at work, report some fundamental considerations. They state that the consequences of negative behaviour which may not at first seem to be serious are, on the contrary, extremely important and affect many areas of the victim's life: personal (psychological, cognitive, physical), interpersonal (aggressive behaviour, family and marital conflicts) and professional (decreasing work satisfaction, increasing absenteeism, changing jobs and retirement). The authors also report that victims of aggressive behaviour can in turn become hostile and aggressive themselves.

Leyman and Gustafsson (1996) found that the most typical psychiatric diagnosis for victims of bullying is post-traumatic stress disorder (PTSD) which was considered to be the best diagnosis in 92% of cases. Matthiesen and Einarsen (2004) confirmed a significant correlation between exposure to bullying behaviour and symptoms of PTSD, with a 72-76% prevalence of the disorder in bullying victims. In literature on occupational medicine, PTSD has been traditionally documented among specific groups of workers who have experienced traumas and accidents at work or in jobs characterised by danger, violence and life-threatening events.

In the last few years mental disorders seem to have become more frequent as conditions in the workplace worsen; the effects on health and general well-being are consequently more serious.

A French study (Niedhammer et al. 2006) confirmed the association between harassment at work and depression in 7694 employees (3132 men and 4562 women). Both male and female victims of psychological violence at work are significantly at risk of depression. The more frequent the episodes of violence, the greater risk there is of mental illness.

In the U.K, a national study has been carried on in order to evaluate incidence of workrelated mental diseases (Cherry et al. 2006). 842 occupational physicians and 878
psychiatrists took part in the project. Occupational physicians collected data regarding
workers with psychiatric problems and diagnosed mental disorders in 2718 cases between 1996 and 2001; between 1999 and 2001, psychiatrists identified 3624 subjects whose mental illness was related to work. Occupational physicians and psychiatrists made their diagnosis following ICD-10 criteria which were used to identify various different pathologies (depression, anxiety, PTDS and other types of stress and mental ilness). Socio-demographic variables, type of employment and events which were thought to be the cause of mental disorders were also investigated.

In patients identified as suffering from psychopathologies, there were diagnoses of anxiety and/or depression in 72 % of cases in diagnoses made by psychiatrists and 60% of cases in diagnoses made by occupational physicians. 3% were diagnosed as suffering from psychosis in cases examined by psychiatrists, while no cases of psychosis was reported in cases examined by occupational physicians. PTSD was indicated in 18% of cases by psychiatrists and in 10% of cases by occupational physicians. In occupational medicine, PTSD is more frequent in men and usually related to work accidents.

Among the precipitating events identified, the most common involved factors intrinsic to the job (above all work overload) followed by problems in interpersonal relationships and changes at work (such as new responsibilities). The incidence of mental disorders was higher in professionals, associated professionals, technical workers and those involved in personal and protective services.

A study conducted in France in 2008 and published in the Journal of Occupational Medicine has found a strong association between stress at work and anxiety/depression suggesting that stress could be an etiologic factor in these mental disorders (Brousse et al. 2008). The study provides evidence that jobs characterized by high demand and low control are associated with serious depression (OR 1.74) in men, while women with stressful jobs are at higher risk for depression or anxiety disorders (OR 1.47).

Melchior et al (2007) suggested that workers with highly demanding jobs involving work overload or too little time to carry out tasks, are doubly at risk of developing serious depressive disorders or general anxiety disorders.

BIOLOGICAL MARKERS OF STRESS

Stress-related psychopathologies are consequences of the extended and excessive activation of the hypothalamic-pituitary-adrenal axis that leads to the exhaustion of the psychic and physical resources.

The biological link between Stress, anxiety and depression has been identified, during a series of experiments at molecular level and therefore on rats, from a group of researchers of the university of the Western Ontario (UWO), (Natures Neuroscience, 2010). The mechanism individualized in this study involves the interaction between the receptor 1 of the corticotropin releasing factor (CRFR1), a hormone produced by the neuroendocrine cells of the hypotalamus that participates in a crucial way to the answer of hypothalamic-pituitary-adrenal axis to different stressors, and specific receptors of the serotonin (5-HTR). Particularly, the study reveals that the CRFR1 increases the number of 5-HTR on the neuron membrane and this can cause an anomalous signal. The CRFR1 activation brings to anxiety as reaction to the Stress, and the 5-HTR is involved in the development of depression.

The diagnosis of Stress is complex and is based on clinical indicators and biological markers such as cortisol, deidroepiandrosterone (DHEA) and prolactin.

Some Authors (Lundberg et to the., 1989; Fox et to the., 1993) have documented the relationship between conditions of chronic stress at work and modifications in cortisol concentrations. In 1996 Melamed and coll. have underlined an association between cortisol concentrations and nervousness at work. The

biological matrices currently used for diagnosis of Stress required invasive methods, a rather high cost in the analysis and a complete adherence of the patient to the established protocol. Nowadays anybody has considereted the potential contribute of the alveolar air in the search on Stress. Alveolar air sampling is simple and non-invasive and the instrumental analysis is not expensive. In the last decade manifold studies have been published documenting the potential role of the alveolar air in the diagnosis of different pathologies (neoplastic, metabolic, inflammatory) through informations on the metabolic state of the patient. Some studies have also applied the analysis of the alveolar air to the psychopathologies, mainly to schizophrenia.

ALVEOLAR AIR

The exhaled air is a biological matrix that contains inorganic compounds like NO, O2, CO2, volatile organic compounds (hydrocarbons, alcohols, ketones, aldehydes, esters) and nonvolatile substances like cytokines, leukotrienes and H2O2 that can be found in the breath condensate (Miekisch and coll, 2004; Buszewski and coll 2007). The alveolar air is that fraction of the exhaled air that reflects the chemical composition of the blood in consequence of phenomena of repartition to level of the pulmonary alveolus. These phenomena allows the attainment of an equilibrium between the concentration of volatile compounds in the haematic phase and in the aerial one. The alveolar air contains thousands of volatile organic compounds (VOCs) deriving from external sources, natural or industrial, or endogenous sources as result of the metabolic processes of the human body. Nevertheless, most of the VOCs in the exhaled breath have typical concentration ranges which are lower than 1 ppb.

About three thousand volatile organic compounds have been detected at least once in the alveolar air (Wei M. and coll, 2006; Ehrwmberg, 2008), but about two hundred VOCs have been found more frequently (Phillips and coll 1999).

Practical application of the alveolar air is wide both in occupational medicine through the biological monitoring of the working exposure to xenobiotics, and in the diagnostic area through the indentification of pathological processes in the body.

Manifold studies have underlined a correlation between the concentrations of some solvents like ethylene, benzene and toluene (Perbellini and coll., 1985; Brugnone and coll., 1986), styrene, trichloroethylene and tetrachloroethylene (Droz Po and coll., 1986) in the expired air and the in the environmental air.

Applications of expired air in the forensic sciences and in the clinical practice are still few. One of the most well-known application is the measuring of ethanol concentrations in the exhaled air after alcohol intake through breathalyzer (Wilson, 1986; Mitsubayashi and coll., 2005; Thrall 2006). Ethanol in the alveolar air reaches equilibrium with ethanol in the blood after 15 minutes from the assumption. The breathalyzer measurements correlates with gas-chromatographic measurements in the blood and it furnishes with good approximation the value of the haematic alcohol concentration through a correction factor (x 2.300).

The 13C-urea or ammonia breath tests are considered to be the most sensitive and specific techniques for diagnosis of Helicobacter pylori infection.

The nitric oxide is used in the diagnosis of the asthma (Ochiai and coll., 2001).

The physiological origin and basis of volatile organic compounds are still not completely known. Some VOCs are polluting substances that are metabolized by the body after absorption via inhalation, ingestion or through the skin and therefore they are excreted by expiration; other VOCs are the product of endogenous metabolic processes or activity of intestinal bacteria. Endogenous markers commonly used in diagnostic area are hydrocarbons like ethane, pentane and isoprene; oxygen-containing compounds like acetone, acetaldehyde, methanol, ethanol and 2-propanolo; sulphur-containig compounds like ethyl mercaptane, dimethylsulfide or dimethyldisulfide; carbon disulfide and nitrogen containing compounds like ammonia and dimethylamine and trimethylamine. Some physiological basis of VOCs endogenous production are known and they maily involve: ethanol metabolism (acetaldehyde), dextrose metabolism and lipolysis (acetone), protein metabolism (methyilamine), lipid peroxidazion of the polyunsaturated fatty acids in the cellular membranes through the oxidative stress (ethane, pentane, ethylene), activity of intestinal bacteria (hydrogen, methane) and cholesterol biosynthesis (isoprene) (Table 1) (Buszewski and coll, 2007) .

Table 1. Volatile compounds physiological origin and basis

Volatile compounds physiological origin	Physiological basis
Acetaldehyde	Ethanol metabolism (Norberg et al., 2003; Turner et al., 2006)
Acetone	Decarboxylation of acetoacetate and acetyl-CoA
Ethane	Lipid peroxidation (Schubert et al., 2004)
Ethylene	Lipid peroxidation (Risby, 2005)
Hydrogen	Gut bacteria (Ledochowski et al., 2001)
Isoprene	Cholesterol biosynthesis (Stone et al., 1993)
Methane	Gut bacteria (Ledochowski et al., 2001)
Methylamine	Protein metabolism (Risby, 2005)
Pentane	Lipid peroxidation (Schubert et al., 2004)

Factors affecting the great variability in the composition of human breath include physical condition, general health of the subject, food intake, environmental influences and overall lifestyle (Ammann and coll., 2004; Libardoni and coll., 2006). The concentrations of VOCs in breath are not generally higher in the diseased state when compared with healthy volunteers, even though this is the case for some prominent molecular species such as for acetone with untreated diabetes or isoprene (in some patients) and ammonia for kidney impairment. (Buszewski and coll., 2007). The oxidative stress is correlated with age and with different pathological states like lung cancer, breast cancer, bronchial asthma, hearth attack, allograft rejection, rheumatoid arthritis (Aghdassi and Allard, 2000; Phillips and coll., 2000; Moretti and coll., 2004). Exhaled ethane and pentane concentrations were elevated in inflammatory diseases; exhaled levels of sulphur-containing compounds were elevated in liver failure and allograft rejection; acetone was increased in patients with uncontrolled diabetes mellitus (Miekisch and coll. 2004). Isoprene

concentration resulted lower in the expired air of subjects with ARDS than in the healthy volunteers (Schubert JK and coll., 1998). Since isoprene is involved in the cholesterol biosynthesis and not in the inflammatory processes and in the lipidic peroxidation, smaller concentrations could be involved to alterations of the reparation processes of the membranes in the alveolar cells. A similar process could explain low concentration of the isoprene in subjects with chronic cardiac impairments (McGrath and coll., 2001).

Table 2. **Some examples for disease markers (Amann and coll., 2005)**

Volatile organic compounds	Disease
Ethane and pentane	Oxidative stress
Methylated hydrocarbons	Lung or breast cancer
Hydrocarbons (especially ethane and pentane)	Oxidative stress
Isoprene	Cholesterol metabolism
Acetone	Diabetes mellitus, ketonemia
Sulfur-containing compounds (dimethylsulfide, methyl mercaptane, ethyl mercaptane)	Liver impairment
Nitrogen-containing compounds (ammonia, dimethylamine, trimethylamine)	Uremia, kidney impairment

Modern breath analysis started in the 1970s when Pauling et coll. have detected more than 200 components in human breath using gas-chromatography. (Pauling and coll., 1971). In the 1980s some studies started to appraise the diagnostic contribution of the expired air in some pathologies like neoplastic diseases, mainly the lung cancer (Gordon e coll., 1985; Phillips e coll., 1999, 2003 e 2008; Deng e coll., 2004; Poli e coll., 2005; Machado e coll., 2005; Mazzone e coll., 2007; Bajtarevic e coll. 2009; Peng e coll., 2009; Taivans e coll., 2009) and the breast cancer (Phillips e coll. 2003, 2006 e 2010) or other disorders like the diabetes

mellitus (Phillips e coll., 2004), the rheumatoid arthritis (Humad e coll., 1988), the schizophrenia (Koraleva e coll., 1989; Phillips e coll., 1993 e 1995), the tubercolosis (Phillips and coll., 2007), the cystic fibrosis (Bennett and coll., 2009). The analysis of the expired air has also been applied to the study of the allograft rejection (Phillips and coll., 2003) and the preeclampsia (Moretti and coll., 2004).

Cross-sectional studies and longitudinal studies were conducted. The common denominator of the researches conducted through the cross-sectional studies was the interest in the identification of one or more molecules that were able to correctly classify sick subjects and healthy volunteers with high sensibility and specificity. In the longitudinal studies the intraindividual modifications of the VOCs concentration were appraised in relationship with the evolution of the pathology or to effects of therapies.

The main analytical technique used in the most of the these studies is the gas-chromatography coupled to the mass spectrometry subject to the sample dehumidification and preconcentration. Initially great volumes of air were necessary for the air sampling in consideration of the low sensibility of the analytical techniques. In 1985 Gordon and coll. have for the first time applied the analysis of the expired air to the study of the lung cancer; 40 liters air for each patient were necessary to sample. In 1999 Phillips and coll. have published the results of their study conducted on the lung cancer in which the volumes of air that were necessary for the analysis of the VOCs were reduced to 10 liters. In this study, the alveolar air sample of 60 subjects with lung cancer and 50 of healthy controls have been submitted to absorption on activated carbon cartridge and analyzed, after thermal desorption, with gas-chromatografy coupled to mass spettrometry. A combination of 22 volatile organic compounds was found in the expired air that could discriminated the subjects with first stadium lung cancerto with a 100% sensibility and a 81,3% specificity. Alkanes were prevailing and they would seem involved in the carcinogesis through production of oxygen free radicals due to the oxidative stress.

Afterwards the preanalytical phase of sampling has been notably simplified performing a single slow vital capacity breath into a one-way valve connected to a Teflon®-bulb, which could trap the last portion of exhaled air (150 ml). (Poli and coll. 2005).

At the same time the sensibility of the analytical techniques has increased and volatile compounds could be identify at very low concentrations (ppb). In 2009 Netzer and coll. have applied ion molecule reaction mass spectrometry (IMR-MS) to a total of 126 human breath gas sample comprising 91 subjects with liver disorders and 35 healthy volunteers; using an algorithm, five molecules have been identified (acetaldehyde, isoprene, M60, M67 and M103) that were able to correctly classify the cases and the controls with high sensibility and specificity.

In the last years other analytical tecniques have been used in addition to the traditional ones like colorimetrical sensor array (Mazzone and coll., 2007) and gold nanoparticles sensor array. In 2009 Peng and coll. have compared a gas-chromatografy coupled with mass spectrometry analysis to a gold nanoparticles sensor array analysis. Gold nanoparticles sensor array was able to measure VOCs concentrations in samples containing high dampening air avoiding samples dehumidification and preconcentration. 96 human breath gas samples (40 subjects with lung cancer and 56 healthy controls) were analysed using the two tecniques one by one; VOCs concentrations were superimposable for 33 molecules. Gold nanoparticles sensor array has however detected nine compounds that gas-chromatografy coupled to mass spectrometry had not identified.

The application of the the alveolar air analysis to the diagnosis of the psychopathologies is not novel and it has been focused on the schizophrenia. The interest was born in order to understand the origin of the characteristic odor of schizophrenic subjects that is not related to little personal hygiene and, according to some studies, it doesn't seem to be emanated with the sweat.

In 1989 Koraleva and coll. have compared the VOCs concentrations in the alveolar air of a group of subjects suffering from schizophrenia and a group of healthy volunteers using GS-MS analysis. The schizophrenic subjects had greater concentrations of pentane than the healthy controls and the concentrations correlated with the severity of the clinical symptoms (Koraleva and coll. 1989). The pentane concentration in asymptomatic schizophrenic patients under pharmacological therapy resulted similar to that of the healthy controls. In 1993 Phillips and coll. have conducted a study also including subjects with other psychophatologies; concentrations of carbon disulfur resulted higher in the schizophrenic subjects than in the subject with other psycophatologies and in the healthy controls. In 1995 Phillips and coll. have analyzed VOCs concentrations in the alveolar air of subjects

suffering from schizophrenia (25), subjects with other psychopathologies (26) and healthy volunteers (38); using statistical algorithms, 11 volatile organic compounds allowed to correctly identify the schizophrenic subjects with a 80% sensibility of and a 61,9% specificity. The 11 identified compounds were: pentane, 2-3-3-trimethylpentane, 2-pentanol, dichloromethane, trichlorofluoromethane, 1-1-1 trichloroethane, 1-1-2-2-tetrachloroethane, 2-methylbutane, 2-2-dimethylbutane, 1-chlorine-2-methylbutane, benzene.

EXPERIMENTAL DESIGN

The aims of this study were:

- identification of the subjects with stress-related psychological disorders among those people that turned to the "Center for the Analysis of Psychosocial Hazards and Pathologies of Working Origin" for a clinical assessment;

- analysis of the prevalence of the different psychiatric disorders correlated with situations of chronic stress at work and of the different types of working uneasiness;

- analysis of the chemical composition of the alveolar air in subjects with psychic and psychosomatic disorders due to conditions of chronic stress at work ("cases") in order to verify potential differences with the alveolar air of healthy subjects ("controls");

- identification of potential biological markers for the diagnosis of Stress-related psychopathologies among the organic volatile compounds (VOCs) in the exhaled air.

MATERIALS AND METHODS

- **Study subjects**

Between 2008 and 2010, 98 subjects with diagnosis of psychiatric pathology correlated with a condition of chronic stress at work have been selected for the analysis of the volatile organic compounds in the alveolar air. The subjects with diagnosis of psychotic pathologies were excluded. 60% were women and the remaining 40% were men with an average age of 44,5. 54% were married, 24% single, 20% separated/divorced and the remaining 2 % widowed.

The majority of the workers examined had a relatively high standard of education: 25 % had a university degree, 50% a senior school diploma; 21% had completed middle school and 4 % primary school. Most subjects (58%) worked in private companies of varying types and sizes (from very small to large businesses)

and 42% were employed in the public sector. The distribution in terms of sector was as

follows: manufacturing and skilled manual work 23%, public administration 22%, health services 12%, wholesale and retail trade 11%, education 4%, transport 2%, other 24%.

The group of control was represented by 80 healthy subjects with negative case history both for psychiatric disorders and working uneasiness. 60% were women and the remaining 40% were men with an average age of 41,5. (Table 3).

The average age of cases and controls was similar in order to reduce the interference given by the age on the oxidative stress that can modify the concentrations of some VOCs. However, in the multivariate analyses age was considered as a possible confounding variable.

Table 3. Main characteristics of the cases and the controls

	Cases	**Controls**
totale	98	80
women	59	48
%	60%	60%
men	39	32
%	40%	40%
average age	44.5	41.5

- **Clinical and diagnostic assessment**

The standardized clinical protocol included a consultation session with an occupational physician and a clinical psychologist, the administration of questionnaires and psychological tests (Romeo and coll., 2010).

The alveolar air was collected in selected workers after acquisition of the informed consent.

The occupational physician collected general information regarding family history, and the patient's pathological, physiological and occupational case history. The working environment, the quality of communication with colleagues and employer, the exact time when the problems at work had begun and the type of harassment (e.g. insult, threat, unreasonable transfers etc.) were established by means of a semi-structured interview. This type of interview is based on a short list of questions and a certain amount of direct questioning, depending on what the physician deems important and what other information is required. We were thus able to reduce the type of false positive responses that may occur using traditional questionnaires. The Val. Mob scale, ISPESL and Karasek questionnaires were also used to evaluate working conditions. As part of the consultation, diagnoses were also acquired from psychologists and psychiatrists.

In order to evaluate psychic distress, we administered the GHQ -12 questionnaire (Goldberg's General Health Questionnaire) which is composed of 12 items (6 positive and 6 negative) dealing with psychic health over the previous 2 weeks. GHQ - 12 seems to be effective in terms of identifying and quantifying mental disorders which are not psychotic (minor psychiatric disorders) and is used for both the general population and psychiatric patients (Piccinelli et al 1993, Goldberg et al. 1997).

The aim of the clinical psychologist was to identify possible outside work factors that could generate or worsen symptoms, to verify general feelings of uneasiness and to establish that there were no serious mental conditions which would affect the patient's ability to provide reliable information.

In a second phase, patients underwent psychological tests (the MMPI-2 and the Wartegg test).

At the end of the diagnostic evaluation, an individual report concerning the relationship between workplace conditions and mental disorders was drawn up for each worker.

Various working conditions were identified: personal bullying, task-related bullying, work distress, and non -specific discomfort.

Psychopathologies in workers who had experienced negative working conditions were represented by various reactive neuroses, in particular, mixed anxiety and depressive disorder, adjustment disorder, depression disorder, anxiety disorder and post-traumatic stress disorder (PTSD).

- **Alveolar air sampling and mass spectrometry analysis**

A total of 178 individuals (98 workers with diagnosis of chronic stress-related psychopathologies and 80 healthy controls) were submitted to the breath sampling.

Patients had to fast overnight in order to avoid potential interferences in the VOCs concentration due to the digestive process of the ingested foods. For the breath sampling, subjects exhaled once through a device called Bio-VOCTM (Health and Safety Laboratory) (Figure 1) into a small glass vial of 20 ml volume conditioned to warm (80° Cs) (Figure 2). The Bio-VOCTM dealt with a special syringe for air of the volume of 250 mls that guaranteed a monodal run of the expired air avoiding reflux phenomena using an in-house valve. The tool was composed besides by a throwaway nozzle and a piston that allowed a sure rinse to air of the room before the use of the device. After completing exhalation, the glass vial was crimped airtight with the appropriate crimp cap (Figure 3).

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Figure 1. Bio-Voc, nozzle and vial

Figure 2. Breath air sampling

Figure 3. Crimped airtight vial

The collected sample was submitted to the flux of air produced by the expiration itself that allowed a more correct measurement of the concentrations of water-soluble VOCs for the presence of smaller concentration of aqueous vapor in comparison to that of the air contained inside the syringe. Two samples of expired air were collected for each subject in order to consider standardized the sampling procedure when the data resulted by the analysis were superposable. The vials were preserved to -20° Cs up to the moment of the Mass spectrometry analysis using "Airsense" (V&F medical development GmbH, Absam, Austria) (Figure 4).

At the beginning Airsense has been used in the industrial and environmental fields for the measurement of traces of gas and in the anaesthesia area for the measurement of exhaled drugs concentration.

The necessary ionization process for the detection of sample molecules is performed via ion beams interacting with the gas sample. Mercury or xenon were first ionized by electron impact. These primary molecule ions then effected a smooth charge exchange with the breath molecules. This procedure is termed Ion Molecule Reaction (IMR). It widely avoids the fragmentation of molecules and similarly allows to distinguish in some cases between mass identical components, e.g. carbon dioxide and acetaldehyde due to the use of different primary energy levels. After this soft ionization the breath ions are separated in a quadrupol mass filter that allowed the quantification of the single compounds.

The vials were placed in the V&F autosampler, heated up to 65°C and dynamically transferred to the V&F Airsense.

The measured gas compounds are given as absolute concentrations (ppb) and volume percent for CO2 and O2. Seven of all components (O2, CO2 acetylene, ethane, ethylene, n-propane, butadiene) were directly calibrated by using calibration gases. Our mixture of calibration (13 components) contained two aldehydes (formaldehyde and acetaldehyde), two acids (acetic ac. and formic ac.), two ketonis (acetone and methylethylketone), three alcohols (methanol, ethanol and propanol), two aliphatic hydrocarbons (pentane and heptane) and two aromatic hydrocarbons (benzene and toluene). The rest of the gases were indirectly calibrated to the sensitivity of one directly calibrated component (benzene).

Since it dealt with low energy ionizations the fragmentation of the molecules that would have allowed a more precise qualitative evaluation of the compounds didn't happen; otherwise the directly injection of the sample in of ionization chamber

allowed the quantification, with excellent sensibility (up to 0,2 ppb-1 ng/liter), of about one hundred compounds in 40 seconds.

The percentages of carbonic anhydride was tested in order to confirm the alveolar origin of the collected air (CO2 > 2%). Samples with inferior levels of CO2 pointed out that the vial was not crimped airtight or that the alveolar air sampling had not correctly been performed.

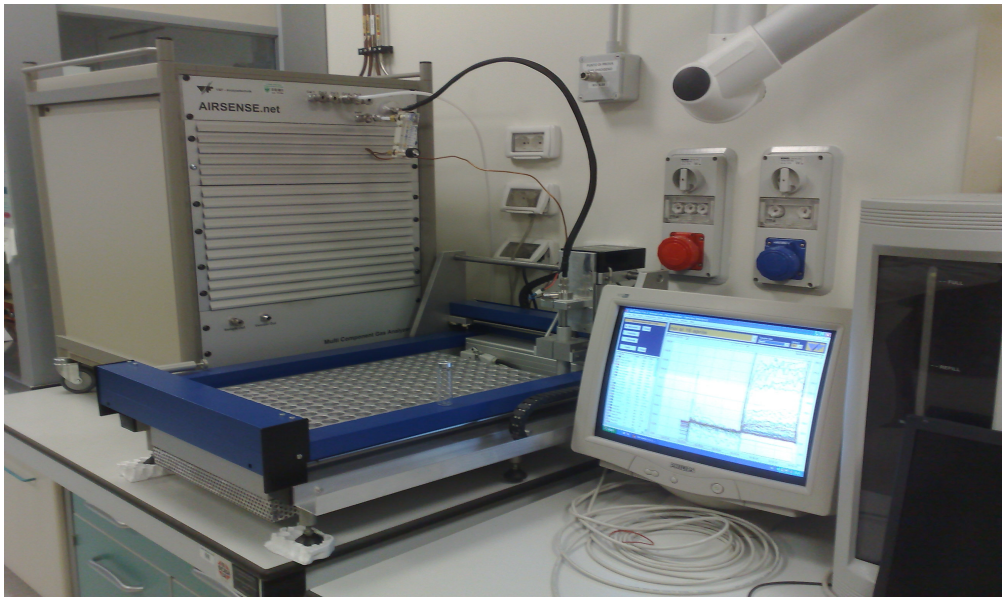

Figure 4. **Airsense mass spectrometry**

- **<u>Statistical analysis</u>**

We computed the Mann-Whitney-Wilcoxon test and the Student's t-test for comparison of medians and means of VOCs concentrations between the two groups (cases and controls); a p value <0,05 was considered statistically significant. The elaboration of the data has been performed through the statistical software "StatGraphics plus".

Subsequently it was necessary to understand what the dynamics between the molecules were in order to produce a high "predictive" model of the pathology under examination, considerating the high number of independent variables (molecules) in relationship with the low number of cases and controls.

First we have performed a non-rotate factorial analysis (principal components). This has allowed us to admit for affinity the molecules in groups (or

factors or latent variables). We have chosen to limit the principal factors to five. We have gathered therefore all the molecules according to the five factors with the following criterion: a molecule has been assigned to the factor which expressed the highest value of correlation with (factor loading) or however a value of factor loading >0,5.

We have built therefore a correlation matrix with all the molecules. The correlations superior to 0,98 are been identified and analyzed and we have decided to exclude the least significant molecule; the motivations of the exclusion of such variables will be given in the results.

Factorial analysis has allowed us therefore to perform some logistic regressions for each group of variables/molecules, with the exclusion of those molecules correlated with other variables/molecules. The logistic regressions have included therefore in the model the molecules that were associated from time to time to the considered factors and they have also included the variables age and sex. In each regression we have adopted a step-down procedure with a level of significance to 0,05. The logistic regression is a model of regression that is applied when the dependent variable is a qualitative and dichotomic one and referable to the values 0 and 1 (in our case affiliation to the cases or to the controls). The factorial analysis, the matrix correlation and the logistic regression have been performed with the program Intercooled Stata 9.0 for Windows (StataCorp LP, College Station, U.S.A.).

RESULTS

Bullying was the working condition most frequently associated with Psychopathology. 39% of the subjects were victims of bullying at work: 26% of the patients suffering from psychiatric disorders reported personal bullying and 13% reported task-related bullying. 37% of the workers had experienced work-related distress, 24% reported non-specific discomfort (Figure 5). The mean duration of the negative working condition was of 30 months.

Psychopathologies in workers who had experienced negative working conditions were represented by various reactive neuroses, in particular, mixed anxiety and depressive disorder (51%), adjustment disorder (16%), depressive

disorder (14%), anxiety disorder (10%), post-traumatic stress disorder (PTSD) (3%); 6% of workers were diagnosed with other non psychotic disorders (figure 6).

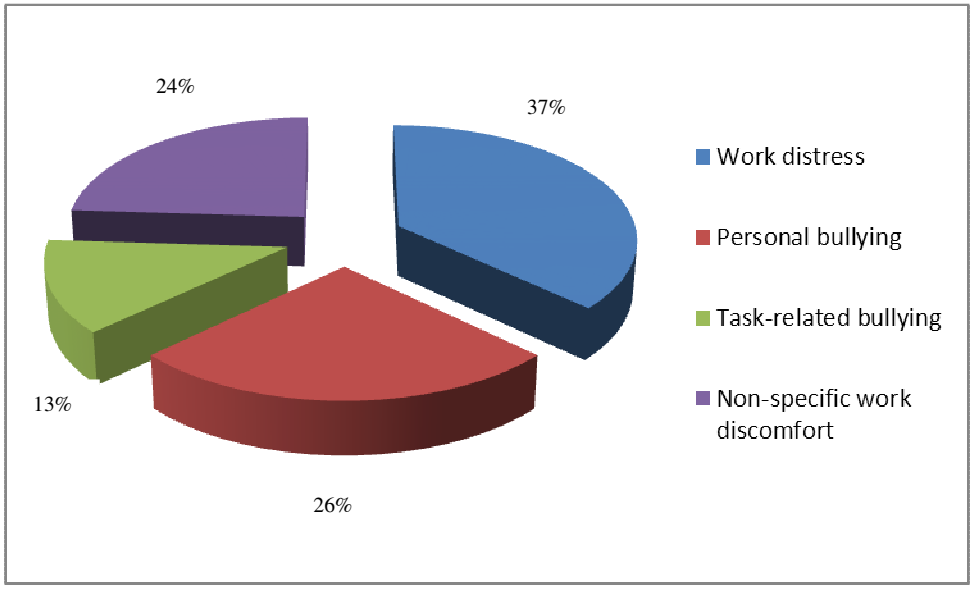

Figure 5: Percentage of work uneasiness

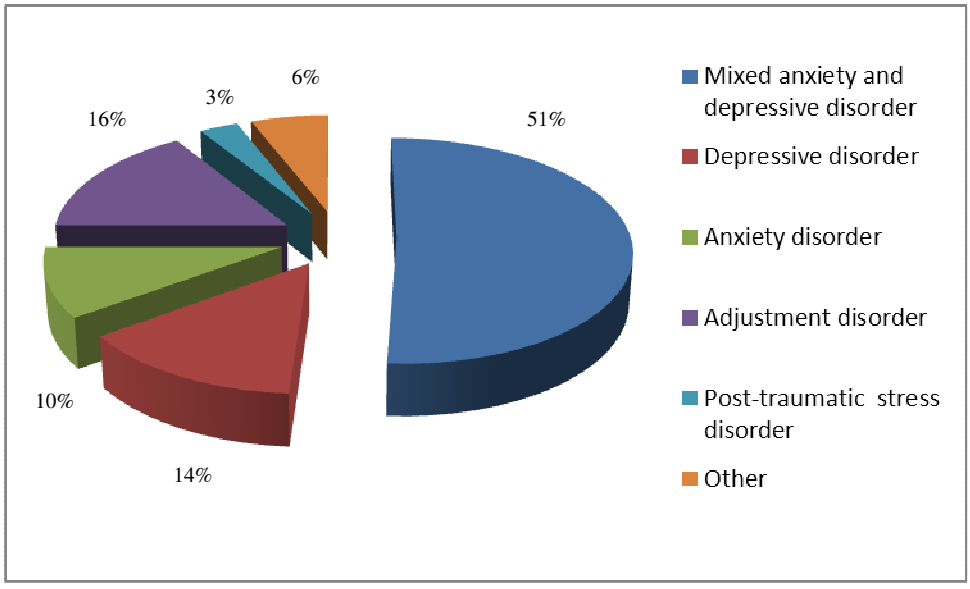

Figure 6. Percentage of psychiatric disorders

Many workers suffered from somatoform disorders. 36% of the subjects complained gastro-intestinal disorders (dyspepsia, hyperemesis and abdominal pain), 34% cardio-vascular symptoms (tachycardia, high blood pressure), 20% eating disorders (mainly hyperfagia with consequent gain in weight). 15% of the workers referred genito-urinary impairment (decline in the libido, menstrual disorders) and 10% suffered from muscle-skeletal disorders (cervical and low back pain) (Table 4).

Table 4. Percentage of psychosomatic symptoms

Apparatus	**%**	**Symptoms**
Gastro-intestinal	36	dyspepsia, hyperemesis, abdominal pain
Cardio-vascular	34	tachycardia, high blood pressure
Genito-urinary	15	decline in the libido, menstrual disorders
Muscle-skeletal	10	cervical and low-back pain

79% of our patients were using psychotropic drugs in association (18%) or less (61%) with non psychotropic drugs when they came to our attention; 6% of the subjects had used psychotropic drugs in the past. 15% of the patients had never used psychotropic drugs (Table 5).

Table 5. Percentage of drugs assumption

Drugs assumption	**%**
Positive	**85**
Psychotropic drugs	61
Psychotropic drugs in association with others	18
Non psychotropic drugs	6
Negative	**15**
Psychotropic drugs assumption in the past	6
Any psychotropic drugs assumption	9

Concentration of 101 volatile compounds (masses from 16 to 123) in the exhaled breath was measured using IMR-MS. 36 compounds were known for their chemical structure, 65 were known only for their masses (Table 6). Most of compounds were at very low concentrations (ppb: parts for billion).

Table 6. Volatile organic compounds detected in the alveolar air samples

VOCs with known chemical structure		VOCs with unknown chemical structure		
Methane	Acetylene	M19	M27	M29
Ethane	Formaldehyde	M32	M33	M37
Methanol	ACN	M43	M46	M47
N2O	Formic Acid	M48	M49	M56
HNO2	Acetic Acid	M60	M61	M62
SO2	HCN	M63	M66	M67
H20	O2	M69	M70	M71
CO2	NH3	M73	M74	M75
Ethylene	NO	M76	M77	M79
CH3NH2	H2S	M81	M82	M83
Propene	Acetaldehyde	M85	M87	M88
Ethanol	Butadiene	M89	M90	M91
MEK	Acetone	M93	M94	M95
Propanol	Isoprene	M96	M97	M98
Pentane	Benzol	M99	M101	M102
Methylcyclopentadiene	Toluol	M103	M104	M105
Heptane	Cyclohexene	M106	M107	M108
n-Hexane	Octane	M109	M110	M111
		M112	M113	M115
		M116	M117	M118
		M119	M120	M121
		M122	M123	

The comparison between medians and means of the concentrations of the molecules respectively through Mann-Whitney-Wilcoxon test and Student't-test has

underlined that a significant difference resulted (p value < 0.05) for the most of the compounds between the cases and the controls to a 95% confidence interval (Table 7). The figure 7 reports the graphic representation of the comparison of some molecules through the system of "Box & Whisker" Plot.

Table 7. Comparison between medians and means of VOCs concentrations (ppb) in the alveolar air of the cases and the controls

VOC	Median	Mean	VOC	Median	Mean	VOC	Median	Mean
Methane			Acetylene		•	M27		•
Ethane		•	HCN			Formaldehyde	•	•
Methanol	•	•	ACN	•		N2O		
Formic Acid	•	•	HNO2			Acetic Acid	•	
SO2	•	•	NH3	•		M19	•	
Ethylene			NO	•	•	CH3NH2	•	
M32	•		M33	•	•	H2S	•	
M37	•	•	Propene	•		M43	•	
Acetaldehyde	•	•	Ethanol	•		M46	•	
M47	•		M48			M49		
Butadiene	•	•	M56	•	•	MEK	•	•
Acetone	•		Propanol	•		M60	•	
M61	•	•	M62		•	M63	•	•
M66			M67			Isoprene		
M69	•	•	M70	•	•	M71	•	•
Pentane	•	•	M73	•	•	M74	•	
M75			M76			M77		
Benzol	•		M79			Methylcyclopentadiene	•	
M81	•		M82	•	•	M83	•	•
Cyclohexene	•	•	M85	•	•	n-Hexane	•	•

VOC	Median	Mean	VOC	Median	Mean	VOC	Median	Mean
M87	•	•	M88	•		M89	•	•
M90	•	•	Toluol			M93	•	
M94	•		M95	•		M96	•	
M97	•	•	M98	•	•	M99	•	•
Heptane	•		M101	•		M102	•	
M103	•		M104	•		M105		
M106	•		M107			M108	•	•
M109	•	•	M110	•	•	M111	•	•
M112	•	•	M113	•		Octane	•	•
M115	•		M119	•	•	M120		
M121			M122	•		M123		

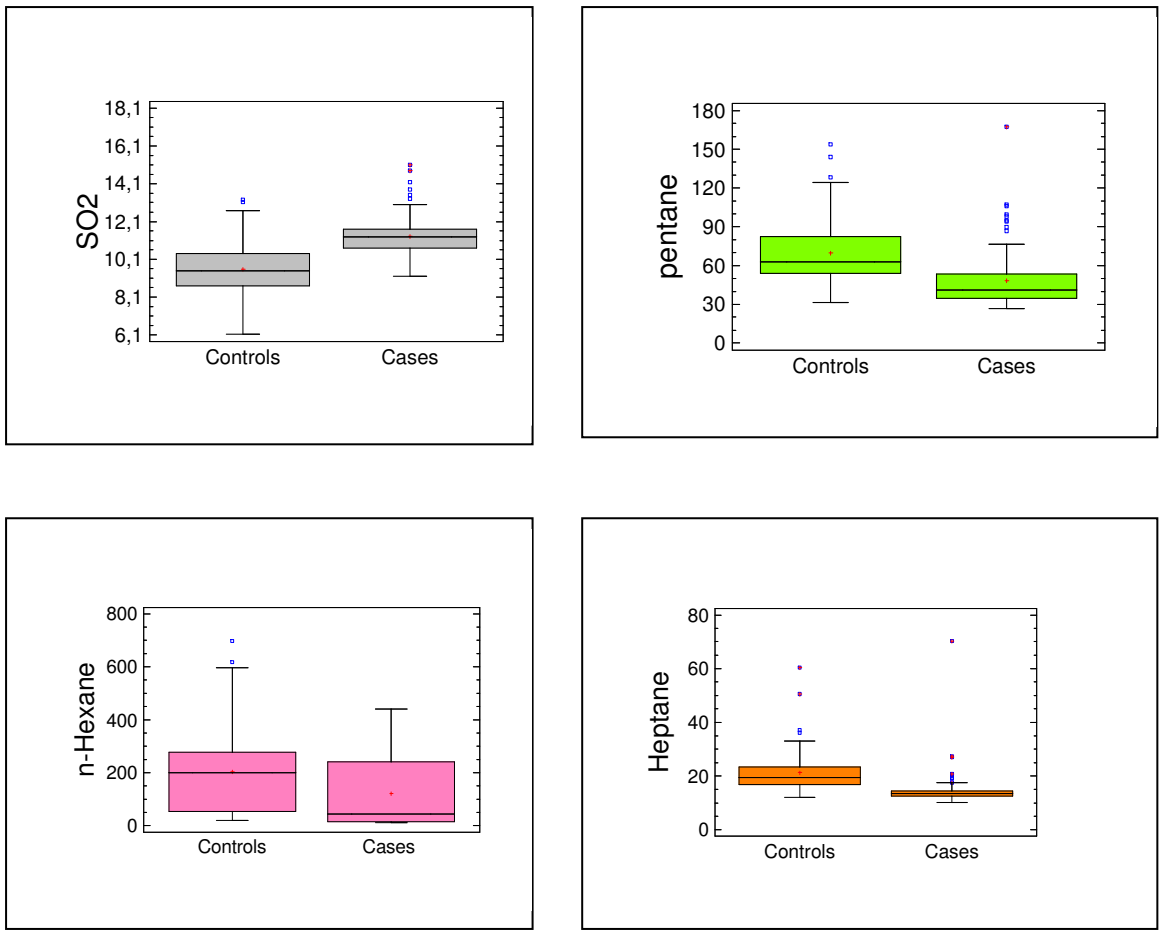

Figure 7. Comparison between some VOCs concentrations (ppb) in the alveolar air (cases and controls)

Factorial analysis has allowed to gather the molecules in five separate groups. We have decided to limit the factors to five because the first five factors express however 69 % of the variability with little increase for the addition of further factors (<4,5%).

For the first factor, having fifty molecules, it has been necessary to subsequently divide the variables in three groups for order of expressed correlation with the factor. Three logistic regressions have been therefore conducted. First we have adopted a step-down procedure on the logistic regression including the least correlated molecules. Only the significant variables have been held and have been therefore added to the next logistic regression. We have adopted therefore the same criterion and the significant variables have been added to the last model. The final model correlated to the first factor contains eleven significant molecules, five compounds were known for their chemical composition (hydrocianic, pentane, cyclopentadiene, acetonitrile, butadiene) and six were known only for their masses (M70, M71, M74, M75, M97 and M123); this model expresses a high predictivity with a Pseudo R2=08887 (Table 8). The logistic regression related to the second factor holds back nine molecules and reaches a Pseudo R2=07021. The regression related to the third factor holds back five molecules (Pseudo R2=07013). The regression related to the fourth factor holds back four molecules and it expresses the highest value of Pseudo R2=09173. The fifth regression holds back three molecules with a Pseudo R2=07927. We have tried to admit the models holding only the significant variables. The aggregation of the fourth and the fifth models reaches a Pseudo R2=0,9371 with only three molecules (analysis ROC-Receiver Operating Characteristic: area under the curve=09986): one compound with a known chemical structure, the heptane, and two molecules known only for their masses (M 27 and M 101). The further aggregation of this model (4+5) with the third one, the second one and the first one makes the predictivity worsen. We have decided therefore to hold two models: the model that is the expression of the fourth and the fifth one and the model expression of the first factor (analysis ROC: area under the curve=09964) (table 8). We have performed therefore a predictive model that, through an algorithm, considered the result of the two models. The model 1 reach in fact a 100,% sensibility with a 86,25% specificity, while the model 4+5 reach a 100% sensibility with a 96.25% specificity. The decisional algorithm establishes that if a subject was not pathological it has a probability expressed by the model 4+5 inferior

to 0,05 or, otherwise, it has a probability expressed by the model 1 inferior to 0,05. The subject must be considered pathological if the two conditions are not respected. This algorithm allows the identification of all the pathological subjects without any false negative and only one false positive (sensibility 100%, specificity 98,75%).

The algorithms are boxed below:

- **Modello 4 + 5** = 1/(1+EXP(-(85,84796 -0.5182649)*[M27] + 8,006762*[M101]-5,544806*[Heptane]+4,137078*sex+0,1146288*age)))
- **Modello 1** = 1/(1+EXP(-(15,11304+0,2658458*age+1,165599*sex-0,6112933*[M97]-0,2654679*[Penthane]-0,4372534*[M74]+1,792575*[M71]-0,0078143*[M29]+0,0951454*[ACN]-1,705469*[Butadiene]+0,5727221*[M70]-1,74183*[M72]+0,564359*[M75]+2,763382*[M123])))
- **Modello 1+ 4+ 5** = 1/(1+EXP(-(17,67192+0,2164149*age+sex*2,183223-0,1247702*[M27]-0,547588*[M97]-0,3706375*[M74]+1,588395*[M71]-0,0065562*[M29]+o,0846187*[ACN]-1,117604* [Butadiene]+0,2642161*[M70]-1,741447*[M72]+2,620559*[M123])))

Table 8. Significant molecules and statisitcal models

Model 1	Model 4+5
Pentane	M27
Cyclopentadiene	Heptane
Acetonitrile	M101
Butadiene	
Hydrocyanic Acid	
M70	
M71	
M74	
M75	
M97	
M123	

The comparison between medians of the concentrations of these molecules through test of Mann-Whitney-Wilcoxon has underlined that a significant difference results (p value < 0,05) for the most of the compounds to a 95% confidence interval between the cases and the healthy controls, except four molecules M27 (p value 0,259), hydrocyanic acid (p value 0,073), M 75 (p value 0,1280) and M 123 (p value 0,0766) (Table 9).

Table 9. Comparison between medians of the VOCs concentrations (ppb) belonging to the models 1 and 4+5 (cases and controls)

	Cases	**Controls**	
	Median		**P value**
Model 1			
Pentane	41,6	62,715	2,64722E-12
Cyclopentadiene	11,45	12,88	0,0454775
Acetonitrile	90,55	150,155	0,000568686
Butadiene	13,05	15,435	1,67036E-7
Hydrocianic Acid	1054,45	1082,8	0,0737524
M70	38,8	51,17	1,60906E-10
M71	19,15	33,42	1,63425E-12
M74	7,9	12,4	0,00165247
M75	5,1	4,4	0,1280
M97	25,6	34,335	0,000058025
M123	6,7	8,1	0,0766
Model 4+5			
M27	49,4	46,655	0,259596
Heptane	1,8	2,515	0.00142498
M101	13,5	19,415	0,0

Environmental air samples that had been collected togheter with the alveolar ones have been also analysed. VOCs in the environmental air can originate from

different sources, human production, vegetable, environmental pollution. The partial pressure of many volatile compounds resulted higher in the expired air than in the environmental air confirming their endogenous origin. In table 10 are reported the medians of the alveolar concentrations and the corresponding environmental ones of the VOCs of the models 1 and 4 +5.

Table 10: Comparison between medians of VOCs concentrations (ppb) in the environmental and in the alveolar air

VOCs	Environmental air	Alveolar air
Model 1		
Pentane	51,66845238	64,07
Cyclopentadiene	11,28071429	12,93
Acetonitrile	119,2816667	150,16
Butadiene	15,25988095	15,47
Hydrocianic Acid	912,4719048	1098,47
M70	44,76595238	51,64
M 71	25,62904762	33,42
M 74	5,3675	12,62
M 75	3,328452381	4,43
M97	27,06345238	34,34
M 123	7,0893	8,06
Model 4+5		
M 27	51,53095238	46,76
Heptane	16,44214286	19,52
M 101	1,539761905	2,51

DISCUSSION

Our data show the wide range of psychopathologies related to the working environment. Depressive disorders, mixed anxiety and depressive disorders, anxiety disorders and adjustment disorders were the most commonly found.

Bullying resulted the prevailing form of working uneasiness; psychic and psychosomatic disorders could also occur through the experience of stress as a result of work distress and non specific discomfort.

Another study on the prevalence of stress related psychopathologies involving 303 workers had already been conducted in the past by the "Center for the Analysis of Psychosocial Hazards and Pathologies of Working Origin". The prevailing pathology was the mixed anxious-depressive disorder (51.6%); 17.9% of the workers suffered from depressive disorders and 12.1% from anxiety disorders, while in the 16.9% of the cases adjustment disorders and only in the 1.5% Post-traumatic Stress disorders had been found (Romeo and coll. 2009 and 2010). These results agree with recent studies (Mausner 2000; Niedhammer and coll. 2006; Melchior and coll. 2007; Brousse and coll. 2008; Cherry 2009) that from a side have underlined the strong prevalence of anxiety and/or depression as stress-related disorders and from the other one they have reduced the prevalence of the PTSD that in previous studies was recognized as the prevailing disorder as a consequence of Bullying (Leymann and Gustavsson 1996; Matthiesen and Einarsen 2001 and 2004). A revaluation of the psychiatric disorders for which a probable working origin is recognized and indemnified should come. Currently INAIL recognizes and indemnifies only adjustment disorders and post-traumatic tress disorders as psychiatric pathologies due to task-related Bullying, while other disorders resulting more frequently correlated with negative conditions at work are not being kept in consideration.

The stress-related psychopathologies arise through the extended and excessive activation of the hypothalamic-pituitary-adrenal axis that leads to the exhaustion of the psychic and physical resources. A recent study has underlined that the corticotropin releasing factor (CRFR1) increases the number of specific receptors of the serotonin (5HTR) on the nervous cells which are involved in the development of depression (Natures Neuroscience 2010).

The contribution of the alveolar air analysis to the diagnosis of different pathologies has been documented from manifold studies conducted in the last decade, but this biological matrix is not yet commonly used for diagnostic purposes. Technical problems in the sampling and the analysis, lacks in the normalization and standardization of the method are responsible of the wide differences found in the different studies and they explain the reason for which the analysis of the expired air has not entered the clinical practice yet (Miekisch and coll. 2004).

In 2009 Netzer and coll. have conducted a study on liver disorders using the same methodic of alveolar air sampling and the same analytical instrument (Airsense) adopted in our search.

Our data have shown that the volatile organic compounds in the alveolar air differ from a quantitative and not qualitative point of view in the two groups (subjects with stress-related psychopathologies and healthy controls).

Factorial analysis has been used for understanding the relationship among the different molecules dividing them in similar groups and finally extracting the most important molecules. A logistic regression using all the molecules contemporarily has not been possible to effect because of the elevated number of the compounds and the small numper of the cases and controls in proportion. Factorial analysis has been conducted limiting the number of factors to five and attributing to each of them the molecules more tightly in relationship with that factor or however with a superior correlation to 0,5. In this way all the molecules were been attributed to one of the five principal factors. A separate logistic regression was carried out for each factor so that to consider in the model only the significant variables to the 95% of confidence. All the significant variables of the five models have finally been reunited. In the logistic regression the binary result is the belonging to the group of the cases or the controls; all the regressions have been corrected for sex and age.

Using two different models with a combination of eleven molecules (pentane, cyclopentadiene, acetonitrile, butadiene, hydrocyanic acid, M70, M71, M74, M75, M97 and M123) and three molecules (M27, heptane and M101) has been possible to classify correctly the cases and the healthy controls with a 100% sensibility and a 98,75% specificity (one false positive and any false negative).

A lot of molecules that characterize these profiles are known only for their masses. This is one of the critical aspects of this study and it is due to the analytical method used (mass spectrometry) that results relatively little specific if compared to

the gas-chromatographic method. Nevertheless the use of mass spectrometry has entailed different advantages: first of all the short time required for the dosing of about one hundred molecules in each sample and the low cost thanks to the possibility to simultaneously analyze hundreds of samples a day; besides the high sensibility of the mass spectrometry analysis allows to quantify molecules at very low concentrations (ppb). The gas-chromatographic analysis of the molecules known only for their masses would allow the identification of their chemical structure and accordingly a great understanding of the physiopathological processes involved in their modifications. In the meantime it is possible to try an identification in terms of probability for some of the organic compounds known only for their masses. M 70 could be identified with the 1-pentene, M 74 with the n-butanolo and M 75 with the nitroethane or the 1-propanolo-2amino.

Manifold studies have identified the main metabolic processes responsible of some compounds production, like the lipidic peroxidation of the cellular membranes, the ethanol metabolism, the cholesterol biosynthesis, the protein metabolism and the activity of intestinal bacteria (Buszewski and coll 2007). The extended and excessive activation of the hypothalamic-pituitary-adrenal axis could influence the metabolic processes responsible for the quantitative modifications of these molecules.

The volatile alkanes, as pentane and the heptane, are markers of oxidative stress and they are produced by the peroxidation of polyunsaturated fatty acids in cellular membranes and then they are exhaled with the breath. Their concentrations in the expired air are also conditioned from other factors such as the age of the subject, drugs assumption or foods intake that can modify the activity of the citocromo P450 enzymes.

Only few subjects with psychopathologies were not using psychotropic drugs when they came to our attention; therefore it has not been possible to appraise the significance of potential differences in the concentration of VOCs inside the group of the cases in relationship with drugs assumption. Nevertheless these subjects came all correctly classified in the group of the cases using the two profiles of molecules.

CONCLUSION

Our study represents an attempt to apply the analysis of the alveolar air, that has already furnished interesting data for the clinical diagnostic of manifold pathologies, to the assessment of stress-related psychopathologies. The analysis of the organic volatile compounds in the exhaled air of subjects with psychiatric disorders and of healthy controls has allowed to indentify two different profiles of molecules that are able to distinguish with high sensibility and specificity the cases and the controls. Some advantages of alveolar in comparison to the use of other biological matrices are primarily referable to the non-invasivity, to the simplicity of the sampling and to the low cost of the analysis.

Future developments will be the close examination of the physiopathologic processes that can modify the concentrations of specific volatile organic compounds in the expired air of subjects with stress-related psychiatric disorders. Primarily it is important the identification of the chemical structure of the compounds known only for their molecular masses associating the mass spectrometry analysis with other methods that can identify the molecular structure (GC-MS); a validation of the analytical method (definition of sensibility, linearity, repeatability, toughness, etc.) is essential to make possible a comparison with the other data in literature and the clinical use of the obteined results.

It will be necessary to widen our case record in order to confirm our results. A close examination of the role of psychotropic drugs in the modifications of VOCs concentrations will be considered.

A follow-up of the subjects suffering from stress-related psychopathologies should be programmed in order to observe the potential modifications of the concentrations of the volatile organic compounds in the alveolar air in relationship with clinical and/or working improvements.

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