

Molecular diagnosis of lung cancer: an overview of recent developments

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Abstract. Health surveillance of workers occupationally exposed to lung carcinogens calls for screening procedures which may not be fully justified, owing to current uncertainties about the outcome of early detection. Indeed, bias-free designs are difficult to set up, and the effects of lead time, length and screening biases can all result in an overestimation of the benefits of screenings, which certainly increase survival, but without any actual reduction of mortality. A major issue with modern imaging techniques is the very high incidence of discovery of lung nodules, usually false positive, but still calling for additional and sometimes painful examinations. Currently, the differential diagnosis is mainly based on additional imaging approaches, particularly positron emission tomography, which is very expensive and also shows limitations in terms of sensitivity and specificity. Therefore, purely morphological criteria seem to be insufficient to distinguish lung cancer at early stages from benign nodules with sufficient confidence. A molecular approach to the diagnosis of lung cancer through biomarkers measured by non-invasive means could greatly improve the specificity of imaging procedures. Extremely sensitive mass spectrometric techniques and polymerase chain reaction-based methods are available to detect, in accessible media, molecular alterations which characterise lung cancer at an early stage, thereby reducing the rate of false positives. The lessons learnt from decades of screening programmes based on imaging and the future prospects possibly enhanced by using biomarkers are briefly discussed in this overview. (www.actabiomedica.it)

Key words: Lung cancer, screening, biomarkers, occupational diseases, smoking

Epidemiology

Lung cancer is the most commonly diagnosed cancer worldwide, but its geographic distribution shows distinct regional variations: it tends to be most common in developed countries, particularly North America and Europe, and less common in developing countries, particularly Africa and South America (1). Almost 900.000 new cases of lung cancer in men and 300.000 in women are diagnosed worldwide each year. More people die of lung cancer than of colorectal, breast, and prostate cancer combined (2).

In Italy, lung cancer is the leading cause of death

due to cancer in men and the second (after breast cancer) in women, and 35.000 people per year die of lung cancer (3). The overall mortality has not significantly changed in decades: only 5 % cured in 1950's, 13% in 1970's, only 15% cured today (4). The prognosis of lung cancer is still poor because of the absence of valid approaches to its early detection, the frequency of metastases at the time of diagnosis, frequent recurrence after surgery, and poor responsiveness to chemotherapy. Furthermore, the increase in average age of the population, smoking and traffic related pollution are all factors which will probably give rise to an additional increase in the incidence of pulmonary tumours (5).

Risk factors

The association between lung cancer and smoking is probably the most intensively investigated relationship in epidemiology. Indeed, cigarettes are the greatest risk factor, accounting for 87% of lung cancers. Absolute risk of lung cancer decreases over time after quitting smoking. Nevertheless, 50% of lung cancers now occur in former smokers (6).

Environmental or second hand tobacco smoke is also implicated in causing lung cancer (6). Environmental tobacco smoke has the same components as inhaled mainstream smoke, although at lower concentrations – between 1% and 10% depending on the constituent. An association between exposure to passive smoking and lung cancer risk in non-smokers has been shown in a number of case-control and cohort studies (7-9). Although tobacco had been widely used throughout the world for centuries, the present pandemic of lung cancer may be due to the introduction of manufactured cigarettes with addictive properties, which resulted in a new pattern of sustained exposure of the lung to inhaled carcinogens (10).

Even though tobacco smoking is now widely recognized to be the main cause, there are other risk factors, some of which act in concert with smoking to synergistically increase the risk. Occupational causes of lung cancer include arsenic, asbestos, chromates, chloromethyl ether, nickel, polycyclic aromatic hydrocarbons, radon daughters, and probably other agents for which evidence is still insufficient. Outdoor air, contaminated by combustion-generated carcinogens, is also considered to contribute to the lung cancer burden in urban dwellers living in polluted areas.

De Matteis et al. (this issue, page 34) reviewed the scientific evidence on the lung cancer burden from occupational exposures. The largest population attributable fractions (PAF) were estimated in highly industrialized areas with a high prevalence of shipbuilding and railroad equipment manufacturing, metal and chemical industries, estimates being similar in different countries. A decreasing trend over the last 30 years is mainly accounted for by improved working conditions and continued effort in identifying carcinogenic agents and managing the associated risk by banning hazardous substances or strictly controlling possible exposure

sources. Despite such a decreasing trend, the etiologic fraction of lung cancer still attributable to occupational exposures remains in the order of 2-3%, or several hundred annual cases in Italy. Asbestos is the single occupational agent causing the highest number of lung cancers, particularly in our country, where hundreds of thousands of workers have been exposed until the 1992 ban, with PAFs ranging from 7% to 19%.

Scarselli et al. (this issue, page 24) provided a contribution to the estimation of oncogenic risks in occupational settings, on the basis of administrative data for all companies recorded in the ISPEL database. The existence of a register of companies with activities linked to lung cancer risk, will allow regional prevention and surveillance programmes to be planned, focussed on industrial sectors at greatest risk. The expected geographical heterogeneity was confirmed, being mainly accounted for by the high prevalence of mechanical and metal fabrication industries especially in northern Italy. The province of Trieste has a higher lung cancer mortality than other Italian provinces, occupational lung cancer accounting for 16% to 25% of the total lung cancer cases, probably due to mechanical, and metallurgical industries, docks and shipyards. The Liguria region, particularly in the provinces of Genoa and Savona, also presents a high lung cancer risk among refinery workers. In particular, maintenance and other blue-collar workers have been found to be highly exposed to hydrocarbons and asbestos, widely used as a thermal insulator for boilers and pipes. Some unexpected findings suggesting a geographical dissociation between the presence of industries implying a greater risk and the incidence of lung cancer may be accounted for by commuting.

Scarselli et al. (this issue, page 24) indicate that 600.000 “industry and services” workers are occupationally exposed to lung carcinogenic agents, corresponding to 4% of Italian workers, an estimate much lower than the 4 million workers estimated to be potentially exposed in Italy by the European study CAREX. However, the difference in these estimates is mainly due to the selection criteria of worker categories considered as exposed, which may be limited to blue collars or may include subjects with low or no exposure.

Indoor air contains several respiratory carcinogens too, including radon. The suspicion that radon

was a cause of lung cancer in underground miners, raised early in the 20th century, led to what was probably the first occupational respiratory carcinogen to be identified; radon in indoor environments is now considered as the second-leading cause of lung cancer in the United States (11). In some developing countries, exposure to fumes from cooking stoves and fires has been found to be associated with lung cancer risk (6).

Lung cancer prevention

Among different approaches to reducing cancer morbidity and mortality, any action limiting cigarette smoking initiation or promoting cessation is an important contribution to lung cancer prevention. This includes tobacco control activities, such as cigarette taxes and smoke-free workplace legislation, as well as interventions at the individual-level to prevent the onset or continuation of smoking. For instance, California lung cancer rates declined by 14%, compared with 2.7% in non-California SEER sites, coincident with declining smoking rates and probably due to California tobacco control initiatives (12, 13). However, although smoking cessation helps, residual risk remains. For example, in people quitting at age 50, a residual excess risk of 6% can be estimated at age 75 (14).

Secondary prevention of lung cancer is based on early detection. The prognosis of lung cancer depends largely on early detection and immediate treatment prior to metastatic spread. When the tumour is confined to the lung without pathological evidence of metastases (stage I), the 5-year survival rate after surgical resection ranges from 80 to 90% if the tumour size is small (4, 14-16). These data confirm that early detection and surgical treatment may improve lung cancer survival, thus suggesting that screening allows the detection of disease at a stage when intervention is still possible and perhaps useful; the target population is asymptomatic persons at risk for a specific disease. However, early diagnosis necessarily implies a longer survival. Unfortunately, early diagnosis also means more time spent in hospitals as a patient, which adversely affects the quality of life. Whether such an individual cost – which adds to the economic costs in-

curred by the National Health Service providing care – is justified by the benefit of a reduced cause-specific mortality, remains a question still waiting for definitive answers after decades of studies and papers alternating hope and expectation with disappointment. Before any screening procedures are usefully adopted, convincing evidence should indicate that early intervention actually changes the course of the disease, thus decreasing cause-specific mortality rates (17).

Screening for lung cancer: the past

Early detection trials of conventional sputum cytology and chest radiography proved unable to decrease lung cancer mortality. Screening with chest X-ray plus sputum cytology appeared to detect lung cancer at an earlier stage, but no reduction in lung cancer mortality has been observed. In the 1950's four non-randomized, uncontrolled screening studies, using chest radiography were performed: (i) Philadelphia Pulmonary Neoplasm Research Project (18); (ii) Veterans Administration Trial (19); (iii) Tokyo Metropolitan Government Study (20); (iv) South London Lung Cancer Study (21). Briefly, the studies demonstrated that lung cancer screening with chest X-ray did show some improvement in survival, which by definition can be attributed to early diagnosis, but mortality was not improved, suggesting that there were no benefits. Between 1950-1970, two nonrandomized but controlled trials using chest radiography were performed: the North London Cancer Study (22-25) and the Erfurt Country Study (26,27), respectively. The main results were that a high number of early-stage and resectable lung cancers were evident in comparison with controls, but no clear reduction in mortality was demonstrated.

In early 1970's, four randomized controlled trials, using chest radiography and sputum-cytology were performed: (i) John Hopkins Lung Project (28-33); (ii) Memorial Sloan-Kettering Lung Project (34-41); (iii) Mayo Lung Project (42-55); (iv) Czechoslovakian Study (56-61). Again, the main results were the detection of early stage lung cancer in the screened group, with an increase in the number of resectable cancers and a 5-year survival higher in screening

group, but the disease-specific mortality was unchanged. Screening and subsequent therapy did not affect the outcome of the disease.

Screening studies have failed to demonstrate that even the very earliest detection of lung cancer has led to improvements in survival. Why did the above trials fail? Difficulties in detecting lung nodules less than 10 mm, difficulties in detecting peripheral lesions or detection limited to advanced tumours might be possible explanations. Early diagnosis with these techniques did not delay death (62). However, several critiques have been provided on these studies. The International Union Against Cancer observed that many of these trials may be invalid owing to the absence of an un-screened study arm; thus no determination of true efficacy could have been made. Moreover, no women were included in these trials, despite their higher susceptibility to lung cancer and the increasing incidence observed in females. People enrolled in these studies were not at high risk for lung cancer from their smoking history, because some studies included subjects with only one pack-year of smoking. Another criticism concerned the inadequate sample size of these trials. In order to determine the role of chest X-ray in screening for lung cancer, a large randomised, controlled, NCI-sponsored trial (the Prostate, Lung, Colorectal and Ovarian - PLCO - Cancer Screening Trial) is ongoing (63).

Screening studies on lung cancer: the present

In more recent times, the advent of low-dose helical computerized tomography (CT scan) has encouraged a resurgence of interest in the secondary prevention of lung cancer. Regardless of whether it is demonstrated ultimately that chest radiographic screening is effective, there are several reasons to consider screening for lung cancer with low-dose CT. First, CT is far more sensitive than chest radiography. In addition, it is possible today to perform a spiral CT of the chest without intravenous contrast in less than 15 s, with a limited radiation dose and costs that are similar to those of screening mammography. Currently, the radiation dose for a single examination is approximately 3.3 mSv, only slightly more than the

United States average annual effective dose equivalent per person from natural sources (64).

A number of studies published in the last 10 years have demonstrated the high sensitivity of spiral CT and the favourable results achievable by systematic use of low dose CT of the chest for early detection in high-risk individuals.

In 1999 the Early Lung Cancer Action Program (ELCAP) enrolled 1000 symptom-free volunteers, aged 60 years or more, with at least a 10 pack-year history of smoking who were fit to undergo surgery. They received yearly CT scan and chest X-ray in a single arm study. The conclusion was that false-positive screening test results were uncommon and usually manageable without biopsy; compared with no screening, such screenings permitted diagnosis at substantially earlier and thus more curable stages. Annual repetition of CT screening was sufficient to minimize symptom-prompted interim diagnoses of nodule-associated malignancies (65-68).

The study of the Mayo Clinic enrolled 1520 subjects, aged 50 years or more, who also underwent annual sputum cytology and DNA analysis. A prospective cohort study was performed of these subjects, all of whom had smoked 20 pack-years or more. This experience showed that multislice CT can depict early-stage lung cancers but a much higher cumulative frequency of non-calcified nodules, giving rise to new questions about the challenge of differential diagnosis, efficacy, and costs of screening (47, 69-71).

In the International Early Lung Cancer Action Program (72), the investigators, in a large collaborative study, screened 31,567 asymptomatic persons at risk for lung cancer using low-dose CT from 1993 through 2005, and from 1994 through 2005; 27,456 repeated screenings were performed 7 to 18 months after the previous screening. The authors concluded that annual CT screening can detect lung cancer that is curable.

A recent study showed that when individuals are screened for lung cancer with CT, the likelihood that they are diagnosed with lung cancer is increased more than 3-fold, and the likelihood that they undergo a thoracic resection for lung cancer is increased 10-fold. However, as for chest X-ray screening, there appears to be neither a meaningful reduction in the number of

advanced cancers being diagnosed nor a reduction in the number of individuals who die of lung cancer (73). Therefore, a firm conclusion on CT scan as screening procedure to be applied in high risk patients for early lung cancer detection cannot be done, at least at this stage of knowledge. Several randomised trials are underway to evaluate the effectiveness of helical CT in lung cancer screening both in Europe and in the USA. The largest one is the U.S. National Lung Cancer Screening Trial as sub-study of PLCO, followed by the Dutch-Belgian NELSON trial. Smaller studies are on-going in Denmark and Italy (74, 75). The control arm receives no intervention in the European trials, while chest X-ray is offered in the US. All trials focus on smokers. While waiting for the results of these trials, the effectiveness of screening based on spiral CT remains unclear.

The reasons why CT scan screening in asymptomatic subjects at risk cannot be recommended are on the contrary straightforward: (i) the high prevalence of surgical interventions on patients who do not actually suffer from lung cancer (70); (ii) evidence of a clear benefit from early diagnosis is still lacking (73); (iii) available evidence suggests that screening for lung cancer with low-dose CT may increase the rate of lung cancer diagnosis and treatment, but may not meaningfully reduce the risk of advanced lung cancer or death from lung cancer (73). Thus, until more conclusive data become available, asymptomatic individuals should not be screened outside of clinical research studies. Such studies include the identification and validation of biomarkers aimed at increasing the specificity of imaging and possibly at anticipating the diagnosis of true (pre)cancerous lesions, whose removal might actually result in reduced mortality.

Molecular diagnosis of lung cancer: current issues and future prospects

An emerging approach to lung cancer diagnosis is based on additional methods complementing imaging techniques. Indeed, purely morphological criteria seem to be insufficient to distinguish lung cancer at early stages from benign nodules with suf-

ficient confidence, so that false positives undergoing surgical resection occur frequently. For example, in the Mayo Clinic report on the outcome of screening procedures, the number of patients showing benign nodules at operation for resection were about one third of stage I malignancies (70). Complementary methods based on alternative approaches would thus be useful not only to pick out lung cancer at screening, but also and especially to exclude its occurrence in cases of pulmonary nodules frequently detected by CT scans.

The widespread use of modern sensitive CT scans in asymptomatic subjects is already posing a new problem to radiologists and chest physicians, owing to the increasing frequency of nodules which are identified. In Italy, many thousand people per year require additional and sometimes invasive diagnostic procedures and a strict follow-up in subsequent years because of false positive images, which have been estimated to occur in 96% of adults undergoing chest CT scan for any reason (70). Complementary techniques would thus be useful not only to pick out lung cancer at screening, but also and especially to exclude its occurrence in cases of pulmonary nodules frequently detected by CT scans.

A pre-invasive phase of lung cancer may last for years. The length of this interval is a window of opportunity for early molecular diagnosis (76). A biomarker-driven approach to signs or symptoms possibly due to lung cancer would represent a complementary tool aimed at ruling out (with known error probability) rather than at diagnosing lung cancer. Easily collected fluids, such as sputum, exhaled breath condensate (EBC), exhaled breath and blood contain molecular alterations associated with lung cancer, and are therefore suitable materials for lung cancer biomarker research.

A note of caution coming from studies on apparently successful screening programmes for prostate and breast cancers is that the enormous amount of resources spent in early diagnosis in asymptomatic subjects, relying on either imaging or biochemical techniques may tremendously increase apparent incidence rates and related medical interventions, without changing the mortality from these causes, which remains only marginally affected.

Exhaled breath

The induced sputum technique allows sampling of the airways in a non-invasive manner and thus offers a unique opportunity to identify biomarkers of potential clinical utility in respiratory medicine. A molecular, rather than cytological evaluation of sputum may represent a promising area of research. Interesting data are available on genetic and epigenetic abnormalities detected in cancer-free heavy smokers, thus being possibly predictive of lung cancer development (77-80). However, induced sputum, although being practicable in a high proportion of patients, is considered moderately invasive, sometimes associated with discomfort. Furthermore, good quality samples are not always available, as some patients are not able to expectorate properly.

Volatile biomarkers

Among totally non-invasive tests, the analysis of exhaled air for volatile organic compounds (VOCs) seems to be very promising: published data have shown that exhaled breath contains a pattern of VOCs which distinguishes patients with and without lung cancer. The first report on exhaled VOCs dates from 1985, when Gordon et al. (81) using a specially developed breath collection technique and computer-assisted gas chromatography/mass spectrometry (GC/MS), identified several VOCs associated with the disease in the exhaled air of lung cancer patients. The selected volatile compounds had sufficient diagnostic power in the GC/MS profiles to allow almost complete differentiation between control and lung cancer patients (although in a limited patient population).

In 1999 Phillips et al. (82) used, for the first time in medicine, the term "breathalyser" for lung cancer. In patients with an abnormal chest radiograph, a combination of 22 VOCs in breath samples distinguished between patients with and without lung cancer.

Recently, Poli et al. (83) showed that a combination of 13 VOCs allowed the correct classification of the cases (lung cancer, COPD, asymptomatic smokers and healthy subjects) into groups defined relying on conventional diagnostic approaches. Furthermore, in this issue of *Acta Biomedica*, Poli et al. extended their

observation showing that the studied VOCs (with the exception of isoprene) did not show significant differences one month after surgery in comparison with baseline values, whereas VOC variations were observed when sampling was done 3 years after surgical removal. Of note, most of the VOCs in lung cancer patients were higher at baseline in comparison with control and this difference was still evident both one month after surgery and 3 years later. Therefore, exhaled VOCs cannot be considered specific biomarkers of lung cancer (they do not seem to be produced by tumor tissue), rather an epiphenomenon which accompanies lung cancer development, probably due to the chronic load and burden of VOCs in overall lung tissue. Factorial and discriminant analysis suggested the separation of VOCs into three categories including: (i) smoke- and environment-related substances, such as benzene, toluene, ethylbenzene and xylenes; (ii) endogenous constituents involved in inflammation, steroid genesis and methylation, such as pentane, isoprene, methyl-pentane; (iii) heterogeneous group of uncertain origin, such as octane, decane, styrene, and pentamethyleptane (83).

The identification of specific VOCs by using mass spectrometric techniques is promising, but GC-MS systems are expensive and require expert interpretation (84, 85). A simpler method of detecting unique patterns of VOCs would permit the broader application of breath testing for the diagnosis of lung cancer.

On the basis of these considerations, gaseous chemical sensing devices have been developed. The premise of these sensors is absorption of gases causing a change in conductivity, mass vibration or colour of the sensor, thus altering the output. Mazzone et al. (86) showed by using a colorimetric sensor array that patients with lung cancer present a unique chemical signature of the breath with moderate accuracy. Colorimetric sensors are inexpensive, reusable and, most of all, easy to use for point-of-care testing. On the contrary, they are not able to identify specific compounds within a mixture, and thus may not be able to identify potentially important VOCs.

Recent advances in odour-sensing technology, signal processing, and diagnostic algorithms have created chemical sensing and identification devices called

“electronic noses”. Electronic noses rely on arrays of chemical vapour sensors that respond to specific stereochemical characteristics of an odorant molecule, particularly VOCs (87). Like the human nose, its electronic counterpart responds in concert to a given odour to generate a pattern, or “smellprint,” which is analyzed, compared with stored patterns, and recognized.

In 2003 Di Natale et al. (88) used an electronic nose, comprising eight quartz microbalance gas sensors, coated with different metalloporphyrins. The application of a partial least squares-discriminant analysis found a 100 % classification of lung cancer-affected patients, while 94 % of the referents were correctly classified. The post-surgery patients were correctly identified in 44% of the cases, while the other samples were classified as healthy referents. The alteration of breath composition induced by the presence of lung cancer was enough to allow a complete identification of the sample of diseased individuals.

These positive studies were supported in 2005 by Machado et al. (89), who showed that the exhaled breath of patients with lung cancer has distinct characteristics that can be identified with an electronic nose.

A further improvement in odour sensors could derive from a better understanding of canine scent detection, due to the extraordinary scenting ability of the dog’s nose, which has detection thresholds as low as parts per trillion. In a recent study (90), five household dogs were trained to detect lung or breast cancer by sniffing the breath of cancer participants. The trial itself consisted of 86 cancer patients (55 with lung cancer and 31 with breast cancer) and a control sample of 83 healthy patients. All cancer patients had recently been diagnosed with cancer through biopsy-confirmed conventional methods. During the study, the dogs were presented with breath samples from the cancer patients and the controls, captured in a special tube. The results of the study showed that dogs can detect breast and lung cancer with sensitivity and specificity between 88% and 97%. Moreover, the study also confirmed that the trained dogs could even detect the early stages of lung cancer, as well as early breast cancer. This suggests that still unidentified substances either unstable or with properties requiring novel an-

alytical approaches can be found in exhaled air and that much work has still to be done prior to their identification and validation as biomarkers of lung cancer.

Non volatile biomarkers

Further biomarkers of lung cancer could derive from the collection and analysis of exhaled breath condensate (EBC). EBC, which is formed by cooling exhaled breath, contains low- or non-volatile markers which reflect, at least to some extent, the components of the airway lining fluid environment (91). The first applications of EBC to patients with lung cancer were in 2002–2004, when Carpagnano et al. (92,93) showed that the concentration of inflammatory markers such as cytokines (IL-6) and vascular stimulating factor (endothelin-1) were higher in EBC of patients with lung cancer than in controls. However, it is difficult to interpret properly these data, as the control group comprised only healthy subjects. As IL-6 and endothelin-1 are not specific to lung cancer, their concentrations should have been also assessed in those clinical conditions which precede or are associated with lung cancer, such as COPD.

More interesting data could be obtained studying genetic markers on EBC, being probably more specific for neoplasm. In this regard, Gessner et al. (94) showed that EBC contains DNA suitable for amplification, and p53 gene mutations were detected in EBC of lung cancer patients, while no mutation was found in healthy volunteers. Recently, micro-satellite DNA alterations that are specific to lung cancer have also been detected in EBC (95, 96).

EBC contains also biomarkers of oxidative stress, which is an imbalance between oxidants and antioxidants, associated with lung cancer development (97). Among different exhaled biomarkers of oxidative stress, hydrogen peroxide has been studied in lung cancer patients (98); however, further validation studies are required before its use.

Methodological issues in biomarker research

Corradi et al. (this issue, page 73) assessed H₂O₂ levels in EBC and bronchoalveolar lavage (BAL) supernatants of subjects with different diffuse interstitial

lung diseases. They concluded that H_2O_2 can be detected in both EBC and supernatants of BAL and that their relative concentrations are similar but not correlated with each other. The positive correlation between H_2O_2 levels in EBC and percentage of epithelial cells leads to the speculation that airway epithelia may be relevant to H_2O_2 production in the airway lumen.

In the view of interpretative issues related to EBC biomarkers, Folesani et al. (this issue, page 79) looked at the concentration of urea in EBC as a possible normalizing factor for EBC non-volatile biomarkers. They showed that urea is a non-volatile diffusible molecule *ex vivo* and that urea concentration in EBC is unaffected by the commonest inflammatory lung diseases, such as asthma and COPD. In patients undergoing hemodialysis, EBC concentrations of urea consistently follow blood levels, with a time lag accounted for by the equilibration between different compartments, thereby indicating that whenever an important intra-individual variation is expected to occur because of changes in hydration or when repeated measurements have to be compared, urea could represent an ideal normalization factor for the variable dilution of droplets derived from the lining fluid. This might become important if suitable methods of air partitioning will allow the collection (and comparison) of fractionated air samples representative of different tracts along the airways.

Identification of protein biomarkers in blood or serum may have further utility for non-invasive lung cancer detection. Direct serum protein profiling by matrix-assisted laser desorption ionization (MALDI) mass spectrometry (99) has uncovered distinct mass profiles in several common types of cancer. However, the technique has difficulties in providing identification of the distinctive proteins. An alternative to mass spectrometry for protein profiling is the use of antibody microarrays. Using protein microarrays, it has been possible to demonstrate significant differences in the serum protein profile of lung cancer patients when compared with healthy controls and subjects with COPD (100). Thus, proteomic techniques could play a role in the early detection of cancer, and could also be useful in identifying particular tumor pathways, which could be targeted for chemotherapy.

Finally, new biomarkers and new mechanistic insights leading to lung cancer development could de-

rive from *in vitro* and *ex vivo* studies; in this issue of the Journal (this issue, pages 87, 97 and 104) *in vitro* models have been used to establish a relationship between exposure to carcinogenic agents (Cr, polycyclic aromatic hydrocarbons and particulate matter) and biomarkers of oxidative stress/DNA damage. In *ex vivo* studies, such as those performed on lung biopsies, toxic metals are directly detectable in lung tissues, thus providing information about the effective dose at the target organ level (this issue, pages 43 and 52). This last approach is of particular significance for some metallic elements, whose intake by inhalation is a recognized risk factors for the development of lung diseases, like COPD, asthma, pulmonary fibrosis and lung cancer (101). Toxic and carcinogenic metallic elements are widespread pollutants dispersed both in the working and the general environment, and as such account for a fraction of chronic lung diseases including cancer. Novel biomarkers accounting for lung exposure rather than for systemic dose seem promising tools for risk assessment and monitoring purposes.

Confirmatory vs. screening tests

The effectiveness of secondary prevention through screening of asymptomatic subjects is still debated. Screening of high risk individuals through CT scan appears promising, but this radiological technique suffers from low specificity. A recent study evaluated the agreement among radiologists on the interpretation of pulmonary findings at low-dose CT screening examinations for lung cancer. Image subsets from 135 baseline low-dose screening CT examinations were classified by four radiologists. The average percentage of reader pairs in agreement on the screening result per case (percentage agreement) was 82%. There was wide variation in the total number of abnormalities detected and classified as pulmonary nodules, with differences of up to more than twofold among radiologists. For cases classified as positive, multi-rater kappa for follow-up recommendations was 0.35 (102), such a result being far from the threshold for good agreement (103).

Diagnostic issues arising from the occasional finding of lung nodules or from symptoms of lung cancer call for costly confirmatory techniques, includ-

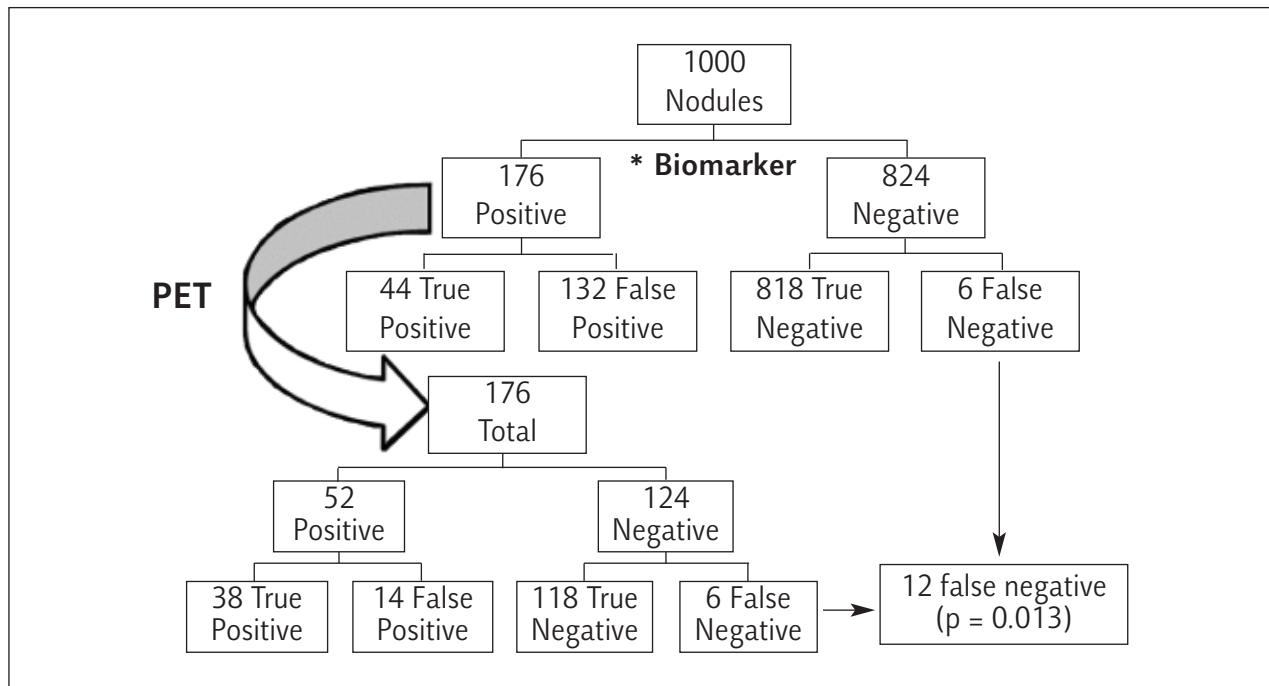


Figure 1. Simulation of a diagnostic tree resulting from the application of a two-step programme based on confirmatory tests: (i) the first one is the application of any of available biomarkers among circulating DNA, exhaled VOCs, electronic nose; (ii) the second one by positron emission tomography (PET), a costly technique which can be saved in more than 80 of patients, without loss of diagnostic power

ing bronchoscopy, serial CT scans, positron emission tomography (PET) that may not be sufficiently accurate. Even such costly imaging techniques show a high rate of false positive results, which means that a sizeable fraction of healthy subjects undergo unnecessary surgical resection indicated by purely morphological criteria. A molecular approach to the diagnosis of lung cancer through the analysis of exhaled breath, induced sputum, and blood could greatly improve the specificity of imaging procedures. Complementary techniques would thus be useful not only to pick out lung cancer at screening, but also and especially to exclude its occurrence in cases of pulmonary nodules frequently detected by CT scans. Biomarkers exploring lung biology provide complementary information for increased diagnostic specificity. Non-invasive techniques would also be both acceptable to the patients and cheaper for the Health Service. A simulation based on characteristics of biomarkers and imaging techniques in terms of sensitivity and specificity taking into account the expected frequency of true posi-

tives (based on annual incidence rates) gives rise to the outcomes depicted in figure 1: techniques already available would make it possible to rule out lung cancer in all subjects with a solitary asymptomatic nodule, but with a negative test for any of the already available biomarkers (circulating DNA, exhaled VOCs, etc.) with a known probability of false negatives. Although low (1%), the probability of misdiagnosis calls for additional investigations, but with limited time pressure and especially with a more relaxed mood, which is an important goal for doctors and patients.

Informed consent

Recent advances in technology and science provide new opportunities to undergo clinical tests which are not fully validated, but whose utility can be suggested by the available body of evidence and weight of potential benefits against possible drawbacks. These methods are therefore different from mandatory tests

applied for biomonitoring purposes and to assess the worker's fitness to the job. They require not only medical judgment, but also the subject's willingness to enter into a diagnostic tree with potential benefits, but also with possible adverse effects or unnecessary and sometimes painful and invasive procedures. Prior to any such invasive procedures, the patient is asked to sign a document known as "informed consent". This is not just a written document ensuring that legal suits will be avoided, but rather it is a form of empathy with the worker/patient, with whom health professionals should share up-to-date information on every procedure which is not mandatory, but that can or cannot be activated, depending on the individual's will.

The occupational health physician is a valuable source of advice and information, though only workers at risk and patients can make the decision. No one – not even medical experts – can predict whether such tests and subsequent care will prove successful. To accept responsibility for his/her decision, the patient must: (i) know what the procedure is; (ii) understand the procedure and subsequent treatment process(es); (iii) know, understand and accept the risks involved in the treatment, including the change of status from "presumably healthy individual" to "possibly affected patient", and knowing that removing a (pre)cancerous lesion may save his/her life, but can also have a negative impact on the quality of life and mood, without affecting the natural history of the disease.

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