LUNG CANCER SCREENING WITH LOW-DOSE CT AND MOLECULAR MARKERS

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Abstract

The US National Lung Screening Trial showed that low-dose computed tomography (LDCT) significantly reduces lung cancer mortality compared to chest X-ray. To confirm that LDCT screening is beneficial, some randomized controlled trials are ongoing in Europe. The largest of these, the Dutch-Belgian NELSON study, recruited 15,422 high risk persons to LDCT versus no screening. NELSON mortality data are not yet available, but the study found volume-based determinations of growth were more reliable than visual two-dimensional assessments. A semi-automated volume-based growth determination method was used, but often needed to be complemented by visual assessment. Preliminary results of the Italian DANTE trial and five years results of the Danish DLCST trial were recently published. These trials showed that the numbers of advanced and lethal lung cancers were not different in the two arms, concluding that screening had no apparent benefit. The Italian Mild study (2208 screened subjects) compared one- with two-year screening intervals: preliminary data support a two-year interval. The Danish DLCST study assessed the impact of screening on participants' quality of life and smoking: screening per se did not favour stopping smoking and effective anti-smoking interventions were considered necessary; however screening did not facilitate smoking continuation either, as claimed by detractors of CT screening. The Italian Cosmos study (5203 screened subjects), introduced a non-invasive diagnostic algorithm and assessed the utility of CT-PET in nodule management with satisfactory results. Cosmos data were used to develop a personalised predictor of lung cancer risk. This appears to reliably stratify participants after baseline so that the screening interval can be reduced in low risk individuals. In addition data from Cosmos also contributed to the development of sensitive and specific serum microRNA signature of early lung cancer, which may be of potential use for first-line screening. Even if lung cancer screening still represents a complex and controversial topic, it seems a promising tool to reduce lung cancer mortality.

Key words: Lung cancer, computed tomography, screening, surgery, micro RNA

Lung cancer is one of the leading causes of death worldwide and the annual incidence continues to grow in women and developing countries (1,2). The high lethality of lung cancer is related to intrinsic biological aggressiveness which makes detection too late in most patients but also to the lack of early detection programs (3). It is known that chances of cure are related to stage at diagnosis (4) and that there is a target population at higher risk because of tobacco use (5). For these reasons every effort should be made to anticipate diagnosis as early as possible.

Screening trials with chest X-ray performed in the late 70 and early 80 failed to demonstrated a lung cancer mortality reduction in the screened group compared to observation even though more and earlier stage cancers were detected, and the rate of radical resection was also higher (6, 7, 8). The excess of lung cancers in intervention groups has been attributed to overdiagnosis-the detection of cancers that will not become symptomatic in the patient's lifetime or will not cause death (9). However the high mortality of untreated small screening-detected cancers is strong clinical evidence against an overdiagnosis bias in lung cancer screening. (10, 11). Molecular evidence against overdiagnosis is represented by the following: most screening detected cancers have marked chromo-

somal alterations even though diagnosed at an early stage; (12) K-ras mutations have similar prevalence in screened and unscreened lung adenocarcinomas; and screening-detected early lung cancers in heavy smokers have a similar gene expression profile (cDNA microarray) than that of symptomatic cancers (13).

Thanks to the ELCAP report at the end of '90, in which the superiority of LDCT compared to X-ray was demonstrated, the research on lung cancer screening found renewed interest. Many single arm or randomised studies on early detection of lung cancer with LDCT including ours have demonstrated how this tool is sensitive to diagnose nodules a few mm in diameter, with low radiation exposure, limited costs, a few seconds of execution and no contrast medium (14-18).

Mortality reduction

The first analysis of long-term outcomes for screening detected lung cancers was published in 2006 as 10-year survival data for the I-ELCAP study (19). A total of 31,567 participants underwent baseline LDCT screening, 27,456 of whom underwent a repeat scan 7-18 months later. Of the 484 lung cancer cases diagnosed, 85% were stage I. For stage I cases, estimated 10-year survival was 88%. For the 301 patients who underwent resection, 10-year survival was 92%. This was the first time that long-term survival for lung cancer had been presented – in sharp contrast to the five-year survival (approximately 70% for resected stage I lung cancer) usually presented. (19)

An important contribution to the controversial issue of the effect of screening on lung cancer mortality has been provided by the results of the large randomised National Lung Screening Trial (NLST), published in 2011 (20). NLST recruited 53,454 persons at high risk of lung cancer (above 55 years; over 30 pack-years smoked) randomized to three annual screenings with either LDCT or chest x-ray. The incidence of lung cancer was 645 per 100,000 person-years (1060 cancers) in the LDCT group, and 572 cases per 100,000 person-years (941 cancers) in the chest x-ray group. There were 247 lung cancer deaths per 100,000 person-years in the LDCT group and 309 deaths per 100,000 person-years in x-ray group. The reduction in lung cancer mortality with LDCT group was 20.0% (95%CI, 6.8-26.7; P=0.004). The any cause death rate was 6.7% (95%CI, 1.2-13.6; p=0.02) lower in the LDCT group than the x-ray group. The authors concluded that screening with LDCT reduces lung cancer mortality.

Other analyses of single arm studies indicate or predict reductions in disease-specific mortality: 28% by Mc Mahon et al., (21) 23% by Chien et al., (22) 40% by our study (23) and 35-50% by the ELCAP study (24). In the latter experience, mortality reduction only became evident 4-5 years after enrolment and if screening was discontinued the death rate increased to the same as that in the absence of screening. In our mortality study the predicted 40% reduction in mortality in the screened population was relative to the expected death rate in an age- and sex-matched population of US smokers (23).

A number of European Randomised trials on the contrary have not supported screening benefit in reducing mortality.

The DANTE (25) study was the first randomised study to publish a mortality analysis for the first three study years. It found no mortality reduction in the screened group. The study design differs from other randomised trials in that all subjects received a chest X-ray before randomisation to LDCT vs. observation (no screening). Only man were included in the trial. A total of 2,472 persons were randomised (1,276 to screening and 1,196 to observation) over five years. The baseline lung cancer detection rate was 0.67%, with 4 of 8 (50%) stage I, in the observation arm; and 2.19%, with 16/28 (57%) stage I, in the screening arm. It is remarkable that stage distribution in the two arms was closely similar. This could have been an effect of the pre-screening chest X-ray. It is also noteworthy that the lung cancer detection rate in the screened arm was higher than in most other series, but this might have been due to the higher average age (64 years) and high number of pack-years smoked. Eighteen point seven percent of thoracotomies were performed for benign lesions. We have indicated some weaknesses in the DANTE study (26): follow-up was short (median 34 months; only 6.5% over 5 years), as the authors acknowledged; and any mortality reduction is unlikely to be evident before 6 years. In addition the DANTE study is characterized by a number of peculiarities: proportions of stage I disease and resectable disease were lower in the screening arm than in other studies (55% stage I compared to 74% and 85% in non-randomised studies (27, 28, 29), resectability of 65% vs. 89% and 85% respectively; there was also a higher proportion of advanced interval cancers in the screened arm than in other single arm LDCT studies. Finally lung cancer-specific mortality was high: with 20/60 (33%) deaths in the DANTE screening arm, compared to 15% in I-ELCAP.

The Danish study (30) and the Italian Mild study (31) both report no benefit of lung cancer screening in the screened arm compared to the control one. In particular in the Danish trial (30) more lung cancers were diag-

nosed in the screening group (69 vs. 24, p<0.001), and more were low stage (48 vs. 21 stage I-IIB non-small cell lung cancer (NSCLC) and limited stage small cell lung cancer (SCLC), p=0.002), whereas frequencies of high-stage lung cancer were the same (21 versus 16 stage IIIA-IV NSCLC and extensive stage SCLC, p=0.509). At the end of screening, 61 patients died in the screening group and 42 in the control group (p=0.059), 15 and 11 died of lung cancer, respectively (p=0.428).

The Mild study (31) included 4099 participants, 1723 randomized to the control group, 1186 to biennial LDCT screening, and 1190 to annual LDCT screening. Lung cancer mortality rates were not different in the three arms. However some limitations of the study can be underlined including that age, gender and duration of follow up were not comparable in the three arms, neither the smoking exposure and the distribution of the predicted FEV1 percentage.

Diagnostic protocols

One criticism with LDCT screening for lung cancer is the high rate of non-calcified indeterminate lung nodules (28, 29). Diagnostic algorithms aim to find a balance between too invasive workup that exposes screened persons to useless invasive procedures and overtreatment, and insufficient surveillance that increases the risks of false negatives or delayed diagnosis. Protocols for the diagnostic work-up of small nodules have been proposed. (32, 27, 28, 29, 33, 34)

Many screening programmes scan have adopted 5 mm as the cut-off between positive and negative nodules on CT (27). The NLST adopted 4 mm as threshold below which no follow-up was needed and resulted in a very high rate of baseline positivity (20). Other investigators consider nodule volumes instead of nodule diameter to provide more reliable assessments of nodule growth (35, 36).

The COSMOS study used 5 mm as the threshold for non-calcified nodules, above which additional short-term follow-up was required. The recall rate was below 10% at baseline and 5-6% at subsequent screening rounds (27).

The NELSON trial adopted volume measurements: nodules less than 50 mm^3 (4.6 mm diameter) were considered negative, those above 500 mm^3 (>9.8 mm diameter) were positive, and those in the 50-500 mm range were indeterminate (35) . Indeterminate nodules underwent repeat LDCT at three months and volume doubling times were used to direct nodules to either further investigation or just follow-up. Using this two-step approach, 2.6% of baseline NELSON scans were positive and a fairly high proportion of positive scans turned out to be lung cancer. However, ground glass nodules pose some assessment problems, as growth may be slow. A 2011 publication by a Danish group proposed that the best predictor of malignancy was a combination of volume doubling time and positron emission tomographic (PET) findings (36).

For the COSMOS study (27) we set up a standardised diagnostic protocol which was modified over time as we gained experience. At the end of five years we found a high overall compliance (82%), and only 8% underwent further examinations after screening CT which was lower than in other non-randomised studies (16, 32) and also lower than the NLST, where 24% of the patients in the CT arm were recalled for further investigations (20). Overall, 34 of COSMOS participants (15% of those with suspicious lung nodules) underwent surgical biopsy for benign disease over the five study years. To consider this in perspective, the proportion was higher among those receiving standard surgical practice in the Division of Thoracic Surgery running COSMOS.

CT-PET may be useful for the further investigation of uncertain nodules detected at screening, particularly when they are solid and 10 mm or more in diameter. Of the 58 lung cancers found on surgical biopsy in the COSMOS study,51 were PET positive (standard uptake value >2.0) and seven were negative (37). Sensitivity was 88% overall, and 100% in those with solid nodules of 10 mm or more. Among the 8 patients with benign disease at surgical biopsy, CT-PET was positive in 6 and negative in 2. We suggested that CT-PET was a promising alternative to invasive procedures for the further investigation of uncertain nodules in the screening setting and that the standard uptake value threshold for positivity could usefully be lowered to >1.5 for nodules <10 mm. However, in our experience of CT-PET for nodules detected after baseline, sensitivity was much lower, in relation to the increased proportions of small size lesions and ground glass nodules and the utility of CT-PET was called into question (unpublished work).

The IASLC Screening Workshop guidelines suggest CT-guided biopsy for suspicious nodules, not in the least because it facilitates the surgical decision process in many cases (38). The ELCAP group reported good diagnostic performance for CT guided biopsy (sensitivity 82%; diagnostic accuracy 88% overall) although results were less good for nodules less than 8 mm (24). However it must not be forgotten that results of CT-guided biopsy are highly operator dependent and other techniques, such as endobron-

chial ultrasound (EBUS), electromagnetic navigation (EMN) and minimally invasive surgical biopsy should be developed as alternatives to increase diagnostic yield of screening-detected lesions.

Guidelines

In 2011 NCCN Lung Cancer Screening Panel published new screening guidelines (39). In particular they recommended LDCT screening for heavy smokers older than 55 years and 30 pack year smoking history (39). They also recommend that smokers should always be encouraged to quit smoking. In addition they provided guidelines for evaluation and follow-up of nodules and described accurately the risk and benefits of screening.

Other prestigious scientific societies such as the American College of Chest Physicians and the American Society of Clinical Oncology recently developed recommendations for LDCT screening. Iin particular they propose that for current smokers or former smokers aged 55 to 74 years who have smoked for 30 pack-years or more and either continue to smoke or have quit within the past 15 years, annual screening with LDCT should be offered over both annual screening with chest radiography or no screening, but only in settings that can deliver the comprehensive care provided to National Lung Screening Trial (NLST) participants. [grade of recommendation: 2B] (40).

Molecular marker for lung cancer early detection

Lung cancer screening with LDCT still represents a complex and controversial topic; in particular, uncertainty exists about the potential harms of screening and the generalizability of results. In such a scenario, the development of blood tests able to detect the presence of lung cancer might be the cornerstone for successful large-scale lung cancer screening. In recent years a number of studies tried to identify serum/plasma biomarkers for lung cancer detection. Some studies focused on detection through an enzyme-linked immunosorbent assay (ELISA) of circulating tumor-associated antigens (TAA) such as p53, NY-ESO-1, CAGE, GBU 4-5, Annexin, or SOX2, with an overall good specificity but unsatisfactory sensitivity [40% sensitivity, 90% specificity] (41). Others have relied on the identification of circulating cancer cells in the blood of patients with metastatic tumors (42). Such chip-based tests show a sensitivity and specificity in detecting cancer cells that is close to 100%, warranting further investigations into the applicability of the detection of circulating cancer cells at earlier stages of disease (43).

Recently, some investigators have identified a group of circulating microRNAs (miRNAs) that accurately detect symptomatic lung cancer (44-47) and, more importantly, LDCT -detected asymptomatic lung cancer (48, 50). MiRNAs are short (about 22 nt) noncoding RNAs that function as endogenous triggers of the RNA interference pathway and that are involved in the regulation of cellular differentiation, proliferation, and apoptosis (51-53). In addition, miRNAs are a very promising class of tumor markers because i) their expression is often deregulated in human cancers in a tissue- and cancer-specific manner (51), ii) they are present in human plasma in a remarkably stable form as they are protected from endogenous RNAse activity (52).

For these reasons the detection of circulating miRNAs may be a valid alternative to LDCT for the early diagnosis of cancer. In a series of experiments described in more detail elsewhere (48), our research group used Real-Time PCR to identify an expression profile of miRNAs extracted from the sera of participants enrolled in a large single-center observational study [COSMOS study] (27). Among the 147 miRNAs detected by Real-Time PCR, 34 showed differences in expression in asymptomatic LDCT-detected lung adenocarcinoma versus normal sera. We developed a multivariate risk-predictor algorithm based on the weighted linear combination of the 34 miRNA expression levels. When the predictor was tested on an independent cohort of patients with asymptomatic LDCT-detected lung cancer, it displayed an overall accuracy of 80% (sensitivity 71%, specificity 90%). Furthermore, we also showed that the miRNA test was able to distinguish between benign nodules detected at LDCT and malignant disease. This finding demonstrates the specificity of the test and highlights its utility in the clinic.

The simplicity of the procedure (it is minimally invasive and requires <1 mL of serum) and its relatively low cost (based on standard Real-Time PCR) should encourage population compliance to screening programs, and its application in the clinic as a 'first line screening test' to identify those high-risk individuals who should benefit of further testing, including by LDCT. In Italy we recently started a multicenter prospective study (Cosmos II) that will enroll 10000 high-risk individuals in one year from 5 Italian hospitals to compare LDCT and miRNA test as 'first line screening tool'.

Lastly, a critical issue relates to the origin of serum circulating miRNAs and their biological function. MiRNAs have been found encapsulated in small membrane vesicles (50-90 nm) named exosomes (52),

which are actively released into the extracellular environment upon fusion of multivesicular bodies with the plasma membrane (52). Strikingly, tumor cells appear to communicate through exosomes with immune cells, leading to immune suppression (53, 54). It is tempting to speculate that lung cancer cells might reprogram the tumor microenvironment, perhaps by altering the expression patterns of surrounding cells, including those of the immune system, through miRNAs contained in exosomes. Undoubtedly, future studies will shed more light on the biological functions of circulating miRNAs and their role, if any, in cancer progression. Such studies will allow researchers to design completely novel strategies for lung cancer therapy.

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