Editorial Commentary

Integration of the blood test into the low-dose computed tomography lung cancer screening: reliable discrimination between malignant and non-malignant radiographic findings

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Lung cancer (LC) is the most common oncological disease worldwide. Its incidence currently exceeds 2 million cases per year, with 1.8 million subjects dying from this malignancy. LC accounts for 11.6% of total cancer diagnoses and 18.4% cancer-related deaths (1). Early-stage LC usually does not cause specific symptoms; consequently, more than 70% LCs are diagnosed at advanced stages (2,3). Recent breakthroughs in targeted and immune therapy resulted in dramatic improvement of LC outcomes (4,5). Nevertheless, LC is a direct cause of death in the majority of patients with this diagnosis (1). LC is often characterized in the scientific literature as a highly aggressive disease; nevertheless, patients diagnosed at stage IA have approximately 80% probability of being cured (6). Furthermore, 5-year overall survival rate for stage IA lung cancer may be close to 90%. In contrast, stage IV LC is associated with dismal prognosis, with 5-year overall survival rate falling below 20% (7). These data suggest that early diagnosis of LC is a key for reducing mortality from this disease. LC screening studies largely support this hypothesis: for example, it was shown that low-dose computed tomography (LDCT) reduces LC mortality by at least 20% in persons at-risk (i.e., in smokers) (8-10).

The major problem of LDCT screening is an unacceptably high rate of false-positive findings. Indeed, many healthy people, particularly elderly subjects and smokers, present with so-called lung "nodules", i.e., some lumps detected by X-rays. These lumps reflect topical increase of the density of lung tissue due to alterations in its structure. The most ominous cause of these nodules is the LC. However, many benign processes, e.g., inflammatory infiltrates caused by smoking-related irritation of bronchi, infections, autoimmune processes etc., manifest with lumps which are largely indistinguishable from LC by imaging. Great efforts have been invested to improve radiographic procedures and their interpretation in order to discriminate between LC and non-LC nodules. Besides careful visual analysis of appearance of these nodules, to be performed by highly-trained specialists with the support of various electronic tools, it is suggested to consider some probability factors, e.g., age of the patient and his/her smoking history. This «clinical» approach, although being wise and intuitively attractive, has a high risk of missing malignant disease at a curable stage. Consequently, many subjects with LDCT-detected nodules end up with invasive procedures aimed to obtain a piece of suspected nodule and to subject it to a morphological analysis. Collection of tissue samples from thoracic cavity is obviously associated with suffering of examined subjects and often results in serious complications, particularly bleeding. Unfortunately, even this invasive intervention cannot guarantee right diagnosis: there are many instances of LC, where tissue biopsy produces false-negative results die to actual failure to obtain tumor sample (11-14).

Liang *et al*. (15) recently presented a test called PulmoSeek, which is based on the detection of LC-specific methylation signatures in circulating tumor DNA

(ctDNA). They recruited 585 patients from 14 hospitals located in China; these patients had lung nodules with a size ranging from 5 to 30 mm, which were detected by LDCT. All included patients had definite pathologic diagnosis discriminating between LC and benign lesions. Approximately one out of ten plasma DNA samples failed to pass quality control, so the study was focused on 529 patients with informative ctDNA test. 309 ctDNA samples (253 malignant and 56 benign) were used for the methylomic study in order to select the most informative loci. The authors utilized AnchorDx next generation sequencing (NGS) platform for the analysis of methylated cytosines; this platform examines 12,899 genomic regions, which demonstrated LC-specific methylation pattern in a previous study (16), and includes 105,844 CpG sites. Liang *et al.* (15) identified the most informative regions (which they called features) and utilized a test set consisting of 80 samples (60 malignant and 20 benign) in order to reveal what would be the optimal size of the PulmoSeek assay. They eventually concluded, that 100 the most informative regions is the optimal size for the ctDNA methylation test.

Liang *et al.* (15) provide thoughtful discussion regarding the requirements for this newly developed assay. They wisely state that it is absolutely inacceptable if the test will miss patients with LC, so they decided to focus on the combination of high sensitivity and high negative predictive value (NPV) instead of considering specificity and positive predictive value. They validated the performance of the PulmoSeek in 140 additional patients (100 LC and 40 non-LC). In this validation cohort PulmoSeek demonstrated 99% sensitivity and 93% NPV; when the patients with nodule size ranging from 6 to 20 mm were considered, both these values achieved 100%. It is important to realize that the ratio between LC and non-LC patients in this validation set does not reflect "natural" frequency of LC in subjects undergoing LDCT screening. Calculations demonstrate that if the frequency of LC in the studied cohort would be 23%, which is still significantly higher than in real-world patient series, the sensitivity and NPV will both approach to 100%.

Although the performance characteristics of the PulmoSeek are impressive, it is necessary to acknowledge that it delivered erroneous results in a small subset of subjects. The important question, how this series of patients would fare if they were managed by currently available tools. The authors evaluated the performance of Mayo Clinic and Veterans Affairs scores, which integrate imaging characteristics of lung nodules and personal risk factors in order to discriminate between LC and non-LC (11,13). These scoring systems were evidently less reliable as compared to the PulmoSeek with the AUC of 0.843 (0.769–0.918) versus AUC of 0.591 (0.482–0.688) for the Mayo Clinic model and 0.544 (0.442–0.640) for the Veterans Affairs model.

Patients with suspicious LDCT finding are recommended to undergo PET-CT, as the latter is more informative for differential diagnosis between LC and non-LC. 26 out of 140 patients included in the validation set had PET-CT records. PulmoSeek correctly classified 8/10 patients with solid nodules, 9/11 cases with part-solid nodules and all 5 subjects with ground-glass nodules; these estimates for PET/CT were 6/10, 7/11 and 0/5, respectively (15).

Several important considerations need to be taken into account while discussing this study. It is clear that the PulmoSeek assay reliably discriminates between LC and non-LC on the level of pathological diagnosis. The current dogma in oncology actually equalizes pathological and clinical diagnosis of cancer disease, so virtually all newly diagnosed malignancies are treated in a rather aggressive way. This attitude relies on the assumption that all detectable cancers will eventually progress if not removed from the body. Screening data obtained for breast cancer suggest that this dogma is not always true. Indeed, breast cancer screening resulted in immediate rise of the number of detected early-stage tumors, but this apparent success was not accompanied by a significant decline of the incidence of metastatic carcinomas. In fact, this effort led to detection of indolent tumors, which would never cause fatal outcome, but largely failed to control potentially lethal cancers (17,18). In any event, right pathological diagnosis is only an intermediate end-point in a cancer screening program, so it is important to ensure that every newly developed approach will indeed eventually result in saved lives.

Many oncological diseases have relatively similar characteristics in patients of different races. This does not apply to lung cancer, which has a number of well-known race-specific features. For example, more than a half of Asian LCs carry EGFR mutations, while this estimate is only around 10% in people of European race (19-21). 387/529 (73%) included subjects in the study of Liang *et al.* (15) were non-smokers, while most of consecutive LC series described in Western countries have significantly higher proportion of smokers. Hence, it is not self-explanatory that PulmoSeek will perform equally well in non-Asian populations. Furthermore, molecular features of smoking-induced and smoking-unrelated LC have very significant

differences (5,22). It is likely that the performance of NGS-based LC screening tests can be further improved by adjusting to smoking history.

The detection of ctDNA is highly complicated, because it is present in the blood in residual amounts, especially in subjects with early-stage cancers. It is of question, what is the reproducibility of serial blood-takes, i.e., whether a single analysis is sufficient, or if the performance of this assay can be improved by analyzing several samples from the same subject. Surprisingly, relatively little attention has been paid to intra-patient replicability of serial ctDNA tests (23).

Physicians are used to deal with straightforward measurements, which produce single, intuitively understandable numerical or qualitative estimates (consider, for instance, leukocyte count or blood group determination). Many modern assays analyze multiple parameters and use sophisticated, usually patent-protected calculations in order to produce a score. It is often difficult for clinical specialists to sense the genuine meaning of these scores. Furthermore, the results of "omics" assays usually cannot be validated in an independent laboratory, in contrast to, say, pathological diagnosis.

Despite the above limitations, the results obtained by Liang *et al.* (15) hold a great promise for breakthrough LC management. The PulmoSeek assay certainly deserves integration into clinical practice in the form of well-controlled prospective LC screening trial.

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