Screen-detected multiple primary lung cancers in the ITALUNG trial

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Abstract: Occurrence of multiple primary lung cancers (MPLC) in individuals undergoing low-dose computed tomography (LDCT) screening has not been thoroughly addressed. We investigated MPLC in subjects recruited in the ITALUNG randomized clinical trial. Cases of cytologically/histologically proven MPLC detected at screening LDCT or follow-up CT were selected and pathologically re-evaluated according to the WHO 2015 classification. Overall 16 MPLC were diagnosed at screening LDCT (n=14, all present at baseline) or follow-up CT (n=2) in six subjects (4 in one subject, 3 in two and 2 in three subjects), representing 0.43% of the 1,406 screenees and 15.8% of the 38 subjects with at least one screen-detected primary lung cancer. MPLC included 9 adenocarcinomas in three subjects and a combination of 7 different tumour histotypes in three subjects. MPLC, mostly adenocarcinomas, are not uncommon in smokers and ex-smokers with at least one LDCT screen detected primary lung cancer.
Introduction

Smoking makes pulmonary tissue diffusely prone to cancer development (“field cancerization” theory) (1,2). Accordingly, smokers and former smokers can develop multiple lung cancers in their lives. Available data on multiple primary lung cancers (MPLC) derive mainly from surgical series (3). However, low-dose computed tomography (LDCT) screening can provide an alternative source of information about MPLC. To the best of our knowledge, so far this has not been thoroughly addressed. We reviewed the occurrence and type of MPLC in subjects undergoing LDCT screening in the ITALUNG randomized clinical trial (4).

Methods

ITALUNG is a randomized clinical trial carried out in Italy evaluating efficacy of LDCT screening in reducing lung cancer mortality as compared to “usual care” (5). The study was conducted in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html) and the study protocol was approved by the Local Ethic Committees of the participating centers (Firenze, approval number 29–30, 30 September 2003; Pisa, number 23, 27 October 2003; Pistoia, number 00028543, 13 May 2004). Each subject provided an informed written consent to participate to the study.

The ITALUNG study design and protocol were previously reported (6,7). Briefly, 3,206 smokers or former smokers identified by general practitioners and invited by mail were randomized to receive four annual LDCT (n=1,613) or usual care (n=1,593). Management protocol for positive LDCT examinations included follow-up LDCT, 2-[18F]fluoro-2-deoxy-D glucose positron emission tomography, and CT-guided fine-needle aspiration biopsy (FNAB).

Follow-up data at 8.5 years in ITALUNG indicated that LDCT screening could reduce lung cancer and overall mortality (4). In the actively screened arm, 1,406 smokers or former smokers (910 men with mean age of 61.1 years and 496 women with mean age of 60.6 years) underwent annual screening LDCT at baseline and in the next 3 years. In ITALUNG the subjects with screen-detected primary lung cancer entered follow-up with contrast-enhanced full-dose head, chest and abdomen CT that was performed every 6 months for the first 2 years and annually for 3 years thereafter. All subjects with screen-detected primary lung cancer had completed the 5 years of follow-up CT at the time of writing.

For identification of MPLC in ITALUNG we applied a three step procedure (see below) and the criteria proposed by Shen et al. (8) that define 3 types of lesions: (I) those that share the same histology but are distributed in different pulmonary lobes, in absence of N2, N3 or systemic metastases; (II) those that show different histological or molecular-genetic characteristics and arise separately from foci of carcinoma in situ; (III) those that share the same histology but are separated by at least 4 years interval and without systemic metastases between the detection of multiple tumors.

The three step procedure included the following:

(I) Step 1: records of all screened subjects with diagnosis of lung cancer based on the results of FNAB or surgical pathology during LDCT screening or full-dose CT follow-up were reviewed searching for cases of multiple primary or secondary lung cancer.

(II) Step 2: one experienced lung pathologist (C.E.C) reviewed and classified all the surgical or fine needle aspiration specimens of the selected cases according to the 2015 WHO criteria taking into account morphology and molecular/genetic features (9,10). The ITALUNG pathology protocol referred to the EU-US shared pathology protocol for CT-screening trials (EU-US Spiral CT Collaborative Group) and is detailed in supplementary material. Pathologic or clinical staging of MPLC was performed according
to the 7th Edition of the American Joint Committee on Cancer Staging Manual (11).

(III) Step 3: two senior chest radiologists (M.M. and F.F.) reviewed all the LDCT and follow-up full-dose CT examinations of the subjects with pathologically confirmed MPLC. They established the first LDCT or follow-up CT showing focal abnormalities that were ultimately diagnosed as lung cancer and described them (12).

Results

Eight subjects were diagnosed with multiple lung cancers during LDCT screening or full-dose CT follow-up. Two had multiple secondary lesions (from renal cancer and colorectal cancer) detected at LDCT and diagnosed at FNAB. Six subjects were ultimately diagnosed with MPLC (overall =16:2 in three subjects, 3 in two subjects and 4 in one subject) (Table 1 and Figures 1,2). They represented 15.8% (6/38) of all subjects with at least one screen-detected primary lung cancer.

The 16 MPLC included 9 morphologically and molecularly heterogeneous adenocarcinomas in three subjects (Figure 1) and combination of different tumor histotypes (2 adenocarcinomas plus 1 carcinoid, 1 adenocarcinoma plus 1 small cell carcinoma, 1 pleomorphic carcinoma plus 1 squamous cell carcinoma) in three subjects (Figure 2). Adenocarcinomas accounted for 75% (12/16) of MPLC.

Fourteen of 16 MPLC were observed during LDCT screening in four subjects and 2 during full-dose CT follow-up in two subjects. All the former were already present at baseline LDCT examination (Figures 1,2) and may be considered as synchronous lesions. The latter appeared during follow-up CT 5 and 6 years after surgical removal of the first lesions and may be considered metachronous lesions. Overall, MPLC appeared in the LDCT or follow-up CT showing focal abnormalities that were ultimately diagnosed as lung cancer and described them (12).

Discussion

Data about occurrence of MPLC in observational or randomized LDCT screening studies are fragmentary and summarized in Table 2. At least one subject with MPLC was reported in 11/33 studies (5,13-18,24-35), with a mean cumulative frequency of 0.18% (136 subjects) in 71,901 screenees and of 11.6% in 1,170 subjects with at least one screen-detected lung cancer (19-23,36-44). The MPLC were considered synchronous in 123/136 (90%) subjects and metachronous in 13 (10%). Unfortunately, in many cases the time of lesions appearance and histological diagnoses were not available. Moreover uncertainties and intervening modifications of the staging system of lung cancer and unavailability of morphologic and genetic/molecular features hinder recollection of information about MPLC in previous reports of LDCT screening (and surgical series). In particular, multiple pulmonary nodules may have been considered stage III or IV tumors (18), and this may have led to under-reporting of cases of MPLC, especially in case of multifocal adenocarcinoma.

In the active arm of ITALUNG, after the 4 years of active screening and 3 years of follow-up we observed few subjects (n=6; 0.43% of the screenees) harboring or developing MPLC. However they represented 15.8% of all those subjects with at least one screen-detected primary lung cancer. These percentages are in line with those reported in LDCT screening studies (Table 2).

In our series, adenocarcinoma was the most frequent histotype. This is in line with the type of primary lung cancers that are discovered by LDCT (Table 1) and in a previous study (18). Despite the small numbers of MPLC cases in our study that indicates need of further studies, two scenarios may be drawn. The first deals with morphological and molecular/genetically heterogeneous multifocal adenocarcinomas. The second deals with combination of adenocarcinoma with others histological types including small cell lung cancer, carcinoid and squamous cell cancer. Only one case of double squamous lung cancer was reported (23). As in previous reports (19,22), most (14/16 lesions in five subjects) of the screen-detected MPLC in ITALUNG were present at baseline and were diagnosed after further LDCT screening rounds, because of increased size or density (12,17,27).

Remarkably, our two patients with synchronous multifocal adenocarcinoma with 4 and 3 lesions, respectively, were alive many years after lesion treatment. This is consistent with the view that multifocal adenocarcinoma...
<table>
<thead>
<tr>
<th>Case/lesion/sex/age/pack-year</th>
<th>Year of CT appearance screening round</th>
<th>Initial CT features: site/size*/shape/margins/density</th>
<th>Time to diagnosis</th>
<th>Histology or cytology</th>
<th>Molecular findings¹</th>
<th>Stage</th>
<th>Therapy/outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/1/male/70/&lt;30</td>
<td>2006 baseline</td>
<td>RUL/32 mm/roundish/spiculated/solid</td>
<td>Immediate</td>
<td>Adenocarcinoma (80% acinar, 15% papillary, 5% solid)</td>
<td>KRAS G12C</td>
<td>pT2N0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>I/2</td>
<td>2006 baseline</td>
<td>RUL/18 mm/round/ill-defined, non-solid</td>
<td>Immediate</td>
<td>Adenocarcinoma (90% lepidic, 10% acinar)</td>
<td>EGFR exon19 deletion</td>
<td>pT2N0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>I/3</td>
<td>2006 baseline</td>
<td>RLL/6 mm/oval/regular/solid</td>
<td>Immediate</td>
<td>Adenocarcinoma (60% acinar, 30% papillary, 10% micropapillary)</td>
<td>EGFR exon19 deletion</td>
<td>pT2N0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>I/4</td>
<td>2006 baseline</td>
<td>LUL, 25 mm/roundish/ill-defined/part-solid</td>
<td>Immediate</td>
<td>Adenocarcinoma (FNAB)</td>
<td>/</td>
<td>T1bN0M0</td>
<td>Radiation therapy; 2016 alive</td>
</tr>
<tr>
<td>IV/1/male/61/&gt;30</td>
<td>2004 baseline</td>
<td>RUL/22 mm/round/regular/solid</td>
<td>Immediate</td>
<td>Adenocarcinoma (70% acinar, 30% solid)</td>
<td>/</td>
<td>pT1bN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>IV/2</td>
<td>2004 baseline</td>
<td>LUL/6 mm/oval/regular/solid</td>
<td>3 months</td>
<td>Small cell carcinoma</td>
<td>/</td>
<td>pT1aN2M0</td>
<td>Surgery, chemotherapy; dead 9 months later</td>
</tr>
<tr>
<td>III/1/male/70/&lt;30</td>
<td>2005 baseline</td>
<td>LUL/10 mm/complex/irregular/solid</td>
<td>24 months</td>
<td>Adenocarcinoma (90% papillary, 10% acinar)</td>
<td>/</td>
<td>pT1bN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>III/2</td>
<td>2005 baseline</td>
<td>RLL/18 mm/round lobulated/part-solid (associated with cystic airspace)</td>
<td>27 months</td>
<td>Adenocarcinoma (60% acinar, 40% papillary)</td>
<td>EGFR exon19 deletion</td>
<td>pT1aN0M0</td>
<td>Surgery; dead 4 days later</td>
</tr>
<tr>
<td>IV/1/male/66/&gt;30</td>
<td>2006 baseline</td>
<td>LLL/10 mm/round/ill-defined/non-solid</td>
<td>12 months</td>
<td>Adenocarcinoma (50% lepidic, 30% solid, 20% acinar)</td>
<td>/</td>
<td>pT1bN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>IV/2</td>
<td>2006 baseline</td>
<td>Lingula/9 mm/round/regular/solid</td>
<td>12 months</td>
<td>Carcinoid</td>
<td>/</td>
<td>pT1aN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>IV/3</td>
<td>2012 follow-up</td>
<td>RUL/4 mm/round/regular/solid</td>
<td>24 months</td>
<td>Adenocarcinoma (FNAB)</td>
<td>KRAS G13D</td>
<td>cT1aN2M1b</td>
<td>Chemotherapy; dead 6 months later</td>
</tr>
<tr>
<td>V/1/female/79/&gt;30</td>
<td>2005 baseline</td>
<td>RUL/14 mm/irregular/spiculated/solid</td>
<td>3 months</td>
<td>Pleomorphic carcinoma</td>
<td>/</td>
<td>pT1aN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>V/2</td>
<td>2010 follow-up</td>
<td>LLL/25 mm/regular/excavated</td>
<td>2 months</td>
<td>Squamous cell carcinoma</td>
<td>/</td>
<td>cT1bN0M1b</td>
<td>Chemotherapy; dead 3 months later</td>
</tr>
<tr>
<td>VI/1/male/70/&gt;30</td>
<td>2005 baseline</td>
<td>RUL/9 mm/oval/regular/solid</td>
<td>24 months</td>
<td>Adenocarcinoma (70% solid, 20% lepidic, 10% acinar)</td>
<td>KRAS G12C</td>
<td>pT1aN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>VI/2</td>
<td>2005 baseline</td>
<td>RLL/12 mm/round/ill-defined/non-solid</td>
<td>24 months</td>
<td>Adenocarcinoma (70% lepidic, 15% papillary, 15% acinar)</td>
<td>/</td>
<td>pT1aN0M0</td>
<td>Surgery</td>
</tr>
<tr>
<td>VI/3</td>
<td>2005 baseline</td>
<td>LLL/10 mm/oval/ill-defined/part-solid</td>
<td>96 months</td>
<td>Adenocarcinoma (70% lepidic, 30% acinar)</td>
<td>/</td>
<td>pT1aN0M0</td>
<td>Surgery; 2016 alive</td>
</tr>
</tbody>
</table>

¹, mean diameter; ², only positive molecular markers are reported.
Figure 1 Case I (Table 1). Four primary adenocarcinomas in a 70-year-old smoker, which were all detected at baseline LDCT screening round. They appeared as a spiculated lung nodule in the right upper lobe (RUL) (A), a ground glass opacity in the same lobe (B), a small solid nodule in the right lower lobe (RLL) (arrow in C) and a ground glass opacity with a small solid component in the left upper lobe (LUL) (D). Haematoxylin and eosin histologic staining (original magnification ×200) demonstrate an invasive adenocarcinoma, acinar predominant (E) in the RUL lesion corresponding to (A), an invasive adenocarcinoma, lepidic predominant (F) in the RUL lesion corresponding to (B) and an invasive adenocarcinoma, acinar predominant (G) in the RLL lesion corresponding to (C). Papanicolaou stain (original magnification ×40) of fine needle aspiration biopsy shows papillary pattern of uniform malignant cells with irregular nuclei consistent with adenocarcinoma (H) in the LUL lesion corresponding to (D).

adenocarcinoma can behave as indolent lesion that should not be confused with aggressive primary lung cancers with intrapulmonary metastases (18). Awareness of this possibility may have significant impact on management of multiple lesions detected at LDCT screening that is currently recommended in the US (39) and is under evaluation in Europe (4).

In ITALUNG after a median follow-up time of 8.5 years, we observed 2 metachronous cancers during full-dose CT follow-up, which were both fatal. Obviously, longer surveillance is expected to increase the yield of metachronous MPLC. In fact one LDCT study reported 6 cases of metachronous MPLC in 2,989 screenees followed for 14 years (23).

Prevalence of MPLC in 18 surgical series outside screening (mean range, 1.1–8.6%) (3) was lower than the frequency in LDCT screening studies reporting at least one such a case. This is not surprising since subjects undergoing LDCT are asymptomatic and prone to show developing cancers in their earlier stages.

Occurrence of MPLC in a single subject per se suggests the possibility of genetic predisposition. However search of genetic features associated with lung cancer (45) was beyond the scope of the present report.

Admittedly, since our study is based on cytopathologically or histopathologically proven primary lung cancer, it is possible that we underestimated MPLC in the cohort of subjects undergoing LDCT screening. In particular, lesions presenting as pure ground glass opacity that can be associated with minimally invasive adenocarcinoma (9) may be missed.

In conclusion, MPLC, mostly adenocarcinomas, are not uncommon in smokers and former smokers with at least one LDCT screen detected primary lung cancer. The
Table 2 Multiple primary lung cancers (MPLC) in low-dose CT (LDCT) screening studies

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of screenees</th>
<th>No. of LDCT rounds</th>
<th>Subjects with screen-detected primary lung cancer (%)</th>
<th>No. of adenocarcinomas</th>
<th>Subjects with MPLC/subjects with screen-detected primary lung cancer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nawa et al. 2002 (13)</td>
<td>7,956</td>
<td>1</td>
<td>40 (0.5)</td>
<td>39</td>
<td>1 (synchronous) 2.5</td>
</tr>
<tr>
<td>Diederich et al. 2002 (14)</td>
<td>817</td>
<td>4</td>
<td>11 (1.3)</td>
<td>5</td>
<td>1 (synchronous) 9.0</td>
</tr>
<tr>
<td>Flieder et al. 2006 (15)</td>
<td>2,968</td>
<td>11</td>
<td>77 (2.6)</td>
<td>81</td>
<td>16 (synchronous) 20.8</td>
</tr>
<tr>
<td>Carter et al. 2007 (16)</td>
<td>10,056</td>
<td>11</td>
<td>250 (2.5)</td>
<td>177</td>
<td>31 (synchronous), 5 (metachronous)</td>
</tr>
<tr>
<td>Lindell et al. 2007 (17)</td>
<td>1,520</td>
<td>5</td>
<td>59 (3.9)</td>
<td>34</td>
<td>1 (synchronous), 1 (metachronous)</td>
</tr>
<tr>
<td>Pelosi et al. 2008 (18)</td>
<td>5,202</td>
<td>3</td>
<td>89 (1.7)</td>
<td>72</td>
<td>10 (synchronous) 11.2</td>
</tr>
<tr>
<td>Vazquez et al. 2009 (19)</td>
<td>27,456</td>
<td>12</td>
<td>338 (1.2)</td>
<td>279</td>
<td>49 (synchronous) 14.5</td>
</tr>
<tr>
<td>van Klaveren et al. 2009 (20)</td>
<td>7,557</td>
<td>3</td>
<td>124 (1.6)</td>
<td>NA</td>
<td>5 (synchronous) 4.0</td>
</tr>
<tr>
<td>Infante et al. 2009 (21)</td>
<td>1,276</td>
<td>4</td>
<td>60 (4.7)</td>
<td>27</td>
<td>2 (synchronous), 1 (metachronous)</td>
</tr>
<tr>
<td>Saghir et al. 2012 (22)</td>
<td>4,104</td>
<td>5</td>
<td>69 (1.7)</td>
<td>48</td>
<td>6 (synchronous) 8.7</td>
</tr>
<tr>
<td>Sanchez-Salcedo et al. 2015 (23)</td>
<td>2,989</td>
<td>14</td>
<td>53 (1.8%)</td>
<td>33</td>
<td>1 (synchronous), 6 (metachronous)</td>
</tr>
</tbody>
</table>

Figure 2 Case II (Table 1). Adenocarcinoma and small cell lung cancer in a 61-year-old smoker both detected at baseline LDCT screening round. They appeared as a rounded lung nodule in the RUL and a subpleural small solid nodule (arrow) in the LUL (A). Haematoxylin and eosin histologic staining demonstrate invasive adenocarcinoma, acinar predominant (original magnification x200) (B) in the RUL lesion and small cell lung carcinoma (original magnification x100) (C) in the LUL lesion.
potential distinction of two subtypes of MPLC, represented by multifocal adenocarcinomas and by combination of adenocarcinoma with others histological types, having different prognoses and treatment implications warrants further study.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was conducted in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html) and the study protocol was approved by the Local Ethics Committees of the participating centers (Firenze, approval number 29–30, 30 September 2003; Pisa, number 23, 27 October 2003; Pistoia, number 00028543, 13 May 2004). Each subject provided an informed written consent to participate to the study.

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Supplementary

ITALUNG pathology protocol

Tumors were sampled as fresh tissue immediately after surgery. The entire tumors, fragments of distant non-neoplastic parenchyma, all detectable lymph nodes, and mediastinal lymph nodes were sampled. Specimens were fixed in buffered formalin for 16–24 hours and processed for standard histological examination. Multiple adenocarcinoma cases underwent molecular analysis for the identification of ALK gene translocation and of the known driver mutations in the following genes: EGFR, KRAS, NRAS, BRAF, PIKCA, ERBB2, DDR2, MAP2K1 and RET. A representative paraffin-embedded block containing about 80% viable tumor was selected for each case. Immunohistochemical staining procedures were conducted on 4 µm-thick sections of paraffin-embedded tissue using the anti-ALK monoclonal antibody (clone D5F3, ready to use, Ventana Medical System). The OptiView DAB Detection kit was used as revelation system, adding the OptiView Amplification kit (Ventana Medical System) for ALK detection. Three consecutive 10 µm-thick sections were performed from the same blocks for molecular analysis. These were conducted by a mass spectrometry-based multiplex assay (MassArray technology, Sequenom, San Diego, CA) using the “Myriapod Lung Status” kit (Diatech Pharmacogenetics, AN, IT) capable of identifying the major mutations of the following genes: EGFR (exons 18, 19, 20, 21), KRAS (codons 12, 13, 61), NRAS (codons 12, 61), BRAF (codons 466, 469, 594, 597, 600), PIKCA (codons 542, 545, 1043, 1047), ERBB2 (exon 20), DDR2 (codons 239, 638, 768), MAP2K1 (codons 56, 57, 67), RET (codon 918).