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Serum biomarkers for lung cancer screening: improving early detection and diagnosis

Biomarcadores séricos no câncer de pulmão: aprimorando o diagnóstico precoce

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ABSTRACT: Lung cancer is by far the leading cause of cancer death among both men and women. Screening patients at high risk of developing lung cancer is a worldwide priority, since it can be cured if diagnosed in early stages. Currently, screening in high risk individuals is made using low dose computed tomography, however, this method may lead to false-positive tests and overdiagnosis. The usefulness of serum biomarkers would be relevant in two situations: 1) the screening of large groups at high risk of developing lung cancer, where the biomarker should be very sensitive and 2) during the investigation of pulmonary nodules, where the biomarker should be very specific. Several serum biomarkers have been tested to work as biomarkers for lung cancer screening. Unfortunately, so far, none of them has come into current clinical practice. In this review, we analyze some of the serum biomarkers described in the last 10 years, evaluating their potential as tools to detect lung cancer, particularly in smokers. The use of serum biomarkers and imaging methods together seems to be a solution to early diagnosis of lung cancer, more efficient treatment and enhanced chance of cure.

Keywords: Biomarkers; Early diagnosis; Tobacco use disorder; Carcinoma, non-small-cell lung; Neoplasias pulmonares/diagnóstico; Neoplasias pulmonares/diagnóstico por imagem.

RESUMO: O câncer de pulmão é de longe a principal causa de morte por câncer entre homens e mulheres. A triagem de pacientes com alto risco de desenvolver câncer de pulmão é uma prioridade mundial, já que pode ser curado se diagnosticado em estágios iniciais. O rastreio em indivíduos de alto risco é feito usando tomografia computadorizada de baixa dose, no entanto, este método pode levar a testes falso-positivos e diagnósticos equivocados ("overdiagnosis"). Vários biomarcadores séricos foram testados para funcionar como biomarcadores para o rastreamento do câncer de pulmão. Infelizmente, até agora, nenhum deles entrou na prática clínica. Nesta revisão, analisamos alguns dos biomarcadores séricos descritos nos últimos 10 anos, avaliando seu potencial como ferramentas para detectar câncer de pulmão, particularmente em fumantes. A utilização de um biomarcador ou de um painel de biomarcadores seria relevante em duas situações: 1) triagem de grandes grupos de indivíduos com alto risco de desenvolver câncer de pulmão, para o qual o biomarcador deve ser muito sensível, e 2) durante a investigação de nódulos pulmonares, em que o biomarcador deve ser muito específico. Portanto, o uso combinado de biomarcadores séricos e métodos de imagem parece ser uma solução para o diagnóstico precoce do câncer de pulmão e, consequentemente, para um tratamento mais eficiente e maior chance de cura.

Descritores: Biomarcadores; Diagnóstico precoce; Tabagismo; Carcinoma pulmonar de células não pequenas; Neoplasias pulmonares/diagnóstico; Neoplasias pulmonares/diagnóstico por imagem.

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INTRODUCTION

Epidemiology of lung cancer

In most of the Western countries, cancer ranks the second most common cause of death following cardiovascular diseases. Tens of millions of people are diagnosed with cancer each year, and more than half of the patients will eventually die from it[1].

Lung cancer is particularly important. With an increasing incidence every year[2], it has the second higher incidence among males (behind prostate cancer) and females (behind breast cancer). However, it ranks first in mortality in both genders.

In Brazil, the National Cancer Institute (INCA), estimated the diagnosis of 18,740 new cases in men and 12,530 in women during the year 2018[4]. Moreover, according to data from the Mortality Information System (SIM), lung cancer accounted for more than 26,000 deaths in the year 2015[4].

As the majority of other cancer types, lung tumors derive from synergy between genetic and environmental risk factors[5]; among the latter, smoking has been described as the most important risk factor for lung cancer development[6]. Other risk factors, such as occupational chemical exposures (asbestos, for example), environmental exposure to radon, personal and family history, are also recorded in the literature[7].

About 90% of lung cancer cases are related to tobacco inhalation[8]. Smoking itself increases the risk of lung cancer 5 to 10 times, presenting a significant dose-response relationship. Not only active smoking, but also passive smoking is a risk factor for lung cancer, and exposure to tobacco accounts for about 20% of cases in nonsmokers[3,6,8-10].

Regarding the histopathological aspect, lung cancer is more commonly subdivided as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC)[7,11]. In the present review, only NSCLC will be addressed, since it is the most common type and its relationship to smoking is better characterized.

Since lung cancer leads to death in a high percentage of the patients suffering from this condition, it is crucial to improve its early detection, when therapeutics is still curative and effective.

Screening for early lung cancer

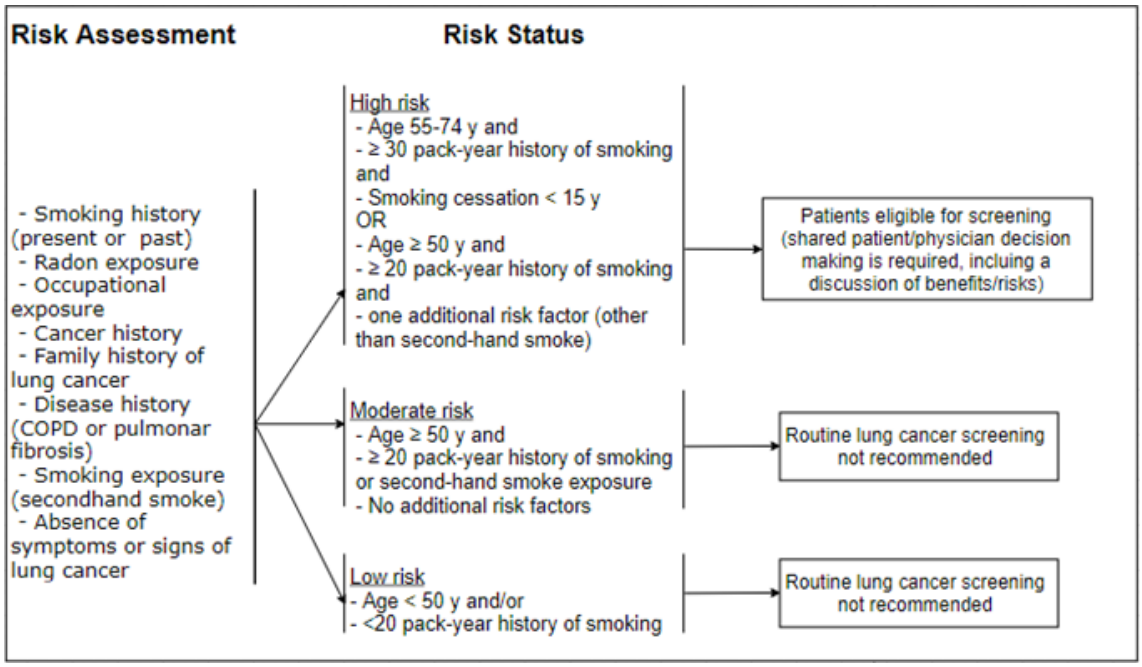
The first large scale study for lung cancer screening was performed by the Mass Radiography Service of the North-West Metropolitan Region of London, in the 1960s[12]. It was a prospective study without randomization, where the test group, composed by 29,723 men, were submitted to chest radiograph exams (CXR) every six months for three years. In parallel, a control group, with 25,311 men, were radiographed at the beginning and at the end of the study, after three years. Lung cancer was diagnosed in more patients in the test group compared to the control group (132 vs. 96, respectively), however, there was no change in mortality between groups (62 vs. 59, respectively). Despite the absence of results in reducing mortality, this study was enough to encourage high-risk groups to be screened regularly since it showed success for the discovery of surgically approachable lung cancers.

In the 1990's, improvements in computerized tomography scanners brought back the interest in screening for lung cancer. The Early Lung Cancer Action Project (ELCAP) was designed to evaluate the usefulness of CT in annual lung cancer screening[13]. The ELCAP obtained chest radiography and low-dose CT (LDCT) of 1,000 asymptomatic individuals, 60 years old or more, with smoking history of at least 10 pack-years. The low-dose CT was more efficient in the detection of noncalcified pulmonary nodules (NCN) than the CXR (23% versus 7%, respectively) and identified the disease in earlier and curable stages (often stage I).

On the other side, this screening method was criticized for the potential overdiagnosis of small lesions that would not fully develop into symptomatic tumors. In order to avoid it, pathologic criteria were carefully used, and an analysis of the histologic specimens from surgeries was done to confirm the previous lung cancer diagnosis. Based on ELCAP and other similar projects, the proportion of overdiagnosis could be empirically estimated. These findings were further confirmed by other studies with high-risk groups[14].

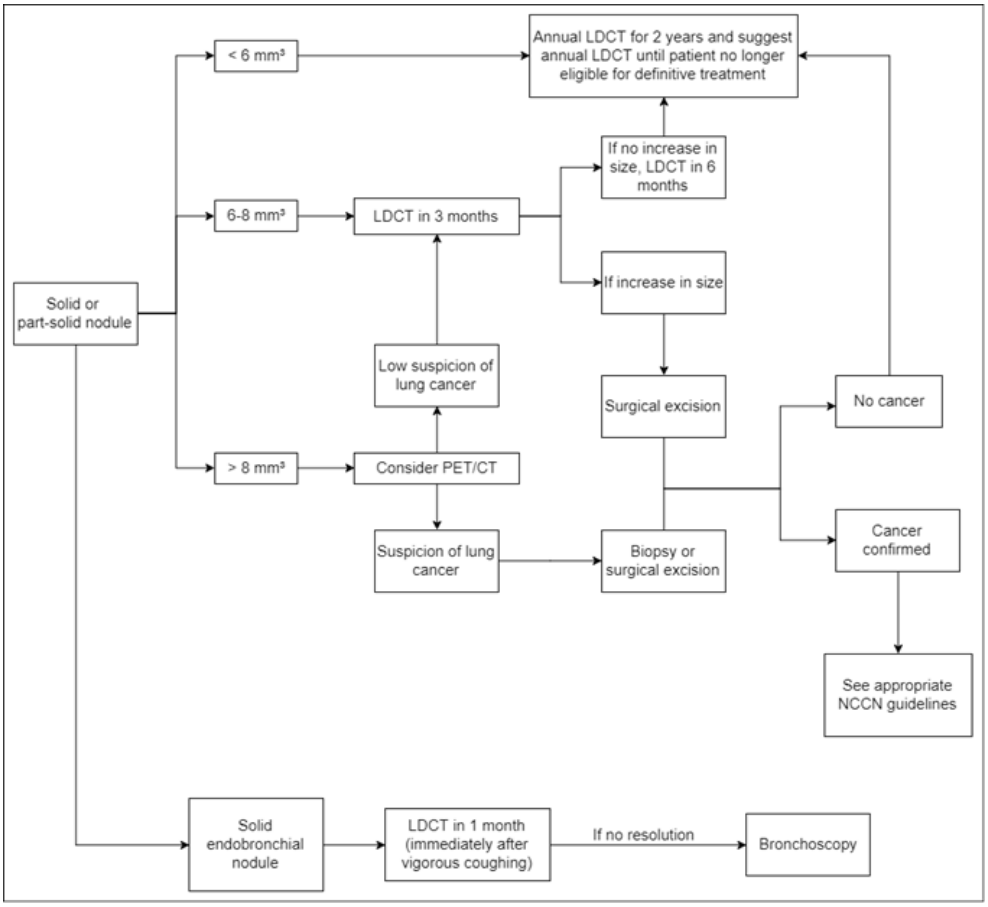
These studies showed that LDCT was adequate to identify early lung nodules, but none of them was capable of demonstrating reductions in mortality related to lung cancer. Hence, a large randomized controlled trial was performed, the National Lung Screening Trial (NLST). In this trial, 53,456 participants underwent screening from 2002 to 2004. This study demonstrated a 20% reduction in disease-specific mortality when low-dose CT (LDCT) was used, compared to chest radiography[10].

These findings prompted the National Comprehensive Cancer Network (NCCN) to release their guidelines for lung cancer screening in 2015[15], with an algorithm to calculate lung cancer risk and indications for CT screening (Figure 1). Smoking history, radon exposure, occupational exposure, cancer history, family history of lung cancer, pulmonary disease history, smoking exposure and absence of symptoms or signs of lung cancer are relevant factors to differentiate the groups. Patients included in the high-risk group are recommended to undergo screening routinely. In case of nodule detection, the algorithm shown in Figure 2 should be followed.



Adapted from: NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Lung Cancer Screening V.1.2015.

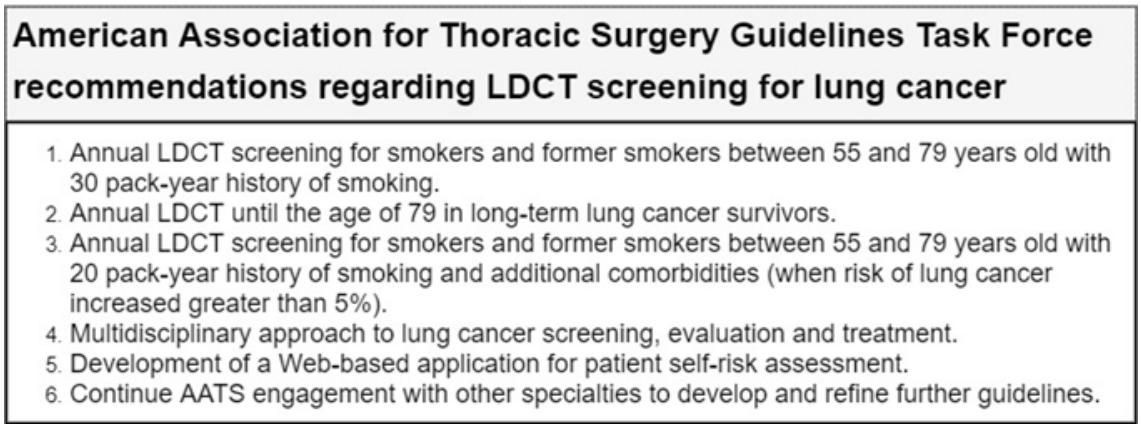
Figure 1: Algorithm for calculating risk of lung cancer and indications for screening



Adapted from: NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Lung Cancer Screening V.1.2015.

Figure 2: Algorithm to investigation of lung nodule detected at CT screening

The American Association for Thoracic Surgery Guidelines Task Force has also released their recommendations to indicate screening with annual LDCT, which are summarized in Figure 3[16].



Adapted from: Stiles BM et al. Surgical Oncology Clinics of North America. 2016 Jul;25(3):469-79 (16)

Figure 3: American Association for Thoracic Surgery recommendations for CT screening for lung cancer

Although the current data show reduced mortality in patients that underwent LDCT screening, particularly due to early surgery to remove suspect nodules, there is still several concerns, mainly about false-positive tests, overdiagnosis and their complications[17].

LDCT is not a very specific method, with a high incidence of false-positive diagnoses; these patients are, then, usually submitted to invasive procedures to confirm the diagnosis of cancer (mediastinoscopy, thoracoscopy, thoracotomy, bronchoscopy or needle biopsy), and complications may arise. Moreover, this kind of investigation can have deleterious effect in patients' mental and physical health, before the cancer diagnosis could be dismissed[18].

In addition, increasing exposure to ionizing radiation from CT screening is also relevant. The amount of radiation in the LDCT is very low, however the repeatedly exposure that the annual screening demands may be clinically relevant, due to its carcinogenic potential[19].

As summary, LDCT screening has proved to be a valuable tool for identifying small nodules and, consequently, lung cancer in early stages. Unfortunately, this method is not highly specific. Therefore, a non-negligible number of patients are submitted to invasive procedures and have a benign histopathological diagnosis. This high incidence of false-positive CTs may lead to undesirable invasive procedures and implies in higher costs to the whole health system[20].

Serum biomarkers for lung cancer screening

LDCT is the method recommended by the recent guidelines to identify early lung nodules but has several pitfalls that points out the necessity to improve or replace it by less invasive methods that might offer a better cost-effectiveness relationship[18-21].

Biomarkers are parameters that can be objectively measured aiming to detect a physiological or pathological process[22]. Biomarkers can be useful for screening, diagnosing, staging or classifying a particular disease, as well as to give a prognosis and to monitor the clinical response to an intervention. They are also a potential tool to provide information about diseases pathophysiology.

Cancer biomarkers are already being used for screening and diagnosis, such as the prostate-specific antigen (PSA) for prostate cancer[23], genetic alterations (BRCA mutations) for breast cancer[24] and the presence of occult blood in the stool for colorectal cancer[25].

Serum biomarkers for lung cancer screening would be less invasive, exposing patients only to minimal risks and they should reflect pathophysiology mechanisms, leading to lower rates of overdiagnosis and false-positives. Beyond that, serum biomarkers could be more accurate when allied to the LDCT regarding the indication of invasive procedures, such as biopsies.

These biomarkers must have specific properties to be considered suitable for screening and diagnosis: they must be involved in carcinogenesis, may be modulated according to disease progression, and be associated with risk factors[26]. Once a biomarker complies with these properties, it may help to more accurately evaluate a disease.

Therefore, in this review, we summarize some of the serum biomarkers that have been studied as potentially useful for screening lung cancer in smokers, a group of patients at high risk to develop the disease. We focused our research in the last ten years literature about serum

biomarkers and did not evaluate studies that show possible genetic traits related to lung cancer.

METHODOLOGY

This study is a non-systematic review, performed to identify published studies that describe serum biomarkers of lung cancer.

A single database was used, PubMed, accessed during October 2016. The terms used for the search were "lung cancer" AND "biomarker" AND (smoker OR smoking OR tabagism).

Initially 306 articles were retrieved, and filters were applied: "humans"; "adults older than 19 years"; "date of publication the past 10 years"; "English or Portuguese language". After this procedure, 138 articles were obtained (Figure 4).

The authors, then, analyzed each article by title and abstract. Articles identified as reviews, articles focused on prognostic or other risk factors different from smoking, sources of biomarkers other than serum, and papers about genetic mutations that cause lung cancer were excluded.

Therefore, 30 articles were included in our review and were carefully analyzed. The selected articles were divided by the three main authors who read the articles and discussed them with the other authors. Table 1 lists all the articles analyzed, while in Results those describing more promising biomarkers are discussed in detail.

RESULTS

Thirty articles describing serum biomarkers for screening lung cancer were evaluated, as shown in Table 1. We divided the serum biomarkers described in each article by their biochemical characteristics: proteins and specific antibodies against antigens expressed by some tumors, micronutrients and metabolites, and nucleic acids, as miRNAs and DNA modifications. Some of them are discussed below.

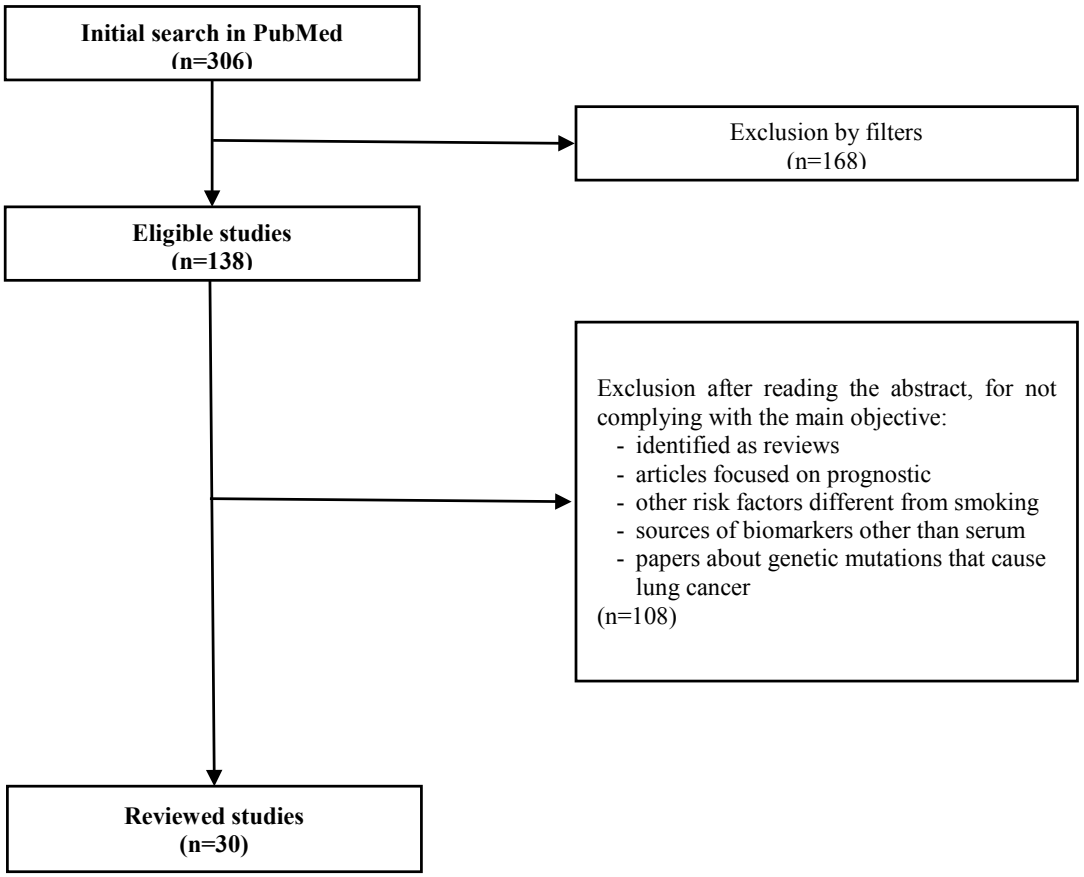


Figure 4. Flowchart describing the studies selection

Table 1: Biomarkers for screening of lung cancer evaluated in this review

Author, Year	Biomarker	Number of patients	Summary	Comments
Ho et al., 2016[31]	Insulin and IGFBP3	1143 cases; 1143 controls	Insulin and IGFBP3: the combination of these biomarkers could be used for screening.	The sample was composed by postmenopausal women.
Guo et al., 2013[32]	Insulin-Like Growth Factor Binding Protein-2	164 cases; 80 controls	Higher serum IGFBP2 levels in patients with lung cancer. In addition, this serum biomarker correlates with clinical and prognostic outcomes.	Alone, the biomarker IGFBP2 is not a sufficient diagnostic value, since sensitivity and specificity have been around 70%.
Sin et al., 2013[35]	Pro-surfactant protein B (pro-SFTPB)	2,485 individuals	Lung cancer tumor cells (mainly adenocarcinomas) have dysregulated Pro-surfactant protein B synthesis leading to the overexpression of it.	Lack of specificity, pro-SFTPB can also rise in other lung diseases.
Taguchi et al., 2013[36]	Pro-surfactant protein B (pro-SFTPB)	188 cases; 337 controls	Levels of circulating mature Pro-surfactant protein B were increased among subjects with lung cancer both at the time of diagnosis and in a pre-diagnostic setting.	Nonspecific - it indicates a pulmonary disease. The sample was composed just by men.
Wikoff et al., 2015[37]	Serum Diacetylspermine + Pro-Surfactant Protein B	208 cases; 415 controls	DAS presented an AUC = 0,657 and pro-SFTPB presented an AUC = 0,682. Combined, the total AUC = 0,808.	Nonspecific - it indicates pulmonary disease.
Shiels et al. 2013[40]	C-reactive protein (CRP)	526 patients; 592 controls	Elevated C-reactive protein (CRP) levels were associated with a two-fold increased risk of lung cancer. Cigarette smoke itself can lead to pulmonary inflammation.	Nonspecific - Serum CRP levels reliably indicate the presence of chronic inflammation (not specific for lung inflammation).
Xu et al., 2013[41]	CRP	96 cases; 124 controls	SNPs associated with CRP level, but not at risk; higher risk for high CRP levels. OR 2.11 for CRP> 5.5.	CRP is an extremely nonspecific marker.
Diamandis et al., 2011[43]	Pentraxin-3; human kallikrein 11 (KLK11) and progranulin	203 patients, 180 heavy smokers, 43 other cancers	Only pentraxin-3 was able to distinguish high risk individuals from lung cancer patients, with 48% sensitivity and 80% specificity. Using specificity of 90%, sensibility declines to 25%.	Pentraxin may be elevated in inflammatory conditions.
Lee et al., 2011[44]	CTAP III	30 patients, 30 high-risk individuals	CTAP III is significantly higher in lung cancer patients.	Small sample.
Yee et al., 2009[45]	CTAP III	16 (1st phase) and 64 (2nd phase)	CTAP III is significantly higher in lung cancer patients and is a good predictive tool, when associated to other methods.	Small sample.
Sen et al., 2008[47]	EMAP II	48 cases; 30 controls	Levels of EMAP II are higher in patients with lung cancer, but it is not able to distinguish high risk group.	Small sample and it is a marker of prognosis, more than diagnosis.
Liu et al., 2012[49]	Antibody anti-ABCC3	275 patients	ABCC 3 was significantly higher only in women with adenocarcinoma.	The result shows restrict use of the biomarker.
Rom et al., 2010[50]	Antibodies anti-TAAs	194 patients	A panel of autoantibodies is used to distinguish healthy controls, high risk and lung cancer and the groups have significant differences.	The sample is too small (only 22 cancer patients).
Chen et al., 2016[51]	Anti-CD25 autoantibody	111 cases; 216 controls	Higher levels of Anti-CD25 IgG in patients with stage IV NSCLC only; not useful for early stages	The observations obtained suggest a more prognostic biomarker than a diagnostic
Church et al., 2009[52]	NNAL and PheT	100 cases; 100 controls	Total NNAL in serum is significantly associated with lung cancer risk.	Nonspecific.
Epplein et al., 2009[54]	Antioxidant biomarkers	207 cases; 414 controls	Association between increasing levels of serum carotenoids and a reduced risk of lung cancer in men.	The biomarkers used are nonspecific for lung cancer; the results of this study diverge from other studies.
Lee et al., 2014[55]	Reactive oxygen species modulator 1 (Romo1)	58 cases; 118 controls	Romo1 expression is higher in NSCLC than in controls. For a corset of 329.7pg / mL, sensitivity of 81.9% and specificity of 89.8%, AUC of 0.847.	ROMO1 may be elevated in other pulmonary diseases.
Chen et al., 2012[57]	10 miRNAs	400 cases; 220 controls	It is possible to distinguish cases and controls based in a panel of 10 miRNAs, with 93% sensibility and 90% specificity.	Promising as a screening method. Calibration of the quantitative PCR method is a concern
Levine et al., 2015[60]	DNA methylation levels at CpG dinucleotides	2029 participants; 43 lung cancer incidences	Having an aging acceleration rate observed by the levels of DNA methylation is associated with a 2.5-fold increase in the risk of developing lung cancer (smoking is seen as a pro-aging factor.	Nonspecific - the methylation was evaluated in blood cells; the sample was composed by women; the predictability is more sensitive for individuals aging 70 years and older; the IEAA has no connection to the exposure (tobacco).
Zhang et al., 2015[61]	F2RL3 methylation	4987 participants; 97 lung cancer incidences	F2RL3 hypomethylation was strongly associated with both lung cancer incidence and mortality. An overexpression of PAR-4 was associated with significantly shorter 3-year survival.	Nonspecific - the methylation was evaluated in blood cells; the predictability is more sensitive for individuals aging 65 years and older.
Greenberg et al., 2007[64]	S-adenosylmethionine (AdoMet)	68 patients	Plasma AdoMet levels had difference between lung cancer, high risk and controls. This may be a useful tool for the diagnosis of early lung cancer, in combination with chest CT.	Nonspecific, difficult to measure, small sample.
Seow et al., 2014[66]	Telomere length in white blood cells	847 cases; 847 controls	The effect of long telomere length in white blood cells and lung cancer is particularly evident for adenocarcinoma, and especially among women.	Nonspecific - the length of the telomeres can indicate different types of cancer; the study was performed using white blood cells.

Table 1: Biomarkers for screening of lung cancer evaluated in this review *continuation*

Author, Year	Biomarker	Number of patients	Summary	Comments
Aujollet et al., 2010[68]	NTproBNP	439 patients	Patients with lung cancer have higher chances of increased levels of NTproBNP.	The objective is to analyze other causes to high levels of the marker.
Köhler et al., 2016[69]	Circulating U2 small nuclear RNA fragments	211 cases; 112 controls	RNU2-1f expression levels were elevated in patients with LC patients treatment naive, compared to controls.	More useful as a prognostic and follow-up biomarker.
Gumireddy et al., 2015[70]	AKAP4	264 cases; 135 controls	In the combined cohort, the AKAP4 relative value of -4.3 distinguished cases from controls with an AUC = 0,9714. Also, distinguished NSCLC from benign pulmonary nodes with an AUC = 0,9825 and accuracy = 93,5%.	Promising as a tool to identify lung cancer from benign nodules. Low sensitivity.
Seder et al., 2015[71]	Angiogenesis growing factors	193 cases; 110 controls	Differences in the concentrations of HB-EGF, EGF, VEGF-A, VEGF-C E VEGF-D were strongly significant (p<0,001), while differences in the concentrations of folistatin, PLGF e BMP-9 were significant (p<0,05).	There was no control of some covariables, such as tobacco history.
Doseeva et al., 2015[72]	Panel of 3 tumor antigens (CEA, CA-125, and CYFRA 21-1) and 1 autoantibody marker (NY-ESO-1)	Training set: 115 cases; 115 controls. Validation set: 75 cases; 75 controls	Training set: in the individual analysis of each biomarker, UAC ranged from 0,60-0,79, with CEA being the largest (0,79). The combined panel had AUC of 0,83. Validation set: UAC 0.81. For a cutoff value of 6.4, it resulted in a sensitivity of 71% and a specificity of 88%. VPN of 99.4% and VPP of 7.2%.	Healthy controls? It did not include in the analysis benign pulmonary diseases or indeterminate nodules. In addition, it does not present the expected distribution of types of cancer, stages, etc.
Li et al., 2015[73]	Mesenchymal-epithelial transition factor (MET)	95 cases; 44 controls	Serum MET is higher in patients with LC compared to controls. MET is even higher in patients with higher smoking load, squamous cell carcinoma, advanced staging. Sensitivity of 72.6% and specificity of 90.9%.	MET levels may be higher in patients with other tumors.
Zhang et al., 2014[74]	Lemur tyrosine kinase-3 (LMTK3)	524 cases; 380 controls	AUC 0.701. In addition, patients with LMTK3> 6.85 presented lower survival, correlating with prognosis as well.	It did not present a high AUC to be considered a diagnostic marker by itself, perhaps it could be associated with other biomarkers. It's more for a prognostic marker.
Weber et al., 2013[75]	RNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1)	45 cases; 25 controls	NSCLC: AUC 0.79; AdCa: 0.75; Squamous cell carcinoma: 0.82; AdCaxSCC: 0.58.	Small sample, no high-risk patient was evaluated.

Proteins

Insulin and Insulin-like growth factor binding protein 3 (IGFBP3)[31,32]

Smoke produced by tobacco cigarettes induces a state of chronic inflammation[8]. It is well known that chronic inflammatory process is usually associated with a reduction in the insulin peripheral sensitivity, inducing a hyper insulinemic status[27-29]. Insulin is a potent mitogen that activates the Ras/MAPK and PI3K pathways. Therefore, hyperinsulinemia can induce cell proliferation that is associated with the pathogenesis of lung cancer[29].

Insulin-like growth factor binding protein 3 (IGFBP3) is part of a family of proteins that serve as carriers for insulin-like growth factors (IGF), enhancing their circulating half-life and modulating their activity[30]. It has been reported that IGFBP3 prevents the activation of the IGF1-induced Ras/MAPK pathways, inhibiting its inflammatory and proliferative actions[30], hence acting as an antitumorigenic molecule.

In a well-designed case-control study[31] it was observed in current smokers that high serum levels of insulin and low levels of IGFBP3 were strongly associated with lung cancer. Serum IGF1 was also associated with lung cancer, however, only moderately. The authors speculated that, among current smokers, both insulin and IGF1 activates proliferative pathways, increasing the susceptibility to lung cancer. In the other hand, the IGFBP3 suppresses these pathways, reducing the risk of lung cancer. Interestingly, authors observed that the effect of insulin in the lung carcinogenesis does not hold a relationship with obesity development.

Pro-Surfactant Protein B (pro-SFTPB)[35, 36, 37]

The pro-surfactant protein B, precursor of protein B, is a hydrophilic 42-kD protein produced by type 2 pneumocytes and non-ciliated bronchiolar cells[33]. Lung tumors, particularly adenocarcinomas, overexpress pro-SFTPB with a muted ability to turn into the mature form[34].

In a prospective study, 2,485 individuals (older than 50 years old) with high risk for lung cancer (2,237 of them were smokers) were followed for at least 2 years and 144 (5.79%) of them developed the disease. It was observed that higher levels of plasma pro-SFTPB, collected at baseline, were significantly and independently associated to presence of lung cancer in smokers. This phenomenon increments the lung cancer prediction calculated by established risk factors[35]. In another study from the same group, it was also noticed that, paradoxically, non-detectable levels of

pro-SFTPB were significantly associated with lung cancer risk in never smokers[36].

Although this protein seems to be suitable as a cancer biomarker, it can also be and indicator of other lung diseases, such as chronic obstructive pulmonary disease (COPD), what limits its clinical usefulness.

C-reactive protein (CRP)[40, 41]

C-reactive protein (CRP) is a circulating protein largely used in the clinical set as a marker on systemic inflammation[38]. High serum levels of CRP have also been identified in various types of cancer[39].

In a nested case-control study, 526 lung cancer patients were matched to 592 control subjects and 77 inflammatory mediators were evaluated[40]. CRP proved to be the most discriminatory of these (with an odds ratio around 2.2). However, association of inflammatory chemokines and cytokines with CRP seemed to be more effective as biomarkers for lung cancer risk among smokers.

Despite this strong association, high serum levels of CRP can also be found in inflammatory lung conditions, such as COPD and pneumonias. Hence, this lack of specificity limits the use of CRP as a valid biomarker for lung cancer screening, unless it is used in combination with other more specific biomarkers.

Pentraxin-3 (PTX3)[43]

Several proteins expressed by NSCLC cells in culture were identified by a proteomics analysis[42]. Three of these proteins were tested in samples from 203 patients with lung cancer, 180 heavy smokers and 43 patients with cancer in other locations[43]. Human kallikrein 11 and progranulin showed to be no informative about cancer. Pentraxin-3, however, was a significant lung cancer biomarker, with considerable ability to separate lung cancer patients from high- risk controls. At 90% and 80% specificity, the sensitivity versus the high-risk and control group were 37% and 48%, respectively. Pentraxin-3 is a protein associated to resistance to pathogens and could be elevated in infections, sepsis and other malignancies. Due to its high specificity, PTX3 would be a very interesting biomarker to differentiate lung cancer from benign pulmonary nodules, however, no study so far has used it in a clinical set.

Connective Tissue-Activating Peptide III (CTAP III)[44,45]

Connective Tissue-Activating Peptide III (CTAPIII) is a chemokine related to angiogenesis and tumorigenesis and was reduced in peripheral blood after surgical resection of the tumor. Using a different approach, Lee et al.[44] evaluated plasma from 30 patients with lung cancer and 30 high-risk individuals using Protein Chip immunoassays. They identified elevation of CTAP III plasma levels in patients' group. Further, they confirmed this hypothesis using an ELISA test in the same population.

In a previous study, it was shown that CTAP III serum levels are increased in patients with lung cancer and decrease after the tumor resection[45]. Unfortunately, both studies enrolled a small number of patients with lung cancer[30,49] and no information was provided about histology or follow up of these patients. However, due to its specificity and relation to cancer pathogenesis, CTAP III persists as an intriguing possibility as a lung cancer biomarker.

Endothelial monocyte-activating polypeptide-II (EMAP II)[47]

Endothelial monocyte-activating polypeptide-II (EMAPII) is a cytokine that has the ability of inhibiting angiogenesis, markedly in solid tumors[46]. The mean EMAPII serum levels were found to be significantly higher in patients' population with untreated NSCLC than the detected in the control group[47]. Serum levels had no significant association with various clinical or pathological features (age, smoking history, performance status, histopathology, tumor stage, lymph node stage, or distant metastasis). However, the authors reported a potential prognostic value, since higher levels were related to poor prognosis.

Even though the difference was significant between patients and controls, this marker could not detect the early stages of the tumor, what limited its use as a screening instrument.

Anti-ATP-binding cassette C3 (Anti-ABCC3)[49]

ATP-binding cassette C3 (ABCC3) is an ATP-dependent transporter that functions as an energy- driven pump to maintain intracellular drug concentrations below a toxic level. Therefore, they are one of the main pathways responsible for tumor multidrug resistance[48]. Since this class of transporters is usually overexpressed by tumors, it is reasonable to conceive that antibodies against them might signal the presence of tumors.

Analyzing 178 men and 97 women diagnosed with lung cancer (adenocarcinoma or squamous carcinoma), authors found that the concentration of IgG against ABCC 3 was significantly higher only in women with adenocarcinoma[49]. This finding restricts its use as a biomarker and suggests that it would be useful only in a panel of autoantibodies, to increase the sensibility of the test.

Anti-tumor associated antigens (Anti-TAAs)[50]

Sera from lung cancer patients contain autoantibodies that react with tumor associated antigens (TAAs) and reflect genetic over-expression, mutation, or other anomalies of cell cycle, growth, signaling, and metabolism pathways.

Following previous studies that identify some of these TAAs, a study was designed to evaluate whether a panel containing ten antibodies against TAAs was able to

differentiate lung cancer form other more benign nodules found on computed tomography[50]. They examined the sera from lung cancer patients (22 subjects); smokers with ground-glass opacities (GGOs) (46 subjects), benign solid nodules (55 subjects), or normal CTs (35 subjects); and normal non-smokers (36 subjects). The authors reported a high specificity for distinguishing patients with lung cancer from smokers with normal CTs, stable solid nodules, ground glass opacities, or normal healthy never smokers.

Although promising, the study sample was small, and no follow-up of the control groups was provided.

Micronutrients and metabolites

There are some circulating molecules that can also have a strong association with lung cancer, such as tobacco-specific carcinogens and antioxidants.

Tobacco-specific carcinogen[52]

Among the many known carcinogens in cigarette smoke, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is specific to tobacco and causes lung cancer in laboratory animals. Exposure to NNK can be measured by serum levels of its metabolites, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL). Therefore, authors evaluated sera from 100 lung cancer patients and 100 matched controls for the presence of NNAL. It was reported that serum total NNAL is significantly associated with lung cancer risk, particularly among long-term heavy smokers[52]. Besides that, there is a positive correlation between serum levels of total NNAL and the presence of lung cancer. Therefore, NNAL may be a valid biomarker of cigarette smoke exposure and consequently, of risk for lung cancer development.

Antioxidants[54, 55]

Some experimental works have demonstrated a relationship between disruption of redox signaling and carcinogenesis[53]. Epplein et al studied the association between circulating levels of antioxidants, such as carotenoids, tocopherols and selenium, and the presence of lung cancer[54]. It was found a strong inverse association between lung cancer risk and total plasma carotenoid levels, albeit only in male individuals. No other more specific marker of redox signaling dysfunction could be associated with the disease. Moreover, there were no other relevant data that can corroborate the use of antioxidants as biomarkers for lung cancer screening.

Nucleic acids

MicroRNA (miRNA)[57]

Micro (mi)RNAs are small RNA species that have an expression frequently dysregulated in cancer. Several studies have focused on the relationship between circulating miRNAs and cancer[56].

Using samples from 200 lung cancer patients and comparing them to 110 healthy controls, Chen et al. found 10 miRNAs differentially expressed from the 91 miRNAs initially tested. Further, these 10 miRNAs were tested in a distinct sample of patients and controls, confirming the initial findings. Finally, the authors obtained serum samples from 20,000 individuals who participated in a community-based screening program and tested for the panel of 10 miRNAs. Seven of these individuals had lung cancer during the medical follow-up and the miRNAs panel was capable of identify six of these seven patients, almost 3 years before the diagnosis was made[57].

These data are very encouraging, since identification of miRNAs is performed by quantitative PCR, an inexpensive and disseminated method.

DNA methylation[60,61,64]

DNA methylation is an epigenetic mechanism that controls the activity of genes and is supposed to play an important role in carcinogenesis[58]. It has also been described that specific methylated motifs may be used to identify the aging process[59].

Based on this premise, Levine et al. studied DNA methylation levels at CpG nucleotides in circulating leukocytes of patients with lung cancer[60]. DNA methylation was classified as positive whether the patient expresses a DNA methylation higher than it would be expected according to the individual chronological age; the negative value expresses a DNA methylation lower than the expected. The smoking history was not taken into account in this study.

It was observed that one standard deviation above the mean indicated a 2.5-fold increased risk of lung cancer. Despite the association found, the use of DNA methylation in a blood cell can indicate an increased risk of cancer in different tissues, not only in the lung tissue, what demonstrates the non-specificity of this biomarker[60].

Methylation of a specific gene, F2RL3, was also studied[61]. F2RL3 gene codifies the production of the coagulation factor II receptor-like 3, also known as protease-activated receptor-4 (PAR4), a thrombin receptor, part of the G-protein-coupled receptor subfamily that plays an important role in tumor development and progression[62]. The PAR4 protein seems to be overexpressed in the majority NSCLC tissues and this overexpression was associated with a shorter 3-year survival[63].

Hypomethylation of the F2RL3 gene induces an enhanced expression of the protein PAR4. Thus, the risk of cancer increased with the decreasing methylation intensity. There was a significant difference in methylation between former smokers and current ones. Former smokers showed intermediate methylation intensity when compared to the current ones. Both hyper and hypomethylation are more prominent in older individuals (65 years and above), what can restrain the use of the DNA methylation as a biomarker[61].

From previous studies, it seems that the association of changes in genes methylation and the development of cancer is more relevant when found in the tumor cells. The use of blood cells in both studies turns this method less specific and sensible, making it less useful in a clinical set.

Another study about methylation was made by Greenberg et al.[64] As many genes related to cell cycle can be methylated, it would be difficult to study each one and this would reduce sensitivity of one possible test. By analyzing S-adenosylmethionine (AdoMet), which is a component of the enzymatic pathway for DNA methylation, it would be possible to identify more alterations[64].

This study measured AdoMet levels in three groups of patients: lung cancer, high risk smokers and healthy nonsmokers, also comparing their CT scans. AdoMet levels were significantly higher in serum from patients who have cancer as compared to high risk smokers with small noncalcified nodules. These findings suggest that using AdoMet levels could help distinguishing suspect and benign nodules in a CT scan, leading to previous diagnosis of lung cancer.

Telomere length

Telomere length has been directly connected with carcinogenesis, since telomerases are more expressed in cancer cells, allowing them to keep an unlimited capacity of proliferation[65].

Seow et al. described a strong association between the telomere length in blood leukocytes and the presence of lung cancer[66]. This association was more evident in adenocarcinomas, particularly among females.

The limitation of using this biomarker is related to its lack of specificity. Telomere length is associated with carcinogenesis in every tissue, not only lung. Therefore, its use as a biomarker should be allied to other markers that are more specific to lung carcinogenesis. Also, the smoking history wasn't relevant in this study.

DISCUSSION

The probability of cure for patients with lung cancer is directly related to the ability to detect the disease in early stages, when both surgical and chemotherapy are more effective. Therefore, early detection is crucial, particularly in patients at high risk, like smokers[2,3,17].

Unfortunately, the available methods are not sensible or specific enough to identify early lesions with the necessary accuracy. Actual guidelines rely heavily on the ability of low dose computed tomography to detect small nodules that may be lung tumors[7]. The main problem of this approach is the large number of false-positive exams or overdiagnosis, leading to invasive procedures and, consequently, increased risks to the patients[17,19].

New tests are necessary and serum biomarkers of lung cancer would be ideal tools to screening large number of individuals.

In this review, we listed several potential candidates as serum biomarkers for early detection of lung cancer in high-risk patients (Table 1). Some of these articles were discussed in more detail above. There are several candidates to perform this task, however, none seems to possess all the necessary attributes.

Looking at the actual guidelines, there are two points where serum biomarkers could be more relevant.

First in selecting patients who could be at higher risk of developing lung cancer. Currently, these patients are submitted to LDCT annually, what leads to an enhanced chance of false-positive exams, besides all the risks of radiation and the costs of the exams[17, 19]. At this step of screening, serum tests should be very sensitive, since our goal would be to recognize all patients at risk of disease and submit them to more specific tests.

Serum biomarkers like NNAL[52], that detect high exposure to cigarette smoke or Pro-Surfactant Protein B[38], that is related with early stages of lung cancer development, could be useful tools to sort patients that would need more frequent or more complex exams.

A second point that serum biomarkers can improve the guidelines is to differentiate between benign and cancerous nodules. Nowadays, once a pulmonary solid nodule is detected, patient is submitted to an invasive procedure (biopsy) or a new exam is performed after some time.

Circulating substances like insulin growth factor binding protein 3[31,32] or the already cited Pro-Surfactant Protein B[35,36,37] could help to identify the nature of the solid nodule, improving specificity of LDCT.

Given the important role of inflammation in lung cancer development[67], the serum levels of some inflammatory mediators (CRP, Pentraxin, EMAP, etc.) could be useful to discriminate between lung cancer and more benign nodules.

CONCLUSION

Unfortunately, no single biomarker seems to be able to identify patients at risk to develop lung cancer, or to differentiate malign nodules from benign ones.

However, some of the serum biomarkers described above appear to be promising, particularly when used in conjunction with other methods, like LDCT.

Large populational studies are yet needed to explore the usefulness of serum biomarkers for lung cancer diagnosis.

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