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Review

Potential biomarkers for the diagnosis of respiratory tract infection and lungs cancer

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Abstract: Major hurdle faced by many physicians in treating various respiratory tract infections and lung carcinomas is their late or mis-diagnosis. In most respiratory tract infections the manner of infection is not completely understood. Similarly, various lung carcinomas are diagnosed at advance stages at which not only the treatment possibilities are narrowed but the chances of survival are also reduced. So, for the sake of better treatment, the quick and improved diagnostic strategies are suggested. Protein biomarkers fully fit the description in this regard as they have shown great potential in specific diagnosis of many respiratory diseases and also have shown capability in timely pin pointing different stages of lung carcinomas. Many serum biomarkers are presently being used for diagnosis but the efficiency for diagnosis of these biomarkers is lower when used alone. So, physicians are suggested to use the combination of different biomarkers. Moreover, genetic biomarkers are also currently studied to indicate the exact stage of disease, the possible damage occurred, the severity of disease and also for the analysis of the possible body response to the therapeutics.

Key words: Respiratory tract infections; Lungs carcinomas; Serum biomarkers; Protein biomarkers; Genomic biomarkers.

Introduction

Biomarkers could be defined as the measurable characteristics which indicate the normal biological processes, pathogenic processes and body's pharmacological response to therapeutics (1). The quantity of biomarkers in body serums varies with changing physiological conditions. For instance, the certain concentration of biomarker alone or in combination with other serum biomarkers can indicate the different stages of the cancer (2), establishment of certain infection, stages of infection or can even indicate the spread of infection from one organ to another.

Lung cancer is the frequently diagnosed and leading cause of cancer-related deaths in males worldwide. And it is the fourth most commonly diagnosed cancer in females, with second highest mortality rate (3). Around 25% of cancer-associated deaths are because of lung cancer and is considered the most life-threatening form of cancer as it readily metastasized due to the highest blood flow towards lungs (4). According to the physical features of the lung carcinomas, World Health Organization classified it into: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC could be treated with radio- and chemo-therapy and is further categorized into small cell carcinoma, mixed small cell/large cell carcinoma, and combined small cell carcinoma(5). 80% of lung carcinomas are NSCLC and its treatment may sometimes include surgery while its major types are squamous cell carcinoma (SCC), adenocarcinoma, bronchoalveolar carcinoma (BAC), and large cell carcinoma (6). Despite all the advancement in its treatment methodologies, its overall survival rate remains low (7). One reason for such poor clinical outcome is the delay in the diagnosis of the disease (2). Most of the cancers when diagnosed are already at terminal stages, making it difficult to be treated with available cures.

Similarly, different respiratory tract infections are also not properly diagnosed or are diagnosed at later stages of disease where recovery from the aberrant condition is nearly impossible. Exacerbation is an event, associated with the decrease in the lung's function in asthma (8) and chronic obstructive pulmonary disease (COPD) (9), commonly caused by community-acquired infections. Although, physicians can identify the exacerbation via history and objective tests (10) but there are no means to explain its patho-physiology which do hinders its complete treatment. The clinical features of lower respiratory tract infections are also misleading and vary according to the viral and bacterial load, virulence and response of host (11).

Many serum proteins are released into blood or in urine at different stages of lungs cancer and at different stages of different lung infections (12). The careful analysis of these serum proteins could provide advantages in timely diagnosis of these life-threatening diseases and the possibility of successful treatment can also be ensured (13). So the objective of this review is to provide the physicians with the overview of the potential biomarkers of the respiratory tract infections and lungs cancer to improve the diagnostic possibilities and also to enhance the chances of effective treatment.

Lung cancer biomarkers

Tumor cells release DNA, metabolites and proteins frequently during their development. This DNA and protein then can be used as biomarkers for screening

Table 1. Types of biomarkers for lung's tumor.

DNA Biomarkers	Protein Biomarkers	References
Serine protease family member-trypsinogen IV (PRSS3)	CEA	(4, 14-18)
Tissue inhibitor of metalloproteinase (TIMP)-3	CYFRA 21-1	
Death associated protein (DAP)-kinase	TPA	
RAR-β mRNA	Tumor M2-pyruvate kinase	
RASSFIA	Haptoglobin-R 2	
IL-8 mRNA	APOA1	
FHIT	KLKB1	
epidermal growth factor receptor (EGFR)	proGRP	
K-ras mutant	Neuron-specific enolase (NSE)	
p53 mutant	Chromogranin A	
COX2	vascular endothelial growth factor (VEGF)	
	BB isoenzyme of creatine kinase (CK-BB)	
	Carbohydrate antigen 125 (CA 125) and Carbohydrate antigen 19-9 (CA 19-9)	
	Annexin II	

test and clinical diagnosis of cancer as the level of these, is directly associated with the cancer stages (14). The understanding of biomarker detection in disease process can be of great help in treating cancers like lungs cancer because 1) it will indicate the risk of cancer 2) it will present the general idea of when the cancer can occur and 3) when will be the best time for intervention (14). Lungs cancer biomarkers are categorized in to nucleic acid and protein biomarkers on the basis of its nature, summarized in Table 1. Thou, there are no specific genomic biomarkers yet known for clinical detection of lungs cancer, despite all the developments in the field of genomics. Every genomic based biomarker is not promising and is not specific, reproducible and sensitive to lung cancer (4).

Contrary to this, biomarkers based on proteins are considerably in use for cancer detection. In the blood samples of cancer patients, the abnormal levels of specific proteins are reported. On the basis of which the diagnosis is specifically made. But for precise and confirm diagnosis the study of metabolites is required (16). Normal function of protein biomarkers in lungs and the aberration in their tissue concentrations are discussed in Table 2.

Protein-based biomarkers

Carcino-embryonic antigen is one of the most widely researched biomarker for lung cancer detection. Its MW is 200 kilo Dalton and is acidic glycoprotein of a cell surface, making it a cellular component. It is involved in cell's recognition and adhesion process as Immunoglobulin super families (IgSF). Normally 2.5 to 5 ng mL^{-1} range of CEA occur in healthy individuals but it's elevated level in the body serum, indicates disturbances in the body (23, 29). Usually it's abhorrent levels are indicator of NSCLC. For instance, CEA level is increased in smokers. In the patients of pulmonary adeno-carcinoma, the level of CEA in the serum is related to genetic level mutations in EGFR (Epidermal growth factor receptor). There is limitation for its use that its efficiency is low when used alone, so it is used with CYFRA for the diagnosis purposes (19-21).

Neuron specific enolase is located as sub-cellular neuro-endocrine molecule. It is highly specific and sensitive biomarker for SCLC diagnosis i.e. shows 74% sensitivity for it. In the serum of healthy person, NSE level is 35 ng mL^{-1} and elevation from this shows occurrence of SCLC. Moreover, in the lung cancer some biomarkers are over expressed such as Annexin II and

Table 2. Different serum markers function in normal cell and their abnormal quantities in different types of carcinomas.

Sr. no	Biomarkers	Cancer type	Function in healthy tissue	Concentration in diseased tissue	References
1	Carcino-embryonic antigen	NSCLC	Involved in cell's recognition and adhesion process	Elevated from 2.5 to 5 ng mL^{-1} range	(19-21)
2	Neuron specific enolase	SCLC	Glycolytic isozyme	Raised levels from 35 ng mL^{-1}	(17, 21-23)
3	CYFRA 21-1	NSCLC	Provide mechanical support	Threshold value 3.3ng/ml	(21, 23, 24)
4	Progastrin-releasing peptide (proGRP)	SCLC	Neuropeptide hormone like activity	Higher concentrations as compared to NSCLC	(21, 23, 25)
5	Pentraxin -3	Both	Apoptotic clearance	Elevated levels indicates different stages of tumor	(26, 27)
6	Chromogranin A	Both	promotes the generation of secretary granules	Elevated from 60 pmol/L	(25)
7	Chromogranin B	SCLC	Precursor for various regulatory proteins	Elevated from 150 pmol/L	(25)
8	Creatine kinase BB	SCLC	catalyses the conversion of creatine to phosphocreatine	Elevated levels in serum indicates advance stages of cancer	(28)

ENO1 (17, 21-23).

CYFRA 21-1 are cytokeratins which is epithelial marker. Increased level of cytokeratin-19 fragment is reported in the lungs cancer (20). Due to specificity and sensitivity, it is efficient biomarker for detecting NSCLC and subtypes of squamous cell. Threshold value of CYFRA is 3.3ng/ml and its sensitivity for NSCLC is 0.59 to 0.94 (23% to 70%), for squamous cell and SCLC is 0.68 and 0.19, respectively. In advanced NSCLC, its concentrations are comparatively higher than early stage carcinomas. Its increased levels are reported in benign lungs cancer patients (21, 23, 24).

Progastrin-releasing peptide (proGRP) is protein in nature and have neuropeptide hormone like activity. It is released from gastrointestinal and respiratory tract by neuro-endocrine tissues. It is 47% to 86% sensitive for SCLC and is a potential biomarker for it. Higher level of pro-GRP in serum before treating NSCLC patients conferred many survival advantages. Its level is comparatively higher in SCLC and pure small cell carcinoma patients than limited diseased and mixed carcinoma patients (21, 23, 25).

Kallikrein B1 is a potential biomarker for lung adeno-carcinoma determination. It is present in the serum of lung cancer patients and discovered while analysis of serum glycoprotein. KLKB1 is similar to family of serine protease-trypsin and KLK3 which produces PSA (prostate specific antigen). It has association with fibrinolysis, inflammation, procoagulation and kinin generation. There are four domains of 18 kilo Dalton fragment of Kallikrein B1, which could be used to detect lungs cancer (30, 31).

Pentraxin-3 is another potential biomarker for lungs cancer patients with 37% specificity. Its role in detecting lungs cancer in multi-parametric panel is still under investigation. Though, it is reported equally efficient in both SCLC and NSCLC detection (26). PTX3 concentration is directly related with tumor stage. Over expression of PTX3 has been seen in several malignancies, for example lipo-sarcomas. It has been reported to be effective in apoptotic cells clearance. Cytokines like IL1b and appears to regulate PTX3 expression. Elevated level in case of tissue damage like myocardial infarction as compared to C-reactive protein. It is not clearly understood why the level of PTX3 is elevated in lung cancer patients, but over-expression is due to macrophages and endothelial cells in case of inflammatory signals (26, 27).

In NSCLC patients, higher the level of Chromogranin A in serum was not favourable diagnostic determinant before carrying out the chemotherapy of these patients. Contrary to this, Chromogranin B is a potential biomarker for the manifestation of immune-histochemical and differentiated neuro-endocrine carcinoids (25). In SCLC patients, Creatine Kinase BB is used as potential biomarker as its level is elevated in there serum. There is a direct relationship between CK-BB amount in serum and the advancement of the lung cancer (28).

Kallikrein 11 is a biomarker for NSCLC determination in lung cancer patients. Its elevated level is found in the serum of cancer patients as compared to healthy individuals (32). Kallikrein 14 is also NSCLC diagnostic biomarker. Its increased quantities are directly associated with the risk of NSCLC. Over expression of its mRNA is reported sufficient for positive detection of tumor(32).

Genetic-based biomarkers

Epidermal Growth Factor Receptor gene plays role in cell growth and multiplication. But the mutated gene result in excessive cell proliferation as in cancers. In 10% patients of NSCLC, this gene is found defective and among these 50% patients were not even smokers (33). Therefore, mutation in this gene is a potential biomarker for the detection of NSCLC induced by EGFR mutation. It is a novel biomarker of targeted tyrosine kinase inhibitors detection. Discovery of EGFR biomarker is a mile for the targeted cancer therapy in different pathways (33, 34).

KRAS gene is involved in the growth and proliferation of cells and is linked with the development of tumor. In NSCLC patients, about 25% mutations occur in KRAS gene. This biomarker is usually used in combination with EGFR for better treatments (34).

Anaplastic Lymphoma Kinase is involved in excessive cell multiplication and tumor existence. Gene rearrangement and mutation occur when different genes fuse and result in alteration of gene function (35).

MicroRNAs are used in cancer diagnosis. miRNAs are kept on circulating in blood which are used as biomarkers for detection of many malignancies as well as lung cancer (23, 24). For instance, serum miRNA can determine survival rate of NSCLC patients. Expression of miRNA can be used as a capable approach in lung cancer diagnosis but to some extent. Further workout is required for clinicaluse.12miRNAs (miR-21, 126, 139, 145, 182, 200b, 205, 210, 375, 429, 486-5p and 708) are identified by using micro array. In tumor tissues, expression level of MiRNAs is two fold increased or decreased compared to noncancerous tissues (36, 37).

Biomarkers for various lung infections

There are several markers which have been discovered and are capable of being used to early diagnose the different conditions of respiratory diseases like interstitial lung disease (ILD), lower respiratory tract infections (LRTI) and M. tuberculosis (T.B). Role of different serum markers in normal cell and their correlation with certain diseases are comprised in Table 3.

Clara cell protein (CC16)

The Clara Cell protein (CC16) which is a secretary type of protein, has a molecular weight of 16 kDa. In humans and rodents, bronchiolar Clara cells (non-ciliated) secrete large amounts of this protein into the respiratory tract's lumen (46). CC16 is not specifically and exclusively the entire product of the Clara cells, or even the lungs, shown by immune-histochemical studies (47, 48). Still the exact function of CC16 is not known, but these are proved to be an important anti-inflammatory mediator in the lung and also important as the immune-suppressant. In addition, CC16 has the ability to inhibit the interferon-γ production capability of PBMCs (peripheral blood mononuclear cells) (49).

Table 3. Different protein biomarkers function in healthy body and their concentrations in various diseases.

Sr. no	Biomarker	Diseases	Function in the body	Value in diseased condition	References
1	Clara cell protein (CC16)	Acute and chronic lung infections	Protects respiratory tract from inflammation and oxidative stress	Elevated from 0.39 ±0.19mg l^{-1}.	(38)
2	Calcitonin Peptides	Commonly acquired pneumonia, COPD, Anemia	Calcium homeostasis	Elevated from range 0.1–0.25 $\mu g l^{-1}$.	(39)
3	Copeptin	Lower respiratory tract infections	Released along with vasopressin and co-regulated osmotic homeostasis	Hundred to thousand fold increase from range 3·5–8·3pmol L^{-1}.	(40)
4	KL-6	collagen vascular disease-associated interstitial pneumonitis, pulmonary alveolar proteinosis, hypersensitivity	Involved in cell signaling	Elevated from 127.1 +/- 69.1 U/ml.	(41, 42)
5	Receptor for Advanced Glycation End Products (RAGE)	Asthma	transdifferentiation of type II alveolar epithelial cell	Increased quantities in BALF	(43, 44)
6	Von Willebrand factor	Lung injury	Mediates adhesion of platelets to sub-endothelial surfaces at sites of vascular injury	Higher the VWF levels, higher the injury	(45)

Thus, in interstitial lung diseases, CC16 blood levels have an association with the degree of involvement and therefore they can be used as a valuable biomarker to check the activity and severity of the disease (38).

Calcitonin peptides

CALC gene is the common ancestral gene that encodes CT peptides having pronounced structural homologies. These peptides include PCT, Adrenomedullin, CGRP-I and –II, and Amylin, and their other respective precursor peptides. During inflammation and infection, except amylin, all these CT peptides have found to be variably increased in serum (50, 51).

These are very effective immune-modulating peptides. Adrenomedullin has antibacterial effect. Procalcitonin has very often early onset and increased concentrations in bacterial infections. Its levels are increased several thousand-folds in systemic infections of high severity like sepsis. Thus elevated serum levels of PCT are correlated with the presence, course and consequence of sepsis in humans. PCT, as a marker, is not perfect, but upon its reliability antibiotic (over)use can be reduced enough in respiratory tract infections. Procalcitonin and other hormokines, due to their considerable increased concentrations can be used as therapeutic target for immune-neutralisation for sepsis in humans (39).

Copeptin

An individual's stress response is revealed by vasopressin which has osmoregulatory, and haemodynamic effects as well. Copeptin is also synthesized along with vasopressin; therefore it reflects the vasopressin levels, and being more stable in serum and plasma is used as a biomarker (52). Lower respiratory tract infections (LRTI) lead to sepsis. With increasing severity of LTRI, the levels of copeptin are also increased, so these levels can be used as a novel biomarker for LRTI patients (40).

KL-6, surfactant protein-A, surfactant protein-D, and monocyte chemoattractant protein-1

KL-6 is a circulating glycoprotein of high-molecular-weight, recently classified as MUC1 mucin in humans (53). KL-6 can be a useful marker for differential diagnosis of ILD, evaluation of the disease activity, and prediction of the disease result. It can also be served as a convenient marker of collagen vascular disease-associated interstitial pneumonitis (CVD-IP), idiopathic pulmonary fibrosis (IPF), radiation pneumonitis, pulmonary alveolar proteinosis, and hypersensitivity and drug-induced pneumonitis (41, 42). KL-6 levels are elevated in both sera and BALF (bronchoalveolar lavage fluid) (41).

SP-A, SP-D and MCP-1 are the other biomarkers that can be useful for diagnosis of ILD but these all are inferior to KL-6. Serum levels of KL-6 have the highest sensitivity, specificity and diagnostic accuracy (54).

Receptor for Advanced Glycation End Products (RAGE)

The receptor for advanced glycation end products (RAGE) is a multiligand binding cell surface receptor belonging to the super family of immunoglobulins (55). RAGE is expressed at low levels (undetectable levels) in adult healthy animal tissues (56, 57). In the lung RAGE expression is higher than the other tissues. RAGE signaling has the capability to trigger the most of the components that are supposed to play a part in the pulmonary fibrosis pathogenesis, but it hasn't been determined that RAGE shows profibrotic responses or not(58). RAGE has been suggested a marker for alveolar epithelial cells (type I) (59) and transdifferentiation of type II alveolar epithelial cell, which is a component of

pulmonary re-epithelialization and repair (43, 44).

Von Willebrand factor

Von Willebrand Factor antigen (VWF), a macromolecular antigen, is produced predominantly by endothelial cells and also by platelets but to lesser extent. In the situation of endothelial injury, this antigen is released into the circulation from preformed stores (60, 61). Elevated levels of VWF in plasma of ALI/ARDS patients have independently been associated with more systemic organ failure, and mortality (45). VWF has been considered as a biomarker of endothelial injury for the patients who are at risk, or have established the condition of ALI/ARDS (62, 63).

Plasminogen activator inhibitor-1

Acute lung injury alters the fibrinolytic system of alveoli. There are very low levels of the plasminogen activator inhibitor- 1 (PAI-1), a fibrinolytic protease inhibitor, in bronchoalveolar lavage to report its prognostic importance.

In both pulmonary edema fluid and plasma, the levels of PAI-1 in ALI are higher than in hydrostatic edema. Therefore in patients with ALI, higher mortality and unassisted ventilation (for fewer days) have been predicted from these elevated levels (64).

Markers for tuberculosis

M. tuberculosis have successfully been detected from the patients with genitourinary TB, using PCR based tests (65). Dying human cells and microorganisms release their broken down nucleic acids into the blood. Some of these DNA and RNA fragments are then, passed through the kidneys to be excreted as urine (66, 67). 16S-rRNA amplification from urine samples is favorable and appears to be a valuable and early biomarker of extra-pulmonary TB (EPTB) (68).

In the patients of M. tuberculosis, antigens MT1721, MT2462, MT3444 and MT1694 are produced in abundant quantities which then sensitize the immune system of the patient and cause the production of specific IgG antibodies. Therefore these are validated as biologically important molecules. MoaA-related protein (MT1721) is unique than the other antigens as it has no homologues. Therefore, this molecule can be a useful biomarker to specifically diagnose the tuberculosis because there are very less chances of cross-reactivity of this protein with other molecules. As all these four molecules are found in M. tuberculosis and because these are present in patients' body fluids, therefore these are potential candidates for the development of diagnostic assays and vaccines (69, 70).

Conclusion

Biomarkers hold the tremendous potential in diagnosis of very lethal diseases. Careful analysis of their presence or absence in the body serum can help physicians conjecture the presence, stage or lethalness of disease. Despite all that one cannot solely really on them for diagnosis due to insufficient available data. Moreover, single biomarker can be released in the blood due to the occurrence of one or more illnesses. So, the analysis of biomarkers in combination or along with other tests could be helpful. In the light of this, there is scope for more research to find out the more accurate biomarkers for lungs cancer and respiratory tract infections. Further, there is a need to characterize the activity of already discovered biomarkers, so the physicians can timely diagnose disease or disease stage via the analysis of one biomarker in the future.

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