Systematic Review

Usage patterns of biomarkers in non-small-cell lung cancer patients in India: Findings from a systematic review and survey

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ABSTRACT

Introduction: Personalized medicine has facilitated improved management of non-small cell lung cancer (NSCLC) patients by identifying predictive and prognostic biomarkers for enhanced efficiency of detection and efficacy of treatment. This systematic review and survey assessed the patterns of biomarker usage, molecular testing techniques to diagnose patients with NSCLC in India and testing techniques recommended by cancer societies. **Materials and Methods:** Studies were retrieved from Embase, PubMed, and Cochrane databases for the last 12 years, using relevant search strategies as per the Cochrane methodology for systematic reviews. Outcomes of interest were biomarkers for NSCLC, patterns of biomarker testing, diagnostic methods, guidelines and cost of biomarker testing. **Results:** In all, 499 studies were identified for screening and 17 primary publications were included in the review. Epidermal growth factor receptor (EGFR) expression and epithelial markers (particularly cytokeratins (CK)) were the most commonly reported biomarkers (7/17) and immunohistochemical (IHC) staining was the most common technique for detection of biomarkers. The frequency of EGFR mutations was higher among women than men. Significantly elevated levels of CK-18 were observed in patients with squamous cell carcinoma and of CK-19 in patients with adenocarcinoma, squamous cell carcinoma, and NSCLC ($P < 0.001$). Prognostic or predictive role of cytokines and angiogenic markers as well as DNA expression were evaluated. The survey also showed that IHC was the most common technique for detection of biomarkers. **Conclusions:** This systematic review and survey provides valuable information on biomarker usage in the Indian population, and highlights the need for initiatives required for future biomarker testing in India.

KEY WORDS: Biomarkers, India, non-small-cell lung cancer, prognosis, tumor markers

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INTRODUCTION

There is a growing preference for personalized or targeted therapies over non-selective chemotherapy, especially in metastatic/advanced non-small-cell lung cancer (NSCLC).[1] The current approach in the treatment of NSCLC is development of corresponding biomarkers and their diagnosis to appropriately inform the treatment decisions. Biomarkers are increasingly being used to improve the management of patients with advanced or metastatic NSCLC by enhancing the efficiency of detection and efficacy of treatment.[2] In order to derive appropriate therapy benefits for NSCLC, predictive and/or prognostic biomarkers should be identified.

NSCLC accounts for 75% to 80% of all lung cancer cases.[3] Histologically, NSCLC can be classified into squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. In India, squamous cell carcinoma has been the most common histological type of NSCLC. However, there is a growing predominance of adenocarcinomas in Indian patients.[3] Treatment options for NSCLC include surgery, chemotherapy, and/or radiotherapy depending upon disease stage (early/advanced). Surgery is the mainstay of treatment for patients with early stages (stage I to III A).[2] The treatment strategy for metastatic or advanced staged patients could be a combination of chemotherapy or

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Website: www.lungindia.com

DOI: 10.4103/0970-2113.135767

radiotherapy or chemotherapy alone.[1] But none of the therapies is completely effective to cure the disease. A number of adverse events have been reported in patients receiving non-selective chemotherapy for treating NSCLC.[4]

Molecular analysis of advanced/metastatic NSCLC involves selection of patients, specimen acquisition and testing methods to determine targeted agents for patients with NSCLC. Routine molecular testing of tumor samples represents an important paradigm shift in NSCLC therapy and would allow for individualized therapy in specific subsets of patients.[1] Recently, an echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) fusion translocation was discovered in advanced/metastatic NSCLC.[5] Testing for mutations of the epidermal growth factor receptor (EGFR), EML4-ALK and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), BRAF (v raf murine sarcoma viral oncogene homologue B1) have been in progress to identify inhibitors for these receptors for targeted therapy.[6,7]

This systematic literature review was conducted to identify patterns of biomarker usage and molecular testing techniques to diagnose NSCLC in India; the review also aimed to report molecular testing techniques recommended by cancer societies. In addition, a physician-based quantitative survey was also conducted to identify patterns of biomarker usage and obstacles for biomarker testing in India.

MATERIALS AND METHODS

Systematic literature review

Search strategy

A systematic literature search from the Embase, PubMed, and Cochrane Library electronic databases was carried out for English language studies published from January 2000 to October 2012. Broadly, the following search terms and their combinations were used: "NSCLC," "non-small-cell lung cancer," "biomarkers," "tumor markers," "diagnostic markers," "EGFR," "KRAS," ALK, "BRAF," "vascular endothelial growth factor (VEGF)." References from systematic reviews and meta-analyses were screened for potentially relevant studies.

Study selection

Studies were included if they met the following criteria: (i) Conducted in patients with NSCLC only, (ii) Randomized clinical trials, observational studies, and economic evaluations (iii) data on usage of biomarkers and testing techniques, (iv) studies conducted in India only.

Validity and data extraction

Relevant data from the published literature were extracted by two independent reviewers and any discrepancies were resolved by a third reviewer. The main outcomes of interest were type of biomarkers, testing techniques used, kits used for assessment of biomarkers, patients diagnosed using biomarkers, and costs associated with biomarkers. The data extraction sheet was reviewed to ensure that all data were captured accurately. Where more than one publication was identified describing a single trial, the data were compiled into a single entry to avoid double counting of patients.

Survey

An online quantitative survey was conducted to identify practice patterns of testing of NSCLC in India. This survey was conducted between April 2011 and May 2012. Oncologists, pulmonologists, thoracic surgeons, pathologists, and geneticists with 2 to 35 years of practice were interviewed using multimodal methodology. Outcomes identified in the survey were general perception regarding use of biomarkers, common techniques for detection of biomarkers, payment options for biomarker tests, and obstacles for biomarker testing in India.

RESULTS

Systematic review

Selection of studies

The initial literature search from all databases resulted in 567 potentially relevant citations. Of these, 68 studies were found to be duplicates (due to overlap of databases), resulting in 499 unique citations. These abstracts were reviewed by two independent reviewers and 28 potentially relevant studies were identified for a full-text review. The remaining studies were excluded as they did not meet the inclusion criteria. Out of the 28 studies, 20 were identified as most relevant for data extraction. Three citations were identified as secondary publications, which were linked to primary publications. Finally, a total of 17 full-text citations were included for qualitative evidence synthesis. A trial flow of the review process (as per PRISMA statement) is presented in Figure 1.

Overview of included studies

An overview of included studies as per category of biomarkers is summarized in Table 1. Most of the studies were conducted in recent years, particularly in the years 2009-2011. Assessment of biomarkers was conducted retrospectively (six studies). Patients aged 27 to 80 years were included in the study and their numbers ranged from 25 to 262.[8,9] In general, patients with stage III or IV NSCLC and without other specifications for disease were included. However, newly diagnosed and untreated patients with advanced stage NSCLC were also included (studies by Kumar *et al.*).[10-13] Some of the studies included patients with newly diagnosed lung cancer (NSCLC and small-cell lung cancer (SCLC); five studies).

Outcomes assessed

Biomarkers and testing techniques

The results from the systematic literature review are presented as per the category of biomarkers. Among the biomarkers reported in the included studies, EGFR was

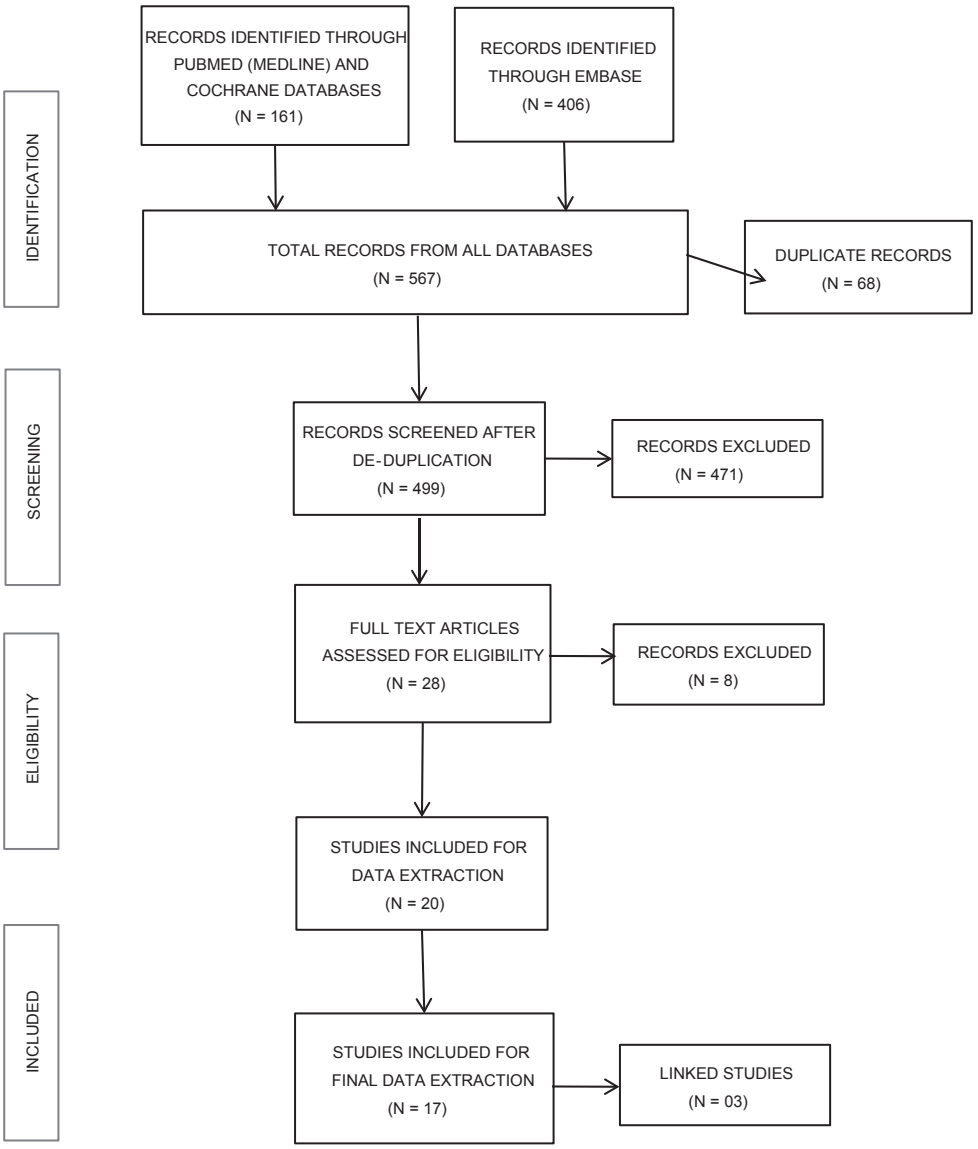

Figure 1: Trial flow as per PRISMA statement

the most common (four studies) followed by epithelial markers (three studies) as shown in Table 1. Epithelial markers included cytokeratins (CKs), carcinoembryonic antigen (CEA), and thyroid transcription factor-1 (TTF-1); CKs were the most frequently expressed. Gene expression was also a useful marker, particularly p63.

For the assessment of biomarkers, specific kits were used such as, EGFR mutation test kit and DxS ARMS-PCR kit for diagnosis of EGFR mutations and Telo TAGGG telomerase PCR kit for detection of telomerase activity using the telomeric repeat amplification protocol (TRAP) method.[14-16] Among testing techniques, immunohistochemical (IHC) staining was identified as the most commonly used technique for the detection of biomarkers. Another technique identified for detection of biomarkers was polymerase chain reaction (PCR). Enzyme-linked immunosorbent assay (ELISA) technique was also used for biomarker detection.

Epithelial growth factor receptor expression and mutations

In a study on 38 NSCLC patients, expression of EGFR in primary and secondary adenocarcinoma was assessed by IHC staining and reported to be 69.6% and 40.0%, respectively; 80% of squamous cell carcinoma expressed EGFR.[17] The frequency of EGFR mutations among women (54%) has been observed to be higher than men (39%).[18] Similar findings were observed in a study by Sahoo *et al.* investigating 220 patients, the EGFR mutation status was 50.9% in women and 49.1% in men, $P = 0.04$.[14]

Expression of epithelial markers

Among epithelial markers, CKs were the most commonly expressed and assessed in two studies.[9,19] CKs belong to a family of keratin containing intermediate filaments that have a role as marker of epithelial differentiation. It comprises of a

Table 1: Overview of included studies and summary of results (as per the category of biomarkers)

Author	Study design	Number of patients included	Name of tumor marker (s)	Biomarker evaluation	Kit (s) used for assay	Name of the company (s) providing kits	Testing techniques	Patients diagnosed using biomarkers, *n*/*N* (%)-overall population	Patients diagnosed using biomarkers, *n*/*N* (%)-histology type/response rate	Others
Epidermal growth factor receptor										
Arcot 2011*[17]	Retrospective study	38 (NSCLC)	EGFR	Diagnostic	NR	NR	IHC staining	NR	Primary ADC: 69.6%; Secondary ADC: 40.0%; SCC: 80.0%	
Dalgliesh 2011*[8]	Unclear	25 (NSCLC)	EGFR	Diagnostic; Screening	Therascreen EGFR Mutation test kit	NR	Therascreen EGFR Mutation test; DNA sequencing	12/25 (48.0)	NR	Out of 12 patients, only 4 detected mutations also evident by DNA sequencing
Pai 2011*[18]	Unclear	46 (NSCLC)	EGFR	Screening	ABI PRISM BigDye Terminator cycle sequencing kit	NR	PCR-sequencing	43% (with deletion in exon 19 (del E746-A750) in 70%)	NR	54% women (*n*=13) had mutations as compared to only 39% of men (*n*=33)
Sahoo 2011[14]	Retrospective study	220 (NSCLC)	EGFR	Screening	DxS ARMS-PCR kit; Qiagen kit RNAse-	Applied Biosystems	Real-time PCR	114/220 (51.8)	ADC: 44.0%	Gender: Female: 50.9%; Male: 49.1% Smoking: Non-smoker: 43.9%; Smoker: 56.1%
Epithelial markers										
Arcot 2011a*[21]	Retrospective study	38 (NSCLC)	TTF-1	Diagnostic	NR	NR	IHC staining	NR	Primary ADC: 23/38 (60.5)	Secondary ADC had not expressed TTF-1 and p63
Mumbarkar 2006[9]	Retrospective study	222 (Lung cancer patients: NSCLC-188; SCLC-34) 40 (Control)	Cytokeratin 19 (Cytokeratins CYFRA 21-1)	Diagnostic	Kit for measuring CYFRA 21-1 (kit not specified)	Boehringer Mannheim immunodiagnostics	ELISA	NR	NR	Significantly elevated levels of TPS (P<0.001) was found in SCC, CYFRA 21-1(P<0.001) was found in ADC, SCC and NSCLC, NSE was elevated significantly (P<0.001) in SCLC whereas CEA levels were elevated in ADC and SCC (P<0.001)
			Cytokeratin-18 (TPS antigen)	Diagnostic	Kit for measuring TPS (kit not specified)	BEKI Diagnostics AB, Sweden	ELISA			
			Carcinoembryonic antigen	Diagnostic	Kit for measuring CEA (kit not specified)	Abbot's Axsym System	MEIA technology			
Kulshrestha 2009[19]	Unclear	29 (NSCLC: 22; SCLC: 7)	TTF-1 Cytokeratin-pan Cytokeratin-7 Cytokeratin-20 CEA Leukocyte common antigen	Diagnostic Diagnostic Diagnostic Diagnostic Diagnostic Diagnostic	NR	Antibodies were procured from Dako, Carpinteria, CA, USA	IHC staining	NR	Tumor positivity: SCC: (*N*=15) TTF-1: 4/15 (26.7) Cytokeratin-pan: 9/15 (60.0) CEA: 7/15 (46.7)	Data reported for patients with NSCLC (*N*=22) only

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Table 1: Contd...

Author	Study design	Number of patients included	Name of tumor marker (s)	Biomarker evaluation	Kit (s) used for assay	Name of the company (s) providing kits	Testing techniques	Patients diagnosed using biomarkers, *n*/*N* (%)-overall population	Patients diagnosed using biomarkers, *n*/*N* (%)-histology type/response rate	Others
									ADC: (*N*=3) TTF-1: 2/3 (66.7) CK-7: 3/3 (100) CEA: 2/3 (66.7) LCC: (*N*=4) CEA: 2/4 (50.0)	
Neuroendocrine markers										
Kulshrestha 2009[19]	Unclear	29 (NSCLC: 22; SCLC: 7)	Chromogranin A (CgA) Synaptophysin	Diagnostic Diagnostic	NR	Antibodies were procured from Dako, Carpinteria, CA, USA	IHC staining	NR	LCC: (*N*=4, based on morphological diagnosis) Cg A: 1/4 (25.0) NSCLC: 0	Synaptophysin was expressed (71.4%) in patients with SCLC only.
Mumbarkar 2006[19]	Retrospective study	222 (Lung cancer patients: NSCLC-188; SCLC-34) 40 (Control)	Neuron specific enolase	Diagnostic	Kit for measuring NSE (name of kit not specified)	Can-Ag Diagnostics	NR	NR	NR	Levels of NSE were elevated significantly (P<0.001) in patients with SCC while lower levels were observed in subtypes of NSCLC, particularly in ADC
Enzymes										
Sundarraj 2010[22]	Retrospective study	76 (NSCLC)	Cytosolic phospholipase A2α (cPLA2α)	Diagnostic	NR	NR	IHC staining	24/76 (32.0)	ADC: 17/36 (47.0); SCC: 6/34 (18.0); LCC: 1/6 (17.0)	Gender: Female: 10/24 (42.0); Male: 14/52 (27.0) Smoking status: Non-smoker: 1/12 (8.0); Smoker: 23/64 (36.0)
Pasrija 2007[15]	Prospective study	42 (NSCLC: 32; SCLC: 10) 30 (Control)	Telomerase activity	Diagnostic	Telo TAGGG telomerase PCR kit	Roche, GmbH, Mannheim, Germany	TRAP method	Biopsy samples: NSCLC: 27/32 (84.4) Sputum samples: NSCLC: 19/32 (59.4)	NR	Telomerase enzyme activity of sputum and biopsy samples of lung cancer patients was found to be statistically significant (P=0.002)
Sen 2001[16]	Unclear	42 (Lung cancer patients) 10 (Control)	Telomerase activity	Diagnostic	TRAP-eze (Pharmingen) Telo-Quant Kit; Telomerase PCR-ELISA kit	Pharmingen; Boehringer Mannheim/Roche Diagnostics	TRAP (Polymerase chain reaction mediated)	29/42 (69.1)	NR	
Gene expression										
Javid 2012*[29]	Unclear	100 (NSCLC) 100 (Control)	p53 expression	Screening	NR	NR	ASO-PCR assay	A 4.256-fold risk (Pro/Pro genotype vs. non-Pro/Pro genotype)	NR	p53 codon 72 polymorphism was found to be associated with NSCLC cancer incidence and progression, but not prognosis

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Arcot 2011a*[21]	Retrospective study	38 (NSCLC)	p63 expression	Diagnostic	NR	NR	IHC staining	NR	SCC: 10/38 (26.3)	Not expressed in secondary ADC
Uke 2010[23]	Retrospective study	100 (NSCLC: 49; SCLC: 51)	p63 expression	Diagnostic	Vectastain ABC kits; Secondary antibody kits	Vector Laboratories, CA, USA	ICH staining	Cytological diagnosis: NSCLC: 20	Cytological diagnosis: SCC: 21 ADC: 7	Histopathological diagnosis identified: SCC: 28; ADC: 7 NSCLC: 20
Sen 2008[30]	Unclear	12 (NSCLC)-(Included patients); 10 (evaluable patients)	Gene expression (Death Inducing protein and geminin expression)	Diagnostic	cDNA subtraction kit; Advantage Klen-Taq Polymerase (for PCR); pGEMT Easy Vector™ (secondary PCR)	BD Biosciences Clontech, Palo Alto, CA, USA; Promega, Madison, WI, USA	Northern blot analysis; Reverse transcription PCR	DIP expression: Increased: 6/10 (60); Decreased: 3/10 (30) Geminin expression: Increased: 5/10 (50); Decreased: 4/10 (40)	NR	
DNA expression										
Kumar 2010[10]	Prospective study	100 (NSCLC) 100 (Control); 42 patients for response assessment	Circulating plasma DNA	Diagnostic; Predictive	QIAamp DNA Blood Mini Kit; PicoGreen dsDNA Kit	Qiagen, Valencia, CA, USA; Molecular Probes, USA	PicoGreen assay	NR	Response: PR: 16/42 (38.1) PD: 14/42 (33.3) SD: 12/42 (28.6)	Patients for response received platinum-based chemotherapy for a minimum of 3 cycles Baseline median plasma DNA levels: 90.3 ng/ml
Kumar 2010a[11]	Prospective study	134 (NSCLC) 100 (Control); 42 patients for response assessment	Plasma nucleosome	Diagnostic; Predictive	Cell Death Detection-ELISAplus	Roche Diagnostics; Mannheim, Germany	ELISA	NR	Response: PR: 16/42 (38.1) PD: 14/42 (33.3) SD: 12/42 (28.6)	Patients for response received platinum-based chemotherapy for a minimum of 3 cycles Baseline median plasma nucleosome levels in NSCLC: 35.2 AU Control: 20.7 AU
Cytokines										
Kumar 2010b[12]	Prospective study	100 (NSCLC) 100 (Control); 42 patients for response assessment	TNF-α	Diagnostic; Prognostic; Predictive	Human TNF-α ELISA Kit	Diaclone, Canton, MA, USA	ELISA	NR	Response: PR: 16/42 (38.1) PD: 14/42 (33.3) SD: 12/42 (28.6)	Patients for response received platinum-based chemotherapy for a minimum of 3 cycles Baseline TNF-α levels in NSCLC: 18.7 pg/ml; TGF-β1 levels in NSCLC: 14.0 ng/ml
			TGF-β1	Diagnostic; Prognostic; Predictive	Human TGF-β1 ELISA kit	RayBiotech Inc., Norcross, GA, USA	ELISA			

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Author	Study design	Number of patients included	Name of tumor marker (s)	Biomarker evaluation	Kit (s) used for assay	Name of the company (s) providing kits	Testing techniques	Patients diagnosed using biomarkers, *n*/*N* (%)-overall population	Patients diagnosed using biomarkers, *n*/*N* (%)-histology type/response rate	Others
Angiogenic markers										
Kumar 209[13]	Prospective study	134 (NSCLC) 100 (Control); 42 patients for response assessment	VEGF	Diagnostic; Prognostic; Predictive	Sandwich enzyme-linked Immunosorbent assay kits	Calbiochem, Darmstadt, Germany	ELISA	NR	Response: PR: 16/42 (38.1) PD: 14/42 (33.3) SD: 12/42 (28.6)	Patients for response received platinum-based chemotherapy for a minimum of 3 cycles Baseline median plasma VEGF levels: NSCLC: 265.7 pg/ml Control: 74.5 pg/ml

*Conference abstract, ADC: Adenocarcinoma, ASO: Allele-specific oligonucleotide, CK: Cytokeratin, DNA: Deoxyribonucleic acid, ELISA: Enzyme-linked immunosorbent assay, EGFR: Epidermal growth factor receptor, ICH: Immunocytochemical, IHC: Immunohistochemical, LCC: Large cell carcinoma, MEIA: Microparticle Enzyme Immunoassay, NSCLC: Non-small cell lung cancer, NR: Not reported, PR: Partial remission, PCR: Polymerase chain reaction, PD: Progressive disease, SCLC: Small-cell lung cancer, SCC: Squamous cell carcinoma, SD: Stable disease, TGF-β1: Transforming growth factor-β1, TNF-α: Tumor necrosis factor-α, TPS: Tissue polypeptide specific, TRAP: Telomeric repeat amplification protocol, TTF-1: Thyroid transcription factor, VEGF: Vascular endothelial growth factor, Screening: Biomarkers used for early detection of a disease in at risk population, Diagnostic: Markers used for identification of a disease (tumor type, stage, grade), Predictive: Biomarkers providing information on the effect of a therapeutic intervention in a patient, Prognostic: Biomarkers providing information for progression of disease in an untreated individual

family of subtypes like CK-5/6, CK-7, CK-14, CK-18, CK-19, etc.[20] In the present review, a study conducted by Mumbarkar *et al*. reported significantly ($P < 0.001$) elevated levels of CK-18 (Tissue polypeptide-specific antigen) in patients with squamous cell carcinoma and of CK-19 (CYFRA 21-1) in patients with adenocarcinoma, squamous cell carcinoma, and NSCLC.[9] Similar results were reported for levels of CEA in this study. In another study, CK-7 was expressed in all patients with adenocarcinoma; however, none of the patients with adenocarcinoma expressed CK-20.[19] In a study by Arcot *et al.*, tumor positivity for TTF-1 was 60.5% in primary adenocarcinoma, while TTF-1 was not expressed in secondary adenocarcinoma.[21]

Expression of gene, enzymes, and neuroendocrine markers

Expression of neuroendocrine marker chromogranin A (CgA) was reported only in patients with large cell carcinoma (25.0%).[19] None of the patients with NSCLC showed positivity for synaptophysin in this study. However, 71.4% SCLC were positive for synaptophysin. The role of cytosolic phospholipase A2α (cPLA2α) enzyme was assessed in the detection of NSCLC.[22] Overall, 32.0% patients expressed cPLA2α, while higher incidence of cPLA2α positivite tumor cells was reported in adenocarcinoma (47.0%) compared to other subtypes. An increased expression of cPLA2α was observed in female (42.0%) as compared to male (27.0%) patients but the difference was not statistically significant ($P = 0.20$). Similar trend was shown for smokers versus non-smokers [Table 1]. One study assessing gene expression found p63 positivity (26.3%) only in squamous cell carcinoma samples.[21] In another study, 21 cases of cytologically diagnosed squamous cell carcinoma showed positivity for p63, followed by 7 cases of adenocarcinoma.[23]

Expression of plasma DNA

In a study by Kumar *et al*., survival analysis according to three tertiles of plasma DNA distribution did not show a correlation between pre-treatment circulating plasma DNA levels and survival. However, circulating plasma DNA levels were comparable in responders (84.8 ng/mL) and non-responders (94.5 ng/mL) to chemotherapy.[10] Findings from another study also suggested that monitoring of plasma nucleosome levels during the course of first-line chemotherapy would help to identify patients who are likely to have insufficient response to therapy and disease progression at an early stage.[11]

Expression of cytokines and angiogenic markers

A study assessed the utility of plasma tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) as predictors of response and survival in advanced NSCLC.[12] In this study, TNF-α and TGF-β1 levels did not correlate with survival as well as response to therapy. However, an elevated plasma level of TNF-α (cut-off 12.9 pg/mL) and TGF-β1 (cut-off 10.45 ng/mL) was associated with a higher risk of NSCLC.[12] In another study by Kumar *et al.* circulating plasma VEGF levels were well correlated with the response to therapy with lower levels being observed in

Table 2: List of institutes or hospitals from included studies and funding source

Study name	Department and name of hospital/institute involved in the study	Type of research center	Funding source
Javid 2012*[29]	Department of Biochemistry, Maulana Azad Medical College, New Delhi; Department of Radiotherapy and Oncology, and Department of Medical Oncology, AIIMS, New Delhi; Department of Radiation Oncology, Sher-I-Kashmir Institute of Medical Sciences, Srinagar	Tertiary hospital	NR
Pai 2011*[18]	Christian Medical College, Vellore, Tamil Nadu	Tertiary hospital	NR
Arcot 2011*[17]	Pathology Department, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai	Research center	NR
Arcot 2011a*[21]	Pathology Department, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai	Research center	NR
Dalgliesh 2011*[8]	Triesta Sciences (I) Private Limited, Bangalore	Research center	NR
Sahoo 2011[14]	Triesta Sciences (I) Private Limited, Bangalore	Research center	Sponsored internally by HCG foundation, Bangalore
Kumar 2010[10]	Department of Medicine, AIIMS, New Delhi; Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida, Uttar Pradesh; Dr. B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi	Tertiary hospital	Funded by the Indian Council of Medical Research, New Delhi
Kumar 2010a[11]	Department of Medicine, AIIMS, New Delhi; Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida, Uttar Pradesh; Dr. B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi	Tertiary hospital	Funded by the Indian Council of Medical Research, New Delhi
Kumar 2010b[12]	Department of Medicine, AIIMS, New Delhi; Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida, Uttar Pradesh; Dr. B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi	Tertiary hospital	NR
Uke 2010[23]	Division of Cytology and Department of Surgical Pathology, Tata Memorial Hospital, Mumbai	Tertiary hospital	NR
Sundarraj 2010[22]	Department of Zoology, Proteomics and Molecular Cell Biology lab, School of Life sciences, Bharathiar University, Tamil Nadu	Research center	Part of the work was funded by UGC and DST-FIST, Government of India
Kumar 2009[13]	Department of Medicine, AIIMS, New Delhi; Division of Molecular Oncology, Institute of Cytology and Preventive Oncology, Noida, Uttar Pradesh; Dr. B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi	Tertiary hospital	Funded by the Indian Council of Medical Research, New Delhi
Kulshrestha 2009[19]	Departments of Pathology and Respiratory Medicine, Vallabhbhai Patel Chest Institute, university of Delhi, Delhi	Research center	NR
Sen 2008[30]	Departments of Biochemistry, Pathology, and Surgery, AIIMS, New Delhi	Tertiary hospital	Supported by Department of Biotechnology, New Delhi
Pasrija 2007[15]	Departments of Pulmonary Medicine, Cytopathology and Cancer Biology Laboratory, and Experimental Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh	Research center	NR
Mumbarkar 2006[9]	Department of Biochemistry, Tata Memorial Hospital, Mumbai	Tertiary hospital	NR
Sen 2001[16]	Departments of Biochemistry, Pathology, and Medicine, AIIMS, New Delhi	Tertiary hospital	NR

*Conference abstract, AIIMS: All India Institute of Medical Sciences, NR: Not reported

patients responding to chemotherapy compared to patients with no change or progression.[13]

Type of research center and funding source

The setting for most of the studies was either tertiary hospitals or research institutes. Of the 17 studies, 10 were conducted in tertiary hospitals, namely All India Institute of Medical Sciences (AIIMS), New Delhi; Tata Memorial Hospital, Mumbai among others [Table 2]. Some of the studies collected data from the medical records of pathology department.[17,21,23] Studies conducted by Kumar *et al*. (prospective design) collected data from the outpatient Department of Medicine of AIIMS, New Delhi.[10-13] While many studies included in this review were funded by the academic institutions where they were conducted, some were sponsored by other government or non-government funding agencies in support of such research endeavors.

Recommendations on usage of biomarkers in NSCLC

Specific and systematic guidelines have been formulated worldwide to serve as recommendations for evidence based and appropriate management of lung cancer patients. The National Comprehensive Cancer Network® (NCCN®) promotes the importance of continuous quality improvement in patients with cancer.[24] This guideline has been updated recently (2012) to include the use of molecular markers in order to individualize therapy for patients. Several biomarkers have emerged as prognostic and predictive markers for NSCLC. These include EGFR, 5' endonuclease of the nucleotide excision repair complex (ERCC1), KRAS oncogene and the new predictive biomarker, ALK fusion oncogene. The guidelines recommend testing for EGFR mutations and ALK gene rearrangements in select NSCLC patients to predict the treatment response. For detection of biomarkers like TTF-1 and p63 expression, IHC staining has been identified as the technique to differentiate primary pulmonary adenocarcinoma from squamous cell carcinoma, and large cell carcinoma. At this point, we do not have a national consensus or guideline recommendations that are specific for Indian patients.

Survey

Outcomes assessed

Patterns of biomarkers and testing techniques

In total, 75 respondents provided information regarding

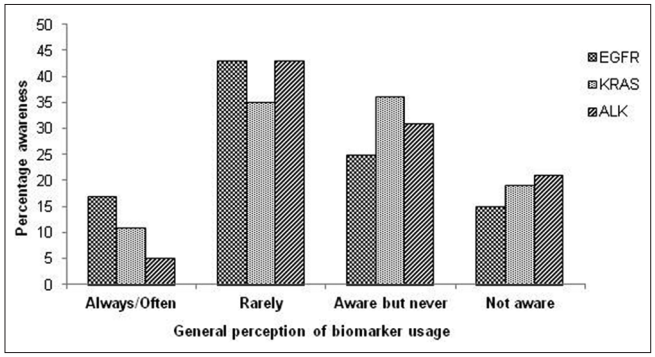

Figure 2: General perception for the usage of biomarkers in India

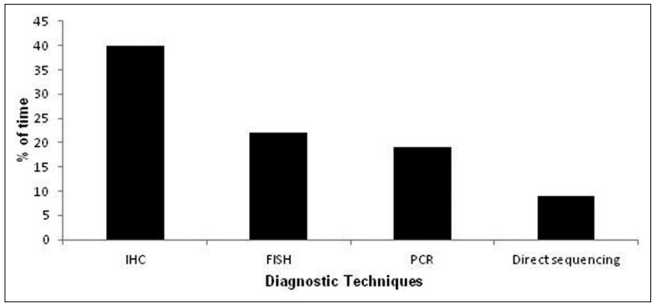

Figure 3: Testing techniques for detection of biomarkers. FISH: fluorescence *in situ* hybdridization; IHC: Immunohistochemical; PCR: Polymerase chain reaction

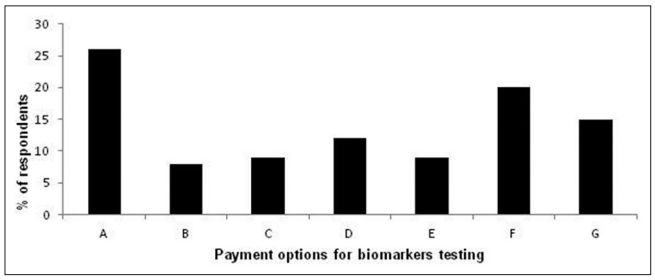

Figure 4: Payment options for testing biomarkers in India. A: Patient out-of-pocket; B: Reimbursed by public health care system; C: It comes out of prescribing oncologist budget; D: Mostly used for research and paid by organization performing the research; E: Supported by pharmaceutical manufacturer; F: Reimbursed by patient' private insurance; G: The lab performing the test pays for it.

general perception on the usage of biomarkers in India. The survey results identified highest responders for the usage of biomarkers as 'rarely/sometime', followed by 'aware but never use' for EGFR, KRAS and ALK biomarkers [Figure 2]. For the testing techniques ($N = 50$), IHC was used as the commonly used technique as per the survey followed by fluorescence *in situ* hybridization (FISH) [Figure 3]. This was irrespective of the biomarker being tested.

Payment options for biomarker testing

Costs for biomarker testing were commonly borne by the patients on their own as shown in Figure 4. This was followed by others who were reimbursed by insurance companies.

DISCUSSION

There is a paucity of data on usage of biomarkers for diagnosis of NSCLC patients in India. To bridge this gap, recent studies have been focusing on the development of reliable diagnostic markers for new therapeutic targets.

This review identified various categories of biomarkers, EGFR being the most commonly expressed followed by epithelial markers. The most commonly used techniques for detection of these biomarkers was IHC staining. For EGFR, IHC staining was used for protein expression, while PCR for detection of mutations. ELISA techniques were used in studies by Kumar *et al*. TRAP method was used in two studies for detection of telomerase activity.[10-13,15,16]

Current therapies targeting EGFR are being extensively used for the treatment of NSCLC. Arcot *et al.* used IHC staining technique to detect EGFR expression.[17] In patients with squamous cell carcinoma, the EGFR expression was found to be quite high (80%); however, the results of this study have not yet been published as a full text article and are not corroborated by other publications from India. Sahoo *et al.* identified EGFR mutation types in stage III or IV NSCLC patients; the authors concluded that screening for EGFR mutations may be useful in deciding response to tyrosine kinase inhibitors (TKI) therapy.[14]

The histological subtype of lung carcinoma is significant for current therapeutic strategies. In the present review, expression of p63 was a useful marker for distinguishing histology of NSCLC into adenocarcinoma and squamous cell carcinomas.[21,23] The frequency of TTF-1 expression was shown in primary adenocarcinoma.[21] The same study concluded that the role of combination of TTF-1 and p63 expression was a useful tool for diagnosis of poorly differentiated NSCLC; expression of TTF-1 only in primary adenocarcinomas and of p63 in squamous cell carcinoma.

Epithelial markers were identified as an important tumor marker. Of the epithelial markers, CK-7 was highly expressed in lung adenocarcinomas. These markers also had a role in differentiation of lung cancer into NSCLC and SCLC as well as to further identify subtypes of NSCLC. In a study by Kulshrestha *et al.*, CK-pan positivity seen in squamous cell carcinoma could be related to cellular differentiation.[19] Further, higher expression of TTF-1 in SCLC patients as compared to NSCLC patients suggested association with the multilineage gene expression of stem cells commonly in SCLC patients.[19]

Studies conducted by Kumar *et al.* evaluated the role of cytokines and angiogenic markers as well DNA expression.[10-13] These studies included patients with advanced NSCLC. Of the total included patients in each study, 42 patients received platinum based chemotherapy for a minimum of three cycles. Levels of different markers

were assessed before each cycle of chemotherapy. The findings from the study by Kumar *et al.* concluded the role of monitoring plasma nucleosome levels to predict response to chemotherapy in patients with remission. Higher levels were observed in patients with no change or progression.[11] However, study by Kumar *et al.* assessing role of TNF-α and TGF-β showed that these did not appear as reliable markers for predicting survival and response to chemotherapy in patients with advanced NSCLC.[12] The prognostic impact of angiogenic factors (VEGF) particularly depend on tumor size.[25] The study by Kumar *et al*. found that VEGF levels were significantly higher in patients with tumor size >3 cm (339.9 pg/ml) as compared to patients with tumor size <3 cm (172.4 pg/ml), $P < 0.001$.

In this review, the identified biomarkers have a role in differentiation of NSCLC into subtypes. Neuroendocrine biomarkers, synaptophysin and CgA have a role in differential diagnosis of lung cancer as well as NSCLC into subtypes.[19] Similarly, findings from another study suggest the role of cPLA2α enzyme in the detection of NSCLC, particularly differentiation into subtypes.[22] Expression of gene p63 may be used for differential diagnosis of lung cancer and for identification of squamous cell carcinoma.[21]

Guidelines specific to diagnosis or treatment in NSCLC are followed in many countries. These guidelines are updated regularly. As per the latest NCCN® guidelines (NCCN Guidelines, version 2.2013), ALK and KRAS are the emerging prognostic biomarkers, in addition to EGFR and ERCC1.[24] The guidelines recommend testing for EGFR mutations and ALK gene rearrangements in NSCLC patients. According to the guidelines, mutational screening assays (e.g. Sequenom/s MassARRAY system, SNaPshot Multiplex System) have been developed for detecting multiple biomarkers that can detect more than 50 point mutations, including EGFR. However, these systems do not detect gene rearrangements because they are not point mutations. The US FDA has approved FISH for the detection of ALK gene rearrangements. The guideline mentions that IHC staining may be used to screen ALK rearrangements but ALK positivity is confirmed using FISH. Guideline from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), and Association for Molecular Pathology (AMP) also recommend molecular testing of EGFR and ALK in lung cancer patients.[26] This guideline suggests that both EGFR and ALK molecular testing should be used to select patients for EGFR- or ALK-targeted TKI therapy and patients with adenocarcinoma should not be excluded from the testing. This guideline further suggests that formalin-fixed, paraffin-embedded or fresh, frozen or alcohol-fixed specimens should be used for PCR-based EGFR mutation testing. However, IHC for total EGFR is not recommended for selection of EGFR TKI therapy. The ALK FISH assay should be used for ALK mutation testing.

In addition, guidelines by other cancer societies such as, the European Society for Medical Oncology (ESMO) for NSCLC pathology and molecular testing and the Lung Cancer Working Group are adhered to in different countries.[27] The later is followed in Asian countries, except Hong Kong, India, Malaysia, Taiwan, and Singapore.[28] There is a need for systematic guidelines to be followed in India. These guidelines provide effective and individualized treatment for patients.

The online survey conducted among physicians provided insight on the awareness of biomarker usage in India and techniques used to detect these markers. IHC staining was identified as the most common technique for detection of biomarkers followed by FISH. This finding was also corroborated in the systematic literature review. Out-of-pocket was the most common payment option for testing biomarkers among the patients in India. This finding is not surprising considering that a large proportion of healthcare spending in India is out of pocket or reimbursed by insurance companies. Since the survey and the review were independent of each other, there were not many synergies in the findings. However, both findings point toward awareness of biomarker testing and their role in disease prognosis.

Our review has several strengths. To the best of our knowledge, this is the most recent systematic review on this important topic particularly in the Indian context. Our review identified a number of studies across different tumor markers. Our findings provide an insight on the direction and focus of research endeavors in our country, while in this area highlighting the unstated need for greater collaboration among academic institutes, government agencies, and industry to do meaningful research on a larger scale in Indian patients. The present review has included a broad range of evidence across different studies but the heterogeneity of data on testing techniques and markers, differing study designs, and inclusion and exclusion criteria has limited the comparability and conclusiveness from such information as might be expected. Another possible limitation of this review could be the quality of studies included. Majority of studies were retrospective in nature, and such studies are prone to bias. However, as with any review of literature, a balance has to be found between having too stringent search criteria and too loose a search strategy to fulfill the question of interest and we have attempted to sketch a baseline understanding of the situation in India for this pertinent area though this systematic review.

In conclusion, this review provides valuable information on biomarker usage in the Indian population. Such information may be useful to inform policy makers and health professionals about the utility of biomarkers in NSCLC. The survey identified the usage of biomarkers and need for initiatives required for future biomarker testing in India.

Further studies are necessary to explore the usage patterns of biomarkers in India, which may provide

valuable information for policy makers to improve disease management in India. A consensus statement or practicing guideline for management of NSCLC in India should be the consequence of such an endeavor.

ACKNOWLEDGEMENT

We would like to thank Hemlata Shukla, Capita India for providing editorial support to the manuscript.

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How to cite this article: Desai C, Mehta A, Mishra D. Usage patterns of biomarkers in non-small-cell lung cancer patients in India: Findings from a systematic review and survey. Lung India 2014;31:249-59.

Source of Support: This study was supported by Pfizer India Private Limited, Mumbai, India, The corresponding author was employed with Pfizer at the time of conduct and analysis of this study. **Conflict of Interest:** None declared.