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). 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. 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. 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. 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). The treatment strategy for metastatic or advanced staged patients could be a combination of chemotherapy or...
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.[9] 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...
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. 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. The frequency of EGFR mutations among women (54%) has been observed to be higher than men (39%). 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, $P = 0.04$.

**Expression of epithelial markers**

Among epithelial markers, CKs were the most commonly expressed and assessed in two studies. CKs belong to a family of keratin containing intermediate filaments that have a role as marker of epithelial differentiation. It comprises of a
| 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 (%) | Patients diagnosed using biomarkers, n/N (%) - histology type/response rate | Others                                                                 |
|-------------------------|------------------------|-----------------------------|-------------------------|----------------------|-----------------------|----------------------------------------|-------------------|--------------------------------------------|-------------------------------------------------------------------------|------------------------------------------------------------------------|
| Desai, et al.           | Retrospective study    | 38 (NSCLC)                  | EGFR                    | Diagnostic           | NR                    | NR                                     | IHC staining      | Primary ADC: 69.6%; Secondary ADC: 40.0%; SCC: 80.0% |                                                                              |                                                                        |
| Arcot 2011*             | Unclear                | 25 (NSCLC)                  | EGFR                    | Diagnostic           | 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*               | 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%) |                                                                              | 54% women (n=13) had mutations as compared to only 39% of men (n=33)        |                                                                        |
| Sahoo 2011              | Retrospective study    | 220 (NSCLC)                 | EGFR                    | Screening            | Dr.SARMS-PCR kit; Qiagen kit RNAse- | Applied Biosystems | Real-time PCR                  | 114/220 (51.8) | ADC: 44.0%                                  |                                                                              |                                                                        |
| Epithelial markers      |                        |                             |                         |                      |                        |                                        |                   | Gender: Female: 50.9%; Male: 49.1%; Smoking: Non-smoker: 43.9%; Smoker: 56.1% |                                                                              |                                                                        |
| Arcot 2011a*            | Retrospective study    | 38 (NSCLC)                  | TTF-1                   | Diagnostic           | NR                    | NR                                     | IHC staining      | Primary ADC: 23/38 (60.5) | Secondary ADC had not expressed TTF-1 and p63 | 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) | Data reported for patients with NSCLC (N=22) only                      |
| Mumbarkar 2006*         | Retrospective study    | 222 (Lung cancer patients: NSCLC-188; SCLC-34) | Cytokeratin 19 (Cytokeratins CYFRA 21-1 Cytokeratin-18 (TPS antigen) | Diagnostic           | Kit for measuring CYFRA 21-1 (kit not specified) | Boehringer Mannheim immunodiagnostics BIKI Diagnostics AB, Sweden | ELISA             | NR                                        |                                                                              |                                                                        |
| Kulshrestha 2009*       | Unclear                | 29 (NSCLC: 22; SCLC: 7)     | TTF-1 Cytokeratin-pan Cytokeratin-7 Cytokeratin-20 CEA Leukocyte common antigen | Diagnostic           | Diagnostic | Antibodies were procured from Dako, Carpentieria, CA, USA | IHC staining | SCC: (N=15) TTF-1: 4/15 (26.7) Cytokeratin-pan: 9/15 (60.0) CEA: 7/15 (46.7) |                                                                              |                                                                        |
| 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 (%)—histology type/response rate | Others |
|--------|--------------|-----------------------------|-------------------------|----------------------|----------------------|--------------------------------------|-------------------|---------------------------------------------------------------------------------|--------|
| Neuroendocrine markers Kulshrestha 2009<sup>[19]</sup> | Unclear | 29 (NSCLC: 22; SCLC: 7) | Chromogranin A (CgA) | Diagnostic | NR | Antibodies were procured from Dako, Carpinteria, CA, USA | IHC staining | NR | LCC: (N=4, based on morphological diagnosis) CgA: 1/4 (25.0) NSCLC: 0 | Synaptophysin was expressed (71.4%) in patients with SCLC only. |
| Mumbarkar 2006<sup>[19]</sup> | 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 | | 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<sup>[22]</sup> | Retrospective study | 76 (NSCLC) | Cytosolic phospholipase A2α (cPLA2α) | Diagnostic | 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) Telomerase enzyme activity of sputum and biopsy samples of lung cancer patients was found to be statistically significant (P<0.002) |
| Pastrija 2007<sup>[15]</sup> | 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) 29/42 (69.1) | NR |
| Sen 2001<sup>[16]</sup> | Unclear | 42 (Lung cancer patients) 10 (Control) | Telomerase activity | Diagnostic | TRAP-eze (Pharminen) Telo-Quant Kit; Telomerase PCR-ELISA kit | Pharminen; Boehringer Mannheim/Roche Diagnostics | TRAP (Polymerase chain reaction mediated) | NR | |
| Gene expression Javid 2012*<sup>[29]</sup> | Unclear | 100 (NSCLC) 100 (Control) | p53 expression | Screening | 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 |
| 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 (%) | Patients diagnosed using biomarkers, n/N (%) - histology type/response rate | Others |
|-----------------|--------------|-----------------------------|--------------------------|----------------------|------------------------|----------------------------------------|-------------------|---------------------------------------------|---------------------------------------------|--------|
| Arcot 2011a*    | Retrospective study | 38 (NSCLC) | p63 expression | Diagnostic | NR | NR | IHC staining | SCC: 10/38 (26.3) | Not expressed in secondary ADC |
| Uke 2010†      | Retrospective study | 100 (NSCLC: 49; SCLC: 51) | p63 expression | Diagnostic | Vectastain ABC kits; Secondary antibody kits | Vector Laboratories, CA, USA | IHC staining | Cytological diagnosis: SCC: 21 ADC: 7 | Histopathological diagnosis identified: SCC: 28; ADC: 7 NSCLC: 20 |
| Sen 2008(*)    | 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); pGEM Easy Vector™ (secondary PCR) | BD Biosciences Clontech, Palo Alto, CA, USA; Promega, Madison, WI, USA | IHC staining | Northern blot analysis; Reverse transcription PCR | | DNA expression |
| Kumar 2010(*)  | Prospective study | 100 (NSCLC)‑Included patients; 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 | 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 Patients for response received platinum-based therapy for a minimum of 3 cycles Baseline median plasma nuclear DNA levels in NSCLC: 35.2 AU Control: 20.7 AU |
| Kumar 2010a(*) | Prospective study | 134 (NSCLC)‑Included patients; 42 patients for response assessment | Plasma nucleosome | Diagnostic; Predictive | Cell Death Detection-ELISAplus | Roche Diagnostics; Mannheim, Germany | ELISA | Response: PR: 16/42 (38.1) PD: 14/42 (33.3) SD: 12/42 (28.6) | | Cytokines |
| Kumar 2010b(*) | Prospective study | 100 (NSCLC)‑Included patients; 42 patients for response assessment | TNF-α | Diagnostic; Prognostic; Predictive | Human TNF-α ELISA Kit | Diacalone, Canton, MA, USA | ELISA | 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 |
Table 1: Contd...

| Author | Study design | Number of patients included | Name of tumor marker(s) | Kit(s) used for assessment | Testing techniques | Patients diagnosed using biomarkers, n/N (%) | Others |
|--------|--------------|-----------------------------|-------------------------|---------------------------|-------------------|-------------------------------------------|--------|
| 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 | 16/42 (38.1) | 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, METIA: 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.

Expression of c-kit and angiopeptin markers

A study assessed the utility of plasma tumor necrosis factors (TNFα) and transforming growth factor-β1 (TGFβ1) as predictors of response and survival in advanced NSCLC.[14] In this study, TNFα and TGFβ1 plasma levels were comparable in responders (84.9 pg/mL) and non-responders (94.5 pg/mL), suggesting that monitoring of plasma nucleosome levels during the course of first-line chemotherapy could help in identifying patients who are likely to have insufficient response to therapy and disease progression at an early stage. However, an elevated plasma TNFα (cut-off 10.45 pg/mL) was observed in all patients with adenocarcinoma, compared to 7.4% in other cancer subtypes.[15] A trend was observed for smokers versus non-smokers (Table 1), with a higher incidence of cPLA2α expression in adenocarcinoma (47.0%) compared to other subtypes (20.0%).[16] An increased expression of cPLA2α was observed in patients with advanced adenocarcinoma (47.0%) compared to early stage disease.[17] In another study, 21 cases of cyclophosphamide-induced squamous cell carcinoma were positive for cPLA2α (95.2%).[18] Overall, 32.0% patients expressed cPLA2α enzyme in the detection of NSCLC.[19]

Expression of new enzymes and neuroendocrine markers

Expression of neuroendocrine marker chromogranin A (CGA) was reported only in patients with large cell carcinoma (25.0%).[20] None of the patients with NSCLC showed positivity (26.3%) only in squamous cell carcinoma.[21] Expression of prostatic acid phosphatase (PAP) was observed in adenocarcinoma (21.0%), while higher levels were seen in squamous cell carcinoma (32.6%).[22] In a study by Arcot et al.,[23] no patients with adenocarcinoma expressed c-erbB-2, while 71.4% SCLC were positive for synaptophysin. However, 74.5% SCLC were positive for chromogranin A.[24] In another study, CK20 was expressed in 94.5% of patients with adenocarcinoma, while none of the patients with squamous cell carcinoma or NSCLC showed positivity for PAP.[25] In a study by Arcot et al.,[26] patients with adenocarcinoma expressed c-erbB-2, while 60.5% of patients with squamous cell carcinoma showed positivity (26.3%) only in squamous cell carcinoma.[27] In another study, 21 cases of cyclophosphamide-induced squamous cell carcinoma were positive for cPLA2α (95.2%).[28] Overall, 32.0% patients expressed cPLA2α enzyme in the detection of NSCLC.[29]
patients responding to chemotherapy compared to patients with no change or progression.\cite{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.\cite{17,21,23} Studies conducted by Kumar et al. (prospective design) collected data from the outpatient Department of Medicine of AIIMS, New Delhi.\cite{10,12} 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.\cite{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, S' 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

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**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*\cite{25} | 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*\cite{13}  | Christian Medical College, Vellore, Tamil Nadu                                                                                    | Tertiary hospital       | NR                                                                             |
| Arcot 2011*\cite{17} | Pathology Department, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai                | Research center         | NR                                                                             |
| Arcot 2011a*\cite{23} | Pathology Department, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Chennai              | Research center         | NR                                                                             |
| Daligleish 2011*\cite{29} | Triesta Sciences (I) Private Limited, Bangalore                                                                               | Research center         | NR                                                                             |
| Sahoo 2011*\cite{14} | Triesta Sciences (I) Private Limited, Bangalore                                                                               | Sponsored internally by HCG foundation, Bangalore | Funded by the Indian Council of Medical Research, New Delhi |
| Kumar 2010*\cite{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*\cite{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*\cite{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       | Funded by the Indian Council of Medical Research, New Delhi |
| Uke 2010*\cite{23}  | Division of Cytology and Department of Surgical Pathology, Tata Memorial Hospital, Mumbai                                            | Tertiary hospital       | NR                                                                             |
| Sundarraj 2010*\cite{23} | 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*\cite{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*\cite{11} | Departments of Pathology and Respiratory Medicine, Vallabhbach Patel Chest Institute, university of Delhi, Delhi | Research center         | NR                                                                             |
| Sen 2008*\cite{10}  | Departments of Biochemistry, Pathology, and Surgery, AIIMS, New Delhi                                                            | Tertiary hospital       | Supported by Department of Biotechnology, New Delhi |
| Pasrija 2007*\cite{11} | 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*\cite{29} | Department of Biochemistry, Tata Memorial Hospital, Mumbai                                                                      | Tertiary hospital       | NR                                                                             |
| Sen 2001*\cite{10}  | Departments of Biochemistry, Pathology, and Medicine, AIIMS, New Delhi                                                          | Tertiary hospital       | NR                                                                             |

*Conference abstract, AIIMS: All India Institute of Medical Sciences, NR: Not reported
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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. 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. The prognostic impact of angiogenic factors (VEGF) particularly depend on tumor size. 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. Similarly, findings from another study suggest the role of cPLA2α enzyme in the detection of NSCLC, particularly differentiation into subtypes. Expression of gene p63 may be used for differential diagnosis of lung cancer and for identification of squamous cell carcinoma.

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. 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. 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. The later is followed in Asian countries, except Hong Kong, India, Malaysia, Taiwan, and Singapore. 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.

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