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Method

Lung cancer: analysis of biomarkers and methods of diagnostic and prognostic value

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Abstract: This paper summarizes the overwhelming evidence that targeted therapy is better than chemotherapy for the treatment of lung cancer. The focus is on lung cancer because of the sheer magnitude of this disease especially in males. In India, lung cancer constitutes 6.9 per cent of all new cancer cases and 9.3 per cent of all cancer related deaths in both sexes (1). Currently the world we reside has a norm of developing cure to an abnormal state of living body (so called disease) based on evidences, recognized during the diagnosis of the disease. It is done to ensure optimized therapy of treatment and maximize the outcome. Personalized medicine is also an uprising norm. In this kind, all the variables such as host, environment, patient, etc. are considered for an individual case and the course of treatment is followed based on the standard options available. Its main objective is the best interest of the patient. Through a survey conducted in the northern belt of the country we tried to determine the biomarkers currently being used and the scope of targeted therapy. Thus, in its most basic form medicine is not only a science but is also an art.

Key words: Biomarkers; Bhemotherapy; Targeted-therapy; Oncology; Prognosis; Non-small cell lung cancer (NSCLC).

Introduction

Lung cancer is one of the deadliest and most commonly diagnosed neoplasms. Early diagnosis of this disease is critical for improving clinical outcome and prognosis. The best chance for successful treatment is offered by surgical resection in the early stages. Because the early stages of lung cancer often produce no symptoms, it is necessary to identify biomarkers for early detection, prognostic evaluation, and recurrence monitoring of the cancer. Current methods for molecular pathology diagnosis, including immunohistochemical analysis (IHC), Fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR), are focused primarily on monitoring changes in macromolecules (i.e., mRNAs and proteins).

A disease is detected with the help of a promising biomarker. A Biomarker is a measurable indicator of a specific biological state, visually one relevant to risk presence, severity and prognosis of diseased condition. Lung cancer in India has the highest incidences among men. Its prevalence accounts to lifestyle habits adaptability like smoking and tobacco consumption (1, 2).

Prognosis is the survival rate or the chances of survival of the patient with the existing conditions and stage. It is predicted statistically based on the survey and studies conducted experimentally. Survival rate tells us about the proportion of people who are alive by the end of certain period of time. For example, Survival rate of 5 years signify number of people who are alive 5 years after being diagnosed. These people may live much longer than 5 years. Duration of 5 years can only be taken as a standard estimate for survival rate. The prognosis period follows different percentages of survival rate with different stages of Non-small cell lung cancer (NSCLC). Different stages of NSCLC are-Stage I (stage IA and IB); Stage II (IIA and IIB); Stage III (IIIA and IIIB); Stage IV.

According to American Cancer Society, survival rate for different stages are

- 5 year survival rate for IA is 49% and for stage IB is 45%. This means that 49 out of 100 people are alive by the end of 5 years who were detected by NSCLC, stage IA.
- For stage IIA of NSCLC, 5-year survival rate is 30% and for stage IIB is 31%.
- For stage IIIA of NSCLC, 5-year survival rate is 14% and for stage IIIB is 5%.
- For stage IV of NSCLC, 5-year survival rate is 1%.

In this project, a survey was conducted among the regional researchers, oncologists and surgeons about some commonly encountered questions of Non-small cell lung cancer. Non-small cell lung cancer (NSCLC) was chosen for study because 85% of the cases of the lung cancer are NSCLC, mean survival time is only 17% and the relevant data was collected from interviews and research paper citations. A comparative study between the most relevant biomarkers used for diagnosis of lung cancer was done. Based on this information, we compared the course of two kinds of treatment that is presently followed, i.e. chemotherapy and targeted-therapy.

Materials and Methods

Survey

The studies were retrieved from a quantitative online survey conducted among total 52 oncologists, consultants and surgeons of Kamla Nehru Memorial Hospital (KNMH) Allahabad, All India Institute of Medical Sciences (AIIMS) Delhi, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS) Lucknow and other regional independently operating clinics, hospitals and Motilal Nehru National Institute of Technology (MNNIT). The survey was conducted from January 22, 2016 to February 1, 2016. The survey was about general perception of the practitioners regarding the features of Non-small cell Lung cancer (NSCLC) patients they face most popularly.

Study

A personal interview was conducted with oncologists and oncology surgeons of KNMH, Allahabad. The interview was focussed on the detection techniques and procedure of the NSCLC, biomarkers used for detection, drugs popularly in use, their dosage, their commercial name and the combinations used frequently.

A systematic data and information were extracted from PubMed, Cancer network and Science direct. Broadly, the following search terms were used: NSCLC, biomarkers in clinical use in India, molecular markers, epidermal growth factor receptor (EGFR), KRAS, Anaplastic lymphoma kinase (ALK), chemotherapy, Afatinib, Erlotinib, Gefitinib, Gemcitabine, Carboplatin and Cisplatin (3-9). References from systematic reviews and meta-analyses were screened for potentially relevant studies.

Study selection was done on the basis of following factors:

(i) Clinical trials, observations and evaluations;
(ii) Data on proper use and dosage of drugs from the proper source;
(iii) Databases on biomarker usage and corresponding treatment techniques;
(iv) Trialscarried out among NSCLC patients only.

Data extraction and its validity- Relevant data were extracted from authentic sources by independent reviewers. The outcome of interests were as following:

(i) Type of biomarkers used popularly against NSCLC in India;
(ii) Techniques used for detection of biomarkers;
(ii) Drugs used for chemotherapy;
(iii) Comparison of drugs combination used in chemotherapy;
(iv) Drugs used in targeted therapy;
(v) Comparison of drugs combination used in targeted therapy;
(vi) Comparison of chemotherapy and targeted therapy.

Results and discussion

Systematic review

Through survey we obtained following results. Based on the interview and online data search, most relevant biomarkers found were EGFR followed with KRAS and ALK. These biomarkers were selected on the basis of their prevalent usage in pathological clinics for diagnosis (Figure 5).

The most prevalent technique used for the detection of these biomarkers are IHC followed with FISH, PCR and direct sequencing.

The results obtained from survey interpret that:

(i) Most of the patients diagnosed with NSCLC are in the age group of 40 to 60 years of age (Fig.1);
(ii) Coughing and weight-loss are common physical features of NSCLC patients (Fig.2);
(iii) Many of the practitioners did not know about the vaccination initiated by Cuba (Fig.3);
(iv) Patients are generally diagnosed at advanced stages (i.e. 3rd and 4th stage) of NSCLC which decreases the survival rate of patients (Fig.4).

Interpretation of results of graphs

(i) In case of squamous carcinoma, EGFR has the highest prevalence (58%), followed by KRAS (5%) and ALK (1.50%) (Figure 5).
(ii) In case of adenocarcinoma, EGFR has the highest prevalence (39%), followed by KRAS (27.5%) and ALK (4%) (Figure 6).
(iii) In case of non-smokers, EGFR has quite high prevalence (77%), followed by ALK (10%) and KRAS (0.5%) (Figure 5).

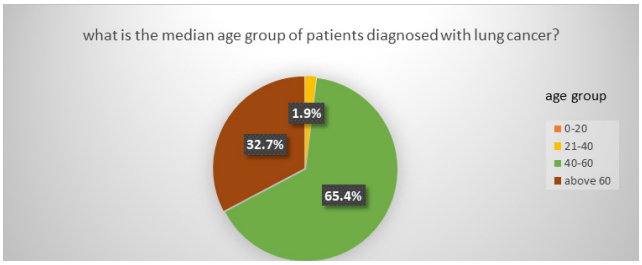

Figure 1. Cases of lung cancer diagnosed in different age groups of patient.

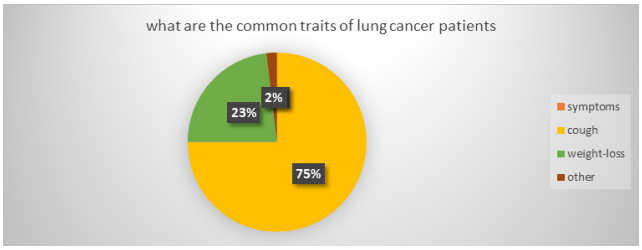

Figure 2. The common traits of lung cancer in patients.

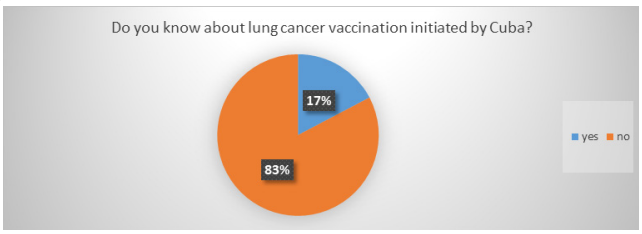

Figure 3. Knowledge of lung cancer vaccination initiated by Cuba.

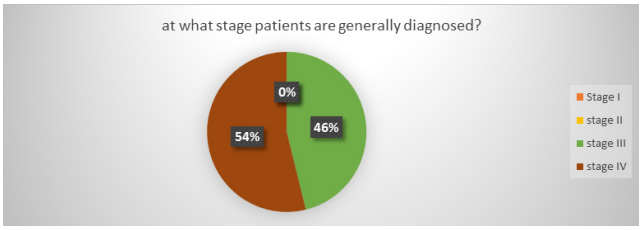

Figure 4. Diagnosis of lung cancer at different stages of cancer progression.

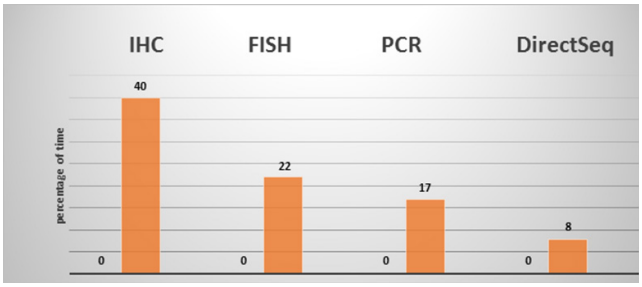

Figure 5. Clinical relevance of different Biomarkers (IHC, FISH, PCR and Direct sequencing) in lung cancer diagnosis.

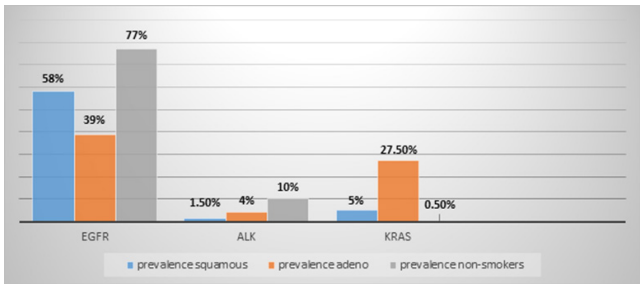

Figure 6. Relative clinical relevance of clinical biomarkers (EGFR, ALK and KRAS) for diagnosis of lung cancer.

(iv) IHC has been used in highest percentage of cases of non-small case lung cancer diagnosis (40%) followed by FISH, PCR and Direct sequencing (Figure 6).

Targeted therapy

The drugs used in targeted therapy are monoclonal antibodies which are specific to particular surface receptors of cancerous cells. The information about dosage of drugs in targeted therapy was done based on data search, study selection and extraction of material from Medscape and the interview. The data is shown in Table 1.

Relative efficacy of drugs targeted against EGFR is comparable but adverse effects and toxicities are maximum for Afatinib and minimum for Gefitinib, as mentioned in Table 2.

Among the drugs undertaken into consideration in our study for targeted therapy, Gefitinib at its highest dosage (Table 1) has minimum percentage of side effects (Table 2) (10).

Chemotherapy

The data search from all databases gave us almost 37 relevant citations. Among them, 7 were identified to be related to our topic of interest. The remaining were excluded. For data extraction of drug dosage and side effects 3 publications (3-5) were accessed directly. The main factors of interest for extraction of data were:

(i) Relative efficacy of drugs (3-5);
(ii) Degree and number of side-effects;
(iii) Different combinations of drugs used;
(iv) Dosage of drugs;
(v) path of administration of drugs;
(vi) The stage of NSCLC at which this therapy is followed.

The most important points related to drug combinations used and cited in literature are (4, 5)

(i) Platinum based chemotherapy is less effective than platinum based doublet combination;
(ii) Drugs used in combination are more effective than the simple formulations except Gemcitabine;
(iii) Gemcitabine-carboplatin is the most efficient combination for chemotherapy in platinum based doublet combination.

Efficacy of drugs (3, 4) was found to be in following order

1. Gemcitabine-Cisplatin > Cisplatin triplet > Cisplatin-Vinorelbin > Cisplatin-Etoposide > Cisplatin
2. Cisplatin-Paclitaxel > Gemcitabine- Cisplatin > Cisplatin-Docetaxel > Carboplatin-Paclitaxel

Table 1. Data of drugs used in targeted therapy (PO qday: by mouth once a day).

Biomarkers	Drug name	Dosage of drugs (in mg PO qday)	Side effects	5-year survival rate
EGFR	Erlotinib	150 (12 hours)	Fatigue, rash, diarrhoea, constipation, stomatitis, anorexia, Paronychia	14.6%
	Gefitinib	250	Liver dysfunction, Nail change, rash, fatigue, diarrhoea, paronychia	14.6%
	Afatinib	40	Rash, fatigue, diarrhoea, paronychia	9-13%
ALK	Crizotinib	200-250 (1-2 hour)	Gastrointestinal side-effects, pneumonitis, Cardiac-toxicity, visual disturbances	5%
	Crizotinib	750	Gastrointestinal side-effects, hepatotoxicity	NA
KRAS	Selumetinib	NA	Diarrhoea, nausea, vomiting, rashes, febrile, neutropenia	10%
	Trametinib	2	Rash, diarrhoea, lymphedema, nausea	NA

Table 2. Grade 3 side effects: sever damage, hospitalization required and patients need extra care.

Drug	Rash and acne (grade ≥ 3) in %	Diarrhea (grade ≥ 3) in %	Fatigue (grade ≥ 3) in %	Paronychia (grade ≥ 3) in %
Afatinib (40/50 mg)	89	95	18	57
Erlotinib (150 mg)	73-80	25-57	5-57	4
Gefitinib (250/500 mg)	66-85	34-54	11-39	14-32

Table 3. Comparison between targeted therapy and chemotherapy.

Serial number	Terms of results	Pooled values for TKIs (in percentage)	Values for standard chemotherapy (in percentage)
1	Overall response rate	66.6	30.9
2	1 year progression free survival	42.9	9.7
3	1 year overall survival	79.2	78.9
4	2 year overall survival	49.7	51.0

3. Gemcitabine-Carboplatin ≈ Gemcitabine-Cisplatin
The main points encountered were-
(i) It is not given daily but in cycles of four to six (4).
(ii) A cycle of chemotherapy (typically about 21 days) refers to the time it takes to give the treatment andthen allow the body to recover from the side effects of the medicines (4).
(iii) A combination (regimen) of two or three chemotherapy drugs is used. Most of the drugs are given into a vein (intravenous, IV) once every three weeks (4).

Comparison between targeted therapy and chemotherapy

Afatinib, Erlotinib, Gefitinib as targeted therapy drugs were used to target EGFR-tyrosine-kinase inhibitor (EGFR-TKIs) and cisplatin in standard chemotherapy was used (15) and compared in terms of overall response rate and overall survival rate. Results are categorized for two broad categories- pooled EGFR-TKIs and standard chemotherapy. These are listed in Table 3.

Since the 5-year survival rate in chemotherapy as well as in targeted therapy is low on cumulative basis. Thus, the early detection of this disease is perceived as a crucial requirement for influential changes in the current medical scenario. Although no clinical practice is universally acknowledged for early detection, but some potential practises at research level have promising and efficient results. These potential practises are based on the type of biomarkers targeted for study. Some suggestions as followed are:

1. The National Lung Screening Trial (NLST) was a large clinical trial that looked at using a type of CT scan known as low-dose CT to screen for lung cancer.Low-dose CT (LDCT) of the chest uses lower amounts of radiation than a standard chest CT.The NLST compared LDCT of the chest x-rays for lung-cancer to see if these scans can help lower the risk of dying from lung cancer. People in the study got either 3 LDCT scans or 3 chest x-rays. After several years, the study found that people who got LDCT had a 16% lower chance of dying from lung cancer than those who got chest x-rays. They were also 7% less likely to die overall (from any cause) than those who got chest x-rays.

2. Studies of different forms of cancer have shown that the use of diagnostic tests based on body fluid protein biomarkers significantly improved patient survival rates. In this regard, urine biomarkers are capable of detecting lung cancer which can potentially aid in early detection. Some protein species up-regulated in lung cancer nodules are present at higher concentrations in the urine of lung cancer patients. The incidence of lung cancer and other types of cancers related to smoking are found to be positively associated with serum ceruloplasmin (CP) levels, which get markedly elevated in urine of lung cancer patients. A significant proportion of the up-regulated urine proteins in lung cancer patients are involved in inflammation, which potentially contributes to the development of lung cancer. Their detection can be a promising tool for detection of lung-cancer (18).

Conclusion

Based on our survey, we concluded that early diagnosis of this disease is critical for improving clinical outcome. On the contrary, because early stages of NSCLC often produce no clinical symptoms, it is difficult to identify biomarkers for early detection, prognostic evaluation and monitoringrecurrence of the cancer. The use of targeted therapy has markedly changed outcomes for some diseases (15, 16). We also claim that targeted therapy is better than chemotherapy for first line treatment as it provides low toxicities and higher mean survival time (Table 3). However due to lack of relevant technology, efficiency of a method and the expenses incurred, both oncologists and patients in India today tend to choose chemotherapy. Therefore considering the benefits of targeted therapy, the need of the hour is to increase its uses and the research in this field.

Among the drugs undertaken into consideration in our study for targeted therapy, Gefitinib at its highest dosage (Table 1) has minimum percentages of side effects (Table 2).In chemotherapy, we determined Gemcitabine (4, 5) to be statistically the best chemotherapeutic drug which is also in clinical use.

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References

1. Lung Cancer focus. India, SIRO Report, Siro 2008 (www.siro-clinpharm.com/).
2. Behera D. and Balamugesh T. Lung Cancer in India. Indian J Chest Dis Allied Sci, 2004; 46: 269-281.
3. Noronha V., Prabhash K., Thavamani A., Chougule A., Purandare N., Joshi A. et al. EGFR Mutations in Indian Lung Cancer Patients: Clinical Correlation and Outcome to EGFR Targeted Therapy. PLOS ONE, 2013; 8(4):e61561.
4. Johnson D.H. Gemcitabine for the Treatment of Non-Small-Cell Lung Cancer.Cancer Network, 2001; (Suppl 6):33-39.
5. Ramalingam S. and Belani C.P. Carboplatin/Gemcitabine Combination in Advanced NSCLC. Oncology (Williston Park), 2004; (8 Suppl 5):21-6.
6. Korpanty G.J., Graham D.M., Vincent M.D., Leighl N.B. Biomar-

kers That Currently Affect Clinical Practice in Lung Cancer: EGFR, ALK, MET, ROS-1, and KRAS. Front Oncol, 2014; 4: 204.
7. Kohler J. and Schuler M. Afatinib, Erlotinib and Gefitinib in the First-Line Therapy of EGFR Mutation-Positive Lung Adenocarcinoma: A Review. Onkologie, 2014; 36(9):510-8.
8. Desai C., Mehta A. and 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(3): 249–259
9. Chan B.A. and Hughes G.M.B.Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer Res, 2015; 4(1): 36–54.
10. Yoshida T., Yamada K., Azuma K., Kawahara A., Abe H., Hattori S. et al. Comparison of adverse events and efficacy between gefitinib and erlotinib in patients with non-small-cell lung cancer: a retrospective analysis. Med Oncol, 2012; 30(1):349.
11. Chidambaram A., Adjei A.A. Second-Generation ALK inhibitors for Therapy of ALK-Rearranged Non-Small Cell Lung Cancer. Targeted Oncology, 2015.
12. Malik P.S. and Raina V. Lung Cancer: Prevalent trends and emerging concepts. Indian J Med Res, 2015; 141(1): 5–7.
13. Gerber D.E. Targeted therapies: A new generation of Cancer treatments. AAFP, 2008; 77(3):311-319.
14. Monika Joshi, Syed M Rizvi, and Chandra P Belani: Afatinib for the treatment of metastatic non-small cell lung cancer. PubMed Central, 2015; 7: 75–82.
15. Wenhua Liang, Xuan wu, Wenfeng Fang, Yuanyuan Zhao, Yunpeng Yang, Zhihuang Hu et.al. Network Meta-Analysis of Erlotinib, Gefitinib, Afatinib and Icotinib in Patients with Advanced Non-Small-Cell Lung Cancer Harboring EGFR Mutations. PLOSONE, 2014; 9(2): e85245.
16. ASCO report, 2015 (https://www.boehringer-ingelheim.com/press-release/asco-2015-superior-os-afatinib-vs-erlotinib).
17. Caroline Helwick. Managing resistance to targeted agents: The future of NSCLC Therapy. (www.ascopost.com/issues/october-15-2014/managing-resistance-to-targeted-agents-the-future-of-nsclc-therapy.aspx).
18. Hongjuan Zhang, Jing Cao, Lin Li, Yanbin Liu et al. Identification of urine protein biomarkers with the potential for early detection of lung cancer. PubMed, 2015, 2;5:11805