Original Article

Audit of Diagnostic Tissue for the Diagnosis of Non-small Cell Lung Cancer

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Keywords: *Lung Cancer, Cancer, Egfr, Alk, Biomarker*

ABSTRACT

Background: Non-small cell lung cancer (NSCLC) harbouring *Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase (ALK)* mutations respond well to tyrosine kinase inhibitors (TKIs). This response is better than that seen with standard chemotherapy. Adequate tissue specimens are necessary for accurate identification of biomarkers in NSCLC to determine subtype and targeted treatment. The aim of this study is to ascertain which biopsy method provides the highest proportion of adequate tissue specimens for biomarker testing.

Methods: TheMosaiq® database was accessed to retrieve information regarding all (164) patients diagnosed with NSCLC between 12/02/2011-15/2/13. The biopsy methods used, patient characteristics and adequacy of tissue obtained for biomarker testing were analysed using the SPSS software.

Result: From the 41 patients tested for biomarkers, surgical resection provided the highest proportion of adequate tissue specimens (100%) compared with fine needle (89%) and core biopsy (61%) respectively.

Conclusion: In conclusion, patients with NSCLC who are unsuitable for surgery, fine needle biopsy can be considered before core biopsy for biomarker testing given the higher proportion of adequate tissue specimens obtained. Larger scale trials are required to assess tissue acquisition, processing and reporting for biomarker testing in order to standardise detection of driver mutations for personalised cancer therapy.

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Introduction

Lung cancer is the leading cause of cancer deaths in Australia and worldwide.[1,2] In Australia, lung cancer accounts for 18.9% of all cancer deaths and approximately 1.35 million deaths worldwide annually.[1-3] Most (approximately 85-90%) lung cancers are non small cell lung cancers (NSCLC).[4-6] In Australia, 61% of males and 64% of females with lung cancer have NSCLC.[7] NSCLC are epithelial cancers and the most common types are adenocarcinoma, squamous cell and large cell carcinoma.[4,5,8,9] Currently the five year survival for NSCLC is poor (less than 15% overall) and worsens with increasing stage (Stage I > 45%, Stage II > 30%, III 5-15%; IV 1%).[1,5,10] Most NSCLC are diagnosed at an advanced stage (40% at stage IV) and are linked to poor survival rates.[11,12] A major challenge is to improve the prognosis of patients with NSCLC, especially those with advanced disease.

Patients diagnosed with NSCLC are treated according to the stage of the disease. Early stage disease is commonly treated with curative intent (surgical resection or radiation therapy and adjuvant chemotherapy) whereas advanced disease is managed palliatively (radiation and/or chemotherapy). In the last decade, newer targeted molecular therapies are paving the way in personalised cancer treatments.[13] Approximately 10-15% of NSCLC are *Epidermal Growth Factor Receptor (EGFR)* positive and 2-7% are *Echinoderm Microtubule Associated Protein Like-4 Anaplastic Lymphoma Kinase (EML4-ALK)* positive tumours[4,6,8,14] Testing for these mutations are important for treatment decision pathway.15 Use of Tyrosine Kinase Inhibitors (TKIs) produce higher response rates, longer progression free survival intervals and significantly improve quality of life in patients with advanced NSCLC with *EGFR* activating mutations compared to chemotherapy.[15-17]

Optimal biomarker testing requires adequate tissue sampling as well as appropriate processing and handling of the tissue specimen. There are differing opinions in the literature regarding adequate tissue sampling and processing. Currently guidelines state when to test for *EGFR* and *ALK* mutations, however, the amount of tissue required for genetic testing is not standardized. [18-22] Without standardized protocols for tissue collection/preparation, it is difficult to determine the number of cancer cells required in a specimen for successful targeted mutation testing. Large diagnostic biopsies are not always possible due patient factors such as age, chronic obstructive pulmonary disease (COPD), body mass index, surgical risk, size of tumour, location of tumour and metastases.[21,23] These factors may limit the amount of tissue obtainable for the diagnosis of NSCLC and biomarker testing. Studies indicate that cytological specimens are adequate and suitable alternatives if tissue samples are insufficient.[18,23-26] Approximately 60-70% patients with NSCLC present at stage IIIb or IV. [19,27,28] In these advanced stages, surgery is not appropriate. With distant metastases, fine needle or core biopsies are often used to obtain tissue specimens for genetic testing. Commonly, they yield insufficient tissue for testing.[19] The critical component of tissue biopsy is to ensure a quality sample that contains adequate numbers of cancer cells to allow for microarray testing irrespective of primary or metastatic tumour origin.

Due to the conflicting recommendations in the literature about adequate tissue retrieval and tissue processing, this study aims to assess and compare which biopsy type provides the greatest proportion of adequate tissue specimens for the diagnostic testing of *EGFR* and *ALK* mutations at the MCTC. Analysing the current practices and examining the proportion of adequate diagnostic tissue specimen via each biopsy modality will inform the development of a diagnostic algorithm for EGFR and ALK testing.

Materials And Methods

Participant Selection: This study is a retrospective audit of all 164 patients on the Mosaiq® database diagnosed with NSCLC at Campbelltown and Liverpool Hospitals between 12/02/2011-15/02/2013. Patients had previously provided written consent to the Macarthur Cancer Therapy Centre for the use of their clinical information. This audit included patients who were diagnosed with NSCLC irrespective of gene testing for *EGFR* and *ALK* mutations. Patients without a histology of NSCLC were excluded from the study. Those who were tested for *EGFR* and *ALK* mutations were grouped together as 'gene mutation tested' patients. Biomarker mutation testing for EGFR testing was limited to exons 18-21 and *ALK*. The use of any recommended biomarker mutation analysis method was permissible. The biopsy methods used for the *EGFR* and *ALK* tested patients were examined for the highest proportion of adequate tissue yield for each biopsy type. Biopsy types were grouped as follows:

- fine needle (fine needle aspiration biopsy and pleural effusion)
- core biopsy (core needle biopsy, EBUS, bronchoscopy, CT guided core needle biopsy and pleural biopsy)
- surgical resection (surgical resection and VATs)

Adequate tissue yield was defined as any biopsy sample that was able to provide a positive or negative result for biomarker mutation analysis.

There were no timeline restrictions as to when the biomarker mutation testing should have been done within the allocated study timeframe. Of the 164 patients diagnosed with NSCLC, the age, gender, smoking status, smoking history and COPD status were documented. Within smoking status, 'never smoker' was defined as smoking less than 100 cigarettes. This data was entered into an Excel Spreadsheet and the data was de-identified during this process to maintain patient privacy. The variables were analysed for their distribution amongst each subtype of NSCLC (squamous cell carcinoma, adenocarcinoma, large cell carcinoma, bronchoalveolar carcinoma, mixed carcinoma and NSCLC NOS). Further analyses of the same variables were conducted amongst patients based on biomarker testing status. The supervisor checked the reliability of obtained data after data collection.

Data Analysis: The de-identified data was initially entered into an Excel Spreadsheet and was later imported into SPSS statistical software for analysis. Chi square was used to calculate the p values for the adequacy of tissue obtained for genetic testing for each biopsy type and to ascertain if there was any relationship amongst different variables against NSCLC subtypes. Unknown or missing variables were not included whilst calculating p values. A p value of ≤0.05 was considered statistically significant. Due to the small sample size, the obtained results were rounded off to whole numbers.

Result

Characteristics of Patients Diagnosed with Nsclc at Macarthur Cancer Therapy Centre: Of the 164 patients diagnosed with NSCLC, adenocarcinoma was the most frequently diagnosed subtype 31% (51) (Table 1). More patients were diagnosed with large cell carcinomas 27% (44) than squamous cell carcinomas 24% (40) (Table 1). A larger number of males 57% (29) were diagnosed with adenocarcinoma compared to females 43% (22) (Table 1).

Which biopsy type provided the highest proportion of tissue samples adequate for biomarker testing at Macarthur Cancer Therapy Centre?: Adequacy of tissue sampling for biomarker testing was highest for surgical resection (100%) compared with fine needle biopsy (89%) and core biopsy (61%) respectively (Table 2). Whilst the difference in sampling adequacy was not statistically different between surgical resection and fine needle biopsy (p 0.30), the difference between surgical resection and core biopsy was statistically significant (p 0.03). There was no statistically significant difference in sampling adequacy between fine needle biopsy and core biopsy (p 0.12).

Table 1: Patient Characteristics amongst the subtypes of NSCLC

Characteristic	Total NSCLC		Squamous		Adenocarcinoma		Large Cell		NSCLC NOS		Bronchoalveolar		Mixed	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Age														
<30 years	1	1	0	0	0	0	1	2.3	0	0	0	0	0	0
30-45.9	5	3	2	5	3	6	0	0	0	0	0	0	0	0
46-55.9	11	7	3	8	3	6	4	9	0	0	1	50	0	0
56-64.9	58	35	12	30	18	35	19	43	8	38	0	0	1	17
65-74.9	65	40	14	35	24	47	15	34	8	38	1	50	3	50
75-84.9	19	12	8	20	2	4	5	11	3	14	0	0	1	17
>85	5	3	1	3	1	2	0	0	2	9.5	0	0	1	17
TOTAL	***164***	***100***	***40***	***100***	***51***	***100***	***44***	***100***	***21***	***100***	***2***	***100***	***6***	***100***
Gender														
Male	101	62	26	65	29	57	30	68	11	52	2	100	3	50
Female	63	38	14	35	22	43	14	32	10	48	0	0	3	50
Smoking Status (total)	**140**	**100**	**34**	**24**	**44**	**31**	**37**	**26**	**20**	**14**	**1**	**1**	**4**	**3**
Current	52	39	15	44	11	25	15	41	11	55	1	100	0	0
Ex-smoker	69	51	17	50	27	61	19	51	6	30	0	0	2	50
Never	14	10	2	6	6	14	3	8	3	15	0	0	2	50
COPD (total)	**83**	**100**	**25**	**30**	**25**	**30**	**21**	**25**	**9**	**11**	*	*	**3**	**4**
Yes	66	80	24	96	17	68	17	81	7	78	*	*	1	33
No	17	20	1	4	8	32	4	19	2	22			2	67
Pack Years (total)	**121**	**100**	**28**	**23**	**33**	**27**	**30**	**25**	**14**	**10**	**13**	**11**	**4**	**3**
0-12	27	22	4	14	10	30	5	17	5	36	0	0	3	75
13-40	38	31	9	32	12	36	10	33	5	36	1	100	1	25
>40	45	37	15	53	11	33	15	50	4	29	0	0	0	0

*Missing data not included in calculations

Table 2: Adequacy of tissue samples by biopsy type amongst patients tested for EGFR and ALK mutation

Characteristic	Fine Needle Biopsy		Core Biopsy		Surgical Resection	
	N	Column %	N	Column%	N	Column%
Specimen adequate for testing	8	89	14	61	9	100
Specimen inadequate for testing	1	11	9	39	0	0
TOTAL	**9**	**100**	**23**	**100**	**9**	**100**

Discussion

Adequacy of Tissue Obtained for Biomarker Testing: The results of this study support Sun et al.'s[29] and Ma et al.'s[30] findings that indicated surgical resections were superior to core and fine biopsies at detecting *EGFR* mutations. In the literature, core biopsies were reported to be superior to fine needle biopsies[29,30]and this may be due to differences in cell block preparation/fixation and sequencing methods. At MCTC, this study has shown fine needle biopsy provided a higher proportion of adequate tissue specimens than core biopsy for biomarker testing. As the majority of biomarker tested patients are stage IIIB and IV NSCLC, fine needle biopsy could mean benefit for both the patient and the healthcare system as it is less invasive, less time consuming, requires less operator training and is cheaper than core biopsy. Thus, it should be the first biopsy method for biomarker testing in late stage NSCLC. In those patients with surgically resectable disease (usually early stage NSCLC), surgically resected specimens will obviate the need for any biopsy. Additionally, fine needle biopsies carry less procedural risk and this supports its use as the primary biopsy method.

Though fine needle biopsy is safer than other biopsy methods, it could mean more patients undergo reflex testing at the time of lung cancer diagnosis. This would result in increased healthcare costs through additional workload, stress, time investment and medication costs for targeted *EGFR* and ALK therapy. Arguments regarding fine needle biopsies hindering biomarker detection have been disproven by already published data[30-34] that illustrated small biopsies provided comparable tissue specimens to surgical resection when adequate gene amplification techniques, tumour enrichment strategies and exfoliative cytology are employed to help increase diagnostic tissue yield.

To optimise fine needle biopsies it will be beneficial to have a multidisciplinary team approach when deciding on initial biopsy method. The initial biopsy method should be the least invasive biopsy method that provides adequate tissue sampling. A pre- biopsy review of imaging to initiate adequate procedural planning, increasing the use of image guided biopsy methods to optimise tissue acquisition and receiving immediate rapid on site evaluation of tissue specimens by a pathologist or cyto-techincian could improve tissue samples retrieved by the interventionist.

Strengths Of The Study: This study has investigated a small population of lung cancer patients in Greater Western Sydney and the proposed algorithm will be used within the same population. Such an individualized approach is a strength of this study. In addition, there is a paucity of evidence in this area from the Australian literature. Though this audit is small, it is Australian and contributes to the data regarding EGFR and ALK biomarker testing. It is my hope that this audit may assist with future studies to improve and guide tissue acquisition methods for efficient biomarker testing to enable timely targeted TKI therapy.

Limitations Of The Study: The obvious limitation is the small sample size. Furthermore, detailed analyses of the data was limited by missing patient information parameters. For example, associations between patient factors and initial biopsy method were difficult to assess. The stage at which patients were diagnosed with NSCLC, the site of the biopsy specimen (primary or metastatic) and any previous cancer treatment were not noted. This information could have shed more light on the appropriateness of the biopsy method chosen for gene testing.

Future Directions: A larger study comparing the adequacy of fine needle biopsy and core biopsy tissue specimens for gene mutation testing should be considered at other cancer services to add to the data from the MCTC. The study should also endeavor to ascertain the quantity of tissue specimen and percentage tumour cellularity required for adequate diagnostic specimens. This information will help devise a diagnostic algorithm that will guide future biopsy practices at the MCTC and optimise gene testing for the local population of patients with NSCLC in the Greater Western Sydney region.

Conclusion

This audit suggests that surgical biopsy provides the most adequate tissue acquisition compared to fine and core biopsies respectively. Surgical biopsies are not always appropriate and this study suggests that fine needle biopsy provides a higher proportion of adequate tissue specimens compared to core biopsy. Using fine needle biopsy as the initial method of tissue acquisition reduces cost, time and

procedural complications, whilst maintaining adequate tissue sampling. Further large scale trials in collaboration with pulmonologists, pathologists, oncologists (medical and radiation), radiologists and respiratory physicians needs to be undertaken to standardise and optimise tissue specimen collection, processing and reporting for biomarker testing at MCTC in order to complement the evolving transition towards personalised cancer therapy.

Acknowledgements
Sincere thanks to A/Professor Wendy Stevens for her generous support during the entire research process.

Funding
No Funding

Competing Interests
No competing interests declared

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