Am J Transl Res 2022;14(9):6696-6702
www.ajtr.org /ISSN:1943-8141/AJTR0144071

Original Article

Risk factor analysis of bone metastasis in patients with non-small cell lung cancer

Yang Li[1], Chongqing Xu[1,2], Qiquan Yu[1]

[1]Department of Thoracic Surgery, Long Hua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China; [2]Department of Orthopaedics, Long Hua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Received May 11, 2022; Accepted July 25, 2022; Epub September 15, 2022; Published September 30, 2022

Abstract: Objective: Bone tissue is the most common metastatic location besides lung and liver. 30%~40% of patients with non-small cell lung cancer (NSCLC) will have bone metastasis (BM) in the development of the disease. This study aims to explore the relevant risk factors through multivariate analysis, in order to provide basis for the prevention of BM and bone related events of NSCLC. Methods: We analyzed 152 patients, with 67 in BM group and 85 in non-BM group. The general clinical data and laboratory indicators (mainly coagulation function) of patients were compared through univariate and multivarijate analysis. Finally, the independent risk factors of BM in patients with NSCLC were screened out. Results: The results of univariate analysis show that thrombosis, clinical stage, tumor-node-metastasis (TNM) stage, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-Dimer (D-D), platelet (PLT) and alkaline phosphatase (AKP) are the risk factors of BM in patients with NSCLC ($p<0.05$). Further multivariate logistic regression analysis suggests that the independent risk factors of BM in patients with NSCLC are clinical stage III-IV, TNM stage T1-T3, TNM stage N2-N3, FIB, APTT, D-D and AKP ($P<0.05$). Conclusion: Clinical stage III-IV, TNM stage T1-T3, TNM stage N2-N3, FIB, APTT, D-D and AKP are the independent risk factors of BM in patients with NSCLC. Meanwhile, patients with these risk factors should be screened in time, which is of great significance to prevent bone related events and relieve pain.

Keywords: Non-small cell, lung cancer, bone metastasis, risk factors

Introduction

At present, lung cancer (LC) ranks first in incidence and mortality of cancer all over the world [1]. Nearly 2 million people die of LC worldwide every year. With the increase of environmental pollution, the incidence of LC is increasing continually. In Asia alone, the rate of new-onset LC patients has exceeded 50% each year [2], which brings huge burden and pain to patients and families. There are about 85% non-small cell lung cancer (NSCLC) in all types of LC, while adenocarcinoma and squamous-cell carcinoma are the most common in NSCLC, accounting for more than 65% of LC patients [3, 4].

LC patients have no significant signs in the early stage. Once diagnosed with LC, they are often in the late stage, so it is very difficult to cure the disease. The death of patients with cancer is usually caused by recurrence or metastasis. Bone tissue is one of the most important hematogenous metastasis sites in the body [5]. It is the most common metastasis location besides lung and liver. 30%~40% of NSCLC patients will have bone metastasis (BM) in the development of the disease, often involving central axis bone and other sites [6, 7]. Moreover, BM can cause bone pain, pathological fracture, spinal cord compression, hypercalcemia and other adverse symptoms, which seriously affect the prognosis of patients. The 3-year cumulative survival rate of LC patients with BM is almost 0% [6].

The factors that can affect the recurrence, BM and survival of patients with NSCLC can be roughly divided into three categories. First, the patients' own clinical and pathological factors, such as age, smoking, tumor stage, pathological type, etc. The second is molecular biomarkers in patients' blood, including coagulation

function indicators and tumor markers. Based on these indicators, it can be decided whether patients can receive targeted treatment. The third is interstitial cells in the tumor microenvironment. It not only provides various growth factors for tumor cells, but also secretes various factors that promote metastasis and resist drugs, which finally assists the dissemination of tumor cells [1, 2, 6, 7]. Thus, the above factors are of great significance in predicting the recurrence or BM, and evaluating the prognosis of NSCLC. Therefore, analyzing the risk factors of BM in patients with NSCLC has important clinical value for the prevention and treatment of BM.

However, there are few studies on multiple categories of risk factors of BM in patients with NSCLC, for example the combination of clinical data and laboratory indicators. This study aims to explore the relevant risk factors through multivariate analysis, in order to provide basis for the prevention of BM and bone related events.

Materials and methods

Patient case selection

We obtained ethical approval exemption from our ethics committee to perform this study since we didn't have direct contact with the participants. Patients treated for NSCLC in database records of our hospital were retrospectively analyzed from Jan 1, 2018, to Feb 28, 2022 (updated medical record system was used since Jan 1, 2018).

Inclusion criteria were as follows: 1) patients diagnosed as lung adenocarcinoma or squamous-cell carcinoma by pathology; 2) patients received bone scintigraphy and were confirmed by CT or MRI to have typical imaging manifestations of BM or not [8]; 3) lower extremity venous ultrasound had been conducted to judge whether there was thrombosis; and 4) no history of anti-tumor treatment within half a year. Exclusion criteria including: 1) patients with liver and kidney diseases or liver adrenal metastasis in the past; 2) patients with endocrine system diseases that affect bone metabolism; 3) patients with blood system diseases such as thrombocytopenic purpura; 4) patients with benign bone lesions such as fractures within 1 year.

All patients were divided into BM group and non-BM group.

Observation indicators

The general clinical data were collected respectively, including age, sex, smoking, hypertension, diabetes, thrombosis, surgical procedure, tumor location, pathological type, clinical stage, and tumor-node-metastasis (TNM) stage. Meanwhile, the laboratory indicators (mainly coagulation function) of patients were compared, including prothrombin time (PT), prothrombin time activity (PTA), international normalized ratio (INR), fibrinogen (FIB), activated partial thromboplastin time (APTT), thrombin time (TT), partial thrombin ratio (PTR), prothrombin ratio (PR), D-Dimer (D-D), blood platelet (PLT), and alkaline phosphatase (AKP).

Statistical analysis

Statistical Packages of Social Sciences (SPSS) software (version 22.0) was used to analyze the collected data. The count data were expressed by percentage (%), and chi-square test was used for comparisons between groups; the measurement data were expressed by mean ± SD, and *t*-test was used for comparison between groups. The risk factors of BM in NSCLC patients were screened out by logistic regression analysis with α=0.05 as the inspection level. Meanwhile, $P<0.05$ was considered statistically significant for all the above.

Result

A total of 152 consecutive cases are involved in the current study, including 103 males and 49 females with a mean age of 63.2±8.5 years (ranges from 34 to 86 years). There are 67 cases in BM group and 85 cases in non-BM group. The detailed patient information is shown in **Table 1**.

Univariate analysis

The comparisons between general clinical data of the two groups show that the age, sex, smoking, hypertension, diabetes, surgical procedure, tumor location, and pathological type of patients have no inevitable correlation with BM ($P>0.05$). However, thrombosis, clinical stage

Table 1. Comparison of general clinical data between the two groups

Item	BM Group (n, %) (N=67)	Non-BM Group (n, %) (N=85)	χ^2	*p*
Sex			0.046	0.812
Male	47 (70.15)	58 (68.24)		
Female	20 (29.85)	27 (31.76)		
Age			2.358	0.194
≤60	26 (38.81)	23 (27.06)		
>60	41 (61.19)	62 (72.94)		
Smoking			0.247	0.605
Yes	32 (47.76)	36 (42.35)		
No	35 (52.24)	49 (57.65)		
Hypertension			0.319	0.562
Yes	29 (43.28)	33 (38.82)		
No	38 (56.72)	52 (61.18)		
Diabetes			0.186	0.709
Yes	20 (29.85)	28 (32.94)		
No	47 (70.15)	57 (67.06)		
Thrombosis			9.725	0.003*
Yes	7 (10.45)	2 (2.35)		
No	60 (89.55)	83 (87.65)		
Surgical procedure			1.364	0.258
Thoracotomy	23 (34.33)	35 (41.18)		
Thoracoscope	19 (28.36)	40 (47.06)		
Without operation	25 (37.31)	10 (11.76)		
Tumor location			0.137	0.723
Left	25 (37.31)	29 (34.18)		
Right	42 (62.69)	56 (65.82)		
Pathological type			3.092	0.095
Adenocarcinoma	50 (74.63)	57 (67.06)		
Squamous carcinoma	17 (25.37)	28 (32.94)		
Clinical stage			12.476	<0.001*
Stage I-II	12 (17.91)	32 (37.65)		
Stage III-IV	55 (82.09)	53 (62.35)		
TNM-T stage			18.851	<0.001*
Stage T1-T3	46 (68.66)	30 (35.29)		
Stage T4	21 (31.34)	55 (64.71)		
TNM-N stage			10.248	0.001*
Stage N0-N1	19 (28.36)	37 (43.53)		
Stage N2-N3	48 (71.64)	48 (56.47)		

BM (bone metastasis), TNM (tumor-node-metastasis). *The result has statistical significance.

(III-IV) and TNM stage (T1-T3, N2-N3) are closely related to BM in NSCLC patients, which have statistical significance (*P*<0.05) (**Table 1**).

The comparisons between relevant laboratory indicators of the two groups show that the PT, APTT and TT in BM group are significantly lower than those in non-BM group, while the FIB, D-D, PLT and AKP in BM group are significantly higher than those in non-BM group (*p*<0.05) (**Table 2**).

Therefore, thrombosis, clinical stage (III-IV), TNM stage (T1-T3, N2-N3), PT, APTT, TT, FIB,

Table 2. Comparison of laboratory indicators between the two groups

Item	BM Group (Mean ± SD) (N=67)	Non-BM Group (Mean ± SD) (N=85)	*t*	*p*
PT	10.12±1.68	11.74±1.95	-5.074	<0.001*
PTA	90.65±21.32	98.93±37.46	-1.125	0.297
INR	1.04±0.28	1.12±0.19	-0.703	0.642
FIB	4.49±1.20	3.75±1.53	2.487	0.035*
APTT	21.73±7.84	27.85±10.29	-10.914	<0.001*
TT	12.97±1.75	14.68±2.01	-3.058	0.010*
PTR	1.01±0.42	0.94±0.28	1.876	0.259
PR	0.97±0.31	1.01±0.25	-0.361	0.728
D-D	0.72±2.54	0.29±1.06	14.475	<0.001*
PLT	319.47±121.62	282.43±113.75	2.246	0.029*
AKP	125.31±72.28	97.06±56.33	6.306	<0.001*

BM (bone metastasis), PT (prothrombin time), PTA (prothrombin time activity), INR (international normalized ratio), FIB (fibrinogen), APTT (activated partial thromboplastin time), TT (thrombin time), PTR (partial thrombin ratio), PR (prothrombin ratio), D-D (D-Dimer), PLT (blood platelet), AKP (alkaline phosphatase). *The result has statistical significance.

D-D, PLT and AKP are the risk factors of BM by univariate analysis.

Multivariate logistic regression analysis

Taking BM as the dependent variable Y, the occurrence of BM is defined as Y=1, and the non occurrence of BM is defined as Y=0. Take the indicator which has significant difference between the two groups as the independent variable X, and assign a value to it. Thrombosis, clinical III-IV stage, T4 stage, N2-N3 stage, PT>13 s, FIB>4.4 g/L, APTT>37 s, TT>18 s, D-D>0.23 mg/L, PLT>300×10^9/L, AKP>135 U/L (female) and AKP>125 U/L (male) are all assigned as 1, and other corresponding items are assigned as 0. The results show that there are 7 independent risk factors in the multivariate logistic regression analysis, including clinical stage III-IV, TNM stage T1-T3, TNM stage N2-N3, FIB, APTT, D-D and AKP, as shown in **Table 3**.

Discussion

BM can be divided into three types according to the pathological characteristics: osteolytic, osteogenic, and mixed type [9]. Osteolytic BM is common in LC, and its predilection sites are spine, ribs, femur and sternum. Most patients with BM will have various complications, which not only affect the quality of life, but also predict the shorter survival of patients. Therefore, clinicians should pay close attention to BM and determine the corresponding prevention and treatment strategies.

At present, there are many factors affecting BM of LC, including age, sex, pathological type, a number of primary focus, number of BM, treatment plan and serum markers [10]. Oliveira et al. [11] conduct a retrospective study on 413 LC patients, pointing out that the risk of BM in adenocarcinoma is higher than in squamous-cell carcinoma. Another cohort study in Brazil finds that the age at diagnosis is negatively correlated with the risk of BM (the risk of BM decreases by approximately 3% for each increased 1 year of age), and adenocarcinoma and minimally invasive resection of NSCLC are correlated with higher risk of BM [12]. Our result show that 41 of 67 patients (61.19%) with BM are older than 60, and the surgical procedure and pathological type have no significant effect on BM, which are inconsistent with the previous study. On the one hand, it may be correlated with the late clinical stage (71.05% stage III-IV) which can increase the risk of BM. On the other hand, it may be related to the regional and ethnic differences of the subjects.

Clinical stage is an important prognostic factor of LC. Studies have confirmed that BM in elderly patients with LC is related to clinical stage and differentiation degree of the primary cancer, while further analysis finds that the later the stage is, the earlier the occurrence of BM [13, 14]. Similarly, TNM stage is correlated with the prevalence of BM in LC patients. With the increase of lymph nodes involvement, the prevalence of BM tends to rise [15, 16]. In addition, Ayan et al. [17] believe that stage T3 is one of the independent factors of BM in NSCLC patients, and stage N2 is correlated with higher risk of BM. Our results show that there are significant differences between the two groups when in stages T1-T3 and N2-N3, indicating that patients are more prone to BM at these stages.

Recent data show that more than 30% of NSCLC patients have BM [18]. Scholars have

Table 3. Logistic regression analysis of BM risk factors

Item	*B*	*SE*	Wald	OR (95% CI)	*p*
Thrombosis	0.582	0.036	2.774	3.688 (1.047-6.329)	0.091
Clinical stage III-IV	0.724	0.289	5.371	2.656 (1.168-4.143)	0.023*
TNM stage T1-T3	1.037	0.341	7.102	3.339 (1.382-5.295)	0.005*
TNM stage N2-N3	0.692	0.215	4.146	4.832 (2.047-7.616)	0.034*
PT	-0.146	0.024	2.259	0.997 (0.986-1.008)	0.117
APTT	-1.282	0.368	8.941	0.604 (0.275-0.932)	<0.001*
TT	-0.091	0.005	1.273	0.998 (0.991-1.005)	0.241
FIB	0.875	0.083	4.912	1.533 (1.128-1.937)	0.018*
D-D	1.469	0.592	21.788	1.896 (1.315-2.476)	<0.001*
PLT	0.008	0.006	0.079	1.008 (0.998-1.017)	0.356
AKP	1.385	0.394	12.041	2.470 (1.274-3.665)	<0.001*

BM (bone metastasis), TNM (tumor-node-metastasis), PT (prothrombin time), APTT (activated partial thromboplastin time), TT (thrombin time), FIB (fibrinogen), D-D (D-Dimer), PLT (blood platelet), AKP (alkaline phosphatase). *The result has statistical significance.

found that the coagulation function of many patients with cancer is abnormal, while part of them are characterized by hypercoagulability and prone to thrombosis, which is the prerequisite for blood metastasis of tumor cells [19]. This study indicates a significant difference in BM between patients with and without thrombus, suggesting that BM is more likely to occur when there is thrombosis.

FIB turns into fibrin monomer under the action of thrombin, and then further crosslinks into fibrin. Study have found that when the content of fibrin increases to a certain level, it will cause irreversible damage to vascular endothelial cells and promote the aggregation of PLT in blood [20]. FIB is a stress protein in the acute phase. Riihimäki et al. [21] point out a close correlation between FIB and metastasis of malignant tumors. This correlation mainly acts as a bridge which can increase the content of leukocytes, promote the adhesion between PLT and tumor cells, and thus protect tumor cells from damage. APTT mainly reflects whether the endogenous coagulation function of the body is normal. The decrease of APTT indicates that the body is in hypercoagulable state and may have a thrombotic disease. D-D reflects the fibrinolytic function. The increase of D-D usually indicates hypercoagulable state, secondary hyperfibrinolysis, disseminated intravascular coagulation and other diseases [22]. The current study finds that there are significant differences in FIB, APTT and D-D between BM group and non-BM group. Therefore, we speculate that patients with NSCLC are more prone to BM when they are in hypercoagulable state.

Osteoblasts will secrete AKP to repair the damaged bone if there is bone-destructive disease (fracture, malignant bone tumor, etc) [23]. Thus, AKP increases significantly when BM occurs. Kang et al. [24] have reported that the level of AKP in LC patients with BM is higher than those without BM. However, many other factors can lead to the increase of AKP, such as liver diseases or liver metastasis. Therefore, those factors are excluded in this study to ensure the inclusion criteria. Our results show that compared with non-BM group, the AKP in BM group is significantly increased, which is consistent with most studies [24, 25].

In this study, the results of univariate analysis show that thrombosis, clinical stage, TNM stage, PT, APTT, TT, FIB, D-D, PLT and AKP are the risk factors of BM in patients with NSCLC. However, after further multivariate logistic regression analysis, it is found that the independent risk factors of BM in patients with NSCLC are clinical stage III-IV, TNM stage T1-T3, TNM stage N2-N3, FIB, APTT, D-D and AKP. These findings suggest that the above factors have important clinical significance in judging BM of NSCLC.

There are some limitations in this study. Firstly, the relatively small sample size (because of our newly updated medical record system). Expanding our sample population will better balance the cases in each clinical stage, eliminate data bias and reduce errors as much as possible. Secondly, this is a study of single center, which relatively limiting the research region. Therefore, high-quality, large sample, and mul-

ticenter studies should be performed in our future clinical work to provide spine surgeons with the best evidence-based information.

Conclusion

In conclusion, clinical stage III-IV, TNM stage T1-T3, TNM stage N2-N3, FIB, APTT, D-D and AKP are the independent risk factors of BM in patients with Patients with these risk factors should be screened in time, which is of great significance to prevent bone related events and relieve pain. In addition, these risk factors may be considered as predictors of BM in patients with LC, providing evidence for the prevention and diagnosis of NSCLC in future work.

Disclosure of conflict of interest

None.

Address correspondence to: Qiquan Yu, Department of Thoracic Surgery, Long Hua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China. E-mail: yqq971151@163.com

References

[1] Nasim F, Sabath BF and Eapen GA. Lung cancer. Med Clin North Am 2019; 103: 463-473.
[2] Popper HH. Progression and metastasis of lung cancer. Cancer Metastasis Rev 2016; 35: 75-91.
[3] Jung K and Lein M. Bone turnover markers in serum and urine as diagnostic, prognostic and monitoring biomarkers of bone metastasis. Biochim Biophys Acta 2014; 1846: 425-438.
[4] Chua KN, Kong LR, Sim WJ, Ng HC, Ong WR, Thiery JP, Huynh H and Goh BC. Combinatorial treatment using targeted MEK and SRC inhibitors synergistically abrogates tumor cell growth and induces mesenchymal-epithelial transition in non-small-cell lung carcinoma. Oncotarget 2015; 6: 29991-30005.
[5] Taniguchi Y, Tamiya A, Nakahama K, Naoki Y, Kanazu M, Omachi N, Okishio K, Kasai T and Atagi S. Impact of metastatic status on the prognosis of EGFR mutation-positive non-small cell lung cancer patients treated with first-generation EGFR-tyrosine kinase inhibitors. Oncol Lett 2017; 14: 7589-7596.
[6] Kuchuk M, Kuchuk I, Sabri E, Hutton B, Clemons M and Wheatley-Price P. The incidence and clinical impact of bone metastases in non-small cell lung cancer. Lung Cancer 2015; 89: 197-202.
[7] Divella R, Daniele A, Abbate I, Bellizzi A, Savino E, Simone G, Giannone G, Giuliani F, Fazio V, Gadaleta-Caldarola G, Gadaleta CD, Lolli I, Sabbà C and Mazzocca A. The presence of clustered circulating tumor cells (CTCs) and circulating cytokines define an aggressive phenotype in metastatic colorectal cancer. Cancer Causes Control 2014; 25: 1531-1541.
[8] Sun Y, Guan Z, Liao M, Yu X, Wang C, Wang J, Niu X, Shi Y, Zhi X, Liu Y, Liu M, Zhang Y, Yang Y, Shen J, Chen G, Zhou Q, Zhou C, Guo Q, Tang L, Duan J, Liang J, Zhang Y and Cheng Y. Expert consensus on the diagnosis and treatment of bone metastasis in lung cancer (2014 version). Zhongguo Fei Ai Za Zhi 2014; 17: 57-72.
[9] Tsuya A, Kurata T, Tamura K and Fukuoka M. Skeletal metastases in non-small cell lung cancer: a retrospective study. Lung Cancer 2007; 57: 229-232.
[10] Zhang L and Gong Z. Clinical characteristics and prognostic factors in bone metastases from lung cancer. Med Sci Monit 2017; 23: 4087-4094.
[11] Oliveira MB, Mello FC and Paschoal ME. The relationship between lung cancer histology and the clinicopathological characteristics of bone metastases. Lung Cancer 2016; 96: 19-24.
[12] da Silva GT, Bergmann A and Thuler LCS. Incidence and risk factors for bone metastasis in non-small cell lung cancer. Asian Pac J Cancer Prev 2019; 20: 45-51.
[13] Zhao JC, Liu JW and Ma JX. Risk factors of lymph node dissection and postoperative bone metastasis in minimally invasive resection of non-small cell lung cancer. Modern Oncology 2020; 28: 1311-1315.
[14] Conen K, Hagmann R, Hess V, Zippelius A and Rothschild SI. Incidence and predictors of bone metastases (BM) and skeletal-related events (SREs) in small cell lung cancer (SCLC): a swiss patient cohort. J Cancer 2016; 7: 2110-2116.
[15] Zhang C, Mao M, Guo X, Cui P, Zhang L, Xu Y, Li L, Han X, Peltzer K, Xiong S, Baklaushev VP, Wang X and Wang G. Nomogram based on homogeneous and heterogeneous associated factors for predicting bone metastases in patients with different histological types of lung cancer. BMC Cancer 2019; 19: 238.
[16] Saito M, Kage H, Ando T, Sawada R, Amano Y, Goto Y, Shinoda Y and Nagase T. Prevalence of bone pain decreases as lymph node stage increases in non-small cell lung cancer patients. Curr Probl Cancer 2019; 43: 86-91.
[17] Ayan AK, Erdemci B, Orsal E, Bayraktutan Z, Akpinar E, Topcu A, Turkeli M and Seven B. Is there any correlation between levels of serum ostepontin, CEA, and FDG uptake in lung cancer patients with bone metastasis? Rev Esp Med Nucl Imagen Mol 2016; 35: 102-106.

[18] Bates JE and Milano MT. Prognostic significance of sites of extrathoracic metastasis in patients with non-small cell lung cancer. J Thorac Dis 2017; 9: 1903-1910.
[19] Tang C, Liao Z, Hess K, Chance WW, Zhuang Y, Jensen G, Xu T, Komaki R and Gomez DR. Prognosis and predictors of site of first metastasis after definitive radiation therapy for non-small cell lung cancer. Acta Oncol 2016; 55: 1022-1028.
[20] Qi Y and Fu J. Research on the coagulation function changes in non small cell lung cancer patients and analysis of their correlation with metastasis and survival. J BUON 2017; 22: 462-467.
[21] Riihimäki M, Hemminki A, Fallah M, Thomsen H, Sundquist K, Sundquist J and Hemminki K. Metastatic sites and survival in lung cancer. Lung Cancer 2014; 86: 78-84.
[22] Nakano K, Sugiyama K, Satoh H, Shiromori S, Sugitate K, Arifuku H, Yoshida N, Watanabe H, Tokita S, Wakayama T, Tatewaki M, Souma R, Koyama K, Hirata H and Fukushima Y. Risk factors for disseminated intravascular coagulation in patients with lung cancer. Thorac Cancer 2018; 9: 931-938.
[23] Renaud S, Seitlinger J, Falcoz PE, Schaeffer M, Voegeli AC, Legrain M, Beau-Faller M and Massard G. Specifc KRAS amino acid substitutions and EGFR mutations predict site-specifc recurrence and metastasis following non-small-cell lung cancer surgery. Br J Cancer 2016; 115: 346-353.
[24] Kang EJ, Lee SY, Kim HJ, Min KH, Hur GY, Shim JJ, Kang KH, Oh SC, Seo JH, Lee SY and Kim JS. Prognostic factors and skeletal-related events in patients with small cell lung cancer with bone metastases at the time of diagnosis. Oncology 2016; 90: 103-111.
[25] Roato I. Bone metastases: when and how lung cancer interacts with bone. World J Clin Oncol 2014; 5: 149-155.