1 **High Postoperative Monocyte Indicated Inferior Clinicopathological**
2 **Characteristics and Worse Prognosis in Lung Adenocarcinoma or**
3 **Squamous Cell Carcinoma after Lobectomy**

4 *Running Title: Postoperative Monocyte and Lung cancer*

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13 **Abstract**

14 **Background:** Peripheral monocyte count is an assessable parameter. Recently, evidence
 15 suggested an elevated preoperative monocyte counts predicting poor prognosis in malignancies.
 16 The aim of this study was to determine the prognostic effect of early postoperative blood
 17 monocyte count in patients with lung adenocarcinoma or squamous cell carcinoma following
 18 lobectomy.

19 **Methods:** We retrospectively reviewed patients with operated lung adenocarcinoma or squamous
 20 cell carcinoma from 2006 to 2011 in Western China Lung Cancer database. Univariate analysis on
 21 disease-free survival (DFS) and overall survival (OS) was performed using the Kaplan-Meier and
 22 log-rank tests, and multivariate analysis was conducted using the Cox proportional hazards
 23 regression model.

24 **Results:** There were 433 patients enrolled in our analysis. High postoperative elevated monocyte
 25 was associated with male gender ($P<0.001$), positive smoking history ($P=0.005$), and higher N
 26 stage ($P=0.002$) and higher tumor stage ($P=0.026$). Two-tailed log-rank test indicated patients
 27 with an early postoperative elevated monocyte count predicted a poor DFS and OS overall
 28 ($P<0.001$, $P<0.001$, respectively) as well as in subgroup analysis, and further presented as a
 29 promising independent prognostic factor for both DFS and OS (HR=2.991, 95%CI: 2.243-3.988,
 30 $P<0.001$; HR=2.705, 95%CI: 1.977-3.700, $P<0.001$, respectively) on multivariate analysis.
 31 However, no significance was detected for preoperative monocyte in multivariate analysis.

32 **Conclusions:** Elevated early postoperative peripheral monocyte count was an independent
 33 prognostic factor of poor prognosis and inferior clinicopathological features for patients with
 34 operable lung adenocarcinoma or squamous cell carcinoma by lobectomy.

35 **Key words:** Lung squamous carcinoma; Lung adenocarcinoma; Peripheral monocyte count;

36 Prognosis

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Introduction

Lung cancer was the leading cause of cancer-related death worldwide, with 5-year survival rates of less than 17% [1]. Among all types, non-small-cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. Adenocarcinoma and squamous cell carcinoma account for approximately 40% and 25-30% of lung cancers, respectively [1,3]. In the aspect of treatment, if operable, surgery provided the best chance to cure NSCLC [2], which also called for a comprehensive perioperative evaluation system.

The hypothesis of the relationship between cancer and inflammation has triggered researcher interests though it was not new. Back to 1863, chronic inflammation was firstly regarded as the origin of tumor cells, and the response of the human body towards malignancies was proven to be closely related with inflammation [3]. Examples included tissue necrosis factor (TNF), interleukin (IL)-1, IL-6, matrix metalloproteinases, vascular endothelial growth factor, etc. As for peripheral blood cells, monocytes/macrophages, neutrophils, dendritic cells, and natural killer cells form the first line of immune defense in normal situations [4], and some of them have been regarded as “superstars” for oncologists. Specifically, in real clinical work, elevated preoperative monocyte counts have recently been shown to predict poor prognosis in various types of malignancies, including hepatic cell carcinoma, malignant lymphomas as well as lung adenocarcinoma [5,6,7]. However, it was still unclear if an elevated early postoperative monocyte count was associated with a poor prognosis in lung adenocarcinoma or squamous cell carcinoma after lobectomy. We focused on the early postoperative period because it was the most turbulent stage of the host immune system which may be caused by the surgical trauma and/or tumor removal effect, and we hypothesized that this change in immune environment related with tumor progression. The aim of this study was to evaluate whether an elevated early postoperative monocyte count predicted a poor prognosis in patients with operable lung adenocarcinoma or squamous cell carcinoma after lobectomy.

Materials and Methods

Study population

The data were retrieved from Western China Lung Cancer database (WCLC). We enrolled patients

with operable lung adenocarcinoma or squamous cell carcinoma treated with lobectomy at West China Hospital, Sichuan University between 2006 and 2011. All patients were >18 years of age, with complete clinicopathological data, and proven to be lung adenocarcinoma or squamous cell carcinoma after surgery. Preoperative evaluation included physical examination, blood routine examination, tumor markers test, chest X-ray and computed tomography, brain magnetic resonance imaging (MRI), bone scintigraphy, and bronchoscopy and integrated positron emission tomography scan and CT (PET/CT) scan when necessary. The eligibility criteria were: 1) lobectomy with no microscopic residual tumor; 2) no preoperative chemotherapy and/or radiotherapy; 3) no previous history of other malignancies; 4) no evidence of infections such as pneumonia; 5) availability of laboratory data and follow-up information. The peripheral venous blood samples were collected from patients within one week before and within 4 days after surgery, the latter meant the first postoperative blood sample taken from the patient must be within 4 days, and only the first sample after surgery was recorded. Absolute peripheral blood count and the percentage were analyzed for each blood sample. Histological classification was made with reference to the latest WHO guideline [8]. The stages of lung cancer were confirmed based on the 7th edition of TNM classification of malignant tumors [9]. The study approval was granted by the Institutional Review Board at the West China Hospital, Sichuan University.

Evaluation of clinicopathological factors

Baseline characteristics included age, sex, underlying diseases, smoking history, pathological stage, pathological tumor status, pathological lymph node status, and peripheral blood counts and the percentages. Blood samples used for analysis were taken within one week before and within 4 days after surgery performed within the clinical laboratory of our hospital via Cell-Dyn 3700 (Abbott Diagnostics, USA). Other data included the surgical date and procedures.

Treatment and follow-up

Lobectomy was performed on all patients with intent to cure, and systematic nodal dissection was carried out. The resection was done both macroscopically and histologically completely with a negative tumor margin and no evidence of distant metastasis. Patients were regularly followed up at outpatient department 1 month after surgery, every 3 months for the first year, every 6 months for the next four years, and once annually thereafter. Patients received a physical examination,

blood routine examination, Chest and brain and upper abdomen CT scan at each follow-up. Bone scintigraphy was performed every 12 months. Particularly, all the patients treated in our department received phone call following-up regularly, during which we recorded their living status, tumor recurrence/metastasis condition, and sequential treatments such as chemotherapy and/or radiotherapy, etc. The patients were followed until August 31, 2017 or until they died.

Statistical analysis

Receiver operating characteristic (ROC) curve was performed to search the best cut-off value for monocyte count to stratify patients at a high risk of tumor recurrence, distant metastasis, or death. In the ROC curve, the point with the maximum sensitivity and specificity was selected as the best cut-off value. Disease-free survival (DFS) was calculated from the date of surgery to the date of recurrence/metastasis or death with any cause, and overall survival (OS) was presented from the date of surgery to the date of death with any cause. DFS and OS were both censored at the date of the last follow-up. Fisher's exact test or χ^2 -test for categorical variables and t-test for continuous variables were used to analyze the clinicopathological features for the two groups divided by the cut-off value of monocyte. Life table method was conducted to calculate 1, 3, 5-year survival rate. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. The prognostic factors of OS and DFS were analyzed by Cox proportional hazard model with univariate and multivariate analysis. Factors proven significant in the univariate analysis were included in the multivariate analysis. Subgroup analysis was used to further discriminate tumor prognosis between monocyte count and other prognostic factors: pathological stage, histological type, etc. All *P* values were two-tailed with less than 0.05 considered to be statistically significant. Statistical analysis was performed using SPSS (SPSS version 19.0, Chicago, IL, USA) and STATA 14.0 (STATA Corporation, College Station, TX, USA). Survival curves were drawn by GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

Results

The selection process of patients was showed in Figure 1. There were 1665 patients identified totally. 1232 patients were excluded for cancer type, pathological stage, or surgical procedure mismatch, previous history of malignancies or chemotherapy/radiotherapy, and those who

combined with infections. Finally, there were 433 patients enrolled in our analysis. Among them, there were 278(64.2%) male and 155(35.8%) female. The average age of year's old and standard difference were 60.6 and 10.0, respectively. Video-assisted thoracic surgery (VATS) was performed in 221(51.0%) patients compared with traditional thoracotomy surgery in 212(49.0%) patients. Specimens were histologically proven to be lung adenocarcinoma in 264(61.0%) patients and squamous cell carcinoma in 169(39.0%) patients. The details were presented in Table 1.

By analyzing the ROC curve, the cut-off value for preoperative monocyte count was $0.375 \times 10^9/L$ with the area under the curve (AUC) of 0.566; while for postoperative monocyte count, the cut-off value was $0.845 \times 10^9/L$ with AUC of 0.692 (Figure 2). The baseline characteristics were stratified by high versus low preoperative monocyte and early postoperative monocyte count (Table 1). The preoperative monocyte was detected to be associated with male gender ($P<0.001$), smoking history ($P<0.001$), T stage ($P=0.014$) and histological type ($P<0.001$). For early postoperative group, compared to the low monocyte count group, male gender ($P<0.001$), positive smoking history ($P=0.005$), and higher N stage ($P=0.002$) and higher tumor stage ($P=0.026$) were more common found in high monocyte count group. The association between preoperative or postoperative monocyte and clinical characteristics were presented in Table 1 in details.

We further performed Kaplan-Meier method to identify difference of survival rates between two groups stratified by monocyte count (low vs. high) of both preoperative and early postoperative group. For preoperative group, the 1-year OS of low and high subgroups were 95% and 93% ($P=0.003$), and the 1-year DFS of low and high subgroups were 82% and 85% ($P=0.106$). The 3-year OS of low and high subgroups were 82% and 74% ($P=0.003$), and the 3-year DFS of low and high subgroups were 67% and 60% ($P=0.106$), respectively. Additionally, the 5-year OS of low and high subgroups were 72% and 58% ($P=0.003$), and the 5-year DFS of low and high subgroups were 62% and 51% ($P=0.106$). As for the early postoperative group, the 1-year OS of low and high subgroups were 96% and 90% ($P=0.004$), and the 1-year DFS of low and high subgroups were 90% and 71% ($P<0.001$). The 3-year OS of low and high subgroups were 89% and 62% ($P<0.001$), and the 3-year DFS of low subgroup was 78% compared to 41% in high group ($P<0.001$). In addition, the 5-year OS of low and high subgroups were 79% and 46% ($P<0.001$), and the 5-year DFS of low and high subgroups were 72% and 33% ($P<0.001$). Overall,

an early postoperative elevated monocyte count was significantly associated with poor OS ($P<0.001$) and DFS ($P<0.001$) (Figure 3). In further analysis, high level postoperative monocyte count was associated with poor OS and DFS in both adenocarcinoma and squamous carcinoma subgroups (Figure 4). These differences were also significant in subgroup analysis when stratified by gender, age, smoking history, stage, T stage, N stage and surgical procedure in postoperative group (Figure 5, Figure 6). On the contrary, no statistical significance was seen in subgroup analysis of preoperative group.

Univariate prognostic analysis was performed to detect the prognostic significance of clinicopathological factors, and preoperative and early postoperative blood monocyte count (Table 2). N stage ($P<0.001$), tumor stage ($P<0.001$), histological type ($P=0.014$), preoperative ($P=0.023$) and postoperative monocyte count level ($P<0.001$) were significantly associated with DFS, while age ($P=0.017$), surgery procedure ($P=0.002$), T stage ($P=0.003$), N stage ($P<0.001$), tumor stage ($P<0.001$), preoperative ($P=0.024$) and postoperative monocyte count level ($P<0.001$) showed significant relationships with OS. The clinicopathological factors proved to be prognostic predictors in univariate analysis were included as covariates in further multivariate analysis (Table 3). Finally, thoracotomy (HR=1.520, 95%CI: 1.117-2.069, $P=0.008$), positive N status (HR=2.506, 95%CI: 1.625-3.864, $P<0.001$), squamous cell carcinoma (HR=0.633, 95%CI: 0.468-0.856, $P=0.003$) and high postoperative monocyte count (HR=2.991, 95%CI: 2.243-3.988, $P<0.001$) were risk factors with statistical significance for DFS. Correspondingly, age (HR=1.022, 95%CI: 1.005-1.038, $P=0.009$), thoracotomy (HR=1.700, 95%CI: 1.163-2.486, $P=0.006$), advanced tumor stage (HR=2.253, 95%CI: 1.178-4.309, $P<0.001$), and high postoperative monocyte count (HR=2.705, 95%CI: 1.977-3.700, $P<0.001$) were observed as risk factors for OS. However, preoperative monocyte was no more associated with either DFS or OS within multivariate analysis.

Discussion

Immune response was an essential component of tumor progression. Studying these responses within tumor microenvironment via several immune factors can further stratify the prognosis of cancer [10]. Back to 1997, Negus et al. had demonstrated a number of cells expressed chemokine

202 receptors could infiltrate to tumor areas [11]. Later on, an increasing number of experimental
203 studies found that inflammation played a crucial role in tumor progression. Inflammatory markers
204 such as C-reactive protein in esophageal squamous cancer [12], Colony-stimulating factor-1 in
205 mammary tumor [13], and inhibitors of metalloproteinases in NSCLC [14], all have been
206 suggested as alternative markers for tumor progression [8]. Likewise, as easy assessable
207 parameters, peripheral blood cell counts have also been regarded as predictors of tumor prognosis.
208 An elevated neutrophil, monocyte and leukocyte counts were proven to be associated with poor
209 survival in patients with metastatic melanoma [15].

210 Monocytes belonged to circulating peripheral blood cells that played the crucial role in immune
211 response with the capability of differentiating into macrophages and antigen-presenting cells
212 (APCs). Thus, they formed the first line of innate immune defense [16]. In addition, monocytes
213 could activate T and B lymphocytes and further produce cytokines such as IL-12 and TNF- α to
214 stimulate the immune response [17]. However, either overstimulation or immunosuppression of
215 monocytes caused by surgical procedure and/or other external factors could disrupt the immune
216 system [18,19]. Previous studies have found an elevated preoperative monocyte count
217 demonstrated a poor prognostic factor in esophageal squamous cell carcinoma, mantle cell
218 lymphoma, follicular lymphoma, and classical Hodgkin lymphoma, respectively [20,21,22,23].

219 Also, for the early postoperative period, Franke et al. discovered an increase in the absolute
220 monocyte count but an impairment of monocyte function, indicating a decreased ability to
221 synthesize IL-12 and TNF- α , to express HLA-DR, and to act as the APC [24]. To the best of our
222 knowledge, this was the first study showing the prognostic significance of the early postoperative
223 monocyte count in patients with lung adenocarcinoma or squamous cell carcinoma after
224 lobectomy.

225 The mechanisms of elevated monocyte count related with poor prognosis of several kinds of
226 tumors were still not clearly elucidated. There were several possible explanations. First, it was
227 hypothesized that monocytes are attracted by several cytokines or chemokines to the tumor site
228 and then differentiated into tumor-associated macrophages (TAMs), which further promoted those
229 invading leukocytes to bring out potentials of angiogenesis, motility, and invasion [25,26].
230 Angiogenic signals from surrounding cells resulted in vasodilatation and increased vascular

permeability [27,28], forming a vicious cycle for tumor progression. Second, human monocyte subsets were differentiated according to their surface CD14/CD16 expression as “majority/classical” (CD14++CD16-), “minority/non-classical” (CD14+CD16+) and the subset with pro-angiogenic feature (CD14++CD16+CCR2+) [29,30]. Among all subsets of the monocytes, the “majority/classical” accounts for approximately 90% of monocytes in healthy people [31]. However, Schauer et al. found that along with the increased number of monocytes, the major subsets shifted from CD16- to CD16+ after liver resection, showing a stronger potential of angiogenesis [32]. Correspondingly, while the classical monocytes were recruited to tumor sites, contributing to tumor macrophage content and promote tumor growth and metastasis, Richard N. Hanna et al. also found out the potential protective role of nonclassical “patrolling” monocytes tumor growth and metastasis [33]. This was also our next step to find out whether it will happen after lobectomy of lung cancer and what the exact type was and the alternatives of the function in immunity. Third, peripheral monocytes grow into TAMs when entering tumor areas. TAMs are classified into two phenotypes: M1 and M2. Activated M1 macrophages have the anti-tumor response, while M2 macrophages, activated by tumor-derived cytokines, were suitable for tumor development [34,35]. A previous study has reported that circulating macrophages predict tumor recurrence after surgery in patients with NSCLC [36], while in this study, an elevated early postoperative peripheral monocyte count was significantly associated with a poor prognosis in patients with lung adenocarcinoma or squamous cell carcinoma after lobectomy. These results might suggest a complex association between peripheral TAMs (M2 type) and monocytes, which still called further studies to verify. Moreover, the reason why we focused on the early postoperative period--the most turbulent stage of host immune system caused by surgical trauma and/or tumor removal effect probably was that we hypothesized that change in this period of immune environment might relate with tumor progression more closely than that of the preoperative data.

In the present study, an elevated early postoperative monocyte count was shown to predict a poor DFS and OS both in the univariate and multivariate analysis. In addition, as shown in the subgroup analysis, the monocyte count was found to be significantly associated with poor prognosis when stratified by gender, age, smoking history, TNM stage, surgical procedure and

260 histological type. All of the above indicated that an elevated early postoperative blood monocyte
261 count to be a very strong prognostic factor in tumor progression.

262 Limitations of the current study are inherent to its design, including the retrospective data
263 collection and several confounding factors when comparing postoperatively. Moreover, the small
264 number of patients, especially the cases with endpoints, also limited the conclusion of the current
265 study.

266

267 **Conclusion**

268 The present study supported the prognostic significance of early postoperative peripheral blood
269 monocyte count in patients with operable lung adenocarcinoma or squamous cell carcinoma after
270 lobectomy in both OS and DFS. This easily measured blood parameter may provide useful
271 information for the clinicians to stratify patients. Further investigations were still needed to figure
272 out the oncological significance of monocyte and its subsets, the association with host
273 inflammatory microenvironment.

274

275 **Abbreviations**

276 OS: overall survival

277 DFS: disease-free survival

278 NSCLC: non-small cell lung cancer

279 TNF: tissue necrosis factor

280 IL: interleukin

281 MRI: magnetic resonance imaging

282 PET-CT: positron emission tomography scan and CT

283 ROC: receiver operating characteristic

284 VATS: video-assisted thoracic surgery

285 AUC: area under the curve

286 APCs: antigen-presenting cells

287 TAMs: tumor-associated macrophages

288

289 **Declarations**

290 **Ethics approval and consent to participate**

291 The study approval was approved by the Institutional Review Board at the West China Hospital,
292 Sichuan University. All participants have signed the consent to patients for participating in this
293 study.

294 **Consent for publication**

295 Not applicable

296 **Availability of data and material**

297 The datasets generated and analysed during the current study are not publicly available due to
298 confidential agreement but are available from the corresponding author on reasonable request.

299 **Competing interests**

300 The authors declare that they have no competing interests.

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304 **Authors' contributions**

305 LXL conceptualized the study and critically read the manuscript. YH, NC, WWW, ZHW, FL, CLG
306 and CWL performed and/or assisted surgery, managed patients, and participated in data analysis.
307 YH and NC wrote the manuscript. All authors read and approved the final manuscript

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311 **References**

- 312 1. Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin. 2018 Jan;68(1):7-30.
- 313 2. Non-Small Cell Lung Cancer. In: Detailed Guide. The American Cancer Society. 2016.
314 <http://www.cancer.org/cancer/lungcancer-non-smallcell/detailedguide/index>. Accessed 25
315 October 2016
- 316 3. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow. Lancet. 2001;357:
317 539–545.

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- 318 4. Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation:
319 a magic bullet. *Science*. 2013;339:286–91.
 - 320 5. Shen SL, Fu SJ, Huang XQ, Chen B, Kuang M, Li SQ, Hua YP, Liang LJ and Peng BJ.
321 Elevated preoperative peripheral blood monocyte count predicts poor prognosis for
322 hepatocellular carcinoma after curative resection. *BMC Cancer*. 2014;14:744.
 - 323 6. Porrata LF, Ristow K, Habermann TM, Witzig TE, Colgan JP, Inwards DJ, Ansell SM,
324 Micallef IN, Johnston PB, Nowakowski GS, Thompson C, Markovic SN. Peripheral blood
325 lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma.
326 *Haematologica*. 2012;97:262–9.
 - 327 7. Kumagai S, Marumo S, Shoji T, Sakuramoto M, Hirai T, Nishimura T, Arima N, Fukui M,
328 Huang CL. Prognostic impact of preoperative monocyte counts in patients with resected lung
329 adenocarcinoma. *Lung Cancer*. 2014;85:457-464.
 - 330 8. Travis WD, Bramvilla E, Muller-Hermelink Hk, Harris CC. Tumours of the lung, pleura,
331 thymus and heart. 4th ed. Lyon: International Agency for Research on Cancer; 2015
 - 332 9. Sobin LH, Gospodarowicz MK, Wittekind C. International Union Against Cancer (UICC):
333 TNM classification of malignant tumours. 7th ed. UK: John Willkey and Sons Ltd; 2010
 - 334 10. Dvorak HF, Weaver VM, Tlsty TD, Bergers G. Tumor microenvironment and progression. *J*
335 *Surg Oncol*. 2011;103:468–474.
 - 336 11. Negus RPK, Stamp GWQ, Hadley J, Balkwill FR. Quantitative assessment of the leucocyte
337 infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. *Am J*
338 *Pathol*. 1997;150:1723–34.
 - 339 12. Badakhshi H, Kaul D, Zhao KL. Association between the inflammatory biomarker, C-reactive
340 protein, and the response to radiochemotherapy in patients with esophageal cancer. *Mol Clin*
341 *Oncol*. 2016;4:643-647.
 - 342 13. Domínguez SA, Sierra FE, Puig KA, Pérez MB, Gómez AF, Corcuera MT, Sánchez MP,
343 Corbí AL. Dendritic cell-specific ICAM-3-grabbing nonintegrin expression on M2-polarized
344 and tumor-associated macrophages is macrophage-CSF dependent and enhanced by
345 tumor-derived IL-6 and IL-10. *J Immunol*. 2011;186:2192-200.
 - 346 14. Gouyer V, Conti M, Devos P, Zerimech F, Copin MC, Créme E, Wurtz A, Porte H, Huet G.

347 Tissue inhibitor of metalloproteinase 1 is an independent predictor of prognosis in patients
348 with nonsmall cell lung carcinoma who undergo resection with curative intent. *Cancer*. 2005;
349 103:1676-1684.

350 15. Gandini S, Ferrucci PF, Botteri E, Tosti G, Barberis M, Pala L, Battaglia A, Clerici A,
351 Spadola G, Cocorocchio E, Martinoli C. Prognostic significance of hematological profiles in
352 melanoma patients. *Int J Cancer*. 2016;139:1618-25.

353 16. Kyoizumi S, Kubo Y, Kajimura J, Yoshida K, Hayashi T, Nakachi K, Young LF, Moore MA,
354 van den Brink MR, Kusunoki Y. Linkage between dendritic and T cell commitments in
355 human circulating hematopoietic progenitors. *Immunol*. 2014;192:5749-60

356 17. Chen HW, Chen HY, Wang LT, Wang FH, Fang LW, Lai HY, Chen HH, Lu J, Hung MS,
357 Cheng Y, Chen MY, Liu SJ, Chong P, Lee OK, Hsu SC. Mesenchymal stem cells tune the
358 development of monocyte-derived dendritic cells toward a myeloid-derived suppressive
359 phenotype through growth-regulated oncogene chemokines. *J Immunol*. 2013;190:5065 -77.

360 18. Nguyen BA, Fiorentino F, Reeves BC, Baig K, Athanasiou T, Anderson JR, Haskard DO,
361 Angelini GD, Evans PC. Mini Bypass and Proinflammatory Leukocyte Activation: A
362 Randomized Controlled Trial. *Ann Thorac Surg*. 2016;101:1454-63.

363 19. Fragiadakis GK, Gaudillière B, Ganio EA, Aghaeepour N, Tingle M, Nolan GP, Angst MS.
364 Patient-specific Immune States before Surgery Are Strong Correlates of Surgical Recovery.
365 *Anesthesiology*. 2015;123:1241-55.

366 20. Kumagai S, Marumo S1, Shoji T, Sakuramoto M, Hirai T, Nishimura T, Arima N, Fukui M,
367 Huang CL. Prognostic impact of preoperative monocyte counts in patients with resected lung
368 adenocarcinoma. *Lung Cancer*. 2014;85:457-464.

369 21. von Hohenstaufen KA, Conconi A, de Campos CP, Zucca E. Prognostic impact of monocyte
370 count at presentation in mantle cell lymphoma. *Br J Haematol*. 2013;162:465–73.

371 22. Wilcox RA, Ristow K, Habermann TM, Inwards DJ, Micallef IN, Johnston PB, Colgan JP,
372 Nowakowski GS, Ansell SM, Witzig TE, Markovic SN, Porrata L. The absolute peripheral
373 monocyte count is associated with overall survival in patients newly diagnosed with follicular
374 lymphoma. *Leuk Lymphoma*. 2012;53:575–80.

375 23. Porrata LF, Ristow K, Colgan JP, Habermann TM, Witzig TE, Inwards DJ, Ansell SM,

376 Micallef IN, Johnston PB, Nowakowski GS, Thompson C, Markovic SN. Peripheral blood
377 lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma.
378 Haematologica. 2012; 97:262–9.

379 24. Franke A, Lante W, Fackeldey V, Becker HP, Kurig E, Zöller LG, Weinhold C, Markewitz A.
380 Pro-inflammatory cytokines after different kinds of cardio-thoracic surgical procedures: is
381 what we see what we know. Eur J Cardiothorac Surg. 2015;28:569-575.

382 25. Wu H, Xu JB, He YL, Peng JJ, Zhang XH, Chen CQ, Li W, Cai SR. Tumor-associated
383 macrophages promote angiogenesis and lymphangiogenesis of gastric cancer. J Surg Oncol.
384 2012;106:462–468.

385 26. Jaipersad AS, Lip GY, Silverman S, Shantsila E. The role of monocytes in angiogenesis and
386 atherosclerosis. J Am Coll Cardiol. 2014;63: 1-11.

387 27. Kimura H, Esumi H. Reciprocal regulation between nitric oxide and vascular endothelial
388 growth factor in angiogenesis. Acta Biochim Pol. 2003;50:49–59.

389 28. Pamukcu B, Lip GY, Devitt A, Griffiths H, Shantsila E. The role of monocytes in
390 atherosclerotic coronary artery disease. Ann Med. 2010;42:394–403.

391 29. González D, Domínguez S, Nieto C. Atypical Activin A and IL-10 Production Impairs
392 Human CD16+ Monocyte Differentiation into Anti-Inflammatory Macrophages. J Immunol.
393 2016;196:1327-37.

394 30. Shantsila E, Wrigley B, Tapp L, Apostolakis S, Montoro-Garcia S, Drayson MT, Lip GY.
395 Immunophenotypic characterization of human monocyte subsets: possible implications for
396 cardiovascular disease pathophysiology. J Thromb Haemost. 2011;9:1056–66.

397 31. Sbrana S, Parri MS, De Filippis R, Gianetti J, Clerico A. Monitoring of monocyte functional
398 state after extracorporeal circulation: A flow cytometry study. Cytometry. 2004;58B:17.

399 32. Schauer D, Starlinger P, Zajc P. Monocytes with angiogenic potential are selectively induced
400 by liver resection and accumulate near the site of liver regeneration. BMC Immunol. 2014;15:
401 50.

402 33. Hanna RN, Cekic C, Sag D, Tacke R, Thomas GD, Nowyhed H, Herrley E, Rasquinha N,
403 McArdle S, Wu R, Peluso E, Metzger D, Ichinose H, Shaked I, Chodaczek G, Biswas SK,
404 Hedrick CC. Patrolling monocytes control tumor metastasis to the lung.Science.2015;

405 350:985-90.

406 34. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization:
407 tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.
408 Trends Immunol. 2002;23:549–55.

409 35. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression andmetastasis. Cell.
410 2010;141:39–51.

411 36. Maeda R, Ishii G, Neri S, Aoyagi K, Haga H, Sasaki H, Nagai K, Ochiai A. Circulating
412 CD14+CD204+ cells predict postoperative recurrence in non-small-cell lung cancer patients.
413 J Thorac Oncol. 2014;9:179–8.

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Figure legends

Figure. 1 Flow chart of patient selection

Figure. 2 Receiver operating characteristic curve for determination of the cut-off value for monocyte. The cut-off value for preoperative monocyte count was $0.375 \times 10^9/L$ with area under the curve (AUC) of 0.566; while $0.845 \times 10^9/L$ with AUC of 0.692 for postoperative monocyte count.

Figure. 3 Disease-free survival(a) and overall survival(b) of high and low postoperative monocyte level.

Figure. 4 Disease-free survival and overall survival of 433 lung cancer patients with different monocyte stratified by histological type of adenocarcinoma(a,b) and squamous cell carcinoma(c,d).

Figure. 5 Disease-free survival and overall survival of 433 lung cancer patients with different monocyte stratified by tumor stage(stage I disease:a,b; stage II disease:c,d; stage IIIA disease:e,f.)

Figure. 6 Disease-free survival and overall survival of 433 lung cancer patients with different monocyte stratified by N stage(N0 stage:a,b; N1 stage:c,d; N2 stage:e,f.)

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Table 1. Basic characteristics of clinicopathological features of patients with different monocyte counts

Category	N (%)	Preoperative monocyte ($10^9/L$)		Postoperative monocyte ($10^9/L$)	
		<0.375	≥ 0.375	<0.845	≥ 0.845
Gender					
Male	278(64.2%)	122	156	155	123
Female	155(35.8%)	128	27	116	39
Age	433(100%)	61.08 $\pm$ 9.74	59.9 $\pm$ 10.47	60.2 $\pm$ 10.05	61.21 $\pm$ 10.08
Smoking history					
Yes	258(59.7%)	115	143	148	110
No	174(40.3%)	135	39	123	51
Location					
Left superior	128(29.6%)	74	54	80	48
Left inferior	62(14.3)	39	23	40	22
Right superior	128(29.6%)	79	49	80	48
Right middle	44(10.2)	24	20	24	20
Right inferior	71(16.4%)	34	37	47	24
Procedure					
VATS	221(51.5%)	139	82	148	73
Thoracotomy	212(49%)	111	101	123	89
T stage					
T1	84(19.4%)	58	26	50	34
T2	279(64.4%)	161	118	178	101
T3	55(12.7%)	26	29	31	24
T4	15(3.5%)	5	10	12	3
N stage					
N0	268(62.0%)	158	110	184	84
N1	71(16.4%)	35	36	42	29
N2	93(21.5%)	56	37	45	48
Stage					
I	205(47.3%)	126	79	139	66
II	107(24.7%)	59	48	68	39
IIIA	121(27.9%)	65	56	64	57
Histological type					
Adenocarcinoma	259(59.8%)	181	78	168	91
Squamous carcinoma	174(40.2%)	69	105	103	71
Differentiation					
Well	11(2.6%)	8	3	9	2
Moderate	261(61.7%)	155	106	159	102
Poor	151(35.7%)	80	71	98	53

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T stage, tumour; N stage, node; VATS, Video-assistant thoracoscopic surgery; N, cases sample size

^a Significance of Fisher's exact test, X²-test or t-test. *P*<0.05 was considered statistically significant.

459 **Table 2.** Univariate analysis of clinicopathological parameters and inflammatory biomarkers influencing prognosis

Category	Variables	Disease-free survival		Overall survival	
		HR (95% CI)	P ^a	HR (95% CI)	P ^a
Gender	Male	Reference		Reference	
	Female	0.963(0.716-1.295)	0.801	0.841(0.581-1.216)	0.358
Age	Per year	1.008(0.993-1.022)	0.311	1.023(1.004-1.042)	0.017
Smoking history	Yes	Reference		Reference	
	No	1.009(0.756-1.348)	0.950	0.838(0.585-1.202)	0.337
Location	Left superior	Reference		Reference	
	Left inferior	0.989(0.634-1.545)	0.962	1.492(0.870-2.560)	0.146
	Right superior	0.875(0.604-1.267)	0.479	1.205(0.758-1.916)	0.431
	Right middle	1.263(0.785-2.031)	0.335	1.486(0.817-2.700)	0.194
	Right inferior	0.655(0.410-1.047)	0.077	0.899(0.506-1.596)	0.715
Procedure	VATS	Reference		Reference	
	Thoracotomy	1.455(1.093-1.937)	0.010	1.760(1.236-2.505)	0.002
T stage	T1	Reference		Reference	
	T2	1.219(0.833-1.783)	0.309	1.268(0.775-2.072)	0.344
	T3	1.559(0.945-2.573)	0.082	2.453(1.361-4.421)	0.003
	T4	1.376(0.609-3.111)	0.443	1.602(0.601-4.271)	0.346
N stage	N0	Reference		Reference	
	N1	2.467(1.695-3.592)	<0.001	2.869(1.833-4.491)	<0.001
	N2	3.349(2.414-4.647)	<0.001	3.442(2.306-5.139)	<0.001
Stage	I	Reference		Reference	
	II	1.847(1.273-2.679)	0.001	2.342(1.460-3.757)	<0.001
	IIIA	3.163(2.260-4.428)	<0.001	4.078(2.658-6.258)	<0.001
Histological type	Adenocarcinoma	Reference		Reference	
	Squamous carcinoma	0.687(0.508-0.928)	0.014	1.117(0.787-1.587)	0.535
Differentiation	Well	Reference		Reference	
	Moderate	6.446(0.899-46.191)	0.064	3.869(0.538-27.831)	0.179
	Poor	7.172(0.996-51.643)	0.050	4.340(0.599-31.455)	0.146
Preoperative monocyte	<0.375	Reference		Reference	
	≥0.375	1.391(1.047-1.849)	0.023	1.490(1.053-2.109)	0.024

	Postoperative	< 0.845	Reference	Reference	
	monocyte				
		≥ 0.845	3.974(2.956-5.342)	<0.001	3.826(2.662-5.499) <0.001
460	T stage, tumor; N stage, node; VATS, Video-assistant thoroscopic surgery; HR, hazard ratio; CI, confidence				
461	interval.				
462	^a <i>P</i> value of univariate analysis. <i>P</i> <0.05 was considered statistically significant.				

Table 3. Multivariate analysis of clinicopathological parameters and inflammatory biomarkers influencing prognosis

Category	Variables	HR (95% CI)	<i>P</i> ^a
<i>Disease-free survival</i>			
Procedure	VATS	Reference	
	Thoracotomy	1.520(1.117-2.069)	0.008
N stage	N0	Reference	
	N1	2.319(1.584-3.396)	<0.001
	N2	2.584(1.845-3.620)	<0.001
Histological type	Adenocarcinoma	Reference	
	Squamous carcinoma	0.561(0.405-0.779)	0.001
Postoperative monocyte	< 0.845	Reference	
	≥0.845	3.684(2.729-4.975)	<0.001
<i>Overall survival</i>			
Age	Per year	1.034(1.014-1.054)	0.001
Procedure	VATS	Reference	
	Thoracotomy	1.700(1.163-2.486)	0.006
Stage	I	Reference	
	II	2.228(1.383-3.592)	0.001
	IIIA	3.592(2.327-5.546)	<0.001
Postoperative monocyte	< 0.845	Reference	
	≥0.845	3.403(2.362-4.902)	<0.001

N stage, node; VATS, Video-assistant thoroscopic surgery; HR, hazard ratio; CI, confidence interval.

^a *P* value of multivariate analysis. *P*<0.05 was considered statistically significant.

ROC curve

(a)

Disease free survival

(b)

Overall Survival

Patients who underwent surgery for lung cancer
during Jan. 2006 to Dec. 2011
n=1665

Metastatic lung cancer: n=56
Small cell lung cancer: n=20
NSCLC with neuroendocrine composition: n=35
Pathological-stage IIIB: n=78
Pathological-stage IV: n=64

Patients who underwent surgery for stage I, II, and
IIIA lung squamous carcinoma or adenocarcinoma
n=1412

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Wedge resection or segmentectomy: n=171
No lymph node dissection: n=103
Lymph node sampling: n=276

Patients with operable lung squamous carcinoma
or adenocarcinoma under lobectomy and lymph
node dissection
n=862

Previous history of chemotherapy/radiotherapy: n=31
Previous history of malignancies: n=56
Blood sample was collected over schedule: n=197
Died from postoperative complications: n=28
Combined with pre/postoperative infections: n=49

Patients with operable lung squamous carcinoma
or adenocarcinoma under lobectomy and lymph
node dissection meeting the above criteria
n=501

Lost to follow-up: n=68

Study population n=433