Format for ANSWERING REVIEWERS

August 25, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 2429-review.doc).

Title: Mucocele of the appendix: An unusual cause of lower abdominal pain in a patient with ulcerative. A case report and review of the literature

Author: Peter Laszlo Lakatos, Gabriella Gyori, Judit Halasz, Peter Fuszek, Janos Papp, Balazs Jaray, Peter Lukovich, Laszlo Lakatos

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 2429

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) ? Please specify the number of analyzed cases (291 vs. 254) in the abstract. Please provide exact numbers of tumor patients in this study. Authors claim that 291 patients have been investigated...however, in table 2 the number of patients is 254? The total number of cases in this study was 291 cases, but after CD15 immunohistochemical staining, only 254 cases of the presence of tumors, so after further analysis, the number of cases in this study is 254, and the subsequent statistical restatistics

(2)? What does off-chip means? Immunohistochemical staining process, the organization separated from the glass slide, leading to reduction in the number of cases

(3)? Please do not use abbreviations in the abstract and explain abbreviations when first used in the manuscript? overall survival time (OS); median overall survival time (mOS); disease free survival time (DFS); median disease free survival time (mDFS); relative risk (RR)

(4)? Abstract is of poor English quality and for the inexperienced reader in this scientific field not easily to understand ? **Abstract**

AIM: To explore the relationship of chinicopathological features, the neutrophils in the tumor microenvironment distribution with prognosis.**METHODS:** Two hundred and fifty-four formalin-fixed and paraffin embedded tissue blocks were analyzed in this study. Including tissues from tumor of cholangiocarcinoma (n = 254), and tumor adjacent tissues (n = 238). Tissue sections were stained for CD15 by immunohistochemical staining. CD15 is one of the marker of mature granulocytes, we observe CD15 expression to reflect the local tumor microenvironment distribution of neutrophils, and speculated that the tumor tissue and the adjacent tumor tissue neutrophil density, in order to reflect the tumor tissue and the adjacent tumor tissue inflammation. Analysis of Two

hundred and fifty-four cases of patients with cholangiocarcinoma tumor microenvironment local inflammatory state. Furthermore, Clinical data and the completed follow-up information of cholangiocarcinoma patients who underwent cholangiocarcinoma operation from January 2004 to December 2010 were analyzed retrospectively the relationship of clinicopathological features, neutrophils, and progonosis of the two hundred and fifty-four cases of patients.**RESULTS:** The positive expression rates of CD15 was significantly only related to the TNM stage. The differences in the positive expression rates of CD15 was significant between tumor tissues and tumor adjacent tissues. High CD15 expression in tumor tissues than in adjacent tissues(73.6% vs 54.6%). In tumor tissues with high expression of CD15 was significantly shorter overall survival time (OS) than low expression in CD15, the difference was statistically significant (median overall survival time (mOS) 39.77 months vs 16.87 months, P = 0.008), CD15 high expression were significantly shorter disease free survival time (DFS) than low expression in CD15, the difference was statistically significant (median disease free survival time (mDFS) 38.27 months vs 16.83 months, P = 0.029). By COX multivariate analysis, high expression CD15 in tumor tissues was an independment risk factor in predicting OS approximately for patients with cholangiocarcinoma(P = 0.012, relative risk (RR) =1.601), but high expression CD15 in tumor tissues was not an independment risk factor in predicting DFS approximately for patients with cholangiocarcinoma(P = 0.073, RR =1.462).**CONCLUSION:** Univariate analysis CD15 in cancer tissues with high expression DFS and OS shorter. Multivariate analysis in cancer tissue, CD15 high-expression is an independent risk factor for OS.

(5)? Please use a consistent way of citation?

[1] Welzel TM, Mellemkjaer L, Gloria G, Sakoda LC, Hsing AW, El Ghormli L, Olsen JH, McGlynn KA. Risk factors for intrahepatic cholangiocarcinoma in a low-risk population:a nationwide case-control study. Int J Cancer 2007; 120: 638–641[PMID:17109384 doi: 10.1002/ijc.22283]

[2] Donato F, Gelatti U, Tagger A, Favret M, Ribero ML, Callea F, Martelli C, Savio A, Trevisi P, Nardi G. Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. Cancer Causes Control 2001; 12: 959–964[PMID:11808716 doi: 10.1023/A:1013747228572]

[3] El-Serag HB, Engels EA, Landgren O, Chiao E, Henderson L, Amaratunge HC, Giordano TP. Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: a population-based study of U.S. veterans. Hepatology 2009; 49: 116–123[PMID:19085911 doi: 10.1002/hep.22606]

[4] Shaib YH, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. Gastroenterology 2005; 128: 620–626[PMID:15765398 doi: 10.1053/ j.gastro.2004. 12.048]

[5] Lee TY, Lee SS, Jung SW, Jeon SH, Yun SC, Oh HC, Kwon S, Lee SK, Seo DW, Kim MH, Suh DJ. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. Am J Gastroenterol 2008; 103: 1716–1720[PMID:18557716 doi: 10.1111/j.1572-0241.2008.01796.x]

[6] Zhou YM, Yin ZF, Yang JM, Li B, Shao WY, Xu F, Wang YL, Li DQ. Risk factors for intrahepatic cholangiocarcinoma: a case-control study in China.World J Gastroenterol 2008; 14: 632–635[PMID:18203300 doi: 10.3748/wjg.14.632]

[7] Sekiya S, Suzuki A. Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J Clin Invest 2012;122: 3914–3918[PMID:23023701 doi: 10.1172/JCI63065]

[8] Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, Takemura S, Tanaka H, Yamazaki O, Hirohashi K, Tanaka T. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci 2004;95: 592–595[PMID: 15245596 DOI: 10.1111/j.1349-7006.2004.tb02492.x]

[9] Chapman MH, Webster GJ, Bannoo S, Johnson GJ, Wittmann J,Pereira SP. Cholangiocarcinoma and dominant strictures in patients with primary sclerosing cholangitis: a 25-year single-centre experience. Eur J Gastroenterol Hepatol 2012; 24: 1051–1058[PMID:22653260 doi: 10.1097/MEG.0b013e3283554bbf]

[10] Bergquist A, Ekbom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S,Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. J Hepatol 2002;36: 321–327[PMID:11867174 DOI: 10.1016/S0168-8278(01)00288-4]

[11] Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ; American Association for the Study of LiverDiseases. Diagnosis and management of primary sclerosing cholangitis. Hepatology 2010; 51: 660–678[PMID:20101749 doi: 10.1002/hep.23294]

[12] Claessen MM, Vleggaar FP, Tytgat KM, Siersema PD,van Buuren HR. High lifetime risk of cancer in primary sclerosing cholangitis. J Hepatol 2009; 50: 158-164[PMID:19012991 doi: 10.1016/j.jhep.2008.08.013]

[13] Boberg KM, Bergquist A, Mitchell S, Pares A, Rosina F, Broomé U, Chapman R, Fausa O, Egeland T, Rocca G, Schrumpf E. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. Scand J Gastroenterol 2002; 37: 1205–1211[PMID:12408527 doi:10.1080/003655202760373434]

[14] Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma.Hepatology 2011; 54: 173–184[PMID:21488076 doi: 10.1002/hep.24351]

[15] European Association for the Study of the Liver. EASL clinical practice guidelines: management of

cholestatic liver diseases.J Hepatol 2009; 51: 237–267[PMID:19501929 doi: 10.1016/j.jhep.2009.04.009]

[16] Razumilava N, Gores GJ, Lindor KD. Cancer surveillance in patients with primary sclerosing cholangitis. Hepatology 2011;54: 1842–1852[PMID:21793028 doi: 10.1002/hep.24570]

[17] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell, 2011; 144: 646-674 [PMID: 21376230 doi: 10.1016/j.cell.2011.02.013]

[18] Hashimoto K, Ikeda Y, Korenaga D, Tanoue K, Hamatake M, Kawasaki K, Yamaoka T, Iwatani Y, Akazawa K, Takenaka K. The impact of preoperative serum C‐reactive protein on the prognosis of patients with hepatocellular carcinoma. Cancer, 2005; 103: 1856-1864 [PMID: 15779015 DOI: 10.1002/cncr.20976]

[19] Porta C, De Amici M, Quaglini S, Paglino C, Tagliani F, Boncimino A, Moratti R, Corazza GR. Circulating interleukin-6 as a tumor marker for hepatocellular carcinoma. Annals of oncology, 2008; 19: 353-358 [PMID:17962206 doi: 10.1093/annonc/mdn290]

[20] Jensen HK, Donskov F, Marcussen N, Nordsmark M, Lundbeck F, von der Maase H. Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. J Clin Oncol 2009; 27: 4709–4717 [PMID: 19720929 doi: 10.1200/JCO.2008.18.9498]

[21] Li YW1, Qiu SJ, Fan J, Zhou J, Gao Q, Xiao YS, Xu YF. Intratumoral neutrophils: A poor prognostic factor for hepatocellular carcinoma following resection. J Hepatol 2011; 54: 497–505 [PMID: 21112656 doi: 10.1016/j.jhep.2010.07.044]

[22] Schmidt H1, Bastholt L, Geertsen P, Christensen IJ, Larsen S, Gehl J, von der Maase H. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer 2005; 93: 273–278 [PMID:1605222 doi: 10.1038/sj.bjc.6602702]

[23] Schmidt H, Suciu S, Punt CJ, Gore M, Kruit W, Patel P, Lienard D, von der Maase H, Eggermont AM, Keilholz U. Pretreatment levels of peripheral neutrophils and leukocytes as independent predictors of overall survival in patients with American Joint Committee on Cancer Stage IV Melanoma: results of the EORTC 18951 Biochemotherapy Trial. J Clin Oncol 2007; 25: 1562–1569[PMID: 17443000 doi:10.1200/JCO.2006.09.0274]

[24] Paesmans M, Sculier JP, Lecomte J, Thiriaux J, Libert P, Sergysels R, Bureau G, Dabouis G, Van Cutsem O, Mommen P, Ninane V,Klastersky J. Prognostic factors for patients with small cell lung carcinoma: analysis of a series of 763 patients included in 4 consecutive prospective trials with a minimum follow-up of 5 years. Cancer 2000; 89: 523–533 [PMID: 10931451 DOI: 10.1002/1097-0142]

[25] Jung MR, Park YK, Jeong O, Seon JW, Ryu SY, Kim DY, Kim YJ. Elevated preoperative neutrophil to lymphocyte ratio predicts poor survival following resection in late stage gastric cancer. J Surg Oncol 2011; 104: 504–510 [PMID: 21618251 doi: 10.1002/jso.21986]

[26] Shimada H, Takiguchi N, Kainuma O, Soda H, Ikeda A, Cho A, Miyazaki A, Gunji H, Yamamoto H, Nagata M. High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer. Gastric Cancer 2010; 13: 170–176 [PMID: 20820986 doi: 10.1007/s10120-010-0554-3]

[27] Yamanaka T, Matsumoto S, Teramukai S, Ishiwata R, Nagai Y, Fukushima M. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology 2007; 73: 215–220[PMID:18424885 doi: 10.1159/000127412]

[28] Donskov F. Immunomonitoring and prognostic relevance of neutrophils in clinical trials. Semin Cancer Biol. 2013; 23: 200-207[PMID:23403174 doi: 10.1016/j.semcancer.2013.02.001]

[29] Kerr MA, Stocks SC. The role of CD15-(Le(X))-related carbohydrates in neutrophil adhesion. Histochem. J. 1992; 24: 811–826[PMID:1362195 doi:10.1007/BF01046353]

[30] de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell 2005; 7: 411–423 [PMID:15894262 doi: 10.1016/j.ccr.2005.04.014]

[31] Coussens LM, Werb Z . Inflammation and cancer. Nature 2002; 420: 860–867 [PMID:12490959 doi: 10.1038/nature01322]

[32] Daniel D, Chiu C, Giraudo E, Inoue M, Mizzen LA, Chu NR, Hanahan D. CD4+ T cellmediated antigen-specific immunotherapy in a mouse model of cervical cancer. Cancer Res 2005; 65: 2018–2025 [PMID:15753402 doi: 10.1158/0008-5472.CAN-04-3444]

[33] Strell C, Lang K, Niggemann B, Zaenker KS, Entschladen F. Neutrophil granulocytes promote the migratory activity of MDA-MB-468 human breast carcinoma cells via ICAM-1. Exp Cell Res 2010; 316: 138–148 [PMID:19747913 doi: 10.1016/j.yexcr.2009.09.003]

[34] Queen MM, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. Cancer Res 2005; 65: 8896–8904 [PMID:16204061 doi: 10.1158/0008-5472.CAN-05-1734]

[35] Bertuzzo VR, Cescon M, Ravaioli M, Grazi GL, Ercolani G, Del Gaudio M, Cucchetti A, D'Errico-Grigioni A, Golfieri R, Pinna AD. Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. Transplantation, 2011; 91: 1279-1285 [PMID:21617590 doi: 10.1097/TP.0b013e3182187cf0]

[36] Huang ZL, Luo J, Chen MS, Li JQ, Shi M. Blood neutrophil-to-lymphocyte ratio predicts survival in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization. Journal of Vascular and Interventional Radiology, 2011; 22: 702-709 [PMID:21514523 doi: 10.1016/j.jvir.2010.12.041]

[37] Chen TM, Lin CC, Huang PT, Wen CF. Neutrophil‐to‐lymphocyte ratio associated with mortality in early

hepatocellular carcinoma patients after radiofrequency ablation. Journal of gastroenterology and hepatology, 2012; 27: 553-561 [PMID:21913982 doi: 10.1111/j.1440-1746.2011.06910.x]

[38] Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci U S A 2006;103: 12493–12498 [PMID:16891410 doi: 10.1073/pnas.0601807103]

[39] Ardi VC, Kupriyanova TA, Deryugina EI, Quigley JP. Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis. Proc Natl Acad Sci U S A 2007; 104: 20262–20267 [PMID:18077379 doi: 10.1073/pnas.0706438104]

[40] Shojaei F, Singh M, Thompson JD, Ferrara N. Role of Bv8 in neutrophildependent angiogenesis in a transgenic model of cancer progression. Proc Natl Acad Sci U S A 2008; 105: 2640–2645 [PMID:18268320 doi: 10.1073/pnas.0712185105]

[41] Nathan C. Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol 2006; 6: 173–182 [PMID:16498448 doi:10.1038/nri1785]

[42] Benelli R, Albini A, Noonan D. Neutrophils and angiogenesis: potential initiators of the angiogenic cascade. Chem Immunol Allergy 2003; 83: 167–181 [PMID:12947984 doi: 10.1159/000071560]

[43] Bergers G1, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000; 2: 737–744 [PMID:11025665 doi: 10.1038/35036374]

[44] Jason E. Faris, Andrew X. Zhu. Targeted therapy for biliary tract cancers. J Hepatobiliary Pancreat Sci 2012; 19: 326- 336[PMID: 22318523 doi: 10.1007/s00534-011-0496-0]

[45] Voss JS, Holtegaard LM, Kerr SE, Fritcher EG, Roberts LR, Gores GJ, Zhang J, Highsmith WE, Halling KC, Kipp BR. Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. Hum Pathol 2013; 44: 1216-1222[PMID:23391413 doi: 10.1016/j.humpath.2012.11.006]

[46] Jesper B. Andersen, Bart Spee, Boris R. Blechacz, Itzhak Avital, Mina Komuta, Andrew Barbour, Elizabeth A. Conner, Matthew C. Gillen, Tania Roskams, Lewis R. Roberts, Valentina M. Factor, and Snorri S. Thorgeirsson. Genomic and Genetic Characterization of Cholangiocarcinoma Identifies Therapeutic Targets for Tyrosine Kinase Inhibitors. Gastroenterology 2012; 142: 1021–1031.e15[PMID:22178589 doi:10.1053/j.gastro.2011.12.005]

[47] D Yoshikawa, H Ojima3, A Kokubu, T Ochiya, S Kasai, S Hirohashi and T Shibata. Vandetanib (ZD6474), an inhibitor of VEGFR and EGFR signalling, as a novel molecular-targeted therapy against cholangiocarcinoma. British Journal of Cancer 2009; 100: 1257- 1266[PMID:19319137 doi: 10.1038/sj.bjc.6604988]

[48] Anthony B. El-Khoueiry, , Cathryn J. Rankin, Edgar Ben-Josef, Heinz-Josef Lenz, Philip J. Gold, R. Darry

Hamilton, Rangaswamy Govindarajan, Cathy Eng, MD, and Charles D. Blanke. SWOG 0514: A phase II study of sorafenib in patients with unresectable or metastatic gallbladder carcinoma and cholangiocarcinoma. Invest New Drugs 2012; 30: 1646–1651[PMID:21748296 doi:10.1007/s10637-011-9719-0]

[49] Takahashi H, Ojima H, Shimizu H, Furuse J, Furukawa H, Shibata T. Axitinib (AG-013736), an oral specific VEGFR TKI, shows potential therapeutic utility againstcholangiocarcinoma. Jpn J Clin Oncol 2014; 44: 570-578[PMID:24755544 doi: 10.1093/jjco/hyu045]

[50] El-Khoueiry AB, Rankin C, Siegel AB, Iqbal S, Gong IY, Micetich KC, Kayaleh OR, Lenz HJ, Blanke CD. S0941: a phase 2 SWOG study of sorafenib and erlotinib in patients with advanced gallbladder carcinoma or cholangiocarcinoma. Br J Cancer 2014; 110:882-887[PMID:24423918 doi: 10.1038/bjc.2013.801]

(6)? There is missing information about the pathogenesis of the studied cases of cholangiocarcinoma. It appears important to report on patients with CCC and underlying Crohn′s disease or colitis ulcerosa?

Cholangiocarcinoma derive from epithelial tumors, diagnosis is late, prognosis is poor. Clinicians and scientists around the world are committed to finding disease genes cholangiocarcinoma, most of cholangiocarcinoma is primary, there are no other risk factors. Research findings from the US and Europe[1-4], hepatitis C is the most important risk factor for cholangiocarcinoma (intrahepatic cholangiocarcinoma in particular), but research from South Korea and China[5-7], found that hepatitis B is intrahepatic cholangiocarcinoma risk factors. A study from Japan confirmed the discovery of Europe and the United States[8]. All of the above studies have confirmed cirrhosis is a risk factor of cholangiocarcinoma. Primary sclerosing cholangitis may develop cholangiocarcinoma (especially hilar cholangiocarcinoma), the disease is characterized by chronic inflammation of the liver damage and possibly merge the proliferation of progenitor cells, these patients the incidence rate of cholangiocarcinoma is 5%-10%[9-12]. After approximately 50% of patients with primary sclerosing cholangitis diagnosis within 24 months was diagnosed with cholangiocarcinoma[9-13]. Primary sclerosing cholangitis patients were diagnosed with cholangiocarcinoma average age is about 40 years old, while the general population is about 70 years old[4.14]. Although there are many risk factors may promote the development of primary sclerosing cholangitis cholangiocarcinoma, but these risk factors is not enough to guide risk stratification of disease surveillance

(7) ? The provided tumor tissue score for CD15 evaluation appears to be based on staining intensity, percentage of positive cells and score calculating formula, however, this is not stated clearly. Please refine and provide profound explanation for how the score and how patient/tumor selection into High and Low was defined! Further, please provide names and initials of the two independent pathologists who evaluated CD15 expression?

The IHC staining scores (percentage of stained cells × staining intensity) CD15 is given for each case after semi-quantitative evaluation by two independent pathologists(Li Ren and Zhi-Yuan Mao). Percentage of stained cells was observed in every 100 positive cells / 100 = percentage of positive cells. Staining intensity was negative (0), weak (1), moderate (2), or strong (3). The final score of the specimen area: percentage of positive cells × staining intensity × 100. Each antibody immunohistochemical staining final score between 0 to 300 points, with a median score of the sector into high expression group and the low expression group, the median value is also included in the high expression group

(8) ? Please re-evaluate with a statistician, if 13 cases are indeed sufficient to make a point about TNM IV-stage and CD 15 expression ? I use SPSS19.0 statistical software, the relation of expression of CD15 with clinicopathological characteristics in tumor tissue use chi-square test, the results of TNM stage and CD 15 expression show that 1 cells (12.5%) have expected count less than 5. The minimum

expected count is 3.43. The standardized statistic is 2.399. Therefore, use Fisher's Exact Test of chi-square test, the value of $\chi 2$ is 8.589, Exact Sig. (2-sided) of P is 0.032, it is statistically significant. Although the cases of TNM IV-stage only 13, after correction fisher exact test, still statistically significant.

(9) ? 74 R1 Patients and 217 R0 resected patients with CCC. How authors evaluated CD15 Expression regarding R classification. How R margin status impacts CD15 significance in CCC ? Now there is evidence to prove the relationship between neutrophil expression in tumor prognosis, and there is no evidence in the R0 and R1 resection of the tumor, the expression of neutrophil incorrect differences. This study is described in R0 and R1 resection of the tumor, the expression of neutrophil no difference, if need to prove the R0 and R1 resection of the tumor, the neutrophils expressing has differences or not, it need further research and experimentation, as well as to expand the sample size confirmed. In Table 1, only appears in the R0 and R1 resection of cholangiocarcinoma, no statistically significant difference (P = 0.900) in CD15 expression, it is R0 and R1 resection of cholangiocarcinoma, there was no difference of neutrophils

(10) ? Of great importance is the fact that the authors claim CD15 proves to be an independent prognostic factor in CCC, because of significant results in the multivariate analysis. However, the data provided in this manuscripts do not support this concept, as in the COX regression only tumor differentiation (p<0.004), margin (R) status (p<0.014) and TNM stage (p<0.001), but not CD15 expression (p=0.073!!), deliver significant p values and, thus present prognostic independence! Why do the authors then claim CD15 to be an independent prognosticator ? my result of the manuscripts is by COX multivariate analysis, high expression CD15 in tumor tissues was an independment risk factor in predicting OS approximately for patients with cholangiocarcinoma(P = 0.012, relative risk (RR) =1.601), but high expression CD15 in tumor tissues was not an independment risk factor in predicting DFS approximately for patients with cholangiocarcinoma(P = 0.073, RR =1.462). So I claim CD15 to be an independent prognosticator in OS, but not in DFS.

(11) ? The discussion gives an overview about published data concerning the relationship between prognosis of different tumor entities and inflammatory markers, mainly CD15 positive granulocytes in the tumor-environment. A review about the clinical value of those findings is missing ? summing up published data concerning the relationship between prognosis of different tumor entities and inflammatory markers, in a variety of tumor infiltrating neutrophils have a poor prognosis, which may act as a preliminary clinical prognosis of cancer patients to provide some help, in addition to infiltration by neutrophils for clinical treatment of cancer provide clues. (has been described in the article)

(12) ? The interpretation of the results is not adequate. It firstly lacks a statement about the incoherence concerning a risk assessment on disease recurrence (multivariate vs. univariate analysis). Secondly, it lacks thoughts about a relation between CD15 positive neutrophils and tumor progression as well as a possible target function. Up-to-date literature about “immune” targeted-therapy in cholangiocarcinoma is missing ? Adjustments were original and incoherence concerning a risk assessment on disease recurrence, and a relation between CD15 positive neutrophils and tumor progression as well as a possible target function, re-inspection "immune" targeted-therapy in cholangiocarcinoma, and added

(13) ? Please correct the key of figure 2 ? I want to say expression of CD15 and TNM staging have relevant, based the correlation, using SPSS statistical software generated histogram, so change to histogram of corrlation between CD15 expression in tumor tissues and TNM staging. The blue means low expression of CD15 and the green means high expression of CD15.

(14) ? The cited literature is not up to date. Please review the literature ? Has been re-check the latest literature, and add

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Zhi-Yuan Mao