Title	Effect of Abdominal Aortic Calcification on Recurrence Following Initial Hepatectomy for Colorectal Liver Metastases
Author(s)	IMAOKA, KOUKI; OHIRA, MASAHIRO; SHIMOMURA, MANABU; HATTORI, MINORU; BEKKI, TOMOAKI; SATO, KOKI; IMAOKA, YUKI; AKABANE, SHINTARO; NAKANO, RYOSUKE; YANO, TAKUYA; SAKAI, HIROSHI; HIRATA, FUMIHIRO; KURODA, SHINTARO; TAHARA, HIROYUKI; IDE, KENTARO; ISHIYAMA, KOHEI; KOBAYASHI, TSUYOSHI; TANAKA, YUKA; OHDAN, HIDEKI
Citation	Anticancer Research , 44 (2) : 649 - 658
Issue Date	2024-02-02
DOI	
Self DOI	
URL	https://ir.lib.hiroshima-u.ac.jp/00056109
Right	
Relation	


Hiroshima University Institutional Repository

Effect of Abdominal Aortic Calcification on Recurrence Following Initial Hepatectomy for Colorectal Liver Metastases

KOUKI IMAOKA[1], MASAHIRO OHIRA[1,2], MANABU SHIMOMURA[1], MINORU HATTORI[3], TOMOAKI BEKKI[1], KOKI SATO[1], YUKI IMAOKA[1], SHINTARO AKABANE[1], RYOSUKE NAKANO[1], TAKUYA YANO[1], HIROSHI SAKAI[1], FUMIHIRO HIRATA[1], SHINTARO KURODA[1], HIROYUKI TAHARA[1], KENTARO IDE[1], KOHEI ISHIYAMA[1,4], TSUYOSHI KOBAYASHI[1], YUKA TANAKA[1] and HIDEKI OHDAN[1]

[1]Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan;

[2]Division of Regeneration and Medicine, Medical Center for Translational and Clinical Research, Hiroshima University Hospital, Hiroshima, Japan;

[3]Advanced Medical Skills Training Center, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan;

[4] Department of Renal Transplant Surgery, Aichi Medical School Medicine, Nagoya, Japan

Correspondence to: Masahiro Ohira, MD, PhD, Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Tel: +81 822575222, Fax: +81 822575224, e-mail: mohira@hiroshima-u.ac.jp

Running title: Impact of AAC on Recurrence of CRLM

Article type: Original article

Word count: 2948

Abstract

Background/Aim: To analyze the association between abdominal aortic calcification (AAC) and patient prognosis following resection of colorectal liver metastases (CRLM).

Summary background data: AAC potentially reflects intrahepatic immunity and is involved in tumor development and progression. However, the clinical effects of AAC on colorectal cancer (CRC) prognosis after curative-intent liver resection for CRLM remain unclear.

Patients and Methods: We evaluated the effect of AAC on the clinical prognosis and metastatic patterns in 99 patients who underwent hepatectomy for CRLM between 2010 and 2019.

Results: The high-AAC group had significantly worse overall survival (OS) and remnant liver recurrence rate (RR) after propensity score matching to adjust for differences in baseline characteristics of patients and tumors. In multivariate Cox regression analyses, high AAC volume was an independent risk factor for poor OS and liver RR, but not poor lung RR. The expression of tumor necrosis factor-related apoptosis-inducing ligand, known as an anti-tumor marker, in liver natural killer (NK) cells was lower in the high-AAC group than in the low-AAC group.

Conclusion: High AAC volume showed a strong relationship with remnant liver RR after curative resection of CRLM. High AAC volume may be responsible for the suppression of anti-tumor activity of liver NK cells, which results in an increased risk of liver recurrence and poor prognosis.

Key Words: Aortic calcification, colorectal liver metastasis, tumor necrosis factor-related apoptosis-inducing ligand, propensity score.

Liver resection is the mainstay of curative-intent treatment for colorectal liver metastases (CRLM) with a 5-year overall survival (OS) of up to 58% and 10-year recurrence-free survival (RFS) of 20% (1, 2). However, recurrence occurs in up to 75% of patients within the first 2 years (3). Therefore, it is important to identify risk factors for CRLM recurrence after liver resection. Tumor morphological factors, including tumor size and number, are important predictors of prognosis in patients with CRLM (4–7). Further, systemic inflammation status related to patient factors, including the neutrophil–lymphocyte ratio (NLR), prognostic nutrient index, and Glasgow Prognostic Score (GPS), predict cancer-specific survival in CRLM, independent of tumor staging (8, 9).

Abdominal aortic calcification (AAC), a known marker of cardiovascular disease, reflects the systemic inflammation status (10). We have preciously reported that an increased AAC has a corresponding increase in postoperative complication severity after major hepatobiliary pancreatic surgery (11). AAC is also potentially associated with intrahepatic immunity and is involved in tumor development and progression (12, 13). Our previous study indicated that the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), an anti-tumor marker, in liver natural killer (NK) cells is associated with atherosclerosis severity based on the presence of AAC in living-donor liver transplantation (14). TRAIL can initiate the apoptosis pathway by binding to its associated death receptors (15), and its expression in immune cells plays a crucial role in controlling tumor growth (16). However, the effect of AAC on prognosis after curative-intent liver resection for CRLM remains unclear. Thus, this study aimed to analyze the association between AAC and prognosis and the phenotypic characteristics of intrahepatic immune cells in patients following liver resection for CRLM.

Patients and Methods

Patients. We evaluated patients with colorectal cancer (CRC) who underwent primary liver resection for CRLM at our institute between 2010 and 2019. Patients who underwent repeat liver resection for CRLM were excluded from the study. Clinical data at the time of colorectal surgery, including age, sex, modified GPS (mGPS) (17), geriatric nutritional risk index (GNRI) (18), fibrosis-4 index (Fib-4 index) (19), total bilirubin (T-Bil), indocyanine green retention (ICG-R), pathological findings of the primary CRC, tumor markers including carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), tumor size and number of CRLM, tumor burden combining the size and number of liver metastases (6), classification of CRLM consisting of a number of liver metastases, size of the largest liver metastasis, mesenteric lymph node metastases, and extrahepatic metastases (20), neoadjuvant chemotherapy, adjuvant chemotherapy, and postoperative complications according to the Clavien–Dindo (CD) system (21), were collected retrospectively from the medical records of the patients.

Patients with resectable CRLM received either immediate resection or neoadjuvant chemotherapy based on the surgeon's discretion, whereas those with unresectable CRLM received chemotherapy including oxaliplatin or irinotecan. Resection of CRLM was indicated when all tumors could be resected with clear margins, and hepatic resection could be performed safely with preserved remnant liver function. All patients with CRLM were assessed by a liver surgeon using the same criterion to assess resectability (22).

Data pertaining to liver or lung recurrence and postoperative long-term survival were also obtained from the clinical records. After surgery, the patients were followed-up using contrast-enhanced computed tomography (CT) and colonoscopy, combined with an evaluation of serum CEA levels at 3-month intervals for 5 years and 6-month intervals for 5 years thereafter.

AAC. CT angiography was performed using a 320-detector row CT scanner (Aquilion ONE ViSION, Toshiba Medical Systems, Tochigi, Japan) applying a standardized examination protocol. The AAC score was calculated using the AZE VirtualPlace Lexus64 Anatomia software (AZE Inc., Tokyo, Japan). Using the Agatston method (23), the AAC volume was automatically calculated from the origin of the renal artery to the iliac bifurcation with an attenuation level. Patients were categorized into two groups according to the AAC volume at a cutoff of 116 mm^3: low-ACC (<116 mm^3; n = 32 [32.3%]) and high-ACC [≥116 mm^3; n = 67 (67.7%)] groups, using the receiver operating characteristic curves for remnant liver recurrence rate (RR).

Parameter measurements. Synchronous metastases were defined as liver metastases detected at the time of diagnosis or within 6 months after radical resection of the primary tumor, whereas metachronous metastases were defined as liver metastases detected >6 months after radical resection of the primary tumor (24). The cut-off value of tumor burden score (TBS) was defined according to a previous study (6). The CEA and CA19-9 levels were measured before hepatectomy for CRLM, and the cutoff values were 10 ng/ml and 100 U/ml, respectively (25).

Collection of liver mononuclear cells and coculture. The phenotypic characteristics of liver mononuclear cells (LMNCs) of patients who underwent liver resection for CRLM were analyzed. Ten additional samples were collected for a sub-analysis of a randomized clinical trial of Hiroshima Surgical Study Group of Clinical Oncology (HiSCO) registered with the National Review Board (HiSCO-01, University Hospital Medical Information [UMIN] 00000378) to investigate the effect of chemotherapy on the function of intrahepatic immune cells in patients with resectable CRLM (26). The clinical trial indicated that neoadjuvant

chemotherapy for the treatment of resectable CRLM induces the activation of liver NK cells (26). LMNCs were obtained by *ex vivo* perfusion *via* the portal vein of the resected livers of patients with CRLM (27). The collected effluent was concentrated by centrifugation, followed by gradient centrifugation using Separate-L (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) to isolate the LMNCs. LMNCs were cultured with interleukin (IL)-2 stimulation (100 Japanese reference units/ml; Takeda) in complete medium for 3 days to prime NK cells with enhanced anti-tumor properties.

Flow cytometric analyses. All analyses were performed using a FACS Calibur cytometer (BD Biosciences, San Jose, CA, USA) and FlowJo 7.6.5 software (TreeStar Inc., Ashland, OR, USA). The monoclonal antibodies used for surface staining of lymphocytes to assess the phenotypic properties of NK cells were as follows (26): fluorescein isothiocyanate-conjugated anti-CD3 and anti-CD56; phycoerythrin (PE)-conjugated anti-NKp30, anti-NKp44, anti-NKp46, anti-CD122, and anti-CD56; allophycocyanin-conjugated anti-CD3 and anti-natural-killer group 2, member D (NKG2D; 1D11), purchased from Becton Dickinson; PE-conjugated anti-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL; RIK-2; eBioscience, Santa Clara, CA, USA) and PE-conjugated anti-signal regulatory protein β (SIRPβ; B4B; BioLegend, San Diego, CA, USA). Mouse immunoglobulin (Ig) G1κ was used as an isotype-matched control. Dead cells were excluded from light scatter analysis and propidium iodide staining.

Statistical analysis. The nonparametric Mann–Whitney U-test was performed to compare differences between the two independent groups; values of $p<0.05$ were considered statistically significant. Values are expressed as median with interquartile range. OS and RR were calculated using Kaplan–Meier analysis and compared using log-rank statistics.

Multivariate analyses were conducted for variables independently related to the liver, using the Cox proportional hazards model. Univariate and multivariate Cox regression analyses were performed to assess the association of RR with the following variables: age, sex, AAC volume (≥ 116 mm^3), mGPS (≥ 2), pT4, pN (≥ 2), tumor location, poorly differentiated histology, TBS (≥ 3.6), liver grade, CEA (≥ 10 ng/ml), CA19-9 (≥ 100 U/ml), neoadjuvant chemotherapy, synchronous CRLM, and postoperative complication (CD class ≥ 3). All variables were included in the multivariate model, and the backward elimination method with a removal criterion of p=0.05 was used to select the covariates. To adjust for differences in baseline characteristics, one-to-one propensity score models were constructed based on each patient's estimated propensity score according to age, sex, and Fib-4 index, *i.e.*, the variables that were associated significantly (p<0.05). One-to-one matching was performed using a 0.20 caliper. All statistical analyses were performed using the JMP statistical software (JMP® 16; SAS Institute Inc., Cary, NC, USA). Statistical significance was set at p<0.05.

Results

Clinicopathological characteristics before and after propensity score matching. The baseline characteristics of patients in the high- and low-AAC groups are presented in Table I. Compared with the low-AAC group, the high-AAC group had a significantly higher proportion of patients with older age and male sex and a significantly high Fib-4 index. No significant differences were found in the mGPS, GNRI, T-Bil, or ICG-R between the two groups. In addition, no significant differences were found in tumor location, pT categories, pN categories, poorly differentiated pathological histology, TBS, tumor size, number of tumors in the liver metastasis, tumor location, or liver grade. The rates of neoadjuvant and adjuvant chemotherapy did not differ significantly between the two groups. No significant differences were noted in postoperative complications (CD class ≥ 3). The relationship

between AAC and clinical factors, such as patient-related, tumor-specific, surgical, and perioperative, in matched cases is shown in Table I. After propensity score matching to adjust for differences in baseline characteristics, no significant differences were observed in any factor between the high- and low-AAC groups (p=0.062–1.00).

Kaplan–Meier survival curve analysis after propensity score matching between the high- and low-AAC groups. The duration of follow-up was 60 months or until death, with a median follow-up of 39.0 months. Kaplan–Meier survival curve analysis showed no significant differences in OS and RFS between the two groups (Figure 1A and B). After propensity score-matched analysis, the high-AAC group had a significantly worse OS but not RFS than the low-AAC group (Figure 2A and B). Recurrence in any organ was observed in 67 of 99 patients (67.7%). The time to recurrence for each organ, including the liver [n = 47 (47.5%)] and lungs [n = 29 (29.3%)], was examined. Kaplan–Meier survival curve analysis showed a significantly worse remnant liver RR in the high-AAC group than in the low-AAC group, while no significant difference in lung RR was found between the two groups (Figures 3A and B). After propensity score-matching analysis, the high-AAC group had a significantly worse remnant liver RR than the low-AAC group (Figures 4A and B).

Factors associated with OS. Univariate analysis revealed that mGPS (≥ 1), TBS (≥ 3.6), and high CEA levels were predictive factors for poor OS. Multivariate analysis revealed that high AAC volume [hazard ratio (HR)=2.22; 95% confidence interval (CI)=1.04–54.74; p=0.040], mGPS (≥ 1) (HR=2.05; 95%CI=1.04–4.04; p=0.037), and high CA19-9 level (HR=2.89; 95%CI=1.09–7.70; p=0.034) were independent risk factors for poor OS (Table II).

Factors associated with remnant liver recurrence. Univariate analysis revealed that high AAC volume, TBS, multiple tumors, liver grade B, high CEA levels, and high CA19-9 levels were risk factors for liver recurrence. Multivariate analysis revealed that high AAC volume (HR=2.14; 95%CI=1.05–4.39; p=0.037), TBS (≥ 3.6) (HR=1.96; 95%CI=1.03–3.71; p=0.039), and high CA19-9 level (HR=2.86; 95%CI=1.25–6.50; p=0.012) were independent risk factors of liver RR (Table III).

Potential depression of NK cell activity in the liver of patients with high AAC volume who undergo hepatectomy for CRLM . We assessed the phenotypic differences in liver NK cells, which play a pivotal role in tumor surveillance, to investigate the effect of AAC on the innate immune system in patients with CRLM. Ten additional samples from the 99 patients were evaluated: six samples in the high-AAC group and four in the low-AAC group. Proportion of TRAIL-positive NK cells was significantly higher in IL-2-stimulated LMNCs in the low-AAC group than in the high-AAC group (p=0.033; Figure 5). No remarkable differences in the expression levels of other surface molecules, including NKp30, NKp44, NKp46, NKG2D, CD122, and SIRPβ in IL-2-stimulated LMNCs were observed between the two groups.

Discussion

The results of this study showed that a high AAC volume was an independent risk factor for reduced OS and remnant liver recurrence after hepatectomy for CRLM. Furthermore, one of the anti-tumor activity markers, TRAIL, in liver NK cells was decreased in patients with higher AAC who underwent hepatectomy. To our knowledge, this is the first study to demonstrate the relationship between AAC and clinical outcomes in patients following hepatectomy for CRLM.

Numerous studies have identified the factors associated with recurrence after hepatectomy in patients with CRLM, including preoperative CEA levels, primary CRC stage, differentiation and lymph node metastasis of the primary CRC, metastatic tumor burden, time interval to metastasis, and administration of neoadjuvant or adjuvant chemotherapy (28–30). Furthermore, tumor size and number are important predictors of survival in patients with intrahepatic recurrence after curative-intent hepatectomy for CRLM (31). In this study, TBS ($\geq$ 3.6) and high CA19-9 level were associated with liver recurrence.

Several studies have focused on patient-related factors of recurrence after hepatectomy, including systemic inflammation and nutritional status (8, 9). We previously indicated that GPS is an independent predictor of RFS in patients with CRLM (25). AAC, a known biomarker of cardiovascular disease, is also associated with systemic inflammation status (10). A systemic inflammatory response can lead to severe malnutrition and reduced preoperative immunocompetence, suggesting that compromised immunocompetence may influence prognosis (32). Our previous studies indicated that AAC is associated with liver metastasis in stage II–III CRC or hepatocellular carcinoma recurrence following hepatectomy (12, 13). This study revealed that a high level of AAC serves as an independent risk factor for intrahepatic recurrence following hepatic resection for CRLM. However, no association was observed between AAC and lung recurrence. Based on these findings, it is postulated that AAC can exert a significant influence on intrahepatic antitumor immunity.

NK cells play a crucial role in the prevention of tumors *via* the innate immune response (33). Especially, NK cells can interact with circulating tumor cells to control cancer metastasis and prevent recurrence (34). Although liver NK cells are essential components of the hepatic anti-tumor immune repertoire, their immunosurveillance role is compromised in patients with recurrent CRLM (35). TRAIL is a hallmark of liver NK cells and exerts strong cytotoxicity against tumor cells through the TRAIL-TRAIL death signaling pathway (27). This pathway

also plays an important role in the clearance of metastatic TRAIL-sensitive tumor cells *in vivo* (36). The treatment with TRAIL is one of the ideal strategies and is often used in CRC. In certain CRCs, the development of resistance to TRAIL during the progression of the malignancy presents a clinical challenge (37). We previously reported that TRAIL expression in NK cells is affected by atherosclerosis severity in living-donor liver transplantation (14). In this study, the expression of TRAIL in liver NK cells was lower in CRLM-bearing patients with high AAC volume than in those with low AAC volume. Our results clarify the mechanism by which AAC is associated with intrahepatic recurrence in several tumors and results in poor prognosis (12, 13). TRAIL has been identified as a cytotoxic ligand for TRAIL-death receptor-expressing tumor cells and is frequently employed as a marker to evaluate the cytotoxicity of liver NK cells in clinical trials (27, 38, 39). This is supported by its robust positive association with other activation markers such as NKG2D, CD69, and NKp44. Accumulating evidence suggests that AAC can enhance intrahepatic recurrence, potentially by attenuating the anti-tumor activity of liver NK cells. This study provides supportive data that aligns with this hypothesis. Further studies are required to elucidate the negative effects of high AAC volume on hepatic immunity and carcinogenesis.

This study has some limitations that should be considered when interpreting our findings. Specifically, the retrospective and non-randomized study design must be mentioned. The small sample size of patients who underwent hepatectomy for CRLM between 2010 and 2019 at a single center may also weaken the conclusion. Furthermore, liver NK cells were not evaluated in all patients who underwent hepatectomy for CRLM; therefore, we reanalyzed the results of our previous clinical study. It should be noted that due to the limited sample size in this study, significant differences in the levels of NKp44 or NKG2D between the high and low AAC groups were not observed. Further validation studies are imperative to elucidate the precise impact of AAC on the anti-tumor activity of intrahepatic NK cells. Additionally, T

cells, which play a central role in the immune response to solid tumors, were not evaluated in this study. Future prospective studies involving a larger number of patients with high AAC volume are needed to analyze the outcomes of hepatectomy for CRLM.

In conclusion, AAC showed a strong relationship with remnant liver RR after curative-intent resection of CRLM. High AAC volume may be responsible for the decrease in anti-tumor activity in liver NK cells, which results in an increased risk of liver recurrence and poor prognosis. ACC could potentially serve as a new clinical tool for predicting remnant liver recurrence in patients after initial hepatectomy for CRLM.

Conflicts of Interest

The Authors declare that they have no competing interest.

Authors' Contributions

KI, MO, MS, KS, and YI conceived and designed the study. KI, TB, KS, SA, RN, and YT acquired the data and calculated AAC scores. KI, MO, MS, and MH analyzed and interpreted the data, and drafted the manuscript. KI, MO, MS, HS, FH, SK, HT, KI, and HO critically revised the article. KI, MO, and HO approved the final version of the manuscript to be published.

Acknowledgements

The Authors thank Editage for the English language review.

Funding

This work was supported in part by JSPS KAKENHI grant numbers JP22K16534,

22K16535, and 23H02981 and AMED grant number 23fk0210108.

References

1 Fernandez FG, Drebin JA, Linehan DC, Dehdashti F, Siegel BA, Strasberg SM: Five-year survival after resection of hepatic metastases from colorectal cancer in patients screened by positron emission tomography with F-18 fluorodeoxyglucose (FDG-PET). Ann Surg 240: 438–447; discussion 447, 2004. DOI: 10.1097/01.sla.0000138076.72547.b1
2 Tomlinson JS, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M, Kemeny N, Brennan MF, Blumgart LH, D'Angelica M: Actual 10-year survival after resection of colorectal liver metastases defines cure. J Clin Oncol 25: 4575–4580, 2007. DOI: 10.1200/JCO.2007.11.0833
3 D'Angelica M, Kornprat P, Gonen M, DeMatteo RP, Fong Y, Blumgart LH, Jarnagin WR. Effect on outcome of recurrence patterns after hepatectomy for colorectal metastases. Ann Surg Oncol. 2011 Apr;18(4):1096-103. DOI: 10.1245/s10434-010-1409-1.
4 Malik HZ, Prasad KR, Halazun KJ, Aldoori A, Al-Mukhtar A, Gomez D, Lodge JP, Toogood GJ. Preoperative prognostic score for predicting survival after hepatic resection for colorectal liver metastases. Ann Surg. 2007 Nov;246(5):806-14. DOI: 10.1097/SLA.0b013e318142d964.
5 Dexiang Z, Li R, Ye W, Haifu W, Yunshi Z, Qinghai Y, Shenyong Z, Bo X, Li L, Xiangou P, Haohao L, Lechi Y, Tianshu L, Jia F, Xinyu Q, Jianmin X. Outcome of patients with colorectal liver metastasis: analysis of 1,613 consecutive cases. Ann Surg Oncol. 2012 Sep;19(9):2860-8. DOI: 10.1245/s10434-012-2356-9.
6 Sasaki K, Morioka D, Conci S, Margonis GA, Sawada Y, Ruzzenente A, Kumamoto T, Iacono C, Andreatos N, Guglielmi A, Endo I, Pawlik TM. The Tumor Burden Score: A New "Metro-ticket" Prognostic Tool For Colorectal Liver Metastases Based on Tumor Size and Number of Tumors. Ann Surg. 2018 Jan;267(1):132-141. DOI: 10.1097/SLA.0000000000002064.
7 Hallet J, Sa Cunha A, Adam R, Goéré D, Bachellier P, Azoulay D, Ayav A, Grégoire E, Navarro F, Pessaux P; French Colorectal Liver Metastases Working Group, Association Française de Chirurgie (AFC). Factors influencing recurrence following initial hepatectomy for colorectal liver metastases. Br J Surg. 2016 Sep;103(10):1366-76. DOI: 10.1002/bjs.10191.
8 Nakagawa K, Tanaka K, Nojiri K, Kumamoto T, Takeda K, Ueda M, Endo I. The modified Glasgow prognostic score as a predictor of survival after hepatectomy for colorectal liver metastases. Ann Surg Oncol. 2014 May;21(5):1711-8. DOI: 10.1245/s10434-013-3342-6.
9 Neal CP, Cairns V, Jones MJ, Masood MM, Nana GR, Mann CD, Garcea G, Dennison AR. Prognostic performance of inflammation-based prognostic indices in patients with resectable colorectal liver metastases. Med Oncol. 2015 May;32(5):144. DOI: 10.1007/s12032-015-0590-2.
10 Okuno S, Ishimura E, Kitatani K, Fujino Y, Kohno K, Maeno Y, Maekawa K, Yamakawa T, Imanishi Y, Inaba M, Nishizawa Y. Presence of abdominal aortic calcification is significantly associated with all-cause and cardiovascular mortality in maintenance hemodialysis patients. Am J Kidney Dis. 2007 Mar;49(3):417-25. DOI: 10.1053/j.ajkd.2006.12.017.
11 Imaoka Y, Ohira M, Sato K, Imaoka K, Bekki T, Nakano R, Kuroda S, Tahara H, Ide K, Kobayashi T, Tanaka Y, Ohdan H. Impact of Abdominal Aortic Calcification After Major

Hepatobiliary Pancreatic Surgery: A Retrospective Cohort Study. Anticancer Res. 2022 Dec;42(12):5983-5989. DOI: 10.21873/anticanres.16109.
12 Imaoka K, Shimomura M, Shimizu W, Akabane S, Ohira M, Imaoka Y, Yoshinaka H, Ono K, Mochizuki T, Matsubara K, Bekki T, Hattori M, Ohdan H. Effect of abdominal aortic calcification on the prognosis and recurrence of colorectal cancer stages II-III: A retrospective cohort study. Int J Colorectal Dis. 2023 Jan 21;38(1):21. DOI: 10.1007/s00384-023-04321-z.
13 Imaoka Y, Ohira M, Sato K, Imaoka K, Kuroda S, Tahara H, Kobayashi T, Ide K, Tanaka Y, Ohdan H. Impact of abdominal aortic calcification on clinical outcomes following initial hepatectomy for hepatocellular carcinoma: A retrospective cohort study. Ann Gastroenterol Surg. 2021 Sep 19;6(1):149-158. DOI: 10.1002/ags3.12508.
14 Imaoka K, Ohira M, Bekki T, Sato K, Imaoka Y, Nakano R, Yano T, Sakai H, Tanimine N, Shimizu S, Doskali M, Kuroda S, Tahara H, Ide K, Kobayashi T, Tanaka Y, Ohdan H. Arteriosclerosis Decreases Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Expression on Liver Natural Killer Cells in Living Donor Liver Transplantation. Transplant Proc. 2023 May;55(4):906-912. DOI: 10.1016/j.transproceed.2023.03.066.
15 Wang S, El-Deiry WS. TRAIL and apoptosis induction by TNF-family death receptors. Oncogene. 2003 Nov 24;22(53):8628-33. DOI: 10.1038/sj.onc.1207232.
16 Cardoso Alves L, Corazza N, Micheau O, Krebs P. The multifaceted role of TRAIL signaling in cancer and immunity. FEBS J. 2021 Oct;288(19):5530-5554. DOI: 10.1111/febs.15637.
17 Hirashima K, Watanabe M, Shigaki H, Imamura Y, Ida S, Iwatsuki M, Ishimoto T, Iwagami S, Baba Y, Baba H. Prognostic significance of the modified Glasgow prognostic score in elderly patients with gastric cancer. J Gastroenterol. 2014 Jun;49(6):1040-6. DOI: 10.1007/s00535-013-0855-5.
18 Buzby GP, Williford WO, Peterson OL, Crosby LO, Page CP, Reinhardt GF, Mullen JL. A randomized clinical trial of total parenteral nutrition in malnourished surgical patients: the rationale and impact of previous clinical trials and pilot study on protocol design. Am J Clin Nutr. 1988 Feb;47(2 Suppl):357-65. DOI: 10.1093/ajcn/47.2.357.
19 Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006 Jun;43(6):1317-25. DOI: 10.1002/hep.21178.
20 Yamaguchi T, Mori T, Takahashi K, Matsumoto H, Miyamoto H, Kato T. A new classification system for liver metastases from colorectal cancer in Japanese multicenter analysis. Hepatogastroenterology. 2008 Jan-Feb;55(81):173-8.
21 Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004 Aug;240(2):205-13. DOI: 10.1097/01.sla.0000133083.54934.ae.
22 Makuuchi M, Kosuge T, Takayama T, Yamazaki S, Kakazu T, Miyagawa S, Kawasaki S. Surgery for small liver cancers. Semin Surg Oncol. 1993 Jul-Aug;9(4):298-304. DOI: 10.1002/ssu.2980090404.
23 Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990 Mar 15;15(4):827-32. DOI: 10.1016/0735-1097(90)90282-t.
24 Mekenkamp LJ, Koopman M, Teerenstra S, van Krieken JH, Mol L, Nagtegaal ID, Punt CJ. Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer. 2010 Jul 13;103(2):159-64. DOI: 10.1038/sj.bjc.6605737.

25 Okimoto S, Kobayashi T, Tashiro H, Kuroda S, Ishiyama K, Ide K, Abe T, Hashimoto M, Iwako H, Hamaoka M, Honmyo N, Yamaguchi M, Ohdan H. Significance of the Glasgow Prognostic Score for patients with colorectal liver metastasis. Int J Surg. 2017 Jun;42:209-214. DOI: 10.1016/j.ijsu.2017.04.068.
26 Hirata F, Ishiyama K, Tanaka Y, Kobayashi T, Hashimoto M, Saeki Y, Ishida N, Taguchi K, Tanaka J, Arihiro K, Ohdan H; Hiroshima Surgical Study Group of Clinical Oncology (HiSCO). Effect of bevacizumab plus XELOX (CapeOX) chemotherapy on liver natural killer cell activity in colorectal cancer with resectable liver metastasis. Ann Gastroenterol Surg. 2018 Jul 18;2(5):383-393. DOI: 10.1002/ags3.12195.
27 Ishiyama K, Ohdan H, Ohira M, Mitsuta H, Arihiro K, Asahara T. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. Hepatology. 2006 Feb;43(2):362-72. DOI: 10.1002/hep.21035.
28 Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaeck D, Mirza D, Parks RW, Collette L, Praet M, Bethe U, Van Cutsem E, Scheithauer W, Gruenberger T; EORTC Gastro-Intestinal Tract Cancer Group; Cancer Research UK; Arbeitsgruppe Lebermetastasen und-tumoren in der Chirurgischen Arbeitsgemeinschaft Onkologie (ALM-CAO); Australasian Gastro-Intestinal Trials Group (AGITG); Fédération Francophone de Cancérologie Digestive (FFCD). Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. Lancet. 2008 Mar 22;371(9617):1007-16. DOI: 10.1016/S0140-6736(08)60455-9.
29 Sorbye H, Mauer M, Gruenberger T, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaeck D, Mirza D, Parks RW, Collette L, Van Cutsem E, Scheithauer W, Lutz MP, Nordlinger B; EORTC Gastro-Intestinal Tract Cancer Group; Cancer Research UK (CRUK); Arbeitsgruppe Lebermetastasen und-tumoren in der Chirurgischen Arbeitsgemeinschaft Onkologie (ALM-CAO); Australasian Gastro-Intestinal Trials Group (AGITG); Fédération Francophone de Cancérologie Digestive (FFCD). Predictive factors for the benefit of perioperative FOLFOX for resectable liver metastasis in colorectal cancer patients (EORTC Intergroup Trial 40983). Ann Surg. 2012 Mar;255(3):534-9. DOI: 10.1097/SLA.0b013e3182456aa2.
30 Son SY, Yi NJ, Hong G, Kim H, Park MS, Choi YR, Suh KS, Kim DW, Jeong SY, Park KJ, Park JG, Lee KU. Is neoadjuvant chemotherapy necessary for patients with initially resectable colorectal liver metastases in the era of effective chemotherapy? Korean J Hepatobiliary Pancreat Surg. 2011 Nov;15(4):206-17. DOI: 10.14701/kjhbps.2011.15.4.206.
31 Sutton TL, Wong LH, Walker BS, Dewey EN, Eil R, Lopez CD, Kardosh A, Chen EY, Rocha FG, Billingsley KG, Mayo SC. Hepatectomy is associated with improved oncologic outcomes in recurrent colorectal liver metastases: A propensity-matched analysis. Surgery. 2023 Jun;173(6):1314-1321. DOI: 10.1016/j.surg.2022.10.019.
32 Sagawa M, Yoshimatsu K, Yokomizo H, Yano Y, Okayama S, Usui T, Yamaguchi K, Shiozawa S, Shimakawa T, Katsube T, Kato H, Naritaka Y. Worse Preoperative Status Based on Inflammation and Host Immunity Is a Risk Factor for Surgical Site Infections in Colorectal Cancer Surgery. J Nippon Med Sch. 2017;84(5):224-230. DOI: 10.1272/jnms.84.224.
33 Jacobs R, Hintzen G, Kemper A, Beul K, Kempf S, Behrens G, Sykora KW, Schmidt RE. CD56bright cells differ in their KIR repertoire and cytotoxic features from CD56dim NK cells. Eur J Immunol. 2001 Oct;31(10):3121-7. DOI: 10.1002/1521-4141(2001010)31:10<3121::aid-immu3121>3.0.co;2-4.

34 Dianat-Moghadam H, Mahari A, Heidarifard M, Parnianfard N, Pourmousavi-Kh L, Rahbarghazi R, Amoozgar Z. NK cells-directed therapies target circulating tumor cells and metastasis. Cancer Lett. 2021 Jan 28;497:41-53. DOI: 10.1016/j.canlet.2020.09.021.
35 Harmon C, Robinson MW, Hand F, Almuaili D, Mentor K, Houlihan DD, Hoti E, Lynch L, Geoghegan J, O'Farrelly C. Lactate-Mediated Acidification of Tumor Microenvironment Induces Apoptosis of Liver-Resident NK Cells in Colorectal Liver Metastasis. Cancer Immunol Res. 2019 Feb;7(2):335-346. DOI: 10.1158/2326-6066.CIR-18-0481.
36 Takeda K, Hayakawa Y, Smyth MJ, Kayagaki N, Yamaguchi N, Kakuta S, Iwakura Y, Yagita H, Okumura K. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. Nat Med. 2001 Jan;7(1):94-100. DOI: 10.1038/83416.
37 Jong KXJ, Mohamed EHM, Ibrahim ZA. Escaping cell death via TRAIL decoy receptors: a systematic review of their roles and expressions in colorectal cancer. Apoptosis. 2022 Dec;27(11-12):787-799. DOI: 10.1007/s10495-022-01774-5.
38 Ohira M, Ishiyama K, Tanaka Y, Doskali M, Igarashi Y, Tashiro H, Hiraga N, Imamura M, Sakamoto N, Asahara T, Chayama K, Ohdan H. Adoptive immunotherapy with liver allograft-derived lymphocytes induces anti-HCV activity after liver transplantation in humans and humanized mice. J Clin Invest. 2009 Nov;119(11):3226-35. DOI: 10.1172/JCI38374.
39 Ohira M, Hotta R, Tanaka Y, Matsuura T, Tekin A, Selvaggi G, Vianna R, Ricordi C, Ruiz P, Nishida S, Tzakis AG, Ohdan H. Pilot study to determine the safety and feasibility of deceased donor liver natural killer cell infusion to liver transplant recipients with hepatocellular carcinoma. Cancer Immunol Immunother. 2022 Mar;71(3):589-599. DOI: 10.1007/s00262-021-03005-3.

Figure legends

Figure 1. Overall survival (OS) and recurrence-free survival (RFS) curves before propensity score matching; A) OS), B) RFS.

Kaplan–Meier survival curve analysis showed no significant differences in OS and RFS between the high- and low- abdominal aortic calcification (AAC) groups after resection colorectal liver metastases.

Figure 2. Overall survival (OS) and recurrence-free survival (RFS) curves after propensity score matching; A) OS, B) RFS.

Kaplan–Meier survival curve analysis showed worse OS but not RFS in the high-abdominal aortic calcification (AAC) group than in the low-AAC group after resection of colorectal liver metastases.

Figure 3. Kaplan-Meier estimated cumulative recurrence rate before propensity score matching

A) Kaplan–Meier survival curve analysis showed significantly worse remnant liver recurrence rate (RR) in the high-abdominal aortic calcification (AAC) group than in the low-AAC group.

B) Kaplan–Meier survival curve analysis showed no significant difference in lung RR between the two groups.

Figure 4. Kaplan–Meier estimated cumulative recurrence rate after propensity score matching (PSM).

A) Kaplan–Meier survival curve analysis showed significantly worse remnant liver recurrence rate (RR) in the high-abdominal aortic calcification (AAC) group than in the low-AAC group after PSM.

B) Kaplan–Meier survival curve analysis showed no significant difference in lung RR between the two groups after PSM.

Figure 5. Phenotypic differences in liver natural killer (NK) cells between the high- and low-abdominal aortic calcification (AAC) groups.

Each point indicates the percentage of interleukin (IL)-2-stimulated liver NK cells in each group that was positive for TRAIL, NKp30, NKp44, NKp46, NKG2D, CD122, and SIRPβ.

*$p<0.05$.

Tables

Table I. Patient characteristics before and after propensity score matching.

	Before propensity score matching			After propensity score matching		
	Low AAC group N=32	High AAC group N=67	*p*-Value	Low AAC group N=29	High AAC group N=29	*p*-Value
Age (years)	55 [46-66]	69 [63-74]	<0.001	56 [44-66]	63 [56-69]	0.062
Sex (Male/Female)	16/16	51/16	0.009	16/13	16/13	1.000
mGPS			0.919			0.398
0	24 (75.0%)	48 (71.6%)		21 (72.4%)	22 (75.9%)	
1	7 (21.9%)	16 (23.9%)		7 (24.1%)	4 (13.8%)	
2	1 (3.13%)	3 (4.5%)		1 (3.5%)	3 (10.3%)	
GNRI	106 [97-110]	103 [94-110]	0.394	105 [97-109]	107 [100-110]	0.423
Fib4 index			0.021			0.547
Low (<1.3)	14 (43.8%)	15 (22.4%)		13 (44.8%)	12 (41.4%)	
Moderate (1.3−2.67)	15 (46.9%)	31 (46.3%)		13 (44.8%)	11 (37.9%)	
High (≥2.67)	3 (9.4%)	21 (31.3%)		3 (10.3%)	6 (20.7%)	
T-Bil	0.6 [0.5-1.0]	0.6 [0.4-0.8]	0.158	0.7 [0.5-1.1]	0.6 [0.5-0.8]	0.140
ICG-R (%)	8.4 [5.3-12.6]	11.0 [7.2-17.0]	0.107	8.5 [6.1-12.7]	10.8 [7.2-15.5]	0.310
Location (colon/rectum)	18/14	38/29	0.965	16/13	17/12	0.791
pT			0.922			0.753
T1	1 (3.1%)	1 (1.5%)		1 (3.5%)	0 (0.0%)	
T2	2 (6.3%)	6 (9.0%)		2 (6.9%)	3 (10.3%)	
T3	20 (62.5%)	41 (61.2%)		19 (65.5%)	19 (65.5%)	
T4	9 (28.1%)	19 (28.4%)		7 (24.1%)	7 (24.1%)	
pN			0.202			0.950
N0	7 (21.9%)	24 (35.8%)		7 (24.1%)	9 (31.0%)	
N1	12 (37.5%)	29 (43.3%)		11 (37.9%)	10 (34.5%)	
N2	12 (37.5%)	13 (19.4%)		10 (34.5%)	9 (31.0%)	
N3	1 (3.1%)	1 (1.5%)		1 (3.5%)	1 (3.5%)	
Tumor burden score	3.7 [2.5-5.8]	3.6 [2.1-5.2]	0.286	3.9 [2.5-5.5]	4.3 [2.3-5.5]	0.762
Number of tumors	2 [1-3]	2 [1-3]	0.860	2 [1-3]	2 [1-4]	0.298
Tumor size (cm)	2.5 [2.0-3.5]	2.0 [1.5-3.1]	0.162	2.7 [2.0-3.8]	2.5 [1.4-4.3]	0.377
Liver			0.143			0.698

metastasis grade						
A	17 (53.1%)	41 (61.2%)		16 (55.2%)	14 (48.3%)	
B	9 (28.1%)	22 (32.8%)		8 (27.6%)	11 (37.9%)	
C	6 (18.8%)	4 (6.0%)		5 (17.2%)	4 (13.8%)	
CEA	5.1 [1.9-20.7]	8.9 [3.2-25.4]	0.223	5.6 [2.4-26.9]	10.7 [3.6-44.7]	0.228
CA19-9	13 [4-69]	12 [3-24]	0.253	15 [5-78]	16 [5-74]	0.839
Pathological differentiation (por/muc)	1 (3.1%)	4 (6.3%)	0.516	1 (3.5%)	3 (10.3%)	0.300
Neoadjuvant chemotherapy	22 (71.0%)	49 (73.1%)	0.823	20 (69.0%)	21 (72.4%)	0.773
Adjuvant chemotherapy	24 (80.0%)	44 (66.7%)	0.183	22 (75.9%)	21 (72.4%)	0.764
Synchronous/metachronous	23/9	48/19	0.981	21/8	22/7	0.764
CD (≥ 3)	4 (12.5%)	7 (10.6%)	0.781	4 (13.8%)	4 (13.8%)	1.000

AAC: Abdominal aortic calcification; mGPS: modified Glasgow prognostic score; GNRI: geriatric nutritional risk index; Fib-4: Fibrosis-4; T-Bil: total bilirubin; ICG-R: indocyanine green retention; pT: pathological tumor; pN: pathological lymph node; CEA: carcinoembryonic antigen; CA19-9; carbohydrate antigen 19-9: por/muc: poorly/mucinous; CD: Clavien–Dindo.

Table II. Risk factors for overall survival.

Factors	Univariate			Multivariate		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (≥ 70)	0.83	0.43-1.61	0.591			
Sex (Male)	1.10	0.56-2.16	0.787			
AAC (≥ 116 mm^3)	1.99	0.95-4.18	0.070	2.22	1.04-4.74	0.040
mGPS (≥ 1)	1.98	1.01-3.86	0.046	2.05	1.04-4.04	0.037
pT4	0.86	0.43-1.72	0.667			
pN (≥ 2)	1.37	0.68-2.76	0.380			
Location of tumor (rectum)	1.10	0.60-2.05	0.752			

Por/muc	0.85	0.20-3.52	0.818			
TBS (≥ 3.6)	1.93	1.00-3.72	0.049			
Liver Grade						
A	1 (reference)					
B	1.86	0.97-3.59	0.064			
C	1.25	0.37-4.26	0.720			
CEA (≥10 ng/ml)	1.91	1.02-3.78	0.045			
CA19-9 (≥100 U/ml)	2.32	0.90-6.00	0.083	2.89	1.09-7.70	0.034
Neoadjuvant chemotherapy	1.37	0.65-2.88	0.411			
Adjuvant chemotherapy	1.15	0.55-2.41	0.718			
Synchronous	1.58	0.77-3.26	0.215			
CD (≥ 3)	1.03	0.40-2.66	0.953			

HR: Hazard ratio: CI: confidence interval; AAC: abdominal aortic calcification; mGPS: modified Glasgow prognostic score; pT: pathological tumor; pN: pathological lymph node; TBS: tumor burden score; CEA: carcinoembryonic antigen; por/muc: CA19-9: carbohydrate antigen 19-9: poorly/mucinous; CD: Clavien–Dindo.

Table III. Risk factors for ruminant liver recurrence.

Factors	Univariate			Multivariate		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Age (≥ 70)	1.28	0.71-2.31	0.417			
Sex (Male)	1.10	0.59-2.05	0.758			
AAC (≥ 116 mm^3)	2.13	1.06-4.31	0.034	2.14	1.05-4.39	0.037
mGPS (≥ 1)	1.43	0.75-2.75	0.277			
pT4	0.98	0.52-1.85	0.945			
pN (≥ 2)	1.72	0.94-3.15	0.077			
Location of tumor (rectum)	1.02	0.57-1.81	0.959			
Por/muc	0.28	0.04-2.06	0.213			
TBS (≥ 3.6)	2.56	1.39-4.70	0.003	1.96	1.03-3.71	0.039
Liver Grade						
A	1 (reference)					
B	1.91	1.03-3.54	0.039			
C	1.73	0.70-4.28	0.239			
CEA (≥10 ng/ml)	2.38	1.34-4.25	0.003			
CA19-9 (≥100 U/ml)	3.32	1.53-7.22	0.003	2.86	1.25-6.50	0.012
Neoadjuvant chemotherapy	0.92	0.48-1.76	0.804			
Adjuvant	1.05	0.54-2.04	0.880			

chemotherapy						
Synchronous	1.60	0.79-3.24	0.190			
CD (≥ 3)	1.24	0.55-2.77	0.609			

HR: Hazard ratio: CI; confidence interval; AAC: abdominal aortic calcification; mGPS: modified Glasgow prognostic score; pT: pathological tumor; pN: pathological lymph node; TBS: tumor burden score; CEA: carcinoembryonic antigen; CA19-9; carbohydrate antigen 19-9: por/muc: poorly/mucinous; CD: Clavien–Dindo.

Figure 1. The over-all survival (OS) and recurrence-free survival (RFS) curve analysis before propensity score matching

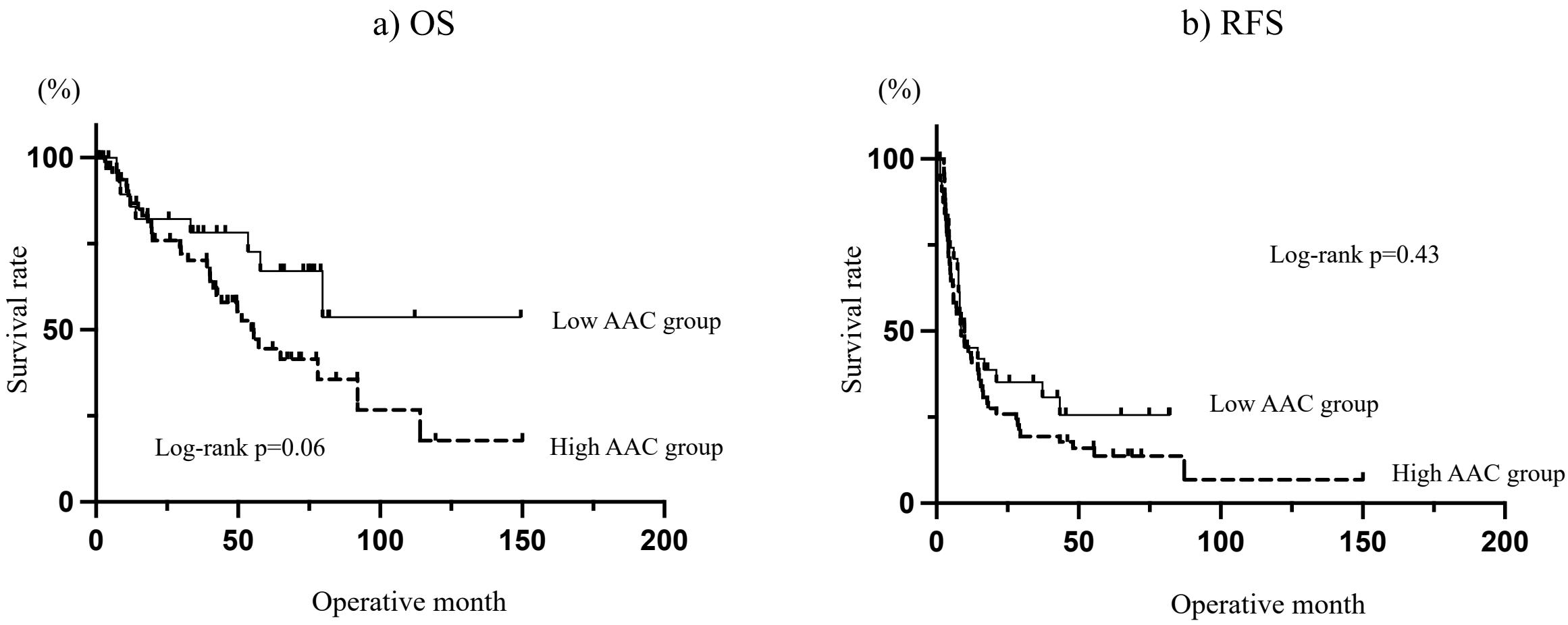
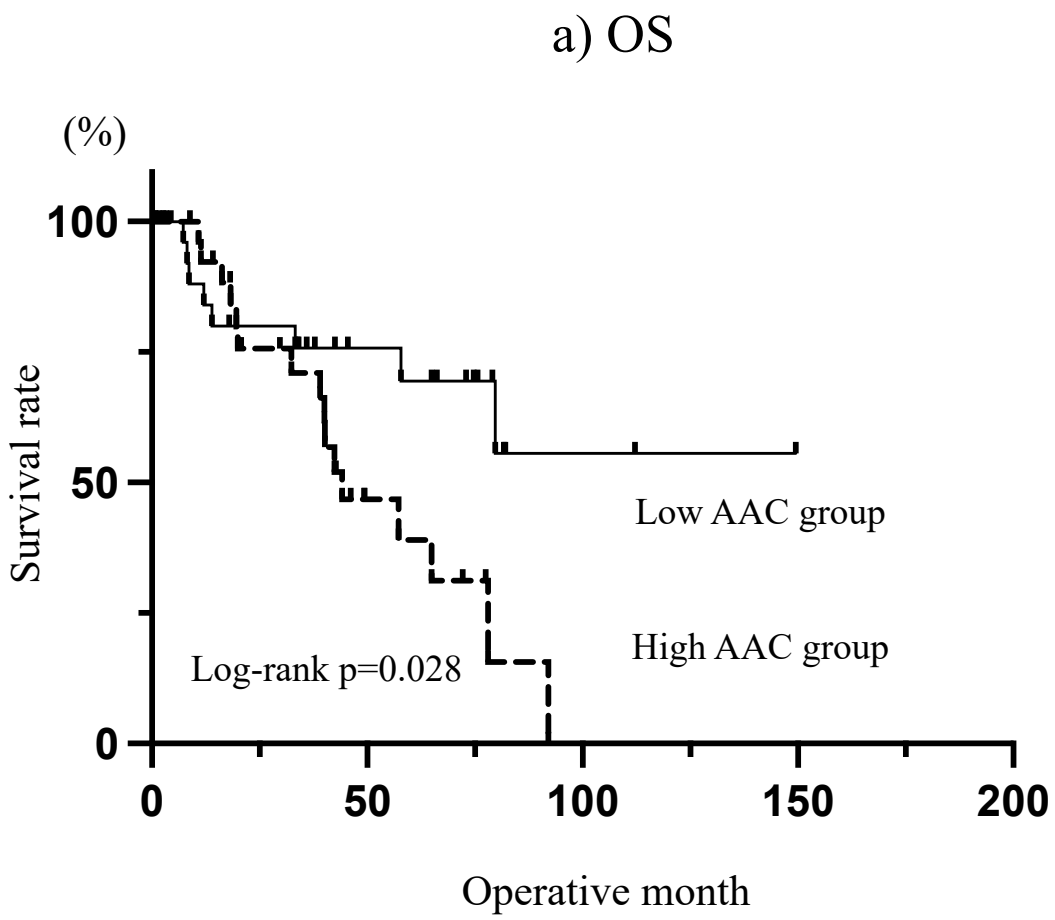


Figure 2. The over-all survival (OS) and recurrence-free survival (RFS) curve analysis after propensity score matching



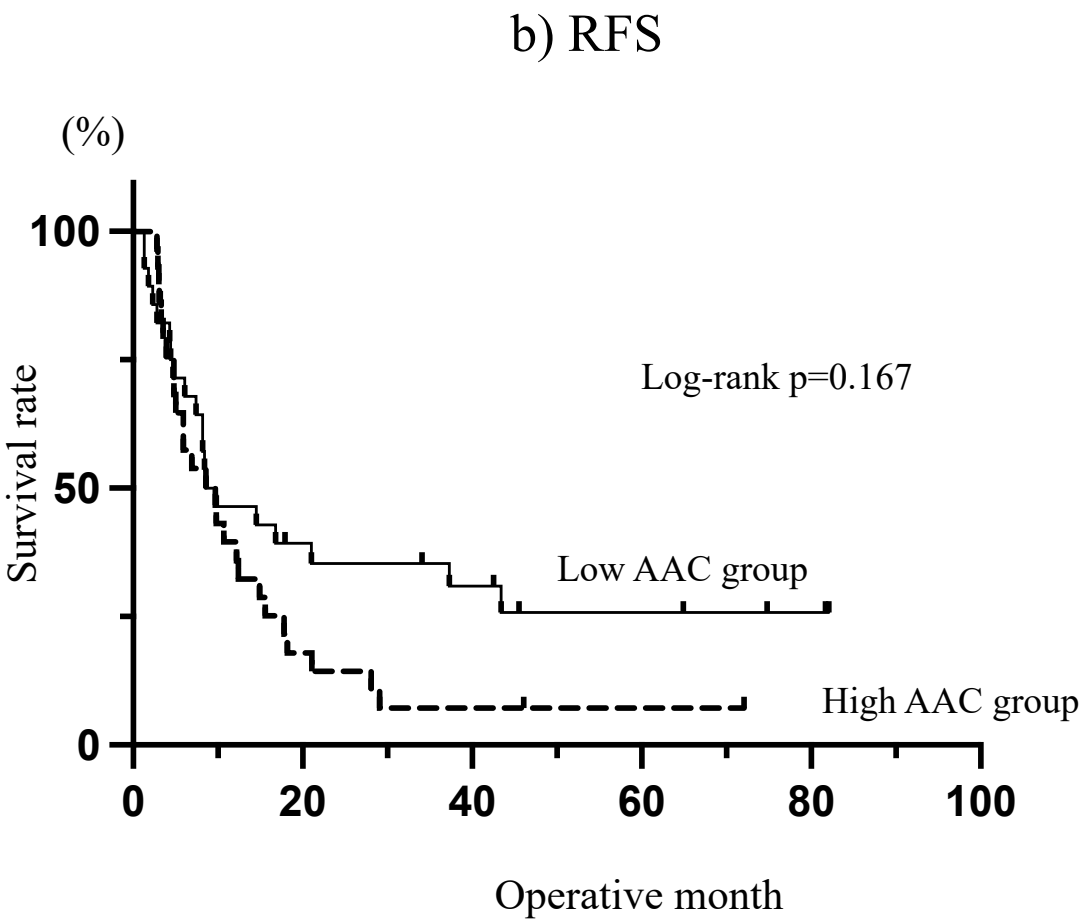


Figure 3. Kaplan-Meier estimated cumulative recurrence rate before propensity score matching

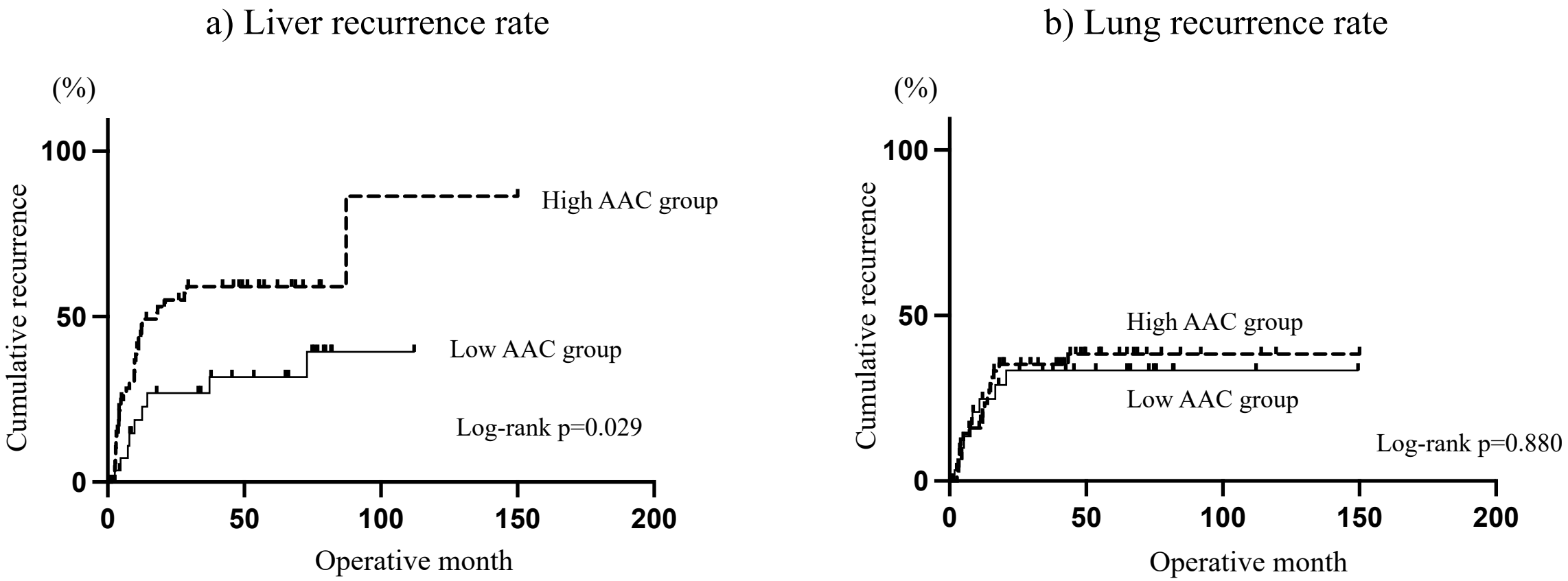


Figure 4. Kaplan-Meier estimated cumulative recurrence rate after propensity score matching

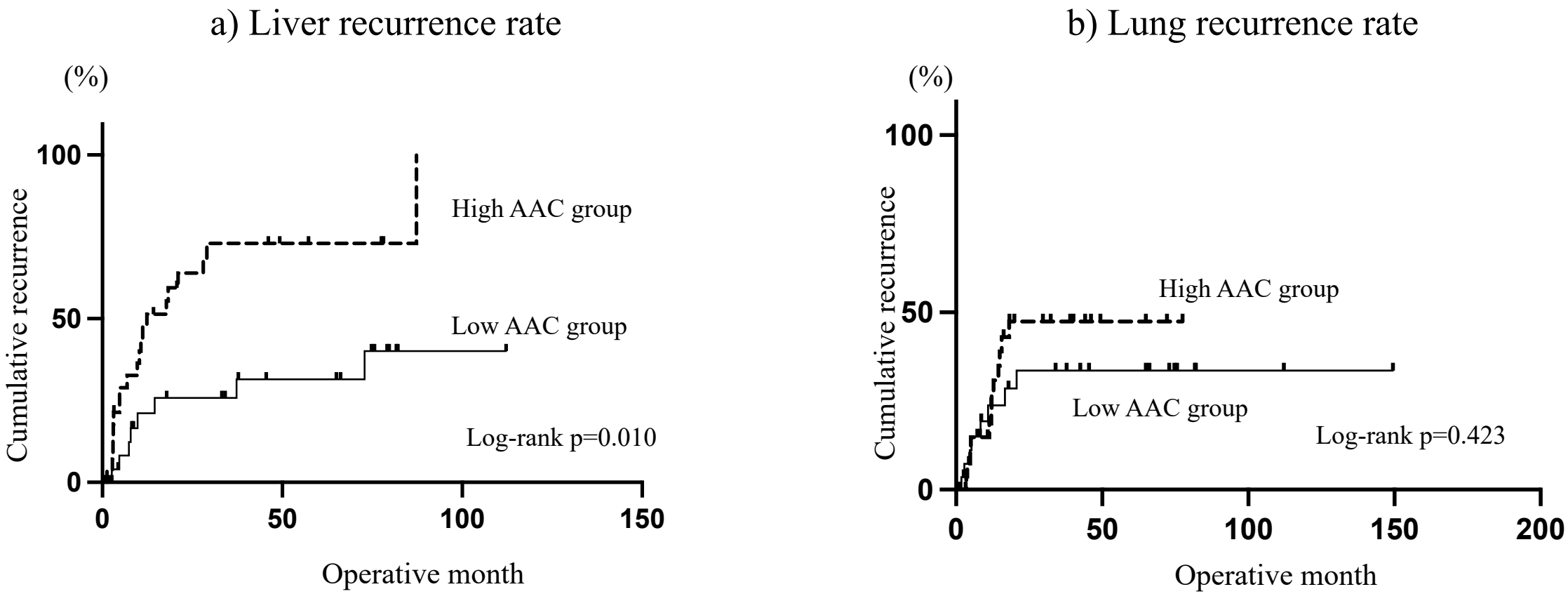


Figure 5. The phenotypic differences in liver NK cells between the high AAC group and the low AAC group