Evaluation of neutrophil-to-lymphocyte, platelet-to-lymphocyte, and lymphocyte-to-monocyte ratios in patients with Klatskin tumors

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AIM: *Our study aimed to evaluate the baseline and early follow-up evolution of three inflammatory ratios, namely neutrophil-to-lymphocyte (NLR), platelet-to-lymphocyte (PLR), and lymphocyte-to-monocyte (LMR) in patients with Klatskin tumors.*

MATERIAL AND METHODS: *A cohort retrospective study was conducted on consecutive patients with Klatskin tumor who presented in a regional surgical department for seven years (1 January 2012 to 31 December 2018). Raw data regarding the patients' characteristics and inflammatory biomarkers were collected from the hospital database. The cohort was divided according to the received treatment as surgical resection or palliative treatment (such as surgical drainage, percutaneous biliary drainage, or endoscopic stenting), and the patterns between groups were compared.*

RESULTS: *Fifty-seven patients, age from 39 to 79 years were evaluated. Neutrophil to lymphocyte ratio (NLR) increased significantly after both procedures (P<0.001). Lymphocytes-to-monocytes ratio (LMR) decreased significantly in the follow-up for patients with surgical resection, for Bismuth class III or IV (P=0.0037), and invasion (P<0.001). The baseline NLR (odd ratio OR=1.23, 95% CI: 1.00 to 1.52, P-value = 0.05) and PLR (OR=1.01, 95%CI: 1.00 to 1.01, P-value = 0.06) ratios could be markers for severity of the disease.*

CONCLUSIONS: *Changes in inflammatory ratios as increases in NLR and decreases of LMR (for patients with resection, higher Bismuth class and invasion) were observed in early follow-up in patients with Klatskin tumors. Baseline NLR and PLR values are potential markers in the identification of advanced hilar cholangiocarcinoma but need further investigation.*

KEY WORDS: Invasive procedures, Lymphocyte-to-monocyte ratio (LMR), Neutrophil-to-lymphocyte ratio (NLR), Platelet-to-lymphocyte ratio (PLR), Klatskin tumor

Introduction

Klatskin tumor is an extrahepatic cholangiocarcinoma situated at the confluence of the biliary tree at the level of liver hilum. Doctor Gerald Klatskin described this type of tumor in 1965 [1,2]. Klatskin tumor is a rare pathologic condition, with a prevalence around 1-4:100.000, more frequently seen in the male population (Male: Female= 1.3:1) from 5th to 7th age decades [3-5]. Klatskin tumor represents about 60% of all bile duct cancers [6] and is the most aggressive one.

The Bismuth-Corlette classification describes four types of Klatskin tumors according to localization: below the bifurcation, at the level of the hepatic duct (type I); at the level of bifurcation without invading the second-order bile ducts (type II); at the level of the right second-order ducts (type III) or only the left second-order ducts (type IIIb); the second-order bilaterally ducts (type IV) [7]. Early lymph

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node involvement, caudate lobe involvement, vascular and perineural invasion are reported in the scientific literature regarding this malignancy [8,9].

Hilar cholangiocarcinoma is very aggressive, had a poor prognosis, and a high stage at the presentation leave only palliative possibilities. The best-desired option in Klatskin tumor is curative surgical resection due to their resistance and modest response to radiotherapy and chemotherapy [10,11]. Resectability is reported in the scientific literature from 26.8% [12] to 80% [13]. Several palliative options are available in case of locally advanced tumors, inoperable cases, or presence of metastasis: percutaneous ultrasound-guided drainage, surgical intraoperative stenting (surgical drainage), endoscopic stenting, standard adjuvant chemotherapy (e.g. gemcitabine cisplatin regime) [5,10,14]. Very often, for curative resection hemihepatectomy is also necessary, and it seems that resection of the quadrate lobe facilitates the dissection of the tumor. However, best results are obtained by combining hilar resection with extended liver resection [15].

Inflammatory biomarkers are involved in the progression of cancer by increasing vascular permeability, increasing cancerous cells infiltration in the lymphatic and blood vessels, and usually associated with poor prognosis regarding survival and tumor progression [16]. The inflammatory response is related to tumor infiltration with lymphocytes and cytokines release [17]. Neutrophils are the primary container of circulating VEGF (vascular endothelial growth factor) among granulocytes.

Monocytes and lymphocytes contain insignificant levels of VEGF. It has been proved using the FACS method (fluorescence-activated cell sorting) that neutrophils and platelets are a physiological pool for VEGF, ready to release the factor in an acute situation like wounds and to increase local angiogenesis.

They also found that in the case of malignancy, neutrophils contain a higher dose of VEGF as compared with healthy volunteers [18]. Furthermore, neutrophils can be activated by pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) to release VEGF [19]. Physiological degranulation and death of neutrophils in the vicinity of the neoplastic process lead to an increased level of VEGF in this area that stimulates the angiogenetic process. Increased vascular supply and permeability can facilitate tumor growth and metastasis.

The neutrophil-to-lymphocyte ratio (NLR) is an inflammatory marker easily calculated and without any additional costs. Gender (lower NLR on women as compared to men: median of 1.96 vs. 1.99, P=0.03), age (higher NLR ≥60 years: median of 2.11 vs, 1.92 P<0.0001), the presence of co-morbidities (median NLR 2.03 vs. 1.91 for healthy participants P<0.0001), marital status (higher median of NLR - 2.17 for widowed and smaller median of NLR – 1.88 for those leaving with a partner, P<0.0001), income (higher median of NLR – 2.01 for low income compared to 1.95 for those with high income, P<0.001) had been reported in the scientific literature [20]. Higher NLR value was reported among smokers, persons with diabetes, cancer, and cardiovascular disease, but proved not significantly associated with cancer mortality (Hazard Ratio = 1.20; 95%CI 0.95–1.51) [21].

Several studies evaluated NLR (neutrophil to lymphocyte ratio), PLR (platelets to lymphocyte ratio), and LMR (lymphocyte to monocyte ratio) in the malignant process, including cholangiocarcinoma. The usefulness of NLR (3.61±1.32 for cases with T2b or higher vs. 2.66±1.41 for cases with T stage <T2b) and PLR (181.56 ± 44.98 for cases with T2b or higher vs. 141.32 ± 43.79 for cases with T stage <T2b) as predictors of the T-stage in hilar cholangiocarcinoma had been investigated by Huang et al. [22]. The cut-off value for NLR was 2.75, and 172.25 for PLR regarding T2b stage [21]. Pre-operative PLR ≥ 185 in patients with extrahepatic cholangiocarcinoma proved associated with higher liver recurrence (P=0.04, n=120), high recurrence in the first year post-intervention (P=0.02), poor prognosis (significantly higher recurrence rate in the first year), and an overall survival reduced with 25% (P=0.008) [23]. A resectability of 78.7% in patients with pre-operative LMR>3.67 as compared to 37.1% in patients with pre-operative LMR ≤ 3.67 for patients with hilar cholangiocarcinoma Bismuth IV type has been reported by Peng et al. [24]. This study also demonstrates that patients with post-LMR ≤4.10 have a significantly higher early recurrence rate than those with LMR>4.10 [24]. Hu et al. reported that PLR≥150, NLR≥3, tumor size ≥ 3 cm, and pre-operative cancer antigen CA125>35 U/ml are rather linked with unresectable tumors on patients with hilar cholangiocarcinoma [6].

Our study aimed to evaluate the pattern of three inflammatory ratios, namely NLR, PLR, and LMR at the presentation as compared to early post-intervention follow-up in patients with Klatskin tumor considering tumor invasion, Bismuth class, and optimal procedure. Furthermore, the association between baseline value of NLR, PLR, and LMR and optimal procedure, Bismuth class, and invasion were also investigated.

Materials and Methods

Setting and Study Design

A cohort study with data collected retrospectively was conducted on patients with Klatskin tumors hospitalized at the "Prof. Dr. Octavian Fodor" Regional Institute of Gastroenterology-Hepatology, Surgery Department Cluj-Napoca, Romania, between 1 January 2012 and 31 December 2018.

The study was approved by the "Iuliu Hațieganu" Ethics Committee (approval no. 121/24.04.2019) and by the

Ethics Committee of the “Prof. Dr. Octavian Fodor” Regional Institute of Gastroenterology and Hepatology (approval no. 8900/10.07.2019).

Participants

All patients with Klatskin tumor at their first presentation into our service and histopathological confirmation (patients with surgical intervention or with clinically and imagistic diagnostic in case of palliative treatment) were enrolled in the study. Patients with unclear diagnosis, presented for reintervention, who followed only medical non-invasive treatment or transferred into other services for intervention were excluded from the study. The diagnosis and Bismuth class were done based on clinical and imagistic characteristics (ultrasound investigation, computer tomography, magnetic resonance, and endoscopic retrograde cholangiopancreatography) for patients with a palliative intervention.

The cohort was classified according to the applied therapeutic strategy as curative surgical resection or palliative intervention (palliative ultrasound-guided transcutaneous biliary drainage, surgical drainage, or endoscopic stenting). Patients with a surgical intervention were considered as resected cases regardless of previous endoscopic stenting (if applicable). Patients with metastasis or locally advanced tumors (extension to the secondary branches of the biliary tree, the involvement of the portal branches or hepatic artery, multiple vessels involvement, a tumor block with the impossibility of dissection at laparotomy) or those unable to tolerate a major surgical procedure due to age, co-morbidities or general poor health received palliative treatment. Whenever a surgical resection was not possible due to invasion, and the case was resumed to an exploration laparotomy, or surgical stenting, the intervention was considered as a palliative procedure.

Data Source and Collection

The medical charts of the eligible patients were reviewed and used as the source of raw data. Demographic data (e.g., age, gender, urban vs. rural settings), clinical data (e.g., main signs and symptoms at the presentation, hepatobiliary and malignant medical history), laboratory data (e.g., absolute number of neutrophils (10^3/L), lymphocytes (10^3/L), monocytes (10^3/L), platelets, and hemoglobin levels, direct and indirect bilirubin levels, alkaline phosphatase (AF), gamma glutamiltranspeptidase (GGT), aspartate amino transferase (AST), alanine amino transferase (ALT), amylase and potassium levels) were collected.

Whenever available, the values of neutrophils, lymphocytes, monocytes, and platelets were collected at the presentation (baseline) and perioperative (3rd to 5th day after the intervention). The NLR (neutrophil to lymphocyte ratio), PLR (platelets to lymphocyte ratio), and LMR (lymphocyte to monocyte ratio) at the presentation (baseline) and early post-intervention were calculated as a ratio of absolute values.

The blood counts were determined on blood samples harvested on EDTA (violet vacutainer - ethylenediaminetetraacetic acid). The dosage method used for complete blood count involved flow cytometry, impedance, and spectrophotometry, were determined with a MINDRAY BC-6800Hematology Analyzer (MINDRAY Headquarters, Shenzen, 518057 P. R. China, SN SH-25000300). For biochemistry analysis, the blood was collected in yellow vacutainer on sodium citrate, and the dosage was determined by potentiometry and spectrophotometry using Konelab PRIME 60 ISE Type 983 (Thermo Fisher Scientific, Vantaa, Finland, SN 23040). The blood samples were analyzed up to two hours from collection.

Statistical Methods

Statistica program (v. 13, StatSoft, OK, USA) was used in statistical analysis. Quantitative data were reported as the median and interquartile range since the number of patients in each group was small. Qualitative data were reported as numbers and frequencies. Wilcoxon test was used to compare baseline with follow-up values by groups of interventions. The quantitative comparisons between groups were made with the Mann-Whitney. Differences in percentages between groups were tested with the Chi-square test. All tests were conducted at a significance level of 5% considering the two-tailed test, and a P-value less than 0.05 was considered statistically significant.

We assumed that inflammatory scores (namely NLR, PLR, and LMR) are significantly different according to the intervention type (surgical resection or palliative intervention), tumor invasion (vascular - arterial or venous, lymphatic, perineural or other invasions such as duodenal, enteral, hepatic, in the gallbladder), and Bismuth class (Bismuth I or II vs. Bismuth III or Bismuth IV). The association of baseline value of inflammatory biomarkers and the optimal intervention, Bismuth class, and invasion was tested using logistic regression analysis.

Results

Sixty-six medical charts of patients with the diagnosis of hilar cholangiocarcinoma registered in the internal database were screened for inclusion in the study. Fifty-seven patients with documented hilar cholangiocarcinoma were included in the analysis. The flowchart of patient selection and the reason for exclusion are given in Fig. 1. The surgical intervention was indicated for 25 patients (43.85%).

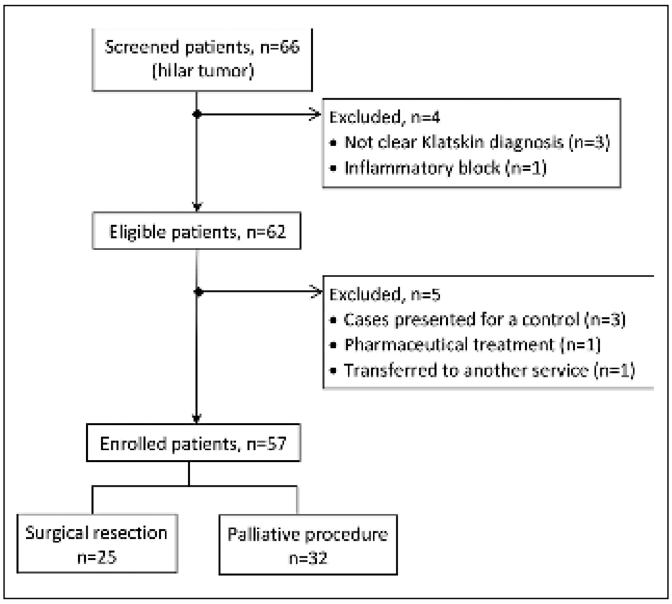

Fig. 1: Enrolment of patients in the study.

TABLE I - *The main characteristic of evaluated patients (n=57)*

Characteristic	Value
Age, years – median (Q1–Q3)	65 (61–70)
Gender, male – no. (%)	36 (63.2)
Urban setting – no. (%)	26 (45.6)
Bismuth – no. (%)	
I	12 (21.1)
II	2 (3.5)
III	18 (31.6)
IV	25 (43.9)
Other diseases – no. (%)	
Gallstone	14 (24.6)
Other malignant tumors history	9 (15.8)
Cirrhosis	5 (8.8)
Hepatic steatosis	2 (3.5)
Signs and symptoms – no. (%)	
Jaundice	47 (82.5)
Pain	21 (36.8)
Fever	11 (19.3)
Ascites	7 (12.3)
Weight loss	6 (10.5)

Q1 = first quartile; Q3 = third quartile; no = absolute frequency

TABLE II - *Laboratory measurements and inflammatory ratios*

Characteristic	Baseline, n=54	Follow-up, n=48	Stat. (P-value)**
Neutrophils (10^3/ L)	6.26 (4.68–8.49)	9.82 (7.76–11.73)	4.19 (<0.0001)
Lymphocytes (103/ L)	1.39 (1.06–1.92)	1.47 (0.92–2.04)	0.67 (0.5050)
Monocytes (103/ L)	0.53 (0.36–0.74)	0.66 (0.49–0.80)	0.92 (0.3572)
Platelets (103/ L)	273 (227–341)*	264 (212–342)	1.04 (0.2973)
NLR	4.13 (2.84–6.51)	7.15 (4.46-10.57)	5.97 (p<0.0001)
PLR	200.00 (127.65–257.38)*	181.08 (124.46–272.05)	0.29 (0.7751)
LMR	2.93 (1.80–4.92)	2.08 (1.61–3.35)	2.55 (0.0108)

Values are expressed as median and interquartile range (Q1–Q3, where Q is the values of the quartile, first and respectively third); NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; LMR = lymphocyte-to-monocyte ratio.* n=53; **Wilcoxon test; Stat = the statistics of the test

The age of the evaluated patients ranged from 39 to 79 years, with more men than women (36 to 21). The characteristics of the patients included in the study are given in Table I.

The majority of patients presented with anemia (43/57, 75.4%), increased liver enzymes (50/57, 87.7% increased GOT- glutamate-oxaloacetate transaminase and GPT-glutamate-pyruvate transaminase), increased values of total bilirubinemia (50/57, 87.7%) and conjugated bilirubinemia (54/57, 94.7%). Fifty-two patients were found with the increased alkaline phosphatases (91.2%), and the values remained over the normal limits at follow-up at 44 patients (77.2%) even if a significant decrease at follow-up was observed (Wilcoxon test: Z-stat=2.09, P=0.0368 regardless the type of intervention – for resection: P=0.0028 vs. palliative intervention: P=0.0019).

Results regarding the values for inflammatory biomarkers are presented in Table II.

A similar pattern with alkaline phosphatases was also observed for GGT (gamma-glutamyl transpeptidase), with elevated values at baseline in 53 patients (93.0%), which remain elevated at follow-up for 44 patients (77.2%). However, GGT significantly decreases at follow-up as compared to baseline values (Wilcoxon test: Z-stat=3.64, P=0.0003) regardless of the type of intervention (resection P=0.0025 vs. palliative intervention: P=0.0280). Only two patients had elevated amylase values at baseline (3.5%). Nine patients (15.8%) presented with hypokalemia.

The majority of patients were with tumors classified as Bismuth type III or IV (43/57, 75.4%). The invasion was documented in 29 patients (50.9%): lymphatic in 16 patients (28.1%), vascular in 15 patients (26.3%), perineural in 11 patients (19.3%), and five patients with other locations of invasion (8.8%) such as the duodenum, gallbladder or enteral invasion.

No significant differences had been observed when the absolute values of NLR, PLR, and LMR were compared

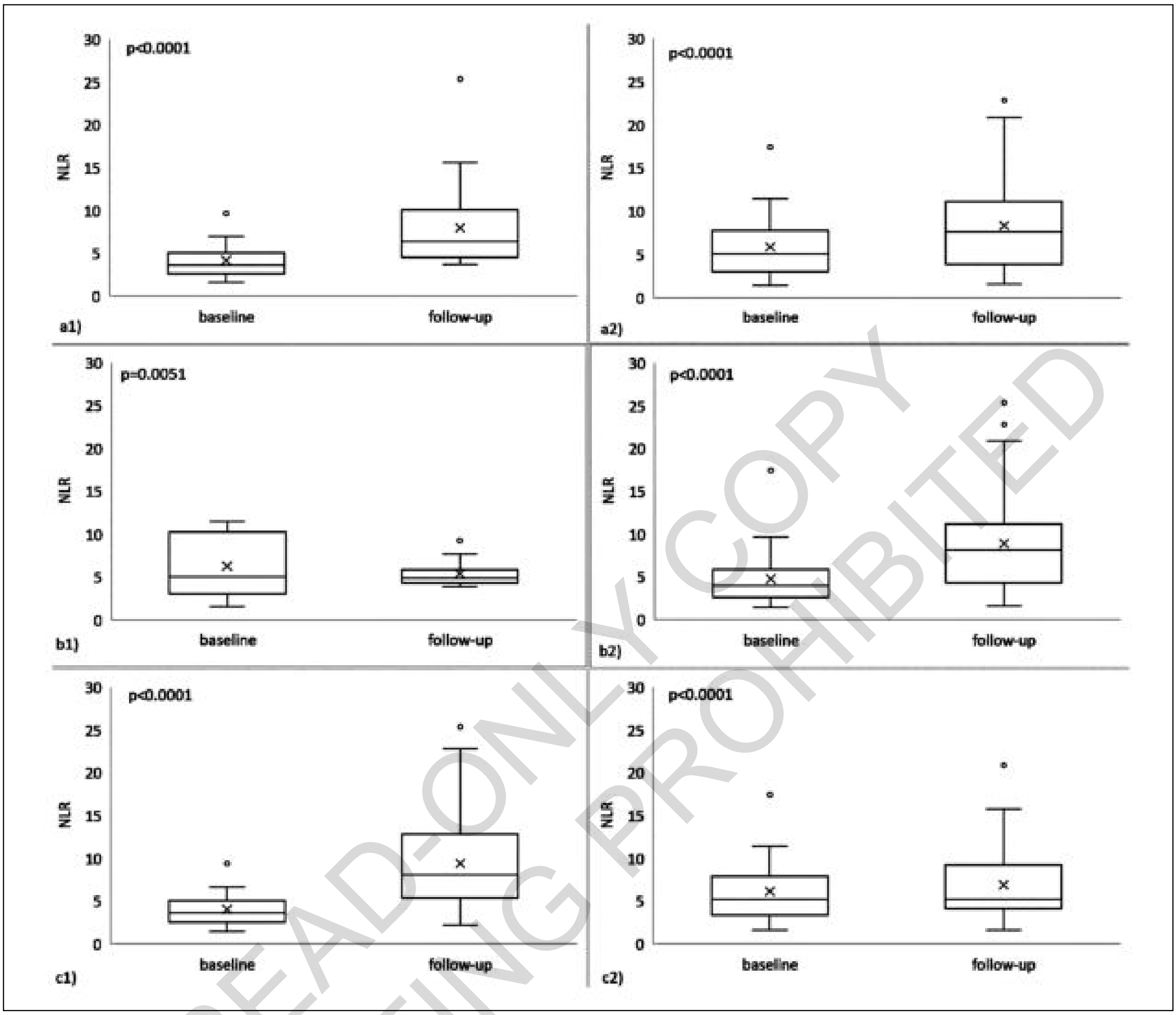

Fig. 2: Variation of neutrophil-to-lymphocytes ratio (NLR) at follow-up compared to baseline values (Wilcoxon test) on different sub-groups: a1) with (n=23) and a2) without resection (n=24); b1) Bismuth I or II (n=11), b2) Bismuth III or IV (n=36); c1) with (n=23) and respectively c2) without invasion (n=24).

on patients with and without resection, Bismuth I or II vs. Bismuth III or IV, or on patients with and without invasion, neither on baseline nor on follow-up (Mann-Whitney test; P>0.06).

With one exception, the neutrophil to lymphocyte ratio significantly increased at follow-up regardless of the intervention (resection in Fig. 2a1, respectively palliative intervention in Fig. 2A2); Bismuth class I or II (Fig. 2b1) vs. III or IV (Fig. 2B2); with (Fig. 2c1) or without invasion (Fig. 2c2). The exception is observed among patients with Bismuth class I or II, with NLR values significantly lower at follow-up compared to baseline (Fig. 2b1).

Lymphocytes to monocytes ratio (LMR) significantly decreased at follow-up as compared to baseline values only for patients with resection (Fig. 3a), those with Bismuth class III or IV (Fig. 3b), and respectively those with invasion (Fig. 3c). No other significant changes in LMR were observed in the investigated cohort (P>0.05).

Logistic regression analysis identified baseline NLR and PLR as candidates for risk factors independent by gender and age (Table III). No other models proved statistically significant (P>0.10).

Discussion

The general pattern of the investigated inflammatory ratios showed significant increases in values at follow-up

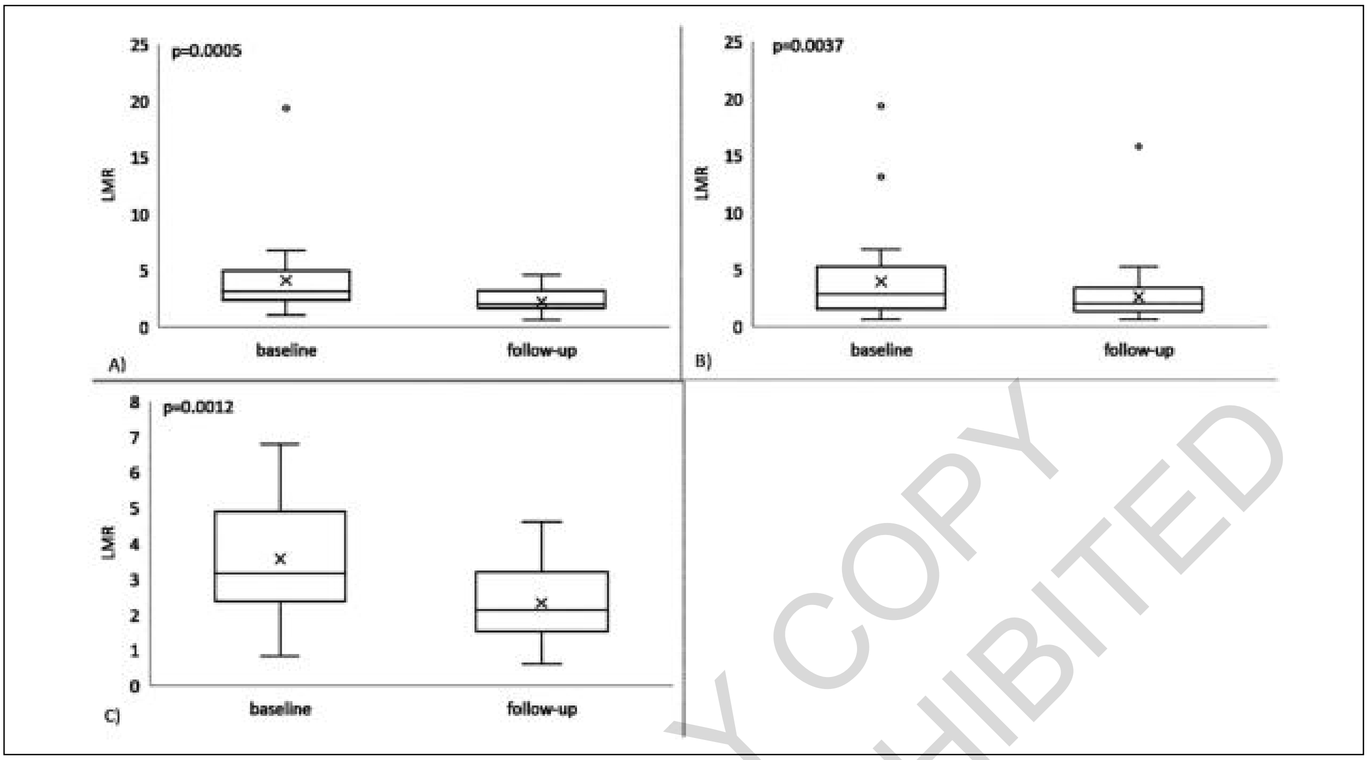

Fig. 3: Variation of lymphocytes-to-monocytes ratio (LMR) at follow-up as compared to baseline values for A) patients with resection (n=24; one extreme value was observed at baseline; the withdrawn of this value does not affect the significance: P=0.0009, Wilcoxon test); B) patients with Bismuth class III or IV (n=37); C) patients with invasion (n=24).

TABLE III - *Association analysis of neutrophil-to-lymphocytes ratio (NLR) and PLR (platelets to lymphocyte ratio) with the type of intervention*

Term	OR [95%CI]	P-value	Model Performances: Hosmer-Lemeshow test: χ^2 (df; P-value)	Model Performances: Nagelkerke's R^2
Type of intervention				
Gender (Male)	1.72 [0.50 to 5.85]	0.3887	8.06 (8; 0.4308)	0.1417
Age (years)	1.03 [0.96 to 1.11]	0.4527		
NLR baseline	1.23 [1.00 to 1.52]	0.0515		
Type of intervention				
Gender (Male)	1.71 [0.50 to 5.90]	0.3921	6.94 (8; 0.5433)	0.1280
Age (years)	1.03 [0.95 to 1.10]	0.5113		
PLR baseline	1.01 [1.00 to 1.01]	0.0588		

All P-values associated with the value of the Constant > 0.25;
OR = Odds Ratio; 95%CI = 95% confidence interval [lower bound to upper bound]; df = degree of freedom

as compared to the baseline values. Furthermore, baseline NLR and PLR showed potential usefulness as prognostic factors for severity independently by age and gender but this result need to be validated in larger cohort. The characteristics of the investigated sample are similar to those reported in the scientific literature, with a slightly higher frequency on men as compared to women (1.7 in our study – Table I vs. 1.3 as previously reported) [25] but in the same age decades [6,26]. Furthermore, the symptoms reported by our sample are similar to those previously reported by patients with Klatskin tumors [6,27]. The late diagnosis and local advanced cases at the presentation are specific to Klatskin tumors and were also observed in our study. The presence of invasion (50.9%) and the association of other medical conditions driven to a high percentage of cases suitable only for palliative treatment as compared to surgical resection that proved suitable only to 25 patients. The most common sites of invasion in the evaluated sample, namely lymphatic, vascular, and perineural, are similar to those referred to the scientific literature [8,9]. However, adiacent structures invasions have also been observed in five patients in our sample.

The efficacy of the therapeutical intervention was observed regardless of the type of intervention, with significantly decrease of the values of cholestasis enzymes, but generally with values higher than the normal upper ranges. The higher significance of the alkaline phosphatases for the palliative group (P = 0.0019 as compared to P = 0.0028 for patients with resection) could be explained by local inflammation associated with the healing process of the biliary tree after anastomosis in the early days after surgery in the group with resection as well as of the overall impact of the resection, a major intervention, on metabolism.

Significantly increased in the neutrophils were observed on follow-up with an increase in the values of lymphocytes and monocytes but without reaching the significance threshold (Table II). The platelet values decrease at early follow-up but, similar to the lymphocytes and monocytes, without reaching the statistical significance (see Table II). Neutrophils are involved in the communication between tumor cells and the microenvironment, increasing the survival, proliferation, and metastasis of cancer cells by secretion of interleukin (IL) 1, IL- 6, and VEGF (vascular endothelial growth factor) [19,28]. The follow-up NLR increase significantly, almost twice as compared to baseline values (Table II). The increase in the evaluated NLR in follow-up was observed on all patients regardless of the type of intervention, surgical resection (Fig. 2a1), or palliative treatment (Fig. 2a2). This pattern was expected for cases with resection considering the invasivity of the procedure, but it was almost comparable even for the groups who received palliative procedures (Fig. 2). Note that the palliative interventions also included the patients with surgical drainage, which could drive the NRL in a similar way as for patients with resection. Increasing in follow-up NLR values were not influenced by tumor invasion (patients - Fig. 2c1 and without invasion - Fig. 2c2). Follow-up NLR was also influenced in our sample by the localization of the tumor (Bismuth classification), with decreasing values in early follow-up for lower Bismuth classes (I and II, Fig. 2b1) and opposite pattern for patients with Bismuth class III and IV (Fig. 2b2). High values of baseline NLR were reported as associated with poor prognostic in patients with cholangiocarcinoma [29]. Higher NLR values were also reported to be associated with a more upper TNM stage in patients with cancer [17,30], including those with cholangiocarcinoma [21]. The PLR slightly decreases at early follow-up without reaching the statistical significance (Table II). The LMR follow the pattern of PLR with decrease values in early follow-up as compared to baseline, but this time the difference of almost one unit proved statistically different (Table II). Statistically significant lower LMR values were observed in the follow-up as compared to the baseline for patients with resection (Fig. 3a). Because of the process of inducing natural killer cells and the release of interferons and TNF-α, lymphocytes are considered one of the most important cells involved in tumor immunity [31]. The significant decrease in LMR after resection procedure (Fig. 3a) could also be explained by the loss of lymphocytes with the tumor tissue. The palliative procedures, which do not involve cytoreduction of tumoral tissue, had no significant changes in LMR (P>0.05). According to Avci and Song, a decrease in lymphocytes number can signify a low immune antineoplastic activity [32,33], which can explain our statistically significant decrease of LMR in the early follow-up among patients with invasion (Fig. 3c) due to the combined of a more aggressive local advanced tumor with the major stress in the early post-intervention period, impairing immune response.

Lymphocytes fight against the cancer cells and a low LMR seems to be correlated with bad prognosis in neoplastic disease, while neutrophils reflects inflammatory activity on the other hand, and an increased NLR value can be associated with poor outcome [16,34].

The association analysis identifies the baseline NLR (P-value = 0.05) and PLR (P-value = 0.06) as possible candidates (the P-values show a tendency to statistical significance) as risk factors to the optimal intervention (Table III). Both, NLR and PLR proved factors independent by gender and age, demographic characteristics previously reported as associated with high values for male and subject of 60 years or older [21]. The absence of significance related to the Hosmer–Lemeshow test suggest that the models presented in Table III fit well the data. However, the risks are closed to one, indicating similar values of NLR and respectively PLR among subjects with different optimal interventions. However, these results need to be validated on a larger sample size ideally in multi-center studies.

Different cut-off values for NLR have been reported in the scientific literature for cholangiocarcinoms (from 2.49 to 5) [28]. Lalosevic et al. reported higher NLR and PLR values for patients with colorectal cancer as compared to healthy volunteers, and the cut-off values were lower than used in our study (2.15 for NLR (AUC = 0.790, 95% CI 0.736-0.884, Se = 74.1%, and Sp = 73%) and 123 for PLR (AUC = 0.846, 95% CI 0.801-0.891, Se = 74%, and Sp = 80%) [34]. Fang et al. reported an increase in the value of NLR and PLR with the stage of the disease and the optimal cut-off values of 2.258 for NLR (AUC=0.715, 95%CI 0.703 to 0.728, Se=48.88%, Sp=83.04%), and 147.368 for PLR (AUC=0.707, 95%CI 0.695 to 0.719, Se=48.2%, Sp=81.79%) on early diagnostic of gastric cancer [35]. However, in a multivariate analysis on patients with gastric cancer, neither NLR (p=0.687) nor PLR (p=0.467) proved associated with mortality [36]. Pirozzolo et al. reported the highest cut-off NLR value equal to 5 and the smallest one of

1.7 in patients with esophageal cancer with higher values associated with poor prognostic [37].
Dal et al. also reported higher NLR values as associated with poor prognosis such as shorter survival time (average of 15 months for higher NLR group versus 29 months for lower NLR group; p=0.0026), higher incidence of anastomosis leakage (20% versus 0%; p=0.048) and longer hospitalization stay (23.9 days versus 13.27 days; p=0.009) and a higher rate of lymph node invasion (93.3% vs 66.7%; p=0.024) in patients with esophageal cancer [38].

Study Limitations

Several limitations of our study can be highlighted. First, data were collected retrospectively, and thus some values were not available in the medical charts. A cohort study with prospective data collection could withdraw the existence of missing data. Second, data were collected in baseline and early follow-up (three to five days), and this could induce between and within groups bias relative to the small-time between measurements of the investigated inflammatory biomarkers. Third, we did not succeed in collecting all confounder factors for NLR, PLR, and MLR (e.g., smoking status, alcohol consumption, co-morbidities, anti-inflammatory medication, etc.). All these variables must be included in the association analysis to validate the possible utility of baseline NLR and/or PLR as risk factors for optimal intervention. Forth, data were collected from one regional center, thus the sample size is limited, and the generalizability of the results is not recommended. Fifth, due to the availability of data, no investigation was conducted in regard to TNM classification. A larger sample size is needed to validate the results of our study with the inclusion of more centers, with a proper reflection of patients regarding the Bismuth classification as well as documented TNM stage to adequate identification of the cut-off NLR for resectability. Future prospective studies should also evaluate the effect of associated inflammatory pathology, anti-inflammatory medication, and/or late follow-up on the inflammatory ratios. The long-term follow-up would be recommended in the investigation of the NLR, PLR, and MLR ratios to eliminate the effect of the invasivity of the applied procedures. The late follow-up evaluation, after the tumor removal and cool down of the associated inflammation process, could also bring insides the pattern of the inflammatory rations between the group with tumor removal and those with a palliative intervention. The combination of different inflammatory biomarkers in a score like Naples prognostic score (NPS) who proved associated with advanced TNM on patients with gastric cancer [39] could also find its usefulness in the evaluation of patients with Klatskin tumors. However, a multicenter study is needed to investigate a representative sample able to capture the heterogeneity of these patients.

Conclusions

A significant increase in neutrophil-to-lymphocyte ratio and a decrease of lymphocyte-to-monocyte ratio for patients with resection, higher Bismuth class, and invasion were observed in early follow-up among patients with Klatskin tumors.
The baseline neutrophil-to-lymphocyte and platelet-to-lymphocyte are potential markers in the identification of advanced disease independent by gender and age but need to be validated on larger samples and in the context of other confounders. Patients with baseline lymphocyte-to-monocyte higher than 3 have a higher frequency of invasion on our sample, but these findings need validation on further studies.

Acknowledgements

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Riassunto

Il nostro studio è finalizzato a valutare la situazione iniziale e l'evoluzione nel followup di tre indici di flogosi, e cioè i rapporti neutrofili-linfociti (NLR), piastrine-linfociti (PLR) e linfociti-monociti (LMR) in pazienti con tumore di Klatskin.
A questo scopo è stato impiantato uno studio retrospettivo su una serie di pazienti con tumore di Klatskin ricoverati consecutivamente per sette anni (dal 1 gennaio 2012 al 31 dicembre 2018) in un reparto chirurgico regionale. I dati sulle caratteristiche dei pazienti e gli indici di flogosi sono stati raccolti dall'archivio dell'ospedale. Il gruppo di pazienti è stato suddiviso in base al trattamento ricevuto come resezione chirurgica o trattamento palliativo (drenaggio chirurgico, drenaggio biliare percutaneo o stent endoscopico) e sono stati confrontati i parametri tra i gruppi.
Risultati: sono stati valutati complessivamente cinquantasette pazienti, di età compresa tra 39 e 79 anni. Il apporto neutrofili.linfociti (NLR) è aumentato significativamente dopo entrambe le procedure (P <0,001). Il rapporto linfociti-monociti (LMR) è diminuito significativamente nel follow-up per i pazienti con resezione chirurgica, per classi di Bismuth III o IV (P = 0,0037) e infiltrazione (P <0,001).
I rapport iniziali NLR (OR=1.23, 95% CI: 1.00 to 1.52, P-value = 0.05) and PLR (OR=1.01, 95%CI: 1.00 to 1.01, P-value = 0.06) potrebbero rappresentare altrettanti markers di gravità della malattia.

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