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Innovative Blood Tests for Hepatocellular Carcinoma: Liquid Biopsy and Evaluation of Systemic Inflammatory Reaction

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Abstract

Hepatocellular carcinoma (HCC) is an aggressive tumour associated with dismal prognosis. To improve the outcome, early diagnostics is important. At present, classical HCC diagnostics is based on evaluation of risk factors, surveillance in cirrhotic patients, preference for non-invasive diagnosis by computed tomography or magnetic resonance imaging and biopsy confirmation in controversial cases. However, ambiguous radiological presentation, biopsy-related complications or insufficient representation of the pathology in the tissue core are well-known problems. Panel assessment of microRNAs has diagnostic and prognostic value; thus, in future, microRNA-based liquid biopsy could partially reduce the need for core biopsies. Systemic inflammatory reaction (SIR), characterised mainly by neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and Glasgow prognostic score, may have prognostic value and can be incorporated in criteria for certain treatment approaches, e.g., becoming an adjunct to Milan criteria. Thus, innovations in HCC diagnostics are expected in the field of miRNA-based liquid biopsy for diagnosis/prognosis and SIR for prognosis/selection of treatment.

Keywords: hepatocellular carcinoma, HCC, liquid biopsy, miRNA, systemic inflammatory response, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, Glasgow prognostic score, prognosis

1. Introduction

Hepatocellular carcinoma is one of the most aggressive human cancers. The total oncological mortality is decreasing in many developed countries, e.g., it has been reduced by 23% since 1991 in the United States of America (USA). In contrast, death rate of HCC is increasing, along with the incidence of this tumour [1, 2]. Positive changes are expected due to risk factor eradication by vaccination against hepatitis B and improved treatment of chronic hepatitis C. The treatment of HCC has also developed significantly, including radiofrequency ablation, transarterial chemoembolisation, liver resection and transplantation as well as molecular targeted treatment by sorafenib. However, SEER-based analysis has revealed that survival has improved in early but not in advanced cases [2]. Thus, timely diagnostics remain an important goal.

Most of the hepatocellular carcinoma cases develop on the background of liver cirrhosis or chronic inflammatory liver disease in precirrhotic stage, e.g. chronic viral hepatitis B or C, alcohol-induced or autoimmune liver disease or non-alcoholic steatohepatitis (NASH). This might facilitate the diagnostics by screening of the risk population. Nowadays, screening by ultrasonography and non-invasive radiological diagnosis by the means of computed tomography or magnetic resonance imaging is the mainstay of HCC diagnostics. However, the radiological findings in early cases can be difficult to interpret. Biopsy is indicated in such controversial cases.

However, the biological course of HCC can result in diagnostic difficulties even in biopsy. HCC frequently develops in a dysplastic cirrhotic nodule. Such early HCC is typically well differentiated. Over time, it progresses to advanced dedifferentiated HCC. The resulting heterogeneity can lead to diagnostic problems and failures in biopsy due to sampling errors. For instance, if a small nodule seemed suspicious but not overtly malignant by radiological imaging, leading to biopsy, the differential diagnosis between dysplastic nodule and HCC will frequently imply the necessity to distinguish between premalignant process and well-differentiated tumour, usually lacking marked cell atypia or clear-cut invasion. In addition, both processes can be adjacent in the tissues. Consequently, early diagnostics of HCC is not straightforward even in biopsy.

In addition, biopsy can cause complications, including arterial hypotension, bleeding, pneumothorax, haemothorax, haemobilia, acute pancreatitis, visceral perforation, biliary fistulas, sepsis and needle breakage. Arterial hypotension is seen frequently (1.1–4.0%), mostly due to vasovagal reaction. In few cases, falling arterial blood pressure might indicate bleeding, if it is unusually severe. Bleeding can develop in the liver tissues or in the peritoneal cavity. It is seen in 4.5% of patients and is more frequent if INR is elevated: frequency of bleeding was 3.3% in patients having INR 1.3–1.5 and 7.1% among those who had INR > 1.5. Pneumothorax and haemothorax have been reported in 0.35 and 0.18% of patients, if the needle has accidentally passed through lung or diaphragmatic and intercostal blood vessels. Haemobilia has been described in 0.1% of patients and can induce acute pancreatitis via biliary obstruction. The frequency of visceral perforation, biliary fistulas, sepsis and needle breakage is 0.01% each. In addition, biopsy can result in pain, experienced in up to 84% of patients. In 40% of cases, pain lasts for 24 hours. It is attributable to skin and liver capsule damage, viscerosomatic irradiation

(to shoulder) or complications that lead to peritoneal irritation (bleeding, bile accumulation in the peritoneum perforation of the internal organs). Few death cases have been reported after liver biopsy: 0.01–0.1% of patients [3].

Non-invasive means of HCC diagnostics would be beneficial. Two promising pathways include so-called liquid biopsy by microRNA analysis in blood and assessment of tumour-induced systemic inflammatory reaction (SIR). MicroRNA spectrum might have diagnostic and prognostic value. Regarding SIR, prognostic aspects have been studied and appear as promising adjunct how to select patients for treatment.

2. miRNAs in the diagnostics of hepatocellular carcinoma

MicroRNAs (miRNAs) are small, double-stranded, non-coding RNA molecules consisting of approximately 22 nucleotides. MiRNAs regulate gene expression at the post-transcriptional level [4, 5] acting as large-scale molecular switches. MiRNAs are found not only in cells but also in body fluids. Due to the stable and relatively simple structure, these molecules are good biomarkers for diagnostic and prognostic evaluation complying with the idea of so-called liquid biopsy—a patient-friendly blood test bringing the same information as a biopsy. In order to increase the diagnostic value of such tests, panels of miRNAs have been advocated. However, the biological course of HCC cause a possible pitfall in the elaboration of such diagnostic tests: as HCC mainly arise on the background of liver cirrhosis, inflammatory or metabolic liver diseases, these preceding pathologies can also alter the levels of miRNAs.

MiRNA-122 is attractive for its wide expression in liver tissues suggesting significant role in liver functions. MiRNA-122 is upregulated in serum of HCC patients and downregulated in HCC tissues suggesting specific excretion of miRNA-122 in blood by HCC. Although miRNA-122 shows high specificity and sensitivity for HCC in comparison with healthy controls (83.3 and 81.6%, respectively), levels of miRNA-122 change in other liver pathologies as well, limiting the diagnostic application [6].

Comparing HCC patients with those having hepatitis B or liver cirrhosis, serum levels of exosomal miRNA-18a, miRNA-221, miRNA-222 and miRNA-224 were increased, while miRNA-101, miRNA-106b, miRNA-122 and miRNA-195 were decreased. MiRNA-16 was decreased in HCC, and the levels significantly differed from those found in hepatitis C virus (HCV) infection because chronic viral hepatitis C and non-alcoholic fatty liver disease are characterised by the contrary changes—increase in miRNA-16. MiRNA-21 is characterised by positive characteristics in meta-analysis showing specificity and sensitivity for HCC diagnosis of 84.8 and 81.2%, respectively. The changes of miRNA-21 serum levels in HCC patients significantly differ from cases of chronic hepatitis; however, other malignant tumours can also yield higher serum concentration of miRNA-21 [7].

Several panels of miRNAs have been recommended. Exploring nine serum miRNAs (miRNA-21, miRNA-30c, miRNA-93, miRNA-122, miRNA-125b, miRNA-126, miRNA-130a, miRNA-193b and miRNA-222) in HCC and chronic viral hepatitis C patients, nine markers were decreased in chronic hepatitis C *versus* healthy controls, while seven markers (miRNA-21,

miRNA-30c, miRNA-93, miRNA-122, miRNA-125b, miRNA-130a and miRNA-222) were significantly ($p < 0.05$) decreased in HCC *versus* chronic viral hepatitis C patients and four (miRNA-93, miRNA-122, miRNA-125b and miRNA-130a) in HCC *versus* non-HCC patients [8]. Panel of three miRNAs (upregulated miRNA-92a-3p, downregulated miRNA-3126-5p and upregulated miRNA-107) could discriminate HCC from healthy controls [9] Evaluating serum levels of 13 miRNAs in HCV-associated chronic hepatitis, liver cirrhosis and HCC *versus* healthy controls, panel of three miRNAs (miRNA-122, miRNA-885-5p and miRNA-29b) in association with serum alpha-fetoprotein (AFP) level could identify HCC *versus* healthy persons, while four miRNAs (miRNA-122, miRNA-885-5p, miRNA-221 and miRNA-22) and AFP were recommended for HCC diagnostics in liver cirrhosis and two (miRNA-22, miRNA-199a-3p) along with AFP—in chronic hepatitis [10]. In another study of HCV-infected patients including cases of HCV-related chronic hepatitis, liver cirrhosis and HCC, serum levels of miRNA-126, miRNA-129, miRNA-155, miRNA-203 and miRNA-223 were significantly decreased in HCC *versus* non-HCC patients [11]. Panel of eight miRNAs was assessed in hepatitis B virus-infected patients diagnosed with HCC or liver cirrhosis as well as in healthy controls. The levels of hsa-miRNA-206, hsa-miRNA-141-3p, hsa-miRNA-433-3p and hsa-miRNA-1228-5p were significantly increased in HCC *versus* control group comprising both healthy and cirrhosis patients, while hsa-miRNA-199a-5p, hsa-miRNA-122-5p, hsa-miRNA-192-5p and hsa-miRNA-26a-5p were downregulated [4].

In addition to the diagnostic role, miRNAs have been evaluated in the prognostic aspect. The influence of miRNAs upon HCC stem cells has been exploited. It has also been suggested that miRNAs could become treatment targets [12].

3. Systemic inflammatory response

Many tumours, including hepatocellular carcinoma, evoke systemic inflammatory reaction (SIR). In the recent years, cancer-induced SIR has become an attractive research area as changes in blood cell counts or ratios or blood levels of certain proteins are associated with the biological potential, course and treatment response in many malignant tumours. Although complex pathogenesis lies behind these changes, SIR can be evaluated by simple, widely available and economically feasible blood tests.

SIR in cancer patients develops through local and central mechanisms. Locally, the invasive growth of malignant tumour injures surrounding parenchyma, connective tissues and endothelium. The tissue damage leads to inflammation necessitating supply of inflammatory cells from the bone marrow through the circulation. The production of acute phase proteins becomes upregulated as well. Endothelial injury activates platelets; indeed, the association between hypercoagulation and advanced cancers is classical. Tumour necrosis and hypoxia are additional causes of local inflammatory response. Cancer can also evoke immune response manifesting by local cellular reactions. In turn, the inflammation can have both tumour-enhancing and tumour-suppressing outcomes. The released cytokines and transcription factors can upregulate the proliferation of malignant cells. Release of metalloproteinases and other enzymes can promote tissue degradation facilitating invasion. Angiogenesis can be upregulated as well. The immune system, in turn, can limit growth of the tumour.

The systemic effects of cancer include alterations in bone marrow function, especially myelo-poiesis. Besides the increased production and release of leukocytes, immature myeloid cells, including the precursors of granulocytes and monocytes, are retained in early stages of differ-entiation. Immature myeloid cells can act as immune suppressors and generate pre-metastatic niches, among other pathogenetic processes [13]. Thus, it has even been stated that cancer is an inflammatory disease [14]. Further, neutrophils can form neutrophil extracellular traps developed from externalised DNA network. These nets are bidirectionally associated with platelet activation and can contribute to cancer progression via several mechanisms; there-fore, neutrophil extracellular traps also represent an attractive treatment target [15].

Cancer-related SIR involves cells of innate and adaptive immunity as well as soluble factors. Macrophages are recruited in tumour by hypoxia and tumour-released molecular agents including growth factors and cytokines [16]. Macrophage phenotype switch from tumour-suppressing classical M1 to tumour-promoting M2 subtype promotes angiogenesis and immu-nosuppression. Platelet activation contributes to cancer progression and patient mortality [15]. Neutrophils are locally recruited in the cancer via chemokine signalling. Neutrophil activation can contribute to angiogenesis and increased blood vessel permeability locally and metastatic spread systemically. In addition, immature myeloid cells and neutrophil extracellular traps might have tumour-promoting activity. These molecular events also highlight the associa-tion between infection or surgery-induced inflammation [17] and cancer relapse or metastatic spread. Thus, innate immunity is generally thought to act as tumour enhancers. In contrast, lymphocytes representing the adaptive immunity are considered to have tumour-suppressing effects [16], although contrary effects have been ascribed to certain subpopulations [18].

HCC can be considered a classical inflammation-induced cancer, as its most common risk factors are hepatitis B and hepatitis C virus infections. Inflammation is also present in liver tissues in patients affected by alcohol-induced hepatitis or NASH. Thus, SIR is not expected to have diagnostic value. Indeed, SIR parameters change before the tumour develops, e.g., increased NLR has been observed in chronic viral hepatitis C [19]. NLR is an independent prognostic factor in liver cirrhosis [20].

Wide variety of inflammation-based markers could be used as indicators of HCC prognosis, tumour recurrence and response to specific treatment. In particular, neutrophil, lymphocyte and platelet counts as well as C-reactive protein and albumin and their combinations, neutro-phil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and Glasgow prognostic score (GPS) have prognostic value.

3.1. Neutrophil-to-lymphocyte ratio in HCC

Neutrophil-to-lymphocyte ratio is the most extensively evaluated parameter of SIR in HCC. NLR, calculated as the ratio between count of neutrophils and lymphocytes in blood, seems to reflect the intensity of systemic pro- and anti-tumour reaction. NLR has shown prog-nostic importance in surgically treated HCC cases, including early stage tumours; in liver transplantation; sorafenib treatment and different ablation techniques.

Several meta-analyses have been devoted to NLR in HCC patients. Thus, baseline NLR was associated with overall survival and recurrence-free survival, while post-treatment NLR was significantly associated with overall survival [21]. In meta-analysis of NLR in HCC patients

treated by liver transplantation, significant association with overall and recurrence-free survival was found [22]. In an early study, NLR was already confirmed as a prognostic factor in HCC. NLR was associated with overall and disease-free survival both generally and after different treatment approaches [23].

3.2. Neutrophil-to-lymphocyte ratio in surgically treated HCC

In HCC patients subjected to curative resection, several research groups have identified high NLR as an independent, significant predictive factor, associated with worse overall survival [24–27], shorter recurrence-free survival [24, 25, 28] and higher frequency of recurrence [29].

The studies show some variability in design, group size and cut-off level. However, the reported differences in survival are not only statistically significant but also biologically important. In 672 HCC patients treated by surgical resection, the recurrence rate within 1, 3 and 5 years was 77.4, 55.2 and 44.8% in those having low NLR (≤2.5), while the corresponding recurrence rates were 64.1, 45.2 and 35.5% (p = 0.016) in high NLR group [29]. In 303 patients who underwent surgical liver resection for HCC, high NLR (>2.0, based on ROC-detected cut-off) was significantly associated both with shorter recurrence-free and overall survival (both $p < 0.001$), but multivariate analysis confirmed it as an independent prognostic factor only for overall survival (hazard ratio (HR) 1.724; 95% confidence interval (CI): 1.241–2.394; p = 0.001). Huang et al. evaluated 1659 HCC patients, stratifying them by NLR quartiles. The 5-year overall survival rate was 60% in the lowest quartile contrasting with 27% in the highest quartile. NLR was associated by HR of mortality 1.031; 95% CI: 1.002–1.060; p = 0.033 [30]. In a large Japanese study, enrolling 958 patients who underwent hepatectomy, the 5-year survival rate was 72.9% in low NLR (<2.81) group *versus* 51.5% in patients with high NLR [24]. In another 256 Japanese patients, NLR was confirmed by multivariate analysis as an independent prognostic factor both for overall and recurrence-free interval. The respective hazard ratios were 2.59; 95% CI: 1.56–4.31; $p < 0.001$ and 2.11; 95% CI = 1.44–3.11; $p < 0.001$ [25]. In a smaller study of 113 patients, the recurrence-free survival was 42.4 months in patients having NLR < 3 but 7.9 months in those having NLR ≥ 3.0. The respective HR was 2.58; p = 0.002 [28].

The findings in Western patients have been less positive. In the largest Western series comprising 370 patients, treated in Memorial Sloan Kettering Cancer Center in New York, USA, PLR, but not NLR, was independently associated with worse recurrence-free survival and overall survival [31].

NLR retains prognostic value in several subgroups of surgically treated patients, including early cases. By multivariate analysis, preoperative NLR (at cut-off 2.8) was the strongest independent prognostic factor for overall survival after liver resection with curative intent for TNM stage I HCC. The HR was 2.69; 95% CI: 1.57–4.59; $p < 0.001$. The 5-year survival in high *versus* low NLR group was 45.0 *versus* 76.4%; $p < 0.001$. Interestingly, the association with survival in stage II or III was not significant, reflected by p = 0.283 and p = 0.155. In stage I patients, high NLR predicted more frequent extrahepatic recurrence (p = 0.006). As growth of HCC is associated with grade progression, these results suggest that NLR reflects the biological potential of HCC [32]. In Chinese cohort of 222 patients, preoperative NLR, using cut-off at

2.1, predicted overall survival in solitary small (≤5 cm) HCC after surgical resection and could discriminate outcome in patients having AFP levels not exceeding 400 ng/mL [33]. Further, in a large study of 963 HCC patients treated by potentially curative surgical resection, high NLR (>2.81) was an independent risk factor for overall and recurrence-free survival (both p < 0.001) in the general group as well as in early or intermediate stage HCC: Barcelona Clinic Liver Cancer (BCLC) stages 0/A or B (both p < 0.05) while no association was found in stage C [34]. NLR was also not associated with early (<1 year) mortality from cancer recurrence after liver resection for huge (at least 10 cm in diameter) HCC in 166 patients [35]. NLR was an independent factor that predicted (p = 0.029) early recurrence after curative resection of HCC presenting as a single focus in 193 Japanese patients [36]. However, contrary results have been reported as well, e.g., NLR had no prognostic significance in early HCC (BCLC stage 0/A) treated by surgical resection in 324 patients [37].

Somewhat contrasting data are reported regarding NLR in surgically treated patients with more advanced HCC. Although limited significance of NLR was previously noted in high-stage or large HCC, some authors have found significant role of NLR in advanced cases. Thus, in 81 hepatectomy-treated HCC patients with portal or hepatic vein tumour thrombosis, high NLR (defined as >2.9) was an independent prognostic factor for worse overall survival, characterised by HR 1.866; 95% CI: 1.048–3.322; p = 0.034. Significant association with recurrence-free interval was found as well. The overall survival in high *versus* low NLR groups was 6.2 months *versus* 15.7 months; p = 0.007, while the recurrence-free survival was 2.2 *versus* 3.7 months; p = 0.039 [38].

Not only baseline NLR but also the dynamic changes of NLR were found to be important. In 189 patients treated by curative resection for small HCC, the temporal change of NLR was an independent prognostic factor for overall (HR 2.637; 95% CI: 1.356–5.128; p = 0.004) and recurrence-free (HR 2.372; 95% CI: 1.563–3.601; p < 0.001) survival. The 1-, 3- and 5-year survival was 92.7, 70.0 and 53.0% in patients experiencing NLR increase *versus* 96.2, 87.5 and 75.9% in those with decreasing NLR (p = 0.003). High preoperative or postoperative NLR had lower prognostic value in this study [39]. Paralleling observations have been reported by Hung et al., studying patients with HCC recurrence after liver resection. The 5-year post recurrence survival was better (45.9%) in those continuously having NLR ≤ 2.5. Patients who had low NLR (≤2.5) at resection but high level (>2.5) at recurrence had 5-year survival of only 24.6%; the difference was significant as reflected by p = 0.013 [29].

3.3. Neutrophil-to-lymphocyte ratio in patients receiving transplantation for HCC

Different aspects of NLR have been evaluated in HCC patients subjected to liver transplantation, including transplantation from living donor. The prognostic value of NLR was revealed already in the early studies. Thus, NLR (applying the cut-off level at 5.0) was an independent predictor of overall and recurrence-free survival in 219 Italian patients after liver transplantation for HCC [40].

By univariate analysis, preoperative NLR was significantly associated by disease-free survival and overall survival in HCC patients after liver transplantation. The 1-, 3- and 5-year overall

survival rate in high *versus* low NLR group was 81.3, 56.7 and 51.0 *versus* 90.9, 74.2 and 66.8% (p = 0.041). Similarly, the 1-, 3- and 5-year recurrence-free survival rate in high *versus* low NLR group was 65.3, 48.5 and 39.4 *versus* 80.0, 68.0 and 65.2%. The difference was also significant as reflected by p = 0.013 [41]. Further, NLR was an independent prognostic factor for overall and recurrence-free survival after liver transplantation for HCC as was shown by multivariate analysis of 160 Western patients [42]. NLR was proved to be an independent risk factor for overall survival (p < 0.001) and recurrence-free survival (p = 0.003) in 248 male patients treated by liver transplantation [43]. Harimoto et al. reported on 213 patients receiving living donor liver transplantation for HCC. High preoperative NLR ≥ 2.66 was an independent predictor of recurrence [44].

NLR can be used in prognostic models to identify patients who exceed Milan criteria but still have good overall and tumour-free survival. In study reported by Wang et al., male patients were enrolled and thus the proposed models were verified in males only [43]. Combination of NLR and Hangzhou criteria has been suggested to identify patients who can be successfully treated by liver transplantation [45]. NLR has been included in the MORAL scores to predict recurrence after liver transplantation, and these scores were superior to Milan criteria [46]. Complex evaluation of NLR along with fibrinogen increases the prognostic accuracy in order to predict disease-free survival and overall survival in HCC patients treated by liver transplantation [41]. Pretransplantation NLR along with levels of C-reactive protein has been combined with Milan criteria to develop new selection criteria for living donor liver transplantation beyond Milan criteria [47].

However, contrasting findings have been published as well. Thus, NLR did not predict post-transplantation recurrence or worse overall survival in 150 patients within Milan criteria [48]. Limited prognostic impact of NLR was found in 124 patients who underwent living donor liver transplantation [49]. In Western patients, NLR was not predictive of treatment success regarding liver transplantation or other tested approaches (hepatectomy, transarterial chemo-embolisation). Although the group was quite small (75), Child-Pugh and Model for End Stage Liver Disease (MELD) scores were informative [50].

3.4. Neutrophil-to-lymphocyte ratio in sorafenib-treated HCC patients

In patients with unresectable HCC, treated by multikinase inhibitor sorafenib, high NLR (>3.1) was a significant independent prognostic factor, associated with worse overall survival. Better treatment response was observed in patients with low NLR [51]. The findings were confirmed by another study, reporting on 442 sorafenib-treated patients (Japan, Italy and United Kingdom) with advanced HCC. High NLR again was an independent prognostic factor, predicting shorter survival with HR 1.218; 95% CI: 1.108–1.322; p < 0.0001 [52].

Regarding combined approach, high pre-treatment NLR (>3.0) was an independent predictor of worse overall survival in 40 patients with unresectable HCC treated by transcatheter arterial embolisation and sorafenib. The median survival in high *versus* low NLR group was 14 months (95% CI = 10.1–17.9) *versus* 26 months (95% CI = 17.4–34.6). The difference was significant (p = 0.001) and biologically remarkable [53]. Other researchers have also confirmed that NLR was independent predictor of overall survival in patients with advanced HCC treated by sorafenib [54].

3.5. Neutrophil-to-lymphocyte ratio in HCC patients undergoing tumour ablation

NLR has been investigated in regard to different embolisation and ablation techniques. High NLR (>3) predicted significantly worse treatment results (p = 0.014) and early disease progression (p < 0.0001) in 86 treatment-naive patients subjected to arterial chemoembolisation or radioembolisation [55]. Elevated pre-treatment NLR (>1.85) was associated with overall survival and disease-free survival in 178 HCC patients subjected to transcatheter arterial chemoembolisation (TACE). The median survival in high *versus* low NLR group was 8 *versus* 17.5 months. The 1-, 3- and 5-year overall survival rates in these groups were 42.1, 19.6 and 9.5 *versus* 57.3, 44.1 and 27.2%, respectively (p < 0.001). Differences in disease-free survival were significant as well (p < 0.001). Multivariate analysis confirmed NLR as a significant (p = 0.04), independent prognostic factor for survival after TACE [56].

In patients with advanced HCC treated by hepatic arterial infusion chemotherapy, high NLR was a significant predictor of lower response rate, worse progression-free and overall survival [57]. Baseline NLR was a significant predictor of treatment response and progression-free survival after hepatic arterial infusion chemotherapy for advanced HCC [58]. In patients receiving hepatic arterial infusion chemotherapy by cisplatin and fluorouracil, response rate and overall survival were associated with NLR [59].

Dynamic changes of NLR had independent prognostic significance (p = 0.035) in HCC with portal vein tumour thrombosis treated by microwave ablation after transarterial chemoembolisation [60]. In 506 patients treated by thermal ablation of recurrent HCC, high pre-treatment NLR (≥2.14) was a prognostic factor for recurrence-free survival, confirmed by Cox multiple regression analysis. The 1- and 3-year recurrence rates in high *versus* low NLR groups were 57.9 and 82.5 *versus* 20.7 and 31.6%. The difference was statistically significant, confirmed by p < 0.001 [61].

Pre-treatment NLR was associated with worse overall survival in early HCC after radiofrequency ablation. Post-treatment NLR was associated both with worse overall survival and recurrence in early HCC after radiofrequency ablation [62]. Similarly, NLR dynamics, but not pre-treatment NLR, was an independent prognostic factor for overall survival and recurrence-free survival in patients with small HCC treated by radiofrequency ablation [63]. In patients treated by radiofrequency ablation for HCC, post-treatment NLR was associated with recurrence and survival. Pre-treatment NLR was associated with recurrence only in patients who had HBV infection and HCC but not in those who developed HCC in association with HCV infection [64]. In unresectable HCC treated by radioembolisation, elevated NLR was an independent predictor of worse survival [65].

3.6. Neutrophil-to-lymphocyte ratio and tumour characteristics

NLR has been mostly assessed in correlation with survival or treatment response. However, some observations are reported on the association between systemic inflammatory response and tumour morphology in gross and microscopic level. Thus, high NLR is observed in patients having larger tumours, multiple HCC foci, higher grade of HCC and vascular invasion [29]. In few studies, the infiltration of neutrophils and macrophages in liver tumours has been assessed. Peritumoural tissues are characterised by higher ratio between neutrophils

and T lymphocytes, and higher ratio also correlates with lower overall survival. The combination of these findings suggests that neutrophils might facilitate tumour progression. They suppress adaptive immunity by death ligand expression [66]. Correlation between NLR and PD-L1 expression in the centre of tumour but not peritumoural tissues has been described [67]. High NLR was associated with CD163-positive tumour-associated macrophages [24]. High NLR was associated with higher peritumoural but not intratumoural CD163 and IL-17-expressing cells [68].

3.7. Platelet-to-lymphocyte ratio in HCC patients

Platelet-to-lymphocyte ratio (PLR) is another frequently assessed estimate of SIR, although fewer publications have been devoted to PLR than to NLR. Nevertheless, prognostic role of PLR has been evaluated in different aspects of HCC patient treatment, including surgery [31, 69, 70], transplantation, sorafenib treatment, TACE and ablation techniques.

In the largest Western series enrolling 370 HCC patients, treated by surgical resection, higher preoperative PLR was identified as an independent risk factor of worse overall survival and recurrence-free survival [31]. Other research groups have confirmed the association between PLR and prognosis. Higher preoperative PLR is an independent predictor of worse overall survival in HCC patients undergoing curative liver resection [69, 70] as was shown in two large Eastern cohorts comprising 778 [70] and 1804 [69] patients, respectively. The significant association with overall survival (p < 0.001) was retained in specific subgroups, e.g., patients having cirrhosis or being positive for HbsAg [70]. In some studies, the associations between PLR and prognosis were statistically significant but not independent. Thus, PLR was significantly associated with disease-free and overall survival in 332 HCC patients after hepatectomy [71]. The association between higher PLR and shorter recurrence-free survival in HCC patients undergoing curative liver resection was statistically significant but not independent [70]. Finally, no association has been found in some studies. Hence, in 113 HCC patients undergoing curative resection, PLR was not confirmed as a significant prognostic factor for recurrence-free survival. NLR was found to be superior in this study [28].

PLR has been analysed in the context of liver transplantation. Already in early studies, statistically significant association was found between pretransplantation PLR and cancer recurrence after liver transplantation. In 146 patients, the recurrence-free survival was 80.7 *versus* 91.6%; the difference was statistically significant as confirmed by p = 0.02 [72].

In 122 Chinese patients undergoing transarterial chemoembolisation (TACE) for HBV-related HCC, high pre-treatment PLR (≥96.13) was an independent, statistically significant (p = 0.001) factor that predicted worse survival [73].

In 414 patients affected by recurrent HCC and treated by thermal ablation, high pre-treatment PLR (≥87.87) was associated with higher risk of recurrence and worse recurrence-free survival. The 1- and 3-year recurrence rates in high *versus* low PLR groups were 56.0 and 79.5 *versus* 39.9 and 54.8%; p < 0.05 [74].

Regarding sorafenib-treated cases, PLR has not shown prognostic value in patients receiving sorafenib for advanced HCC. Although the negative result could be attributed to the small group size (16 patients), significant association with NLR was still confirmed [75].

Several meta-analyses have recently been carried out to evaluate PLR in hepatocellular carcinoma. Significant association between higher PLR and increased risk of death was reported by Hu and Yu, reporting odds ratio for death 1.59; 95% CI: 1.15–2.20 on the basis of meta-analysis of six studies with 1446 HCC patients [76]. The association between high PLR and worse overall survival was confirmed by Ma et al., Song et al., and Zhao et al. [77–79]. In a meta-analysis of nine studies including 2017 patients, high PLR was associated with poor overall survival (HR 1.63; 95% CI: 1.42–1.88; p < 0.001) as reported by Ma et al. [77]. Evaluating 2507 patients in 11 studies, high PLR was significantly associated with worse overall survival, as reflected by hazard ratio HR = 1.78; 95% CI: 1.36–2.34; p < 0.001 [78]. In a meta-analysis of 10 studies including 2315 patients, high PLR was associated with the HR for worse overall survival of 1.60; 95% CI: 1.23–2.08; p = 0.0005 [79].

Controversial findings are reported regarding PLR and recurrence-free survival. The association has been confirmed by some research groups [77, 78] but denied by others. In a meta-analysis of nine studies including 2017 patients, high PLR was significantly (p < 0.001) associated with poor recurrence-free survival: HR = 1.32; 95% CI: 1.15–1.52. In a meta-analysis of 11 studies comprising 2507 patients, elevated PLR was significantly associated with worse recurrence-free survival, as reflected by hazard ratio HR = 1.82; 95% CI: 1.56–2.13; p < 0.001. In contrast, by meta-analysis of 2315 patients in 10 studies, high PLR was not significantly associated with worse recurrence-free survival as the HR was 1.21; 95% CI: 0.87–1.67; p = 0.26 [79].

High PLR also showed correlation with high tumour size exceeding 3 cm, TNM stage, lymph node metastases and distant metastases [78]. The findings regarding vascular invasion are controversial again, as the association is confirmed in some studies [78] but not others [77]. No association between PLR and tumour multifocality and higher grade has been confirmed [77].

3.8. Glasgow prognostic score in hepatocellular carcinoma

The prognosis and treatment options of HCC patients depend not only on tumour progression but also on the extent of liver dysfunction. As a consequence, several staging systems have been proposed to predict prognosis for HCC, including Glasgow prognostic score (GPS), based on the levels of C-reactive protein and albumin (**Table 1**). GPS in HCC patients with hepatocellular carcinoma is an independent prognostic predictor after hepatic resection, with higher score indicating worse prognosis [80]. Thus, among 144 patients who underwent surgical resection for HCC, GPS 2 was associated with worse disease-free (HR = 2.527; 95% CI: 1.163–5.490; p = 0.019) and overall (HR = 8.012; 95% CI: 2.818–22.784; p < 0.001) survival, but GPS 1 with shorter overall (HR = 2.277; 95% CI: 1.029–5.039; p = 0.042) survival than seen in patients preoperatively presenting with GPS 0 [80]. The independent prognostic role of GPS for overall survival was confirmed by other research teams [81] and meta-analysis [82]. Modified GPS score and dynamics of GPS score have also shown prognostic value. Thus, elevated modified GPS was associated with overall survival (HR = 2.21; 95% CI: 1.73–2.82; p < 0.05) in a meta-analysis of 2047 HCC patients [83]. Dynamics of GPS (assessed in association with hepatitis B infection status) was an independent predictor of overall survival in 247 patients treated by liver resection [84]. In addition, GPS was related to blood transfusion requirement and postoperative pulmonary complications after liver resection for HCC [85]. In patients undergoing liver transplantation for HCC, elevated GPS, reaching 1 or 2, was

Score	Definition
Glasgow prognostic score	
0	C-reactive protein (CRP) < 10 mg/L AND albumin ≥35 g/L
1	One high-risk finding: CRP ≥ 10 mg/L OR albumin <35 g/L
2	Both high-risk findings: CRP ≥ 10 mg/L AND albumin <35 g/L
Modified Glasgow prognostic score	
0	CRP ≤ 10 mg/L irrespective of albumin level
1	Increased CRP on the background of normal albumin level: CRP > 10 mg/L AND albumin ≥35 g/L
2	Increased CRP and hypoalbuminaemia: CRP > 10 mg/L AND albumin <35 g/L

Table 1. Glasgow prognostic score and its modifications [30].

significantly associated with poor overall (p = 0.018) and recurrence-free (p = 0.030) survival. Complex scoring system has been created on the basis of Milan criteria and GPS [86].

Regarding advanced HCC, GPS retained independent prognostic value for survival (HR = 1.410; 95% CI = 1.060–1.874; p = 0.018). In addition to the statistical significance of results, the biological survival differences by GPS were also remarkable, e.g., median survival was 480 days in patients presenting with GPS 0 and 154 days in those having GPS 1 or 2 [87]. GPS was an independent prognostic factor (HR = 5.483; 95% CI: 2.563–11.729; p < 0.001) for overall survival in patients undergoing sorafenib treatment: the median survival in those having GPS 0 *versus* elevated GPS 1 or 2 was 18.1 *versus* 5.2 months; p < 0.001 [88].

In HCC patients undergoing transarterial chemoembolisation, GPS was an independent prognostic factor for overall survival (HR = 1.697; 95% CI: 1.325–2.174; p < 0.001). It was also found to be superior to other inflammation scores as NLR, PLR or modified GPS. Based on these findings, the authors proposed a new complex score that included Child-Pugh stage, number of tumour nodules and proportion of affected liver, AFP, presence or absence of portal vein thrombosis and GPS. The new score was also a significant predictor of survival as the HR was 1.724; 95% CI: 1.347–2.285; p < 0.001 [89].

4. Conclusions

HCC is a frequent and aggressive malignant tumour. Longer survival can be reached in early diagnosed and properly treated cases. In the future, diagnosis of HCC might be based on combination of radiological features and miRNA profile representing liquid biopsy. MiRNA profile can have both diagnostic and prognostic value. Thus, further lowering of the needs for core biopsy might be expected, leading to patient-friendly examination and avoiding the rare but still possible complications. After the diagnosis of HCC has been confirmed, SIR assessment by NLR and PLR represents innovative means of prognostic evaluation.

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