Validation and refinement of PROSASH model using the neutrophil-to-lymphocyte ratio in patients with HCC receiving sorafenib

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Abstract
The recently developed PROSASH model is proving to be a useful tool in risk-group discrimination in hepatocellular carcinoma (HCC) patients treated with sorafenib. Several studies highlighted that the neutrophil-to-lymphocyte ratio (NLR) is one of the most important predictors of survival in HCC patients treated with sorafenib. The aims of the present study were to validate the PROSASH model and determine whether the incorporation of inflammatory markers can improve risk stratification.

This study included 438 patients. According to the four categories of the PROSASH model, median overall survival (OS) was 20.0, 14.9, 8.5 and 3.0 months respectively (P < .001). The Harrell’s c for this categorized model was 0.621. NLR (cut-off 3) stratified OS in each of the PROSASH categories. After reclassification, median OS was 21.0, 15.1, 8.2 and 4.1 months (P < .001). The Harrell's c increased from 0.621 to 0.673 (P = .001). Integrating NLR into the PROSASH model allowed a more accurate classification of the patients in the risk groups.

KEYWORDS
alpha-fetoprotein, BCLC, extrahepatic disease, hepatitis C, macro-vascular portal vein invasion, prognosis
INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide.\(^1\) Despite the adoption of surveillance programs, most patients are diagnosed at intermediate and advanced stages, which bear a high mortality. Sorafenib is the recommended therapy for patients either at an intermediate stage unsuitable for locoregional therapies or at an advanced stage.\(^2\) However, patients receiving sorafenib display significant tumour and clinical characteristics heterogeneity, which impacts on the variability of prognosis. Consequently, if most patients fall around a median overall survival (OS) of about 10 months, there is also a limited, yet significant, subset of patients (<10%) achieving long-term survival (>3 years).\(^2\)

This heterogeneity underscores the need of a tool capable to stratify HCC patients in different prognostic groups, in order not only to provide patients more accurate information, but also to obtain necessary data for future clinical trials design. Different scoring systems have been proposed for prognostic stratification of advanced HCC patients treated with sorafenib.\(^3-7\) Among them, the ‘PRediction Of Survival in Advanced Sorafenib-Treated HCC’ (PROSASH) model has been recently released, showing promising risk-group discrimination ability.\(^8\)

The authors used data collected by two phase III randomized clinical trials (RCT) that compared sorafenib with brivanib\(^9\) and sunitinib.\(^10\) PROSASH model, composed by vascular invasion, age, ECOG score, AFP, albumin, creatinine, AST, extra-hepatic spread and aetiology, highlighted that it is possible to predict the survival of patients before to start sorafenib. PROSASH model identified four risk categories with a range of survival between 4 months (high-risk category) to 20 months (low-risk category).\(^2\)

Nevertheless, the PROSASH model still lacks an external validation in routine clinical practice. In addition, two studies highlighted that neutrophil-to-lymphocyte ratio (NLR) with the cut-off of 3 is one of the most important predictors of survival in HCC patients treated with sorafenib.\(^11,12\) but it remains unknown whether this prognostic factor could help furtherly refining the PROSASH model.

The aims of the present study were to validate the PROSASH model in a cohort of HCC patients treated with sorafenib in daily clinical practice, and to verify whether the incorporation of inflammatory markers can determine an improvement of its risk stratification ability.

MATERIALS AND METHODS

The present multicentric retrospective study included 438 advanced- (n = 336) or intermediate-stage (n = 102) HCC patients consecutively treated with sorafenib between 2008 and 2018 at four Italian Hospitals.

Patients with advanced-stage and intermediate-stage HCC refractory to or unsuitable for locoregional therapies, receiving sorafenib according to standard schedule, were eligible for our analysis. None of the patients had received previous systemic therapies. Eligibility criteria were the same as those of Llovet’s pivotal study on sorafenib in HCC.\(^2\)

Sorafenib dose reductions were applied as clinically indicated. Follow-up consisted of CT/MR every 8 weeks or when clinically indicated. Treatment was continued until disease progression (evaluated by modified Response Evaluation Criteria in Solid Tumors) or unacceptable toxicity.

Several lines of information on neutrophil, lymphocyte and platelet counts were retrieved from blood tests carried out at baseline (the day before the start of treatment). Complete blood counts have been carried out with XE-2100 (Sysmex).

NLR was obtained by dividing the absolute neutrophil count by the absolute lymphocyte count and the cut-off was set at 3.\(^11,12\)
Patients were classified according to the PROSASH model. The linear predictor was calculated using the published formula\(^8\) and the proposed cut-offs were then applied to generate the four risk categories: \(\leq 2.898\) (low-risk category), \(>2.898\) to \(\leq 3.666\) (intermediate/low-risk category), \(>3.666\) to \(\leq 4.559\) (intermediate/high-risk category) and \(>4.559\) (high-risk category). An online tool was available for rapid calculation at https://jscalc.io/calc/oGSDLHDsDg9g2XBF.

The study was approved by the local ethics committee (CEIIAV: comitato etico unico Area Vasta Romagna e Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori), study number IRST B041 protocol and performed in accordance with the Declaration of Helsinki.

3 | STATISTICAL ANALYSIS

Patients forming the study population were consecutively treated from 2008 to 2018 at four tertiary referral hospitals. Since this was a retrospective observational study, without any intervention comparison, no sample size was required. Overall survival was the main outcome measure and was estimated using Kaplan-Meier methods; differences between subgroups were assessed through log-rank test. The first aim of the study was to validate the PROSASH model. To accomplish this task, the Harrell's c-statistic was calculated for both the categorization into four different risk classes and the linear predictor, by fitting a Cox regression on overall survival, thus obtaining also the 95% confidence intervals.\(^3\) C-indexes were compared when necessary using the methodology proposed by Kang et al.\(^4\) The second aim was to verify whether the introduction of NLR can improve accuracy in the present validation cohort. Initially, the PROSASH model and the NLR values were checked for potential collinearity through the variance-covariance matrix of the parameters of the model used in the estimation command after Cox regression analysis including both variables. Once collinearity was excluded, we reclassified groups of patients according to their observed survival in different subgroups. With this empirical approach a group of patients belonging to the initial PROSASH lower risk group, but with unfavourable NLR, would experience a median survival similar to a higher risk group, providing a ’stage migration’ (as well as possible vice versa). After reclassification of groups of patients, C-indexes were recalculated as previously. Finally, to verify the validity of log-rank test between subgroups, the Freedman method was applied, which suggested that for subgroups lower than 36 patients, the log-rank would be biased. (StataCorp. Stata Statistical Software: Release 14) and R-Project (R version 3.5.1; R Foundation for Statistical Computing) were used for all statistical analysis.

4 | RESULTS

Characteristics of the cohort (\(n = 438\)) are reported in Table 1. The median follow-up was 9.8 months (5.1-19.9 months), 331 patients had died (75.6%) and the median OS was 14.1 months (95% CI: 12.4-16.2).

| Characteristic                        | Value       |
|--------------------------------------|-------------|
| Age [years; (median, IQR)]           | 67 (60-74)  |
| >67 y (%)                            | 214 (48.9)  |
| Male (%)                             | 361 (82.4)  |
| Hepatitis C infection (%)            | 227 (51.8)  |
| Hepatitis B infection (%)            | 85 (19.4)   |
| Alcohol (%)                          | 70 (20.0)   |
| ECOG (%)                             |             |
| 0                                    | 267 (61.0)  |
| >1                                   | 171 (39.0)  |
| Macrovascular portal vein invasion (%) | 163 (37.2) |
| Extrahepatic disease (%)             | 161 (36.8)  |
| BCLC stage (%)                       |             |
| B                                    | 102 (23.3)  |
| C                                    | 336 (76.7)  |
| Alpha-fetoprotein [ng/mL; (median, IQR)] | 51.8 (7.5-950) |
| >400 ng/mL (%)                       | 143 (32.7)  |
| NLR (median, IQR)                    | 2.76 (1.93-4.1) |
| >3 (%)                               | 199 (42.1)  |
| Albumin (g/L)                        | 37 (34-40)  |
| Creatinine (µmol/L)                  | 75.1 (62.6-90.0) |
| AST                                  | 53 (34-91)  |
| History of locoregional therapies or resection (%) | 271 (61.9)   |
| Patients receiving full dose         | 389 (88.8)  |

Abbreviations: AST, aspartate aminotransferase; BCLC, Barcelona-clínic liver cancer; ECOG, Eastern Cooperative Oncology Group; NLR, neutrophil-to-lymphocyte ratio.

4.1 | Validation of the survival model

According to the PROSASH model, 163 patients were classified at ’low risk’ (37.2%), 175 at ’intermediate/low risk’ (40.0%), 86 at ’intermediate/high risk’ (19.6%) and 14 at ’high risk’ (3.2%). Median survivals were as follows: 20.0 months (95% CI: 15.9-22.6), 14.9 (95% CI: 11.6-18.0), 8.5 (95% CI: 5.7-10.8) and 3.0 months (95% CI: 1.7-5.7) respectively \((P < .001)\). None of the survival curves for each category overlapped with the other, providing good stratification of prognosis (Figure 1A).

The Harrell’s c for this categorized model was 0.621 (95% CI: 0.586-0.656) whereas that for the model considered as continuous was 0.652 (95% CI: 0.615-0.689), proving that categorization decreased predictive ability.

4.2 | Systemic inflammatory indicator

The median OS of 239 patients with NLR \(\leq 3\) was 18.1 months (95%CI:16.4-21.1), higher \((P < .001)\) than that of the 199 patients

### Table 1 Characteristics of the cohort

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with NLR >3, which was 8.8 months (95% CI: 7.1-11.1). This inflammatory indicator was not related to the PROSASH model in the regression model ($P = .741$), proving that it was a stand-alone determinant of survival.

As detailed in Figure 2 NLR stratified OS in each of the PROSASH categories. Consequently, patients were reclassified by comparing the original median OS with that resulting by NLR stratification. As an example, the 71 patients initially deemed at ‘low risk’ but with a NLR >3 had a median survival of 15.1 months, that was similar to that of all the 175 patients initially belonging to the ‘intermediate/low-risk’ group. Thus, these 71 patients moved to this higher risk class and so on. When the median survival of a specific subgroup was ≤5 months all patients were defined at ‘high risk’.

After reclassification, 187 patients belonged to the ‘low-risk’ group (42.7%) with a median survival of 21.0 months (95% CI: 17.9-23.0), 114 belonged to the ‘intermediate/low-risk’ group (26.0%) with a median survival of 15.1 months (95% CI: 11.0-17.8), 80 belonged to the ‘intermediate/high-risk’ group (18.3%) with a median survival of 8.2 months (95% CI: 7.0-11.9), and the remaining 57 patients belonged to the ‘high-risk’ group (13.0%) with a median survival of 4.1 months (95% CI: 3.0-5.6; $P < .001$; Figure 1B). It is noteworthy that the new confidence intervals narrowed compared to the original categorization. The Harrell’s c increased from 0.621 to 0.673 (95% CI: 0.634-0.708; $P = .001$) and the inclusion of NLR categories into the continuous PROSASH model increased the c-statistic from 0.652 to 0.693 (95% CI: 0.658-0.783; $P = .001$).

### DISCUSSION

This study provided an external validation of the PROSASH model in a cohort of patients treated with sorafenib in daily clinical practice and highlighted that NLR can increase its discriminatory ability. PROSASH score includes the major factors capable to predict sorafenib benefit (extra-hepatic spread, vascular invasion, AFP, aetiology, albumin and AST). Subanalyses of the two pivotal randomized trials of sorafenib, along with other retrospective studies, highlighted that NLR is one of the most important predictors of
survival in HCC patients treated with sorafenib.\textsuperscript{11,12} Nevertheless, the PROSASH model does not contain it.

Previous research has revealed the critical role of lymphocytes and neutrophils in tumour response and prognosis.\textsuperscript{3} Several inflammations and immune-based prognostic scores, such as systemic immune-inflammation index (SII) and NLR, have been tested to predict survival in several types of cancer.\textsuperscript{15-20} The exact mechanism that explains the poor survival outcomes reported in patients with high NLR is not currently determined. A higher NLR may reflect an increased level of circulating cytokines, favouring immunodepression, angiogenesis, peri-tumour stroma formation and negatively regulated PD-L1/PD-1 signalling pathway.\textsuperscript{21}

We believe that adding NLR to PROSASH allows us to enrich the model with the inflammatory status of the patient. The improvement was not the consequence of a further discrimination between subgroups rather in a more homogeneous classification into each of the risk groups.

Recently, the results of IMbrave 150 trial\textsuperscript{22} (Phase 3 trial of sorafenib versus atezolizumab plus bevacizumab) were presented. Unfortunately, in terms of OS it is not possible to make a comparison with the atezolizumab + bevacizumab arm of the IMbrave 150 trial (OS not reached) but 6-month OS rate was 94% in patients at low risk in our recategorization and 85% in the atezo + beva arm of the IMbrave 150 trial. Although it is inappropriate to directly compare findings from different studies (our study and the IMbrave 150), we can globally speculate that if we select the patients population according to the genotypes investigated in the present analysis it would be possible to improve the selection of patients more likely to benefit from sorafenib in this specific group of patients. We believe that this would be relevant for the management of HCC patients receiving systemic therapy and for the design of future clinical trials.

The main limitation of this study was its retrospective nature. However, cases were consecutively selected and this may have reduced potential bias. Furthermore, we do not have data on sequence treatment because this cohort included patients treated before the results of all second-line studies.

6 | CONCLUSIONS

To conclude, PROSASH model did not show good accuracy in prognostication of HCC patients treated with sorafenib in routine clinical practice. Present results suggest that the integration of NLR index in PROSASH model might increase its accuracy, but its final role in a refined prognostic model would be assessed more adequately in dedicated larger studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AC and ACG conceived and designed the study, performed the experiments and analysed the data; ACG, GC, VG, SL, DD, CE, GR, MR, PA, VZ, MS, PJ, SC and AC collected and assembled the study data. All the authors contributed to the drafting and revision of the manuscript and approved the final version.

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REFERENCES

1. Villanueva A. Hepatocellular carcinoma. N Engl J Med. 2019;380(15):1450-1462.
2. Llovet JM, Ricci S, Mazzaferro V, et al Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378-390.
3. Marisi G, Cucchetti A, Ulivi P, et al Ten years of sorafenib in hepatocellular carcinoma: are there any predictive and/or prognostic markers? World J Gastroenterol. 2018;24(36):4152-4163.
4. Rovesti G, Orsi G, Kalliopi A, et al Impact of baseline characteristics on the overall survival of HCC patients treated with sorafenib: ten years of experience. Gastrointest Tumors. 2019;6(3-4):92-107.
5. Casadei-Gardini A, Solaini L, Riggi L, et al Prognostic role of a new index (RAPID index) in advanced hepatocellular carcinoma patients receiving sorafenib: training and validation cohort. Gastrointest Tumors. 2019;6(3-4):71-80.
6. Cabibbo G, Cucchetti A, Cammà C, et al Outcomes of hepatocellular carcinoma patients treated with sorafenib: a meta-analysis of Phase III trials. Future Oncol. 2019;15(29):3411-3422.
7. Di Costanzo GG, Casadei Gardini A, Marisi G, et al Validation of a simple scoring system to predict sorafenib effectiveness in patients with hepatocellular carcinoma. Target Oncol. 2017;12(6):795-803.
8. Berhane S, Fox R, Garcia-Fiñana M, Cucchetti A, Johnson P. Using prognostic and predictive clinical features to make personalized survival prediction in advanced hepatocellular carcinoma patients undergoing sorafenib treatment. Br J Cancer. 2019;121(2):117-124.
9. Johnson PJ, Qin S, Park J-W, et al Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. J Clin Oncol. 2013;31(28):3517-3524.
10. Cheng A-L, Kang Y-K, Lin D-Y, et al Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. J Clin Oncol. 2013;31(32):4067-4075.
11. Gardini AC, Scarpi E, Faloppo L, et al Immune inflammation indicators and implication for immune modulation strategies in advanced hepatocellular carcinoma patients receiving sorafenib. Oncotarget. 2016;7(41):67142-67149.
12. Bruix J, Cheng AL, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: analysis of two phase III studies. J Hepatol. 2017;67(5):999-1008.
13. Harrell FE, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. JAMA. 1982;247(18):2543-2546.
14. Kang L, Chen W, Petrick NA, Gallas BD. Comparing two correlated C indices with right-censored survival outcome: a one-shot non-parametric approach. Stat Med. 2015;34(4):685-703.
15. Margetts J, Ogle LF, Chan SL, et al Neutrophils: driving progression and poor prognosis in hepatocellular carcinoma? Br J Cancer. 2018;118(2):248-257.
16. Casadei Gardini A, Foschi FG, Conti F, et al Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. Dig Liver Dis. 2019;51(5):681-688.
17. Casadei Gardini A, Scarpi E, Orlandi E et al Prognostic role of aspartate aminotransferase-lymphocyte ratio index in patients with metastatic colorectal cancer: results from the randomized ITACA trial. *Onco Targets Ther*. 2018;11:5261-5268.

18. Casadei Gardini A, Conti F, Foschi FG, et al Imbalance of neutrophils and lymphocyte counts can be predictive of hepatocellular carcinoma occurrence in hepatitis C-related cirrhosis treated with direct-acting antivirals. *Gastroenterology*. 2018;154(8):2281-2282.

19. Chen L, Yan Y, Zhu L et al Systemic immune-inflammation index as a useful prognostic indicator predicts survival in patients with advanced gastric cancer treated with neoadjuvant chemotherapy. *Cancer Manag Res*. 2017;9:849-867.

20. Fan Z, Luo G, Gong Y et al Prognostic value of the C-reactive protein/lymphocyte ratio in pancreatic cancer. *Ann Surg Oncol*. 2020.https://doi.org/10.1245/s10434-020-08301-3. [published online ahead of print, 2020 Mar 6].

21. He G, Zhang H, Zhou J et al Peritumoural neutrophils negatively regulate adaptive immunity via the PD-L1/PD-1 signalling pathway in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2015;18(34):141.

22. Cheng A-L, Qin S, Ikeda M et al IMbrave150: efficacy and safety results from a Ph 3 study evaluating atezolizumab (atezo) + bevacizumab (bev) vs sorafenib (Sor) as first treatment (tx) for patients (pts) with unresectable hepatocellular carcinoma (HCC). *Ann Oncol*. 2019;30(suppl_9):ix183-ix202.

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