We would like to thank reviewers and editors for their valuable comments that enriched and enhanced the manuscript quality

Here in a point by point response to reviewers comments (Changes made in the manuscript are shown as **bold and underlined**):

Reviewer #1:

Comment 1: The data that was lost of follow-up needs to be shown, whether any of the patients had an end-point event. It is necessary to consider whether the exclusion of these cases has a potential impact on the outcome.

**Response: The patients who were excluded from the analysis didn't bleed until they are lost, but we don't have an evidence about how they finished their follow-up (some of them died during the COVID-19 pandemic, others we have lost contact with them and didn't attend for endoscopic follow up), and we couldn't know if they had end-point or not. However, some of their data are provided as supplementary excel file.**

Comment 2: Please use a scale to indicate the size of the picture, not the magnification.

**Response: Scales is added to the immunohistochemistry pictures (Figure 1)**

Comment 3: In table 1, the frequency of large should be 36.7%

**Response: corrected, but actually, the number (percent) for small, medium, and large varices for the responders group (n=60) was 10 (16.666%), 28 (46.666), 22 (36.6666) respectively, and the summation should equal 100% so we had to approximate the fractions.**

Comment 4: Page 10, the sentence "There was a significant change in the antral expression of β-Arr-2 after treatment with NSBB compared to baseline. The change was 42.9% vs. 18.6% respectively for low expression, 37.4% vs. 36.3% respectively for

moderate, and 19.7% vs. 45.1% respectively for strong expression, (McNemar Bowker's $\chi 2$ =16.18, P = 0.001). ", it is difficult to read, the author needs to indicate the change in the proportion of all cohort before and after treatment.

**Response: It has been rephrased as following [At baseline, 18.6% of cases have low expression, 36.3% have moderate expression, and 45.1% have strong expression. After NSBB treatment, 42.9% of cases have low expression, 37.4% have moderate expression, and 19.7% have strong expression respectively (McNemar Bowker's $\chi^2$ =16.18, *P* = 0.001)]**

Comment 5: Page 10-11: Multinomial regression showed that NSBB dose (P = 0.02, OR = 0.95, 95% CI: 0.91-0.99) and the ΔPVCI (P = 0.005, OR = 1.58, 95% CI: 0.001- 0.002) were the only independent predictors of reduced β-Arr-2 expression. For multivariate regression, the author needs to list the result as tables, including which factors are included in the multiple regression.

**Response: Data were listed in table 2**

Comment 6: Please indicate the full name for the abbreviation in the figure legend.

**Response: This has been done (for figure 2 and figure 3, 4)**

Comment 7: We have doubts about the APRI and Platelets values of patients. All patients are cirrhotic patients with esophageal varices. Why is the average APRI value around 1.75? I hope the author can check the data, and what is the reference range of AST?

**Response:**

- **The reference value for AST in our study is 35 IU/l.**
- **Value were revised in table 1**
- **APRI for all cohort (mean ± SD, min-max) was [1.94±0.46, (1.03-3.41)]**

- **there was no difference among our patients indicating matching of the study subgroups**
- **some of our cases have low AST below the cut-off value which is 35 IU/l in our study (AST min-max = 28-78 IU/l)**
- **In previous studies, a threshold of 1.0 was 76% sensitive and 72% specific (Lin ZH et al, Hepatology. 2011 Mar;53(3):726-36). However, higher values by Cidci S. et al were documented but with lower sensitivity (60%) and specificity (52.1%) and 55% diagnostic accuracy (doi.org/10.5946/ce.2021.028).**
- **Moreover, in a recent study: APRI score showed low diagnostic accuracy for prediction of varices in liver cirrhosis (Rajesh Pandey Rahul Pathak, Arun Gnawali, et al JAIM 18 (Volume 9, Number 2 |July - December 2020).**
- **In addition, APRI is not an independent factor for the prediction of EV. Its sensitivity, specificity and predictive values are insufficient for the index to be used for the screening of EV in cirrhotics in a study by De Mattos et al (Annals of Hepatology 2013;12:810–4. doi:10.1016/s1665-2681(19)31324-9.), AND in Wang L, Feng Y, Ma X, et al. PLoS One. 2017;12(8): e0182969.**

Comment 8: During follow up, NSBB non-responders represented 82.4% of patients with low β-Arr-2 expression (n=14/17), 45.5% for moderate expression (n=15/33), and 4.9% for strong expression (n=2/41), ($\chi 2$ =35.10, $P < 0.001$). This was hard to read. There were only 31 patients in non-responders group ? What about 41 and 33 patients ?

**Response: These calculations were referred to the number of patients in each expression grade (Low = 17 patients, moderate = 33 patients, strong = 41 patients, total = 91), the total number of non-responders is 31 (14 patients within the low expression category,15 in moderate expression, and 2 in strong expression, total = 31), however, we rephrased the text as followings: *((At baseline, 17 patients had low β-Arr-2 expression, among them, 14 patients (82.4%) were bleeders (NSBB non-responders). Also, 33 patients had moderate β-Arr-2 expression, among them, 15 (45.5%)***

***patients were bleeders, and 41 patients had strong β-Arr-2 expression, among them, two (4.9%) patients experienced variceal bleeding, ($\chi^2$ =35.10, P < 0.001)).***

Comment 9: The name of section of "Survival analysis", which may make readers mistakenly believe that the author performed a survival analysis. But the author only did a Kaplan-Meier curve to analyze the bleeding time. In addition, the author should analyze the bleeding of all cohort during follow-up based on the cut-off value.

**Response: The title has been changed into:** ***Kaplan-Meier and regression analysis (page 11-12), the analysis and corresponding figures were modified based on all cohort (figure 4a-b) page 12***

Comment 10: By Cox-regression analysis, a low serum β-Arr-2 level (P < 0.001, OR= 0.13, 95% CI: 0.09-0.13), a low grade of β-Arr-2 expression in the gastric antrum (P < 0.001, OR= 0.15, 95% CI: 0.1- 0.3), and platelet count (P = 0.008, OR= 0.91, 95% CI: 0.85-0.98), were the only independent predictors for variceal bleeding. Cox regression needs to show the factors included in the regression analysis in the form of a table.

**Response: Text has been checked and a table has been added (table 4)**

Comment 11. In addition, a low grade of β-Arr-2 expression in the gastric antrum (P <0.001, OR= 0.15, 95% CI: 0.1- 0.3). How is this classified? The grade of β-Arr-2 Expression is handled as a three-category variable or a binary variable.

**Response: We mean that the lower antral expression is associated with higher risk of bleeding, the grading of expression was expressed as (low-moderate, and strong), however, to avoid this difficult to read text, we rephrased it (page 12)**

Reviewer #2:

Comment 1. The enrolment period was not reported

**Response: The enrolment period is added to the manuscript (patient selection section, page 6)**

**The enrollment started from December 2017 to November 2019 with the last follow-up visit on April 2021**

Comment 2: The Authors said that the starting propranolol dose was 40 mg BID, with progressive titration until a dose of 360 mg when appropriate. The mean daily dose was only of 60 mg, however.

**Response: in our methodology we described the planned dose arrangement for our cohort as per guidelines, but in practice, we didn't reach the full dose of beta blockers due to variable patient tolerance. The dose ranged from 30 mg to 100 mg as a maximum tolerated dose among our cohort.**

Comment 3: Why the patients underwent upper GI endoscopy every 12 wks?

**Response: This was done for 3 reasons, 1st: to follow the progression/regression in varices, 2nd: to detect any sign of impending/ or recent bleeding which may be missed by the patient (sometimes patients do not notice minor changes in stool color " melena" due to some cultural and educational factors in our community; 3rd: ensure regular follow up and to reduce the number of losses.**

Comment 4: The Authors said that NSBB responders were those who did not bleed during the period. Nevertheless, they considered also an endoscopic NSBB response (at what time period, considering that each patient performs 6 EGD during the observational period). What was the leading indication to response?

**Response: We evaluated the response on the bases of variceal control and prevention of variceal bleeding (the primary end point), and by endoscopic visualization as regards the progression/regression of varices over the treatment duration and follow up.**

Comment 5. How was β-arrestins-2 measured in serum?

**Response: Serum β-Arr-2 levels were measured using an ELISA kit. we added a reference for the methodology (reference number 12)**

Comment 6. The Authors proposed two different cut-off values for β-arrestins-2 (one able to identify patients with low likelihood of bleeding, the other able to identify a longer bleeding-free interval time). What is the best cut-off to use in the clinical setting?

**Response: This was a typing mistake (there is only one cut-off (2.23 ng/ml) as obtained from ROC analysis, and we corrected this mistake) page 12**

Comment 7. There are few typos which should corrected

**Response: We revised the manuscript again and we requested another language correction service**

Comment 8. The Authors discussed the potentially dangerous role of NSBB in patients with SBP and refractory ascites in the discussion section of this paper. This point is largely debated until now, since controversial data have been reported in literature. Notwithstanding, how many patients experienced refractory ascites and SBP in this cohort?

**Response: we have 7 (7.7%) cases with SBP (data not shown in the manuscript):**

- **They have Child score B (2 patients) and C (5 patients).**
- **In addition, only one patient (14.3%) of them had strong β-arrestins-2 expression**

- **they also have a serum β-arrestins-2 of 2.15±0.3 ng/ml (vs. 2.60±0.47 ng/ml for patients without SBP, P = 0.006)**
- **This subgroup of patients received a mean dose of NSBB = 60 ± 19.1 mg (vs. 65.23 ± 14.26 mg, P = 0.36)**

**Also, we have 5 (5.5%) cases developed refractory ascites:**

- **They had Child score B (1 patients) and C (4 patients).**
- **In addition, 2 patients of them had low β-arrestins-2 expression and 3 patients had moderate expression (no strong expression)**
- **they also had a serum β-arrestins-2 of 2.10±0.19 ng/ml (vs. 2.59±0.48 ng/ml for patients without SBP, P = 0.002)**
- **This subgroup of patients received a mean dose of NSBB = 70 ± 10 mg (vs. 64.5 ± 14.85 mg, P = 0.42)**

**In the current study, few patients developed SBP and refractory ascites. The lack of significant difference in NSBB dose in these events among subgroups may reflect the possible explanation of readiness of β2-AR receptors rather than a dose-dependent effect. This again, emphasizes the role of β-arrestins-2 as a marker to select those who will tolerate NSBB without complications. However, the number of cases with SBP and refractory ascites in our cohort is too low to provide a conclusion as regards this point and more validation on a wide scale is recommended.**

**This paragraph has been added to the manuscript, results section (page 12) and in the discussion section page 15**

Comment 9. Among the potential pitfalls of this study, the Authors should add the need to externally validate these results in larger cohorts

**Response: we added this point in the conclusion "In addition, we recommend future studies to validation of the current results on a larger scale of patients." page 15**

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## 4 LANGUAGE POLISHING REQUIREMENTS FOR REVISED MANUSCRIPTS SUBMITTED BY AUTHORS WHO ARE NON-NATIVE SPEAKERS OF ENGLISH

As the revision process results in changes to the content of the manuscript, language problems may exist in the revised manuscript. Thus, it is necessary to perform further language polishing that will ensure all grammatical, syntactical, formatting and other related errors be resolved, so that the revised manuscript will meet the publication requirement (Grade A).

Comment: **Authors are requested to send their revised manuscript to a professional English language editing company or a native English-speaking expert to polish the manuscript further. When the authors submit the subsequent polished manuscript to us, they must provide a new language certificate along with the manuscript.**

**A language polish was addressed and a certificate is associated with the resubmitted files**

## 5 ABBREVIATIONS

In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.

The basic rules on abbreviations are provided here:

**1-Title:** Abbreviations are not permitted. Please spell out any abbreviation in the title.

**Response: No abbreviations in the title**

**2-Running title:** Abbreviations are permitted. Also, please shorten the running title to no more than 6 words.

**Response: Short title has been modified**

**(3) Abstract:** Abbreviations must be defined upon first appearance in the Abstract. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*).

**Response: Rule is followed**

**(4) Key Words:** Abbreviations must be defined upon first appearance in the Key Words.

**Response: Rule is followed**

**(5) Core Tip:** Abbreviations must be defined upon first appearance in the Core Tip. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

**Response: Rule is followed**

**(6) Main Text:** Abbreviations must be defined upon first appearance in the Main Text. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

**Response: Rule is followed**

(7) **Article Highlights:** Abbreviations must be defined upon first appearance in the Article Highlights. Example 1: Hepatocellular carcinoma (HCC).

**Response: Rule is followed**

Example 2: *Helicobacter pylori* (*H. pylori*)

**(8) Figures:** Abbreviations are not allowed in the Figure title. For the Figure Legend text, abbreviations are allowed but must be defined upon first appearance in the text. Example 1: A: Hepatocellular carcinoma (HCC) biopsy sample; B: HCC-adjacent tissue sample. For

any abbreviation that appears in the Figure itself but is not included in the Figure Legend textual description, it will be defined (separated by semicolons) at the end of the figure legend. Example 2: BMI: Body mass index; US: Ultrasound.

**Response: Rule is followed**

**(9) Tables:** Abbreviations are not allowed in the Table title. For the Table itself, please verify all abbreviations used in tables are defined (separated by semicolons) directly underneath the table. Example 1: BMI: Body mass index; US: Ultrasound.

**Response: Rule is followed**

**6 EDITORIAL OFFICE'S COMMENTS**

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

***(1) Science editor:***

In this article, the author described the changes in expression and role of β-Arrestin-2 in predicting the response to non-selective beta-blockers in cirrhotic portal hypertensive patients. They concluded that higher serum levels were associated with longer bleeding-free intervals. It is a study that is fit for the content of the journal. However, there are concerns about this article, as reviewers noted.

Comment 1: The author should present the change in the proportion of all cohorts before and after treatment.

**Response: At baseline, the frequency of small, medium, and large esophageal varices between patients (n=91) was 18.7%, 52.7%, and 28.6% of cases, respectively. At the end of the study, the frequency of small, medium, and large esophageal varices was changed to 31.9%, 45.1%, and 23.1% of cases, respectively ($P$ = 0.049, $\chi^2$ =7.6)**

**This paragraph was added to the results (page 10), supplementary figure 3.**

Comment 2: It is doubtable about the APRI and platelets values of patients.

**Response: Data were revised and managed table 1**

Comment 3: A Kaplan-Meier curve to analyze the bleeding time is not suitable because such survival analysis may make readers mistakenly believe that the author performed the objective survival analysis.

**Response: This was changed according to reviewers recommendations**

Comment 4: The starting dose of propranolol is inadequate, and they should describe the reason for endoscopic interval.

**Response: This point has been explained and addressed**

Comment 5. They presented two different cut-off values for β-arrestins-2. The best cut-off to use in the clinical setting is unclear.

**Response: This was a typing mistake, we have only one cut-off for B- arrestion-2 which is 2.23 ng/ml and we clarified this and corrected in the text.**

Comment 6. They should need larger cohorts to validate these results.

**Response: We added this recommendation to the conclusion section in the text**