Comments for ESPS Manuscript NO 53452

Reviewer #1: Comments for ESPS Manuscript NO 53452 This article is interesting. However, a small sample size makes it difficult to draw a convincing and reliable conclusion.

Many thanks for your comment. We confirm that this is only a small, pilot study and findings will need to be confirmed in a larger cohort. We did control for gender and excluded conditions known to be associated with disturbances in angiogenic levels, which strengthens our findings.

Reviewer #2: The authors demonstrated that serum Ang1 levels were decreased and Ang2 were increased in patients having GI angiodysplasia. Although these findings seem to be interesting, there are some issues to be clarified.

1. The Ang/Tie receptor signaling cascades are involved in fundamental angiogenesis events. Both angiopoietins exerts angiogenesis-regulating effects via the angiopoietin tyrosine-kinase receptor (Tie-2). So I think the Tie2 level needs to be verified. Can you measure Tie-2 levels?

That's an interesting point. We would like to measure the receptor for these factors, Tie-2 in our next study.

2. Figure 1 only shows the levels of Ang1 and Ang 2 in the disease group but not the levels in the control group. Comparisons with the control group may explain the expression levels of Ang1 and Ang 2 in several diseases more clearly.

We did actually compare (SBA, PHG, GAVE) to controls. All three groups have similar age range and our real interest was variation in angiopoietin levels in disease group as shown in Figure 1.

3. In the Results section, the mean age (range) for the control group is shown as 49 years (range 20-74), but it is shown as 49 (range 24-60) in Table 1. The authors need to correct it.

Many thanks for your note. The typo in Table 1 has been amended accordingly**.**

4. In the Results section, the mean age (range) for 44 patients is shown as 60 years (range 18-93). Which group have 18 years old patient and which group have 93 years old patient, please explain it.

Many thanks for your note. This typo has been amended. The Controls had the 20years old patient while the GAVE group had the 85years old patient.

5. Abstract is redundant. Please revise it.

Abstract has been revised.

6. I am not sure if these angiopoietin changes are related to GI angiodysplasia. Are these produced at local angiodysplastic lesions and secreted into systemic circulation? To address this issue, I recommend to conduct immunostaining for ANG1/2 in surgically- or endoscopically-removed angiodysplastic lesions.

Many thanks for the brilliant suggestion. Now we know that there are alterations in the angiopoietin levels in cases versus controls, we would like to carry out the immunostaining technique as part of our future studies to detect the signalling protein location. Previous study by our colleague, Holleran et al [8] observed an increased mucosal expression of levels of Ang-1, Ang-2, and their receptor Tie2 in biopsies from areas of angiodysplasia compared with normal small bowel. In addition to SBA, we hope to measure expression levels of angiopoietins in PHG and GAVE lesions using the same techniques.

7. The weakest point for publication is too small sample size. Can you increase the number of samples?

Many thanks for your observations. We acknowledge that this is a pilot study but our findings are novel to the scientific community; and we agree warrant further exploration in a larger cohort.

Reviewer #3: This pilot study in three small groups of patients with neovascularisation in the GI-Tract compares serum levels of angiogenic factors with those of a control group. The concept of the study is novel and so are the results and their interpretation by the authors. As discussed also by the authors, the main limitation are the small sample sizes of the 3 cohorts of patients and a significant difference of 17 years between the ages of patients and control subjects.

Beyond that, several aspects need attention: The TITLE should point out the character of this study as a PILOT STUDY.

We appreciate your suggestion. Title has been revised to: ‘’Shared changes in angiogenic factors across distinct gastrointestinal vascular conditions, a pilot study”.

The ABSTRACT would gain in readability by omitting the detailed numbers for Ang-1 levels, similar to the description of Ang-2 levels.

Many thanks for your suggestion. Abstract has been revised.

METHODS: Paragraph 1 describes exclusion criteria ..."known chronic liver disease _of_ non-liver-related portal hypertension". Most likely this should read ..._or_...? ...

Many thanks for your observation. The typo has been amended accordingly.

" 2 red tubes of blood were taken" ... What is the meaning of "red tubes"?

Many thanks for your observation. This should have read ‘’red-top tubes” i.e. serum separator tubes. This has now been rephrased for clarity**.**

RESULTS: A current hemoglobin level was available in only 66% of subjects, which significantly lowers the validity of interpreting relevant results in the a priori small cohort of patients. Why could blood samples not be ascertained in about a third of patients?

Patients were recruited directly from endoscopy and as a tertiary referral centre, this included patients referred from other units and as such access to their original blood works was not available for every patient. Our ethical approval did not cover additional bloods for the purpose of our study. As part of our larger cohort we hope to take additional bloods.

The Para "Serum levels of angiogenic factors" redundantly describes almost all numbers given in Table 1 anyway. Presentation either in the body of the manuscript or in the Table is suggested.

Many thanks for your suggestion. Table 1 represents the study population while the paragraph in question describes serum levels of each group. Table 2 (If this is what is referred to) represents the serum levels of angiogenic factors in tabular form to aid readability and comprehension for different readers.

The statement "Of interest, the median SBA Ang-1/2 ratio was similar to controls 21. Table 2" is not clear and should be checked and possibly rephrased.

Many thanks for your note. It was a little confusing. We have altered the sequences to read more clearly as below:

The relative expression of Ang-1 and Ang-2 was calculated for each group. Controls as expected had a higher ratio; 35696/2803 ng/ml =13 for SBA, 23111/4298 ng/ml =5 for PHG, 30753/4232 ng/ml =7 for GAVE vs 53114/1899 ng/ml =28 for controls. However, the difference between SBA and control Ang1/Ang2 ratios did not reach statistical significance which was not unexpected as SBA Ang-2 levels while higher, was not statistically different from controls. Table 2.

In addition, the median Ang-1/Ang-2 ratio for all patients was found to be significantly lower compared to controls, 8 versus 28 respectively, p= 0.001, 95%CI -27.55 to -7.12.

DISCUSSION: The statement in Para 2 "Ang-2 is known to be expressed ... etc." should be referenced.

Reference has been included.

TABLE 1: As hemoglobin levels were ascertained in only 66% of subjects, the calculation and comparison of subjects with anemia of 13 patients (4+6+3) versus 1 Control is meaningless nd should be omitted.

Thank you for your suggestion. We considered it still valuable to control for anaemia because these conditions are a major cause of recurrent bleeding and anaemia.

FIG 1: The number on top of the second bar is reproduced incomplete (4678...) and this should be checked.

Many thanks for your observation. This has been corrected.

Science Editor: Recommend for potential acceptance. 1 Scientific quality: The manuscript is a study of common angiogenic profile across distinct gastrointestinal vascular disorders. The topic is in the scope of WJG. (1) Classification: 1C and 2D. (2) Summary of the peer-review report: This pilot study in three small groups of patients with neovascularisation in the GI-Tract compares serum levels of angiogenic factors with those of a control group. The concept of the study is novel and so are the results and their interpretation by the authors. As discussed also by the authors, the main limitation are the small sample sizes of the 3 cohorts of patients and a significant difference of 17 years between the ages of patients and control subjects.

Abstract is redundant. Please revise it.

Abstract has been revised.

The number of the table 1 and figures 1 should be checked.

Many thanks for your note. Corrections have been made where necessary.

#00054993 reviewer recommend to conduct immunostaining for ANG1/2 in surgically- or endoscopically-removed angiodysplastic lesions.

This is a helpful suggestion and fits into our plan to apply this technique in a larger cohort. Previous study by our colleague Holleran et al [8] observed an increased mucosal expression of

levels of Ang-1, Ang-2, and their receptor Tie2 in biopsies from areas of angiodysplasia compared with normal small bowel. In addition to SBA, we hope to measure expression levels of angiopoietins in PHG and GAVE lesions using the same technique.

(3) Format: one figure and two tables. Fifteen references were cited, including one reference published in the last four years. No self-citation. 2. Language quality: 1A and 1B. 3. Academic norms and rules: Case Control Study. The authors provided biostatistics review certificate and STROBE statement. The authors signed the copyright license agreement. The institutional review board approval form was uploaded. The written informed consent was provided.

"Conflict-of-Interest Disclosure Form" is not qualified and need to be provided again.

Conflict of disclosure form has been resubmitted.

Bing search is eligible. The highest single-source similarity index is 17%.

The authors need to revise the repetitive parts according to the CrossCheck report.

All revisions have been made**.**

4: Others: Without financial support. Corresponding author has not published articles in WJG. Unsolicited Manuscript