Dear Editors,

On behalf of my co-authors, we thank you for giving us a chance to revise and improve the quality of our article. We have read the reviewers'and your comments carefully and have made revision which marked in red in the paper. We have tried our best to revise our manuscript according to the comments. Here, we would like to explain the changes and the problems briefly as follows:

For First peer-review (minor review):

Problem 1: The main limitation of the study was the exclusion of patients who relapse or who were dose escalated. If you want to know the predictive capacity of IFX levels, this should also include patients who have secondary loss of response or complete loss of response, not only those who maintain remission.

Answer 1: Thanks for the reminder. Our study collected these patients but the study mainly focused on the treatment course when patients received IFX 5mg/kg and AZA therapy regularly. What's more, the main study content of this article is long-term endoscopic outcomes of CD patients with clinical remission. Our study excluded CD patients with intensive treatment because the therapeutic strategy of these patients changed and the change may influence the blood concentration of IFX. Our study excluded CD patients with disease relapse because these patients suspended IFX therapy and converted to hormone therapy in our center, which means they would not receive IFX blood concentration later.

Problem 2: I suggest making other corrections in the manuscript: Abbreviations such as ITL are not defined in the abstract

Answer 2: Thanks for the reminder. I have corrected.

BACKGROUND ↵

Infliximab trough level severely affect therapeutic outcome of Crohn's Disease (CD) patients under Infliximab (IFX). Recently frontier researches have focused on identify IFX trough level based on different therapeutic targets. Although previous studies have elaborated clinical value of IFX trough level (ITL) monitoring on short-term outcome in CD patients during therapy, studies contraposing the predictive value of ITL on long-term endoscopic outcomes in CD patients are still scarce in the domestic and overseas. ↵

Problem 3: The discussion is cumbersome, too much data and not very clear concepts, I recommend simplifying it.

Answer 3: Thanks for the reminder. I have corrected.

CONCLUSION↵

Combination of ITL, CRP and FCP contribute to long-term endoscopic prognosis monitoring. During IFX maintenance treatment, low ITL, high CRP level and high FCP level were independent risk factors of CD patients with clinical remission in adverse endoscopy outcomes within one-year follow-up. ↵

For Second peer-review (major review):

Problem 1: High inflammatory load affects the pharmacokinetics of IFX, inducing secondary nonresponse by decreasing blood drug concentration. Currently, it is believed that inflammatory biomarkers are good predictors of disease activity. FCP or CRP?
Answer 1: Thanks for the reminder. Both FCP and CRP are good predictors in previous studies.

Problem 2: In my opinion, the term “phase I study” let someone have a wrong intuition and the word “phase” should be replaced by “part” or “step” .
Answer 2: Thanks for the reminder. The word “phase” has been replaced by “part” or “step”

Problem 3: Materials and methods It is not clear how the patients were selected. How many patients had received infliximab? How many of them had done colonoscopy at week 14? How many of them had measured the ITL at week 14?
Answer 3: Thanks for the reminder. 181 CD patients underwent IFX treatment. 153 CD patients underwent enteroscopy as well as serum concentration monitoring at weeks 14 after the third dose of IFX induction therapy.I have corrected.

A single-center retrospective research has been implemented in the first affiliated hospital of Zhejiang Chinese Medical University. Crohn’s diseases patients under IFX therapy from January 2012 to December 2020 were collected. 181 CD patients underwent IFX treatment. 153 CD patients underwent enteroscopy as well as serum concentration monitoring at weeks 14 after the third dose of IFX induction therapy. Inclusion criteria, (I) endoscopic remission at week 14 (CDEIS Score ≤ 2 points or Rutgeerts' ≤ i1), (II) clinical remission after IFX

Problem 4: In Study Subjects Design, there are a statement that Clinical, laboratory, endoscopic and imaging evaluation were implemented every two months after IFX induction therapy in all patients. Have they done colonoscopy every 2 months? or that they had done the colonoscopy 8 weeks after the 3rd dose at week 6?
Answer 4: Thanks for the reminder. Blood drug concentration, clinical, laboratory, endoscopic and imaging evaluation had been implemented every two months since the third dose of IFX induction therapy in all patients. I have corrected（Blue Part）.

A single-center retrospective research has been implemented in the first affiliated hospital of Zhejiang Chinese Medical University. Crohn’s diseases patients under IFX therapy from January 2012 to December 2020 were collected. 181 CD patients underwent IFX treatment. 153 CD patients underwent enteroscopy as well as serum concentration monitoring at weeks 14 after the third dose of IFX induction therapy. Inclusion criteria, (I) endoscopic remission at week 14 (CDEIS Score ≤ 2 points or Rutgeerts' ≤ i1), (II) clinical remission after IFX induction therapy without corticosteroids more than 6 months, (III) therapeutic strategy during maintenance stage was designed as IFX 5mg/kg every 8 weeks combined with AZA 50mg every day. Therapeutic strategic would be modulated if CD patients were confronted with clinical relapse or secondary lose of response (LOR) and data analysis would focus on the treatment course when patients received IFX 5mg/kg and AZA therapy regularly. Secondary lose of response (LOR) means a recurrence of the disease during IFX maintenance therapy. Two criteria should be met to determine LOR: the recurrence of symptoms of IBD in clinical remission after induction therapy, and symptoms caused by the inflammatory activity of IBD itself. Clinical relapse means CDAI＞150 points. Blood drug concentration monitoring, clinical, laboratory, endoscopic and imaging evaluation had been implemented every two months since the third dose of IFX induction therapy in all patients. The study was divided into two

Problem 5: Excluding patients who had their therapeutic strategy changed, or who had clinically relapsed of the final analysis, will not bias the results?

Answer 5: Thanks for the reminder. I admit wrong statement that may mislead scholars' understanding. Actually, this study collected these patients but data analysis would focus on the treatment course when patients received IFX 5mg/kg and AZA therapy regularly. Hence, I deleted the statement that excluding patients who had their therapeutic strategy changed or who had clinically relapsed of the final analysis.

Problem 6: Let's imagine a patient who have a high ITL at week 14 and had a clinical relapse at weeks 48. Even if you decided to analyze only patients in clinical remission, per protocol, it is important to show the data of those who had clinical relapse during the study with an intention-to-treat analysis where the last observation is carried forward (LOCF). Who had clinical relapse? Any of them had surgery? Or had changed to another biologic because of disease activity?

Answer 6： Thanks for the reminder. Our co-authors considered these problems belonged to the same aspect that the result of CD patients with disease relapse. Our study contraposed to CD patients with clinical remission and according to inclusion criteria, these patients has been satisfied with clinical remission after IFX induction therapy without corticosteroids more than 6 months.

Study Subjects Design

A single-center retrospective research has been implemented in the first affiliated hospital of Zhejiang Chinese Medical University. Crohn's diseases patients under IFX therapy from January 2012 to December 2020 were collected. 181 CD patients underwent IFX treatment. 153 CD patients underwent enteroscopy as well as serum concentration monitoring at weeks 14 after the third dose of IFX induction therapy. Inclusion criteria, (I) endoscopic remission at week 14 (CDEIS Score ≤ 2 points or Rutgeerts' ≤ i1), (II) clinical remission after IFX induction therapy without corticosteroids more than 6 months, (III) therapeutic strategy during maintenance stage was designed as IFX 5mg/kg every 8 weeks combined with AZA 50mg every day. Therapeutic strategic would be modulated if CD patients were confronted with clinical relapse or secondary lose of response (LOR) and data analysis would focus on the treatment course when patients received IFX 5mg/kg and AZA therapy regularly.

So in study stages, only 12 patients appeared clinical relapse due to secondary non-response of IFX. They all received hormonotherapy firstly for several reasons. Firstly, before June 2021, our center located in China just had Infliximab and other new biologics as Vedolizumab or Ustekinumab couldn't be obtained. Secondly, these 12 patients didn't satisfy indications of operation. So we chose hormonotherapy as the primary choice for patients to alleviate disease. I have corrected.

Characteristics of study subjects

The study cohort totally collected 112 CD patients achieving clinical remission after IFX induction therapy. In step I study, 19 CD patients were excluded due to data absence (N=1, 5.26%) and endoscopic activity at week 14 (N=18, 94.74%) while 93 CD patients with endoscopic remission at week 14 were included. In step II study, 58 CD patients were excluded due to course of therapy shorter than two years (n=10, 17.24%), secondary non-response of IFX (n=12, 20.69%), suspension of IFX therapy within two years for disease remission (n=10, 17.24%)

and endoscopic activity at week 54 (n=26, 44.83%) while 54 CD patients with endoscopic remission at week 54 were included. These 12 patients didn't satisfy indications of operation and received hormonotherapy as the primary choice to alleviate disease for our center lacked other biological agents at that time. (FIGURE 1) All CD patients under IFX maintenance therapy combined with azathioprine. The dose of IFX was 5mg/kg every 8 weeks and the dose of AZA was 50mg every day. Characteristics of CD patients included in study was shown in TABLE 1.

Problem 7: In Data Collection, there are the information that anti-infliximab antibody (ATI) was collected, but there is no data about that in the paper. The same happens with the CDAI score (CDAI is a score for activity and not severity of the disease) .

Answer 7: Our study made Qualitative detection of ATI and CD patients included study ATI was all negative. The reason of collecting CDAI score is to exclude CD patients with clinical activity and the study solely included CD patients with clinical remission, CDAI scores are all less than 150 points. So these data did not influence the results.

Problem 8： The therapeutic strategy during maintenance stage was designed as IFX 5mg/kg every 8 weeks combined with AZA 50mg every day. Nevertheless, it is well known that dose below 2mg/kg is ineffective. Why have you chosen this low dose of azathioprine?

Answer 8： Although the effective dose of AZA is 2mg/kg, the main purpose of AZA in IBD patients receiving AZA co-therapy is to improve the plasma concentration of IFX. Relevant studies show that the dose of AZA is not necessary to reach 2mg/kg. Another reason is that AZA metabolism in Chinese population is different from foreign population, and the required dose of AZA is less than 2mg/kg. Therefore, although AZA dose is insufficient for treatment, it can effectively maintain the plasma concentration of IFX and reduce the generation of ATI.

Problem 9： In Outcome Definition, the colonoscopy was evaluated at week 52 and week 104 after IFX initial therapy, but in other part of the text the time point used was 54 and 108.

Answer 9： Thanks for the reminder. The definition should be changed to weeks 54 and 108. I have corrected this problem.

Problem 10： What exactly means " were evaluated by specialist physicians on IBD under electronic colonoscopy"? Did they review pictures or movie of the original colonoscopy?

Answer 10： The doctors who perform colonoscopy and disease evaluation for IBD patients in our hospital are trained IBD specialists, which means that only specialists are qualified to

perform colonoscopy and follow-up for IBD patients regularly. The department of gastroenterology in our hospital is specialized in IBD outpatient service and IBD diagnosis and treatment group. They review both pictures and movie of the original colonoscopy.

Problem 11： Results In Characteristics of study subjects, this part is a little confuse. In the 1st part of the study 93 patients were included and in the 2nd part 54 patients, is that correct?
Answer 11： Thanks for the reminder. The data is correct. In the 1st part of the study, only 93 patients sticked to receive IFX 5mg/kg and AZA more than one year. In the 2nd part of the study, only 54 patients sticked to receive IFX 5mg/kg and AZA more than two years.

Problem 12： What exactly you mean with secondary non-response of IFX?
Answer 12： Secondary lose of response (LOR) means a recurrence of the disease during IFX maintenance therapy. Two criteria should be met to determine LOR: the recurrence of symptoms of IBD in clinical remission after induction therapy, and symptoms caused by the inflammatory activity of IBD itself.

Problem 13： Why some patients had the course of therapy shorter than two years?
Answer 13： Because these patients started IFX therapy after December 2018 but our data analysis suspended at December 2020.

Problem 14： In Correlation between Infliximab Trough Level, Inflammatory Biomarkers and Endoscopic Outcomes, it is not clear when the infliximabe trough level, CRP and FCP were measured, every 8 weeks or just at week 14?
Answer 14: Thanks for the reminder. The infliximabe trough level, CRP and FCP were measured, every 8 weeks since the third dose of IFX induction therapy in all patients.

A single-center retrospective research has been implemented in the first affiliated hospital of Zhejiang Chinese Medical University. Crohn's diseases patients under IFX therapy from January 2012 to December 2020 were collected. 181 CD patients underwent IFX treatment. 153 CD patients underwent enteroscopy as well as serum concentration monitoring at weeks 14 after the third dose of IFX induction therapy. Inclusion criteria, (I) endoscopic remission at week 14 (CDEIS Score ≤ 2 points or Rutgeerts' ≤ i1), (II) clinical remission after IFX induction therapy without corticosteroids more than 6 months, (III) therapeutic strategy during maintenance stage was designed as IFX 5mg/kg every 8 weeks combined with AZA 50mg every day. Therapeutic strategic would be modulated if CD patients were confronted with clinical relapse or secondary lose of response (LOR) and data analysis would focus on the treatment course when patients received IFX 5mg/kg and AZA therapy regularly. Secondary lose of response (LOR) means a recurrence of the disease during IFX maintenance therapy. Two criteria should be met to determine LOR: the recurrence of symptoms of IBD in clinical remission after induction therapy, and symptoms caused by the inflammatory activity of IBD itself. Clinical relapse means CDAI>150 points. Blood drug concentration monitoring, clinical, laboratory, endoscopic and imaging evaluation had been implemented every two months since the third dose of IFX induction therapy in all patients. The study was divided into two

I wish this revision will be acceptable for publication in your journal.
Thank you for your consideration. I am looking forward to hearing from you.
Yours Sincerely,
Wan-ting Cao

Email: 2334420162@qq.com