Reference: Rebuttle Letter - manuscript NO. 43461

Dear Ruo-Yu Ma, dear Ladies and Gentlemen,

on behalf of all authors I thank You very much for reviewing our manuscript NO. 43461 - "Triggers of histologically suspected drug-induced colitis." and the valuable comments that have helped to improve the work substantially. Accordingly, we have revised the manuscript. All changes have been highlighted in red.

In addition to the revised manuscript, we would like to answer the reviewer's considerations in a point-by-point manner as follows:

Reviewer 1:

1. There are no specific, commonly agreed upon histopathologic features of DiC and different drugs may induce different histopathologic changes. Given the common absence of strictly specific histopathological features, the diagnosis of DiC often relies upon thorough clinicopathological correlation, but in clinical practice it is difficult to establish the correlation between a certain medication and a particular pattern of injury. For example specific patterns – crypt epithelial cell apoptosis can be induced by fluorouracil, NSAIDs (diclofenac, mefenamic acid), cyclosporin, colchicine and ranitidine. With other drugs DiC may represent a diagnostic challenge (see below PDF) and may occur concomitantly with IBD.

We totally agree with these arguments. Nevertheless, there are some histopathological features that raise the suspicion of drug-induced aetiology. In our study, diagnosis of DiC was based on mixed, predominantly neutrophilic or lymphocytic inflammatory infiltrates, erosions, absence of granulomas, absence of basal plasmacellular infiltration and absence of crypt architectural distortion. Laxative-, corticosteroid- or gold compound-associated damage and well-defined drug-induced conditions, such as microscopic, infectious (including clostridium-associated colitis) and neutropenic colitis, were not regarded as suitable for inclusion.

We furthermore agree that overlap with IBD is possible, but assume that it is unlikely at least in the vast majority of cases. Firstly, we collected all preexisting co-morbidities; IBD was not present in any case. Secondly, first manifestation of IBD might have occurred, but there are some arguments against: usually IBD starts during second and third decade while the mean age of our cohort ranged within the seventh decade; diagnosis of IBD does

to rely upon histology solely, but includes history, laboratory parameters, stool testing, endoscopy, MRI scan etc.; as gastroenterologists we are particularly aware of IBD and focus to clarify the differential diagnoses.

2. *Was histopathological reassessment performed by the same or different pathologist(s)? What was agreement rate between these different pathologists?*

The histological assessment was performed by the same team of pathologists. During histological reassessment we did not verify the diagnosis drawn from histopathology, but gathered three different groups from a clinical perspective, evaluated the slides unaware of the former result and the respective group and aimed to define the histopathological characteristics. Therefore, we are unable to report the rate of agreement.

In the methods section we added the phrasing: "The same team of pathologists evaluated the slides unaware of the former result and the respective group. "

3. *Histologic slides are not good quality and should be replaced with ones of better quality and specific changes should be labeled with arrows and/or asterisks*

We have reviewed the slides and decided to stick with the submitted images, but the pathological features have been marked with arrows.

4. *Some statements e.g.*

a)"Written, informed consent was obtained from all patients before specific examinations. Due to its retrospective character informed consent was neither practicable nor necessary" should be better clarified and rephrased e.g., Written, informed consents were obtained from all patients before specific examinations and procedures such as colonoscopy and biopsy. For this retrospective study informed consent was neither practicable nor necessary and was exempted by ……..IRB?

We have changed the phrasing according to the proposal and thank for the linguistic assistance: "Written, informed consents were obtained from all patients before specific examinations and procedures such as colonoscopy and biopsy. For this retrospective study informed consent was neither practicable nor necessary and was exempted by the IRB of the Ruhr-university. "

b) "Statistics were realized" should be rephrased

We have changed the phrasing to: "Statistical analysis was carried out with …".

Reviewer 2:

1. *In Table 6-S, two titles of 'Drug-induced colitis without atherosclerosis' were seen. Please correct the titles.*

Thank You for the attention! We have corrected the title of the 2nd column to: "Drug-induced colitis with atherosclerosis".

Reviewer 3:

1. *In tables 6S and 9S 1st and 2nd column is the same? DiC without atherosclerosis? Probably column 2nd is <<DiC with atherosclerosis>>>*

Thank You for Your attention! We have corrected the title of the 2nd column to: "Drug-induced colitis with atherosclerosis".

2. *The study is based on histological examination of colorectal biopsies. More specific definition of histological diagnosis of DiC and differential diagnosis from ischemic colitis is necessary. Please give in details, which are the histological features specific for DiC and which are the histological features of both groups.*

There are no specific, commonly agreed upon histopathologic features of DiC and different drugs may induce different histopathologic changes. Nevertheless, there are histopathological features that raise the suspicion of drug-induced aetiology. In our study, diagnosis of DiC was based on mixed, predominantly neutrophilic or lymphocytic inflammatory infiltrates, erosions, absence of granulomas, absence of basal plasmacellular infiltration and absence of crypt architectural distortion. More specific patterns that can be attributed to certain aetiologies like laxative-, corticosteroid- or gold compound-associated damage and other well-defined drug-induced conditions, such as microscopic, infectious (including clostridium-associated colitis) and neutropenic colitis, were not regarded as suitable for inclusion.

3. In patients with atherosclerosis and multiple anti-atherosclerotic medications, it is not easy to prove etiologically the connection of specific drug category to DiC. A comment is necessary.

Please see the answer to the following comment.

4. A major conclusion is that: <<In univariate analysis, DiC was associated with diuretics, dihydropyridines, glycosides, ASS, platelet aggregation inhibitors, NSAIDs, statins and fibrates.>> However, the majority of these drugs are also used in atherosclerotic patients, who are also candidates for ischemic colitis. So it is difficult to prove the DiC. The definition of DiC should exclude atherosclerosis.

We totally agree with these comments and tried to focus these arguments in the discussion section. Nevertheless, this study is the first one that systematically investigates associated drugs in the case of histologically suspected DiC. The association of DiC with drugs that are indicated for the treatment of cardiovascular and related diseases is a completely novel and unexpected finding. The potential role of atherosclerosis in the pathogenesis of suspected DiC was unclear while designing the study. Therefore, the study design is not suitable to distinguish the role of atherosclerosis on the hand and the particular drugs on the other hand. We have added a specifying phrase to the discussion section:

"The overall results support the idea of disturbances in microperfusion at least in a subset of patients, maybe those patients with underlying atherosclerosis."

Further studies that focus on this particular differentiation are needed. Thorough detection and exclusion of atherosclerosis might be a suitable option for future study designs. Similarly, in the Conclusion section we stated (and hope that this phrasing supports the idea clearly enough):

"Prospective studies including larger cohorts with clearly defined cardiac function, pattern and severity of atherosclerosis and related comorbidities, such as hyperlipoprotenaemia, are warranted to unravel the underlying aetiology and pathophysiology of this under-recognised entity. "

5. It is very important to differentiate ischemic colitis from drug-induced colitis histologically. <<….DiC patients with atherosclerosis exhibited histological features from both other groups..>> Did the patients have two etiologies for colitis? DiC plus ischemic? This is a little confusing.

Patients with clear features of ischaemic colitis were regarded suitable for inclusion in one the two control groups (inflammatory controls), but were included in the DiC group. On the other hand, there are no specific, commonly agreed upon histopathologic features of

DiC and different drugs may induce different histopathologic changes. In our study, diagnosis of DiC was based on mixed, predominantly neutrophilic or lymphocytic inflammatory infiltrates, erosions, absence of granulomas, absence of basal plasmacellular infiltration and absence of crypt architectural distortion.

After first analysis of the data we reassessed the histological slices as described above and found that patients with DiC and atherosclerosis showed patterns of both, DiC and IC. Therefore, we propose that focal disturbances of the microcirculation play a substantial role in the pathogenesis of a subgroup of DiC patients. Maybe these patients are more susceptible to drug-induced damages. Unfortunately the study design is not suitable to confirm this hypothesis.

In order to point that out we have added a specifying phrase to the conclusion section:

"The distribution of drug intake further supports this hypothesis, but the study design is not suitable to prove it."

Again, we thank You for the valuable discussion and the comments and hope that we were able to use them properly in order to improve the manuscript so that it is eligible for publication now. We are looking forward to Your esteemed feedback,

Best regards

Thorsten Brechmann