Role of viruses in biliary atresia: news from mice and men

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Abstract: Biliary atresia (BA) is still an enigmatic disease of unknown etiology and cryptic pathomechanism. Despite the fact that BA is rated among rare diseases, it represents the most frequent indication for pediatric liver transplantation. Although every effort is made to elucidate the origin of the ongoing deterioration of liver function, no breakthrough has so far been achieved, which switches the surgical but symptomatic therapy to a cause-oriented approach. The nowadays leading hypothesis focuses on hepatotropic virus as a triggering agent for an autoimmune self-limiting inflammatory process along the entire biliary tree. The present review highlights the current state of research on the factor “viruses in biliary atresia” in both patients undergoing the Kasai procedure and the virus-induced BA mouse model.

Keywords: animal model; biliary atresia; liver transplantation; neonatal cholestasis.

Abbreviations: BA, biliary atresia; CBD, common bile duct; CMV, cytomegalovirus; IFN, interferon; LTx, liver transplantation; PCR, polymerase chain reaction; RRV, rhesus rotavirus.

Introduction

Neonatal jaundice is a common phenomenon that occurs in approximately 50% of newborns. The process starts with yellowing of the sclera and skin; however, in the majority of cases, the symptoms rapidly subside until full health returns. However, a small number of newborns remain cholestatic, and several rare pediatric liver diseases hide behind this unspecific symptom. In those cases, the fact that the patient may be suffering from one or more of the numerous neonatal liver diseases needs to be taken into consideration. Many of these diseases are relatively harmless and resolve spontaneously or after appropriate therapy. However, some hereditary liver diseases present with early symptoms and require lifelong medical assistance and care. In some cases, liver transplantation (LTx) is the only option for survival [1, 2]. Among these entities, which are all ranked as rare diseases, biliary atresia (BA) is the only condition that requires specific attention in terms of timely diagnosis and treatment. In addition to the clinical signs of hyperbilirubinemia, the color of the stools of these patients become pale, and they stop thriving [3]. They also exhibit persistent cholestasis, an ongoing inflammatory process that transforms the liver tissue into a state of fibrosis, and liver function deteriorates rapidly. The course of this particular disease destroys the liver of neonates within weeks and months. For this reason, BA is the most frequent indication for LTx in children, although the incidence in the Western world is just 1 in 19,000 live births. The only option for patients with BA to survive with their native liver is to diagnose the problem as early as possible and to perform a hepatoportoenterostomy, which was named after Morio Kasai. The principle of this surgical procedure is based on removal of the atretic extrahepatic bile ducts, meticulous excision of a fibrotic plate in the porta hepatis, and the creation of a particular biliodigestive anastomosis. However, even in the most experienced centers, the mid-term jaundice-free survival of BA patients with their own liver does not exceed 50%, while, in the long-term, about 80% of those affected finally require LTx [4, 5].

Besides incomplete screening for BA, belated diagnostics, and inappropriate surgery in some places, the crucial problem is that the etiology of BA remains unknown [2, 6]. This problem can be illustrated by the fact that BA is defined as atresia of the extrahepatic bile ducts but is characterized by changes along the intrahepatic and extrahepatic biliary tree. In other words, we use the term “biliary atresia” as an antonym for a non-understood...
entity. Nevertheless, from the very beginning until today, clinicians and researchers have speculated about the origin, etiology, and pathomechanism of this disease. Historically, BA was thought to be a congenital malformation until Landing presented the hypothesis that BA and other neonatal cholestatic diseases “… are different results or permissible outcomes of a single basic process…” He also assumed that hepatotropic viruses could play a crucial role in this process [7]. Similar, but rather not as concrete, Perlmutter and Shepherd [8] proposed that BA is not a single disease but a phenotype of an absolutely different pathomechanism. Simultaneously, a new theory about an autoimmunologically driven course was discussed because similarities between BA and other liver diseases, like autoimmune hepatitis, were observed [9].

Today, clinical and basic researchers are working hard to identify new ways and directions by which it is possible to access deeper insights into the origin of this obscure disease. Herein, the hypothesis that hepatotropic viruses might play a crucial role in BA is still favored and, since 1985, approximately 250 papers have been published on this topic. The following discussion presents a summary of the current status of research that has focused on BA in humans and in murine models and, finally, a question about translational validation is raised.

**Viruses in human BA**

A few years after “Landing’s theory” was recognized, the first observations describing the sporadic findings of hepatotropic viruses in BA patients were published. Between 1994 and 2005, a total of 11 studies reported 28% positive findings of hepatotropic viruses across a population of 165 patients. The liver specimens of the patients were taken at the moment of the Kasai procedure and, on average, one to two out of eight different viruses were detected by polymerase chain reaction (PCR). The most frequent viruses were human papilloma and parvovirus B19, which were detected in about 15 patients per study [10–12].

Our research group also ran real-time and nested PCR in liver biopsies; however, we tested for 12 DNA/RNA hepatotropic viruses simultaneously in 74 BA patients. Overall, only 42% of the specimens tested positive for at least one virus, predominantly cytomegalovirus (CMV) and the reovirus. Two different viruses in the same biopsy were detected in five patients, which seems to imply that this could represent a secondary infection. In 59 biopsies of the same series, we looked for Mx-A by immunohistochemistry, which is a strong indicator of previous or active viral infections. We found that 92% of the specimens were Mx-A positive (Figure 1), while PCR revealed reovirus, CMV, and enterovirus in only 30% of the cases. From this study, we concluded that PCR studies in liver biopsies come late when they are performed at the moment of the Kasai procedure [13]. No study has generated sufficient evidence about the etiological role of hepatotropic viruses in BA. In other words, it was impossible to distinguish between primary and potentially etiologically relevant and secondarily acquired infections. However, we learned that Mx-A protein along the bile ducts, in hepatocytes, and in endothelial cells can be seen as a footprint of a viral infection, even when the virus itself is not measurable.

Our decision to suspend performing PCR in BA liver biopsies was confirmed by a review that was performed by Saito et al. in 2015. They concluded: “Although a considerable number of PCR studies have sought to clarify a viral role in the pathogenesis of BA…, the findings… have not succeeded in achieving an obvious differentiation between causative and accidental infection….” [14]. In the future, new techniques in genetic engineering may enhance the understanding of this important issue.

**Viruses in murine BA**

Viral-induced cholestasis in mice was first reported by Phillips et al. [15]. Newborn pups from different mouse strains were intraperitoneally inoculated with reoviruses. All these studies were able to induce inflammation of the liver and bile ducts with consequent jaundice; however, they failed to simulate the pattern of human BA [16–18].

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Figure 1: Immunostaining for Mx-A protein of a liver biopsy taken at the Kasai procedure. Positive findings in hepatocytes (asterisk), bile ducts (arrows), and endothelial cells (diamond).
The turning point was reached when newborn Balb/c mice were infected with rhesus rotavirus (RRV) and then developed BA-like obstruction of the extrahepatic bile ducts. Riepenhoff-Talty et al. [19] orally inoculated 2-day-old mice with simian RRV. Half of the RRV-infected pups developed cholestasis, and most died within 3 weeks. Dissection of the diseased pups revealed an obstruction of the common bile duct (CBD) and BA-like changes in the liver, e.g. inflammatory infiltrates and bile duct proliferation. Our BA research group modified the study design and injected RRV intraperitoneally between 24 and 48 h of life, with about 60% of the affected Balb/c pups showing jaundice, alcoholic stool, and the oily fur syndrome. Minimal weight gain of the affected pups occurred by day 14, while recovering animals put on weight and showed normalized clinical symptoms. Dissection beyond day 14 of RRV infection showed a spotted surface of the liver, edema of the hepatobiliary ligament, and long- and short-distance atresias of the CBD with or without hydrops of the gallbladder [20, 21]. Lymphocyte infiltration was observed in expanded portal triads, with bile duct proliferation that also resembled the features of human BA. However, even in 3-week-old pups, resultant liver fibrosis was uncommon, which represents a crucial difference between murine BA and human BA [22, 23].

The three key variables in this particular model are the mouse strain, time of infection, and virus dosage. Thus, the occurrence of BA is associated with early postnatal infection but is inversely related to the infective viral dosage. Prenatal infection does not induce jaundice but prevents the offspring from developing cholestasis after postnatal RRV infection. It can be summarized as follows: Balb/c mice are the most susceptible mouse strain, high dosage of RRV is still the only known agent that induces definite atresia of the extrahepatic bile ducts, and the first 24 h postpartum is the optimal time point for intraperitoneal injection of the virus (Figure 2). The versatility of the model is somewhat limited, as sequential investigations cannot be performed in the same animal. Diseased pups are extremely unstable and too small for repetitive biopsies or blood sampling. To try to overcome this, groups of mice are usually scheduled for sacrifice [24–27].

In the liver of jaundiced mice, a significant virus load peak is observed at day 7 after infection. However, in most pups, the virus is cleared from the liver by day 14, although the cellular and humoral immune responses persist [28]. The innate immune system concentrates natural killer cells around bile ducts, and antigen-presenting cells induce a T-cell-mediated immune response. Interferons (IFNs) seem to play a crucial role in the murine BA model.
because Mx protein, which is an IFN type I-specific indicator, persists in the hepatocytes, bile ducts, and intrahepatic endothelial cells of cholestatic mice beyond the second week of RRV infection [29].

Apoptosis has been shown by terminal deoxynucleotidyl transferase dUTP nick end labeling assay and mRNA expression for caspase 1 and 4 with a peak at day 7 in the liver and extrahepatic bile ducts. However, it is still unclear whether apoptosis is part of the clearing mechanism after viral infection or, rather, demonstrates the hyper-responsiveness of immature immunity. Regulatory T cells may play a pivotal role, as they seem to be involved in the determination of autoimmune processes. They are absent in newborn mice and become activated during the first week of life. A first attempt to substitute regulatory T cells in the mouse model reduced the incidence of BA and shifted the focus to natural killer cells [30–37].

It is possible to attenuate the destructive process of experimental BA. For instance, repeated administration of IFN in pups, starting individually at the onset of jaundice, was curative [38, 39]. It is not clear whether this boosts the clearance of the virus or if IFN modulates the immune response. Immunization of dams with RRV before mating and during pregnancy also protects their offspring from developing cholestasis and BA [40]. These preliminary results are far from ready for clinical application in humans; however, they do open the door to prophylactic strategies in BA research.

**Translational research in BA**

Taking into consideration the fact that the etiology and early pathomechanism of BA in babies is still unknown, and ongoing fibrosis of the liver and consecutive sequelae of portal hypertension cannot be reproduced in the artificial BA mouse model, it remains debatable whether this particular animal model is suitable for translational research in BA. As shown in Figure 3, there is a time mismatch between the course of the human and the murine disease in terms of the starting point of the inflammatory process that finally leads to BA. It cannot be determined because the onset of the disease is always hidden behind the unspecific symptoms of neonatal cholestasis. Moreover, as far as BA is defined as irreversible fibrotic changes of the extrahepatic bile ducts, our observation period in humans starts shortly before or at the moment of the Kasai procedure. In other words, the triggering agent and the immune response of the early phase cannot be retraced and remains unclear. However, this exact period is subject to basic research in the murine BA model. Hence, the crucial point is whether findings in the animal model can be translated to the human disease to elucidate its unperceivable early course (Figure 3).

According to the individual point of view, this issue remains controversial. Critics argue that the animal model only resembles human BA because mid- and long-term...
courses, including liver cirrhosis and its clinical sequelae, cannot be simulated in diseased mice. However, we and other research groups argue that the correspondence of BA and BA-like changes between “mice and men” is so strong that continuation of this approach is definitely justified. Furthermore, we are actively working on a concept that makes longer survival of diseased pups possible and through which we can observe the ongoing process of artificially induced BA.

In conclusion, as long as no other BA animal model is available, we and other international research groups will continue working with the RRV-Balb/c mouse model and remain optimistic that we will gradually shed more light on the etiology of this obscure disease.

**Author Statement**

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**Author Contributions**

Claus Petersen: conceptualization; investigation; methodology; project administration; writing – original draft. Omid Madadi-Sanjani: methodology; visualization; writing – review and editing.

**References**

[1] Sokol RJ, Mack C, Narkewicz MR, Karrer FM. Pathogenesis and outcome of biliary atresia: current concepts. J Pediatr Gastroenterol Nutr 2003;37:4–21.
[2] Petersen C, Davenport M. Aetiology of biliary atresia: what is actually known? Orphanet J Rare Dis 2013;8:128.
[3] Wang L, Yang Y, Chen Y, Zhan J. Early differential diagnosis methods of biliary atresia: a meta-analysis. Pediatr Surg Int 2018;34:363–80.
[4] Petersen C, Madadi-Sanjani O. Registries for biliary atresia and related disorders. Eur J Pediatr Surg 2015;25:469–73.
[5] Verkade HJ, Bezerra JA, Davenport M, Schreiber RA, Mielii-Vergani G, Hulscher JB, et al. Biliary atresia and other cholestatic childhood diseases: advances and future challenges. J Hepatol 2016;65:631–42.
[6] Sokol RJ, Mack C. Etiopathogenesis of biliary atresia. Semin Liver Dis 2001;21:517–24.
[7] Landing BH. Considerations of the pathogenesis of neonatal hepatitis, biliary atresia and choledochal cyst – the concept of infantile obstructive cholangiopathy. Prog Pediatr Surg 1974;6:113–39.
[8] Perlmutter DH, Shepherd RW. Extrahepatic biliary atresia: a disease or a phenotype? Hepatology 2002;35:1297–304.
[9] Lakshminarayanan B, Davenport M. Biliary atresia: a comprehensive review. J Autoimmun 2016;73:1–9.
[10] Morecki R, Glaser JH, Cho S, Balistreiri WF, Horwitz MS. Biliary atresia and reovirus type 3 infection. N Engl J Med 1982;307:481–4.
[11] Riepenhoff-Talty M, Gouvea V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, et al. Detection of group C rotavirus in infants with extrahepatic biliary atresia. J Infect Dis 1996;174:8–15.
[12] Fischler B, Ehrnst A, Forsgren M, Orvell C, Nemeth A. The viral association of neonatal cholestasis in Sweden: a possible link between cytomegalovirus infection and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr 1998;27:57–64.
[13] Rauschenfels S, Krassmann M, Al-Masri AN, Verhagen W, Leonhardt J, Kuebler JF, et al. Incidence of hepatotropic viruses in biliary atresia. Eur J Pediatr 2009;168:469–76.
[14] Saito T, Terui K, Mitsunaga T, Nakata M, Ono S, Nise N, et al. Evidence for viral infection as a causative factor of human biliary atresia. J Pediatr Surg 2015;50:1398–404.
[15] Phillips PA, Keast D, Papadimitriou JM, Walters MN, Stanley NF. Chronic obstructive jaundice induced by Reovirus type 3 in weaning mice. Pathology 1969;1:193–203.
[16] Papadimitriou JM. The biliary tract in acute murine reovirus 3 infection. Light and electron microscopic study. Am J Pathol 1968;52:595–611.
[17] Szavay PO, Leonhardt J, Czech-Schmidt G, Petersen C. The role of reovirus type 3 infection in an established murine model for biliary atresia. Eur J Pediatr Surg 2002;12:248–50.
[18] Mack CL, Sokol RJ. Unraveling the pathogenesis and etiology of biliary atresia. Pediatr Res 2005;57:87R–94R.
[19] Riepenhoff-Talty M, Schaelkel K, Clark HF, Mueller W, Uhnnoo I, Rossi T, et al. Group A rotavirus produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res 1993;33:394–9.
[20] Petersen C, Biermanns D, Kuske M, Schaelkel K, Meyer-Junghanel L, Mildenberger H. New aspects in a murine model for extrahepatic biliary atresia. J Pediatr Surg 1997;32:1190–5.
[21] Petersen C, Grasshoff S, Luciano L. Diverse morphology of biliary atresia in an animal model. J Hepatol 1998;28:603–7.
[22] Keyzer-Dekker CM, Lind RC, Kuebler JF, Offerhaus GJ, Ten Kate FJ, Morsink FH, et al. Liver fibrosis during the development of biliary atresia: proof of principle in the murine model. J Pediatr Surg 2015;50:1304–9.
[23] Nadler EP, Li X, Onyedika E, Greco MA. Differential expression of hepatic fibrosis mediators in sick and spontaneously recovered mice with experimental biliary atresia. J Surg Res 2010;159:611–7.
[24] Leonhardt J, Kuebler JF, Turowski C, Tschernig T, Geffers R, Petersen C. Susceptibility to experimental biliary atresia linked to different hepatic gene expression profiles in two mouse strains. Hepatol Res 2010;40:196–203.
[25] Mohanty SK, Shivakumar P, Sabla G, Bezerra JA. Loss of interferon-12 modifies the pro-inflammatory response but does not prevent duct obstruction in experimental biliary atresia. BMC Gastroenterol 2006;6:14.
[26] Kuebler JF, Czech-Schmidt G, Leonhardt J, Ure BM, Petersen C. Type-I but not type-II interferon receptor knockout mice are susceptible to biliary atresia. Pediatr Res 2006;59:790–4.
[27] Leonhardt J, Stanulla M, von Wasielewski R, Skokowa J, Kubler J, Ure BM, et al. Gene expression profile of the infective murine model for biliary atresia. Pediatr Surg Int 2006;22:84–9.

[28] Czech-Schmidt G, Verhagen W, Szavay P, Leonhardt J, Petersen C. Immunological gap in the infectious animal model for biliary atresia. J Surg Res 2006;101:62–7.

[29] Wehrmann F, Kuebler JF, Wienecke S, Al-Masri AN, Petersen C, Leonhardt J. Functional Mx protein does not prevent experimental biliary atresia in Balb/c mice. Eur J Pediatr Surg 2008;18:318–21.

[30] Mack CL, Tucker RM, Sokol RJ, Karrer FM, Kotzin BL, Whittington PF, et al. Biliary atresia is associated with CD4+ Th1 cell-mediated portal tract inflammation. Pediatr Res 2004;56:79–87.

[31] Jafri M, Donnelly B, Allen S, Bondoc A, McNeal M, Rennert PD, et al. Cholangiocyte expression of α2β1-integrin confers susceptibility to rotavirus-induced experimental biliary atresia. Am J Physiol Gastrointest Liver Physiol 2008;295:G16–26.

[32] Shivakumar P, Campbell KM, Sabla GE, Miethke A, Tiao G, McNeal MM, et al. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-γ in experimental biliary atresia. J Clin Invest 2004;114:322–9.

[33] Mohanty SK, Ivantes CA, Mourya R, Pacheco C, Bezerra JA. Macrophages are targeted by rotavirus in experimental biliary atresia and induce neutrophil chemotaxis by Mip2/Cxcl2. Pediatr Res 2010;67:345–51.

[34] Saxena V, Shivakumar P, Sabla G, Mourya R, Chougnet C, Bezerra JA. Dendritic cells regulate natural killer cell activation and epithelial injury in experimental biliary atresia. Sci Transl Med 2011;3:102ra194.

[35] Tucker RM, Hendrickson RJ, Mukaida N, Gill RG, Mack CL. Progressive biliary destruction is independent of a functional tumor necrosis factor-α pathway in a rhesus rotavirus-induced murine model of biliary atresia. Viral Immunol 2007;20:34–43.

[36] Miethke AG, Saxena V, Shivakumar P, Sabla GE, Simmons J, Chougnet CA. Post-natal paucity of regulatory T cells and control of NK cell activation in experimental biliary atresia. J Hepatol 2010;52:718–26.

[37] Feng J, Li M, Cai T, Tang H, Gu W. Rotavirus-induced murine biliary atresia is mediated by nuclear factor-κB. J Pediatr Surg 2005;40:630–6.

[38] Harada K, Nakanuma Y. Biliary innate immunity in the pathogenesis of biliary diseases. Inflamm Allergy Drug Targets 2010;9:83–90.

[39] Petersen C, Bruns E, Kuske M, von Wussow P. Treatment of extrahepatic biliary atresia with interferon-α in a murine infectious model. Pediatr Res 1997;42:623–8.

[40] Bondoc AJ, Jafri MA, Donnelly B, Mohanty SK, McNeal MM, Ward RL, et al. Prevention of the murine model of biliary atresia after live rotavirus vaccination of dams. J Pediatr Surg 2009;44:1479–90.

Supplemental Material: The online version of this article offers supplementary material (https://doi.org/10.1515/iss-2018-0009).
Reviewer Assessment

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Reviewers’ Comments to Original Submission

Reviewer 1: anonymous
Feb 25, 2018

Reviewer Recommendation Term: Accept with Minor Revision
Overall Reviewer Manuscript Rating: 70

Custom Review Questions
Response

Is the subject area appropriate for you? 5 - High/Yes
Does the title clearly reflect the paper’s content? 4
Does the abstract clearly reflect the paper’s content? 4
Do the keywords clearly reflect the paper’s content? 4
Does the introduction present the problem clearly? 4
Are the results/conclusions justified? 3
How comprehensive and up-to-date is the subject matter presented? 3
How adequate is the data presentation? 3
Are units and terminology used correctly? 4
Is the number of cases adequate? N/A
Are the experimental methods/clinical studies adequate? N/A
Is the length appropriate in relation to the content? 3
Does the reader get new insights from the article? 3
Please rate the practical significance. N/A
Please rate the accuracy of methods. 3
Please rate the statistical evaluation and quality control. N/A
Please rate the appropriateness of the figures and tables. 3
Please rate the appropriateness of the references. 2
Please evaluate the writing style and use of language. 4
Please judge the overall scientific quality of the manuscript. 3
Are you willing to review the revision of this manuscript? Yes

Comments to Authors:
With interest I have read this paper regarding the role of viruses in biliary atresia. The authors group is undoubtedly the most experienced research group regarding the mouse model for biliary atresia, and I have to compliment them for their work. However, there are some issues that should be adressed prior to publication of the present paper.
1) Can the authors elaborate on similarities and differences in immunological responses as observed in mice and men? Could this be presented in a new figure to present the reader with a clear overview?
2) I am missing the role of CMV in human BA. There is data that CMV+ BA behaves differently from CMV- BA. The authors should discuss this more in detail, including the necessary references (e.g. Zani et al)
3) How does the virus model of BA compare to other recent developments, e.g. the discovery of biliatresone? I realize that this might be slightly beyond the scope of the present paper - yet it would be informative.
4) The authors point out several issues paramount for the development of BA in the mouse model (timing of infection, dosage, mouse strain). Could the authors speculate on the mechanisms behind these issues, as they could shed light on the development of the disease. Eg: why does late inoculation not induce BA? Do the authors have any suggestions about why some animals recover?

Reviewer 2: anonymous

Mar 06, 2018

Reviewer Recommendation Term: Accept
Overall Reviewer Manuscript Rating: 95

Custom Review Questions
Is the subject area appropriate for you? 5 - High/Yes
Does the title clearly reflect the paper’s content? 5 - High/Yes
Does the abstract clearly reflect the paper’s content? 5 - High/Yes
Do the keywords clearly reflect the paper’s content? 5 - High/Yes
Does the introduction present the problem clearly? 5 - High/Yes
Are the results/conclusions justified? 5 - High/Yes
How comprehensive and up-to-date is the subject matter presented? 5 - High/Yes
How adequate is the data presentation? 5 - High/Yes
Are units and terminology used correctly? N/A
Are the experimental methods/clinical studies adequate? N/A
Is the length appropriate in relation to the content? 4
Does the reader get new insights from the article? 5 - High/Yes
Please rate the practical significance. 5 - High/Yes
Please rate the accuracy of methods. N/A
Please rate the statistical evaluation and quality control. N/A
Please rate the appropriateness of the figures and tables. 5 - High/Yes
Please rate the appropriateness of the references. 4
Please evaluate the writing style and use of language. 4
Please judge the overall scientific quality of the manuscript. 5 - High/Yes
Are you willing to review the revision of this manuscript? Yes

Comments to Authors:
This is an excellent review on a complex subject. Authors are able to highlight the importance of animal models for the understanding of this rare disease but also show that animal models are not an overall solution to clarify all aspects of the clinical situation.

Authors’ Response to Reviewer Comments

Mar 19, 2018

The comments and suggestions for amendments by the reviewers are in principle meaningful and welcome. However, they overlap in content with the article, which is referred as reference 2. A repetition of the detailed discussion would therefore offer no new content. For this reason, I do not want to make any significant changes to the manuscript.