Reviewer #1

I read with great interest the manuscript from Loosen et al concerning the role of circulating miRNAs in liver disease. The manuscript is summarizing the multiple short non coding RNAs involved in liver disease, and it is a well-written analytical review focused on this item. However I think the review lacks of a profound analysis of sufficient literature data. Minor revisions might be suggested to Authors to improve the scientific impact. It is recommended to describe wich technological platform was used in each study, where possible, (microarray, Nanostring, digital PCR, Next Generation Sequencing- NGS), because there are a lot of papers concerning this issue and it's interesting to evaluate also the approach used to select the paper. Maybe a table could be useful for the readers. Moreover, they didn't mention the miRNAs present in the exosomes, and I suggest to reporter at least one paper concerning this point. Interestingly, in the conclusion the authors speculate concerning a standardization of sample collection and it will be interesting also to discuss the role of the new platforms in this kind of study.

We thank reviewer #1 for the fair evaluation of our manuscript. As the reviewer suggested, we have now included a table that explicitly describes the technological platform used in the reviewed paper (see last pages of the revised manuscript). To highlight the role of exosomal miRNAs in liver diseases we furthermore have added a respective paragraph in the introduction:

"Circulating miRNAs can be either bound to serum proteins and lipoproteins or be encircled in extracellular vesicles including exosomes, microvesicles or apoptotic bodies [17, 19]. As exosomes can be released by various hepatic cells (e.g. hepatocytes and Kupffer cells) and can be transferred to other recipient cells to regulate expression profiles in these cells, they were suggested to play an important role in hepatic cell-cell-communication and in the pathophysiology of different liver diseases. Findings that miRNAs encircled in these vesicles are well protected from degradation furthermore highlight the potential of exosomal miRNAs to serve as potent biomarkers [20-22]."

Moreover, we included the following sections on published data about exosomal miRNA with regard to alcohol-induced liver injury and HCC patients:

"In the context of alcohol induced liver injury, microarray based screening of exosomal miRNAs revealed an up-regulation of miRNA-192, miRNA-122, miRNA-30a, miRNA-744, miRNA-1246, miRNA 30b and miRNA-130a in blood sera of chronic alcohol-fed mice compared to healthy controls [42]. Moreover, ROC curve analyses indicated a diagnostic potential of miRNA-192, miRNA-122, and miRNA-30a for the identification of alcohol-induced liver injury [42]."

"Moreover, serum levels of exosomal miR-18a, miR-221, miR-222 and miR-224 were significantly higher whereas exosomal miR-101, miR-106b, miR-122 and miR-195 were significantly lower in patients with HCC compared to patients with chronic hepatitis B or liver chirrosis [61]."

Finally, we have added a paragraph about sample standardization and the contribution of next generation sequencing into the conclusion:

"As qPCR and microarray based measurements naturally depend on the design of miRNA specific primers or microarray probes, similarities between different miRNAs might result in further difficulties regarding the comparison between studies. Moreover, data normalization issues mainly arise from the lack of a valid intrinsic RNA housekeeping gene for human serum samples and high inter-platform differences in miRNA quantification efficacy contribute to a poor comparability between studies. Finally, most studies are carried out as single center study including only a small number of patients. Therefore, next generation sequencing might have an important impact on the validation of miRNA profiles, as it allows mostly sequence independent, parallel measurement and detection of overall numbers of a broad spectrum of different miRNAs (reviewed e.g. in [79])."

Reviewer #2

The authors reviewed the role of circulating miRNAs in the most important liver diseases, including acute liver failure, liver fibrosis, liver cirrhosis and liver cancer. It will be beneficial to the diagnostic and prognostic evaluation of live diseases.

We thank reviewer #2 for the evaluation of our manuscript and the statement that the manuscript is suitable for publication.

Reviewer #3

In this review, the authors have conducted a comprehensive summary on the clinical significance of circulating miRNAs for various acute or chronic liver diseases, such as acute liver failure, liver fibrosis and cirrhosis, liver cancers, etc. Also, they have made the assessment on the merits and existing problems of clinical application of circulating miRNAs for liver diseases. The review is of interest and significance for readers. However, the review may be more comprehensive and valuable, if the summary on the data of the role of circulating miRNAs in autoimmune liver diseases are added, including primary biliary cholangitis, autoimmune hepatitis and primary sclerosing cholangitis, because they also represent a large class of liver disease, distinct from other liver diseases. Another minor point is that there is no space between words in some parts, making the reader confused.

We are very thankful for the comments of reviewer #3. We have addressed the issue of missing spaces between words in the whole manuscript. According to the reviewer's suggestion, we have now included a new section on the role of circulating miRNAs in autoimmune liver diseases (AIH, PSC, PBC):

"Although autoimmune liver diseases have gained rising importance in the field of hepatology due to its increasing incidence over the last decades [46], only very few studies have

evaluated the involvement of circulating miRNAs in autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC).

To our knowledge only one study investigating circulating miRNAs in patients with AIH exists to date. In this study, serum samples of 46 type-1 AIH patients were screened for 2555 miRNAs using a microarray system and compared to patients with chronic hepatitis C and healthy controls. Circulating levels of miR-21 and miR-122 were significantly higher in AIH patients compared to both control groups. Interestingly, the authors observed a strong decrease of miR-21 and miR-122 levels after treatment with glucocorticoids, indicating a potential role of these miRNA not only as a diagnostic marker but also as a marker to assess treatment response [47].

In PSC patients, serum levels of miR-1281 and miR-126 were shown to be significantly increased compared to healthy controls. Importantly, the elevation of these miRNAs in PSC patients was also significantly higher compared to CCA patients, arguing that miR-1281 and miR-126 might reflect disease-specific processes of PSC that do not or to a lesser extend occur during malignant transformation of bile duct cells into CCA [48]. Moreover, Bernuzzi and co-workers described miR-200c as significantly down-regulated in patients with PSC in large screening approach including 667 miRNAs [49].

In PBC patients, a deep sequencing approach revealed circulating levels of miR-505-3p and miR-197-3p as significantly decreased when compared to healthy controls [50]. However, this study was performed in a very small cohort of patients (n=10) and needs further validation. In another study, Tan and colleagues establish a diagnostic serum miRNA panel in a cohort of 207 PBC patients using a stepwise logistic regression model. The panel, consisting of miR-122, miR-141 and miR-26b, had an AUC of 0.905 for the discrimination between PBC patients and healthy control, which was superior to established biomarkers for PBC such as AP and ANA [51].

In summary, the role of circulating miRNA in autoimmune liver disease has so far only been analyzed in a very limited number of studies with comparatively small cohort sizes. Thus, further studies are needed to make a clear statement on the potential role of serum miRNAs as a biomarker for AIH, PSC and PBC."

Reviewer #4

Emerging interests in the application of circulating biomarkers including targeted cells (such as tumor cells), metabolites, and nucleic acids (such as cell-free DNA, exosomal microRNAs) have opened a new avenue for early diagnosis and prediction of prognosis of diseases. Of them, microRNAs (miRNAs) are of particularly promising because of the relatively high content and easy detection in the circulation. This review summarizes currently available data on the role of circulating miRNAs in the potentially clinical purposes, namely diagnosis and prognosis, of liver diseases including acute hepatic failure, fibrosis and cirrhosis, and cancers. Moreover, it also provides the challenges that currently impede the clinical use of circulating miRNAs as biomarkers for liver diseases. Overall, the subject is of clinical interest and potential significance and is in a right timing for both investigators and clinicians. Suggestions: 1. Abstract: MicroRNAs … the translation or transcription of their mRNAs. ? turnover 2. Tables summarizing currently reported circulating miRNAs in the diagnosis and/or prognosis of liver diseases (including the sample size used in each study) will be very helpful for the readers. 3. If the concept of "liquid biopsy" included in Introduction, it will be more appealing to the readers. 4. More about the current limitations or bottleneck in the application of miRNAs as biomarkers in liver diseases in the last section or Summary will be further substantiated the impact of this review on the field.

We also thank reviewer #4 for the valuable suggestions to further improve the quality of our manuscript. We have included the reviewer's suggestions in our manuscript and highlighted them accordingly.

Suggestion 1: We changed the abstract section as follow:

"*MicroRNAs (miRNAs) are small RNAs regulate gene expression by inhibiting the turnover of their target mRNAs."*

Suggestion 2: We have included a table (see Table 1) that summarizes currently reported circulating miRNAs for the diagnosis of liver diseases including the sample size and the method of analysis.

Suggestion 3: We have included the concept of "liquid biopsy" in the introduction as follow:

"With respect to the concept of "liquid biopsy" which has recently been suggested as a novel detection tool for malignant diseases [23, 24], miRNA might thus function as a potential "liquid biopsy" not only for malignant but also benign liver disease."

Suggestion 4: We have replenished the paragraph about the current limitations of miRNAs to serve as biomarkers in liver diseases in the conclusion:

"As qPCR and microarray based measurements naturally depend on the design of miRNA specific primers or microarray probes, similarities between different miRNAs might result in further difficulties regarding the comparison between studies. Moreover, data normalization issues mainly arise from the lack of a valid intrinsic RNA housekeeping gene for human serum samples and high inter-platform differences in miRNA quantification efficacy contribute to a poor comparability between studies. Finally, most studies are carried out as

single center study including only a small number of patients. Therefore, next generation sequencing might have an important impact on the validation of miRNA profiles, as it allows mostly sequence independent, parallel measurement and detection of overall numbers of a broad spectrum of different miRNAs (reviewed e.g. in [79]).”