

Dear Jin-Xin Kong and all reviewers,

On behalf of myself and my co-authors, I would like to thank you for this opportunity to revise our manuscript entitled: Drug-induced Liver Injury: Do we know everything? ESPS manuscript number 29702.

We have taken all of the reviewer comments and suggestions into account, and have made the necessary changes to our manuscript, which are highlighted. Please find below our responses to peer-review comments.

#here means comments of reviewers #here means our response

Reviewer: 02445121

In this review, the author provided an overview of the current evidence-based information on drug-induced liver injury. The author emphasized that drug-induced liver injury has gained a great amount of interest in the past decade, raising the question of whether we know everything. Various global registries have been established and the first guidelines for diagnosis and management have come to define the state of the art. The identification of risk factors and predictors of injury, novel mechanisms of injury, refined causality assessment tools, and targeted treatment options have amplified our understanding of the impact of drug-induced liver injury; however gaps in our knowledge still remain. This review is described in detail, which, as valuable information, could help the readers that have better understand the first-hand knowledge of this topic to start novel studies.

Thank you for your positive review, and encouraging comments.

Reviewer: 03660337

A great job

Thank you for taking the time to review our manuscript, and for your positive review.

Reviewer: 00503536

The review written by Alempijevic et al. describes current understanding on drug-induced liver injury. The manuscript tries to achieve comprehensive review, but overall description is superficial and some important issues are lacking. Major points, 1. The description of possible mechanisms responsible for DILI is missing. 2.

In the diagnosis of DILI, differential diagnosis with autoimmune hepatitis is important and sometimes difficult. The role of liver biopsy for the differentiation should be mentioned. 3. The readers cannot understand the practical strategy for the diagnosis of DILI from this review. 4. In the treatment of DILI, management for DILI with hepatocellular type is missing.

Thank you for taking the time to carefully review our manuscript.

1) Thank you for your comment. Prior to revision, we already mentioned under the section “defining, recognizing and predicting dili”, a comment on the mechanism of acetaminophen injury. We have now added a small paragraph outlining the mechanisms involved in idiosyncratic DILI, however, a detailed description of the many different mechanisms involved is outside of the scope of our review. Please find the revised content as: “The mechanisms of idiosyncratic DILI on the other hand, have a far more complex nature and are the focus of the majority of current research. Broadly speaking they may be divided into two categories, hypersensitivity-type reactions (also known as immunologic), and metabolic types of injuries[10]. Hypersensitivity-type reactions, occurring due to reactive metabolites covalently binding proteins, forming drug-protein adducts, and thus triggering immune-mediated reactions or direct hepatic toxicity[12], account for 23%-37% of all idiosyncratic DILI cases[10]. In addition, lipophilicity combined with dose, also known as the “rule-of-two”[27,28], is known to enhance the risk of developing DILI, due to increased blood uptake into hepatocytes, forming greater amounts of reactive metabolites and subsequently interacting with hepatocanalicular transport and mitochondrial membranes[12]. As such the other identified mechanisms include oxidative stress, mitochondrial liability and inhibition of hepatobiliary transporters[12]. In the case of INH induced DILI, hepatocellular injury may result from the creation of covalent drug-protein adducts, leading to hapten formation and an immune response, and/or through direct mitochondrial injury by INH or its metabolites, leading to mitochondrial oxidant stress and energy homeostasis impairment[54]. If such mitochondrial deficiencies are already present, even non-toxic concentrations of INH, may trigger marked hepatocellular injury, due to underlying impairment of complex I function[54]. Other examples of mitochondrial injury include: impaired beta-oxidation, and mitochondrial respiration, membrane disruption and mtDNA damage, usually caused by tamoxifen, valproic acid, diclofenac and tacrine, respectively[12].”

2) This is a very valid point, and we thank you your comment. We have addressed this in the revised manuscript. Please find the added descriptions, highlighted under the section “defining, recognizing and predicting dili “,: “With this in mind, according to the first guidelines for DILI diagnosis and management^[69], liver biopsy is integral in differentiating drug-induced autoimmune hepatitis (DI-AIH) from idiopathic autoimmune hepatitis (AIH) (Table 1). Histopathological evidence of portal neutrophils, and intracellular cholestasis, favours the diagnosis of DI-AIH over AIH ^[7,69], and therefore one may employ biopsy in such cases.

Furthermore, Table 1 also mentioned the use of liver biopsy in such cases in the original manuscript.

3) The practical strategy for diagnosing DILI is multifaceted, with causality assessment being central to reaching a correct diagnosis. It remains a diagnosis of exclusion, and as such, a clear diagnostic algorithm cannot be easily employed. In our review, we have, however, discussed the difficulty in diagnosing DILI, and Tables 1 and 5 are of particular use to the readers. The fact remains, that once other identifiable causes of liver injury have been excluded, the diagnosis of DILI is likely, if drugs received by the patient are known hepatotoxins. Therefore, the key to reaching a diagnosis is recognizing the clinical picture of DILI, which we summarized in Table 4. For a comprehensive practical strategy to DILI diagnosis, please refer to:

Chalasani NP, Hayashi PH, Bonkovsky HL, Navarro VJ, Lee WM, Fontana RJ; Practice Parameters Committee of the American College of Gastroenterology. ACG clinical guideline: the diagnosis and management of idiosyncratic drug induced liver injury. *Am J Gastroenterol* 2014; **109**: 950-966 [PMID: 24935270 DOI: 10.1038/ajg.2014.131]

4) Thank you for your comment. Currently the most effective treatment for DILI is removal of the offending drug. Indeed, for cholestatic DILI ursodesoxycholic acid and steroids have shown to be so what successful, however, in the case of hepatocellular DILI, no targeted treatment option currently exists, and therefore we outlined the various treatment options for this type of DILI, ranging from NAC to bioartificial liver assist devices such as molecular absorbant recirculating systems. We hope this clarifies your comment.

Reviewer: 01490291

the authors have done an excellent job on 'argument hepatic drug toxicity' only suggest to add in the "defining, recognising and .." section a comment and a mention of the possible presence of a HEV infection.

Thank you for your positive review.

We have added a comment regarding the possible presence of HEV infection. Please find it in the revised manuscript under the section “defining, recognising and predicting dili”: “With our growing clinical expertise, newly identified viral causes, including hepatitis E virus (HEV), have made clear recognition even more arduous[7]. Mimicry by HEV should therefore be on the clinician’s mind when forming a differential diagnosis of DILI[7,60].”

Reviewer: 00037846

The authors reviewed the current state of knowledge regarding drug-induced liver injury with focus on idiosyncratic liver injury. Overall, the review is well written and the content is very informative and useful for the readers of WJH. A few minor issues need to be addressed: 1. P.10: The authors refer to various enzyme activities as absolute numbers, which is not very informative. The enzyme activities should be given as IU/mg protein (if referred to liver enzyme activities) or IU/L (if plasma levels are mentioned). 2. P.15: Conclusions: The phrase in the second sentence "..., we know how they cause damage,..." is only correct for acetaminophen hepatotoxicity but not for idiosyncratic DILI, which is mostly discussed in this review. This should be rephrased.

Thank you for your positive review, and relevant comments.

1. As plasma levels were in question, we added IU/L, thank you for pointing this out.
2. As another reviewer mentioned the need to discuss mechanisms of idiosyncratic DILI, we have added this in the section "defining, recognizing and predicting dili", and have also slightly rephrased our conclusion phrase. Please find the highlighted changes in our revised conclusion as: "We have an extensive amount of knowledge about which drugs are responsible and how to detect them, our understanding of the various mechanisms involved is constantly expanding, and we are identifying which patients are most at risk, however our knowledge is far from complete."

Thank you all once again for this opportunity and we look forward to your final decision,

Kind Regards,

Prof. Dr Tamara Alempjevic, MD, PhD

University of Belgrade, School of Medicine, Clinical Center of Serbia, Clinic for Gastroenterology and Hepatology, 2 Dr Koste Todorovica Street, 11000 Belgrade, Serbia.

Email: tamara.alempjevic@med.bg.ac.rs

Telephone: +381113628582

Fax: +381113628582