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CLINICAL STUDY PROTOCOL

Project title: Analysis of liver injury risk factors in a multiethnic population treated with antituberculosis drugs

Protocol code: TUBILI

Study Drug: Isoniazid

Version: 1.0 / 26.01.2024

Principal Investigator: Dott. Alessandro Torre

Statement Of Principal Investigator

I have read this protocol and I agree to conduct this clinical study in accordance with all stipulations of the protocol and in accordance with the current regulations.

Principal Investigator	Signature	Date
Alessandro Torre		19/02/2024

Summary

List of abbreviations

5-OH-PA	5-Hydroxy Pyrazinoic Acid
5-OH-PZA	5-Hydroxy-Pyrazinamide
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATDH	Anti-Tuberculosis Drug-Induced Hepatotoxicity
BIL	Bilirubin
BMI	Body Mass Index
COVID-19	Corona Virus Disease 19
CYP2E1	Cytochrome P450 2E1
CYP3A	Cytochrome P450 3A
CYP3A4	Cytochrome P450 3A4
CYP450	Cytochrome P450
DILI	Drug-Induced Liver Injury
DNA	Deoxyribonucleic Acid
eCFR	electronic Case Report Form
EMB	Ethambutol
FDA	Food and Drug Administration
GDPR	General Data Protection Regulation
GWAS	Genome Wide Association Study
HAART	Highly Active Anti-Retroviral Therapy
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
INH	Isoniazid
NAD	Nicotinammide adenina dinucleotide
NAT2	N-acetyltransferase 2
OR	Odds Ratio
PA	Pyrazinoic Acid

PI	Principal investigator
PK	Pharmacokinetic
PXR	Pregnane X Receptor
PZA	Pyrazinamide
RMP	Rifampicin
STR	Streptomycin
TB	Tuberculosis
TBI	Tuberculosis Infection
ULN	Upper Limit of Normal range
WHO	World Health Organization
XO	Xanthine Oxidase

Abstract

Tuberculosis is the world's second leading cause of death from a single infectious agent in 2022, after COVID-19 [1]. In 2022, TB disease was estimated to affect 10.6 million people, from which 1.3 million dead because of it, despite the WHO adoption of the End TB Strategy [1] [2]. The standardised therapy involves a treatment regimen of INH, RMP, PZA, EMB, for 2 months, then INH and RMP for at least an additional 4-7 months [3]. Even though 85% of TB cases are successfully treated, significant morbidity from treatment-related adverse events, such as hepatotoxicity, skin reactions, gastrointestinal, and neurological disorders, reduces the efficacy of therapy [4] [5] [6]. In 11% of patients receiving INH, RMP, and PZA in combination, hepatotoxicity is the most frequent adverse effect that results in drug discontinuation [5] [7] [8]. One of the most prevalent subtypes of idiosyncratic hepatotoxicity is caused by anti-TB drugs [4] [9] [10]. Numerous variables, including the features of the specific cohort, the drug regimens used, the employed threshold to define hepatotoxicity, and the monitoring and reporting procedures, influence the incidence of anti-TB drug-induced hepatotoxicity. Overall, 5%–28% of patients receiving anti-TB drugs have experienced hepatotoxicity associated with the drug [4] [10]. It is difficult to determine how many of these fit the recent international consensus definition of DILI [9]. The majority of reports have defined hepatotoxicity as an elevated level of AST or ALT that is three times the ULN when symptoms (such as abdominal pain, nausea, vomiting, unexplained fatigue, or jaundice) are present, or five times the ULN when symptoms are absent [5]. Up to 20% of patients receiving INH as monotherapy or combination therapy for TB may experience a transient, asymptomatic elevation in liver enzymes; however, it usually resolves without drug discontinuation [11] [12] [13]. ATDH may manifest as asymptomatic elevations in liver enzymes or even as fulminant liver failure [5] [14]. In accordance to these results, the most commonly observed DILI pattern is the hepatocellular one [15]. Anti-TB drugs related hepatotoxicity is of great concern not only because of its frequency but also because of its severity and outcome. The median interval between starting a course of anti-TB treatment and the onset of clinical symptoms is 16 weeks (range 6 weeks-6 months) [16] [17] [18]. Despite decades of use and a large number of patients exposed to anti-TB drugs worldwide, the pathogenesis underlying

hepatotoxicity remains poorly understood. Further research investigating drug-related, host genetic, and environmental factors associated with susceptibility to hepatotoxicity, as well as additional studies searching for potential mechanisms leading to DILI, may be able to help clinicians develop strategies that reduce the incidence of hepatotoxicity and other adverse effects.

Risk factors associated with hepatotoxicity

Factors Associated with Drug

Since most patients receive a combination of medications during their anti-TB therapy, it can be difficult to estimate the incidence of hepatotoxicity caused by individual agents. INH, RMP, and PZA are known to cause hepatotoxicity, whereas EMB is not thought to be hepatotoxic. Low risk of DILI has been shown by studies where RMP has been used alone in the treatment of TB infection [19] [20]. The most commonly reported drug associated with liver toxicity is INH [21]. According to four large population-based observational studies, the incidence of INH hepatotoxicity ranges from 0.1% to 0.56% when used as monotherapy to treat TB infections [16] [22]. A review based on FDA data estimated that 23.2 deaths per 100,000 patients receiving prophylactic therapy based on INH have occurred [23]. In a meta-analysis, INH was more likely to be associated with hepatotoxicity (odds ratio, OR 1.6), even in the absence of RMP [24]. However, when compared to each drug alone, the combination of these two was associated to a higher rate of hepatotoxicity (OR 2.6) [24]. The risk of hepatotoxicity has not been demonstrated to be higher for daily dosing regimens than for regimens that are administered three times a week [25].

Drug Biotransformation, Detoxification, and Elimination

Reactive metabolites formation has been associated with several types of clinical toxicities, including some of those classified as idiosyncratic DILI [26]. In general, reactive metabolites are electrophiles, and, to escape detoxification, they interact with nucleophilic groups located on cellular proteins, especially at the level of lysine and cysteine [27]. Immune-mediated injury can also result from the production of reactive metabolites following covalent protein binding [28]. Cellular proteins with

covalent modifications should be degraded or repaired: failure of these processes results in the manifestation of target organ injury due to drug-metabolite adduct formation that compromises important cellular functions. An elevated concentration or an enhanced activity of the enzymes catalysing the biotransformation of a drug into a reactive metabolite may increase the level of reactive metabolites formation in an individual. Generally, these are phase I CYP450 enzymes involved in oxidation, reduction, or hydrolysis. Another reason of drug toxicity may be ascribed to low concentrations or reduced activity of those enzymes required to detoxify reactive metabolites. Generally, detoxification occurs through the processes of acetylation, sulfation, glucuronidation, or glutathione conjugation, which are mediated by phase II enzymes. Water soluble metabolites are excreted into bile or in the systemic circulation with the contribution of transporter molecules or proteins, which mediate phase III of drug disposition. Since the majority of first-line anti-TB drugs are lipophilic, they need to be biotransformed into water soluble compounds before being eliminated. Rather than being the direct result of the parent drug's action, hepatotoxicity seems to be related to the production and accumulation of reactive metabolites [29] [30].

Isoniazid

INH is primarily metabolised in the liver. NAT2 and the microsomal enzyme CYP2E1 are the principal enzymes involved in INH metabolic pathway and therefore they are crucial in determining the greatest risk of hepatotoxicity. NAT2 converts INH to acetyl isoniazid, followed by hydrolysis to acetyl hydrazine. This latter could be oxidised by CYP2E1 to produce N-hydroxy acetyl hydrazine, which then undergoes additional dehydration to produce acetyl diazine. Acetyl diazine can be a toxic metabolite in and of itself, or it may breakdown into reactive acetyl radicals, acetyl onium ions, and ketene, which, in turn, can covalently bind to hepatic macromolecules and cause injury to the liver. Additionally, acetyl hydrazine is further acetylated by NAT2 to produce nontoxic diacetyl hydrazine [31] [32]. Thus, mono acetyl hydrazine and the parent compound accumulate as a consequence of slow acetylation [33]. INH itself further inhibits the acetylation of acetyl hydrazine. Furthermore, the production of hydrazine through direct hydrolysis of INH without acetylation increases the risk of liver injury [34], as observed in slow acetylators which show ten-fold increased INH metabolism through

this pathway, especially when combined with rifampicin [35]. The human hepatic NAT2 enzyme exhibits a highly polymorphic nature. One or more wild-type, referred to as NAT2*4, alleles are associated with rapid acetylator status, whereas slow acetylators are characterised by the presence of any two of several variant alleles [36]. By *in vitro* experiments, it has been observed that acetylation activity gradually decreases according to the presence of a specific allelic variant, in the following order: NAT2*4 > NAT2*7 > NAT2*6 > NAT2*5 [37]. Genetic polymorphisms in metabolism account for 88% of the drug's PK variability, with a linear relationship between isoniazid clearance and number of high-activity NAT2 alleles [38] [39]. Due to the reduced metabolism, slow acetylators show higher plasma levels and lower drug clearance than rapid acetylators [40] [41] [42]. In several studies [43] [44] [45] [46] [47], NAT2 slow acetylator profiles (or two slow NAT2 alleles) have been associated with an increased risk of hepatotoxicity/liver injury/hepatitis caused by anti-TB drug treatment when compared to rapid acetylators. Due to the existence of common loss of function polymorphisms in NAT2 and the important contribution of acetylation to INH metabolism, there have been a large number of studies examining these variants as INH-induced hepatotoxicity risk factors in tuberculosis patients [48] [49] [50]. With an overall OR ranging from 1.59 to 6.42 and, although the risk varied depending on the specific genotypic definition of slow acetylation and the population under study, at least four meta-analyses, including a significant number of cases, have concluded that slow acetylators have an increased risk of DILI [46] [49] [51] [52] [53] [54]. Furthermore, slow acetylators were more likely to experience severe hepatotoxicity, with respect to rapid acetylators. In particular, compared to other genotypes, NAT2*6/6 and NAT2*6/7 variants are associated to reduced enzymatic activity and significantly higher risk of liver injury. In accordance with many candidate gene studies in several ethnic groups, a recent GWAS identified NAT2 as the most important risk factor for ATDH in the Thai population [55]. Based on available data, it appears that reactive metabolites are the cause of INH hepatotoxicity. Several studies on the mechanism of INH hepatotoxicity have highlighted the role of immune-mediated idiosyncrasy, described as the mechanism of the liver's adaptive responses to INH, and the heterogeneity of the clinical picture of INH hepatotoxicity [56] [57].

Rifampicin

After oral administration on an empty stomach, RMP is rapidly and completely absorbed. In the liver, RMP is metabolised into desacetyl rifampicin by desacetylation [58] [59], and a separate pathway of hydrolysis produces 3-formyl rifampicin [59] [60]. The metabolic derivative, desacetylrifampicin, is more polar than the parent compound, and microbiologically active. This metabolite is indeed responsible for the majority of the antibacterial activity in the bile. RMP is almost equally excreted in bile and urine, with recovery rates on the same order of magnitude. All RMP metabolites are non-toxic, however, RMP is considered able to induce hepatocellular patterns of DILI and to increase toxicity of other anti-TB drugs, like INH [58] [59] [61]. RMP is known as the activator of PXR, a ligand-activated transcription factor that plays a crucial role in the metabolism of xenobiotics and endobiotics in mammals [58] [62]. Indeed, PXR regulates the expression of drug-metabolising enzymes and transporters, as well as genes involved in endobiotic metabolism, by binding to its DNA response elements. In particular, PXR positively influences the expression of the major Phase I enzymes, CYP450s. Via PXR, RMP induces several metabolic enzyme pathways, particularly the CYP3A4 system [63] [64]. CYP3A4 activation produces, indeed, an increased metabolism of INH, resulting in toxic metabolites, therefore explaining the anti-TB drug induced hepatotoxicity of RMP. When used in combination with INH, RMP also induces INH hydrolysis, leading to increased hydrazine production, and increasing its toxicity, especially for slow acetylators [35].

Pyrazinamide

PZA is an analogue of nicotinamide, which is a vitamin B3 (nicotinic acid, also called niacin) precursor. PZA is mainly metabolised in the liver by amidase, which converts PZA to PA [65]. XO further oxidises PA to 5-OH-PA [66] [67]. Alternatively, PZA can be hydrolysed by amidase to form 5-OH-PA, after first being oxidised by XO to 5-OH-PZA. Moreover, trace amounts of pyrazinuric acid can be produced when PA and glycine conjugate. Kidney excretion is the primary route of elimination for PZA and its metabolites [68] [69]. PZA has a longer half-life than both INH and RMP; moreover, it has been observed that PZA half-life increases, when taken with other drugs that inhibit XO, such as allopurinol, and in presence of an underlying liver disease [68] [69]. The extent of PZA

hepatotoxicity is associated with its hepatic metabolism, suggesting a direct toxic effect rather than an immunological or hypersensitive response [5] [69] [70]. PZA hepatotoxicity is also dose-dependent, particularly at daily doses exceeding 40 mg/kg. In murine models, PZA inhibited CYP450 activity [71] and changed NAD levels [72], leading to free radical species-mediated hepatotoxicity.

Fluoroquinolones

According to current WHO guidelines, fluoroquinolones are the most valuable second line anti-TB agents in case of multi-drug resistant TB and hepatotoxicity from first line agents [73]. Quinolones are either excreted unaltered by the kidneys (for example, levofloxacin) or metabolised in the liver (such as ciprofloxacin). Except for trovafloxacin, which is currently withdrawn, fluoroquinolone-induced hepatotoxicity is extremely rare and can only be identified by extensive research or international pharmacovigilance reporting [74]. There have been isolated reports of ciprofloxacin, levofloxacin, and gatifloxacin-related severe hepatotoxicity [75] [76], and hypersensitivity reactions, which are frequently associated to peripheral eosinophilia and fever, are assumed to be the cause of hepatotoxicity [77]. When fluoroquinolones were administered to patients with underlying chronic liver disease whose hepatitis was brought on by first-line anti-TB drugs, there was no further hepatotoxicity [78]. It has been also observed that ofloxacin is safe and effective when used in patients who already have liver diseases [79].

Factors Associated with Hosts

Several risk factors for the development of hepatotoxicity have been suggested from a multitude of studies, but robust conclusion cannot be drawn due to significant variations in study design, cohort size and case definition.

Age

A correlation between age and a higher risk of DILI has been traced. Age over 60 has been associated to a 3.5-fold increased risk of DILI in a study involving 519 patients receiving standard anti-TB drugs [80]. In another study with 430 patients, the incidence of pyrazinamide-related adverse events, such as DILI, was 2.6 times greater in over 60 individuals [78]. Furthermore, among a cohort

of more than 3000 patients receiving INH monotherapy, those with 50 years of age or older showed a higher frequency of DILI [16]. There have also been reports of increased mortality rates associated with INH hepatotoxicity beyond the age of 50 [23] [29] [81]. Patients who experienced DILI with anti-TB drugs were found to be older (39 years) in a case-control study than those who did not (32 years) [82]. A multivariate analysis revealed that the only independent variable that could predict anti-TB DILI in over 35 patients was their age, with a rate of 17% respecting a rate of 33% in patients over 35 [83]. Moreover, advancing age is characterised by pharmacokinetic changes, including a reduction in renal and hepatic clearance and an increase in volume of distribution of lipid soluble drugs [84]. All these changes lead to a prolongation of plasma elimination half-life. In contrast, an older meta-analysis [24] found that children receiving INH and rifampicin had a higher incidence of clinical hepatitis (6.9%) than adults (2.7%). The inclusion of three small studies, each with 22–60 patients, however, significantly contributed to the high frequency of DILI in children by reporting a high frequency of "clinical hepatitis" in 25–52% of all patients.

Gender

Anti-TB therapy has been shown to increase the risk of DILI in women [78] [85] [86], with a reported 4-fold increase [87]. Females are indeed more susceptible to hepatotoxicity due to higher CYP3A activity [88]. Moreover, it has been observed that in the third trimester of pregnancy and in the first three months after giving birth, there is a trend towards an increased risk of INH hepatotoxicity [89].

Status of Nutrition

Malnutrition is common in TB patients and has been related to an increased risk of anti-TB drugs hepatotoxicity [90]. According to a recent retrospective observational study [91], a highly significant independent risk factor for DILI is a weight loss of 2 kg or more occurring during the first 4 weeks of TB treatment. Given that fasting and malnutrition have an impact on cytochrome P450 enzyme system, TB drug detoxification is ensured by receiving adequate amounts of nutrients, therefore preserving liver metabolism integrity [92].

Alcohol Intake

Liver injury may be caused by both the direct toxicity of alcohol metabolic by-products and the inflammation induced by the by-products themselves. Numerous studies showed that alcohol extended the hepatotoxicity caused by anti-TB drugs [93] [94] [95]. Even patients receiving rifampicin as a preventative treatment have been shown to be at risk [96] [97].

Concomitant Infection

It has been suggested that mild inflammatory reactions caused by co-existing viral infections can frequently function as a "danger signal", allowing the development of early events in the pathophysiological process into a full-blown hepatotoxic reaction; this can be explained by the increased level of drug hypersensitivity in these patients [98]. Several cohorts have been examined to determine whether chronic infections other than tuberculosis raise DILI risk during anti-TB therapy. The risks of drug- and virus-induced hepatitis were significantly higher in patients with high initial viral load than those with low initial viral load and those without viral hepatitis (39% vs 10.5% of HBV patients and 33% vs 16.7% of HCV patients, $p<0.001$) [99]. This study has also demonstrated a direct correlation between the viral load at the onset of anti-TB therapy and the severity of hepatotoxicity [99]. Comparable to HBV infection, 30% of all HCV-infected patients receiving anti-TB medication developed hepatotoxicity, whereas 11% of controls had a 5-fold relative risk of hepatotoxicity development and severity directly related to the viral load [100]. Additionally, there is a significant increased risk of hepatotoxicity from anti-TB drugs when HIV infection coexists. This has been observed both prior to and during the highly active HAART era. Hepatotoxicity ranged from 4 to 15% in the pre-HAART period and from 4 to 27% in the HAART era [101]. Nevertheless, a number of confounding factors have been identified, including the use of intravenous drugs, alcoholism, viral hepatitis, hepatotoxicity due to HAART therapy, drug–drug interactions, and liver damage caused by immune reconstitution [101].

Rationale and Aims

Patient's susceptibility to DILI during treatment with anti-TB drugs is influenced by several drug- and host-related factors [102]. The most extensively studied potential causes of INH-induced DILI are related to those mechanisms. As to drug-related factors, acetylhydrazine, which is generated by NAT2, is widely considered as a crucial INH metabolite that contributes to INH-induced DILI. Given the importance of acetylation in INH metabolism, several studies have investigated into NAT2 loss of function polymorphisms as possible risk factors for DILI. In addition, advanced age [94], female gender [86], poor nutritional status [103], HBV or HCV infection [100] [104], HIV infection [78] [105], chronic liver disease, and alcoholism are environmental, physiological, and pathological factors contributing to DILI incidence. Moreover, the influencing variables differ according to racial and geographic characteristics.

Studies conducted in China demonstrated that approximately one-third of DILI patients had no symptoms, including some cases with severe liver injury [106] [107]. It has been demonstrated that screening patients for risk factors is useful in preventing disease progression, improving the outcome of therapy, and reducing mortality. However, rapid acetylators are very common in the Chinese population, so they are characterised by a lower susceptibility to develop DILI. Our patient cohort is multi-ethnic, with a prevalence of Caucasians and so a higher frequency of slow acetylator status. For this reason, our objective is to corroborate the conclusions drawn by the previous studies through the examination of our complementary data.

Outcome measures and assessments

The primary outcome is to determine the association between risk factors (drug- and host-related factors) and the incidence of DILI.

The primary outcome measures are the occurrence of anti-TB DILI (number of participants with DILI), which was defined by: 1) AST or ALT level > 5 times the ULN in patients with absence of symptoms or with total BIL level > 2 times the ULN, or 2) AST or ALT level > 3 time the ULN and total BIL level > 3 times the ULN in patients who show symptoms compatible with hepatitis.

The secondary outcome is to evaluate the characteristics of patients who first experienced anti-TB DILI. The secondary outcome measures are the prevalence of early hepatotoxicity experience.

Methods

Study design

This is a retrospective observational study, no profit, to determine environmental, physiological, and pathological factors associated with the incidence of DILI during anti-TB treatment. Data collected from patients with confirmed tuberculosis, enrolled at the Department of Infectious Diseases of Luigi Sacco Hospital in Milan, Italy, between July 2020 and September 2023, will be analysed. Subjects were all followed up as outpatients at Tuberculosis Clinic at Luigi Sacco Hospital, some of them with previous inward stays at various hospitals in the Lombardy Region.

Study population

The following are the inclusion criteria: 1) adult patients (>18 years), 2) patient who received standard initial therapy including INH (5mg/kg), RMP (10mg/kg), and PZA (25mg/kg) for patients with active TB disease, 3) treatment with first line anti-TB drugs, including rifampicin and isoniazid for patients with latent TBI, 4) normal serum ALT and bilirubin levels, no symptoms related to abnormal liver function prior to anti-TB drug treatment and 5) informed consent. Patients with any of the following conditions are excluded from the study: 1) liver dysfunction, including biliary origin, before anti-TB therapy, 2) patients receiving non-standard treatment regimen initially (e.g., patients with severe pulmonary or extrapulmonary TB receiving large doses or more than four anti-TB drugs), 3) modified treatment regimen due to drug resistance or intolerance excluding first line anti-TB drugs, 4) lactation or pregnancy, 5) concomitant use of hepatotoxic drugs, 6) abnormal hepatic function on laboratory testing before anti-TB, 7) disease that was resistant to INH at the start of treatment or 8) patients refusing to sign informed consent.

The participants were given standard anti-TB treatment in line with international guidelines (RMP 10 mg/kg, INH 5 mg/kg), primarily administered orally, although intravenous administration was

employed for inpatients as needed, and modified to oral administration as soon as possible. The other drugs of the standard regimen included standard daily doses of PZA (15-30 mg/kg) and EMB (15-20 mg/kg).

Data collection

Data that will be collected include clinical, biochemical, and genotyping findings that are typically used in clinical practice to manage and monitor tuberculotic patients at Luigi Sacco Hospital. The complete list of parameters (host- and drug-related factors) is provided below:

- Clinical data: age, weight, height, gender, ethnicity, hepatotoxicity or alcoholism, duration of anti-TB treatment, tuberculosis site (pulmonary or extra), adverse event, concomitant infection (HIV, HAV, HCV, HBV), past medical history, concomitant drugs. Patient`s nutritional status was reported as BMI, which was calculated based on height and body weight;
- Biochemical data: ALT, AST, GGT and BIL;
- Laboratory data: NAT2 acetylator status. The acetylation status of patients will be assigned like as suggested by earlier research:
  - rapid acetylator: NAT2*4 is a wild-type allele (defined by absence of slow alleles);
  - intermediate acetylator status: carry one copy of NAT2*4 and one defective variant allele (NAT2*5, *6, *7, and *14);
  - slow acetylator: two copies of slow variant allele (NAT2*5, *6, *7, and *14).

Statistical analysis

Sample size calculation

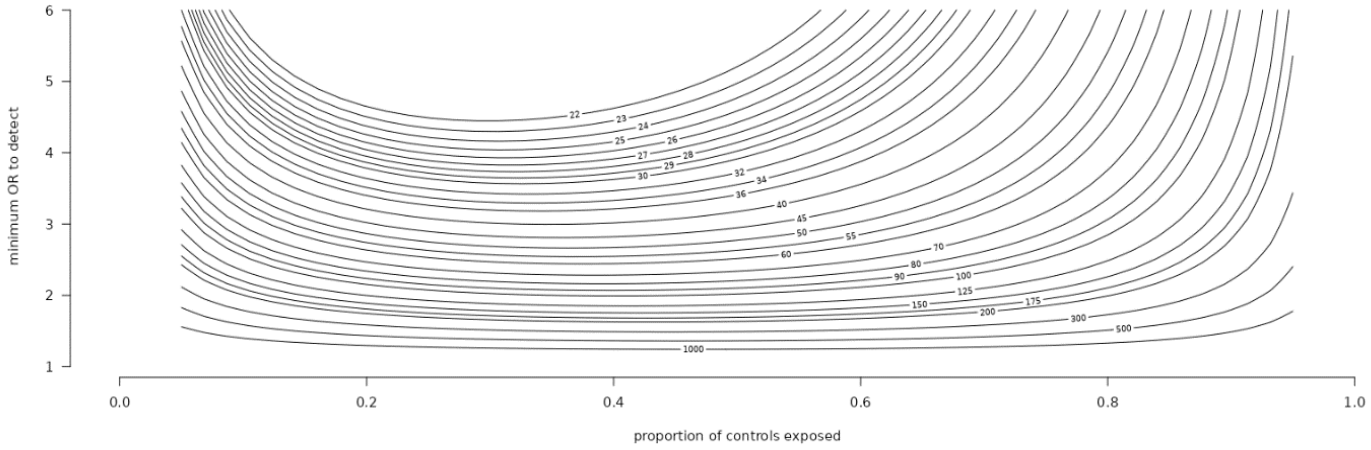
We are planning a study of matched sets of cases and controls with 2 matched controls per case. Prior data indicate that the probability of exposure among controls is 50%, and the correlation coefficient for exposure between matched cases and controls is -0.2 [10] [108] [109] [110] [111]. If the true odds ratio for disease in exposed subjects relative to unexposed subjects is 3, we will need to study 40 case patients with 80 matched controls to be able to reject the null hypothesis that this

odds ratio equals 1 with a power of 80%, assuming that the Type I error probability associated with the test of this null hypothesis is 5%.

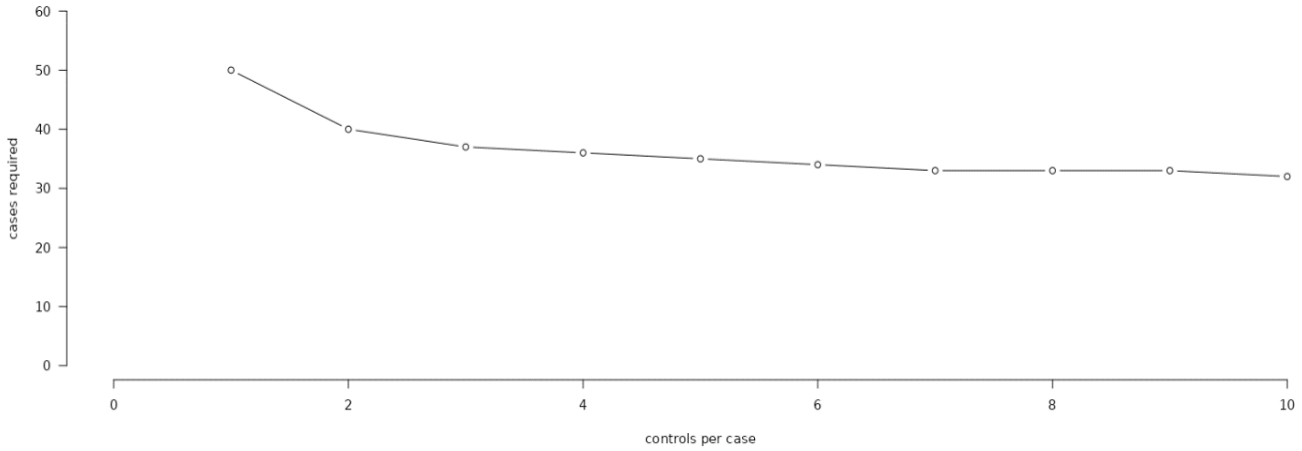
Parameters: Exposure in cases = 75%; Exposure in controls = 50%

Absolute difference = 25%; Relative difference = 50%; Odds ratio = 3

Sample sizes across range of OR and control exposure, with inputted values:



Number of cases required varying ratio of controls per case, other inputs constant:



We will use a nested case-control study design that takes into account time of events occurrence in sampling of controls. For each case, one or more controls are selected from the risk set at the time in which the event of interest occurred in the case. The risk set means the set of all individuals that are still “events-free” and not censored. Therefore, they are still included in the study at that time, thus, eligible to experience the event now observed in the case. As a consequence, individuals can

be sampled as controls for more than one case and, in turn, individuals sampled as controls may subsequently become cases. Cases and controls will be matched according to baseline variables, including age, sex, and BMI, and then conditional regression analysis (a type of matched statistical analysis) will be performed to draw associations.

Data management

All data are retrieved from the patient's medical record. Variables will be entered into the eCRF by site staff, who will be trained on how to use the eCRFs and capture the data required by the protocol. The eCRF is an electronic data entry media and should not constitute the original (or source) medical record. Data derived from source documents and reported in the eCRFs should be consistent with the source documents, or the discrepancies should be explained in these source documents. The investigator must complete the eCRF for each participant. The investigator’s signature on the eCRF will attest to their accuracy and completeness.

Data collected from each participant will be anonymized: each subject will be assigned a unique code so that only the investigator will be able to identify the subject. The file associating the participant’s code with their identifying data will be stored separately on a password protected computer. The study database will be password protected and uploaded to a computer that is also password protected and accessible only to study personnel designated by PI. Identification of the data will be done in such a way that individuals accessing the database will not be able to trace the identity of the subjects in any way.

The study will involve statistical calculations on collected data using literature standard methods to identify and correlate variables (Chi-square, Fisher's exact, Mann-Whitney U-test, conditional regression). The results will be published in medical journals and presented at conferences. Study data will be retained for 7 years starting from the study's conclusion. Data processing will be done in strict compliance with GDPR and any applicable national legislation.

Potential Benefit of the Study

The data collected will provide important new perspectives on the management and prevention of DILI. Preventing serious hepatic failure requires early detection and prompt drug withdrawal, which is also a crucial step in managing adverse reactions. The diagnosis of DILI is based on the index of suspicion, the careful assessment of the temporal correlation between the exposure to a specific drug and the particular clinical event, and the exclusion of other possible diagnoses.

For patients undergoing anti-TB treatment, hepatotoxic side effects represent significant obstacles. Indeed, DILI development can compromise treatment compliance, or lead to treatment interruption, or, again, result in disease relapse, or induce drug resistance; all these conditions will finally lead to treatment failure. Screening patients for risk factors is of great importance in preventing disease progression, improving therapeutic outcomes, and reducing mortality.

Furthermore, patient's re-exposure to the same drug regimen leads to DILI recurrence in 11%–30% of cases [112]. Compared with the initial event, DILI after rechallenge occurs more rapidly (generally after days or weeks) than after initial exposure [113]. DILI on rechallenge leads to jaundice in 64%, hospitalisation in 52%, and mortality in 13% of cases [114].

Combining host- and drug-related risk factors associated with the development of DILI might generate better tools to pre-empt or diagnose DILI, leading to better regimen tolerability and ultimately to a better treatment outcome.

Study Management & Coordination

PI with support from the working group's different specialists, is responsible for coordinating and accurately executing the study in compliance with the procedures outlined in this protocol. All study-related documentation (including protocol, amendments, eCRF, database, partial and final reports) must be archived by PI. Each component of documentation will be preserved electronically.

Study Timeline

The study will start after obtaining approval from the Ethical Committee. Consequently, the duration will be computed for a total of three months, starting from the approval of this study protocol.

Ethical Considerations

The responsible investigator will ensure that this study will be conducted in accordance with the protocol, following the instructions and procedures described, adhering to the principles of Good Clinical Practice E6 (R2) and in compliance with principles established by the 18th World Medical Assembly (Helsinki, 1964 and further amendments) and with the laws and regulations of the country in which the research will be carried out, whichever offers the greatest protection to the individual. The observational study and related documentation will be submitted to the relevant Ethics Committee.

The study will begin only after obtaining the required authorisations, in accordance with the internal procedures of the institution.

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