PROSPECTIVE EUROPEAN DRUG-INDUCED LIVER INJURY REGISTRY

PRO-EURO-DILI REGISTRY

PROGRESS REPORT: EASL REGISTRY RESEARCH GRANT
(year 1 April 2014-April 2015)

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PROGRESS REPORT: EASL REGISTRY RESEARCH GRANT
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Objectives

The EASL Registry Research Grant was awarded to Prof Raúl J Andrade in March 2014 to establish a Prospective European Drug-Induced Liver Injury (DILI) Registry (Pro-Euro-DILI Registry) together with Prof Guru P Aithal at the Nottingham University. The main objectives for this registry is to build up a large cohort of prospectively identified DILI cases, record detailed corresponding phenotype data and collect serial biological samples (blood, urine, stool and liver biopsy) over the course of the hepatotoxicity event. Drug-matched controls (exposed to the same medications without developing DILI) will similarly be identified, recorded, and biological samples will be collected. The Pro-Euro-DILI Registry data and sample collection will be a valuable tool for future DILI mechanistic and biomarker studies.

Resource distribution

The EASL Registry Research Grant (50 000 Euros) has been allocated to Málaga (34 834 Euros) and Nottingham (15 166 Euros) to cover expenses for setting up the Pro-Euro-DILI Registry. The Málaga quota will be used for database expenses and personnel responsible for supervising case enrollments and database entries. The Nottingham quota will be used for biobank facilities and sample shipment expenses.

Participant status

The initial research proposal included three additional collaborators, who had confirmed their interest in participating in this registry: Prof Helena Cortez-Pinto (Portugal), Prof Einar Björnsson (Iceland) and Prof John Dillon (UK). Since April 2014 several European hepatologists and clinical pharmacologists have been approached and asked to participate in the Pro-Euro-DILI Registry. Hence, the Pro-Euro-DILI Registry now also includes collaborators from Belgium (Prof Yves Horsman), France (Prof Dominique Larrey), Germany (Profs Alexander Gerbes and Christian Trautwein), Italy (Profs Antonio Gireco and Piero Almasio) and Switzerland (Prof Gerd Kullak-Ublick). Profs Felix Strickel (Switzerland) and Mia Wadelius (Sweden) have accepted to participate as consultants, but are unable to contribute with DILI cases. It is anticipated that each country will establish an internal DILI network organised by a country coordinator. This task is currently underway in Spain and the UK, with 3 hospitals in Spain (Seville: M Romero-Gomez, Barcelona: E Montane-Esteve, La Laguna: A Aldea) and 6 hospitals in the UK (Newcastle: A Daly, Brighton: S Verma, Birmingham: A Elsharkawy, Leicester: A Grant, Warwick: J Shearman, Dundee: J Dillon), in addition to the coordinating centres

in Málaga and Nottingham respectively, now having accepted to form part of the Pro-Euro-DILI Registry. More English collaborators are anticipated once the Pro-Euro-DILI project is adopted by the NIHR Clinical Research Network (process underway). Similarly, the Pro-Euro-DILI project was presented in the Spanish DILI Registry's reunion during the Spanish Society for the Study of the Liver conference (AAEH 2015) in February 2015 to encourage participation.

Achievements during the year

The first year's activities in the Pro-Euro-DILI Registry are depicted in Figure 1.

1. Study protocol

The first task undertaken in the establishment of the Pro-Euro-DILI Registry was the preparation of the study protocol, a joint effort by members from Prof Aithal and Andrade's groups completed in October 2014 (see Annex 1). In addition to ensure uniformity in DILI case identification and sample collection, the protocol will serve as underlying documentation for ethical clearance. The protocol has been sent to all the participating groups, which will have to adapt it with regards to language requirements prior to submitting it to their local Ethical Committees.

Ethical approval status in the Pro-Euro-DILI Registry

Prof RJ Andrade (Málaga, Spain): Approved (November 2014)
Prof H Cortez-Pinto (Lisbon, Portugal): Approved (April 2015)
Prof GP Aithal (Nottingham, UK): Submitted (pending on study sponsorship clarification, which has now been approved)
Prof P Almasio (Palermo, Italy): Submitted (ethics approval expected within the next two weeks)
Prof A Grieco (Rome, Italy): Subimitted protocol to local ethics committee
All other members are currently preparing the protocol for submission

2. DILI case identifications

Due to the requirement of ethics approval prior to case identification, only Prof Andrade's group in Málaga has initiated case enrolments. Seven DILI cases have been enrolled to date with blood and urine samples collected and stored at -80°C. All potential DILI cases enrolled in the Pro-Euro-DILI Registry will be adjudicated by an adjudication committee prior to being given a confirmed DILI status. The adjudication committee has not yet performed its role, but will do so once the number of enrollments improves.

Table 1. DILI cases enrolled in the Pro-Euro-DILI Registry

Number of cases	Suspected causative agent	Type of liver injury	Hospital group
1	Amoxicillin-clavulanate	Mixed	Málaga, Spain
1	Ibuprofen / Metamizole	Hepatocellular	Málaga, Spain
1	Aceclofenac / Metamizole	Cholestatic	Málaga, Spain
1	Amoxicillin	Hepatocellular	Málaga, Spain
1	Clarithromycin	Hepatocellular	Málaga, Spain
1	Clomipramine	Hepatocellular	Málaga, Spain
1	Paracetamol	Hepatocellular	Málaga, Spain

3. Biobanking facilities

A Pro-Euro DILI biobank for sample storage has been set up within the NIHR Nottingham Digestive Diseases BRU at the Nottingham University Hospitals NHS Trust and University of Nottingham and is ready to receive samples. Shipment instructions as well as sample preparation and labelling information have been prepared and included in the study protocol (see Appendix 1).

4. Database development

An online database for DILI case recording has been developed in Málaga in collaboration with IT consultant R Hidalgo from Malaga University and Coresoft Clinic SL (http://www.coresoft.es). The database collects relevant medical data required to diagnose DILI and additional phenotypic data that could be of interest for epidemiological studies. Furthermore the database records biological sample information in a manner that will facilitate the biobank in identifying the number of samples to be included. All information required for the CIOMS/RUCAM causality assessment scale is incorporated into the data entry, which enables the database to automatically calculate a CIOMS/RUCAM score for each DILI case. The database is password protected to ensure that only participating units will have access to the data. Due to unforeseen circumstances the database development has taken longer than predicted, however it is expected that the database will be operative in two to three months. A practical database tutorial organised by Prof Andrade's group will be offered to all collaborators prior to starting case entries.

5. Meetings

The Pro-Euro-DILI Registry has met three times since April 2014 (two face-to-face meetings and one videoconference). The first face-to-face meeting took place in Boston, US the 10/11/2014 during the AASLD conference. Yves Horsmans (Belgium), Helena Cortez-Pinto (Portugal), Einar S. Björnsson (Iceland), Oren Shibolet (Israel),

Dominique Larrey (France), John Dillon (UK), Alexander Gerbes (Germany), Christian Trautwein (Germany) participated in the meeting in addition to Profs Andrade and Aithal. Issues discussed in the meeting included: overview of registry structure, case/control inclusion/exclusion criteria and ethical approval. The meeting minutes are present in Appendix 2.

A videoconference took place the 26/01/2015 with seven participants in addition to members from Profs Aithal and Andrade's groups. Other three participants could not get through into the TC. Issues discussed in this meeting included: study protocol presentation, sample collection clarifications, biobanking procedures and a potential COST grant application. The meeting minutes are present in Appendix 3.

The second face-to-face meeting took place during the EASL 2015 conference in Vienna. The participants were Profs A Gerbes (Germany), M Biolato (representing A Grieco), P Almasio y A Licata in addition to representatives from Profs Andrade y Aithal's groups. Issues discussed in this meeting included: situation of ethical approval of the study protocol in each center, clarifications of sample collections and control subject definition (Appendix 4).

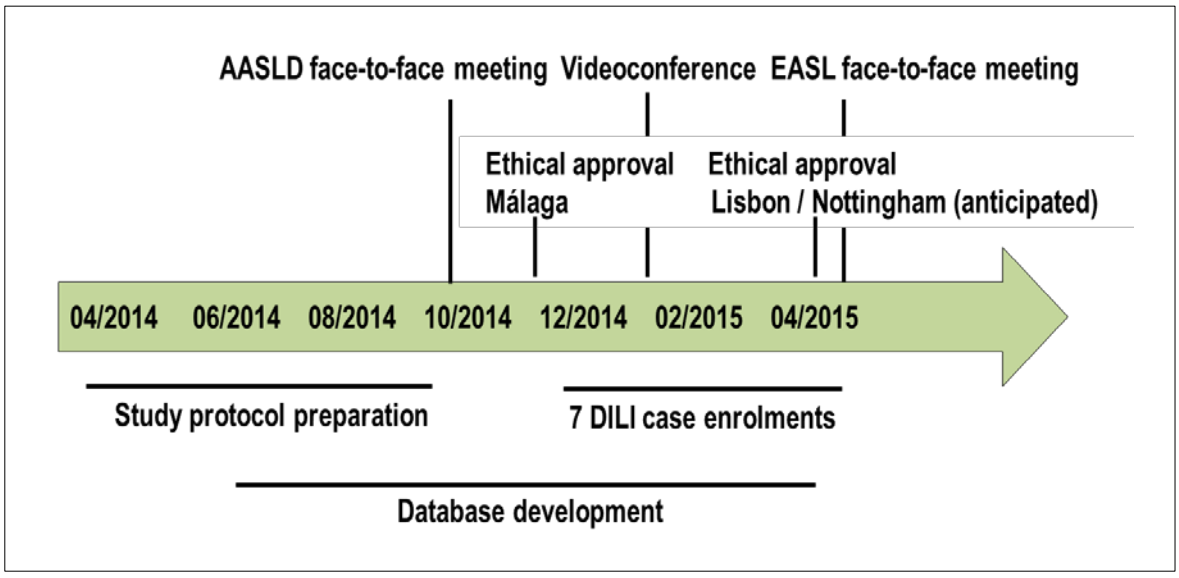

Figure 1. First year activities in the Pro-Euro-DILI Registry

6. Communications

In addition to Prof Andrade, Profs J Drenth and H Wedemeyer were also awarded EASL Registry Research Grants in April 2015. On the initiative of Prof Drenth, the three award winning Profs have prepared a manuscript discussing requirements and benefits

in forming hepatic disease registers. Hence, a manuscript titled “Creating a registry for rare hepatic disorders” is pending submission.

Action Plan 2015

The following tasks are planned to be initiated during the second year (April 2015- April 2016)

Database tutorial: A telephone conference will be organised to provide a database tutorial. It will be prepared and presented by members of Prof Andrade’s group, who have been involved in designing the online database. The tutorial will outline the use of the database and explain the required patient information. The tutorial will take place once each participating group has received a username and password information. This information will be sent to the person nominated as database entry manager at each site.

Pro-Euro-DILI Registry logo design: A Pro-Euro-DILI Registry logo contest was opened earlier this year and remains open for a few more months. The entry best representing the registry and its objectives will be selected. The chosen logo will be incorporated in all future communications including conference communications, websites etc.

Web site design: A Pro-Euro-DILI Registry website will be designed and launched. This website will include information such as the study protocol, meeting and telephone conference announcements and minutes. In addition, links to other websites that could be of interest and ‘DILI news’ will also be included.

Setting up a governance structure: To maximize the organization of Pro-Euro-DILI Registry a governance structure will be adopted including a network board (representatives from all collaborating centers) as well as an executive board (nominated representatives). An adjudication committee will also be selected. The role of this committee is to revise all the enrolled DILI cases and adjudicate them as DILI or non-DILI cases. The adjudication committee will arrange adjudication session by telephone regularly once recruitment starts among the participating centers. The adjudication committee members will vary slightly between sessions to ensure that all cases are adjudicated by independent DILI experts.

Preparation of funded research proposals: In order to expand and continue the Pro-Euro-DILI network new funding is required. Depending on the available open calls, we will take the opportunity to prepare and submit proposals to other large scale funding research programs (ERC, Horizon 2020, COST grants).

Appendix 1

Pro-Euro-DILI Registry: Creation of a multicentre and multidisciplinary European registry of prospective drug-induced liver injury cases.

Final Version 1.0

06 Oct 2014

Short title: ***Pro-Euro-DILI Registry***

Study Sponsor: **Raúl J Andrade**

Funding Source: **European Association for the Study of Liver**

STUDY PERSONNEL AND CONTACT DETAILS

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European Consortium Collaborating Centre:
NIHR Nottingham Digestive Diseases Biomedical Research Unit, E Floor, West Block, Queens Medical Centre, Nottingham, NG7 2UH, UK

European Consortium Chief Investigator/Collaborator: Professor Raul J. Andrade / Professor Guru P Aithal

Appendix 1

STUDY SYNOPSIS

Title	Pro-Euro-DILI Registry: Creation of a multicentre and multidisciplinary European registry of prospective drug-induced liver injury cases.
Short title:	Pro-Euro-DILI Registry
Chief Investigators	Professor Guruprasad P. Aithal Professor Raul J. Andrade
Study centre(s)	<u>Coordinating Centres:</u> -Nottingham Digestive Diseases Biomedical Research Unit, UK -UGC Ap Digestivo, Servicio Farmacología Clínica, Biomedical Research Institute of Málaga ,University Hospital Virgen de la Victoria, Málaga University, Málaga, Spain <u>European participating sites:</u> • Landspitali University Hospital, Iceland (national coordinator: Einar Björnsson) • University Hospital of Santa Maria, Portugal (national coordinator: Helena Cortez-Pinto) • University of Dundee, Scotland, United Kingdom (national coordinator: John Dillon) • University Hospital, Zurich, Switzerland (national coordinator: Gerd Kullak-Ublick) This list is not exhaustive and the study will allow additional national institutes to participate.
Objectives	• To set-up an international European registry of patients with idiosyncratic DILI, (iDILI) enrolled prospectively with the collation of in-depth phenotyping: including details of drug dose, duration, concomitant medications, host demography, comorbidity including insulin sensitivity as well as the course of the event. • To enrol a similarly well-characterised control group of people who are exposed to medications matched for those implicated in DILI. • To collect and store biological samples (blood, urine, stool and liver biopsy) from patients with idiosyncratic DILI through the course (at onset and follow-up) of the event.
Number of participants	We aim to recruit up to 50 participants with acute DILI and 50 similar case controls from Spain over a 2 year period.

	Europe-wide (incl.Spain) we are aiming to collect samples from 540 participants (a total of up to 3000 biological samples).
Diagnosis and main criteria for inclusion	<u>Both DILI patient group and control patient group:</u> Aged 18 years and over Able to give written informed consent or Potential participants who lack capacity to give written informed consent and have a consultee (personal or nominated) giving consent <u>DILI Patients:</u> Adults diagnosed with acute DILI (within 4 weeks of onset) and meeting the following criteria will be included: 1. Meets <u>one</u> of the following analytical thresholds at enrolment (day 0) • alanine transaminase (ALT) exceeding 5 times upper limit of normal (ULN) • alkaline phosphatase exceeding 2 times ULN • ALT exceeding 3 times ULN plus bilirubin exceeding 2 times ULN 2. Exposure to drugs including any prescription drug, over-the-counter drug, recreational drug, herbal remedies or dietary supplements prior to the DILI onset. 3. Absence of other known causes of liver injury after detailed investigations <u>Control Group:</u> For each drug found to contribute to cases of DILI, the local Investigator will access hospital pharmacy databases to identify patients taking specific drugs of interest to identify appropriate unaffected control patients. These patients exposed to particular drugs without developing DILI will be invited to participate in the study as a control group. We aim to recruit a similar number of control patients exposed to the same drugs as those with DILI recruited to the study.
Duration of study	Recruitment will be over a two year period.

	-DILI patients: 3-5* visits, (each visit lasting for up to 20mins). Each patient will be in the study for up to 6 months duration. Follow-up: Each patient will have a clinical follow up until the event resolves or 12 weeks after the first event whichever occurs later. **Exceptions -Patients presenting alterations in their liver profile on visit 3 will be scheduled to have an extra visit:- (Visit 4) scheduled for 3mths after visit 1* *-Any cases with a cholestatic type injury will be considered for an additional visit (visit 5) six months after the initial visit 1, (depending on the hepatic profile status at visit 4).* -Control participants: A single study visit of up to 30mins duration. We will seek consent to store and use samples in a research tissue bank for at least 10 years.
Description of interventions	DILI patients and Controls: Blood, urine and stool samples will be collected at each visit. -For DILI patients only; we plan to collect any surplus liver biopsy samples taken as part of clinical care (and any archived paraffin sections taken previously as part of clinical care).

Appendix 1

ABBREVIATIONS:

AE: adverse event
ALT: alanine transaminase
ALP: alkaline phosphatase
ATC: anatomical therapeutic chemical
CI: Chief Investigator
CMV: cytomegalovirus
DILI: Drug-Induced Liver Injury
EBV: Epstein–Barr virus
eCRF: electronic case report form
HIV: Human immunodeficiency virus
iDILI: Idiosyncratic drug-induced liver injury
INR: international normalized ratio
REC: Research Ethics Committee
RUCAM/CIOMS: Roussel Uclaf Causality Assessment/Council of International Organization of Medical Sciences
SAE: Serious Adverse Event
SAFE-T: Safer and Faster Evidence-based Translation Consortium
TBL: total bilirubin
ULN: upper limit of normal
WHO: World Health Organisation

STUDY BACKGROUND INFORMATION AND RATIONALE

Idiosyncratic drug-induced liver injury (iDILI) is an acute adverse hepatic reaction to a medication used in its therapeutic dose and that is unexpected from the known pharmacological action of the agent. The incidence of DILI is between 2.4 and 8.4 per 100,000 person years in population based studies [1, 2]. Although a recent study reported an annual incidence of 19.1 per 100,000 people [3], the study used a lower threshold for case definition than that was recommended by the 'phenotypic standardization project' [4]. The 'European Commission' on Public Health defines rare diseases as a life-threatening or chronically debilitating disease which affects fewer than 1 in 2,000 people, so that special combined efforts are needed to address them. Despite its rarity, idiosyncratic DILI accounts for 7-15% of the cases of acute liver failure in Europe and is the most frequent reason for the market withdrawal of an approved drug. In addition, DILI occurs in association with a large number of drugs and shows heterogeneity. There are no markers that can effectively pre-empt and prevent DILI or monitor the severity and course of the adverse event.

Case of need: One of the biggest hurdles in DILI studies is the limited number of identified cases. Due to the relative rarity of this condition it is unlikely that a single hospital will be able to identify a sufficient number of cases to perform well-powered studies. Hence, a collaborative effort is practically a prerequisite for these kinds of studies. National multicentric DILI registers, such as the Spanish DILI Registry and the US Drug-induced liver injury network (DILIN) have demonstrated the advantage of collaborations [5,6]. Furthermore international consortia, such as SAFE-T (www.imi-safe-t.eu) and iDILIC / iSAEC (www.saeconsotrium.org) have also emerged with the goal of developing new more sensitive and specific DILI markers than the routine liver profile markers currently used in

clinical practice to determine liver injury, as well as genetic variations that can predict DILI susceptibility.

In addition, majority of cases of DILI in the published clinical and genetic studies have been enrolled retrospectively; hence, the data set is reliant on clinical investigations and the setup of health services which are incomplete. In addition, the majority of the studies have used healthy populations as control groups (rather than those exposed to the drugs). Despite the discovery of genetic factors such as the association of specific HLA genotype with DILI, a low positive predictive value of the genotype in predicting DILI is limiting its use in clinical practice [7,8]. Age, smoking, metabolic syndrome, co-morbidity and other yet unidentified factors may generate an environment of oxidative stress that contributes to DILI. Therefore, we need 'in-depth phenotyping' together with data from an exposed control group to develop refined algorithms incorporating drug-related factors, host genetic and environmental risk factors that would enable us to pre-empt DILI. Deeply phenotyped cohorts with biological samples are essential for the development and validation of novel diagnostic/ prognostic markers [9].

We hypothesise that the severity of DILI is determined by factors that modulate cellular response to oxidative stress and the transition of adaptive response to the development of serious DILI can be recognised by a combination of markers that reflect the balance between pro- and anti-inflammatory responses to oxidative stress. So, we will develop a panel of markers that assess the severity of DILI. As the prospective in-depth phenotyped DILI cohort and controls are being recruited, the network infrastructure can also support any evaluation and validation of novel tests with potential application in DILI.

There are no evidence-based treatments for DILI; rarity of the condition and heterogeneity of the manifestations prevents the conception and successful completion of clinical trials. Multi-centre Pro-Euro-DILI registry will provide both the infra-structure to conduct clinical trials, and also a sufficient number of well characterised patients to evaluate the efficacy of novel anti-cholestatic agents in the resolution of DILI [10].

Participants will be asked to give consent for their samples to be stored and used for the purposes of this study and to be stored in a research tissue bank and used for future research.

Each participating study centre will collect and process biological samples as per SOP; samples will be kept at -80^{0}C at the IBIMA-University Hospital Virgen de la Victoria (HU VV) Research Biobank (Andalusian Public Health System Biobank, Biobanco del Sistema Sanitario Público de Andalucía) and shipped in regular batches to Nottingham Digestive Diseases BRU tissue bank located in room WE1392 E floor west block already designated as a HTA authorised location. Samples will be stored long-term under tissue licence no.12265.

STUDY OBJECTIVES AND PURPOSE

PURPOSE

The purpose of this study is to set up an international interdisciplinary consortium to obtain a better understanding of the mechanisms underlying drug-induced liver injury (DILI) and to develop methods of preventing DILI and its consequences. The consortium including the clinicians and scientists from European countries covering a wide area of expertise will allow the development of mature hypotheses that can be tested using a robust study

design. The consortium will facilitate the development of strong translational research proposals based on our hypothesis.

PRIMARY OBJECTIVE

To, collect, store and catalogue biological samples and clinical data, to form a large international patient/control cohort available for subsequent detailed analysis by consortium members which can also be used in current and future epidemiologic and mechanistic studies.

STUDY DESIGN

STUDY CONFIGURATION

This is a case controlled multi-centre study for the collection and storage of biological samples and data (medical, demographic and clinical)
We will prospectively develop a cohort of patients with liver injury induced by prescription drugs, over-the-counter medications, herbal remedies or dietary supplement intake identified by the participating clinical centres. Each patient will be followed from the identification of hepatotoxicity until normalization of the analytical liver profile.
We will also develop a control group cohort which will consist of patients who have been exposed to similar medications as the DILI group without having developed liver injury.

Coordinating Centres:
This is a European multicentre collaboration consisting of two coordinating centres; Biomedical Research Institute of Malaga (IBIMA) Spain and Nottingham Digestive Diseases BRU (NDDC BRU) UK. Both coordinating centres will be responsible for the adjudication of each potential DILI case entered into the Pro-Euro-DILI Registry database.
Both will share responsibility for the overall data monitoring, causality assessment of cases and the organisation/scheduling of regular conference call meetings and annual meetings.

Participating European Centres: (Incl. both coordinating centres)
Each Centre will obtain ethical approval for the study, nationally and locally and form links with their local regional liver transplant unit.
Each centre will be responsible for the collection of biological samples (patients & controls) Samples will be stored at -80^{0}C in the IBIMA-HU Virgen de la Victoria Biobank and thereafter shipped to Nottingham UK in batches.
Each Centre will also be responsible for entering the corresponding patient/control data into the Pro-Euro DILI Registry database (developed by Coresoft Clinic S.L, http://www.coresoft.es), available online. Tutorials on data entry and database implementations will be organized by the co-ordinating centres (Málaga and Nottingham) during the pilot phase and later if required.
During the pilot phase, the web based application and data will be hosted at Malaga University servers, with automated data backups on a daily basis, a report system for successful backup monitoring and reliable network connectivity. The software strictly follows the regulations imposed by the European Union ensuring that registries are secure and protected by the law. Furthermore, the software follows the Declaration of Helsinki

requirements to ensure protection of patient's rights. No data that can reveal the patients identity will be entered into the database. Internet access to the registry platform is password protected and all stored passwords will be encrypted.

PRIMARY ENDPOINT

Acquisition of approximately 270 DILI patients and 270 control participants' biological samples and appropriate clinical data.

SECONDARY ENDPOINT

We will collate detailed and comprehensive data using an electronic case report form (eCRF) to achieve in-depth phenotyping.

Causality assessment: Causal relationships between the event and the drug will be assessed using the Roussel Uclaf Causality Assessment/Council of International Organization of Medical Sciences (RUCAM/CIOMS). DILI will be phenotyped and severity graded as recommended by the phenotyping standardization project [4].

Standard variables in DILI, such as type of liver damage, severity and chronicity, will be classified as recommended by the phenotyping standardisation project ([4]). The type of liver damage will be defined as hepatocellular, cholestatic or mixed based on the ratio (R) between alanine transaminase (ALT) and alkaline phosphatase (ALP) expressed in times the upper limit of normal (ULN), R = (ALT/ULN) / (ALP/ULN). Hepatocellular damage is defined as R≥5, cholestatic damage ≤2 and mixed damage as 2<R<5. The R value will be calculated from the first available analytical values after DILI initiation.

Severity of DILI will be graded as follows:
Mild: Elevated ALT/ALP concentration reaching criteria for DILI but total bilirubin (TBL) concentration <2x ULN
Moderate: Elevated ALT/ALP concentration reaching criteria for DILI and TBL ≥2x ULN, or symptomatic hepatitis
Severe: Elevated ALT/ALP concentration reaching criteria for DILI, TBL ≥2x ULN and one of the following:

- international normalized ratio (INR) ≥1.5
- ascites and/or encephalopathy, disease duration <26 weeks and absence of underlying cirrhosis
- other organ failure considered to be due to DILI

Fatal or transplantation: Death or liver transplantation due to DILI

Disease duration will be graded as follows:

- **Persistent DILI**: Evidence of continued liver injury >3 months for hepatocellular or mixed type of liver damage and >6 months for cholestatic liver damage

- **Chronic DILI**: Evidence of persistent liver injury at >1 year after the onset of DILI

Appendix 1

Causal DILI agents will be classified according to World Health Organisation (WHO) anatomical therapeutic chemical (ATC) classification system. Associated diseases will be classified according to the WHO tenth edition of the international classification of diseases, ICD-10.

STUDY MANAGEMENT

The Chief Investigator has overall responsibility for the study and shall oversee all study management.
The data custodian will be the Chief Investigator.

DURATION OF THE STUDY AND PARTICIPANT INVOLVEMENT

PARTICIPANT DURATION:

Recruitment will be over a two year period.

-For DILI patients: 3-5 visits, (each visit lasting for up to 20mins). Each patient will be in the study for up to 6 months duration.

Follow-up: Each patient will have a clinical follow up until the event resolves or 12 weeks after the first event whichever occurs later.

Enrolment:
We expect to enrol each case at the earliest opportunity possible, following the DILI event (up to a maximum of 4 weeks from onset). Each patient will be followed from the identification of hepatotoxicity until normalization of the analytical liver profile.
Enrolment will begin once a patient has been clinically diagnosed with DILI and gives consent to take part in the study. In all instances the visit schedule should be adapted to routine hospital procedures in order to facilitate patient participation as well as avoid additional hospital costs.
-Control participants: A single study visit of up to 30minutes duration. Controls will be required to provide biological samples, demographic and medical data will also be collected.
Study Duration:
Enrolment will end at two years from the start of the study or once the recruitment target has been met (i.e. x270 cases of DILI and x270 case controls); whichever is soonest.

END OF THE STUDY

This study involves the collection and storage of data and tissue. We will be seeking consent to collate, store and use samples and data for a minimum of 10 years.
Collated biological samples will be stored at the IBIMA-HU VV Biobank in addition to being shipped to the REC-approved tissue bank located on HTA licenced premises at Nottingham University for subsequent analysis by members of the consortium and collaborators in future projects for at least 10 years.

Appendix 1

SELECTION AND WITHDRAWAL OF STUDY PARTICIPANTS

DILI Patients:
DILI patients to be included are defined as:

1. One of the following analytical thresholds is met:

- alanine transaminase (ALT) exceeding 5 times upper limit of normal (ULN)
- alkaline phosphatase exceeding 2 times ULN or
- ALT exceeding 3 times ULN plus bilirubin exceeding 2 times ULN

2. Exposure to drugs including any prescription drug, over-the-counter drug, recreational drug, herbal remedies or dietary supplements prior to the DILI onset.
3. Absence of other known causes of liver injury after detailed investigations.

Control Group:
Adults who are exposed to the same drugs (as those responsible for DILI) without developing DILI will be identified. The Chief Investigator will access hospital pharmacy databases to identify patients taking specific drugs of interest. Then patients exposed to particular drugs without developing DILI will be invited to participate in the study as a control group.

Withdrawal of study Participants

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time from the study but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we may still use the anonymised data in the final analyses. Remaining tissue samples can be destroyed/disposed of if the participant so wishes. Similarly, the information about them will be deleted so that it cannot be used again. We will explain to the participant that if they decide to withdraw after a long period of time, the samples may already have been used and we cannot recall samples or information from researchers if this is the case.

Based on previous studies involving the participating centres; we initially aim to enrol a total of 270 cases with acute DILI over a 2-year period. The patient assessment schedule is outlined in appendix 1.

Recruitment

Will take place in a secondary care hospital setting.

Potential participants will be recruited from the acute and in-patient services as well as outpatient clinics at the Hospitales Universitarios Regional y Virgen de la Victoria de Málaga. Patients who may have DILI event will be identified and approached.The initial

approach will be from a member of the patient's usual care team (which may include the investigator and his research team), and information about the study will be on display in the relevant clinical areas.
Patients will be identified as having DILI / possible DILI from their medical records and laboratory test results from samples taken as part of their standard clinical care. The study will be explained and potential participants will be given a copy of the Participant Information Sheet to read and asked if they would be interested in taking part in the study. If a patient agrees to take part in the study, they will be asked to sign a copy of the consent form. Once signed, the patient will have a blood sample taken, the results of this blood test taken at enrolment will be used to assess/determine eligibility; if eligible, the patient will continue in the study. In all instances the visit schedule should be adapted to routine hospital procedures in order to facilitate patient participation as well as avoid additional hospital costs.

The investigator or their nominee, e.g. from the research team or a member of the participant's usual care team, will inform the participant (or their nominated representative if they are unable to give informed written consent) of all aspects pertaining to participation in the study.

Control Group:
Adults who are exposed to the same drugs (as those responsible for DILI) without developing DILI will be identified. The Chief Investigator will access hospital pharmacy databases to identify patients taking specific drugs of interest. Then patients exposed to particular drugs without developing DILI will be invited to participate in the study as a control group.

Eligibility criteria

Inclusion criteria:

Both DILI patient group and control group:
Aged 18 years and over
Able to give written informed consent
Potential Participants who lack capacity to give written informed consent and have a consultee (personal or nominated)

DILI Patients:
Adults diagnosed with acute DILI (within 4 weeks of onset) and meeting the following criteria will be included:

1. Meets one of the following analytical thresholds at enrolment (day 0)
   - alanine transaminase (ALT) exceeding 5 times upper limit of normal (ULN)
   - alkaline phosphatase exceeding 2 times ULN OR
   - ALT exceeding 3 times ULN plus bilirubin exceeding 2 times ULN

2. Exposure to drugs including any prescription drug, over-the-counter drug, recreational drug, herbal remedies or dietary supplements prior to the DILI onset.
3. Absence of other known causes of liver injury after detailed investigations

Control Group:
Adults similarly matched to the DILI group i.e. who are similarly exposed to the same types of drugs including any prescription drug, over-the-counter drug, recreational drug, herbal remedies or dietary supplements without developing DILI.

Exclusion criteria:

Anyone with any of the following conditions will not be included in the study:

- Acute viral hepatitis due to hepatitis A, B or reactivation of B, C, E, CMV, EBV, HIV
- Acute presentation of auto-immune hepatitis unrelated to the drug.
- Confirmed acute liver injury that explains the clinical manifestation eg: ischemic hepatitis, acute ascending cholangitis.
- Acute exacerbation/ decompensation of known chronic liver disease that explains the acute event.
- Biliary obstruction explaining cholestasis.
- On the judgement of CI that the person has certain alternative explanation to the acute event (rather than DILI).

Informed Consent
All participants will provide written informed consent. If the potential patient lacks capacity, a suitable person will be identified who can act as consultee and advise the researcher on whether the person who lacks capacity would want to be involved in the project. The researcher will first approach a personal consultee; however a nominated consultee will be appointed if the patient does not have a personal consultee. Patient information sheets and consent forms will be submitted for review and approval by the Research Ethics Committee. The Informed Consent Form will be signed and dated by the participant before they enter the study or on their behalf if they lack capacity and a suitable person has been identified to act as their consultee. The Investigator will explain the details of the prospective clinical study and provide a Participant Information Sheet, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.
Informed consent will be collected from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant, one will be kept by the Investigator, and a third will be retained in the patient's hospital records.
Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended Consent form which will be signed by the participant.

Appendix 1

STUDY TREATMENT AND REGIMEN

DILI patients

Biological samples will be collected from each enrolled case on at least 3 occasions. These visits will take place as soon as possible following acute presentation; there will be a time lag of up to 4 weeks between identification of an episode of DILI by the team responsible for the clinical care of the patients and the patient giving consent to take part in the study. Patients' blood tests must meet the eligibility criteria on the day they are enrolled (Day 1 visit) in order to participate in the study.
Visit 2 will be 7 ± 2 days from the Day 1 visit
Visit 3 will be 30 ± 7 days after the initial visit (Day 1 visit).
For those who are in-patients (hospitalised at the time of acute DILI) we will secure additional data and samples on up to 2 further time points during the period of the patient's hospitalization. In such cases samples will be collected as part of clinical care and saved for research purposes.
At the first visit the Chief Investigator (CI) or a delegated member of the research team will collect 5 ml of whole blood (for leucocyte DNA), 10 ml of clotted blood for extracting serum, 10 ml of anti-coagulated blood for separating plasma, 30 m of urine and if possible a stool sample. (The 5 ml whole blood sample can be collected at a later time point should the physician in charge consider it to be more appropriate).
During the following second and third visits, we will collect 10 ml of blood for serum, 10 ml for plasma and 50ml of urine. Patients will be asked to bring a stool sample to these visits. An additional 5 ml of blood (serum extraction) for a routine biochemical analysis will be extracted during each visit.
All biological samples will be processed in each collaborating centre according to the agreed standard operating procedures (Appendix 2), labelled and stored at -80°C at the IBIMA-HU Virgen de la Vicotira Research Biobank until sent to the sample depository in Nottingham.

If a liver biopsy is obtained for clinical reasons any samples available (when the liver biopsy core is >25 mm) will be collected and stored for research purposes. (We will also aim to obtain and store any archived liver sections that were taken as part of clinical care).

Controls
During a single visit lasting for approximately 30 mins, biological samples will be collected (blood, stool and urine sample) and processed as for the first DILI patients. Demographic data and medical and pharmacological history will also be collected.

Risks: The study will be conducted with close attention to patient safety. Patients recruited will have undergone venepuncture to collect blood samples. This procedure will be carried out by trained, competent health professional.
Donating a blood sample via venepuncture has a low risk of slight bruising and tenderness.

Sample size and justification: Considering that DILI is a rare disease we have based our recruitment target on the feasibility.
Based on previous studies involving the participating centres, we initially aim to enrol a total of 270 cases with acute DILI and 270 controls over a 2-year period.
We aim to recruit up to 50 acute DILI participants and 50 controls at our centre and estimate that we will achieve for the whole study a total of around 3000 separate samples.

ADVERSE EVENTS

Definitions

An adverse event (AE) is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.
However, this is a case control study and does not include any clinical or experimental interventions other than blood sampling. In addition, natural history of DILI includes progressive worsening of the liver injury even after offending agent/ medication/ drug has been withdrawn. Acute liver failure and death are recognised complication. Therefore, while we will record accurately the severity of DILI formally based on the international consensus criteria [4],
AE does not include a/an:
1. exacerbation of a pre-existing illness.
2. increase in frequency or intensity of a pre-existing episodic event or condition.
3. condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.
4. continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
5. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.
6. pre-existing disease or conditions present or detected at the start of the study that did not worsen.
7. situation where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).
8. disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.
9. overdose of concurrent medication without any signs or symptoms.
A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the treatment or intervention that results in any of the following outcomes. However, acute liver failure, liver transplantation and death are recognised consequences of DILI and these will be recorded as part of the outcome of DILI as recommended by consensus criteria [4]. Therefore, following will NOT be considered as SAE:
1. Liver related death
2. Acute liver failure
3. Liver transplantation
4. Inpatient hospitalisation or prolongation of existing hospitalisation
5. A disability / incapacity

In the current study we will be performing venepunctures for blood sampling. So, we will report major bleeding leading to hypotension, drop in Hb, requirement of resuscitation, transfusion or hospital admission will be reported as adverse events.
Any adverse events reported will be assessed for seriousness, expectedness and causality: A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to trial treatment / intervention administration which makes a causal relationship incompatible or for which other treatments, chemicals or disease provide a plausible explanation**.** This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment / intervention administration which makes a causal relationship a reasonable possibility, but which could also be explained by other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment / intervention administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other interventions, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment / intervention administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation. The Chief Investigator shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.
The Chief Investigator shall be responsible for all adverse event reporting to the Regional Pharmacovigilance Centre.

TRANSPORT AND STORAGE OF THE TISSUES

Serum, plasma, whole blood (EDTA) urine, and stool samples will be stored in aliquots at -80°C in the IBIMA-University Hospital Virgen de la Victoria Research Biobank. From an ethical and regulatory point of view, the samples will be managed through the IBIMA-

University Hospital Virgen de la Victoria Research Biobank (Andalusian Public Health System Biobank, Biobanco del Sistema Sanitario Público de Andalucía), belonging to the National Biobank Platform (project PT13 / 0010/0033), thus ensuring integral treatment of the samples and associated data according to the procedures contained in the Operating System, developed based on Law 14/2007 for biomedical research, and the development according to Royal Decree 1716/2011 of biobanking and biological sample treatment, law 41/2002 on patient autonomy and rights and obligations for clinical information and documentation, and Law 15/1999 on protection of personal data, among other applicable regulations, and taking into account international standards.

Furthermore, the "Tumour Bank" within the the Biobank is authorized to form part of the Andalusian Tumour Bank Network (Red de Bancos de Tumores Andaluza, RBTA) and operates according to the Andalusian Tumour Bank Network care process (countersigned by a SAS authorization, R.S.C 786/05), approved by the Andalusian Ethics Committee.

Samples to be used in future DILI biomarker studies will be shipped from the IBIMA-University Hospital Virgen de la Victoria Research Biobank to the Nottingham BRU tissue bank (an agreement between the biobanks will be signed) where they will be held under HTA licenced premises (DI Prof Jim Lowe- Licence Number 12265) for subsequent analysis by members of the consortium and collaborators in future projects for at least 10 years.

We will also receive samples of plasma, serum, whole blood, urine and stool from the respective national collaborators in batch shipments by courier as frequently as required. All shipments will comply with HTA/EUTCD and will contain a complete inventory of all samples, along with the name of the person responsible for sending the samples and a copy of the signed MTA. Samples will be stored within the IBIMA-University Hospital Virgen de la Victoria Research Biobank for future research if participants are agreeable and sign the optional clause on the consent form.
Where participants do not agree to the future use of the samples they will be destroyed in accordance with the Human Tissue Act, 2004.

LABORATORY ANALYSES

All routine laboratory analysis will be done by appropriate laboratory routine services as part as normal patient care. Blood will be processed for storage of serum and plasma as outlined in the SOP.
All subsequent analysis will form part of any separate future applications.

ETHICAL AND REGULATORY ASPECTS

Ethical Issues

The study will not be initiated before the protocol, informed consent forms and participant information sheets have received approval / favourable opinion from a Research Ethics Committee (REC). Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed

consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC. A protocol amendment intended to eliminate an apparent immediate hazard to participants or researchers may be implemented immediately providing that the REC is notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki; the principles of Good Clinical Practice and Good Laboratory Practice, the DH Research Governance Framework, 2nd ed, 2005 and in accordance with the Human Tissue Act, 2004.

Informed Consent and Participant Information

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or their nominee and the participant or other legally authorised representative shall both sign and date the Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Study Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the study.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No study-specific interventions will be done before informed consent has been obtained.

If the Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

Withdrawal

It will be explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time from the study but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we may still use the anonymised data in the final analyses. Remaining tissue samples can be destroyed/disposed of if the participant so wishes. Similarly, the information about them will be deleted so that it cannot be used again. We will explain to the participant that if they decide to withdraw after a long period of time, the samples may already have been used and we cannot recall samples or information from researchers if this is the case.

The Investigator may also remove a subject if, in his / her opinion, it is in the best interests of the subject. If a patient permanently withdraws from the study, or is lost to follow-up, the reason will be recorded.

RECORDS

We will collate detailed and comprehensive data using an electronic case report form (eCRF) to achieve in-depth phenotyping.

Data collection

Data to be collected from each patient are outlined in Appendix 3. Data to be collected from controls will follow the same protocol, with the exclusion of information directly relating to a DILI episode. The data will include demographic characteristics, current and past medical history and drug exposure. Any medications taken in the last 6 months prior to the DILI episode will be recorded, including start and stop dates, doses and indications. In addition, clinical data (such as abdominal ultra sound results and biopsy findings if available) and analytical data (including serological testing for viral hepatitis and presence of positive autoantibody titres) corresponding to the DILI episode will also be recorded. Blood analysis information to be recorded include: glucose, urea, creatinine, total protein, albumin, alpha-1, alpha-2, beta, gammaglobulines, total bilirubin, direct bilirubin, aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase, triglycerides, total cholesterol, high-density lipoprotein, low-density lipoprotein, thyroid stimulating hormone, iron, transferrin, copper/ceruloplasmin, immunoglobulin M, immunoglobulin G, immunoglobulin A, prothrombin activity, international normalized ratio, haemoglobin, haematocrit, mean corpuscular volume, platelets, erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Principal investigator in each collaborating hospital will be responsible for entering the corresponding patient/control data into the Pro-Euro DILI Registry database (developed by Coresoft Clinic S.L, http://www.coresoft.es), available online. Tutorials on data entry and database implementations will be organized by the co-ordinating centres (Málaga and Nottingham) during the pilot phase and later if required.

Each participating institute will only be able to access its corresponding patient/data entries in the database, with the exception of the national coordinator, who will have access to all cases from the corresponding country when there are multiple national institutes involved. The two coordinating centres, Málaga and Nottingham will have full access to all patient/control entries. The master database will be held by Malaga in a password encrypted file.

Documents relating to the study that contain personal data that may disclose the identity of the subject should remain with the Chief Investigator in a locked filing cabinet. The Investigator should not provide any personal data that may identify the subject to any third party at any time during or after the study. Subject confidentiality will be further assured by utilising unique subject identification code number. The link between the patient's name and code will be broken and the sample completely anonymised when the study is completed and all clinical data have been obtained.

The following identifiers along with the trial ID will be detailed in the study recruitment log by the CI to allow identification of the participant's tissue samples or when chasing data queries with participating remote sites.
Each participant will be assigned a trial identity code number, allocated at randomisation if appropriate, for use on eCRFs other trial documents and the electronic database.
eCRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number and Participant Study Number (the Study Recruitment Log), to permit identification of all participants enrolled in the clinical study, in accordance with regulatory requirements and for follow-up as required.
eCRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Study Delegation Log.'
All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.
The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the eCRF.

Sample Labelling

Samples will be stored in linked anonymised format at the IBIMA-University Hospital Virgen de la Victoria Research Biobank.

Each participant will be assigned a trial identity code number for use on the samples, consent forms and other study documents and the electronic database. All samples (whether from Málaga or elsewhere in Europe) will be labelled according to an agreed system compatible with the database developed by Coresoft as outlined in Appendix 4.

Samples for pathology analysis will be labelled in accordance with local University Hospital Virgen de la Victoria procedures.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and records. eCRF's may also completely serve as its own source data. Only study staff as listed on the Delegation Log shall have access to study documentation other than the regulatory requirements listed below.

Direct access to source data / documents

Study documents, including progress notes and copies of laboratory and medical test results shall be made available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities.
The Principal investigator in each collaborating hospital will be responsible for entering the corresponding patient/control data into the Pro-Euro DILI Registry database (developed by Coresoft Clinic S.L, http://www.coresoft.es), available online. Tutorials on data entry and

database implementations will be organized by the co-ordinating centres (Málaga and Nottingham) during the pilot phase and later if required.
During the pilot phase, the web based application and data will be hosted at Malaga University servers, with automated data backups on a daily basis, a report system for successful backup monitoring and reliable network connectivity. The software strictly follows the regulations imposed by the European Union ensuring that registries are secure and protected by the law. Furthermore, the software follows the Declaration of Helsinki requirements to ensure protection of patient's rights. No data that can reveal the patients identity will be entered into the database. Internet access to the registry platform is password protected and all stored passwords will be encrypted.

All study staff and investigators will endeavour to protect the study participants' rights to privacy and informed consent, and will adhere to the Data Protection Act, 1998. Only the minimum required information for the purposes of the study shall be collected. Documents will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the study staff and investigators and relevant regulatory authorities (see above). Computer held data including the study database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).
Information about the study in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

STUDY CONDUCT

Study conduct may be subject to systems audit of the study files for inclusion of essential documents; permissions to conduct the study; CVs of study staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, timeliness of visits); and equipment calibration logs.
The Chief Investigator, or where required, a nominated designee, shall carry out a site systems audit at least yearly and an audit report shall be made to the Study Sponsor.

STUDY DATA

Monitoring of study data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Chief Investigator, or where required, a nominated designee, shall carry out monitoring of study data as an ongoing activity.

Data entries will be verified by inspection against the source data. A sample (10% or as per the study risk assessment) will be checked on a regular basis for verification of all entries made. In addition the subsequent capture of the data on any study databases will be checked. Where corrections are required these will carry a full audit trail and justification.

Study data and evidence of monitoring and systems audits will be made available for inspection by the regulatory authority as required.

RECORD RETENTION AND ARCHIVING

The Chief Investigator will maintain all records and documents of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The study documents held by the Chief Investigator shall be finally archived at secure archive facilities in IBIMA-HU Virgen de la Victoria. This archive shall include all study databases and associated encryption codes.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to study data in the computer files.

Data generated as a result of this study will be available for inspection on request by the REC and representatives of the Human Tissue Authority.

PUBLICATION POLICY

All potential manuscripts deriving from Pro-Euro-DILI Network samples and patient data must be presented to the scientific committee for acceptance prior to submission. Publication authorship will be dependent on study participation and case recruitment, with centres having enrolled a greater number of cases appearing earlier in the list of authors than those with fewer case enrolments. In the event of patent applications resulting from the Pro-Euro-DILI Network data the authorship will be determined based on the same strategy as outlined for publications.

Plan of diffusion

A Pro-Euro-DILI Network web site will be developed in order to disseminate the presence of the consortium and its work. The coordinating centres (Málaga and Nottingham) will be responsible for the initiation and maintenance of the web site. All collaborating centres are encouraged to present the Pro-Euro-DILI Network at national conferences, but any potential work to be presented must be provided to the scientific committee prior to submission. If a group wants to present multinational data, i.e. data relating to cases from other countries, permission must be obtained from the scientific committee

STUDY FINANCES

European Association for the Study of Liver has supported the development of registry by Prof Raul J. Andrade (Malaga University) and a biorepository at the NIHR NDDC BRU,

Nottingham University Hospitals NHS Trust by Prof G. P. Aithal. Based on the cohort that we develop and the biorepository of samples that we collate, the Chief Investigators and collaborators will discuss proposals using the Pro-Euro-DILI registry, seek funding opportunities and develop grant proposals to carry out future investigations.

Participant stipends and payments

Participants will not be paid to participate in the study. Travel expenses will be offered for any hospital visits in excess of usual care.

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APPENDICES:

Appendix 1. Patient assessment schedule

	Day 1	1< Day < 8	Day 7 ±2	Day 30 ±7	Day 90 ±7	Day 180 ±7
	Visit 1	**Optional[§]**	**Visit 2**	**Visit 3**	**Optional[§§]**	**Optional[§§§]**
Inclusion/Exclusion criteria	X					
Medical history/current medical condition	X					
Demography	X					
Body height	X					
Body weight	X					
Drug history in past 6 months, including OTC, herbal, dietary supplement, recreational drugs	X					
Smoking history	X					
Alcohol, quantitative assessment	X					
Viral hepatitis screen	X					
Autoantibody* screening	X			(X)**		
Liver ultrasound	X					
Hematology, Blood chemistry, Urine analysis	X		X	X	X	X
Blood sample collection	X		X	X	X	X
Urine sample collection	X		X	X	X	X
Stool sample collection if possible	X		X	X	X	X
Study completion information				X	X	X

*Autoantibodies: antinuclear (ANA), antismooth muscle (ASMA), antimitochondrial (AMA) and liver kidney microsomal type 1 (LKM-1)

**Repeat autoantibody screening if positive in week 1

[§]Only if patient is hospitalized, [§§]Only if patient has elevated liver profile values at visit 3, [§§§]Only chlolestatic patients with elevated liver profile values at visit 4

Appendix 2.

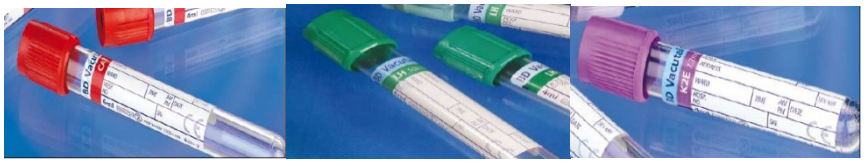
Pro-Euro-DILI Registry 2014-2017 (G. P. Aithal)

Standard Operating Procedures (SOP)

A. Collection

1. Suppliers and Ordering Information:

Vacutainers – BD https://www.bd.com/resource.aspx?IDX=10155



BD vacutainer plastic serum with red hemoguard closure: Cat no. 367837 (6ml) or 367895 (10ml)

BD vacutainer plastic lithium heparin with green hemoguard closure: Cat no. 367885 (6ml)

BD vacutainer plastic K2EDTA with lavender hemoguard closure: Cat no. 367839 (4ml) or 367873 (6ml)

BD urine sterile specimen collection cup: Cat no. 364941 or equivalent eg 30ml Universal

Sample containers – thermo fisher scientific (sterilin) or alpha labs or equivalent products

http://www.sterilin.co.uk/internet/content.nsf/framesetter/TMAY5BQDST?OpenDocument

polypropylene 30ml Universal: Cat no. 128B/P

polypropylene spoon Universal: Cat no. 128SB/P

polystyrene/polypropylene bijoux 7ml: Cat no. 129B

1.2ml polypropylene cryogenic vial (screw cap): Cat no. 1CRIS

http://www.alphalabs.co.uk/home.aspx

polypropylene 30ml Universal: Cat no. CW3890

polypropylene spoon Universal: Cat no. SC4874

polystyrene/polypropylene bijoux 7ml: Cat no. SC2122

1.2ml polypropylene cryogenic vial (screw cap): Cat no. CPS318WL or LW3432

2. Sample Labelling

An 11 digit code [A]-[B]-[C]-[D] will be assigned as follows:

[A] Participants to be sequentially given number by recruiting centre:

Patients (DILI): D001 onwards;

Controls (non-DILI): N001 onwards.

[B] 3 digit site code:

NOT = Nottingham

MAL = Malaga

[C] Sample type:

SE = serum

PH = plasma heparin

PE = plasma EDTA

WB = whole blood (EDTA)

UR = urine

ST = stool

LB = liver biopsy

[D] Visit number: 01 onwards

Example: Dili patient 6 Nottingham urine visit 2 D006 NOT UR 02

3. Collection of patient samples

Urine, stool and blood collection (up to 28ml blood in total)

1. For Serum Samples

Collect 2 x 6ml blood into red (no additive) collection tubes.

Allow to coagulate for 60 minutes at ambient temperature (18-22°C).

a. Then centrifuge the samples at 2000g for 10 minutes at room temp. (samples can be kept at 4°C for no more than 4h before centrifugation if necessary)

b. The serum forming the top yellow coloured layer, should be carefully collected by aspiration or pipetted and put in 500ul aliquots into labelled cryovials (usually 2-4 tubes). Note the volume of the final aliquot on the tube if less than 500ul.

c. Aliquots should be frozen immediately (with liquid nitrogen if available).

d. Samples should be stored in freezer at –80^{o}C until shipped to UK.

2. For Plasma Samples

a. Collect 1 x 10ml blood into green lithium heparin tubes (**pre chill on ice**) OR into purple EDTA tubes.

b. Samples should be inverted gently x 5 to mix and kept on ice.

c. Centrifuge the samples within 15 minutes of sample collection, at 2500g at 4°C for 10 minutes, to obtain platelet-free and haemolysis-free plasma. If haemolysis is apparent please record. (samples can be kept at 4°C for a maximum of 4h before processing if necessary)

d. The plasma (top yellow layer) should be carefully collected by aspiration/pipetting and 500ul aliquots put into labelled cryovials (usually 3-4 tubes). **Please indicate whether it is from Heparin or EDTA** (This is crucial for several biomarker assays). Note the volume of the final aliquot on the tube if less than 500ul.

e. Aliquots should be frozen immediately (with liquid nitrogen if available).

f. Samples should be stored in freezer at –80^{o}C until shipped to UK.

3. For DNA

a. Collect 1 x 6ml blood into purple K_2EDTA tubes.

b. Store tubes in storage boxes in freezer at –80^{o}C until shipped to UK.

4. Urine

a. Collect approximately 50ml of midstream urine (not first urine of the day).
b. Transfer into 5x 1ml labelled polypropylene cryogenic tubes and upto 10 x 5ml bijoux containers.
c. Aliquots should be frozen immediately (with liquid nitrogen if available).
d. Samples should be stored in freezer at –80^{o}C until shipped to UK.

5. Stool

Collect stool using appropriate standard methods /kit
Avoid contact with urine.

a. Transfer using 'spoon' into Universal container.
b. Transfer to bijoux container if possible.
c. Store tubes in storage boxes in freezer at –80^{o}C until shipped to UK.

A. Stool collection protocols

1.Faecal sample collection procedure

Faecal sample collection

Subjects should be provided with a stool specimen collection kit and collection advice sheet. Stool specimens are to be collected within a 24-hour period before a clinic visit/collection (as previously discussed)

Stool Collection Advice for Patients

1. Place or hold a cardboard tray to collect stool as passed – avoid urine.
2. Remove the screw cap and spatula from the stool container and collect a small piece of stool (about the size of a cherry)
3. Replace the screw cap and close the container tightly.
4. Place container in a plastic bag.
5. Flush any leftover stool down the toilet.
6. Dispose of cardboard into household waste/ normal dustbin.
7. Bring the sample to your healthcare appointment.

Thank you.

Appendix 1

Pro-Euro DILI Collection Summary

Sample	Collection	Container	Volume	Processing	Storage
1. Serum	Blood ambient	Coagulation (red) 2x6ml	10ml	60 min coagulation, centrifuge, 500ul aliquots.	-80°C
2. Plasma	Blood chilled	Lithium heparin (green) **or** EDTA (purple) 1x10ml (please record which)	10ml	Immediate chilled centrifugation, 500ul aliquots. Record if heparin or EDTA.	-80°C
3. DNA	Blood	EDTA (purple) 1x6ml	6ml	No.	-80°C
4. Urine	Urine	30ml Universal polypropylene/polystyrene	10-50ml	Aliquot into 5x1ml and 5ml tubes	-80°C
5. Stool	Faeces	30ml spoon Universal	1-10g	Transfer to bijoux.	-80°C

B. Shipping

When samples from approximately 5 patients has been collected and stored ready for shipping, contact your preferred courier and arrange shipment. Please let us know when you do this.

Shipment must be compliant with the HTA and EUTCD, and MTA should already be in place.

Samples should be clearly labelled and in sealed tubes inside a sealed bag/box.

A 40x30x30cm polystyrene transport box or similar can be used filled with approx. 5kg dry ice.

The recipient contact is: **Jane Grove/Mel Lingaya**

The recipient address is:

Nottingham Digestive Diseases Centre BRU.

E floor West block,

Queens Medical Centre

Nottingham

NG7 2UH

UK

Tel: 44 115 924 9924 ext. 64429

Mobile: 44 7952638335

Email jane.grove@nottingham.ac.uk or melanie.lingaya@nottingham.ac.uk

** Please contact us to confirm details before sending shipment **

Please contact **jane.grove@nottingham.ac.uk** **or** **melanie.lingaya@nottingham.ac.uk** for any further information about sample collection, processing or shipment.

Appendix 1

Code of practice for the shipping of category B specimens:

To ensure that all staff involved in the transport of biological materials, from the sender to the receiver are aware of their responsibilities and know the procedures to take in order to ensure their safe and efficient transport.

Patient specimens are human materials, collected directly from humans, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Samples collected for this clinical study are considered:

Category B infectious substances are any that do not meet the criteria of category A and included any human or animal material including but not limited to excreta, blood and its components, tissue and tissue fluids transported for purposes such as research, diagnosis, investigational activities, disease treatment or prevention.

These are assigned to UN 3373 Biological Substance Category B [formerly referred to as Diagnostic Specimens]

RESPONSIBLITIES & ORGANISATION

The efficient transport and transfer of infectious materials requires good co-ordination between the sender, the carrier and the receiver (receiving laboratory), to ensure that the material is transported safely and arrives on time and in good condition. Such co-ordination depends upon well established communication and a partner relationship between the three parties. All have specific responsibilities to carry out in the transport effort.

Consent from the donor to transport the tissue must be in place prior to transportation, whether this is to establishments within England, Wales and Northern Ireland or exported to establishments outside England, Wales and Northern Ireland.

Appendix 1

All processes involving the handling and transportation of human tissues must be subject to risk assessment and made available to everyone involved. Risk assessments must cover risks to the tissue in addition to the individual Workers handling the tissues must have training and health screening as appropriate.

Care must be taken when handling heavy or bulky containers, personnel involved in their transport should be trained in handling techniques.

Suitable labelling including biohazard warnings must be affixed to each container.

Reagents used in preserving and fixing tissues are harmful and consideration must be given to the safe handling of these reagents.

A Material Transfer Agreement (MTA) will be in place for any outgoing or incoming tissue. The MTA will define how the human tissue will be preserved, any potential contamination risks associated with it and who will be responsible for disposal. The MTA must be signed on behalf of the University/UHB not by an individual researcher.

Appendix 1

The sender must:

1. Ensure the correct designation, packaging, labelling and documentation of all infectious substances.
2. Make advance arrangements with the receiver of the specimens including investigating the need for an import permit.
3 Make advance arrangements with the carrier to ensure:

- that the shipment will be accepted for appropriate transport
- that the shipment (direct transport if possible) is undertaken by the most direct routing, avoiding arrival at weekends;

4. Prepare necessary documentation including permits, dispatch and shipping documents;
5. Notify the receiver of transportation arrangements once these have been made, well in advance of expected arrival time.
6. Ensure the School or Departmental Safety Officer is informed and involved in the transport arrangements.

The carrier must:

1. Provide the sender with the necessary shipping documents and instructions for their completion;
2. Provide advice to the sender about correct packaging;
3. Assist the sender in arranging the most direct routing and then confirm the routing;
4. Maintain and archive the documentation for shipment and transport;
5. Monitor required holding conditions of the shipment while in transit;
6. Notify the sender of any anticipated (or actual) delays in transit.

The receiver must:

1. Obtain the necessary authorisation(s) from national authorities for the importation of the material;
2. Provide the sender with the required import permit(s), letter(s) of authorisation, or other document(s) required by the national authorities;
3. Arrange for the most timely and efficient collection on arrival;
4. Immediately acknowledge receipt to the sender.
5. Where the University is the recipient, arrangements should have made to ensure that the consignment is received at a suitable location and time so as to ensure adequate control and security of the material.

Shipments should not be dispatched until:

- advance arrangements have been made between the sender, carrier and receiver
- consent forms are available for the samples
- an MTA agreement has been arranged

Appendix 1

PACKAGING, LABELLING AND DOCUMENTATION FOR TRANSPORT

Primary Containment

Tissues must be held in suitable containment able to withstand the planned mode of transport and possible accidents, in conjunction with secondary containment or further packaging. Primary containers must be break resistant and leak proof. The nature of the tissue and the project will largely determine the optimum choice of container.The primary container must bear appropriate labelling.

Secondary Containment

Secondary containers must be able to hold the primary containers securely and be able to withstand the planned mode of transport. Secondary containers must also be suitable for the containment of any refrigerants being used, e.g. liquid nitrogen, dry ice, water ice.

The container must bear appropriate labelling, including hazard information and emergency contact details. Secondary containers must be leak proof and have adequate locks, seals or catches, able to prevent the accidental release of contents during transport.

There should be sufficient absorbent material to absorb the fluid form all the primary containers in the case of breakage. Processes must be in place to ensure that there is no contamination to any external surfaces of secondary containment.

Outer Packaging

Outer packaging must be sufficiently robust to withstand the planned mode of transport. The outer packaging must bear appropriate labelling, including hazard information and emergency contact details.

Appendix 1

Documentation

The following shipping documents are required.

- To be prepared and signed by the shipper:

  • For Air: the shipper's Declaration for Dangerous Goods • a packing list/proforma invoice that includes the receiver's address, the number of packages, detail of contents, weight, value (Note: for international transport, a minimal value shall be indicated, for customs purposes, if the items are supplied free of charge)

  • an import and/or export permit and/or declaration if required.

- To be prepared by the shipper or the shipper's agent:
  • An air waybill for air transport or equivalent documents for road, rail and sea journeys.

USE OF REFRIGERANTS IN TRANSPORT

Dry Ice is the normal and preferred means of refrigeration. Where dry ice is used as a refrigerant the following ICAO/IATA Packing instruction 904 must be met.

- Dry ice should be placed **outside** the secondary receptacle. It must not be placed inside the primary or secondary receptacle because of the risk of explosion.
- The secondary receptacle must be secured within the outer packaging to prevent damage after the ice has dissipated.
- Packaging must permit release of gas to prevent pressure build up.
- The carrier [courier or taxi] must be aware that the package contains dry ice and that procedures are arranged to ensure adequate ventilation.

Additional labelling for dry ice

The package must bear the following labelling [in addition to the labelling for the biological material]

- Nett weight of dry ice.
- Miscellaneous hazard label UN class 9
- If dry ice is used for infectious substances, the details must appear on the shipper's Declaration for Dangerous Goods.

MODES OF CARRIAGE

Use of a reputable company experienced in the transport of dangerous goods is the preferred means of transport as this will assist in ensuring that all regulatory requirements are met. It is also the most reliable way of ensuring that the goods reach their destination safely and within a reasonable time frame.

Disclaimer

When using this document, please ensure that the version you are using is the most up to date either by checking on the GOVRN/HTA website for any new versions or contact the HTA Governance Officer to confirm the current version

Appendix 3.

DRUG-INDUCED HEPATOTOXICITY REPORT FORM

(Clinical history number) .....

Pro-Euro-DILI code.....

Age ……. .....

Gender: 1. Male 2. Female.....

Weight (kg) .....

Height (cm) .....

Ethnicity……..

Country of origin……

Institution: 1. Hospital 2. Primary Care centre …….....

City: .....

DRUG(S) SUSPECTED TO HAVE CAUSED THE REACTION

(active ingredients)

PHARMACEUTICAL SPECIALITY (brand name)

Posology (mg/h)…….

Total daily dose (mg)…….....

Length of dose interval (h)……. .....

Drug administration: 1. Oral 2. I.V. 3. I.M. 4. Sublingual

5. Rectal 6. Inhaled 7. Others .....

Reason for the prescription (Indication)

Appendix 1

Time of treatment from: (day/month/year).....
to: (day/month/year)…….....

Duration of treatment (days) .....

During this time when did the reaction appear? (nausea, anorexia, abdominal pain, jaundice…….....

Did the reaction disappear after discontinuation of the drug?

1. Yes 2.No 3. Don't know .....

Did the reaction reappear after readministration of the drug?

1. Yes 2. No 3. Don't know .....

Time to recovery (days): [normalisation of TBL, ALT, AST, ALP]

Underlying hepatic conditions? 1. Yes 2.No
Specify……..

Is this a second DILI episode? 1. Yes 2.No

Specify drug in first episode……

DESCRIPTION OF THE ADVERSE REACTION(S)

(Including symptoms (hepatic and extrahepatic) and relevant laboratory finding)

Date of initiation …….

Appendix 1

Blood Pressure:

Heart rate:

Fever: 1.Yes 2.No 3.No data **Jaundice**: 1.Yes 2.No 3.No data

Rash: 1.Yes 2.No 3. No data **Eosinophilia**: 1.Yes 2.No 3. No data

Arthralgia: 1.Yes 2.No 3.No data

Lymphopenia (< 1000/µL): 1. Yes 2.No 3. No data

CONCOMITANT DRUGS PRESCRIBED OR SELF-MEDICATED (EXCLUDING THOSE FOR TREATING THE ADVERSE REACTION)

Prescription	Daily dose	Administration	Dosage	Duration
_____	_____	_____	_____	Start date……...
				Stop date……...
_____	_____	_____	_____	Start date……...
				Stop date……...
_____	_____	_____	_____	Start date……...
				Stop date……...
_____	_____	_____	_____	Start date……...
				Stop date……...

Appendix 1

OTHER RELEVANT CLINICAL INFORMATION

Smoking (cigarettes/day) :

Known allergies: 1.Yes 2.No

Diabetes : 1.Yes 2.No

Hypertension: 1.Yes 2.No

Metabolic syndrome: 1.Yes 2.No

Diagnosis of metabolic syndrome (MS) according to the International Diabetes Federation: Abdominal obesity defined by a waist circumference >94 cm for men and >80 cm for women of European origin (specific values according to ethnicity) and at least 2 of the following criteria: hypertriglyceridemia (>150 mg/dL), low HDL (<40 mg/dL for men and <50 mg/dL for women), hypertension (>130/85 mmHg) and elevated fasting glucose (>100 mg/dL).

Other associated diseases:

DIAGNOSTIC TEST: (abdominal ultrasound, computed tomography, colangiography, etc.).

Date :

BIOPSY: (if performed, date and description, anatomical pathology classification, see attached table at the end)

Appendix 1

OUTCOME OF THE REACTION (indicate as appropriate)

Spontaneous recovery.....

Treatment required..

.....

Persistent injury…….....

Hospitalization required.....

Admission date…… Discharge date…….. ……

Need to prolong hospitalization …….....

Permanent or significant disability.....

Recovery…….....

Life threatening reaction …….....

Death.....

Any other relevant information about the outcome

Physician in charge .....

Date .....

Signature .....

Phone: .....

Fax .....

Appendix 1

	Before treatment (date)(fecha)	Onset (date)	Follow-up (date)	Follow-up (date)	Follow-up (date)
BIOCHEMICAL DATA					
Glucose (mg/dL)					
Urea (mg/dL)					
Creatinine (mg/dL)					
Total Proteins (g/dL)					
Albumin (g/dL)					
alpha-1 (g/L)					
alpha-2 (g/dL)					
Beta (g/dL)					
Gammaglobulines (g/dL)					
Total bilirubin (mg/dL) n= (n=					
Direct bilirubin (mg/dL)					
AST (IU/L)(range)					
ALT (IU/L)(range)					
GGT (IU/L)(range)					
Alk phosp (IU/L)(range)					
Triglycerides (mg/dL)					
Cholesterol (mg/dL)					
HDL-cholesterol (mg(dL)					
LDL-cholesterol (mg/dL)					
Iron (µg/dL)					
Transferrin (mg/dL)					
Copper/Ceruloplasmin					
Immunoglobulin M (g/L)					
Immunoglobulin G (g/L)					
Immunoglobulin A (g/L)					
BLOOD PANEL					
Erythrocytes ($x10^6$/µL)					
Hemoglobin (g/dL)					
Hematocrit (%)					
MCV (fL)					
Platelets ($x10^3$/µL)					
Prothrombin activity (%) INR					
Leukocytes ($x10^3$/µL)					
Neutrophils (%)					
Lymphocytes (%)					
Monocytes (%)					
Eosinophils (%)					
Basophils (%)					

Appendix 1

	Onset (date)	Follow-up (date)	Follow-up (date)
MARKERS			
IgM anti HAV			
HBsAg			
Anti HBs			
Anti HBc IgM			
Anti HCV			
PCR HCV			
Anti HEV IgM			
Anti HEV IgM (PCR)			
CMV IgM			
PCR CMV			
Epstein Barr virus IgM			
Others			
AUTOANTIBODIES			
ANA			
ASMA			
AMA			
Anti LKM-1			
Others			

Appendix 1

OTHER FACTORS OF EXCLUSION

* Viral infections
    - Hepatitis A, B, C, E (endemic in areas of Mexico)
    - AIDS
    - Infectious mononucleosis
    - Others
* Bacterial infections
    - Sepsis
* Parasitis
    - Leishmaniasis, malaria, yellow fever, Dengue hemorrhagic fever, schistosomiasis, Q fever
* Hepatic diseases
    - Previous toxic hepatitis
    - Systematic diseases that change liver functions:
        Inflammatory bowel disease
        Rheumatoid arthritis
        Other autoimmune diseases: SLE, polyarteritis nodosa
        Cardiac/Heart failure
        Thyroid dysfunction
        Severe trauma with multiple blood transfusion
        . Severe hypotension
    - Other hepatic diseases, diffuse neoplasm infiltration, granulomatous liver diseases
* Previous surgical interventions
    - Anesthesias
* Previous radiologic exams: PIV, angiography, others.
* Cancer (Hodgkin's)
* Pregnancy

Appendix 1

* Tatoos
    - Blood transfusions
    - Herbal products and alternative medicines
* Toxins
    - Intoxication with paraquat (herbicide), adhesives, etc.
    - Alcohol consumption
    - Drug addictions

ALCOHOL CONSUMPTION

TYPES OF DRINK:.....

QUANTITY (gram conversion) Cahalan, 1981.....

- a glass of beer	(300 mL)	=	7.7 gram alcohol
- a glass of beer	(200 mL)	=	5.1 g "
- a bottle of beer	(330 mL)	=	8.5 g "
- a glass of wine	(200 mL)	=	14.4 g "
- a small glass of wine	(100 mL)	=	7.2 g "
- a glass of liquor	(45 mL)	=	12.6 g "

DURATION (months) .....

Appendix 1

Table 1: Histopathological terms to be used for liver biopsy reports in patients with suspected drug-induced hepatotoxicity.

CODE	DESCRIPTION
1	HEPATOCELLULAR NECROSIS
11	FOCAL NECROSIS
12	BRIDGE NECROSIS
13	ZONAL NECROSIS
14	MASSIVE NECROSIS
2	STEATOSIS / FATTY LIVER
21	ACUTE STEATOTIC CHANGES
22	STEATOHEPATITIS (NASH)
3	GRANULOMATOUS REACTION
4	ACUTE COLESTASIS
41	CHOLESTASIS WITHOUT HEPATITIS
42	CHOLESTASIS WITH HEPATITIS
43	CHOLESTASIS WITH DUCTAL LESION
5	CHRONIC CHOLESTASIS
51	PROLONGED CHOLESTASIS
52	DUCTOPENIA ("VANISHING BILE DUCT SYNDROME")
53	SCLEROSING CHOLANGITIS
6	CHRONIC HEPATITIS
61	CHRONIC ACTIVE HEPATITIS
62	HEPATIC FIBROSIS AND CIRRHOSIS
7	VASCULAR ALTERATIONS
71	SINUSOIDAL DILATATION (FLARING) AND PELIOSIS
72	NON-CIRRHOTIC PORTAL HYPERTENTION
73	OBSTRUCTION TO THE VENOUS HEPATIC FLOW (Budd-Chiari syndrome)
74	OTHERS
8	HEPATIC TUMORS
81	HEPATOCELLULAR ADENOMA
82	HEPATOCELLULAR CARCINOMA
83	OTHER CARCINOMAS
84	ANGIOSARCOMA
9	UNSPECIFIC CHANGES

Minutes of the Face-to-Face Meeting Pro-Euro-DILI Registry

John B. Hynes Veterans Memorial Convention Center. Boston, 10th of November, 2014.

Attended:

Raúl J. Andrade (Spain); Guruprasad P Aithal (UK); Yves Horsmans (Belgium), Helena Cortez-Pinto (Portugal), Einar S. Björnsson (Iceland), Oren Shibolet (Israel), Dominique Larrey (France), John Dillon (UK), Alexander Gerbes (Germany), Christian Trautwein (Germany)

Apologies:

Antonio Grieco (Italy), Gerd A. Kullak-Ublick (Suiza), Mia Wadelius and Pär Hallberg (Sweden), Sumita Verma (UK).

Current status:

ProEURO DILI Registry was funded by EASL; the funding 50K Euros would be used to develop the database and storage of samples.
Guruprasad Aithal (Nottingham) will set up a system for sample transfer and bear the costs of transportation. At present, all other costs will be born by the participating centres.

Structure:

Raul Andrade (Spain) will lead as the co-ordinating centre from Malaga and is developing the database which is web- based. Database will be ready at the latest by the end of Dec 2014.

Guruprasad Aithal (UK) from Nottingham has developed the fulll protocol ready for ethical submission which will be circulated soon. Nottingham willreceive samples in batches and store them suitable for future studies.

It is expected thatPI for each country will in turn develop network within the respective country and acts as a co-ordinating centre for that particular country.

Objectives:

Our objectives were to develop a long-term registry beyond 2 years; but we need to demonstrate that we can as a network enroll reasonable number of cases in the first year. If we do hen we aim to maintain the registry for years to come.

We would like to focus on deeply phenotyped/ very well worked up cases and we would like them to be identified at the time of acute DILI. Quality of cases and comprehensive set of samples should be the focus with less emphasis on just the number.

In the long term we would aim to apply for grants and run clinical trials as a network depending upon our effectiveness in enrolling.

Inclusion criteria:

These were agreed as detailed in the protocol. It was clarified that paracetamol was NOT included. But clear-cut cases of herbal, complemetary and alternative medications ARE included.

One idea discussed was to get from Biochemistry Dept from the hospital a list of patients who meet the specific liver biochemistry criteria and then an expert reviewing these patients to identify those who are having acute DILI. It was recognized that the review has to be done by an expert to be sure of the diagnosis.

Controls:

These were important element of the registry. One way of identifying control is-whenever a DILI is enrolled,ask Hospital Pharmacy for the list of patients on that particular drug and invite them to give sample. Controls DON't need any other matching (other than the drug).

It is possible that if we enroll patients in acute phase, some may turn out to have alternative pathology which comes to light later on. We agreed that there will be a proportion of patients who will turn out NOT to be DILI, but, these can be used as controls for studies.

Standard operating procedures for all the sample collections were in the protocol including the tubes to be used and processing. Stool samples were particularly interesting, so, an extra effort should be made to collect these.

Ethical approval:

Guruprasad P Aithal was ready to submit for ethical approval. Once that is complete then others could follow as it may be easier for other centres. But, an alternative put forward was that if the protocol is forwarded, then some centres would like to start ethical approval process in parallel.

Meeting was more informal face to face meeting.

Next step:

It will be necessary to have a Teleconference once we were ready to enroll so that all the issues regarding sampling and coding are clear.

We will aim to meet again in EASL in April 2015.

Contact information:

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Biomedical Research Institute of Malaga (IBIMA)- University of Málaga
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Minutes of the Kick-off TC Meeting Pro-Euro-DILI Registry

Málaga, 26th January, 2015.

Attended:

Spain: Raúl J Andrade, M Isabel Lucena, Camilla Stephens, Aurelie Papineau, Mercedes Robles, Inmaculada Medina, Judith Sanabria, Andres Gonzalez
UK: Guruprasad P Aithal, Jane Grove, Ann Daly
Portugal: Helena Cortez-Pinto
Germany: Andreas Benesic
Italy: Anna Licata, Antonio Grieco
Sweden: Mia Wadelius
Switzerland: Kullak Gerd

Apologies:

Alexander Gerbes (Germany), German Soriano (Spain); John Dillon (UK); Felix Stickel (Suiza); Einar S. Björnsson (Iceland); Sumita Verma (UK)

Yves Horsmans (Belgium); Dominique Larrey (France); Christian Trautwein (Germany) tried to join in the meeting but could not get through.

Objective:

Introduction of the study and protocol overview

Study design, methodology and sample collection:

Presented by Camilla Stephens (Spain)

Brief introduction of the study protocol

►The issue of cases with liver profile elevations reaching the DILI criteria at enrolment (DILI recognition) but not at the first sample collection was raised. It was concluded that it is desirable that the first sample collected from each enrolled case fulfils the DILI criteria in terms of liver profile elevations. Hence, the first patient visit for blood extraction should occur as close as possible to the DILI recognition date. However, cases with liver profile elevations not reaching

the DILI criteria at the first sample collection date will not be excluded at this moment. Furthermore, it is desirable to start sample collections of enrolled cases prior to obtaining final results excluding alternative causes. Cases adjudicated as non-DILI could potentially be used as a form of control samples in future biomarker studies.

► Prof Ann Daly suggested increasing the collection of whole blood for DNA extraction to 2 x 5 mL tubes. The suggestion was accepted and should be implemented to the extent it is possible. The two tubes of whole blood (instead of only one as described in the protocol) do not necessarily have to be collected at the same visit, but can be divided between two separate visits.

► Modifications to the original study protocol were presented in the meeting:

• Blood extraction tubes for serum and plasma containing gel separators can be used if more convenient. New SOPs for blood manipulation using these tubes will be sent to all collaborators.

• Whole blood samples should be transferred into 1.2 mL polypropylene cryogenic tubes prior to freezing as vacutainer tubes may break at -80°C.

► The issue of drug-matched controls was raised. It was concluded that controls corresponding to DILI cases with multiple causative agents do not necessarily have to have taken all the causative agents. It is sufficient if the control has taken at least one of the suspected causative agents without developing DILI. Furthermore, it may be difficult to find drug-matched controls for some causative agents, such as herbal and dietary supplements.

Ethical committee approval:

Presented by Camilla Stephens (Spain)

The study protocol sent to the collaborating units in December last year needs to be adapted to the corresponding groups in terms of group leader names and affiliated hospitals. Likewise, distributed patient information sheet and consent form need to be translated into the native language of each collaborating group prior to being presented to local Ethics Committees.
We urge you to start this task as soon as possible in order to not delay the initiation of case enrolments. At this stage only Profs Aithal (Nottingham) and Andrade's (Málaga) groups have obtained Ethical clearance and subsequently initiated case enrolments. The Malaga group has enrolled three potential DILI patients to date.
Any collaborating group that has not received the study protocol, patient information and consent forms should contact the Malaga group (spanishdili@uma.es)

Biobanking issues:

Presented by Jane Grove (UK)

Overview of patient samples to be sent to the Nottingham biobank and shipment procedure. Collaborators should contact Nottingham (jane.grove@nottingham.ac.uk or melanie.lingaya@nottingham.ac.uk) when having complete sample sets of 4-6 cases in order to arrange for shipments to the biobank.

Data registry:

Presented by Camilla Stephens (Spain)

The online database only requires some final touches before being ready for data entry. The Málaga group requested that each collaborating group appoints a group member responsible for data entry and that this person's name and contact details be sent to Málaga (spanishdili@uma.es).
A database tutorial is planned for February. Further information about this tutorial will be sent once the date is finalized and database access has been distributed to the each group representative.

Logo Contest 2015:

Presented by Aurelie Papineau (Spain)

Aurelie suggested creating a logo contest in order to obtain a suitable logo for the Pro-Euro DILI Registry. The winning logo will be used to identify our group and promote all our future activities and communications, such as website, events, flyers etc.

Prize: 400 €
Deadline: 15, March 2015
Evaluation committee: Principal investigator of each group

Application of COST grant:

Presented by Maribel Lucena (Spain)

Prof Lucena gave an overview of the COST (European Cooperation in Science and Technology) grant and application requirements.

Collaborating groups interested in participating in the COST grant application should contact Málaga (spanishdili@uma.es).
Any proposals with regard to the set up of Meetings, Conferences, Workshops, Short-term scientific exchanges, Training schools and Dissemination Activities are welcome.
The deadline for an application submission is 24 March, 2015.

Other issues:

We have a new email account for Pro-Euro DILI issues:

spanishdili@uma.es

Next step:

Next meeting: The EASL Meeting @Vienna (Austria) – April 22-26, 2015
A brief presentation will be done by Profs Guru Aithal and Raul Andrade at the conference to report on the progress of the Pro-Euro-DILI Registry

Contact information:

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Minutes of the Meeting
Pro-Euro-DILI Registry

Vienna, 23rd April, 2015.

Attended:

Raúl J. Andrade, Mª Isabel Lucena and Aida Ortega Alonso (Spain); Guruprasad P Aithal (UK); Marco Biolato (Italy), Alexander Gerbes (Germany), Anna Licata y Pierro Almasio

Apologies:

Antonio Grieco (Italy) who was represented by Marco Biolato; John Dillon (UK); Helena Cortez-Pinto (Portugal); Christian Trautwein (Germany); Gerd A. Kullak (Switzerland); Einar S. Björnsson (Iceland)

Objective:

Feedback on achievements since our last TC in January and an overview of future actions.

Study design, methodology and sample collection:

Questions regarding practical issues of DILI case identification and collection of serial biological samples were clarified. The following agreements were made:
A detailed list of biological samples to be collected at each time, including volumes and number of aliquots to be prepared at each sample collection, will be prepared by the coordinating groups and distributed to all collaborators. A productive discussion around the definition of control subjects led to the agreement that a description of control cases will be prepared with examples for clarification included and distributed to all collaborators.

Ethical committee approval:

In Italy, the Palermo Centre is expected to have its ethical approval of the study protocol in two weeks and will then be able to start recruitment of DILI cases and controls.

Prof A Grieco's group in Rome has submitted the protocol to the local ethics committee and is expecting an approval within the next couple of weeks.

Prof Gerbes is waiting to receive a copy of the ethical committee approval from Notthingham prior to submission to his local ethics committee.

Biobanking issues:

It was discussed and agreed that samples will be collected, alliquoted and stored at -80ºC until shipment to Nottingham. The issue of sample guarantee in case of shipment failure was also commented on. It was suggested that all centres store backup aliquots of their samples to circumvent potential delivery problems.

Data registry:

The first action to accomplish will be to appoint a representative from each collaborating centre, who will be responsible for data entry into the online database registry. A tutorial on how this database is working is planned to be held via telephone before the database is ready for use. The tutorial is expected to take place in the next couple of months.

Logo Contest 2015:

The Pro-Euro-DILI Registry logo contest is still open.

Other issues:

Prof Gerbes commented on the importance of preparing a grant proposal for the next Horizon 2020 health call. He will send contact details of different research organizations that could help us to prepare the grant on a free basis, i.e. a fee is only paid if the proposal is funded.

Profs Aithal and Andrade's groups are reviewing the different topics that will be proposed in the next call to determine which would be best suited for our research area..

Next step:

Each collaborating centre should provide ERB approval feedback
Coordinating groups will send detailed information about samples aliquots and volumes to all collaborating centres

To clarify the definition of DILI controls
To provide a database tutorial prior to data entry initiation
Determine the feasibility of preparing a grant proposal for Horizon 2020

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