Patterns of Drug-induced Liver Injury

Stefan Hübscher,
Institute of Immunology & Immunotherapy, University of Birmingham
Department of Cellular Pathology, Queen Elizabeth Hospital, Birmingham
Drug-induced Liver Injury

1. Incidence

2. Causative Agents

3. Diagnostic Features

4. Patterns of Liver Injury
Drug-induced Liver Injury

1. Incidence

2. Causative Agents

3. Diagnostic Features

4. Patterns of Liver Injury
Drug-induced Liver Injury - Incidence

Å Difficult to determine, due to vast number of drugs consumed (including non-prescribed medications)

Å One prospective population-based study over 3 year period in Dijon, France:
    - annual incidence of 14 per 100,000 inhabitants (Sgro 2002)

Å Another prospective population-based studies over 2 year period in Iceland:
    - annual incidence of 19 per 100,000 inhabitants (Bjornsson 2013)

Å Lower rates (2-3 cases/100,000/year) reported in other studies from U.K. and Sweden probably underestimate true frequency (de Abajo 2004, de Valle 2006)

Å Many other cases of DILI are likely to be asymptomatic or not recognised
Drug-induced Liver Injury - Incidence

<table>
<thead>
<tr>
<th>Liver injury</th>
<th>Incidence of drugs as likely cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaundice</td>
<td>2-10%</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>10-40%</td>
</tr>
<tr>
<td>Acute liver failure</td>
<td>15-50%</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>1%</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>Rare (&lt;1%)</td>
</tr>
</tbody>
</table>
Drug-induced Liver Injury

1. Incidence

2. Causative Agents

3. Diagnostic Features

4. Patterns of Liver Injury
Drug-induced Liver Disease - Causative Agents

1. At least 600 drugs have been implicated in causing liver damage

2. Most of the commonly prescribed drugs are potentially hepatotoxic

3. Some drugs available “over the counter” are potentially hepatotoxic (including serious reactions)

4. Many potentially hepatotoxic agents may not be recognised as drugs or admitted to
<table>
<thead>
<tr>
<th>“RECOGNISED” DRUGS</th>
<th>OTHER AGENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANALGESIC/ANTI-INFLAMMATORY DRUGS</td>
<td>HERBAL MEDICINES</td>
</tr>
<tr>
<td>e.g. aspirin, NSAIDs, paracetamol</td>
<td></td>
</tr>
<tr>
<td>ANTIBIOTICS</td>
<td>ILLICIT DRUGS</td>
</tr>
<tr>
<td>phants, ecstasy</td>
<td>e.g. Cocaine, solvents, ecstasy</td>
</tr>
<tr>
<td>ANTICONVULSANTS</td>
<td>INDUSTRIAL /DOMESTIC CHEMICALS</td>
</tr>
<tr>
<td>e.g. methyldopa, amiodarone</td>
<td>e.g. carbon tetrachloride</td>
</tr>
<tr>
<td>CARDIOVASCULAR AGENTS</td>
<td>VITAMINS</td>
</tr>
<tr>
<td>e.g. methyldopa, amiodarone</td>
<td>e.g. Vitamin A</td>
</tr>
<tr>
<td>ENDOCRINE AGENTS</td>
<td>FOODS</td>
</tr>
<tr>
<td>e.g. oestrogens, oral hypoglycaemics</td>
<td>e.g. mushroom poisoning</td>
</tr>
<tr>
<td>IMMUNOSUPPRESSIVE AGENTS</td>
<td></td>
</tr>
<tr>
<td>e.g. methotrexate, azathioprine</td>
<td></td>
</tr>
<tr>
<td>CHEMOTHERAPEUTIC AGENTS</td>
<td></td>
</tr>
<tr>
<td>NEUROPSYCHIATRIC DRUGS</td>
<td></td>
</tr>
<tr>
<td>e.g. phenothiazines, tricyclic antidepressants</td>
<td></td>
</tr>
</tbody>
</table>
Identifying/Confirming Drugs as Causing Liver Injury
(Bjornsson & Hoofnagle, Hepatology 2016)

Â Causality analysis of 671 drugs identified in the NIH LiverTox website ([http://livertox.nih.gov](http://livertox.nih.gov))

Â Drugs categorised according to number of convincing published reports

<table>
<thead>
<tr>
<th>No of Reports</th>
<th>No of Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 50</td>
<td>48</td>
</tr>
<tr>
<td>12 ÷ 49</td>
<td>76</td>
</tr>
<tr>
<td>4- 11</td>
<td>96</td>
</tr>
<tr>
<td>1-3</td>
<td>126</td>
</tr>
<tr>
<td>0</td>
<td>318</td>
</tr>
</tbody>
</table>

7 drugs known to cause direct dose-dependent liver injury classified separately

Â includes paracetamol, which is commonest drug-related cause of death due to liver failure
Commonest Drugs Implicated in Causing Liver Injury
(Bjornsson, Arch Toxicol 2015)

Table 2  Top ten implicated drugs in three prospective studies on DILI, DILIN study from the USA (Chalasani et al. 2008), a prospective study from Iceland (Björnsson et al. 2013) and Spain (Andrade et al. 2005)

<table>
<thead>
<tr>
<th>DILIN study</th>
<th>Icelandic study</th>
<th>Spanish registry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin–clavulanate</td>
<td>Amoxicillin–clavulanate</td>
<td>Amoxicillin–clavulanate</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Diclofenac</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Azathioprine</td>
<td>RIP + INH + PIZ</td>
</tr>
<tr>
<td>SMZ/TMP</td>
<td>Infliximab</td>
<td>Flutamide</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Nitrofurantoin</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Isotretinoin</td>
<td>Ebrotidina(^a)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Atorvastatin</td>
<td>Diclofenac</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Doxycycline</td>
<td>Atorvastatin</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>Herbalife products</td>
<td>Ticlopidine</td>
</tr>
</tbody>
</table>

RIP + INH + PIZ: rifampin, isoniazid and pyrazinamide
SMZ/TMP sulfamethoxazole/trimethoprim
\(^a\) Withdrawn from the Spanish market in 1998 due to hepatotoxicity
Herbal Medicines

- Review of 185 publications identified at least 60 herbal agents with potential hepatotoxicity (Teschke, Liver International 2012)

- Rising prevalence as cause of liver injury
  - 20% of DILI in US now attributed to herbs/dietary supplements (HDS) (Navarro, Hepatology 2014)
  - >70% of DILI in Singapore and Korea due to HDS (Wai 2007, Suk 2012)

- Patterns of injury ascribed to herbal hepatotoxicity
  - Fatty change
  - Acute hepatitis (including rare severe cases with acute liver failure)
  - Chronic hepatitis
  - Fibrosis
Acute Liver Failure due to Chinese Herbal Medicine

Clinical Summary - Male, age 32
- Sudden onset of jaundice with rapidly progressive liver failure
- Viral and autoimmune serology negative. No other drug history
- Recent treatment with Chinese herbal medicine for subcutaneous lipomatosis
- Liver transplantation 6 weeks after first presented
  - Shrunken liver, weight 730g with areas of collapse

[Image: Panacinar necrosis]
Drug-induced Liver Injury

1. Incidence

2. Causative Agents

3. Diagnostic Features

4. Patterns of Liver Injury
Drug-induced Liver Disease – Clinical Diagnosis  

<table>
<thead>
<tr>
<th>Diagnostic Feature</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Biochemical injury</td>
<td>ALT/AST &gt; 3-5x ULN, Alk Phos &gt; 2xULN, Bilirubin &gt; 2xULN</td>
</tr>
<tr>
<td></td>
<td>Â  Hepatocellular (ALT/Alk Phos ratio ≥ 5)</td>
</tr>
<tr>
<td></td>
<td>Â  Cholestatic (ALT/Alk Phos ratio &lt;2)</td>
</tr>
<tr>
<td></td>
<td>Â  Mixed hepatocellular/cholestatic (ALT/Alk Phos ratio 2-5)</td>
</tr>
<tr>
<td>2. Drug known to be hepatotoxic</td>
<td></td>
</tr>
<tr>
<td>3. Time to onset</td>
<td>Â  Typically 5 days ÷ 3 months</td>
</tr>
<tr>
<td></td>
<td>Â  In some cases liver injury only apparent after months/years (e.g. minocycline, amiodarone, nitrofurantoin)</td>
</tr>
<tr>
<td>4. Exclusion of other known causes of liver injury</td>
<td></td>
</tr>
<tr>
<td>5. Time to recovery</td>
<td>Â  Usually rapid (starts within 1-2 weeks and resolves completely in 2-3 months)</td>
</tr>
<tr>
<td></td>
<td>Â  Occasional cases progress to chronic liver injury, which is less readily reversible (e.g. chronic cholestasis or fibrosis)</td>
</tr>
<tr>
<td>6. Response to re-exposure</td>
<td>May be inadvertent or intentional.</td>
</tr>
</tbody>
</table>
Drug-induced Liver Disease – Clinical Diagnosis
Role of Liver Biopsy

Liver biopsy rarely diagnostic in isolation and no longer considered mandatory for diagnosis (NIDDK/NIH Clinical Research Workshop Ŧ Fontana, Hepatology 2010; 52: 730-742)

May provide pointers towards a likely drug aetiology in cases where this hasn't already been suspected
  Ŧ Includes identifying and classifying the nature of liver injury in patients receiving novel drugs not known to be hepatotoxic

Helpful in identifying the presence/severity of underlying/co-existent liver disease

May identify features relevant for prognosis:
  Ŧ Severity of necrosis predicts progress to liver failure in cases of drug-induced acute hepatitis (Bjornsson 2007 & 2010)
  Ŧ Bile duct injury/loss predicts progression to chronic cholestasis (Degott 1992)
Drug-induced Liver Injury

1. Incidence

2. Causative Agents

3. Diagnostic Features

4. Patterns of Liver Injury
Drug-induced Liver Disease - Patterns Of Liver Injury

(1) Most of the common morphological patterns of liver damage may be caused by drugs

(2) Histological distinction from other causes of liver damage is frequently difficult or impossible
   - Drug-induced liver injury therefore diagnosed by exclusion

(3) For some patterns of liver injury drugs should be considered near the top of the differential diagnosis
Clinico-Pathological Phenotypes of Drug Induced Liver Injury
NIH LiverTox website http://livertox.nih.gov

1. Acute hepatic necrosis
2. Acute hepatitis
3. Cholestatic hepatitis
4. Mixed hepatocellular-cholestatic hepatitis
5. Enzyme elevations without jaundice
   - Hepatocellular
   - Cholestatic
   - Mixed
6. Bland cholestasis
7. Hepatic steatosis and lactic acidosis
8. Nonalcoholic fatty liver
9. Chronic hepatitis
10. Sinusoidal obstruction syndrome (veno-occlusive disease)
11. Nodular regenerative hyperplasia
12. Hepatic adenoma and hepatocellular carcinoma
Liver biopsies from 249 patients with suspected DILI
48 separate histological features assessed
Overall histological features classified into 1 of 18 patterns
5 main patterns accounted for 83% of biopsies:

1. Acute hepatitis 21%
2. Chronic hepatitis 14%
3. Acute cholestasis 9%
4. Chronic cholestasis 10%
5. Cholestatic hepatitis 29%

Prognostic Significance of Histological Findings

Adverse - degree of necrosis, fibrosis stage, microvesicular steatosis, ductular reaction, neutrophils, cholangiolar cholestasis

Favourable – granulomas, eosinophils
Common Patterns of Drug-Induced Liver Injury

1. Cholestasis/cholestatic hepatitis
2. Chronic hepatitis
3. Fatty liver disease
4. Zonal necrosis
5. Granulomas
Common Patterns of Drug-Induced Liver Injury

1. **Cholestasis/cholestatic hepatitis**
2. Chronic hepatitis
3. Fatty liver disease
4. Zonal necrosis
5. Granulomas
Drug-induced Cholestasis – 3 Main Patterns

1. Pure (bland) cholestasis

2. Cholestatic hepatitis

3. Chronic cholestasis (+ duct loss)
“Pure” (bland) Cholestasis

**Histological Features**
- Bilirubinostasis (severe, perivenular)
- No significant inflammation or features to suggest large duct obstruction

**Common examples**
- Oral contraceptive pill
- Anabolic steroids
- Some antibiotics

**Differential diagnosis**
- Bile transporter defects (e.g. BRIC, PFIC)
- Occult malignancy (e.g. Hodgkin’s)
- Sepsis
- Early large duct obstruction
Cholestatic Hepatitis

**Histological Features**
- Bilirubinostasis and lobular hepatitis
- Resembles other forms of acute (cholestatic) hepatitis

**Common examples**
- Numerous (e.g. NSAIDs, anti-convulsants, anti-infective agents, statins etc)

**Differential diagnosis**
- Acute viral hepatitis
- Acute autoimmune hepatitis
- Seronegative hepatitis

**Features favouring a drug reaction**
- Disproportionately severe bilirubinostasis, with only mild inflammation
- Sharply-circumscribed areas of centrilobular necrosis
- Eosinophils, granulomas
**Chronic Cholestasis**

**Histological Features**
- Ductopenia (some cases may be preceded by inflammatory duct lesions)
- Ductular reaction/chronic cholestasis
- Biliary fibrosis/cirrhosis

**Common examples**
- Phenothiazines
- Some antibiotics (e.g. augmentin)
- Carbamazepine

**Differential diagnosis**
- PBC & PSC
- Other ductopenic chronic biliary diseases
  - Portal inflammation, ductular reaction and fibrosis less prominent in drug-induced chronic cholestasis
  - Ductopenia may be reversible if causative agent removed (Vuppalanchi 2006, Watkins 2006)
Common Patterns of Drug-Induced Liver Injury

1. Cholestasis/cholestatic hepatitis

2. **Chronic hepatitis**

3. Fatty liver disease

4. Zonal necrosis

5. Granulomas
Drug-induced Autoimmune Hepatitis

- Biochemical, serological and histological features closely resembling classical autoimmune hepatitis
  - Mostly type 1 AIH (ANA, SMA positive)
  - Less commonly type 2 AIH (LKM positive)

- At least 15 drugs implicated
  - Common examples include methyldopa, minocycline, nitrofurantoin
    - 24/261 (9%) of patients with AIH at Mayo Clinic = drug-induced (Bjornsson 2010)
    - 11 nitrofurantoin, 11 minocycline

- Liver injury may only become apparent after years of use

- Response to treatment similar to AIH
  - No relapse after treatment withdrawn (up to 65% in AIH)
Drug-induced Autoimmune Hepatitis – Histological Features

Similar to classical AIH
- Plasma cell rich portal inflammation
- Interface hepatitis
- Hepatocyte rosettes
- Emperiploesis

- Fibrosis tends to be absent/mild
  - Advanced fibrosis/cirrhosis not seen in 31 cases reported by Bjornsson (2010) and Susuki (2011) compared with up to 30% of cases with classical AIH
Common Patterns of Drug-Induced Liver Injury

1. Cholestasis/cholestatic hepatitis
2. Chronic hepatitis
3. Fatty liver disease
4. Zonal necrosis
5. Granulomas
Drug-Induced Fatty Liver Disease
Macrovesicular Steatosis

- Numerous drugs implicated in causing "simple steatosis"

- Some may also be associated with steatohepatitis
  - directly (e.g. amiodarone) or as a co-factor (e.g. methotrexate and NAFLD)

- Overall drug-induced steatosis/steatohepatitis much less common than alcoholic and metabolic (non-alcoholic) fatty liver disease
Drug-Induced Fatty Liver Disease

Microvesicular Steatosis

Aetiology

Drug-related
- tetracycline, valproate, nucleoside analogues (e.g. didanosine, stavudine, zidovudine)

Other causes
- Reye’s syndrome (?salicylates as precipitating factor), acute fatty liver of pregnancy, congenital mitochondrial cytopathies

Clinical Course & Outcome

Often associated with features of severe liver dysfunction, including acute liver failure and other metabolic problems (e.g. lactic acidosis)

Mitochondrial injury with impaired beta oxidation is important mechanism
- Direct inhibition of mitochondrial function (e.g. tetracycline) presents early (7-28 days)
- Inhibition of mitochondrial DNA synthesis (e.g. nucleoside analogues) present later (2-3 months)
Microvesicular Steatosis - Histological Features

- Hepatocytes with ballooned/foamy/clear cytoplasm
- Lack of inflammation
- Glycogen depletion
- Presence and severity may be difficult to assess in H&E sections
- Stains for lipids may be helpful to confirm the presence/severity of microvesicular steatosis
- Mitochondrial abnormalities (enlarged mitochondria with loss of cristae) can be seen on electron microscopy
Common Patterns of Drug-Induced Liver Injury

1. Cholestasis/cholestatic hepatitis
2. Chronic hepatitis
3. Fatty liver disease
4. Zonal necrosis
5. Granulomas
Zonal Necrosis due to Toxic Liver Injury

- Severe cases associated with:
  - rapidly progressive liver failure (usually unsuitable for liver biopsy)
  - massively elevated transaminase levels (100-1000x normal)
## Patterns of Zonal Necrosis

<table>
<thead>
<tr>
<th><strong>Distribution of Necrosis</strong></th>
<th><strong>Examples</strong></th>
</tr>
</thead>
</table>
| Perivenular (zone 3)        | Paracetamol (acetaminophen)  
Carbon tetrachloride  
Mushroom poisoning  
Some herbal medicines |
| Periportal (zone 1)         | Ferrous sulphate  
Phosphorus |
| Mid-zonal                   | Bacillus cereus toxin |

Zonal distribution may reflect:

- heterogeneous distribution of enzymes involved in drug metabolism (e.g. P450 enzymes and paracetamol)
- factors related to blood supply (e.g. highest concentrations of ferrous sulphate in periportal regions)
### Toxic injury in acute liver failure

<table>
<thead>
<tr>
<th></th>
<th>TOXIC (e.g. paracetamol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pattern of necrosis</strong></td>
<td>Coagulative</td>
</tr>
<tr>
<td></td>
<td>(may appear lytic later)</td>
</tr>
<tr>
<td><strong>Distribution of necrosis</strong></td>
<td>Uniform</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td>+/-</td>
</tr>
</tbody>
</table>
Acute Paracetamol Toxicity
Uniform Severity of Necrosis (perivenular & mid-zonal regions)
Acute Paracetamol Toxicity
Coagulative Necrosis with Little/No Inflammation
Acute Paracetamol Toxicity
Sublethal Injury in Periportal Hepatocytes – Ballooning, Fat, Cholestasis
Acute Paracetamol Toxicity
Intact Reticulin Framework
### Toxic versus hepatitic injury in acute liver failure

<table>
<thead>
<tr>
<th>Pattern of necrosis</th>
<th>TOXIC (e.g. paracetamol)</th>
<th>HEPATITIC (e.g. viral, drugs, autoimmune)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coagulative (may appear lytic later)</td>
<td>Lytic (with collapse)</td>
</tr>
<tr>
<td>Distribution of necrosis</td>
<td>Uniform</td>
<td>Patchy</td>
</tr>
<tr>
<td>Inflammation</td>
<td>+/-</td>
<td>+++/+++</td>
</tr>
</tbody>
</table>

**BUT**

1. Some hepatotoxic drug agents may induce a "second wave" of inflammatory/immune mediated liver injury
2. Some hepatitic drug reactions may be associated with disproportionately severe zonal necrosis, suggesting a component of cytopathic injury (e.g. halothane)
Common Patterns of Drug-Induced Liver Injury

1. Cholestasis/cholestatic hepatitis
2. Chronic hepatitis
3. Fatty liver disease
4. Zonal necrosis
5. Granulomas
Drug-induced Hepatic Granulomas

At least 60 drugs implicated

- Commoner examples include phenylbutazone, sulphonamides, allopurinol, phenothiazines, penicillins
- Many others are single case reports

Account for 2.5% - 10% of granulomas in studies of liver biopsies (Gaya 2003, Drebber 2008)

Various patterns of granuloma formation described

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Examples of drugs implicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioid</td>
<td>allopurinol, carbamazepine, phenylbutazone</td>
</tr>
<tr>
<td>Fibrin-ring</td>
<td>allopurinol</td>
</tr>
<tr>
<td>Mineral-oil</td>
<td>mineral oil ingestion</td>
</tr>
<tr>
<td>Foreign body</td>
<td>chronic metal exposure, IV drug abuse</td>
</tr>
<tr>
<td>Necrotising</td>
<td>BCG (may also contain acid-fast bacilli)</td>
</tr>
</tbody>
</table>
Drug-induced Hepatic Granulomas

- Difficult to assess due to numerous other possible causes
- May occur as isolated finding
- Often associated with other forms of liver injury (e.g. cholestatic, hepatitic)
- Generally indistinguishable from other causes of hepatic granulomas
- Presence of eosinophils or vasculitis (rare) may point to a drug aetiology
Drug-induced Granulomatous Vasculitis
Phenytoin-induced
Summary
Drug-induced Liver Disease - Liver Biopsy Interpretation

Always consider drugs in the differential diagnosis of any pattern of liver injury
- most drugs are potentially hepatotoxic
- most forms of liver injury are potentially drug-induced

Index of suspicion should be increased if:
- liver biopsy shows a pattern of damage for which drugs are a more likely cause (e.g. "pure" cholestasis)
- atypical features are present in another pattern of liver injury (e.g. acute hepatitis with disproportionately severe cholestasis or necrosis)
Final diagnosis depends on clinico-pathological correlation, including exclusion of other diseases which may produce a similar pattern of liver injury.

Remember that a number of potentially hepatotoxic agents may not be identified by routine history taking:

- Natural/herbal remedies
- Over the counter non-prescribed drugs
- Illicit drugs
- Recently administered drugs, no longer being taken (e.g. antibiotics)
LiverTox provides up-to-date, accurate, and easily accessed information on the diagnosis, cause, frequency, patterns, and management of liver injury attributable to prescription and nonprescription medications, herbs and dietary supplements. LiverTox also includes a case registry that will enable scientific analysis and better characterization of the clinical patterns of liver injury. The LiverTox website provides a comprehensive resource for physicians and their patients, and for clinical academicians and researchers who specialize in idiosyncratic drug induced hepatotoxicity.

LiverTox content produced by the NIDDK and NLM is in the public domain and its free use is encouraged. It is requested that any subsequent published use be given appropriate acknowledgement.

For more information about LiverTox, see About Us.