Il danno d’organo alcol correlato: Da Morgagni alla genetica

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The Pathology applied to the clinical medicine

- **Giovanni Battista Morgagni (1682-1771, Forlì).**
- He belonged to the school as a derivation Galilean, because he was a pupil of Antonio Maria Valsalva (1666-1723), who in turn was a pupil of Malpighi, Malpighi of Borelli, and he was a pupil of Galileo.
- Morgagni had a iatrofisic conception, but he was not using the microscope, used the eye: he had a tendency to try to discover the human body as a machine.
Giovanni Battista Morgagni

- Doctor, poet, archaeologist, classicist
- Chair of Anatomy at the University of Padua in 1629, remaining for fifty-six years
- Unitary vision of the anatomy and pathology of the one part and the other part of the clinical data
- Link between autopsy and clinical data
- Founder of modern pathological anatomy
Giovanni Battista Morgagni

- Clinical history and necropsy description of at least 700 cases
- Original findings: liver cirrhosis, hepatisation lung in pneumonia, many tumors
- First to demonstrate that the brain abscess is a consequence and not the cause of purulent otitis
HEPATIC CIRRHOSIS
Morphological alterations

- Diffuse process
- Fibrosis
- Nodules
- Alteration of the normal architecture
- Rigeneration

- Micronodular (<3mm)
- Macronodular (>3mm)
- Mixed micro/macronodular
- Septal incomplete
- “Cardiac Cirrhosis”
Pubblicazioni scientifiche

Pubblicazioni scientifiche “storie” su Pancreas ed Alcol

Il danno d’organo alcol correlato

- Sistema nervoso centrale (Wernicke’s syndrome; Korsakoff’s syndrome; degenerazione cerebellare; disturbi del comportamento e psichiatrici)
- Sistema nervoso periferico (neuropatia)
- Apparato gastrointestinale (esofago, stomaco, pancreas, fegato)
- Tumori
- Sistema emopoietico
- Sistema cardiovascolare
- Sistema genito-urinario, funzione sessuale
- Sviluppo fetale
- Apparato muscolare e scheletrico
- Modificazioni ormonali (surrene, tiroide)
Prolonged consumption of moderate doses of alcohol and
in vitro gastro-duodenal and ileal contractility in the rat

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Abstract. The effects of chronic feeding with moderate
doses of ethanol (3% vol/vol in drinking water for 8
weeks), which do not induce tolerance, dependence
and withdrawal, on the contractility of gastric, duodenal and ileal strips from rats were investigated. Only

It is concluded that, in the rat, moderate doses of ethanol given chronically impair both spontaneous
and tonic contractility of the stomach and duodenal muscle without affecting ileal contraction. It is possible that motility defects in the gut exposed to ethanol concentrations which do not cause tolerance, dependence or withdrawal in the rat may be due to a local rather than a systemic effect on the smooth muscle.
Liver transplantation in Europe. Indications in cirrhosis. Data from European Liver Transplantation Registry (ELTR) 2010
Natural history of alcoholic liver disease (ALD). The spectrum of ALD is comprised of steatosis, steatohepatitis, fibrosis, cirrhosis, and superimposed hepatocellular carcinoma.
Spectrum of Alcohol-Induced Hepatic Pathology

- Normal
- Steatosis: 80-90%
- Steatohepatitis: 30-40%
- Repair and regeneration
- Hepatocellular carcinoma: 1-2%
- Cirrhosis: 15-20%

Factors:
- Gender
- Race/ethnicity
- Genetic factors
- Nutrition
- Chronic diseases
- Obesity
Alcoholic Liver Disease

Robert S. O’Shea, Srinivasan Dasarathy, Arthur J. McCullough, and the Practice Guideline Committee of the American Association for the Study of Liver Diseases and the Practice Parameters Committee of the American College of Gastroenterology

Clinical Practical Guidelines

EASL Clinical Practical Guidelines: Management of Alcoholic Liver Disease

European Association for the Study of the Liver* †
Pathogenesis of ALD

• Although many individuals consuming more than 60 g of alcohol per day develop steatosis, only a minority of the patients with steatosis progress to ASH and 10–20% eventually develop cirrhosis.

• Genetic and nongenetic factors modify both the individual susceptibility and the clinical course of ALD.

• The mechanisms of ALD are not completely understood and the pathogenesis varies in different stages of the disease.
Alcoholic fatty liver: pathogenetic factors

(1) Increased generation of NADH caused by alcohol oxidation, favouring fatty acid and triglyceride synthesis, and inhibiting mitochondrial $b$-oxidation of fatty acids.

(2) Enhanced hepatic influx of free fatty acids from adipose tissue and of chylomicrons from the intestinal mucosa.

(3) Ethanol-mediated inhibition of adenosine monophosphate activated kinase (AMPK) activity resulting in increased lipogenesis and decreased lipolysis by inhibiting peroxisome proliferating-activated receptor $\alpha$ (PPAR$\alpha$) and stimulating sterol regulatory element binding protein 1c (SREBP1c).

(4) Damage to mitochondria and microtubules by acetaldehyde, results in a reduction of NADH oxidation and the accumulation of VLDL, respectively.
Oxidative Pathways of Alcohol Metabolism
ALD: CONSEQUENCES OF INCREASED NADH/NAD+ RATIO:

• Alcoholic hypoglycemia
• Alcoholic acidosis
• Hyperuricemia
• Hypertriglycerideridemia
• Hypoxia
Alcoholic steatohepatitis - 1

- Alcoholic fatty livers can develop parenchymal inflammation (mainly by PMN cells) and hepatocellular damage, a prerequisite for progress to fibrosis and cirrhosis.

- Various factors may contribute to the development of ASH:
  - Acetaldehyde-induced toxic effects (protein, DNA, mitochondria, glutathione)
  - Impaired ubiquitin–proteasome pathway leading to hepatocellular injury and hepatic inclusions of aggregated cytokeratins (i.e. Mallory–Denk bodies)
  - Reactive oxygen species (ROS) generation and the resulting lipid peroxidation with DNA adduct formation
  - Pro-inflammatory cytokines
Alcoholic steatohepatitis – 2
ROS generation and DNA adduct formation

• Main sources of ROS include CYP2E1-dependent MEOS, mitochondrial electron transport system of the respiratory chain, NADH-dependent cytochrome reductase, and xanthine oxidase.

• Moreover, chronic alcohol intake markedly up-regulates CYP2E1, which metabolizes ethanol to acetaldehyde and parallels the generation of ROS and hydroxyl–ethyl radicals.
Alcoholic steatohepatitis – 3
Pro-inflammatory cytokines

- Alcohol metabolites and ROS stimulate signaling pathways such as NFκB, STAT-JAK, and JNK in hepatic resident cells, leading to the local synthesis of inflammatory mediators such as TNFα and CXC chemokines (e.g. interleukin-8), as well as osteopontin.

- Alcohol consume also results in changes in the colonic microbiota and increased intestinal permeability, leading to elevated serum levels of lipopolysaccharides that induce inflammatory actions in Kupffer cells via CD14/TLR4.

- The resulting inflammatory milieu in the alcoholic liver leads to PMN infiltration, ROS formation and hepatocellular damage.
Fibrosis progression

• Alcohol metabolites such as acetaldehyde can directly activate hepatic stellate cells (HSC), the main collagen producing cells in the injured liver. HSC can also be activated paracrinically by damaged hepatocytes, activated Kupffer cells and infiltrating PMN cells.

• These cells release fibrogenic mediators such as growth factors (TGFβ1, PDGF), cytokines (leptin, angiotensin II, interleukin-8, and TNFα), soluble mediators (nitric oxide), and ROS.

• ROS stimulate pro-fibrogenic intracellular signaling pathways in HSC including ERK, PI3K/AKT, and JNK, up-regulate TIMP-1 and decrease the actions of metalloproteinases, thereby promoting collagen accumulation.

• Cells other than HSC can also synthesize collagen in ALD. They include portal fibroblasts and bone-marrow derived cells.
Fibrosis progression in ALD

**CHRONIC ALCOHOL ABUSE**

- ↑ GUT permeability
- ↑ serum LPS
- ↑ Acetaldehyde
- ↓ NK cell activity
- Decreased antioxidant defense
- ↓ IFN-γ
- Mitochondrial damage
- Oxidative stress
- TGFβ

**Inflammation**

- TLR4 activation
- NF-κB activation
- CB1 receptor
- Cannabinoids

**Apoptosis**

- TGFβ

**FIBROSIS**

- ↓ adiponectin
- ↑ leptin

**Obesity**
Risk factors for disease progression in alcoholic liver disease

• Non-genetic or environmental factors:
  – amount and type of alcoholic beverage,
  – the duration of consume
  – patterns of drinking.

• Genetic or host factors:
  – Gender, ethnicity,
  – coexisting conditions such as metabolic syndrome, iron overload, and infection with chronic hepatitis viruses
  – Host genetic factors
Factors of progression in ALD
Diet related risk factor for fibrosis progression

• Obesity and metabolic syndrome

• Several studies show that obesity is the single most important risk factor determining the risk of cirrhosis in heavy drinkers.

• The synergy between obesity and heavy alcohol intake presumably reflects similar mechanisms of disease for both ALD and non-alcoholic fatty liver disease, along with the direct fibrogenic effects of expanded larger mass of adipose tissue (via high levels of noradrenaline, angiotensin II and leptin, and low levels of adiponectin).
Genetic susceptibility to ALD

• Comparison of the allelic and/or genotypic frequencies of certain genetic variants (i.e. single nucleotide polymorphisms; SNP) between alcoholic cirrhosis and alcoholics without liver disease or healthy controls

• While there were significant associations between certain genetic variants and the risk of alcoholism, no overall association of any of the tested SNPs with alcoholic cirrhosis was detected.

• Recently, two candidate gene case control studies in alcoholics found a significant association between the risk of alcoholic cirrhosis and carriage of genotype PNPLA3 rs738409 (GG) in Mestizo subjects and Caucasians.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Patients</th>
<th>Results</th>
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<tbody>
<tr>
<td>Tian et al. *</td>
<td>Genetic case control</td>
<td>482 cases (alcoholic cirrhosis)</td>
<td>rs738409 GG associated with cirrhosis when compared to controls (OR 2.25, 1.74-2.9; 1.7 \times 10^{-9}) and to non-cirrhotic ALD (OR 1.43, 1.15-1.78, 1.0 \times 10^{-9}) Association robust after ancestry correction (OR 1.81, 1.36-2.41; 4.7 \times 10^{-8})</td>
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<td>434 non-cirrhotic ALD</td>
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<td>305 alcoholics w/o liver enzyme elevations</td>
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<td>Seth et al. *</td>
<td>Genetic case control (British Caucasians)</td>
<td>266 cases (alcoholic cirrhosis)</td>
<td>rs738409 G homozygosity associated with alcoholic cirrhosis (OR 7.34, 2.19-24.52, p=0.0012)</td>
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<td></td>
<td>182 controls (heavy drinkers w/o clinical ALD)</td>
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<td>Carriage of rs738409 G allele associated with alcoholic cirrhosis (OR 1.95, 1.34-2.84, p=0.00002)</td>
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<td>Trépo et al. *</td>
<td>Genetic case control (Belgium Caucasians)</td>
<td>330 cases (97% biopsy-proven ALD; 283 cirrhotics)</td>
<td>rs738409 G associated with ALD (OR 1.54; 1.12-2.11, p=0.008) and alcoholic cirrhosis (OR 2.08; 1.15-3.77, p=0.02)</td>
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<td>328 controls (healthy individuals without ALD)</td>
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<td>PNPLA mRNA expression inversely correlated with cirrhosis and portal pressure</td>
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<td>Stickel et al. *</td>
<td>Genetic case control (German Caucasians)</td>
<td>Multicenter sample with 1,043 alcoholics (210 cirrhosis, 394 non-cirrhotic ALD, 439 alcoholic controls Population-based sample with 376 alcoholics (269 non-cirrhotic ALD, 107 alcoholic controls Non-alcoholic healthy subjects (n=162)</td>
<td>Genotype rs738409 GG associated with alcoholic cirrhosis (OR 2.79, 1.55-5.04, p=1.18 \times 10^{-5}, cirrhosis vs. controls) Genotype rs738409 GG associated with alanine aminotransferase elevation (OR 2.33, 1.27-4.26, p=0.0085) Confirmation of association in separate replication sample (OR 4.75, 1.08-20.9, p=0.04, ALD vs alcoholic controls) Population-attributable risk of rs738409 to cirrhosis 26.6%</td>
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Risk factors for ALD and fibrosis progression

(1) Large genome-wide association studies should identify the genetic determinants implicated in individual susceptibility to develop ALD.

(1) E.g.: Whether PNPLA3 genotype represents a marker that will assist decision-making in clinical practice remains to be shown, as well as whether it could serve as a therapeutic target.

(2) The interaction between environmental and genetic factors should be investigated.

(3) Additional studies are required to identify the factors influencing disease regression after drinking cessation and long-term outcome in abstinent patients.
ALD pathogenesis: from Morgagni To genes and DNA damage

Oxidative stress

\[ \uparrow \text{p66Shc} \]

\[ \text{ERK} \rightarrow \text{Nrf2} \rightarrow \downarrow \text{ARE genes} \]

\[ \text{ROS} \rightarrow \uparrow \text{DNA Damage} \rightarrow \text{Tissue Cell Injury} \]

\[ \text{Akt} \rightarrow \text{FoxO3a} \]

Perrini, Palmieri, Giorgino, Palasciano, 2013, submitted