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Biochemical examination of liver function

General Medicine

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Biochemical examination of liver function

The liver has an indispensable role in the intermediary and energetic metabolism of sugars, lipids and nitrogen-containing compounds; it provides inactivation and excretion of many endogenous or exogenous substances, as well as synthesis of plasmatic proteins and blood clotting factors. Diseases of the liver alter many biochemical parameters in the serum, and so, in turn, biochemical examination is important for diagnostics of liver diseases.

Understanding the liver morphology is critical for adequate interpretation of the liver biochemical tests. About 72 % of the liver tissue is hepatocytes; the Kupffer cells, endothelium and adipose cells together form another 8 %; bile tracts contribute 1 %; and the rest is extracellular fluid. While in an acute liver damage mainly hepatocytes are affected, in other diseases impairment of bile flow leading to predominant damage to bile capillary cells may prevail.

1. Overview

Tests for examination of function of the liver and biliary tract can be divided into several groups:

- **Markers of hepatocyte damage:**

If the hepatocyte cell membrane is damaged e.g. by inflammation or hypoxia, it becomes more permeable and allows leakage of hepatocyte **cytosolic enzymes** into the extracellular space. The most sensitive indicator is leakage of **alanine aminotransferase (ALT)**; other enzymes leaving hepatocytes for circulation are **cytosolic isoenzyme of aspartate aminotransferase (cAST)** and **liver isoenzyme of lactate dehydrogenase (LD5)**. Increase of these cytosolic enzymes in serum is a marker of reversible damage of hepatocytes.

In more serious liver diseases accompanied by destruction (necrosis) of hepatocytes not only the cytosolic enzymes, but also **enzymes localized to mitochondria** find their ways to the circulation. Especially **glutamate dehydrogenase (GMD)** and **mitochondrial isoenzyme of aspartate aminotransferase (mAST)** have diagnostic significance. Elevated activities of these mitochondrial enzymes suggest severe, irreversible liver damage.

- **Markers of cholestasis:**

Cholestasis (bile accumulation) can result from a **mechanical cause** (obstruction of the biliary tract by a concrement or tumor), or from a **functional alteration** due to e.g. inflammation, effects of some drugs or hereditary disorders. Cholestasis is accompanied by impairment of bilirubin secretion, and also by increase in activities of enzymes localized to bile duct endothelium and canalicular membrane of hepatocytes. In clinical practice, **bilirubin in serum and urine**, **urobilinogen in urine** (see also the lesson on bile pigments), and enzymes **alkaline phosphatase (ALP)** and **γ -glutamyl transferase (GGT)** are commonly examined.

- **Markers of biosynthetic liver function:**

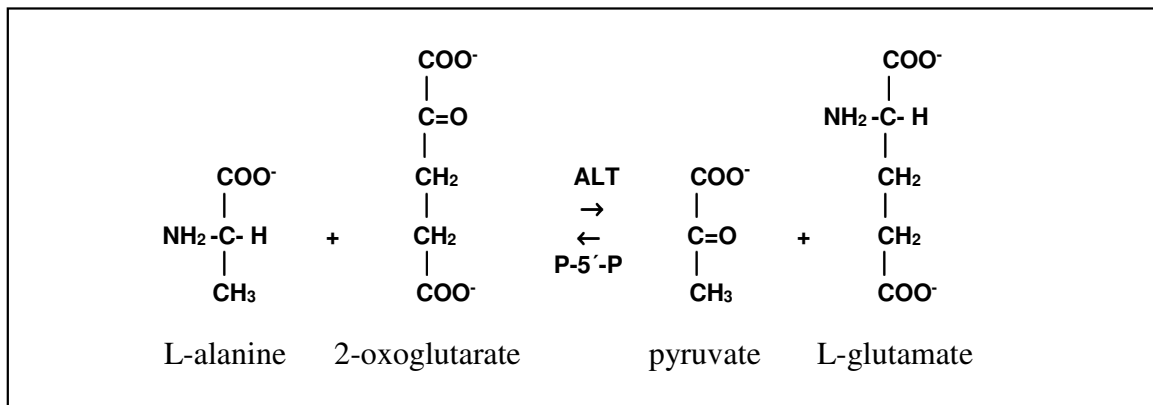
Intensity of proteosynthetic liver function reflects predominantly condition of the granular endoplasmic reticulum in the hepatocytes, where synthesis of most plasma proteins takes place. Unlike the cytosolic and mitochondrial enzymes whose activities rise due to liver damage, markers of liver proteosynthesis decrease under pathological condition. For diagnostic purposes, **serum cholinesterase**, **serum albumin**, **transferrin** and **coagulation factors** are estimated.

2. Aminotransferases

Aminotransferases are enzymes that catalyze reversible transfer of the amino group from an amino acid to a keto acid. Pyridoxal-5'-phosphate serves as a cofactor. In clinical biochemistry, **alanine aminotransferase** and **aspartate aminotransferase** are the most significant.

2.1. Alanine aminotransferase (ALT):

ALT catalyzes a reversible transfer of amino group from alanine to 2-oxoglutarate:



ALT is present mostly in the **liver**; its activity in other organs (skeletal muscle, myocardium, etc.) is much lower. Unlike AST it localizes **only to the cytosol**.

Estimation of ALT is a sensitive and relatively specific test for hepatocyte damage. Its activity in serum rises even in a **small damage of the liver cell**, caused by **increased permeability of the cell membrane**. In inflammation of the liver (viral hepatitis), for instance, elevation of ALT is the earliest indicator that hepatocyte cell membrane integrity is compromised. Repeated ALT estimation is suitable for monitoring course of the disease.

Reference values:

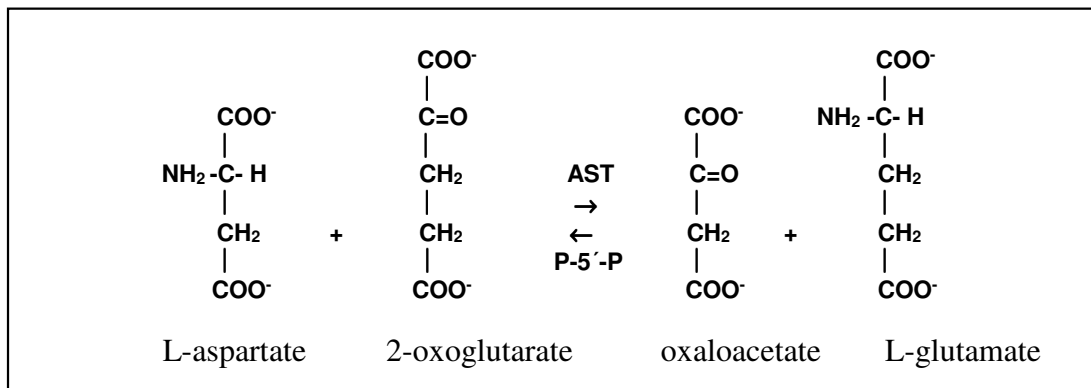
Catalytic concentration of serum ALT (S-ALT):

Men up to 0.80 μkat/l

Women up to 0.60 μkat/l

2.2. Aspartate aminotransferase (AST):

AST catalyzes a reversible transfer of amino group from aspartate to 2-oxoglutarate:



AST occurs in numerous organs: **liver, heart, skeletal muscle, kidney, pancreas, and red blood cells.**

It exists in two isoenzymes: **mitochondrial** (about 70 %), and **cytosolic** (about 30 %). Cytosolic fraction is readily released into circulation due to mild alterations of hepatocyte cell membrane permeability. In contrast, the mitochondrial fraction is released only after **destruction (necrosis) of the hepatocyte**. Therefore, a high increase of serum AST is a marker of hepatocyte destruction, because both isoenzymes are likely to participate in the increase.

Since AST is not specific for the liver tissue, it can be elevated also in **damage of skeletal muscle and myocardium**. AST in blood rises in acute myocardial infarction (heart stroke) and following heart surgery, but also due to a long lasting strenuous physical exercise.

Hemolysis of the sample can cause false positive results of AST estimation, since quite high levels of the enzyme are present in the erythrocytes.

Reference values:

Catalytic concentration of serum AST (S-AST):

Men up to 0.85 $\mu\text{kat/l}$

Women up to 0.60 $\mu\text{kat/l}$

2.3. Evaluation of examination of aminotransferases

Aminotransferases are widely used in the diagnostics of liver diseases. In the **acute viral hepatitis**, typically both AST and ALT activities are markedly increased. Two to three fold elevation is noted as early as in the prodromal stage, it peaks (up to 100 $\mu\text{kat/l}$) the 7th – 12th day after appearance of icterus, and normalizes usually the 5th – 8th week. Strong (even 10-fold) but transient increase of aminotransferases is observed in severe gallstone colics. Other hepatic lesions are usually accompanied by rather moderate elevations (max. 5-fold) of ALT and AST. In chronic liver diseases, such as liver cirrhosis, the activities of aminotransferases are often only slightly above the upper reference limits.

In general the extent of aminotransferase increase correlates well with severity of liver damage. However, if a large part of hepatic tissue has been lost due to e.g. liver cirrhosis, the number of cells that can still release the aminotransferases may be so low that serum levels of aminotransferases do not exceed the reference limits even in a severe liver damage.

Moderate non-specific increase of both aminotransferases can also be observed after strenuous physical exercise (release from skeletal muscle), and also in obese persons.

The severity of hepatocyte damage can also be assessed from the ratio AST/ALT, also known as **De Ritis Quotient**. A value higher than 1 is considered as a sign of unfavorable prognosis.

2.4. Methods for estimation of aminotransferases

Aspartate aminotransferase (Fig.1):

The estimation of AST is based on the Warburg optical test. In the first enzyme reaction, catalyzed by AST from the sample, oxaloacetate is formed. In the next, indication reaction, malate dehydrogenase reduces oxaloacetate to malate and simultaneously oxidizes NADH to NAD⁺. Activity of AST is determined kinetically as decrease in absorbance of reduced NADH at 334, 340 or 365 nm.

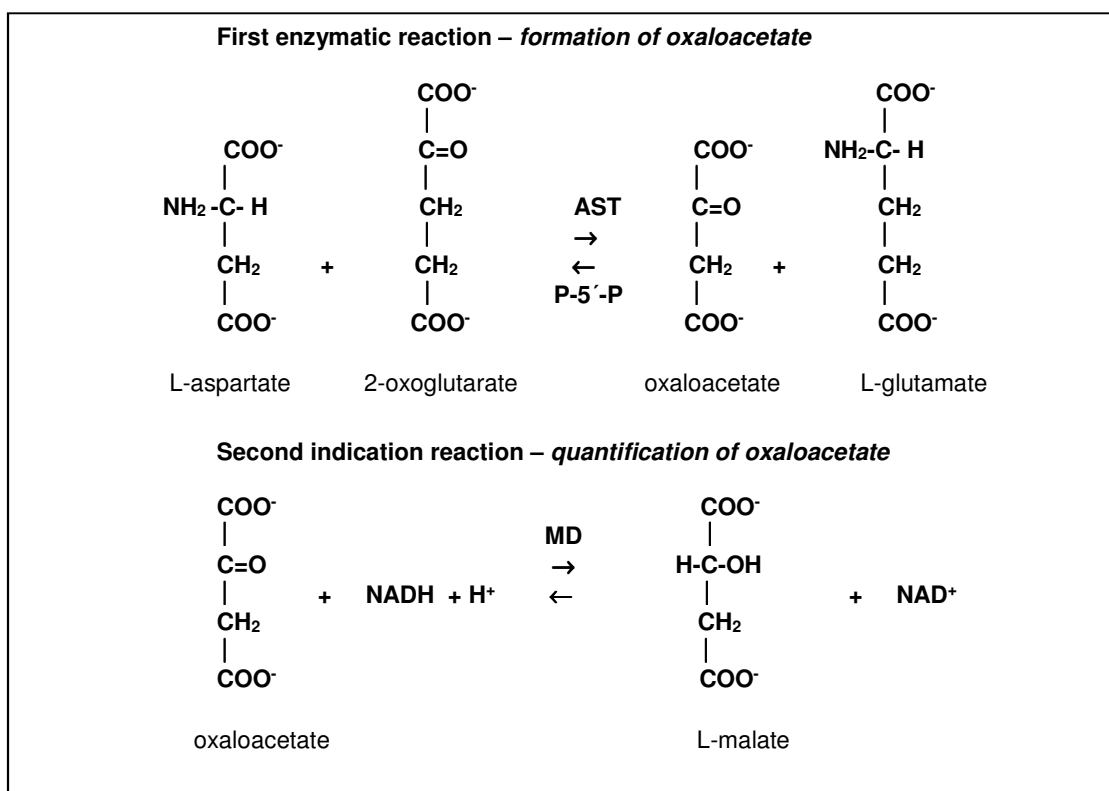


Fig. 1: Principle of estimation of aspartate aminotransferase

The reaction mixture for AST assay contains in addition to the substrates (L-aspartate and 2-oxoglutarate), NADH and malate dehydrogenase also pyridoxal-5'-phosphate and lactate dehydrogenase. Addition of pyridoxal-5'-phosphate saturates the enzyme with its essential cofactor and ensures full enzyme activity. Lactate dehydrogenase is necessary for reduction of any other NADH-dependent keto acids in the sample, otherwise false, higher values could be obtained. These reactions take place during 5–15minute pre-incubation of the mixture without 2-oxoglutarate. Next, the AST reaction is started with 2-oxoglutarate and the change in absorbance (ΔA) is read in one-minute intervals for several minutes.

Alanine aminotransferase (Fig. 2):

The assay for ALT is again based on the Warburg optical test and is in principle very similar to the estimation of AST, except that in the first enzyme reaction, alanine serves as donor of amino group instead of aspartate, and pyruvate is formed. The indication reaction is catalyzed by lactate dehydrogenase that simultaneously reduces any endogenous keto acids. Just as for AST, the procedure requires 5–15 minutes of pre-incubation, then the reaction is started with 2-oxoglutarate.

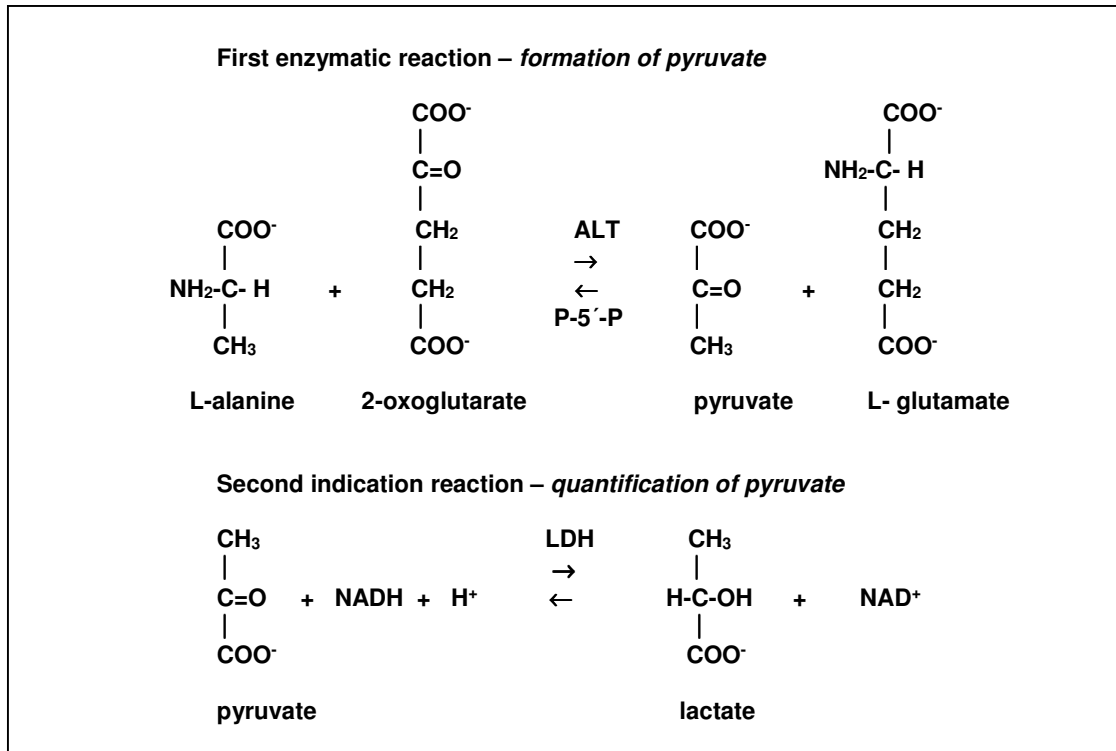
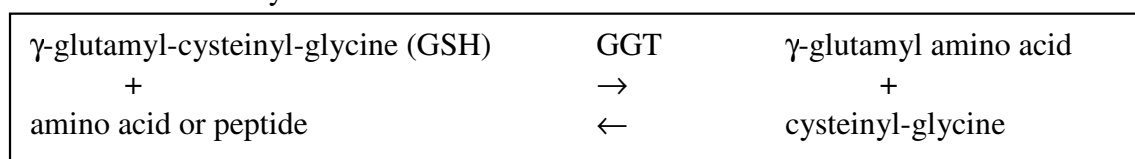


Fig. 2: Principle of estimation of alanine aminotransferase

3. γ -Glutamyl transferase (GGT)

γ -glutamyl transferase (GGT, formerly also GMT) is a key enzyme of the **γ -glutamyl cycle** that provides transport of some amino acids and peptides through the cell membrane from extracellular fluid to the cells. GGT catalyzes **transfer of γ -glutamyl moiety** from γ -glutamyl-peptides to other peptides, amino acids, or water. The physiologic donor of γ -glutamyl is the tripeptide glutathione in the cytosol, and the acceptor is an amino acid localized extracellularly. Cysteinyl-glycine remaining from glutathione following cleavage of γ -glutamyl is converted by other reactions back to glutathione.

The basic GGT-catalyzed reaction:



GGT occurs in **membranes of cells with high secretion or absorption capacity**. In the liver it is present in the microsomal fraction and in the membranes of biliary tract endothelium. High activity of GGT is also found in proximal tubules of the kidney, in enterocytes, and in the pancreas. Synthesis of GGT can be induced by some drugs (barbiturates, antidepressants, alcohol). It is possible to release GGT from membranes by detergent action of bile acids or alcohol.

Increase in GGT is especially characteristic for damage of the hepatobiliary tract:

- **intrahepatic or extrahepatic cholestasis** – in these cases alkaline phosphatase is increased as well
- **hepatocellular damage** – acute and chronic liver diseases
- **chronic alcohol abuse** – a high isolated elevation of GGT is characteristic. Its increase in alcoholics precedes the liver damage (induction of GGT synthesis)
- tumors of the liver and pancreas

Principle of GGT estimation

The assay for GGT (Fig. 3) originates from the reaction that GGT catalyzes in the body. The enzyme transfers γ -glutamyl from a substrate-donor to glycyl-glycine as an acceptor. Either L- γ -glutamyl-p-nitroanilide, or L- γ -glutamyl-3-carboxy-p-nitroanilide are used as the substrate donors; the enzyme-catalyzed reaction converts them to colored **p-nitroaniline** or **5-amino-2-nitrobenzoate**, respectively. Increase in the colored product is monitored photometrically.

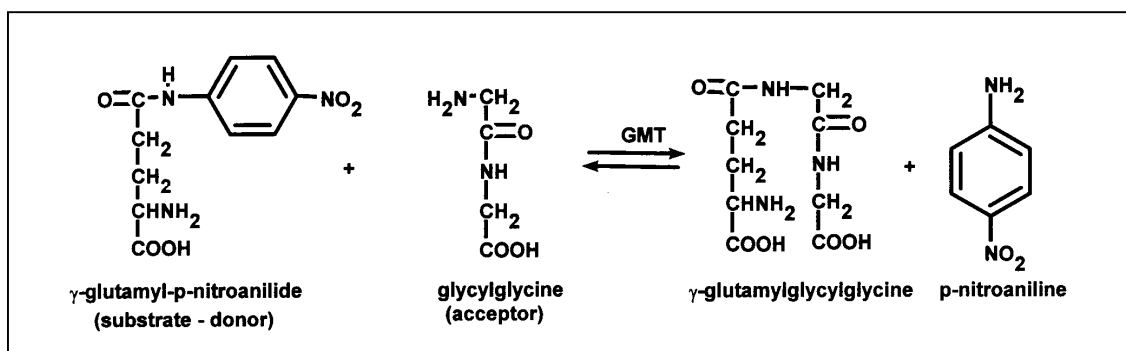


Fig. 3: Principle of γ -glutamyl transferase estimation

Reference values:

Catalytic concentration of serum GGT (fS-GGT):

Men: 0.14-0.84 μ kat/l

Women: 0.14-0.68 μ kat/l

GGT is higher in men since the enzyme is relatively abundant in prostate gland.

4. Alkaline phosphatase and its isoforms

Estimation of alkaline phosphatase (ALP) and its isoenzymes is useful as a marker of cholestasis in diseases of the liver and biliary tract, and also in bone diseases. Therefore it will be discussed in detail in the lesson on markers of bone metabolism.

5. Carbohydrate-deficient transferrin

Up to recently, the γ -glutamyl transferase (GGT) was considered as the best marker of one of the commonest causes of liver disease, the chronic alcohol abuse. Nowadays, however, a new parameter has appeared: **carbohydrate-deficient transferrin (CDT)**.

Transferrin is a glycoprotein that contains in its molecule usually from four to six moieties of sialic acid. In case of chronic alcohol abuse (60 g of alcohol daily for period at least two to three weeks) the fraction of transferrin lacking the sialic acid moieties – the carbohydrate-deficient transferrin (CDT) – increases. If the fraction of CDT exceeds 6 % of total transferrin, it indicates a chronic alcohol abuse. The CDT remains elevated for about 2 weeks following discontinuation of alcohol intake.