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Before the Analysis: Rules for Interpretation of Hepatic Cytology

After several years of daily diagnostic practice, I have come to believe that, on the one hand, there is cytology based on classic criteria, which are listed in dedicated chapters of books on the subject, as well as being updated in specialized press articles and discussed at professional meetings. On the other hand, there is liver cytology, which is based on criteria that can frequently differ from standardized, shared, and consolidated concepts applied to several systems. The criteria I am referring to are represented by variations from classic, widely acknowledged issues and interpretations that cannot ignore the clinical context within which the liver disease is being investigated. I believe that the cytological diagnostic approach must be accompanied by in-depth knowledge of microanatomy, as well as cytological and histopathological aspects of the organs being investigated. This statement is particularly important when dealing with an organ such as the liver, since it justifies the differences from classic cytology. Aside from these mandatory bases, I firmly believe there are initial conditions, preexisting situations to the analysis and diagnostic obstacles that must be known in order to modulate one's diagnostic skills. Based on thousands of cases analyzed and diagnosed – frequently supported by histological assessment and further corroborated by clinical course, compatibility with data provided by laboratory and imaging diagnostics or response to therapy – I have been able to draw a few conclusions about the primary skills pathologist must develop to interpret this very complex organ. I have summarized them in a short collection of rules, which I believe should be memorized and taken into due consideration before producing any cytological diagnostic conclusion.

Canine and Feline Liver Cytology, First Edition. Carlo Masserdotti.

1.1 The Rules for Cytological Diagnosis of Hepatic Diseases

1.1.1 Rule 1

The diagnostic value of cytopathology in evaluation of hepatic diseases ranges from 30.3% to 82.1% agreement with histopathological diagnosis [1, 2]. This discrepancy is mostly due to the fact that the samples used for cytological investigation represent a very small percentage of a potentially pathological liver, and thus may not be indicative of some lesional processes (low diagnostic sensitivity). Moreover, according to Wang, the diagnostic agreement between cytology and histology should be high in cases of so-called "vacuolar hepatopathy," although this is, in my view, not a specific diagnosis and just morphological evidence of hepatocellular damage. Even a large number of cytological samples from a pathological liver may not detect any alteration, as in some cases of vascular disturbances; in others instances, the tip of the sampling needle collects cells which may not be affected by the primary pathological process occurring or may be affected by aspecific changes. Especially in the course of widespread pathological processes, it is always preferable to sample from many different parts of the liver, as this will increase the chance of collecting samples and therefore data that are morphologically useful for diagnostic purposes. Similarly, when evaluating nodular lesions, comparison between cells from the lesion site and those from the surrounding nonnodular parenchyma may give good diagnostic results (widely described in relevant chapters).

1.1.2 Rule 2

There are some pathological processes, such as amyloidosis or extrahepatocytic cholestasis, whose cytological identification is always extremely useful in terms of diagnosis (very high specificity), even if not corroborated by other tests. For example, amyloidosis in the cat may not be associated with a significant increase in serum amyloid [3]; similarly, cholestasis, in rare instances, may not necessarily be associated with an increase in the concentration of total bilirubin [4]. Indeed, some unmistakable morphological signs may be the result of focal phenomena in a progressive phase and therefore precede certain alterations of other diagnostic parameters.

1.1.3 Rule 3

Many pathological processes can only be successfully interpreted if a histopathological architectural context is available. Furthermore, cytology provides only nonspecific aspects of these processes; for example, fibrosis or inflammation does not provide any information about the causes, extent or distribution but they may

highlight an important morphological aspect, which is a valid and sufficient reason to carry out an in-depth histological analysis.

1.1.4 Rule 4

Liver diseases are often not evaluable by cytology. They are often better assessed by histopathology and, in that case, recognition of a specific disease is the result of a morphological diagnosis based on a biopsy of tissue fragments, carried out according to established, accepted, and shared criteria [5]. Cytology is a diagnostic aid that may render histological examination unnecessary (for example, when recognizing amyloidosis or several neoplastic conditions, such as hepatic large cell lymphoma), but in most cases, the information it provides is nonspecific, as in many cases of mixed inflammation or aspecific reversible change. The role of cytology is often limited to excluding other potential suspected pathologies or to reducing or contextualizing the possible differential diagnoses, which must undergo histopathological evaluation.

1.1.5 Rule 5

Sometimes, it is hard not to feel defeated but I will always be determined to persuade clinical colleagues that a morphological diagnosis of cellular or tissue characteristics is, in many cases, impossible without a comparison with all data resulting from clinical and anamnestic investigations, collateral tests, laboratory and imaging diagnostics. The readers of this book will understand that a specific morphological characteristic may correspond to several different clinical conditions (each with its own therapy and prognosis); furthermore, if they have had firsthand experience in making a diagnosis through cytological morphology, they are also likely to understand the importance of being sufficiently informed about data relating to the lesion being analyzed. Given the above, I call on anyone reading this book to join me in this battle: to accept that collaboration between clinicians and pathologists is essential if we are to succeed in improving the management of a disease.

1.1.6 Rule 6

There is an urgent need for cytologists to translate every morphological characteristic of the sample into a diagnosis that is clinically useful in order to come to terms with the relatively scanty information that a liver sample can provide. Cytological samples must be approached with humility, refraining from drawing any diagnostic conclusion when the signs are insufficient or the correlation with clinical indications is incomplete or missing.

1.1.7 Rule 7

Romanowsky-type stains are represented by a group of different stains, such as Hemacolor®, Diff-Quik®, May Grünwald Giemsa and others [6]. These are normally used as routine stains in veterinary cytology, but chromatic results can differ from one stain to the other; consequently, what can stain deeply basophilic or red with one stain can appear as black or brown with another; for example, bile can appear variously as deeply basophilic, greenish or black on the base of the selected stain. Sometimes a color may appear darker or lighter due to the time the slide is exposed to the stains, mostly in cases when a stain procedure is not standardized; always remember that the colors frequently are subjective and must be interpreted carefully.

I believe these rules provide a solid base on which to start discovering the diagnostic secrets hidden in liver cytology. I have attempted to explore them in this book.

1.1.8 Rule 8

Always consider that, after the therapy is initiated according to cytological features, the pathological process can change and histopathologic examination, when done weeks or months after the cytological examination, can result in different findings. For example, an inflammatory process could initially be evident on cytologic preparations but disappear or appear attenuated on histopathologic examination if this latter is done after adequate therapy has started.

1.2 Diagnostic Approach to Liver Disease

In my view, the diagnosis of liver disease is the result of an algorithm that provides an evaluation of the patient based on:

- historical, clinical, and anamnestic signs
- hematochemical investigation
- ultrasonographic investigation
- cytological investigation
- histopathological investigation.

The above is summarized in the diagnostic pyramid shown in Figure 1.1, which clearly shows that only by going from the bottom up is it possible to refine a diagnostic investigation aimed at identifying the causes. Each step contains the indications necessary to proceed to the next diagnostic phase and, eventually, to reach the diagnostic perception of a specific liver disease. With the exception of the first step, which generally provides nonspecific clinical signs, each step can potentially contribute to acquiring information concerning the liver disease in question.

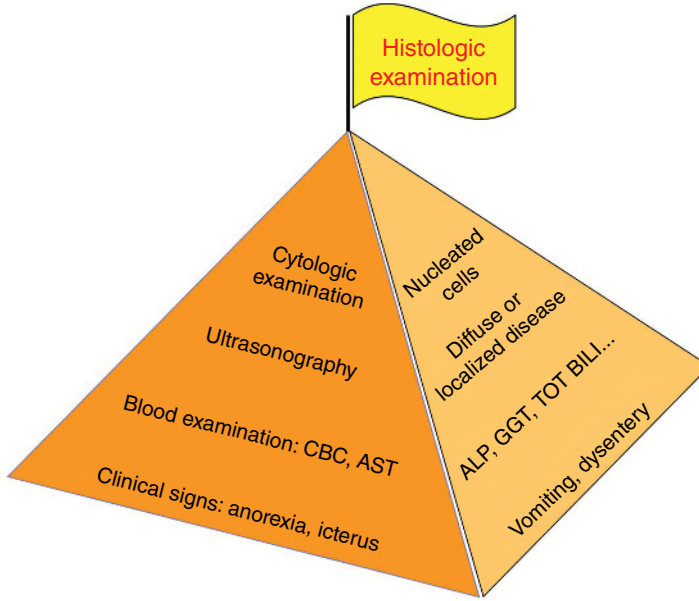

Figure 1.1 The "diagnostic pyramid" of hepatic diseases describes cytology and histopathology as the last steps in clinical, laboratory, and ultrasonographic evaluation and that they need the support of all these data to provide a reliable diagnosis.

In the final levels, cytology and especially histology allow identification of the nature of the pathological process.

1.2.1 Clinical and Anamnestic Signs

The symptomatology of liver disease is generally very nonspecific, as it is represented by generic clinical signs such as malaise, dysorexia or anorexia, vomiting or dysentery. With the exception of jaundice, a consequence of hyperbilirubinemia caused by several liver lesions (may also be caused by prehepatic conditions, such as hemolytic, or posthepatic forms, such as obstructive), hepatic disease has no distinguishing clinical signs. Generally speaking, the symptoms of liver disease are so nonspecific that only an additional assessment (supported by clinical investigation) can confirm with certainty that the ongoing pathological process is localized in the liver.

1.2.2 Hematochemical Investigation

1.2.2.1 Pathological Bases of Liver Damage

When damage to the liver cells occurs, some enzymes contained in the cytoplasm or located on the plasma membrane are released by the damaged cells and

consequently enter the circulatory system, a phenomenon that can be measured and utilized as a diagnostic tool if an increase of the said enzymes is found.

The release of cytoplasmic enzymes can result in reversible or irreversible damage. In *reversible damage* (Figure 1.2a), there is a release of small portions of cytoplasm containing the diagnostic enzymes (a phenomenon called "blebbing") even if the cells are not subject to destructive alterations [7]; the small portions of cytoplasm containing the diagnostic enzymes released into the circulatory system undergo lysis and liberation of the enzymes. In contrast, in *irreversible damage* (Figure 1.2b), the release of cytoplasmic enzymes occurs by destruction of the cell (lethal damage), represented by necrosis or apoptosis, although in smaller degree, which results in total leakage of the cytosol into the extracellular space. Detection of increased cytoplasmic enzyme activity (leakage of enzymes) in the serum indicates the presence of damaged hepatocytes (Figure 1.3). Such increase depends on the number of damaged cells, as well as the extent of the damage, so a given increase may correspond to reversible or widespread damage (mild damage involving many cells), as well as to irreversible but localized damage (involving few cells but with severe damage and leakage of all cytoplasmic contents).

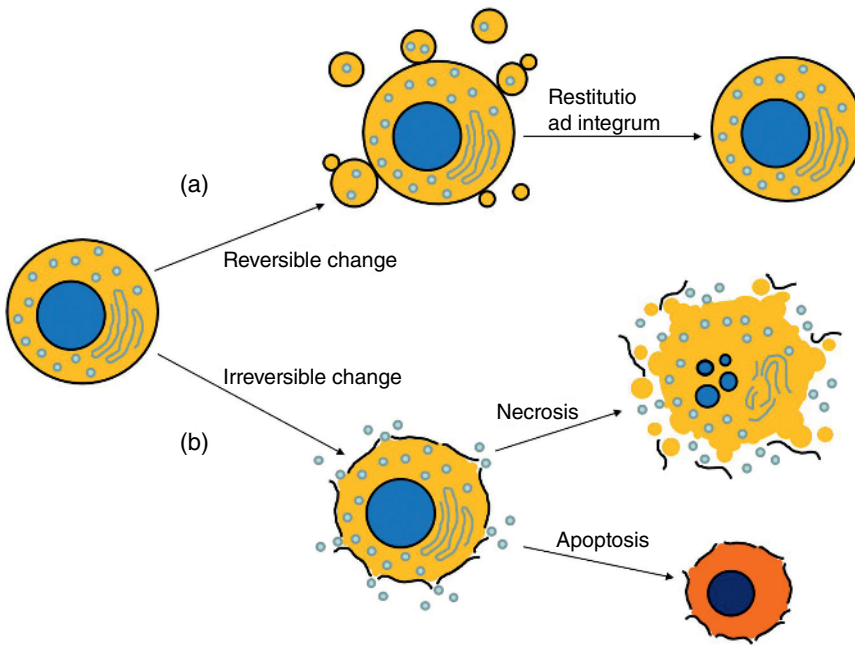

Figure 1.2 (a) Reversible change in the hepatocyte, which recovers completely after the causative process ceases. (b) Irreversible change in the hepatocyte, causing necrosis or apoptosis of the cell.

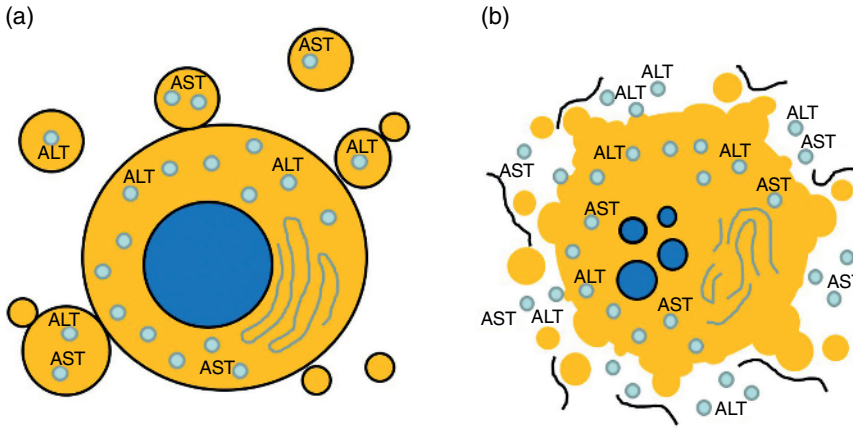

Figure 1.3 Leakage of ALT and AST, which is valuable for biochemical investigation of the plasma, is aspecific and not related to primary causes, since it can occur as a consequence of reversible (a) or irreversible (b) damage of the hepatocyte.

This induced increase in enzymes present in the serum is not correlated to the extent of damage to the parenchyma (focal or widespread). Furthermore, it does not indicate the severity of the damage (reversible versus irreversible) either – it only suggests the presence of nonspecific hepatocyte damage.

In cases of localized irreversible damage, the increase in enzymes may be transitory, although high, since parenchyma is repaired by *restitutio ad integrum*, following substitution of dead cells from regenerative hepatocytes (Figure 1.4a). In cases of widespread irreversible damage, the increase in enzymes may be high and persistent as well, but regeneration is not able to repair the necrotic parenchyma and fibrosis, as a cicatricial process occurs with progression of the damage to cirrhosis (Figure 1.4b). In other cases, an increase in specific enzymes indicates the presence of a pathological process affecting the liver (directly or indirectly), without damage to the hepatocyte membranes. Alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) are enzymes located respectively on the membrane surface of the canalicular side of the hepatocyte (mostly ALP) and the cholangiocyte (mostly GGT). Both are released into the bile as a consequence of detergent action of bile salts; both can increase during acute and chronic cholestasis, leaking back into the plasma. There may also be a condition, referred to as "induction," during which the cell produces an overabundance of certain enzymes (inducible enzymes); induction, stimulated by endogenous or exogenous cortisol or many drugs, stimulates increased production of the enzymes' protein via modified transcription, translation or other processes [8]. Such increased synthesis is followed by an increase in enzymes localized on the membrane surface, as well as an increase in the amount that enters the circulatory system.

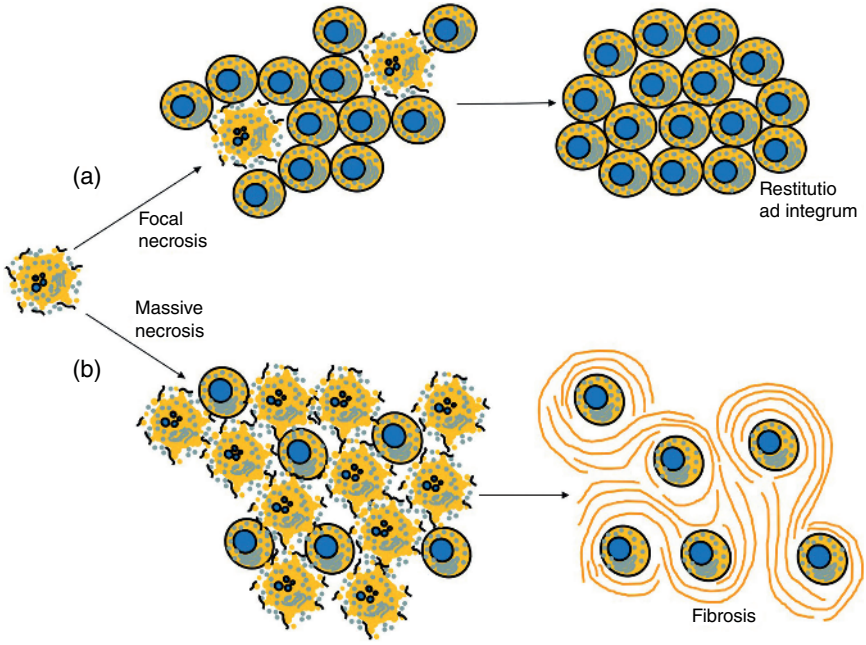

Figure 1.4 (a) When focal irreversible change affects a few cells, *restitutio ad integrum* may be obtained by replacement of dead cells by adjacent proliferative hepatocytes or immature cells. (b) When necrosis occurs extensively, *restitutio ad integrum* is not possible and the only outcome is fibrosis.

1.2.2.2 Diagnosis of Liver Damage

Determination of the increase in the circulation of enzymes used by laboratories to identify liver damage is based on biochemical reactions in which the enzyme being investigated plays a key role. The resulting chemical reaction yields a metabolite whose increase is identified through spectrometric techniques and the concentration of which is directly proportional to the concentration of the enzyme. For this reason, the concentration of liver enzymes is measured in international units (IU), which indicates the activity of the enzyme and not its true quantity, i.e., not in mg/dl. In this respect, it should be remembered that the concentration of a certain enzyme in the circulatory system is a direct consequence of its release, which is caused by either damage to the cell that produces it or the clearance activity of the enzyme itself, which in turn may be altered by pathological processes. It is quite significant how some enzymes, such as lipase or amylase, can increase in the circulatory system not only as a consequence of damage to the exocrine pancreas but also, for example, as a result of alterations in renal excretion function, which is the metabolic process that eliminates these enzymes.

1.2.2.3 Useful Enzymes for Recognition of Damage to Hepatocytes and Cholangiocytes

Useful concepts for interpreting the activity of enzymes include the following.

- Plasma half-life, namely, the survival of the enzyme in plasma. Some enzymes undergo faster degradation in plasma after sampling and consequently, they may appear artificially low and therefore provide unreliable data.
- If evidence of hepatocytic damage suggested by evaluation of biochemical parameters has no correspondence with the cytohistopathological evaluation and vice versa.

Alanine Transaminase (ALT) A cytoplasmic enzyme resulting from reversible or irreversible hepatocytic damage, whose increase is a consequence of any type of pathogenic insult.

Aspartate Transaminase (AST) The mitochondrial and cytoplasmic enzyme indicates reversible or irreversible hepatocellular damage but it is also present in the cytoplasm of the striated muscle and therefore, it can increase in the course of muscle damage. To better differentiate the causes of the AST increase, it is advisable to measure creatine phosphokinase (CPK, also known as creatine kinase – CK), an enzyme contained in the cytoplasm of the muscular and cardiac striated muscle cell. Should the increase in AST be consistent with the increase in CPK, the damage is likely to concern the striated myocell and not the hepatocyte. Eventually, *in vitro* hemolysis might cause falsely increased ALT activity.

Alkaline Phosphatase (ALP) Alkaline phosphatases are a group of isoenzymes located on the outer layer of the cell membrane; they catalyze the hydrolysis of organic phosphate esters present in the extracellular space. At least two isoenzymes are known: intestinal ALP (I-ALP) and nonspecific ALP, with the latter including the ALP isoform of hepatocyte production and that of bone production. Due to its short half-life, I-ALP does not contribute to the serum increase in ALP, which is consequently almost entirely of hepatic (70–90%) and bone (10–30%) origin, with the exception of the so-called C-Canine ALP (corticosteroid-induced ALP), namely the ALP produced by hepatocytes when stimulated by corticosteroids or other drugs (e.g., phenobarbital).

In the liver, ALP, involved in processing bile content, is cytosolic and present on the canalicular membrane of hepatocytes and on the membrane of cholangiocytes. The response of the liver to some primary causes induces an increase in the synthesis of ALP; some of the newly formed enzyme enters the circulation to

increase the enzyme activity in plasma. The main reasons for the increase of hepatic ALP are as follows.

- Cholestasis, obstructive (intra- or extrahepatic) and functional (associated with sepsis).
- Drug induction (Figure 1.5), by endogenous or exogenous corticosteroids or other drugs (in dogs).

ALP may also increase as a consequence of bone tissue remodeling phenomena (diseases or bone reworking, growth period of puppies, especially large breeds) or placental production in the final period of cat pregnancy.

Gamma-glutamyltransferase (GGT) This is a membrane enzyme found mainly on cholangiocytes, as well as in smaller quantities on hepatocytes, exocrine pancreas, and renal tubular epithelium. Its plasma increase is mainly due to cholestasis (Figure 1.6), which is probably secondary to stimulation enhanced by bile acids. Some experimental data suggest hyperplasia of the biliary epithelium as one of

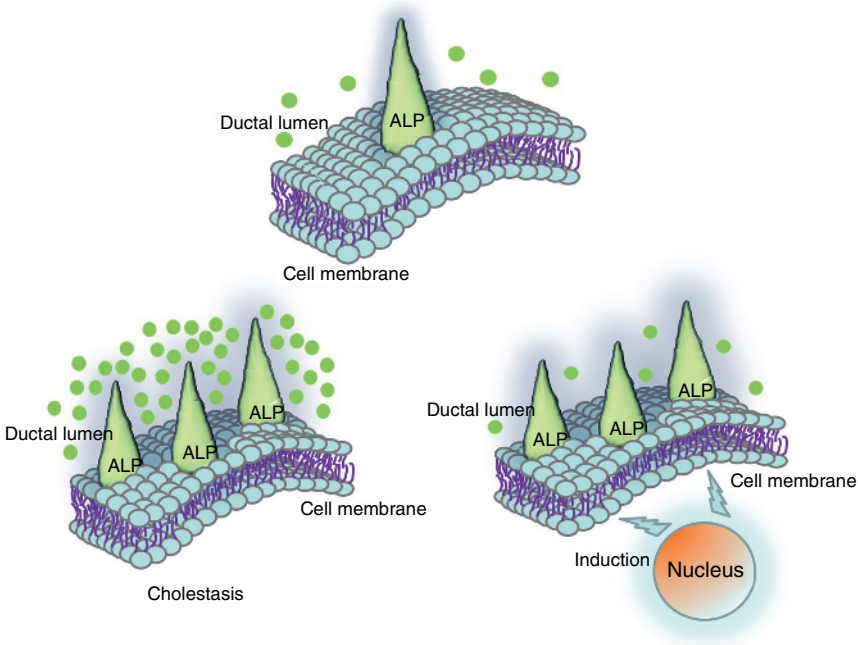

Figure 1.5 ALP is an enzyme located on the external surface of the cell membrane. Many causes, for example cholestasis (left) or induction from exogenous or endogenous steroid (right), may induce increase of ALP molecules on the surface.

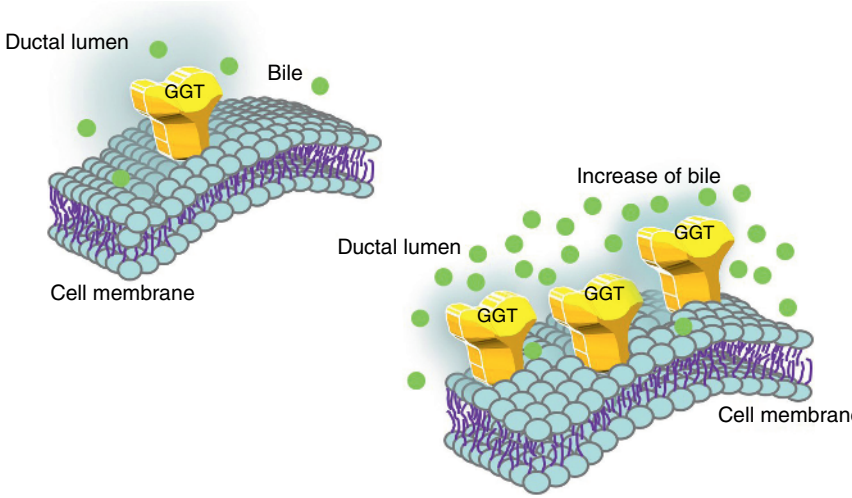

Figure 1.6 GGT, as ALP, is located on the external surface of the cell membrane; many conditions, for example increase of bile into the canalicular surface of the hepatocyte, can induce increase of this enzyme.

the possible causes of this increase. Similarly to ALP, it can increase during pharmacological stimulation or administration of exogenous and endogenous corticosteroids.

Lactate Dehydrogenase (LDH) Lactate dehydrogenase is an enzyme composed of numerous isoforms and produced by tissues found in several organs, such as the heart, muscles and liver, which limits its use as a marker of liver damage. It may also increase in the course of hemolysis, as it is contained in the cytoplasm of erythrocytes.

1.2.2.4 Liver Failure Diagnosis

Liver damage, whose main evaluation parameters have been listed in the previous paragraph, can manifest as an altered ability of the parenchyma to perform synthesis, excretion, storage, and detoxification functions. Liver damage can alter liver function but if such damage does not exceed at least 70% of its functioning mass, the liver is capable of compensating, showing no alterations and continuing to function normally. If the damage exceeds such compensatory capacity or if hepatic functional mass is decreased, liver failure occurs. In this case, the liver does not fully carry out its normal functions and these deficiencies can be measured with various functional parameters.

Synthesis The liver synthesizes numerous proteins, including albumin, clotting factors, some globulins and lipoproteins; carbohydrates, especially glycogen, the main carbohydrate for intrahepatocyte storage; and lipids, including cholesterol, triglycerides, and fatty acids.

Detoxification Through intrahepatocytic metabolic processes, including conjugation, hydrolysis, oxidation, and reduction, the liver modifies and degrades numerous potentially harmful endogenous and exogenous chemical species, such as ammonium ions, which come from the intestine. Hydrophobic molecules are metabolized to a hydrophilic state for excretion in the bile or urine.

Storage The liver stores glycogen, triglycerides, and trace elements, including copper and iron.

Secretion The liver is involved in some excretory functions, mostly represented by the production of bile and bile acids.

Diseases that can lead to liver failure are not necessarily associated with hepatocyte or biliary damage. In fact, there are serious vascular disorders, such as portosystemic shunt (PSS), in which hepatocellular and biliary damage is minimal or even absent.

1.2.2.5 Parameters of Liver Failure

The substantial difference between liver failure and liver damage is mostly due to the fact that hepatic insufficiency is evaluated on the basis of parameters used to measure its metabolic potential.

- *Reduced synthesis*: decreased concentration of albumin, glucose, cholesterol, and fibrinogen in plasma.
- *Reduced detoxification capacity*: hyperammonemia is one of the most common alterations of this function, which is the result of the liver's inability to metabolize intestinal-derived ammonia.
- *Alterations in secretion*: bile is an important metabolite that is secreted by the liver. Restriction of secretion or retention of bile can occur, due to the conditions described below in relation to jaundice, as well as obstructive events affecting the biliary outflow.

Bilirubin Bilirubin is a catabolism derivative of the heme molecule and, to a small extent, of myoglobin or other enzymes, such as cytochromes. The liver captures the circulating bilirubin bound to albumin (called nonconjugated) and

combines it with glucuronic acid (conjugated bilirubin), a process by which, after becoming water soluble, it is eliminated with the bile salts into the bile. Increase in plasma bilirubin may be the result of increased production (hemolysis), decreased hepatocyte capacity to capture or eliminate bilirubin, or occlusion of biliary outflow.

Urea The liver metabolizes ammonium ion and transforms it into urea; hypoazotemia may occur as a consequence of hepatic failure, hepatocellular diseases, portosystemic shunts or urea cycle enzyme deficiencies.

Ammonium Hyperammonemia may occur as a result of decreased clearance from portal blood or due to a decrease of functional hepatic mass, mostly as in diffuse hepatocellular disease, congenital or acquired portosystemic shunt.

Albumin Albumin is synthesized by the hepatocyte and has a long plasma half-life (8.2 days in dogs [9]); its decrease can be associated with chronic hepatic insufficiency.

Cholesterol The liver synthesizes cholesterol and the proteins necessary for its plasma transport. Hypocholesterolemia can be associated with forms of severe insufficiency.

Proteins Involved in Coagulation Pro- and anticoagulant proteins are synthesized by the liver, therefore hepatic failure can lead to disturbed clotting.

Glucose Glucose is stored by the liver in the form of glycogen and released when needed. Consequently, hepatic insufficiency can be associated with forms of moderate hypoglycemia, a consequence of decreased gluconeogenesis activity.

Bile Acids Bile acids are synthesized by the liver and released with the bile. They are reabsorbed in the intestine by the portal circulation and subsequently transported back to the liver, with the exception of a small amount which remains in the circulating blood and is eliminated with the urine or stool.

Abnormal biliary acid levels can be assessed in two ways.

- *Measurement of pre- and postprandial bile acids*: this is an effective way to compare the amount of bile acids in the circulation in a fasting state compared to levels following gallbladder emptying. Abnormally high postprandial levels indicate that the liver cannot clear the bile acids, due to either shunting of portal blood or hepatocyte injury. High fasting bile acid levels are also an indication of shunting of portal blood or hepatic failure. Obtaining unambiguous data may be difficult.

- *Levels of urinary bile acids*: the one-time collection of urine, compared to fasting and postprandial blood collection, makes this assay attractive for assessing bile acids.

1.2.3 Ultrasonographic Investigation

When the presence of a liver disease can be ascertained through the diagnostic investigations listed above, evaluation of the parenchyma through diagnostic imaging becomes essential, as this can establish the type of lesions on the hepatic parenchyma or biliary tree. A complete and exhaustive evaluation of ultrasonographic diagnostics and its merits is beyond the purpose of this book so I will only underline the key role of diagnostic imaging, especially when it is necessary to identify characteristics that, in turn, render subsequent investigations necessary.

Ultrasonographic examinations allow us to establish the existence of a pathological process spreading to the whole parenchyma, as in the course of suspicious pathological accumulations of lipids or glycogen [10]. This investigation is crucial, especially if performed with Doppler and contrast ultrasonography, as it identifies portal circulation disorders, such as PSS [11]. Ultrasonographic examination allows us to highlight the presence of nodular lesions, such as in the course of primary or metastatic neoplasia and even with the contrast-enhanced ultrasonography (CEUS) method, and investigate the vascular characteristics of some nodular lesions, as well as to correlate the pattern to any malignant behavior [12].

In addition, ultrasonographic examination can identify peculiar characteristics of biliary swelling and primary diseases, such as cystic hyperplasia of the gallbladder. Although ultrasonographic investigations rarely result in conclusive diagnoses, the value of this tool is undeniable, especially its ability to establish whether the liver damage is widespread or localized and, consequently, to identify possible differential diagnoses. Ultrasonographic examination is also an essential support for subsequent cytological or histopathological analysis; depending on the appearance of the lesion found, it is possible to sample by means of fine needle capillary suction (FNCS) both the parenchyma affected by widespread processes and nodular lesions, even those of small dimensions, as this technique allows us to target precise parts of the parenchyma.

Finally, the use of ultrasonography for sampling the hepatic parenchyma with a cutting needle allows us, on the one hand, to obtain suitable samples for histopathological investigation and, on the other, to monitor any hemorrhagic incidents.

1.2.4 Cytological and Histopathological Investigation

If a diagnosis has not been made, the progression upward through the levels of the diagnostic pyramid (Figure 1.1) ultimately results in morphological assessments of the lesion process based on cytological observations, which are widely discussed in this book using histological terminology, which has only been touched upon in some chapters and is abundantly dealt with in the material included in the references.

1.2.4.1 Sample Collection

While an extensive review of sampling methods is beyond the scope of this book, a brief summary of sampling issues should be useful for the reader. Given that the target audience of this book is the skilled and expert cytologist, I will not provide a complete description of the sampling process, which is already extensively described in available cytology books.

Fine needle aspiration (FNA) is widely used to collect cytology samples, usually performed using a 6 or 12 cc syringe and a 22 gauge, 1.5 to 3.5 in. disposable hypodermic or spinal needle. FNCS [13], conducted with ultrasonographic guidance (Figure 1.7), is widely used to collect cytology samples from the liver, typically using a 25 gauge, 1.5 to 3.5 in. disposable hypodermic or spinal needle. Although

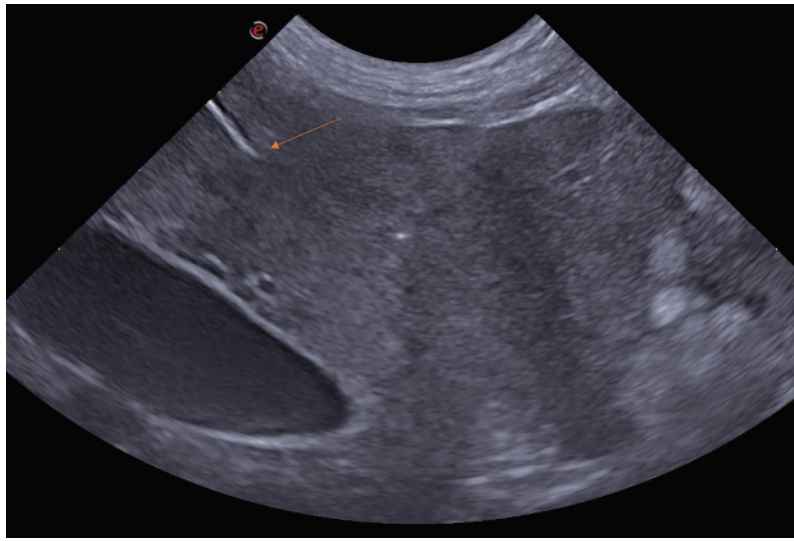

Figure 1.7 Ultrasonographic sampling of liver; notice the tip of the needle that enters the parenchyma (yellow arrow).

FNA has been used for a long time, FNCS is my preferred method of collection because of the high number of cells retrieved, the integrity of arrangements, and the minimal blood contamination.

The needle is inserted into the liver via a percutaneous transabdominal approach in small animals or transthoracically in large animals. FNA, FNCS, Tru-Cut®, and Menghini needles, laparoscopic forceps, and surgical wedge biopsy can all be used to sample hepatic tissue, the former by direct smear of the sample, the others by touch imprint of the sampled hepatic tissue on a slide [14]. Large-scale studies of complications associated with liver aspirates in dogs, cats, and horses are lacking, although hemorrhage and accidental perforation of large biliary ducts or the gallbladder are possible [15]. A small amount of blood loss is always expected, which can be visualized ultrasonographically or during laparoscopy or surgery. The average amount of blood loss from a liver biopsy is reported to be around 2 ml in healthy dogs [15, 16]. The risk for prolonged or excessive bleeding should be assessed by evaluating coagulation times before performing the procedure [17, 18]. Studies of ultrasound-guided and laparoscopic liver biopsies in dogs, cats, and horses suggest that this procedure is generally safe [19, 20].

1.2.4.2 Cytological Approach to Hepatic Diseases

Clinical pathologists should always bear in mind that cytology should be considered a preliminary evaluation of hepatic diseases and that cytological sampling may produce one of the following outcomes.

- *Nondiagnostic*: many cytological samples, on the basis of the previously discussed rules, are not useful for diagnosis, mostly because the primary disease site has not been sampled or because architectural or vascular changes are not evaluable. In these cases, just a description of the aspecific changes of hepatocytes, inflammatory conditions or other aspecific changes should be given in the report.
- *Suggestive of differential diagnosis*: in some cases, although not definitively diagnostic, cytological changes may be indicative of a specific pathological process, as for example the presence of fibrosis or cholestasis, although histopathological investigation is necessary to correctly address this change.
- *Diagnostic*: in some cases, cytology is definitively diagnostic and histopathological investigation is not necessary to correctly manage the patient, for example in cases of amyloidosis or in many cases of primary or metastatic neoplasm.

1.3 Key Points

- Liver diseases can be correctly identified only if all data, from clinical, historical, laboratory, and ultrasonographic investigations, are available.
- Clinical data are mostly aspecific.

- Biochemical abnormalities are mostly represented by increased plasma concentrations of ALT, AST, ALP, GGT, and total bilirubin, that are the most useful biochemical parameters to recognize a hepatic disease.
- Liver can be affected by diffuse or focal pathological processes; these processes may be recognized by ultrasonographic investigation.
- Cells sampled by cytological methods can be diagnostic, helpful for final diagnosis or aspecific; this diagnostic power is correlated with the nature of the primary process.
- Diagnostic power may also be correlated with the likelihood that the tip of the needle samples an area where a primary pathological process occurs and with the number, appearance, and integrity of diagnostic cells.

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