

Lecture

Ladies and Gentlemen!

Before I begin with my remarks on the topic of

Autoimmune hepatitis - diagnosis in collaboration between clinicians and pathologists

I would like to tell you the following story from my childhood: About 60 years ago I read a book of young children's stories from Armenia. The story went that the women there hatched hens' eggs under their breasts when the hens refused to sit on them. In the meantime – down to the present day - the anatomy of these women must have undergone some fundamental changes: When I arrived at the airport in Yerevan, I didn't see a single woman who would have been suitable for hatching eggs. The women of today are far too attractive - as attractive as the diagnosis of autoimmune hepatitis to the pathologist - and I think for clinicians as well – our subject for this session.

Introduction

1.1. General remarks on hepatic diagnostics

It is generally true that the morphological diagnosis of inflammatory liver diseases represents one of the most difficult tasks confronting pathologists. The reasons for this are:

- The relatively low frequency of liver biopsies. Due to improvements in clinical, laboratory chemical and molecular biological methods, fewer liver biopsies are performed in many cases. Some clinicians believe that one can dispense with liver biopsies entirely. That is a fundamental error to be addressed later.
- The risk of a lack of practice and experience in individual pathologists.
- Frequent isomorphism of the inflammatory response forms and reactivity to highly heterogeneous stimuli or damage. But therein lies the charm of histological liver diagnostics as well.

On the one hand there are very clear patterns of damage in the liver - such as toxic alcoholic liver damage, specific signs of inflammation associated with confirmed pathogens, for instance tuberculosis, Brucellosis ect. On the other hand, the pathologist relies entirely on a knowledge of clinical parameters for liver biopsy tissue diagnostics.

The clinician in such cases has a double debt to pay - not only in terms of obtaining and supplying adequate liver biopsy tissues, but also by providing comprehensive information on the clinical and laboratory findings. It can also be formulated like this: The better the information provided by the clinician to the pathologist, the better the morphological diagnosis will be. Both diagnostic partners - the clinician and the pathologist - are interdependent. The diagnosis must in any case be a consensus diagnosis supported by both partners. Within the framework of this effort, the pathologist must have a knowledge of the clinical issues and the clinician must possess a certain degree of morphological understanding.

The earlier opinion of some clinicians that all that was needed was to send liver biopsies to the pathologist without any clinical information, in the belief that pathology had to be a completely neutral and impartial judge of the liver tissue, is now completely obsolete.

It might be smugly remarked that there is a "conflict of interest " between clinical medicine and pathology: The pathologist has to rely on representative and significant biopsy material and the clinician has to consider the potential complications of such an invasive measure.

The quality of a liver biopsy cylinder must meet certain requirements. A biopsy cylinder should be at least 15-30 mm long and 1.2-2 mm thick and contain at least 6-8 portal fields. There is a strong correlation between the number of portal tracts and the validity of the morphological diagnosis. As a rule, biopsy cylinders longer than 30 mm do not increase the information content.

Some clinicians think they can do without liver biopsy because of very good non-invasive methods available. But fact that liver biopsies are at the top of the list in the "revised descriptive criteria for diagnosis of autoimmune hepatitis" issued by the International Autoimmune Hepatitis Group (Alvarez F, 1999), to be discussed later, is not without justification.

Today, the liver biopsy remains the safest method of recognizing and diagnosing the fine structural changes and reactivity of hepatic cells. Liver biopsy remains the gold standard in diagnostics of autoimmune hepatitis.

Of course it remains the duty of the clinician to consider the indication for a liver biopsy carefully, taking into consideration the overall clinical picture. He must weigh the risks of a liver biopsy against expected diagnostic benefit.

1.2. Laboratory requirements for morphological diagnostics at an institute of pathology

Apart from the expertise of the pathologist doing the hepatic diagnostics, an institute of pathology must meet certain technical requirements:

- Fixation of liver tissue immediately after extraction of biopsy material in 2.5 - 4% neutral buffered formaldehyde. The formalin solution should be provided by the pathologist.
- Realization of at least 8 - 10 sectional levels
- Staining techniques to assess the liver tissue compartments (hematoxylin-eosin, van Gieson, iron staining, reticulin staining, D-PAS; also optionally PAS, Congo red, Ladewig, etc.)
- Special methods: immunohistochemistry, molecular pathology, electron microscopy

In addition to the "standard stains", the selection of optional tissue stains may sometimes vary among institutions and pathologists.

Just as the pathologist has something like "specifications" for his laboratory and mental tasks, the clinician is required to comply with certain "standards " applying to his diagnostic procedures.

Proposals for such procedural standards were first made at the international level in 1999 by the "International Autoimmune Hepatitis Group" working with Alvarez (1999), after interested clinicians and pathologists had met in previous years on several occasions. Two basic categories were identified:

- First of all the "Revised criteria for diagnosis of autoimmune hepatitis" defining the typical characteristics of liver biopsies, serum biochemistry, immunoglobulins, autoantibodies, viral hepatitis serology and anamnestic factors such as alcohol consumption and medication.
- Later we will address histological characteristics - such as portal and lobular inflammation signs, interface hepatitis, severity of inflammatory responses, etc. - in the individual case discussions.
- Important biochemical parameters include mainly ALP (alkaline phosphatase), AST (aspartate aminotransferase) and ALT (alanine aminotransferase), GOT (glutamyl oxalate transferase) and GPT (glutamyl pyruvate transferase).
- The serum immunoglobulin content should be at least 1.5 times the norm.
- Important serum autoantibodies include ANA (antinuclear autoantibodies), SMA (smooth muscle autoantibodies), AMA (antimitochondrial autoantibodies) and anti-KLM autoantibodies.
- Viral hepatitis serology is an important parameter in the definition of autoimmune hepatitis.
- A knowledge of person habits such as alcohol consumption and medication also play an important role in understanding liver damage.
- Depending on the extent of these findings, a statement concerning the validity of the diagnostic confirmation can be arrived at as "definite for autoimmune hepatitis" or "probable for autoimmune hepatitis".
- Secondly, the International Autoimmune Hepatitis Group supplemented these "Revised criteria for diagnosis of autoimmune hepatitis" with a "Revised scoring system for diagnosis of autoimmune hepatitis". In this scoring system, the previously mentioned parameters and individual deviations are scored with a point system. The following criteria are evaluated in the system: patient gender, level of serum globulins, levels of the autoantibodies ANA, SMA and KLM, detection of AMA, positive or negative hepatitis serology, history of medications and alcohol consumption, results of histologically determined inflammatory changes in liver tissue, history of autoimmune diseases in family or first-degree relatives, optionally serum changes for other serum antibodies – e.g. positivity for HLA (human lymphocytic DR3 or DR4 antibodies) as a general indication of an allergic diathesis and - last but not least - of response to therapy and disease recurrence. Applied prior to therapy, 15 points or more indicate "definite for autoimmune hepatitis" and 10-15 points "probable for autoimmune hepatitis". When the scoring system is used post treatment, 2 points are added in each case.
- After this "Revised criteria for diagnosis of autoimmune hepatitis" and the "Revised scoring system for diagnosis of autoimmune hepatitis" were in use for nearly 20

years, the "Simplified Criteria for the Diagnosis of Autoimmune Hepatitis" were proposed in 2008 (Hennes EM, 2008). This system is based only on the behaviour of the autoantibodies ANA, SMA, KLM, SLA (soluble liver antibodies), immunoglobulins, the liver histology and the diagnosis of viral hepatitis serology. Seven or more points indicate "definite for autoimmune hepatitis" and six points "probable for autoimmune hepatitis".

Generally speaking, the following clinical parameters and symptoms accompany autoimmune liver damage:

- The female gender predominates
- Occurrence in all age classes
- Occurrence of two peaks in adolescence and in the 4th - 6th decade (see Table XY on distribution of AIH into types 1 and 2)
- Prototype: young patients with endocrine disorders
- Insidious onset
- General prodromal symptoms in the form of fatigue, anorexia, tiredness, pallor
- Alternating jaundice
- Abdominal pain
- diarrhoea
- Weight loss
- Arthralgia
- Non-specific skin changes
- Mild hepatomegaly and splenomegaly
- Highly variable symptoms in individual patients – some developing more pronounced symptoms and others virtually asymptomatic
- Simultaneous occurrence of other autoimmune diseases in the same patient +
- Autoimmune diseases in first-degree relatives
- Determination of serum levels and changes
- IgG level
- The importance of biochemistry and antibodies was already mentioned in the discussion of the revised criteria and the revised scoring system. In recent years, more and more antibodies have of course been added to the list of aids in the diagnosis of autoimmune hepatitis – e.g. pANCA (perinuclear anti-neutrophil cytoplasmic antibody), cANCA (cytoplasmic anti-neutrophil cytoplasmic antibody), ASGPR (asialoglycoprotein receptor) and many more.
- Despite all the advances in diagnostics of autoimmune liver disease, the statement made in 1999 still holds today: "There are nowadays no specific symptoms, signs, specific liver tests or serum abnormalities, that are of sufficient evidence to be considered part of the diagnostic criteria" (Alvarez F, 1999)

Before we discuss the pathological histomorphology of autoimmune liver damage, we should briefly recall an insight into the **normal histology** of liver tissue; for without a knowledge of the normal one cannot recognize the diseased.

- The liver regions at the focus of autoimmune hepatitis are the
- Periportal / portal unit
 - The periportal / portal unit or portal tract (Glisson's tract) consists of the small branches of the portal vein, the branches of the hepatic artery, the bile ducts and the connective tissue (**Figure**. Pardon me for labelling this schematic drawing in German but the English and German nomenclature is quite similar). This image shows schematic cross-sections through several hepatic lobules. These points are the locations of the Glisson's triangles with the branches of the portal vein, hepatic artery and bile ducts. While the directions of flow in the portal vein and the hepatic artery are lobular-centrifugal, the flow direction in the bile ducts is the opposite: lobular-centripetal.
- Sinusoidal unit
 - This image presents in schematic form the region of the sinusoids, sinusoidal endothelial cells and hepatocytes and in between them the tiny bile canaliculi. The Kupffer cells (macrophages, here entered later) are located both on and beneath the perforated sinusoidal endothelia. The Kupffer or stellate cells play a prominent role in the immune defences as well as in the inflammatory reactions of the liver in general. They are components of the monocytic macrophage system. They are capable of migration, replication, and thus expansion. As macrophages they are involved in the primary phase of the cell-mediated immune response and tolerance induction. They migrate, so-to-speak, to the battle zones in the liver, i.e. to where the individual liver compartments are damaged.

Central vein

The central vein system is mentioned here for the sake of completeness only. The blood in the hepatic sinusoids comprising a mixture of blood from the hepatic artery (about 25%) and the portal vein (about 75%) is transported via the hepatic sinusoids through the sublobular veins and thus into the inferior vena cava.

The components of the liver may respond to damaging influences individually and/or in concert, resulting in local inflammations which may then spread to the surrounding structures. This then meets the criteria for an "-itis ", i.e. hepatitis.

Having demonstrated **the importance of the preconditions** for collaborative diagnostics of autoimmune hepatitis involving both clinicians and pathologists and how important the following aspects are:

- shared appreciation of the problems confronting both clinician and pathologist
- sufficient knowledge of the mutual problems of the clinician and the pathologist

- flow of information
- discussion of findings, the objective being collaborative interpretation and formulation of a
- consensus diagnosis,

...we can now address autoimmune hepatitis itself:

Classification of autoimmune hepatitis

Autoimmune hepatitis is a chronic progressive inflammation of the liver, which if left untreated will ultimately result in progressive destruction of the organ. The exact aetiology has not been sufficiently clarified. There are two different types of autoimmune hepatitis. The earlier definition of a Type 3 has been dropped.

Type 1 autoimmune hepatitis

Autoimmune hepatitis is classified according to its age-dependent occurrence and based on the antibody constellation. Type 1 occurs in childhood and adolescence and in adults or in all age groups and is characterized by alternating detection of anti-nuclear antibodies (ANA), smooth muscle antibody (SMA) and anti-SLA/anti-LP.

Type 2 autoimmune hepatitis

Most cases of type 2 are already present in childhood and adolescence. In type 2 autoimmune hepatitis, anti-liver-kidney microsomal antibodies (KLM) are detected. The other serum antibodies mentioned above do not occur in type 2.

The table (Figure) provides a synopsis of autoimmune hepatitis types 1 and 2; it emphasizes once again the detection of LKM/LC antigen (liver- kidney microsomal antigen / liver cytosol) for type 2, which occurs predominantly in childhood.

Classification of autoimmune hepatitis					
Type	AIH	ANA	SMA	Anti-SLA/LP	Anti-LKM1/LC1
1	ANA	+	(+)		
	SAM		+		
	Anti-SLA		(+)	+	
2	Anti-LKM1/LC1				+

Incidence level of autoimmune hepatitis

The literature does not contain sufficiently accurate data on the incidence of autoimmune hepatitis. According to Wirth, there are about 170 cases of disease per 1 million persons (Wirth, 2010). Sukerek assumes incidence levels of 0.1-1.2 cases/100,000 inhabitants in Western Europe, but only 0.08-0.015 cases/100,000 inhabitants in Japan (Sukerek, 2010).

Concomitant autoimmune diseases of autoimmune hepatitis

It is an empirical fact that autoimmune hepatitis can be associated with other autoimmune diseases. The possibility has even been discussed that autoimmune hepatitis may be quasi a parallel phenomenon to a generalized autoimmune disease. Experience has shown that AIH may be associated with the following extrahepatic autoimmune diseases:

- Primary sclerosing cholangitis (PSC)
- A primary biliary cirrhosis (PBC)
- Overlap syndrome of AIH / PBC and AIH / PSC
- Rheumatoid arthritis
- Glomerulonephritis
- Haemolytic anaemias
- Thyroid diseases (e.g. Hashimoto's thyroiditis)
- Diabetes mellitus
- Vitiligo and other skin manifestations
- Coeliac disease
- Chronic inflammatory bowel diseases

Concomitant non-autoimmune diseases of autoimmune hepatitis

In addition to the concomitant autoimmune diseases that may occur together with autoimmune hepatitis there are a number of non-autoimmune concomitant diseases that may also occur in association with autoimmune hepatitis:

- Alcohol damage
- Medical drug damage
- Combined alcohol and drug damage
- Viral hepatitis
- Non-toxic alcoholic steatohepatitis (NASH)
- Metabolic disorders, etc.

Morphology of autoimmune hepatitis

Autoimmune hepatitis is an inflammation of the liver with a highly variable e.g. unspecific pattern of damage. As noted above, because of the possibility of an isomorphic damage profile in liver tissue in the presence of heterogeneous noxae, clinical and laboratory information are required for classification and interpretation of morphologic findings accordingly.

The histological findings characteristic of an autoimmune hepatitis may comprise the following:

- **Periportal inflammation**, primarily lymphocytes, plasma cells and possibly eosinophilic granulocytes. Widened, lighter portal field. It contains a cellular infiltration, composed predominantly of lymphocytes. In terms of immunohistology there are T – lymphocytes with CD3 and CD8 positivity, thus indicative of a T cell-mediated immune response. In between are individual plasma cells and histiocytes. The small bile ducts are normal. No portal fibrosis.
- Interface hepatitis with inflammatory changes in the boundary zone between portal fields and adjacent liver tissue. The border between the portal field and the adjacent liver tissue, i.e. the "interface region" is disjunctive, with individual hepatocytes undergoing apoptosis or destruction. They are in most cases surrounded by lymphocytes, or lymphocytes are migrating into the hepatocytes - a process that is referred to as emperipolesis. Individual necroses with rosette formation and so-called piecemeal necrosis may occur in some cases in the interfacial zones.
- Inflammatory cells, with rosette-like formations - formerly the piecemeal necroses – gather around the hepatocellular single-cell necroses at the interfaces between portal fields and adjacent liver tissue.
- Hydropic swelling of periportal hepatocytes: Mainly in areas near the periportal zone, the hepatocytes may swell, show a lightened cytoplasm and blurred nuclei.
- Lobular single-cell necroses. Intralobular necroses of hepatocytes occur that are characterized by acidophilia, cell debris and isolated inflammatory cells. In the vicinity, the above-mentioned activated Kupffer cells occur in increased numbers and always migrate towards cellular damage.

Kupffer cell hyperplasia: The so-called Kupffer cells are part of the immune system of the liver and can be found wherever there is an immune reaction. In cases of autoimmune hepatitis they are very often present as hyperplastic cell proliferates.

- Necrotic and collapse fields: These may be bridge-like, porto-portal and porto-central. The presence of several of these areas of necrotic changes results in so-called collapse fields.
- Fibroses, porto-portal and portal in chronic disease: In longer disease courses, the lobules react with fibrosis, which may react in both portal and intralobular forms. The maximum variant of such a fibrosis would be hepatic cirrhosis.

Ladies and gentlemen,

in this overview we gained an impression of the various possible reactions of liver tissue in autoimmune hepatitis. It must be emphasized that normal cases rarely show all of these morphological changes at any one time. The rule is that the various forms of reaction in liver tissue are accentuated in varying degrees: Sometimes portal inflammatory parameters predominate, sometimes the lobular regions.

If the bile ducts are also affected in a case of autoimmune hepatitis, showing changes consistent with PBC (primary biliary cirrhosis) or as in PSC (primary sclerosing cholangitis), we speak of a so-called overlap syndrome. These situations of course complicate the diagnostics, just as the occurrence of AIH in childhood represents a challenge to the medical

disciplines contributing to the diagnostic process due to the variations in laboratory parameters.

Summary

The first major summary study of autoimmune hepatitis at the international level essentially goes back to 1999 (F Alvarez, 1999). More than 800 publications appear on the subject annually. A search for "AIH and autoimmune hepatitis" in the database of the German Institute for Medical Documentation and Information in Cologne turned up 449 publications for the year 2010 alone (DIMDI, 2011).

A diagnosis of autoimmune hepatitis is based on clinical, laboratory, molecular biology and pathohistological findings. A liver biopsy is needed to confirm the diagnosis. All of the specialist disciplines involved contribute equally to the diagnostics of autoimmune hepatitis – all are needed and interdependent. No single key diagnostic sign has been determined for this clinical picture to date. A timely diagnosis is crucial in terms of the course and prognosis of the disease.

Ladies and Gentlemen,

I would like to end my remarks with the words with which I began it: The better the information provided by the clinician to the pathologist, the better the morphological diagnosis will be. Both diagnostic partners - the clinician and the pathologist - are interdependent.

You, ladies and gentlemen, are an audience comprising various different specialities. I hope nonetheless that my remarks have satisfied the expectations of at least some of you.

Thank you for your patience and attention.

Since we still have a few minutes, I would like to show you some examples of the wide variety of histological changes from several AIH cases:

1.2. Case studies of autoimmune hepatitis

- Case A: Autoimmune hepatitis: 24-year-old woman with the following laboratory results
- Case B: Autoimmune hepatitis: 48-year-old woman with the following laboratory results
- Case F: Autoimmune hepatitis: 63-year-old woman with the following laboratory results
- Case C: Overlap syndrome (AIH/PBC): 16-year-old boy with the following laboratory results

- Case D: Overlap syndrome (AIH/PBC): 38-year-old man with the following laboratory results

See also:

Power point Presentation with the same title (**Autoimmune hepatitis - diagnosis in collaboration between clinicians and pathologists**)