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STEPS OF PATHOLOGICAL DIAGNOSIS IN THE DIAGNOSTIC PROCESS ASSOCIATED WITH ADULT LIVER TRANSPLANTATION

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В статье описаны последовательные шаги проведения патоморфологического обследования, связанного с выполнением трансплантации печени у взрослых пациентов: 1) морфологическая оценка биоптата печени, полученного у кандидатов на трансплантацию; 2) исследование образца донорской печени, полученного перед и во время выполнения трансплантации; 3) исследование всей удалённой во время трансплантации печени пациента; 4) оценка результатов биопсии печени после пересадки. Кроме того, одной из наиболее частых проблем, с которой сталкиваются патоморфологи при пересадке печени, является принятие решений о перспективах и пригодности донорского органа. В принятии таких решений играют роль не только объективные данные патоморфологического исследования, но и собственный опыт специалиста.

Ключевые слова: трансплантация печени, патоморфологический диагноз

Consecutive steps of pathological diagnosis related to adult liver transplantation were shown herein: (1) assessment of liver biopsy taken prior to transplantation from the transplant candidates, (2) examination of donor's liver biopsy taken prior to and during liver transplantation procedure, (3) assessment of the whole native liver removed during transplantation, and (4) examination of the graft biopsy. Additionally, basic information on pathological diagnostics of the liver transplant with its opportunities and limitations as well as, basing on own experience, the most common problems encountered in pathologist's practice.

Keywords: liver transplantation, pathomorphological diagnosis

Introduction

As surgical technique and medical care of the patient after liver transplantation progress, the group of patients who require continual medical supervision to monitor graft function and diagnose post-transplant complications early grows bigger. Thus, the role of the pathologist as a member of a therapeutic team increases as well, as often on his/her thorough and precise judgment the choice of therapy relies. The aim of this notion is to present basic tasks of the pathologist and prospects and restrictions of the histopathologic methods in liver transplantation.

Stages of pathomorphological diagnostics

Pathomorphological assessment is inevitable for appropriate patient qualification for

transplantation, performing the transplant and monitoring the recipient. Pathological diagnosis has subsequent steps:

1. Assessment of liver biopsy in patients awaiting liver transplantation.
2. Urgent microscopic analysis of donor's liver.
3. Assessment of donor liver after reperfusion (so called «zero biopsy»).
4. Evaluation of the recipient's liver removed during transplantation.
5. Assessment of the transplanted liver biopsy.

Assessment of liver biopsy in patients awaiting liver transplantation

Today, liver biopsy is not done routinely in transplant candidates. In selected, clinically dubious cases a core needle biopsy of the liver is taken, usually under ultrasound guidance to:

- a. determine stage of the disease (so called clinical staging, i.e. degree of liver fibrosis);
- b. assess of histological grading of the disease;
- c. find or confirm etiology of the disease.

Biopsy is usually not done in fulminant hepatitis of various etiologies, when patient's clinical condition is so grave, that only immediate liver transplantation gives any chance of survival. In very rare situations with more favorable course (subfulminant hepatitis) a biopsy can be performed to assess regenerative potential and chance to recover the function of the liver. However, as distribution of the changes (mostly necrosis) is not uniform, taken specimen may not reflect an actuarial condition of the whole organ and histopathology can be misleading.

In remaining cases of chronic liver insufficiency with signs and symptoms of portal hypertension, liver cirrhosis is usually diagnosed. In Poland, majority of Western Europe countries and the U.S. most common etiologies are viral (B, C and D) hepatitis and alcoholic liver disease. Less common are autoimmune diseases: primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH) and so called overlap syndromes, which have features of two or three of the above. The next group in a row are metabolic liver diseases, with Wilson's disease and hemochromatosis being most common.

Despite extensive diagnostics, there still remains a small group of patients with cryptogenic cirrhosis, i.e. final diagnosis cannot be established. Many researchers believe that the disease is caused by an unknown hepatotropic virus. In some innumerable cases liver transplantation is offered to patients without cirrhosis or even with no signs of portal hypertension, for instance patients infected with *Echinococcus multilocularis*, polycystic liver disease, primary portal hypertension, Budd-Chiari syndrome, early primary biliary cirrhosis with itching being a primary symptom affecting patient's vital activity.

Causes of fulminant liver failure in adults is

most often concomitant infection with B and D viruses, intoxication with acetaminophen or *Amanita phalloides* as well as Wilson's disease.

Urgent microscopic analysis of donor's liver

Next task for pathologist is to evaluate donor's liver and okay it or discharge from transplantation. In the beginning of transplantation program in each center pathologist's opinion on qualification of the organ for transplant is sought almost every time. With accumulating experience, macroscopic assessment of the liver by a transplant surgeon becomes sufficient to determine whether the graft is suitable for transplantation. Only in innumerable cases pathologist is asked for microscopic evaluation. This occurs for instance when major fatty degeneration, fibrosis or necrosis is suspected. Sometimes during organ retrieval surgeon finds a small spot of pathologic tissue – usually benign, e.g. cholangiocellular adenoma, microhamartoma, haemangioma, focus of non-neoplastic ductal hyperplasia etc. Such situation requires calling a pathologist for definite verification of the lesion. In very rare instances a surgeon discovers focal lesion in extrahepatic organ missed by visual studies. Just as within the liver, such changes are usually small and benign, e.g. renal adenoma, ovarian cyst, pancreatic cyst, myoma of the uterus etc. Microscopic evaluation of the finding is however necessary.

Assessment of the donor's liver is an emergency procedure. Specimens sent in physiologic saline solution are processed exactly as any intraoperative material. They are frozen on a cryostat, sliced into thin slices, dyed with hematoxylin and eosin and placed on a glass slide. As other emergency assays, these preparations are of inferior quality than paraffin sections obtained from formalin-fixed samples. Assessment of these samples is more difficult, as they often look «worse» than they really are. An «overdiagnosis», i.e. evaluation of small changes as significant, is more common than «underdiagnosis», i.e. qualification of the liver for

transplantation despite severe morphological abnormalities. There is always an opportunity to compare freeze-section samples with the same formalin-preserved, paraffin-embedded specimen processed later. Of course in such case pathologist's mistake is irreparable, and either good liver is lost or a patient receives suboptimal graft. Classical histologic contraindications for liver transplantation are: macrovesicular steatosis exceeding 60% of hepatocytes (Fig. 1, see colour sheet) and obvious pathology such as extensive necrosis, severe fibrosis of the organ, significant inflammatory infiltrates and cancer. It is generally accepted, that microvesicular steatosis does not affect graft function [1], although there are notions connecting it to graft failure [2]. Additional obstacle is, that sometimes on frozen section artifacts can be seen (attributable to both organ preservation and sample preparation), mimicking image of microvesicular steatosis. As these changes are extensive and affect nearly all hepatocytes, they can cause significant difficulty to pathologist assessing frozen section. After formalin fixation, «microvesicular steatosis» recedes. It should be stressed, that qualification criteria for elective transplantation are more stringent than for life-threatening conditions, i.e. in acute organ failure.

Assessment of donor liver after reperfusion (so called «zero biopsy»)

Post-reperfusion, i.e. zero biopsy is a useful tool for assessment of transplanted liver quality. This biopsy allows diagnosing potential donor's disease (if no frozen sections were run to disqualify an organ with significant pathology) and additionally describes changes associated with pre-operative period and surgical procedure. A sample is taken by a surgeon after completion of vascular anastomoses (inferior vena cava, porto-portal and hepatic artery) and anastomosis of biliary tract, i.e. practically after finishing liver transplant, prior to wound closure. Many factors affect quality of donor's liver at this moment, from donor's primary disease and therapy, via organ retrieval, transportation, preparation for transplant

to operation in a recipient. Preservation injury is a name given to factors influencing status of the donor's liver from the moment of cessation of circulation (and beginning of flush with preservation solution through portal vein and abdominal aorta) till the end of vascular anastomosis (so called cold ischemia). It should be noted that a number of external factors as well as those dependent on a patient (both donor and recipient) affect transplanted liver.

Morphological images of frozen sections and those retrieved after reperfusion do not differ substantially. Usually mild or moderate inflammatory granulocyte infiltrates within the hepatic lobule (as a result of surgical intervention), minute subcapsular hemorrhages, pericentral necrosis of hepatocytes, apoptosis of liver cells and features of its regeneration are found. It is usually believed, that exactly as frozen section, post reperfusion liver biopsy not always does reflect true hepatocyte injury, which in early period can be visible only in ultrastructural studies.

Evaluation of the recipient's liver removed during transplantation

At this stage pathologist examines the whole liver removed from the recipient during the transplantation procedure. Macroscopic description is a must, and then microscopic evaluation should be based on analysis of numerous samples from various regions of the liver (both lobes, gallbladder, and focal lesions if present). Conclusions summarize the results of examination.

According to etiology of liver disease (either confirmed or suspected), special attention ought to be paid on particular regions of the liver, i.e. when PSC is suspected, specimens from large extra- and intrahepatic bile ducts should be taken. Suspected neoplasm, usually hepatocellular carcinoma (HCC) (Fig. 2, see colour sheet) calls not only for sampling well visible, different from surrounding tissue tumor, but for meticulous slicing the liver in search for satellites or multi-foci as well. As patients with viral hepatitis C and alcohol abuse

are at the highest risk of developing HCC [3], and patients with hemochromatosis form the second group, they should be screened especially thoroughly for focal lesions. Microcellular dysplasia of hepatocytes is considered preneoplastic lesion [3]. It is rarely seen, and if unaccompanied by a cancer, means that the transplantation preceded progression to invasive cancer. Macrocellular dysplasia is seen more often, yet it is not considered preneoplastic stage.

Despite the whole recipient's liver available for studies, pathologist is not always capable of answering all the questions clinicians put forward. Most often they inquire about etiology of the disease, however unfortunately there are no true characteristic morphological features of each nosological unit. Histological diagnosis is often based on probability and statistical prevalence of some features over the other in a given pathology of the liver. Hence, clinical data are extremely important and when unavailable, pathologist ought to stop at description of the visible abnormalities and suggestion of differential diagnosis. When clinical diagnosis was established with serological and visual studies, histopathology allows for its confirmation. In dubious cases it can help coming to final diagnosis. Knowledge of etiology is important, as different liver primary diseases have different prognosis. It relates to both acute and chronic rejection (more frequent in patients with autoimmune etiology of cirrhosis) [4, 5] and recurrences of disease (for instance hepatitis C recurs in nearly all patients) [6, 7] (Fig. 3, see colour sheet).

Pathologist encounters particular difficulties in some liver tumors with histological image of hepatoma and/or highly differentiated hepatocellular carcinoma. Nevertheless there are some points of morphological difference described in the literature and didactic monographies, it is not always possible to distinguish between above changes. Due to «radical» removal of usually small tumor, further follow up as to potential of disease recurrence is not able to define the character (malignancy) of the primary lesion.

Assessment of the transplanted liver biopsy

The role of histopathological examination in monitoring of the transplanted liver function is humongous; in many cases microscopic evaluation is inevitable. Due to excellent results of liver transplantation (80% of patients survive one year after transplantation) [6, 8] and more and more effective immunosuppression methods the number of patients after transplantation grows. Nearly all of them at some point of follow up need core needle biopsy of the graft. In transplant centers, which began their programs years ago, there were protocols including routine periodical biopsies of the graft regardless of clinical course and occurrence of post-operative complications. In most cases, «0», seven-day, one year and 5 years after transplantation biopsy were taken [9]. Of course this considered only patients with no significant symptoms of disease, in other cases biopsy was performed more often. Hence, many patients after transplantation had numerous microscopic studies of the liver, which rarely influenced medical treatment. They had undoubted scientific value and we owe them some knowledge of the picture of transplanted liver in various time points after surgery.

Nowadays, routine biopsy of patients with normal liver function was given up. Sometimes it is done many years (usually five) later to assess the graft before eventual weaning of immunosuppression.

When graft function is abnormal, microscopic evaluation of liver biopsy is nearly always necessary. A cause of graft dysfunction could be a complication of liver transplantation or recurrence of primary disease. Most frequent complications are:

- a) infections – viral (cytomegalovirus, Epstein-Barr virus, rarely adenovirus, herpes simplex, primary hepatitis B and C), bacterial (when biliary stricture is present), fungal;
- b) vascular – stricture or thrombosis in vascular anastomosis (hepatic artery, portal vein and its branches, hepatic veins), haemorrhagic

complications. Thrombosis of the hepatic artery can result in ischemia and necrosis of large biliary ducts and their subsequent stricture;

c) biliary-involving anastomosis strictures and strictures resultant from biliary tree ischemia (ischaemic cholangitis).

According to criterion of frequency of recurrence after transplantation, liver pathology can be divided as follows:

a) diseases almost always and often recur in transplanted liver (malignant neoplasms, hepatitis C and B);

b) those, hardly ever recurring, or those with course so benign, that they never call for retransplantation (PBC, alcoholic liver disease – approximately 10–30% of patients relapse into previous drinking habits [6];

c) those, which can result in significant graft dysfunction, but frequency of their diagnosis is not quite determined. There are no certain diagnostic criteria and diagnosis can be very difficult and often subjective (autoimmune liver diseases, hemochromatosis, Budd-Chiari syndrome [6, 7].

Among many complications of liver transplantation there are ones that can be diagnosed solely on biopsy, as there are no specific markers allowing for diagnosis. The best example is graft rejection. In other cases, for instance in suspicion of viral hepatitis, diagnosis can be confirmed with serological tests, however biopsy is necessary for determination of activity and staging of the disease.

Unfortunately liver morphology is rarely unequivocal and pathologist can rarely undoubtedly determine the cause of its malfunction. Often specimen morphology suggests coexistence of at least two different pathologies. Similarly, when native liver is studied to determine etiology, hardly ever a histopathological feature can be attributed to singular pathology. Usually it is present in a number of diseases, with variable occurrence. In differential diagnosis greatest similarity of morphological features of liver images can be found in:

a) hepatitis C and acute rejection

b) PBC and chronic rejection

c) Recurrence of PSC and ischemic biliary stricture.

Additional factor changing image of the liver is immunosuppressive therapy, which blunts immune response, diminishing inflammatory infiltrates and itself can be toxic to the organ.

In an early post-operative period, during the first month, a question asked most often by a clinician is whether acute rejection is present. Up to 80% of patients have histological features of acute rejection, and 20–50% of them need additional immunosuppression [4, 6]. Magnitude of acute rejection in majority of centers is assessed in Banff classification [10], based on a classic Snover's triad [11]. It covers evaluation of three basic parameters: inflammation of the walls of portal and central veins (venulitis), inflammation of bile ducts wall (ductitis) and extent of inflammation of the portal space (Fig. 4, see colour sheet). Biopsy before modification of immunosuppression is inevitable. Otherwise morphological picture changes greatly, inflammation recedes and morphological description can be unreliable. Core needle liver biopsy is prepared for assessment with abbreviated histological technique, just as are other small tissue specimens (for instance taken on gastroscopy) and pathologist usually is able to judge if there is an acute rejection or not at most on the following day. In many centers apart from eosin and hematoxylin additional stains are made (Gomori, azan, trichrome, PAS after diastase treatment, orcein) to visualize reticulin fibers, vascular walls, histiocytes, copper deposits, viral particles within hepatocyte cytoplasm).

Apart from a number of described pathologies there are also patients, who in spite of morphological changes seen in liver biopsy have no symptoms, and their biochemical tests are normal or nearly normal. This concerns mostly biopsies taken late after transplantation, as a part of transplant function monitoring protocol. This is considerably large group of patients (20–40%) [6], in whom liver morphology resembles viral

hepatitis, yet it cannot be confirmed with any laboratory studies.

Summary

In conclusion, transplant pathologist has a very significant role in liver transplantation process:

- decisive in donor liver acceptance for transplant in clinically dubious cases
- determination of the need for additional immunosuppression when graft rejection is diagnosed
- cooperation in diagnosis of post-transplant complications: infection, blood flow disturbances, biliary complications, de novo carcinogenesis (for instance skin cancers or post-transplant lymphoproliferative disorder)
- cooperation in diagnosis of recurrence of primary disease: hepatitis (usually C), autoimmune diseases, alcoholic liver disease, neoplasms, some metabolic diseases and other rare diseases
- determination of activity and progression of the disease with decision when to retransplant according to histopathological features.

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Поступила 5.10.2010 г.