used less often.[174] Shikata-type stains, such as orcein and aldehyde fuchsin, detect increased copper-binding protein(s) and may be used as a surrogate for detection of increased copper. Because histochemical stains for copper in general have poor sensitivity and a negative stain does not exclude the diagnosis of WD,[174] quantitative copper analysis of the liver biopsy is preferred. Histochemically detected hepatocellular copper provides a clue to diagnosis of WD and may be particularly helpful in the context of ALF.

Ultrastructural abnormalities in hepatocellular mitochondria are highly characteristic of WD,[175] notably in pediatric patients.[176] Typical findings include variability in size and shape, increased density of the matrix material, numerous inclusions including lipid, and fine granular material, which may be copper and copper-binding proteins.[177] The most striking alteration in mitochondrial structure is increased intracristal space with dilatation of the tips of the cristae, creating a cystic appearance. At later stages of the disease, dense deposits within lysosomes are present. With adequate treatment, these mitochondrial changes may resolve.

Although hepatocellular carcinoma (HCC) has been regarded as a rare complication of WD, it may be more frequent than previously recognized. Liver biopsy has a role in characterizing hepatic neoplasia complicating WD, including distinction of HCC and intrahepatic cholangiocarcinoma (CCA).

Hepatic parenchymal copper concentration

Normal hepatic copper content is <50 μg/g dry weight. In most but not all cases of WD, hepatic copper content is greatly elevated: >250 μg/g dry weight is generally the reference value. A threshold value of 75 μg/g was proposed to increase test sensitivity.[30] Another large study proposed 209 μg/g dry weight as having a sensitivity of 99% and specificity of 96%.[178] The concentration of hepatic copper in simple heterozygotes, although frequently elevated above normal, is usually <250 μg/g dry weight.[179] Hepatic copper also accumulates with chronic cholestatic disorders and cirrhosis, typically not to WD levels.[180] Indian childhood cirrhosis and other idiopathic copper toxicosis syndromes are also characterized by very high levels of hepatic copper.[181,182]

Biopsies for quantitative copper determination should be taken with a disposable suction or cutting needle and placed without additional liquid in a copper-free container. To prevent autolysis, a core (or part of a biopsy core) of liver should be frozen immediately or vacuum-dried for shipment to a laboratory for quantitative copper determination, according to the direction of the local laboratory. Paraffin embedded specimens can be analyzed for copper content but require additional laboratory processing.

Because hepatic copper is irregularly distributed in the parenchyma in WD, analysis of liver biopsies may result in underestimation of the copper content, especially in the setting of cirrhosis. To overcome the problem of sampling error, performing two biopsy passes has been proposed,[183] with one core devoted entirely to analysis of hepatic copper concentration.[178] Accuracy and precision of measurement are related to sample size; a decrease in accuracy of 15% has been reported for sample weights of less than 3 mg, with further decreases with decreasing weight of the biopsy.[184] Reported pediatric experience indicates that measuring hepatic copper content is reliable, based on data with a single biopsy core.[185] A minimum biopsy dry weight of 3 mg[183] or at least 1–2 cm long[186] should be submitted for analysis. Transjugular liver biopsy has largely circumvented technical problems associated with obtaining a liver biopsy in patients with decompensated cirrhosis or severe coagulopathy; however, multiple samples may be required due to the small diameter of the biopsy needle. Measurement of hepatic parenchymal copper concentration is most important in younger patients in whom hepatocellular copper is mainly cytoplasmic and thus undetectable by routine histochemical methods.

The most commonly used technique for measuring hepatic copper has been atomic absorption spectroscopy, but this method is being supplanted in many reference laboratories by quantitative inductively coupled plasma mass spectroscopy (ICP-MS). Laser-ablation ICP-MS, used in the research setting, allows imaging of the spatial distribution of copper within liver tissue[187,188] and highlights its heterogeneous distribution.

Imaging of the brain

Magnetic resonance imaging (MRI)

MRI of the brain can be helpful in establishing a diagnosis for patients with unexplained neurological and psychiatric symptoms and frequently reveals findings consistent with WD. MRI findings include signal changes in the basal ganglia, thalami, pons, and white matter, as well as atrophy.[189] The so-called “face of the giant panda sign,” which consists of increased T2 signal in the midbrain (Figure 1), has been considered pathognomonic for WD, but several other findings are more commonly seen. MRI findings typical of WD must be distinguished from changes associated with end-stage chronic liver disease. Patients with extensive changes on brain imaging, such as evidence of tissue cavitation, are less likely to improve with treatment.[190] In general, repeated MRIs are not useful for determining prognosis or monitoring neurological progression.