Biomarkers to Monitor Graft Function Following Liver Transplantation

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Abstract

Liver transplantation (LT) has become the only curative treatment for end-stage liver disease. Patient survival has improved drastically over the years, but poor initial graft quality and complications following transplantation still limit patient and graft survival. Monitoring and evaluation of graft quality during follow-up is achieved by routine biomarker measurements in recipients' blood, starting directly following surgery and in the months and years thereafter. This allows clinicians to early detect complications following LT, like early allograft dysfunction and biliary complications. They are also used as a tool for deciding on further diagnostics or interventions. Classic biomarkers are able to assess liver injury (aspartate and alanine aminotransferase, lactate dehydrogenase), biliary injury and obstruction (gamma-glutamyl transferase, alkaline phosphatase), and liver function (albumin, bilirubin, prothrombin time). Novel genetic markers such as microRNAs also show potential as more accurate or specific biomarker for various types of injury and functions. Some of these serum biomarkers were shown to be promising in predicting disease or severity of injury when measured in bile, though widespread implementation in clinical practice is not implemented yet. Therefore, liver biopsy remains the gold standard for diagnosing acute cellular rejection, even with less invasive serum biomarkers that are currently available. Future applications of biomarkers should enable early assessment of marginal graft function when applied to preservation solution in both simple cold storage and during ex situ machine perfusion. In the future, these developments could help to increase the donor pool for LT by optimizing and allocating grafts based on favorable biomarker profiles from donors with unfavorable clinical characteristics.

Keywords

Serum markers • Transaminases • Complications • Graft dysfunction • Biliary strictures • Cholestasis • Recurrence of disease • microRNAs • Machine perfusion • Risk factors

List of Abbreviations

ACR	Acute cellular rejection
AFP	Alpha fetoprotein
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AS	Anastomotic biliary stricture
AST	Aspartate aminotransferase
CA 19-9	Cancer antigen 19-9
CCA	Cholangiocarcinoma
CDmiR	Cholangiocyte-derived miRNA
DCD	Donation after circulatory death
EAD	Early allograft dysfunction
ERCP	Endoscopic retrograde cholangiopancreatography
GGT	Gamma-glutamyl transferase
HCC	Hepatocellular carcinoma
HDmiR	Hepatocyte-derived miRNA
LT	Liver transplantation
MiRNA	microRNA
MP	Machine perfusion.
MRCP	Magnetic resonance cholangiopancreatography
mRNA	Messenger RNA
NAS	Non-anastomotic biliary stricture
PNF	Primary nonfunction
PSC	Primary sclerosing cholangitis
SNP	Single nucleotide polymorphism

Key Facts of microRNAs

- MicroRNAs (also called miRNAs or miRs) are 20–23 nucleotide-long, hairpin-shaped RNA. Up to 30% of the human genes is regulated by miRNAs via inhibition of mRNA translation.
- A single miRNA is responsible for the regulation of multiple genes.
- The first reports on the presence of miRNAs in *Caenorhabditis elegans* date from 2001, and since then, over a 1,000 different miRNAs have been discovered in mammals.
- Various cell types express distinct sets of miRNAs that are related to metabolism, oncology, endocrinology, the vascular system, and infection.
- Tissue-abundant miRNAs are released from cells into the circulation and other body fluids under different (patho)physiological conditions via active and passive mechanisms.

– In contrast to mRNA, extracellular miRNA is protected from degradation in fluids, making them attractive for noninvasive biomarker research.

Definitions of Words and Terms

Anastomotic stricture (AS)	Isolated benign tapering of the biliary anastomosis following LT.
Cholangiocarcinoma (CCA)	Malignancy of the hepatic bile ducts and cholangiocytes.
Cholestasis	Accumulation of bile due to obstruction flow to the duodenum or altered bile composition.
Donation after brain death (DBD)	Procurement of donor organs after disappearance of brain stem functions (brain death), while the circulation is still intact. Organs are usually of better quality compared to DCD.
Donation after circulatory death (DCD)	Procurement of donor organs after circulatory arrest of the donor. Associated with warm-ischemic injury of organs.
Early allograft dysfunction (EAD)	Poor graft function in the first week post-LT, based on AST or ALT >2,000 IU/L, or total bilirubin serum levels >10 μg/L on day 7 post-LT, or INR >1.6 on day 7 post-LT.
Hepatocellular carcinoma (HCC)	Malignancy of liver parenchyma and hepatocytes.
MicroRNAs	Small, noncoding RNAs involved in posttranscriptional gene regulation. Potential novel biomarkers.
Non-anastomotic strictures (NAS)	Benign tapering of the intrahepatic and (perihilar)-extrahepatic bile ducts following LT.
Preservation	Storage of organs at cold temperature and suitable fluids to prevent deterioration of the grafts, for optimal quality and functioning following transplantation.
Primary sclerosing cholangitis (PSC)	Autoimmune disease in which there is a progressive fibrosis of the intra- and extrahepatic bile ducts.

Introduction

The liver is the largest visceral and most multifunctional organ of the human body. It produces and drains bile, which is responsible for digestion. Furthermore, the liver metabolizes glucose, proteins like albumin and coagulation factors, amino acids, and lipids. Detoxification is achieved by the breakdown of hormones like insulin and drugs. Cells in the livers' reticuloendothelial system are responsible for immunological effects and protection against certain antigens (Burroughs and Westaby

2005). This enumeration describes only part of all liver functions but also illustrates the livers' diverse and essential role for the body. Under stable conditions, the liver has 60–70% overcapacity. This allows for resection in healthy individuals of up to 70% of liver volume (Kishi et al. 2009). After such surgery, the liver will regenerate to its normal volume within weeks. However, an absent liver function due to acute liver failure or chronic end-stage liver disease is not compatible with life and can only be cured by liver transplantation (LT).

It took 4 years for Thomas Starzl to perform the first successful LT in human in 1967, after several unsuccessful attempts since 1963, with most patients dying on the operation table (Starzl et al. 1963, 1968). Still, the first LT series in human reported a 1-year survival rate of only 25%, illustrating the complex surgical technique and severe complications that could occur early following LT in those days. One of the major complications limiting patient and graft survival was acute rejection of the transplanted organ against the recipient. A decade later, survival rates of LT recipients improved drastically after Sir Roy Calne introduced cyclosporine, an immunosuppressant drug, into the clinic (Calne et al. 1979).

Nearly 50 years later, LT is regarded standard treatment for end-stage liver disease and performed worldwide in various populations suffering from different pathologies. Because of optimized surgical techniques and immunosuppressant regimens, graft survival can now reach beyond 20 years with excellent graft function in some recipients (Jain et al. 2000). This has also led to an expansion of the designated indications for LT; on-going trials investigate the benefit of LT in selected patients with cholangiocarcinoma (Darwish Murad et al. 2012a), hepatocellular carcinoma (Mazzaferro et al. 1996), and colorectal liver metastases (Dueland et al. 2015). However, while the list of patients awaiting LT is getting longer, the number of transplantable organs remains scarce. Moreover, the quality of transplantable organs is deteriorating due to increasing donor age, liver steatosis, viral hepatitis of the donor, and prolonged ischemia times following donation after circulatory death (DCD) (Durand et al. 2008). All these factors can cause a wide range of complications threatening graft and patient survival following LT. Early complications mainly consist of infections, graft primary nonfunction (PNF), early allograft dysfunction (EAD), biliary complications (i.e., leakage and anastomotic and non-anastomotic biliary strictures), and acute rejection. Besides biliary complications, other complications at the intermediate and long-term usually consist of recurrence of liver disease that initially required LT (like hepatitis C viral infection and primary sclerosing cholangitis), the development of malignancies, chronic rejection, and liver fibrosis (Verhoeven et al. 2014).

In order to discover these complications in LT recipients timely, monitoring of graft function with suitable biomarkers is required. Routine monitoring of minimally or noninvasive biomarkers enables early recognition of complications to which physicians can adapt their medical policy. Two examples are to obtain histology in the case of suspicion of allograft rejection or to perform imaging/endoscopic treatment in the case of suspicion of biliary complications. Therefore, LT recipients are subjected to protocol (blood) measurements depending on their clinical status during follow-up, varying from daily monitoring at the intensive care unit directly after

surgery to yearly routine measurements at the outpatient clinic. Different patients and underlying diseases require personalized or precision monitoring with established biomarkers in liver disease.

The following paragraphs provide an outline on the definition of biomarkers in the field of LT and the different types of biomarkers that are used in clinical practice for short- and long-term monitoring of graft function. Finally, potentially interesting novel biomarkers are discussed, and recommendations are given regarding future applications of biomarkers in the context of LT.

Definition of Biomarkers in Liver Transplantation

The term "biomarker," an amalgamation of the words "biological marker," was defined in 1998 by a working group of the National Institutes of Health, describing it as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working Group 2001). Since that time, however, multiple other definitions have been introduced that further expanded the interpretation of the term biomarker. This was, for instance, done by a collaboration of the World Health Organization, the United Nations, and the International Labor Organization, who defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease"(WHO 2001). Based on the descriptions above, one can conclude that biomarkers can be used to measure the effect of treatment as well as predict or be related to a clinical endpoint. Biomarkers are also increasingly being used as a primary or secondary outcome measure in experimental or clinical studies and therefore sometimes applied as a surrogate endpoint (Strimbu and Tavel 2010). Especially in LT, definitions like EAD or PNF are mainly defined by persistently elevated transaminase levels in serum, often combined with perturbed coagulation function of the liver.

Furthermore, the previously described definitions on biomarkers allow to distinguish "dynamic" markers from "static" markers. In the context of LT, dynamic markers are usually molecular markers and liver enzymes that can be measured in serum and which levels fluctuate depending on the functional status or degree of injury of the liver graft. As an example, immediately after LT, ischemia-reperfusion injury of the graft causes elevation of serum aspartate and alanine aminotransferase levels (AST, ALT) above 200 IU/L, while a more gradual rise in gamma-glutamyl transferase (GGT) or alkaline phosphatase (ALP) starts approximately 24–48 h after LT (Fig. 2). When a patient has been transplanted because of viral hepatitis as underlying pathology, routine measurements of viral load during follow-up are part of regular clinical practice. This is because of the reasonable chance of recurrence of disease in the new liver graft (Al-Hamoudi et al. 2015). Depending on the type of complication, treating the cause will ultimately result in normalization of

serum levels of dynamic markers. Therefore, dynamic markers are variable markers that can be suitable for determining whether treatment or interventions are successful.

Static markers on the other hand are less subjected to change by the (patho) physiological status of the liver graft. One could think of genetic polymorphisms like single nucleotide polymorphisms (SNPs) of either donors or recipients that are related with certain outcomes following LT. Genetic markers or SNPs are more often fixed factors that do not fluctuate or change by graft injury. However, certain polymorphisms do make LT recipients more susceptible for certain complications; several SNPs involved in the innate immunity system have been correlated to a higher incidence of severe infections post-LT (de Rooij et al. 2010). Also in recipients that were transplanted for cholestatic diseases like primary sclerosing cholangitis (PSC), certain SNPs were identified that cause earlier recurrence of severe biliary injury after LT (op den Dries et al. 2011). Because of the predicting capacity for outcome rather than their monitoring capacities, in literature, SNPs are more often referred to as "risk factors" instead of biomarkers.

A separate category of markers are histological markers or markers measured in liver biopsies. Up to a decade ago, many transplant centers monitored graft injury and rejection by evaluating histological changes in by-protocol liver biopsies during follow-up. Most dynamic markers for liver injury in serum are related to histological changes of the liver parenchyma and bile ducts (Giannini et al. 2005). However, it usually takes more time to detect histological and morphological changes in liver tissue and puncture of the liver is not harmless. Therefore, taking liver biopsies is nowadays mainly indicated to confirm suspected graft rejection and recurrence of disease or malignancy based on changes in serum biomarkers and imaging.

The next chapters will focus mainly on dynamic markers in blood and serum and the most important histological markers associate with liver injury and function following LT.

Different Biomarkers for Different Cell Types

In liver disease, biomarkers are divided in predominantly hepatocellular or cholestatic markers. Liver enzymes as AST and ALT are indicative of hepatocellular injury, while GGT and ALP reflect biliary injury or obstruction. Besides these two categories, markers of liver function are also of importance for the evaluation of graft quality, especially in the first days following LT. Very often, the liver enzymes AST and ALT are used to indirectly asses liver function. Strictly spoken they do not represent liver function but are more indicative of liver cell death. Thus, for this purpose it is more useful to analyze products that are normally metabolized or synthesized by the liver, like proteins such as albumin and certain coagulation markers. Table 1 provides an overview of classic biomarkers per cell type, injury or function, which are discussed more extensively in the following paragraphs.

Table 1 Conventional biomarkers used in liver transplantation for graft monitoring

Category	Biomarkers
Hepatocellular injury	AST, ALT, LDH
Cholangiocyte injury and cholestasis	GGT, ALP, bilirubin
Liver function	Albumin, bilirubin, PT, INR
Recurrence or new onset HCC	AFP
Recurrence or new onset CCA	CA 19-9

Biomarkers for Hepatocellular Injury

Aspartate Aminotransferase (AST)

AST is an enzyme involved in the production of proteins and catabolization of amino acids, allowing them to cross membranes and enter the citric acid cycle. In humans, AST is present in a descending concentration in the following tissues: the heart, liver, skeletal muscle, kidney, pancreas, spleen, lungs, brain, and erythrocytes. Current clinically applied techniques however do not trace tissue origin from which AST was released. Therefore, it is often necessary to involve other markers as well for the interpretation of serum AST in the clinical setting. AST can be measured in serum and plasma obtained through venipuncture, remaining stable for at least 24 h at room temperature. Halftime is approximately 12 h. Two iso-enzymes of AST can be distinguished that occur in separate cellular compartments, namely, in the cytoplasm (c-AST) and in the mitochondria (m-AST). Following mild tissue injury, particularly c-AST can be elevated in serum, while severe injury will also lead to a release of m-AST (Kirsch et al. 1984). In adult healthy individuals, the range of AST varies between 31 and 35 U/l but usually depends on sex and age (Hooijkaas et al. 2013a).

Following LT, peak AST in serum is usually reached within the first 24–48 h after surgery, sometimes being a 100-fold increased or higher. In particular when a liver graft is of poor quality, for instance, due to increased warm ischemia time, high donor age, or liver steatosis, peak AST can reach extreme values during the first week post-LT (>1,000 U/l). Although transaminase levels usually decrease quickly following LT, one should be careful with interpreting this as graft recovery. Massive hepatocellular necrosis can result in hepatic failure, which should be evaluated based on the capacity of the graft's coagulation function and bile production. Therefore, both markers for hepatocellular injury (AST, ALT) as well as cholestatic markers (ALP, GGT) and functional markers (PT, INR, albumin, bilirubin) should always be evaluated together directly following LT.

Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) catalyzes the transfer of the amino group L-alanine to α-ketoglutarate, resulting in the production of pyruvate and L-glutamate. High

concentrations occur in the hepatocyte cytoplasm, whereas only low concentrations are found in heart and kidney tissue (Wroblewski 1958). Therefore, ALT is considered to be more liver specific compared to AST. However, because of their differences in intralobular distribution, elevation of AST levels is usually faster than ALT. Nevertheless, serum or plasma ALT has proven to be of value in the diagnostic process of various liver diseases. For instance, in acute viral hepatitis, serum ALT can quickly rise up to 20-fold its normal range, while levels of AST remain lower or show only mild increase. At the same time, the ALT/AST ratio, which is <1 in healthy individuals, becomes >1 (De Ritis et al. 2006). Chronic (viral) hepatitis results in milder elevations of AST and ALT. When levels of AST become higher than ALT, one should be aware of cellular necrosis.

Despite being markers of hepatocellular injury, biliary obstruction can also result in liver injury and therefore increased levels of AST and ALT. Furthermore, peak serum ALT levels in the first week following LT have been associated with the development of severe biliary complications (den Dulk et al. 2015). A possible explanation for this finding could lay within the distribution of ALT in the liver acinus; the bile ducts and hepatic artery are located periportally (zone 1). Ischemic injury in this zone will cause release of ALT into the serum. Zone 3 on the other hand is located pericentrally, is less oxygenated, and contains higher concentrations of AST (Giannini et al. 2005). It remains unclear whether serum levels of AST are also related to the development of biliary complications.

Just like AST, the reference value of ALT depends on sex and age but normally does not rise above 50 U/l. Be aware that halftime of ALT in plasma or serum is however longer, approximately 50 h.

Lactate Dehydrogenase (LDH)

This enzyme catalyzes the conversion of lactate into pyruvate and vice versa. Pyruvate, the product of glycolysis, is converted to lactate under anaerobic conditions. The inverse reaction takes place in the liver and results in gluconeogenesis. LDH is present in the cell cytoplasm of practically all organs in the human body, making it widely applicable but thereby also less attractive for diagnostic purposes. Also distinguishing between the five different isotypes of LDH, which differ in characteristics as halftime, does not seem to give additional diagnostic benefit. Furthermore, hemolysis can give an overestimation of LDH activity in serum. The normal range of LDH in healthy adults is <225 U/l (Hooijkaas et al. 2013b).

Despite these apparent shortcomings, LDH is still applied as a clinical biomarker in the follow-up of liver transplant recipients. Strong elevations of LDH in serum or plasma directly after liver transplantation are usually indicative for the severity of ischemia-reperfusion injury of the graft. When strong elevations of LDH prolong and are accompanied with high levels of other transaminases, one should be aware of serious complications, like hepatic artery thrombosis (Cassidy and Reynolds 1994). But experimental studies also suggest the measurement of LDH in bile to assess the

amount of biliary or cholangiocyte injury (Op den Dries et al. 2014). However, this novel application of LDH is currently not used in standard clinical practice.

Biomarkers for Biliary Obstruction or Cholestasis

Gamma-Glutamyl Transferase (GGT)

The enzyme GGT is a carboxypeptidase located in cellular membranes. It transfers gamma-glutamyl glutathione to acceptor amino acids, peptides, or water. Furthermore, it transfers amino acids across the cellular membrane. The hepatopancreatobiliary system is the largest contributor of GGT levels in serum, but high concentrations of GGT are also present in kidney tubular epithelium and prostate tissue. Lower tissues are found in the spleen, brain, and heart. The liver excretes GGT via the bile. Therefore, biliary obstructions can cause strong elevations of GGT in serum (Goldberg 1980). Together with alkaline phosphatase (ALP), GGT is useful to screen whether recipients have developed significant biliary complications following LT, in particular anastomotic and non-anastomotic strictures (AS and NAS, respectively). In contrast to ALP, GGT is not elevated in bone disease (Lum and Gambino 1972). Increased serum levels of cholestatic markers are an indication to perform further imaging to determine the cause of obstruction, generally via endoscopic retrograde cholangiopancreatography (ERCP) or via magnetic resonance cholangiopancreatography (MRCP).

In healthy adults, GGT serum levels are below 35–40 U/l. Directly following LT, levels of GGT are often not elevated but start to rise within the first postoperative days. If a recipient develops AS, levels of GGT and ALP are expected to be high, up to 400–500 U/L. Stenting of the biliary anastomosis will give a rapid normalization of serum levels, as illustrated in the right panel of Fig. 2. When a liver graft is affected by NAS, levels of GGT and ALP can strongly fluctuate, but will increase over time, since these strictures are more stubborn to treat by stents or percutaneous drains. When a mild rise in cholestatic markers is accompanied by a rise in hepatocellular markers, one should also think of (recurrence of chronic) hepatitis (Huang et al. 2014).

A paradoxical finding confirmed by multiple researchers is that higher levels of GGT early following LT are associated with improved 90-day survival in recipients, while recipients who died before the 90th postoperative day had lower GGT serum levels (Eisenbach et al. 2009). After the first 90 days, however, high levels of GGT are associated with impaired 5-year survival. It has been suggested that high levels of GGT early following surgery are the result of a proper systemic response to reactive oxygen species that are released after graft reperfusion. A different hypothesis states that the increase of GGT is correlated to regeneration of hepatocytes following LT (Alkozai et al. 2014). Direct evidence for this hypothesis is however not available.

Alkaline Phosphatase (ALP)

The enzyme ALP is responsible for dephosphorylation of multiple types of molecules. It is bound to plasma membrane lipoproteins of tissues throughout

the entire body. Serum ALP is mostly derived from liver parenchyma, biliary epithelium (cholangiocytes), and bone osteoblasts. To a lesser extent, serum ALP can also originate from intestinal mucosa, placenta, and kidney tissue (Kaplan 1972). The isoenzymes of intestinal and placental ALP are different from ALP in other tissues. It is possible to distinguish between the different isoenzymes, for instance, by elektropheresis. In clinical practice, however, ALP is generally tested together with GGT to differentiate. Strong elevation of both ALP and GGT indicates biliary obstruction, whereas extrahepatic obstruction causes a stronger rise in ALP compared to intrahepatic obstruction. Other hepatic causes for elevation consist of alcoholic abuse, hepatitis, and cholestatic disease. A sole elevation of ALP without rise in GGT levels indicates extrahepatic pathology, like bone disease or hyperthyroidism. In adults, serum values of ALP are $<$125 U/l. The halftime of most ALP isoenzymes is 3–7 days, while the halftime of intestinal ALP is $<$8 h.

Biomarkers to Assess Graft Function

Albumin

Albumin is one of the most abundant proteins in human serum and plasma besides blood coagulation factors. It is involved in pH homeostasis, maintaining oncotic pressure, and the transportation of blood compounds, hormones, and drugs. Synthesis takes place in the liver, and therefore, serum albumin is considered to be an important marker for liver function. Over 20 structural variants of albumin exist and its halftime is approximately 20 days. In healthy adults, serum/plasma levels are usually between 35 and 55 g/l, but levels can be influenced by body fluid distribution, for instance, by dehydration (Johnson 2006).

In particular hypoalbuminemia has been associated with liver disease and, following liver transplantation, with impaired graft function. A higher degree of graft injury, mirrored by high postoperative transaminase levels, often negatively affects liver graft function. However, the increased use of marginal grafts for liver transplantation has gained more interest for pure functional markers; because despite extensive injury, some marginal grafts manage to function well in recipients. Therefore, experimental studies with graft machine preservation focus on the assessment of liver function already prior to graft implantation in recipients (Bruinsma et al. 2014). But also following liver transplantation, early allograft dysfunction is estimated by a lack of markers that normally result from good liver function, like conjugated bilirubin and INR (coagulation). However, serum albumin is not included in this definition (Olthoff et al. 2010). Though albumin could be of use for assessing graft function, one should also be aware for other causes of hypoalbuminemia, like inflammation, malnutrition/malabsorption, malignancies, and hypothyroidism. Furthermore, albumin levels can remain in the normal range when patients suffer from biliary obstruction.

Bilirubin (Indirect and Direct)

Bilirubin is the yellow-colored breakdown product of hemoglobin when erythrocytes are degraded. A vast majority of bilirubin is derived from aged erythrocytes (over 85%), but ineffective erythropoiesis by bone marrow and certain hepatic enzymes can also contribute to bilirubin formation. When heme is degradated by splenic macrophages, unconjugated bilirubin is formed, which is not soluble in water and cannot be excreted. Subsequently, unconjugated bilirubin is bound to albumin and is transported to the liver, where hepatocytes conjugate bilirubin with glucuronic acid (90% diglucuronic, 10% monoglucuronic). This step makes bilirubin soluble in water and suitable for excretion via the hepatobiliary system. Once transported to the intestine and colon, conjugated bilirubin is hydrolyzed and reduced to urobilinogen by bacteria and excreted via the feces. A small part of the urobilinogen (2–5%) is resorbed into the enterohepatic circulation and excreted via the urine (Fevery 2008).

Human plasma or serum contains four fractions of bilirubin: unconjugated bilirubin (~27%), unconjugated bilirubin bound to albumin (~36%), monoconjugated bilirubin (~24%), and di-conjugated bilirubin (~13%). "Indirect" bilirubin consists of unconjugated bilirubin and the fraction of bilirubin not covalently bound to albumin. "Direct" bilirubin usually refers to fractions of conjugated bilirubin and bilirubin that is covalently bound to albumin. In clinical practice, total bilirubin and direct bilirubin are measurable in human serum or plasma. Total bilirubin consists of conjugated as well as unconjugated forms of bilirubin. Based on these measurements, the indirect bilirubin can be calculated with the formula: indirect bilirubin = total bilirubin – direct bilirubin. In healthy adults, total bilirubin levels are <20 μmol/l, and direct bilirubin levels are <5 μmol/l. Jaundice usually occurs when serum bilirubin exceeds 50 μmol/l (Marshall and Bangert 2005).

Based on total and direct bilirubin, one can distinguish different causes for hyperbilirubinemia. Strong elevation of unconjugated bilirubin indicates prehepatic pathophysiology like hemolysis or dysfunction of hepatocytes and conjugation at the hepatic level. However, most complications that can occur following liver transplantation will cause conjugated hyperbilirubinemia. At the hepatic level, hepatocyte injury due ischemia-reperfusion injury, EAD or PNF, is accompanied by a rise in direct bilirubin and liver transaminases. These changes can occur early after liver transplantation. In the case of intrahepatic cholestasis, for instance, due to biliary strictures, but also extrahepatic bile duct obstruction (post-hepatic level), hyperbilirubinemia is accompanied by a rise in ALP and GGT. Recurrence of (viral) hepatitis can elevate both conjugated and unconjugated serum bilirubin. Thus, by measuring conjugated and unconjugated hyperbilirubinemia and comparing serum levels with hepatocellular and cholestatic markers, one can distinguish between different complications following liver transplantation. When hepatocellular function is impaired, bilirubin levels also become measurable in urine and are per definition pathologic (Klatskin and Bungards 1953). When possible, collection of bile following liver transplantation can also be used for determining biliary bilirubin levels that can mirror hepatocyte function but also cholangiocyte injury (Verhoeven et al. 2015).

Prothrombin Time (PT) and International Normalized Ration (INR)

Synthesis of tissue factors for sufficient blood coagulation is an important function of the liver. A lack of tissue factors in blood plasma could indicate severe liver disease or, in the case of transplantation, graft failure. To assess the degree of graft failure or graft (dys)function following liver transplantation, one could measure individual coagulation factors, but instead, PT and INR are commonly used as general indicators.

Prothrombin time measures the time it takes for blood plasma to form a fibrin clot after adding tissue factor (III). In healthy individuals, PT is usually between 12 and 15 s but it depends on the standards of the laboratory performing the analysis. A prolonged PT could indicate a deficiency in the production of coagulation factors I (fibrinogen), II (prothrombin), V, VII, and X, which are all part of the extrinsic coagulation cascade. Logically, the use of anticoagulant drugs should be taken into account when interpreting PT. Immediately after liver transplantation, PT is usually prolonged and can reach up to 100 s. When PT does not decrease or normalize in the first postoperative week, this could indicate severe graft dysfunction with risk of developing serious complications and impaired patient survival. Urgent re-transplantation can be lifesaving in these cases. As mentioned before, the analysis and subsequent interpretation of PT is very institutionally dependent (Northup and Caldwell 2013).

Therefore, a standardized PT ratio, also known as the international normalized ratio (INR), is used more often to determine early allograft dysfunction. Outside the context of liver transplantation, INR is often used as a tool to monitor patients on vitamin K antagonists. The INR standardizes PT values of patients by calibrating reagents to an international sensitivity index (ISI) and by comparing patients' PT value with the mean PT of healthy individuals (normal), with the formula $INR = (PT_{patient}/PT_{normal})^{ISI}$(Kirkwood 1983). At 1 week following liver transplantation, INR is used as one of the parameters to evaluate early allograft dysfunction; an $INR \geq 1.6$ is considered to be a risk factor for shortened graft and recipient survival (Olthoff et al. 2010). Importantly, the cutoff of 1.6 seems to be a predictor of graft failure for grafts that were obtained from brain death donors as well as those obtained from circulatory death donors. Therefore, it has been suggested to give more weight to INR as a predictor of graft failure following liver transplantation (Croome et al. 2012).

Biomarkers for Recurrence of Disease Following Liver Transplantation

Besides the threat of cellular damage due to severe ischemia-reperfusion injury, biliary injury, and rejection, the recurrence of disease for which recipients were transplanted is also an important factor for graft loss. In particular PSC, HCC, and viral hepatitis B and C are notoriously recurring diseases in the transplanted graft (Kotlyar et al. 2006). Furthermore, over the last years, patients with unresectable cholangiocarcinoma are transplanted, but survival rates are limited due to recurrent or metastatic disease (Darwish Murad et al. 2012b). Several biomarkers are clinically

available to monitor recurrence of the abovementioned diseases in liver transplant recipients, which are described shortly in the following paragraphs.

Cholestatic Markers in Recurrence of PSC

Primary sclerosing cholangitis (PSC) is an autoimmune-related disorder that causes chronic inflammation and strictures of the (mainly intrahepatic) bile ducts. This progressive disease occurs more frequently in men compared to women and has been associated with ulcerative colitis (Lindor et al. 2015). Incidence is highest in the USA and north European countries. The time of onset until end-stage liver disease is approximately 12 years, and currently, LT is the only curative treatment for PSC. Unfortunately, recurrence of disease occurs in up to 20% of the PSC recipients, sometimes requiring re-transplantation (Hildebrand et al. 2015).

Clinical symptoms of recurrence of PSC consist of obstructive jaundice, bacterial cholangitis, fever, and fluctuating elevations of liver enzymes and cholestatic serum markers. Cholangiography shows typical intra- or extrahepatic strictures, beading, and irregularities. Histological features consist of fibrous cholangitis or fibro-obliterative lesions. Because of the overlap in clinical presentation with NAS, one of the criteria of recurrent PSC prescribes this diagnosis should be excluded if it develops within the first 90 days following LT (Graziadei et al. 1999). Besides recurrence, PSC patients also have an increased risk to develop CCA. Therefore, it could be plead to monitor these recipients for cancer antigen 19-9 (CA 19-9), a potential marker of CCA. Table 2 illustrates expected serum levels of classic biomarkers in PSC.

Cancer Antigen 19-9 (CA 19-9) in Recurrence of CCA

Cholangiocarcinoma is a rare disease that accounts for less than 3% of all gastrointestinal malignancies, but which has a poor prognosis due to its aggressive nature. Transplant centers recently started exploring the success of LT for perihilar CCA, either with or without the use of neoadjuvant chemo(radio)therapy (Darwish Murad et al. 2012a). In particular patients suffering from PSC have a 398-fold increased risk to develop CCA compared to the general population (Boonstra et al. 2013).

A potential serum marker to screen for (recurrent) CCA in PSC patients is CA 19–9. This carbohydrate structure is found in pancreatic tissue as well as on epithelial cells of the stomach and gallbladder. It can be secreted into serum by cancer cells. Besides cholangiocarcinoma, increased serum levels of CA 19-9 have been associated with pancreatic and colon cancer but also with benign causes of biliary obstruction. Therefore, when assessing the risk for a malignancy based on CA 19-9 serum levels, one should take into account whether cholestasis or cholangitis is present (preferring a cutoff value of $\geq$300 U/mL) or absent (better discrimination with a cutoff of $\geq$37 U/mL) (Kim et al. 1999). It is recommended to evaluate CA 19-9 levels after recovery of cholangitis. However, the optimal cutoff value for CA 19-9 remains inconclusive. A lower cutoff at 37 U/mL can be undesirable in terms of specificity, but higher cutoff values are at the

Table 2 Serum biomarkers during different pathophysiological states of the liver graft following LT. Values represent which serum levels can be expected for the various outcomes or diagnoses following LT. Except for PNF and EAD, these values are an indication and can diverge between different LT recipients

Complication following LT							
Biomarker	Healthy liver	PNF/EAD[a]	AS	NAS	ACR	Rec PSC	(Rec) HCC, CCA
AST (U/L)	<50	>2,000 within 7 days post-LT	=	50–400	100–1,000	50–400	=↑
ALT (U/L)	<50	>2,000 within 7 days post-LT	=	50–200	100–1,000	50–200	=↑
Total bili (μmol/L)	<20	≥170 on day 7 post-LT	= ↑	↑, up to 300	↑, up to 300	↑, up to 300	=↑
Albumin (g/L)	35–55	↓	=	=↓	↓	=	=↓
GGT (U/L)	<40	= ↑	↑ up to 500	↑, up to 200	↑, up to 300	↑, up to 200	=↑
ALP (U/L)	<125	= ↑	↑ up to 400	↑, up to 200	↑, up to 300	↑, up to 200	=↑
INR or PT (sec)	12–15	≥1.6 on day 7 post-LT	=	Prolonged	Prolonged	Prolonged	=↑
AFP (mcg/L)	<10–15	<10–15	<10–15	<10–15	<10–15	= ↑	↑
CA 19-9 (U/mL)	Neg	Neg	↑ prior to stenting	↑	Neg	If >100, higher risk of CCA	↑

[a]EAD (and PNF) are defined by serum biomarkers in the first week post-LT and consist of one of the following: serum AST or ALT > 2,000 U/L in the first postoperative week, total bilirubin levels ≥10 mg/dL (=170 μmol/L) on day 7 post-LT, or INR ≥1.6 on day 7 post-LT

Legend: Rec, recurrence = normal levels, ↑ increased levels, ↓decreased levels

expense of sensitivity (Levy et al. 2005). Current guidelines recommend a cutoff between 100 and 127 U/mL. Another important limitation of CA 19-9 is that its biosynthesis depends on the activity of fucosyltransferase-2 and fucosyltransferase-3 (FUT2 and FUT3, respectively). Individuals with inactive FUT3 do not express CA 19-9 on their epithelial cells. In contrast, FUT2 inactivity increases CA 19-9 expression. These genetic variations in FUT2 and FUT3 are not uncommon and strongly influence the optimal cutoff level for CA 19-9 in individuals (Wannhoff et al. 2013).

Finally, one could plea for use of CA 19-9 during follow-up after LT for cholangiocarcinoma, since posttransplant CA 19-9 levels are predictive of recurrence of cholangiocarcinoma (HR 1.8). This could influence the timing of adapted medical policy (Darwish Murad et al. 2012b).

Alpha-Fetoprotein (AFP) in Recurrence of HCC

The glycoprotein AFP is mainly produced in the fetal liver and yolk sac during gestation. In the first months after birth, plasma levels of AFP decrease and become undetectable at the age of approximately 1 year. In healthy adults, AFP levels are usually $<$10–15 μg/L (Tomasi 1977). Experimental animal studies have shown a role of AFP in estradiol transport and preventing virilization of female fetuses, but its function in humans remains largely unknown. After malignant degeneration, cells from various tissues are able to produce AFP. These cells can originate from the yolk sac, the gonads, hepatocytes, and certain gastric cells (Liu et al. 2010).

In patients with HCC, pre-transplant levels of AFP were shown to be predictive for recurrence of HCC during follow-up. Therefore, it has been suggested to incorporate pre-transplant AFP levels in the Milan criteria, which are currently used for screening of HCC patients to undergo LT (Duvoux et al. 2012). A rise in AFP levels during follow-up has also been associated with the recurrence of disease (Chaiteerakij et al. 2015; Macdonald et al. 2015). However, no clear correlation exists between AFP levels and tumor size, stage, or prognosis. Current guidelines advise to measure AFP every 3–6 months for 2 years combined with imaging in patients transplanted for HCC. After that, annual monitoring is sufficient. If AFP levels show a strong elevation, further diagnostics for possible recurrence should be undertaken.

Patients with chronic HBV or HCV infection have an increased risk to develop HCC. Serum levels of AFP can be elevated without the presence of an intrahepatic malignant process. However, AFP levels $>$500 mcg/L increase the risk of HCC (Wu 1990). Half-life of AFP is 5–7 days and is expected to decrease within 25–30 days after effective therapy.

Biomarker Dynamics in Various Complications Following LT

After discussing the specific markers for recurrent disease, the next paragraphs will provide an outline on biomarker dynamics that can be expected for common complications that can occur following LT.

Graft Primary Nonfunction (PNF) and Early Allograft Dysfunction (EAD)

Incidence of PNF is 5–8%, and despite being one of the most severe complications following LT, no formal definition of PNF exists. Usually, the diagnosis of PNF is ascertained by exclusion, and in retrospect, the transplanted liver fails to start functioning in the first postoperative days and requires liver re-transplantation or otherwise will inevitably result in the patients' death (Ploeg et al. 1993). Risk factors of PNF can be, for instance, donor related (high donor age, steatosis, small for size) or procedure related (prolonged cold or warm-ischemia times, donation after circulatory death, thrombosis (hepatic artery)) (Braat et al. 2012; Durand et al. 2008). However, in up to 50% of the cases, the exact cause of PNF remains unknown. Complete failure of the graft in PNF results in extremely elevated liver enzymes in serum, impaired or absent bile production, encephalopathy, and coagulopathy within the first 72 h following LT.

A complication similar to PNF is early allograft dysfunction (EAD). In 2010, Olthoff et al. formulated and validated criteria in order to determine EAD based on one or more of the following serum biomarker levels in the first week posttransplant: bilirubin $\geq$10 mg/dL on day 7, INR $\geq$1.6 on day 7, and ALT or AST levels $>$2,000 IU/L within the first 7 days. Though EAD is a risk factor for impaired graft and patient survival, in contrast to PNF, it will not inevitably result in liver re-transplantation or patient death. One could consider PNF as an excessive form of EAD, and therefore it might be questioned whether the two definitions should be fused. Furthermore, liver grafts obtained by donation after circulatory death (DCD) usually have poor immediate function and elevated serum biomarker levels, compared to donation after brain death (DBD). It has been suggested to adjust the definition of EAD for this category of LT in order to better assess the risk for graft failure (Croome et al. 2012). Especially since DCD is responsible for a significant contribution of the donor pool in many (particularly Western) countries, early prediction of EAD for this category could benefit graft and patient outcome. The median panel of Fig. 2 shows examples of biomarker dynamics during the first postoperative week in LT recipients suffering from PNF and EAD. Such dynamics are usually accompanied with extensive ischemic necrosis at the histological level.

Acute Cellular Rejection (ACR)

As explained before, the introduction of cyclosporine significantly improved graft survival by lowering the degree of cellular rejection. Nevertheless, in individual patients, it remains a challenge to lower immunosuppressant's use in order to avoid related complications, on one hand, and to prevent acute cellar rejection (ACR), on the other hand. ACR is the result of a T-cell-mediated immune response directed against tissue of the donor graft and mostly occurs within the first 90 days following LT (early ACR). However, low serum levels of immunosuppressant drugs have also been associated with ACR even years after transplantation (Mor et al. 1992). Clinical

symptoms in recipients consist of, fever, abdominal pain, hepatomegaly, and sometimes ascites. Laboratory test can show increased serum levels of hepatocellular and cholangiocyte-injury markers as well as bilirubin. The golden standard for diagnosing ACR however remains liver biopsy.

In 1995, experts formulated the so-called histological Banff criteria to evaluate the degree of ACR in liver biopsies, also known as the rejection activity index (Banff 1997). This index, outlined in Table 3, scores the extend of inflammation and lymphocytic infiltration into (i) the portal triads, (ii) the bile ducts, and (iii) the venous endothelium. To date, this index is used as part of standard clinical practice. In the early days, tissue biopsies were taken frequently post-LT to monitor for ACR but are now only indicated based on clinical symptoms.

Because of the low specificity of regular laboratory tests for ACR and the invasiveness of liver biopsies, many other surrogate biomarkers have been investigated to monitor for ACR, among which are interleukins, intercellular adhesion molecules, and many others. None have made it into clinical practice jet. A potential novel biomarker reported for ACR but also for other complications following LT is microRNAs (miRNAs), which will be discussed separately later.

Biliary Complications

Biliary complications are very common after liver transplantation and can vary in nature, location, and time of onset. The most common biliary complications consist of biliary leakage, anastomotic biliary strictures (AS), and non-anastomotic biliary strictures (NAS), which will all be discussed shortly.

Leakage of the biliary anastomosis usually occurs early following LT, and the cause is either technical or because of insufficient blood supply to the biliary tree resulting in biliary necrosis. Suspicion for biliary leakage rises when patients have pain and feel ill due to irritation of the peritoneum. Abdominal-free bile collections can be imaged by ultrasound but is more sensitive with ERCP, which is also useful for therapeutic stenting (Arain et al. 2013). Biliary leakage is often accompanied by AS.

Benign local narrowing or tapering at the site of the biliary anastomosis, also known as AS, occurs in approximately 5–10% of LT recipients. Shortly after LT, the biliary anastomosis can be edematous due to surgical trauma and/or ischemia. The development of AS does not depend on the type of biliary anastomosis (Verdonk et al. 2006). It is usually detected by elevated cholestatic markers in serum combined with clinical symptoms in recipients. Diagnosis and therapy of AS are accomplished by ERCP (Fig. 1a), and depending on the severity of the stricture, the bile duct can be cannulated by single or multiple stents (in the case of duct-duct) or by percutaneous drains (in the case of hepaticojejunostomy). If repeated attempts via the endoscopic or percutaneous route fail, AS can also be treated surgically (Balderramo et al. 2012). AS can occur early but also later following LT. Some recipients have recurrence of AS for which they need progressive stenting (Poley et al. 2013). Successful treatment of AS will result in a rapid decrease of cholestatic markers in serum and

Table 3 Banff scoring criteria or rejection activity index to evaluate histological graft rejection

Category	Description	Score
Portal inflammation	Mostly lymphocyte involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils, and eosinophils	2
	Marked expansion of most or all triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the peripheral parenchyma	3
Bile duct inflammation/ damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear-cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts are infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity, and cytoplasmic vacuolization of the epithelium	2
	As the above for two, with most or all of the ducts showing degenerative changes or focal luminal disruption	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority, of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules as above for two, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

patients can recover without residual symptoms. An example of cholestatic biomarker dynamics in AS is provided in the right panel of Fig. 2.

Besides the biliary anastomosis, some liver grafts develop strictures of the intrahepatic bile ducts or extrahepatic hilar region, which are called NAS (Buis et al. 2007). The method of postmortem donation strongly influences the risk for a liver graft to develop NAS: ~10% of DBD grafts versus ~30% of DCD grafts (Howell et al. 2012; O'Neill et al. 2014). Furthermore, it is known that thrombosis of the hepatic artery, the major supplier of blood to biliary tree, will inevitably lead to NAS. Therefore, warm ischemia is thought to play a key role in the pathophysiology of NAS. In contrast to AS, the (multiple) strictures in NAS and their anatomical localization are often less accessible for biliary stents or drains (Fig. 1b). Therefore, liver re-transplantation is indicated in 10–15% of all LT recipients due to NAS (Dubbeld et al. 2010). Large HAT usually indicates immediate liver re-transplantation. In serum, NAS give elevation of cholestatic markers, and only in few cases, normalization of biomarker levels to baseline is achieved. Eventually, NAS will lead to such severe cholestasis that patients will become ill and liver function will be affected.

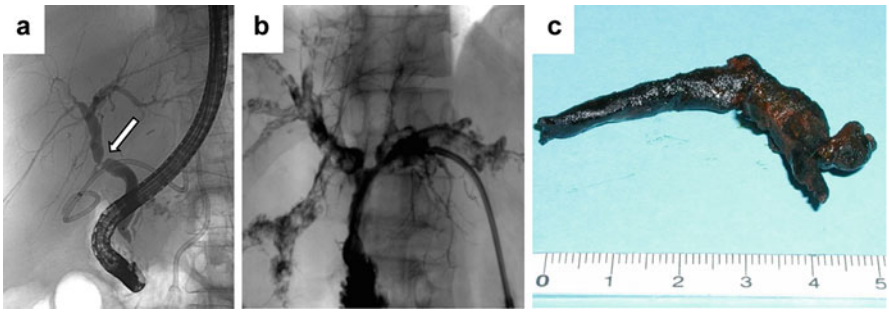

Fig. 1 Visualization of biliary complications following LT. (**a**) ERCP showing an isolated stricture at the biliary anastomosis, pointed out by the white arrow, with dilatation of the common bile duct and slim intrahepatic bile ducts. (**b**) ERCP showing dilated intrahepatic bile ducts throughout the entire liver graft with loss of normal architecture due to NAS. (**c**) Biliary cast removed from the hilar region of the liver graft that was formed due to obstruction and which is often seen in NAS. The length of the cast is displayed in cm. Pictures are derived from the database of the Erasmus Medical Center Rotterdam, The Netherlands

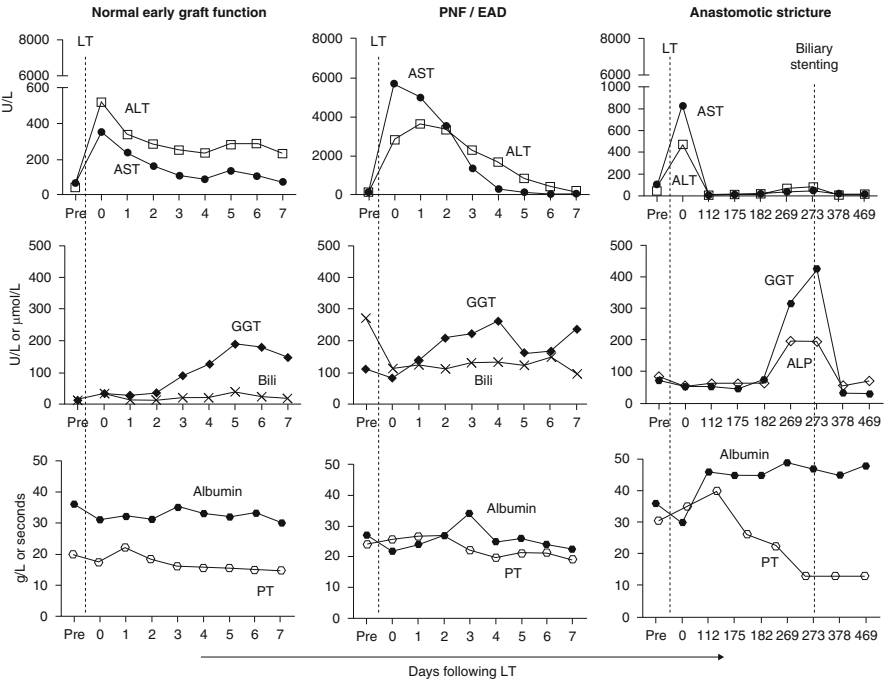

Fig. 2 Biomarker dynamics in blood and serum following LT. The *left* panels show biomarker dynamics in recipients with normal early graft function, the median panels show elevated biomarker levels in serum during PNF/EAD, and the *right* panels show increased cholestatic markers in AS. Data represent biomarker serum levels of individual patients following LT and were derived from the database of the Erasmus Medical Center Rotterdam, The Netherlands

Novel Biomarkers in the Field of Liver Transplantation

MicroRNAs (miRNAs) as Novel Biomarker

In the last decade, miRNAs have gained interest in the field of biomarker research. MicroRNAs are short, hairpin-shaped RNAs with the potential to regulate gene expression by inhibiting messenger RNA translation (Fig. 3). miRNAs are highly cell-type abundant and can be released via active and passive mechanisms into the circulation and other body fluids in which they remain stable up to 24 h. These characteristics make miRNAs attractive candidate biomarkers for various diseases. Besides their biomarker potential, the knowledge regarding miRNA-induced gene expression and regulation is increasing, though not yet fully understood (Farid et al. 2014).

For various liver diseases, particularly miR-122 has been related to hepatocellular liver injury. Serum levels of miR-122 increase earlier than conventional transaminase levels, which was shown in patients with viral hepatitis as well as in LT recipients who developed ACR (Farid et al. 2012; van der Meer et al. 2013). Therefore, hepatocyte-derived miRNAs (HDmiRs) might be suitable early markers for severe hepatocellular injury following LT, as is the case in grafts developing EAD or PNF. In contrast to liver transaminases, which are mainly injury markers, HDmiR-122 secretion into bile has also been correlated to good bilirubin excretion of hepatocytes into bile (Verhoeven et al. 2015). Therefore, HDmiR-122 and perhaps other HDmiRs might also be suitable markers for graft function.

Cholangiocytes have a different expression of miRNAs compared to hepatocytes (Chen et al. 2009). Therefore, cholangiocyte-derived miRNAs (CDmiRs) could be more sensitive or specific in the detection of biliary complications. Already at time of graft preservation, CDmiRs are released in response to ischemia-induced biliary injury that causes severe complications in LT recipients during follow-up (Verhoeven et al. 2013). Besides changes in expression, also the composition of miRNAs in bile is changed during biliary obstructions (Lankisch et al. 2014).

Despite the growing evidence of their utility, miRNAs as biomarker are currently not part of clinical practice in liver disease. Future research should focus on validation of sensitivity and specificity of previously identified CDmiRs and HDmiRs. Another challenge for implementing miRNAs as a routine laboratory test lies within the technical aspect of measuring miRNAs. This is now done by real-time quantitative polymerase-chain reaction (RT-qPCR), which takes approximately 3 h before miRNAs are isolated and analyzed. This issue could be facilitated by improving accelerated PCR techniques. Because of the highly sensitive analysis of qPCR, mild elevations of miRNA levels in blood or other body fluids can be determined quite accurately. Despite the fact that much is still unknown about miRNAs as therapeutic target, the first clinical series in human showed that inhibition of HDmiR-122 reduces viral load in HCV patients (Janssen et al. 2013). Whether CDmiRs are potentially interesting in (prevention of) cholestatic disease needs to be explored by future research.

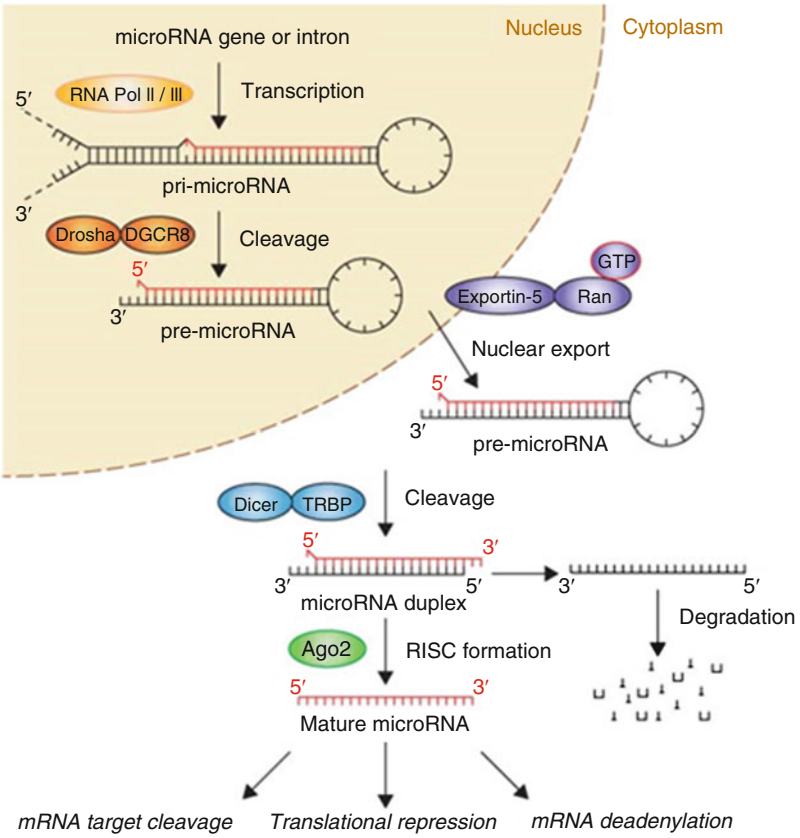

Fig. 3 MicroRNA structure and biogenesis. Biogenesis of miRNAs by cells. Immature precursor miRNAs are formed inside the cell nucleus. In the cell cytoplasma, miRNAs reach their mature form and are able to inhibit mRNA translation, thereby regulating gene expression. Illustration from (Winter et al. 2009)

Potential Application to Prognosis, Other Diseases, or Conditions

The previous paragraphs provided an overview of different types of biomarkers that are regularly used in liver disease and how these should be interpreted in the context of LT. Routine monitoring of graft quality based on biomarkers helps clinicians to decide whether or not to perform additional (mostly more invasive) tests like ERCP or liver biopsy. Biomarker levels can be the reason to adjust therapy, for instance, to increase immunosuppressant dosage when high transaminase levels indicate cellular rejection. But also as a definition of outcome, biomarkers play an important role in predicting prognosis early after LT.

Some important complications that can occur following LT, like EAD and biliary strictures, are often related to marginal quality of the liver graft already at time of

transplantation. As mentioned before, grafts obtained by DCD have a higher risk to develop EAD and NAS. For this reason, DCD liver grafts from elderly donors (over 60 years of age) are often rejected for LT. However, some of the rejected DCD grafts might have functioned well in recipients. With the increasing number of marginal grafts for LT, there is a need to improve and simultaneously to objectify graft quality in an earlier phase of LT. The prolonged time window between graft procurement and graft implantation, known as the preservation period, is in particular useful for this purpose. Many studies showed that during static cold storage, liver grafts can still release some injury markers that have been associated with outcome. A novel technique designed to preserve and improve graft quality is machine perfusion (MP) (Schlegel et al. 2015). With MP, the liver graft is flushed ex situ on a pump that recirculates preservation solution (perfusates) before implantation into the recipient. Many different techniques of MP have been investigated with variations in solutions, temperature, oxygenation, single-portal or dual portal-hepatic artery perfusion, flow pressure, and more. The first clinical studies with MP show promising results regarding prevention of hepatic and biliary injury (Dutkowski et al. 2015). However, during MP it remains a challenge to objectify that marginal grafts show enough recovery to be transplanted and which should still be rejected for LT. Multiple options are available to assess graft quality during MP with the use of biomarkers in graft perfusates and produced bile, depending on the applied technique (Verhoeven et al. 2014).

Despite the potential of biomarkers to assess graft quality during preservation, their clinical application is still experimental and the decision to accept a graft for LT is mainly driven by clinical donor variables and the macroscopic aspect on inspection by the donor surgeon. Besides donor variables, some researchers plea for the implementation of recipient variables as well in allocation algorithms, since recipient factors as age, MELD score, and gender can strongly influence survival (Blok et al. 2015). Because of the limited number of performed LTs annually in transplant centers, many biomarker studies omit validation of potential biomarkers in multiple cohorts. This will however delay the implementation of biomarkers to assess graft quality during preservation. Furthermore, criteria for EAD should be adapted for DCD liver grafts; despite their worse biomarker profile post-LT, multiple DCDs show good recovery during follow-up. The current criteria might be insufficient to distinguish grafts that will eventually function properly in recipients from the ones that actually cause PNF. This could also be the case for other types of donation, like living donor liver transplantation, for which another literature is recommended.

Summary Points and Discussion

To conclude, this overview discussed routinely measured biomarkers and more novel ones for evaluation of graft injury and function in the follow-up of LT recipients and their dynamics at time of various complications and (recurrence of) disease. It is evident that biomarkers can indicate hepatocellular injury, biliary obstruction, and liver function. Evaluation of biomarkers can play a key role in the

early recognition of complications and provide an objective tool to monitor graft quality after transplantation. As in recent years, many new potential biomarkers have been discovered; therefore this overview is incomplete and limited to established serum biomarkers. Furthermore, it should be emphasized that experienced clinical knowledge and imaging techniques of the liver are two other key factors in clinical decision making, and determining the need of intervention will rarely be based on biomarkers solely. Much likely, LT recipients will start with monitoring of graft function through biomarker measurements in the home situation as part of individualized medicine. Finally, novel application of biomarker measurements during graft preservation seems promising in the early evaluation of graft quality that could help extend the donor pool for LT.

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