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A Comprehensive Review of Liver Allograft Fibrosis and Steatosis: From Cause to Diagnosis

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Abstract. Despite advances in posttransplant care, long-term outcomes for liver transplant recipients remain unchanged. Approximately 25% of recipients will advance to graft cirrhosis and require retransplantation. Graft fibrosis progresses in the context of de novo or recurrent disease. Recurrent hepatitis C virus infection was previously the most important cause of graft failure but is now curable in the majority of patients. However, with an increasing prevalence of obesity and diabetes and nonalcoholic fatty liver disease as the most rapidly increasing indication for liver transplantation, metabolic dysfunction-associated liver injury is anticipated to become an important cause of graft fibrosis alongside alloimmune hepatitis and alcoholic liver disease.

To better understand the landscape of the graft fibrosis literature, we summarize the associated epidemiology, cause, potential mechanisms, diagnosis, and complications. We additionally highlight the need for better noninvasive methods to ameliorate the management of graft fibrosis. Some examples include leveraging the microbiome, genetic, and machine learning methods to address these limitations. Overall, graft fibrosis is routinely seen by transplant clinicians, but it requires a better understanding of its underlying biology and contributors that can help inform diagnostic and therapeutic practices.(Transplantation Direct 2023;9: e1547; doi: 10.1097/TXD.0000000000001547.)

Liver transplantation (LT) is considered the only curative treatment for chronic liver disease.[1] Although short-term outcomes within 1 y post-LT have drastically improved because of the implementation of immunosuppression, improvement in surgical techniques, and postoperative care, long-term outcomes beyond 1-y long-term outcomes beyond 1 y have not significantly improved the past 3 decades.[1] More than 37% of LT recipients develop graft fibrosis (GF) because of various insults that include recurrent disease and biliary complications exacerbated by the metabolic side effects of immunosuppressants.[2-6] Without timely intervention, LT recipients who develop stage 2 GF within 1 y post-LT have reduced patient survival, leading to compromised long-term graft survival and increased need for retransplantation.[7,8] Previous reviews on this topic had a strong focus on recurrent hepatitis C virus (HCV) infection; however, advances in direct-acting antivirals (DAAs) have led to better management of recurrent HCV and will no longer compromise graft health prospectively.[9] With the rising epidemic of nonalcoholic steatohepatitis (NASH) as an indication for LT, this disease is anticipated to have a major impact on post-LT patients, with 2% to 20% of recipients developing advanced fibrosis,[10,11] highlighting the need to summarize the changing landscape of the literature. Furthermore, the underlying predictors of GF are not well understood, especially because GF is more accelerated and progressive compared with pretransplant liver fibrosis.[12] Here, we provide a comprehensive overview of the different aspects of GF, covering cause, prevalence, risk factors, clinical implications, and diagnostic assessment.

EMERGING CAUSE AND EPIDEMIOLOGY OF GF

Various diseases and insults can lead to GF, developing into end-stage liver disease. Chronic disease creates a vicious cycle of inflicting graft damage and activating excessive

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healing responses that both contribute to graft dysfunction. The most common diseases are recurrent HCV, primary sclerosing cholangitis (PSC), and primary biliary cirrhosis (PBC). Emerging diseases that will have a significant impact in the next 5 to 10 y are NASH, autoimmune hepatitis (AIH), and alcohol-related liver disease (ALD).

De Novo and Recurrent NASH

Both de novo and recurrent NASH are becoming increasingly prevalent in LT recipients, largely because of the presence of other metabolic syndromes such as obesity and diabetes.[11,13-20] Patients who are transplanted for NASH are at a higher risk of developing recurrent disease.[11] Although several studies reported differing prevalence, they tend to be between 10% and 40% with one study observing 56% with recurrent disease.[11,17,20] More commonly, 48% of LT recipients will develop steatosis within 10 y, with close to 40% of patients transplanted for non-NASH indications and 60% to 70% of recipients transplanted for NASH.[14,15] It was reported that patients with recurrent HCV with metabolic syndromes had worse fibrosis progression.[4,21] A similar correlation may also be seen in patients with recurrent NASH because risk factors reported in 2 studies include higher post-LT body mass index, fasting triglyceride levels, insulin use, hypertension, and any presence of pre- or posttransplant metabolic syndromes.[17,18] The occurrences of pre-LT diabetes were also associated with recurrent NASH.[22] However, most of these studies were in small heterogenous cohorts where the diagnosis is often by imaging or nonprotocol biopsies with selection bias that may underestimate prevalence.

The increasing prevalence of NASH also suggests that it is projected to become the leading cause of GF in the near future. One review determined that 5% to 10% of patients with recurrent NASH will progress to ≥F3 fibrosis compared with 2% to 4% of recipients with de novo NASH within 5 y.[11] Similar prevalence rates were also seen in a recent meta-analysis because most patients across the 14 studies had low-stage fibrosis for both recurrent and de novo NASH (estimated 40%–90% with F0/F1 stage).[23] Because no consensus has been reached regarding the prevalence of fibrosis because of posttransplant NASH, further population-based studies are required to determine its role in contributing to GF.

Discussion of fibrosis-related patients and graft survival is limited in the literature; thus, studies often report the progression rate that may provide insight into the severity of fibrosis and the prevention of severe complications. Several articles noted differences between de novo and recurrent NASH fibrosis progression with recurrent disease leading to severe fibrosis; however, one noted that no difference was found, showing a similar rate of 0.43 stages/y in de novo and recurrent NASH.[11,14,15,24]

Autoimmune Liver Diseases

This group of diseases consists of AIH, PSC, and PBC. Incidence of this disease type as a primary indication for LT is rare, but post-LT outcomes for these patients are good. However, recurrence can occur post-LT that depends on disease type. AIH recurs in approximately 7% to 42% of recipients, followed by PSC recurring in 8% to 25%.[25-28] PBC recurrence is rare and has a wide incidence range, 11% to 53%; however, this incidence is center specific, particularly if the center does protocol biopsies or not because PBC can be missed without a biopsy.[26,27,29]

Risk factors for developing recurrent autoimmune liver disease are similar but can vary between disease subtypes. The most common one is the type of immunosuppression regimen used for these patients. For AIH, early withdrawal of corticosteroids and low-maintenance immunosuppression, high immunoglobulin G serum levels, and HLA mismatching between donor and recipients were important factors.[26,27,30,31] Recurrent PSC was present in individuals who had intestinal bowel diseases, colectomy, acute cellular rejection, and use of mycophenolate mofetil and calcineurin inhibitors (CNIs).[26,27,30] There is no confirmation that CNI, either tacrolimus or cyclosporine A, leads to worse progression for PSC. Because PBC is rare, there is some conflicting evidence regarding the appropriate risk factors; however, it was found that tacrolimus use led to PBC progression.[26,28,29]

In terms of GF, there was some discussion in the context of AIH. One study determined that there is a modest association between AIH and fibrosis progression.[32] GF staging also differed based on recurrent versus de novo AIH, in which patients with recurrent AIH had worse fibrosis (>F3) compared with those with de novo disease.[33] This is similar to the NASH population in which most studies found worse fibrosis progression in recurrent disease. AIH is associated with progressive fibrosis, particularly de novo disease, with one review citing that approximately 20% may require retransplantation.[25]

Alcohol-Related Liver Disease

ALD is increasingly becoming one of the most common indications for transplant, especially among younger individuals (aged <40 y).[34] Unlike other recurrent diseases, ALD progression post-LT is predominantly dependent on patient characteristics. If alcohol use is identified early, clinicians can implement strategies to decrease disease progression and GF.[35] The post-LT outcome that clinicians have to be mindful of is relapse especially if the relapse leads to significant alcohol intake because it can lead to alcoholic cirrhosis. Patients who have alcohol relapse post-LT (16% relapsed) are more likely to develop advanced fibrosis (stage 3 or higher) when matched with patients who had HCV.[36] A similar trend was seen in another study that matched patients with NASH to those with ALD and found that recipients with ALD tend to have higher fibrosis stages, but the difference was not statistically significant.[15]

Recurrent HCV

With the introduction of DAAs, the management of HCV recurrence has significantly improved and is no longer the leading cause of GF development. This has also led to the increasing consideration of HCV-positive donors to address the organ demand.[37] For a complete discussion of all causes of GF, we provide a brief summary of the role of HCV recurrence in GF. Before the introduction of DAAs, some of these patients undergo LT for survival but can subsequently develop graft reinfection with a severe inflammatory response and rapid fibrosis development.[2,4,38] Historically, up to 16% to 43% of LT recipients with recurrent HCV had severe fibrosis with an incidence rate of 9.3/100 person-y (95% confidence interval, 8.1-10.7).[39-41] Once HCV cure is achieved, a small proportion of individuals can develop immune-mediated graft dysfunction, causing liver injury.[42] This was noted in elevated liver enzymes (ie, alanine transaminase [ALT], alkaline phosphatase) in which untreated liver damage can lead to further progression of GF.[42]

Other Hepatic Injuries and Diseases

Other types of injuries can also lead to the development of GF (Table 1). Biliary complications (eg, anastomotic and nonanastomotic strictures) stimulate fibrogenesis because portal fibroblasts can be activated and differentiate into myofibroblasts that play a role in extracellular matrix (ECM) deposition in biliary fibrosis.[43,44] Bile duct dilatation proximal to anastomotic strictures can lead to fibrosis in adjacent tissue areas because of injury as well.[44,45] Chronic rejection can also lead to GF, particularly in the periportal area, mimicking biliary complications.[2] For both acute and chronic rejections, central perivenulitis lesions can progress to centrilobular fibrosis and some chronic rejection cases can develop bridging fibrosis.[2,46] Other viral infections, such as hepatitis B, can also lead to the development of fibrosis, particularly if not treated with immunoprophylaxis or antivirals.[46] This list of causes is not comprehensive because any insult that results in chronic hepatic injury can lead to GF; however, it does provide insight into the myriad of diseases that contribute to a highly inflammatory and fibrotic environment in the liver allograft.

Pediatric Populations

In pediatric LT populations, the progression of GF may be of significant concern, especially because pediatric recipients require a graft that can last their entire lifetime (8–9 decades). Unlike in adult populations where the primary disease is a strong contributor to GF, histological changes in pediatric patients are not attributable to any definite cause other than chronic immune-related damage or idiopathic hepatitis.[47,48] Within 6 mo posttransplant, one study found that 74% of children had GF, with most experiencing mild fibrosis.[49] Fibrosis grade was stable across the 5-y follow-up, with only 36% of children progressing to moderate fibrosis within this time.[49] In terms of the presence of advanced fibrosis 5 to 10 y post-LT, 55% of children had moderate fibrosis with 3% experiencing severe fibrosis.[50] This prevalence did not change significantly at the 10-y mark, suggesting that after a certain period, GF stabilizes for most children.[50] Because of the long-term consequences of immunosuppression, pediatric recipients may undergo a complete withdrawal of immunosuppression, which can lead to GF in nontolerant patients. One pilot study noted only mild fibrosis in tolerant patients after 2 y post-withdrawal, whereas nontolerant recipients developed allograft dysfunction, which regressed after the reintroduction of immunosuppression.[51]

POTENTIAL MECHANISMS OF GF

Understanding the important factors involved in fibrosis can be applied to developing new diagnostic methods and treatments for LT recipients with GF (Figure 1). Currently, our understanding of the molecular mechanisms involved in fibrosis comes from immunocompetent patients. However, the role of immunosuppression and other unique transplant-related factors are not considered in these studies that play an important role in LT recipients and can influence GF.

Role of Alloimmunity and Immunosuppression

GF can be a response to (1) alloimmunity driving graft rejection and (2) side effects from chronic immunosuppression exposure. As previously mentioned, acute and chronic rejection can cause GF, particularly through donor-specific alloantibodies (DSAs). HLA-specific DSAs are known risk factors of severe GF, particularly in patients taking cyclosporine who present with higher levels of DSAs.[52,53] Additionally, certain non-HLA DSAs (ie, angiotensin II receptor type 1 and endothelin type A receptor) also contribute to fibrosis progression, particularly in combination with HLA DSAs, suggesting that direct allospecificity may not be required to contribute to GF.[54]

Immunosuppressants play a vital role in prolonging graft survival by preventing immune-mediated rejection. Despite their need to manage LT patients, they have adverse side effects that can affect graft survival. From the limited studies

TABLE 1.

A comprehensive list of causes/pathologies that contribute to GF

Time period	Etiology/pathology	Estimated prevalence	Comments
Pretransplant	Steatosis	30%	Specifically in donor grafts
	AIH	–	
	Acquired HCV/HBV	–	Specifically, from donor grafts
Perioperative	Ischemia-reperfusion injury	–	
Posttransplant	Recurrent HCV	16%–43%	Previously most common cause of GF
	De novo or recurrent NAFLD/NASH	10%–70%	
	ALD	16%	Differs based on length and severity of relapse period
	De novo or recurrent AIH	7%–42%	
	Recurrent HBV	–	
	Recurrent HCC	13%	
	Acute rejection	–	
	Chronic rejection	–	
	Biliary strictures (ie, anastomotic, nonanastomotic)	–	
	Non-HCV or -HBV viral hepatitis	–	
	Budd-Chiari syndrome	–	
	Recurrent PBC	11%–53%	
	Recurrent bile duct carcinoma	–	
	PSC	8%–25%	
	Immune-mediated graft dysfunction	–	Rare occurrence post-DAA therapy for HCV

ALD, alcohol-related liver disease; AIH, autoimmune hepatitis; DAA, direct-acting antiviral; GF, graft fibrosis; HBV, hepatitis B virus; HCC, HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

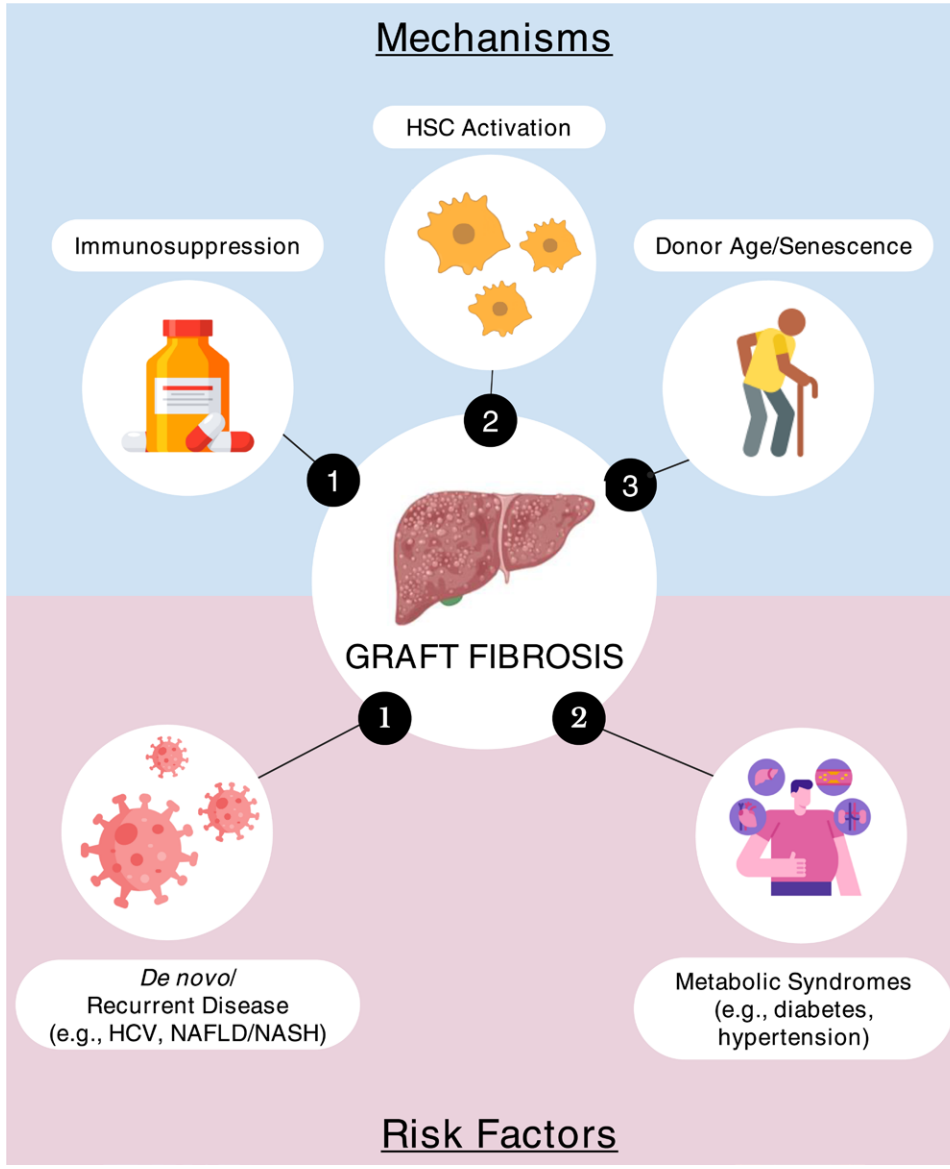

FIGURE 1. | Potential mechanisms and risk factors of liver allograft fibrosis. HCV, hepatitis C virus; HSC, hepatic stellate cell; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

describing the role of immunosuppression in GF, different research groups observed variable results. Specific CNIs were associated with fibrosis progression in recurrent HCV LT recipients. One study found that receiving tacrolimus monotherapy was associated with developing F4 fibrosis compared with recipients who received a cocktail of tacrolimus, azathioprine, and prednisone.[55] A meta-analysis comparing the outcomes of cyclosporine A and tacrolimus found that both can cause severe fibrosis 1 y post-LT for recurrent HCV.[56] However, with these accounts alone, it is difficult to identify whether the effect was solely due to immunosuppressants or due to worsening viral hepatitis. As previously mentioned, this was also seen with PSC and PBC in which certain CNIs were associated with GF. Another study found that in pediatric and adult LT recipients, 62% experienced progressive fibrosis even when treated with tacrolimus for recurrent AIH.[25] This suggests that in the context of AIH, lower immunosuppression trough levels can contribute to faster disease progression and GF. Although the underlying molecular mechanisms of immunosuppression concerning GF are not known, these studies suggest that they may play a role, warranting further investigation.

Donor Age and Cellular Senescence

Although the effect is not well understood, increasing donor age affects fibrosis progression in liver grafts. One study found a higher rate of 0.8 fibrosis stages/y with a strong association between male gender (median fibrosis progression rate of 0.92 for male individuals versus 0.45 for female individuals) and older donor age (specifically in recipients who had a progression rate >0.8 stages/y) with increased fibrosis progression.[57] Two clinical studies determined that increasing donor age correlates with fibrosis progression.[58,59] This correlation may be rooted in specific age-related changes in the liver: reduced number of mitochondria and increased hepatocyte size correlated with polyploidy, a marker of cellular senescence.[60] In nontransplant mouse models, hepatocytes of older livers had higher staining of γ-H2AX, a marker of

double-stranded breaks, which indicates a diminishing ability to repair DNA.[61] This may render aging hepatocytes unable to respond to injury and are more susceptible to developing fibrosis. Shortening telomeres and damaged DNA are all signs of senescence and have been associated with increased fibrosis. One study sampled cirrhotic histological samples and determined that these samples have short telomeres regardless of cause and high hepatic senescence in patients with severe fibrosis compared with mild fibrosis.[62] Similar observations were made in murine models whereby NASH-afflicted hepatocytes had shortened telomeres, high γ-H2AX, increased levels of CDK4, and decreased levels of Mcm2, cyclin A, and PH3, which are all indicative of G1/S arrest.[63] The same study also investigated a telomerase knockout model and observed that the mice experienced increased progression to cirrhosis and reduced survival. Further understanding of the role of donor age and cellular senescence may, therefore, provide insight into organ allocation and management of LT recipients, such as determining age-related GF risk factors.

Hepatic Stellate Cell Activation

A common player in hepatic fibrosis is hepatic stellate cells (HSCs), which, on activation, differentiate into myofibroblasts, leading to excess ECM production.[12,64] One study compared LT recipients and non-LT patients with HCV and found that both had comparable levels of alpha-smooth muscle actin, a marker of HSC activation, and transforming growth factor-β1, a marker of inflammation, stainings for each fibrosis stage.[65] Several clinical articles have also shown similar associations of higher HSCs activation with increased fibrosis and progression to cirrhosis.[66-69] Although most of these studies investigated recurrent HCV LT patients, a similar trend was also seen in nontransplant patients with NASH, observing a statistically significant association between HSC activation and fibrosis.[70] Therefore, the extent of HSC activation may provide insight into the prognosis of patients and can be used to identify rapid progressors.[66,70] Nevertheless, the mechanism of HSC activation and GF is incompletely understood, and thus, more studies are required to understand the underlying molecular mechanisms.

CLINICAL IMPLICATIONS OF GF

The long-term clinical implications of GF are not well known. With a higher fibrosis progression rate and lack of standardization of diagnostic assessment of GF, LT patients with GF are at higher risk of progressing to cirrhosis, which could subsequently lead to the need for retransplantation. The prevalence of cirrhosis is variable based on the disease type.

NASH-related graft cirrhosis is quite rare as one study found that 2% of its study population had graft cirrhosis, whereas another study reported none.[15,71] Yalamanchili et al[72] determined that 5% and 10% developed severe fibrosis/cirrhosis 5 and 10 y post-LT, respectively.[72] Time to cirrhosis post-LT was also variable with a meta-analysis reporting the development of cirrhosis from 15 mo to 4 y.[73] As noted in patients with HCV, retransplantation was low with one study of 143 patients citing only 1 procedure, whereas another retrospective cohort study of 226 patients reported none.[16,71] However, the correlation between recurrent disease and retransplantation is mixed. One study looking at NASH or cryptogenic cirrhosis recurrence reported that NASH recurrence was strongly associated with retransplantation.[18] Conversely, Dooghaie et al[20] determined that patients with recurrent NASH are less likely to be retransplanted compared with patients with de novo disease.[20]

In autoimmune liver diseases, progression to graft cirrhosis and subsequently retransplantation is rare and may also be dependent on disease type. For AIH, some studies have noted that approximately 50% of patients with recurrent AIH can progress to cirrhosis, leading to retransplantation. However, the use of triple immunosuppression and reintroduction of corticosteroids can help with disease regression, preventing the need for retransplantation.[26,27] Recurrent PSC does pose a significant risk to retransplantation, and unlike AIH, there are no interventions that can be used to prevent disease progression.[26] Finally, for PBC, 15% of patients progress to cirrhosis during a 10-y period with retransplantation being rare.[27]

In the context of treating recipients for fibrosis, the focus is to address the underlying disease and inflammation that contribute to GF. For certain causes, such as recurrent HCV and ALD, there is a possibility for fibrosis regression once the insult is removed. This may involve prescribing DAAs for HCV recurrence, adjusting immunosuppression doses for autoimmune recurrence, reducing alcohol consumption for ALD, and recommending lifestyle changes to address NASH. However, once graft cirrhosis has been established, fibrosis regression is less feasible since the ability of the liver to regenerate becomes compromised. Although retransplantation is rare, for severe cases of GF, this procedure may be the only available option for recipients.

DIAGNOSTIC ASSESSMENT OF GF

LT recipients are required to undergo routine diagnostic tests to monitor graft function (Figure 2). Firstly, this requires close surveillance of the graft by monitoring liver enzymes that correlate with liver damage. The investigation of elevated liver enzymes starts with ultrasound (US) imaging. Because US imaging may miss lower stages of fibrosis, biopsies are subsequently scheduled to quantify the histological markers of fibrosis or liver damage, informing treatment options. Thus, accurate and timely diagnosis is crucial for treating patients efficiently and preventing further graft damage.

Liver Biopsy

The clinical gold standard for diagnosing fibrosis is a liver biopsy in both pre- and post-LT populations. Post-LT patients predominantly undergo their first biopsy within 1 y post-LT or earlier if they have abnormal laboratory values. Despite its invasiveness and sampling limitations, liver biopsy remains the gold standard for fibrosis staging. Fibrosis is staged using various semiquantitative scales, such as Batt-Ludwig, Ishak, METAVIR, and Scheuer.[74-77] The liver allograft score was developed for pediatric populations and follows a similar framework.[78] We continue to use these scales because there are no specific histopathological scoring systems specifically for post-LT fibrosis.

The main advantage of liver biopsy is that it directly measures fibrosis. A pathologist can quantify the degree of fibrosis from tissue samples, which can inform clinical decision-making. Many patients with abnormal histological biopsies routinely present with seemingly normal laboratory results.[79-81] In fact, one study found that in 33 patients with

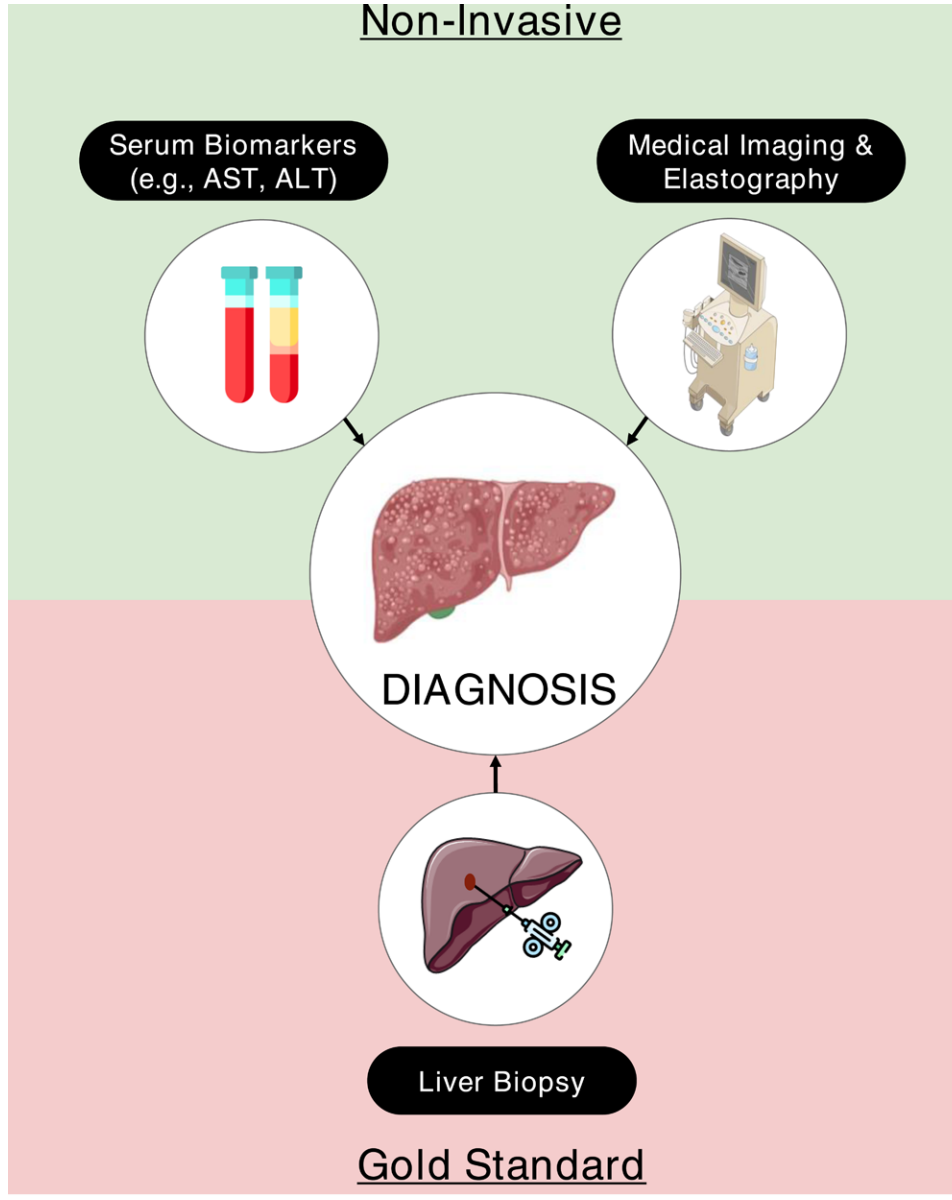

FIGURE 2. | Current methods for diagnosing liver allograft fibrosis. ALT, alanine transaminase; AST, aspartate aminotransferase.

normal liver biochemistry, 82% had abnormal histological findings.[79] Another study estimated that regardless of disease type, 25% of recipients with normal laboratory values have some degree of fibrosis.[82] One limitation of liver biopsy is that it only samples a small area of the liver that can lead to a sampling bias rather than looking at the distribution of GF throughout the entire liver. Additionally, clinicians are required to balance the risk of using liver biopsy and its utility, which can render it impractical for longitudinal follow-up. As patients survive beyond several y post-LT, the need for liver biopsy decreases and protocol biopsies are done less frequently. Although this careful approach is necessary to prevent patients from undergoing unneeded tests, it can also lead to suboptimal management of patients, especially if they develop recurrent disease. Thus, we may need to reconsider the limited use of protocol biopsies for long-term follow-up of LT recipients. Ultimately, liver biopsy may not be completely replaced in clinical settings, but further work is needed to better use noninvasive alternative methods in the post-LT setting.

Noninvasive Methods

To address the limitations of liver biopsy, many studies are developing and validating noninvasive methods that can diagnose or predict fibrosis in post-LT patients, regardless of cause. The 2 main subtypes include serum biomarkers and their associated scores and imaging, particularly elastography. The main aim of developing noninvasive tools is to guide management while reducing the need for liver biopsy.

Serum Biomarkers

The most common and relatively inexpensive method is to quantify relevant biomarkers in the blood. Routine laboratory tests quantify aspartate aminotransferase (AST), ALT, albumin, gamma-glutamyl transferase, and other liver enzyme levels to assess liver function and damage. Additionally, fibrotic markers can be quantified, including type 3 and 4 collagen, amino-terminal propeptide of type III procollagen (PIII3NP), matrix metalloproteinases , and tissue inhibitor of metalloproteinases.

Generally, different proteins are integrated into specific indices and/or statistical modeling (Table 2). Common, cheap, and readily available scores are the AST-to-platelet ratio index (APRI), AST/ALT ratio, fibrosis 4 score (FIB-4), nonalcoholic fatty liver disease (NAFLD) fibrosis score (NFS), and Forns index. The accuracy of these indices varies widely among studies: for APRI and FIB-4, Pissaia et al found an area under the curve (AUC) of 0.87 and 0.78, respectively, independent of cause post-LT for differentiating ≥F2 fibrosis. However, Kabbany et al[83] determined AUC values of 0.64 for APRI and 0.59 for FIB-4, in HCV and NASH populations. Values similar to the latter results were also seen in several studies with both APRI and FIB-4 achieving AUCs of approximately 0.66 to 0.70.[84-87] AST/ALT ratio also varied in accuracy, with one study citing an AUC of 0.52 and an insignificant association with fibrosis, whereas another study found an AUC of 0.749.[83,88,89] For NFS, they are highly correlative in NASH patients with advanced fibrosis, with 3 different studies reporting an AUC range of 0.75 to 0.89.[83,84,90] However, these scores are not as predictive in the post-LT setting because of prolonged thrombocytopenia and other causes that can lead to elevated AST and ALT. Additional indices include the London Transplant Center score and FibroTest (BioPredictive, Paris, FR) with similar accuracy.[91-93]

Several studies also independently investigated the accuracy of different fibrotic markers; however, this was primarily developed for the recurrent HCV population. Certain examples include the enhanced liver fibrosis score that integrates hyaluronic acid, PIII3NP, and tissue inhibitor of metalloproteinase-1 variables,[94] hyaluronic acid and YKL-40, an inflammatory glycoprotein for predicting rapid fibrosis progression for patients with HCV.[95] Serum globulins, cytokines, markers associated with chemotaxis, endothelium activation and collagen synthesis (ie, HA), chemokines (ie, CXC motif chemokine 10/11), and decreased cell-mediated immunity were highly associated with fibrosis and can be incorporated into models to predict progression.[96-100] These biomarkers may be cause-independent because they are fibrosis-specific and thus may be applicable to NASH- or ALD-origin GF.

Other studies have also noted serum immunological markers that can predict fibrosis in various post-LT causes. One study looked at the ECM formation and degradation proteins in LT patients' sera that progress to cirrhosis and found that type IV collagen formation and matrix metalloproteinase-9 degradation had AUCs of 0.90 and 0.88, respectively, in differentiating rapid progressors from nonprogressors but not intermediate progressors.[101] Iacob et al[102] were able to predict late allograft dysfunction in HCV and non-HCV populations using 7 parameters: serum ALP, ALT, AST, gamma-glutamyl transferase, soluble major histocompatibility complex class I polypeptide-related sequence A and B, interleukin-6, and albumin, with an AUC of 0.91. Mac-2 binding protein glycosylation isomer, a glycoprotein, was also associated with fibrosis but overall performed similarly to other serum markers, with an approximate AUC of 0.60.[103] This biomarker is higher in recipients with recurrent HCV; thus, we can identify patients with cause-specific GF.

Using available demographic, clinical, and laboratory values, certain studies developed statistical models for predicting fibrosis progression. One study included albumin, prothrombin time (PT), AST, and time since LT in a logistic regression model and achieved an AUC of 0.84 in the validation set.[104] The same group validated their model in a prospective study but found that only AST and PT were significant, with an AUC of 0.77, lower than their initial study.[105] Bhat et al[39] found that using a Cox multivariate regression model, chronic HCV infection, hypoalbuminemia, and hyponatremia were predictive of advanced fibrosis.

Although the serum markers, indices, and models performed moderately well, they are currently not well validated and may be specific to certain diseases because many methods only analyzed fibrosis in patients with recurrent HCV. However, certain biomarkers that are fibrosis-specific may be applicable to a wider range of diseases despite being developed in the recurrent HCV era. Furthermore, some of the ECM and immunological protein analyses are not routinely available, require validation, and are not ready for clinical use. Inevitably, the modest results highlight that serum markers

TABLE 2.

Overview of cited serum biomarkers and corresponding formulae

Type	Formula	AUC[a]
AST/ALT ratio	$\frac{[AST]}{[ALT]}$	0.52–0.79
APRI	$\frac{[AST]}{[AST]_{Upper\ normal} \times Platelet_{Count}} \times 100\%$	0.64–0.87
Fib-4	$\frac{Age \times [AST]}{Platelet_{Count} \times \sqrt{[ALT]}}$	0.59–0.78
NFS	$-1.675 + (0.037 \times Age\ (y)) + (0.094 \times BMI\ (kg/m^2)) + (1.13 \times IFG/Diabetes) + (0.99 \times [AST]/[ALT]) - (0.013 \times Platelet_{Count}) - (0.66 \times Albumin)$	0.75–0.89
Forns index	$7.811 - 3.131 \times ln\ (Platelet_{Count}) + 0.781 \times ln\ (GGT) + 3.467 \times ln\ (Age) - 0.014 \times (Total\ cholesterol)$	0.71
FibroTest	Uses logistic regression of serum markers and estimates parameters	0.70–0.79
LTC score	$TFLT\ (mo) \times AST \times \frac{INR}{Platelet_{Count}}$	0.78
ELF score	$2.494 + 0.864 \times ln\ ([HA]) + 0.735\ ln\ ([PIIINP]) + 0.391 \times ln\ ([TIMP - 1])$	0.67–0.78 (at 3, 6, and 12 mo post-LT)

[] denote serum concentration. ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AUC, area under the curve; BMI, body mass index; ELF, enhanced liver fibrosis; Fib-4, fibrosis 4 score; HA, hyaluronic acid; IFG, impaired fasting glucose; INR, international normalized ratio of prothrombin time; LTC, London Transplant Center; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score; PIII3NP, amino-terminal propeptide of type III procollagen; TFLT, time from liver transplant; TIMP, tissue inhibitors of matrix metalloproteinase.

[a]AUC range was provided for scores that was reported in numerous studies; AUC calculations across the various scores and studies were determined at F≥2 (significant fibrosis) or F≥3 (advanced fibrosis).

alone cannot replace liver biopsy for fibrosis staging and may be used to confirm biopsy results.

Imaging Modalities

Increasing liver stiffness is positively correlated with fibrosis. A common imaging modality used to quantify liver stiffness is US-based elastography. Transient elastography (TE) is routinely used in the clinic to noninvasively diagnose fibrosis because it is shown across various studies to be effective in HCV and increasingly in patients with NASH.[106-108] Compared with serum biomarkers, TE has a higher accuracy as noted by the AUCs of several studies with values ranging from 0.84 to 0.95.[8,109-115] Multiple studies have also shown that fibrosis staging has greater accuracy than serum markers such as APRI with the highest AUCs for differentiating no fibrosis (F0–F1) from advanced fibrosis (F3–F4) being approximately 0.95+.[87,113,114,116,117] One recent study reported a lower AUC of 0.87 for differentiation of F0 to F1 and F3 to F4 fibrosis but it is considerably higher in accuracy compared with standard serum biomarkers.[115] Several studies have also shown that liver stiffness decreases with the introduction of therapy and can effectively monitor fibrosis regression with TE, such as after DAA treatment for HCV.[115,118-120] In the context of acute rejection, Crespo et al[121] determined that liver stiffness is higher in individuals with severe acute rejection and the values were not statistically different for recipients with recurrent HCV. TE was also used to track acute rejection regression after treatment, and it was found that liver stiffness improves in mild rejection for most patients within 30 d, whereas with severe rejection, improvement in liver stiffness is seen in 90 d.[121] For patients with NASH, the controlled attenuation parameter (CAP) score is measured using TE to detect the presence of steatosis accurately, which may be correlated with fibrosis progression. Using CAP as a surrogate marker for steatosis, 2 studies found conflicting results that question the predictability of fibrosis/cirrhosis using CAP, whereas another study found no independent correlation between CAP values and fibrosis progression.[115,122,123]

Other examples of US-based elastography include acoustic radiation force impulse (ARFI) elastography and shear wave elastography (SWE). ARFI can provide stiffness measurement using B-mode US scanners by detecting the wave propagation speed in the liver.[124] In addition to its practical use in the clinic, studies have shown that ARFI performs comparably to TE and is applicable to both adult and pediatric populations.[125-133] Meanwhile, SWE quantifies stiffness similarly to TE with the advantage of measuring liver stiffness in several regions of interest in real time.[134] However, SWE tends to have lower performance metrics compared with ARFI and TE in LT recipients; thus, its use may be limited in the clinic.[135-139] Nevertheless, one study showed that SWE can be used reliably like TE to monitor fibrosis regression in response to DAA therapy.[140]

Magnetic resonance elastography (MRE) uses mechanical waves to assess tissue deformation and generate an elastogram to measure liver stiffness in the entire liver.[141] One study found that MRE has a higher correlation with histological assessment, unlike TE and ARFI.[142] Nonetheless, several studies found variable performance in diagnosing and staging fibrosis with AUCs ranging from 0.75 to 0.97.[142-146] The variability noted in MRE and other imaging modality studies is similar to studies investigating serum biomarkers, which may be explained by differences in LT populations examined as well as the sample size and the number of patients with advanced fibrosis. Hence, more prospective validation studies are needed for better comparisons. Because most studies diagnosed fibrosis in recurrent viral hepatitis patients, further work must be done to evaluate its performance for NASH, ALD, and other causes. However, because elastography determines liver stiffness, it may be that this parameter is cause-independent and can be applied to various GF causes as seen with acute rejection. Although the use of elastography is favorable in the post-LT setting, its utility lies in being integrated with other diagnostic tests for confirming the diagnosis of fibrosis.

Omics Approaches

Additionally, omics approaches are of significant interest in the realm of precision medicine. Proteomics, and specifically metabolomics, can provide a systematic approach to understanding disease progression in the context of specific molecular pathways and determine their association with patient prognosis.[147] Two studies investigated the role of metabolomics in diagnosing fibrosis in recurrent HCV patients, particularly differences in phospholipids, sphingophospholipids, and global changes in oxidative and proinflammatory pathways with rapid fibrosis progression.[148,149] Metabolomic approaches were also considered for patients with recurrent NASH. Patients with recurrent NASH had a higher concentration of free fatty acids and triglycerides compared with controls and patients with rejection.[150] However, when solely considering fibrosis, no metabolites were found to differentiate normal liver from any fibrosis stage.[150]

As highlighted, various noninvasive methods can be used as a surrogate for liver biopsy; however, there are some discrepancies in performance that may be cause-based. Moving forward, further refinements to noninvasive diagnostic tools can be used as a screening tool to stratify fibrosis severity, using liver biopsy as a confirmatory method for severe fibrosis and ambiguous cases. For example, patients transplanted for any etiology may be routinely screened for progressive fibrosis with TE and only undergo liver biopsy if there is a significant progression. This approach could potentially reduce the reliance on liver biopsy and its associated complications.

FUTURE DIRECTIONS

To develop more accurate and robust noninvasive diagnostic tools for GF, several different avenues are currently being considered. These studies are in response to current methods that are not well validated and are not comparable in performance to liver biopsy. Most of the proposed methods have few studies in the post-LT setting but highlight the different ways to improve noninvasive diagnosis. Ultimately, these new methods aim to facilitate improved management of LT recipients, particularly enabling closer monitoring of post-LT complications and tailoring immunosuppression to reduce further graft damage.

Microbiome Methods

Changes in the microbiome may reflect graft dysfunction, particularly in the context of metabolic liver diseases such as NASH. We found one study that investigated changes in the microbiome of LT patients who developed recurrent NASH.[151] Using the NAFLD activity score for stratification, several genera were lost (ie, Clostridiales, Propionibacterium, and Rikenella), whereas others were elevated with increasing

NAFLD activity score (ie, Veillonella, and Faecalibacterium, Bilophila). Based on the liver biopsy NASH clinical research network score, a decreased number of genera, specifically Lachnospiraceae, Coprococcus, Ruminococcus, and Bacillus, were associated with stage 2 fibrosis.[151] The authors noted that the Firmicutes/Bacteroidetes ratio was important in predicting recurrent NASH, whereby a lower ratio was associated with a stronger presence of NASH. Dysbiosis is an emergent field of analysis, especially in post-LT; thus, further studies are needed to understand its role in predicting recurrent NASH and, ultimately, GF.

Genetic Methods

Another method of interest for diagnosing and predicting GF is leveraging genetic mapping in the post-LT population. Studies have investigated the role of changes in genetic markers, particularly with single nucleotide polymorphisms, and transcriptomic profiles in specific hepatic diseases, such as HCV and NASH. These considerations may lead to the development of new biomarkers[152] or targets for therapies. Additionally, microRNAs (miRNAs) for post-LT fibrosis have been previously reviewed, especially in fibrosis-related genes expressed in HSCs.[153] Interestingly, both donor and recipient genetic profiles can contribute to graft outcomes by influencing key immune and metabolic pathways.[154] This dysregulation is also disease-specific because various genes and miRNAs can be upregulated or downregulated depending on whether the patient has HCV or NASH.[153] One study also determined that certain miRNAs (ie, miR-34a, miR-122, and miR-210) can also differentiate between acute rejection and HCV recurrence.[155] There have been an extensive number of studies that have investigated and determined genetic signatures for recurrent HCV studies. Because these signatures are disease-specific and the current graft pathology landscape has changed, we will be focusing on NASH and other disease-related signatures.

Because NASH is increasingly a disease of interest, several studies focused on deriving gene signatures, particularly in steatotic livers that can contribute to GF. One study investigated the gene profile of steatotic and nonsteatotic liver samples and found that the most downregulated genes were *P4HA1*, *IGF1*, or fetuin B, the most upregulated were *PLIN1* and *ME1*, and important upstream regulators were *HNF1A*, *RXRA*, and *FXR*.[156] Some of the pathways affected by these changes include cholesterol and bile acid metabolism, inflammation, lipid metabolism, blood coagulation, and oxidative stress.[156] Association of the *PNPLA3* genotype with a higher risk of fibrosis (ie, increased CAP value and NFS) was also reported although this effect was not noted with *IL-28* genotypes.[157] The variability of these results highlights the complexity of gene regulation in diseases, and thus, future studies should investigate and consolidate gene expression signatures that can be used in diagnosis, particularly for GF, graft loss, recurrent disease or malignancies to better manage LT recipients.

One study investigated the molecular profiling of acute rejection, identifying 13 genes that are upregulated in extracellular remodeling and activation of Kyoto Encyclopedia of Genes and Genomes canonical pathways by IFN-gamma along with ATAGC PBT gene sets.[158] The following gene sets were upregulated in this group: *QCAT*, *QCMAT*, *GRIT1*, *AMAT1*, *ENDAT*, *BAT*, *IRITD3,* and *IRITD5*.[158] Although there was some overlap with HCV-related signatures, there were notable differences in the signatures that are specific to acute rejection. The study also noted that recipients with elevated gene expression had discernable differences in clinical parameters, such as higher fibrosis score, AST, and bilirubin but lower platelet count.

Artificial Intelligence Methods

With recent interest in incorporating artificial intelligence (AI)-based tools in the clinic, a growing number of studies are investigating the uses of AI with large data sets and integrating different data types (ie, serum, imaging, clinical, and pathological factors) to improve the diagnosis and management of chronic liver disease. Although applying these tools shows promise, few studies are currently investigating the applications of AI in GF.[159] Piscaglia et al[160] trained an artificial neural network using serum markers, particularly serum sodium and PT, to predict significant fibrosis in patients with recurrent HCV and observed an AUC of 0.93 in the validation set. A recent study by Azhie et al[161] used a weighted long short-term memory deep learning model that can predict F2 fibrosis using longitudinal demographic, serum, and clinical data in the post-LT setting. The model's AUC was 0.79 and performed better than serum markers such as APRI and FIB-4, which had an AUC of 0.68 and 0.65, respectively.[161]

In the context of ALD, one study investigated the role of AI to predict post-LT alcohol relapse using 13 psycho-social variables.[162] They identified that the gradient boosting model performed the best with a validation AUC of 0.69. The top-ranked features were variables associated with social support and substance abuse as highly predictive of post-LT alcohol use. Although this model does not specifically target GF, this tool does provide some promise in identifying patients who may be more likely to relapse and provide the necessary interventions to prevent significant GF for this subpopulation.

CONCLUSIONS

Recurrent graft disease in LT recipients is common and ultimately results in GF. Understanding the underlying mechanisms of GF can inform diagnostic methods that can facilitate the implementation of preventative measures to safeguard graft survival. Current methods for diagnosing GF are limited that can affect the timely intervention of therapies. New emerging methods include mapping the gut microbiome, identifying key genetic markers, and leveraging machine learning tools, all of which are promising. With increasing organ demand but insufficient organ supply, better management of LT recipients is required to prevent graft dysfunction and ultimately, the need for retransplantation. Addressing GF and other contributors to graft dysfunction is the first step in improving the long-term outcomes of LT recipients.

METHODOLOGY AND SEARCH STRATEGY

A comprehensive search strategy was developed for the molecular mechanisms and diagnostic methods sections using a combination of database-specific subject headings and text words for the main concepts of post-LT and fibrosis (our inclusion criteria). A sensitive search filter for diagnosis from Haynes and Wilczynski[163,164] was applied to the base set. Results were limited to humans, adults, and the English language. Conference materials and books were omitted where applicable. Searches were executed in the following databases:

Ovid MEDLINE; Ovid Embase; Cochrane Database of Systematic Reviews (Ovid); and Cochrane Central Register of Controlled Trials (Ovid). The reference lists of retrieved publications were also searched and considered for inclusion. Additional searches were also conducted using Google Scholar for subtopics which made it difficult to build an effective search strategy. Because of the broad scope of this narrative review, it was not possible to follow a specific search strategy and inclusion criteria for all the review's sections.

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