MRI-serum-based score accurately identifies patients undergoing liver transplant without rejection avoiding the need for liver biopsy: a multisite European study

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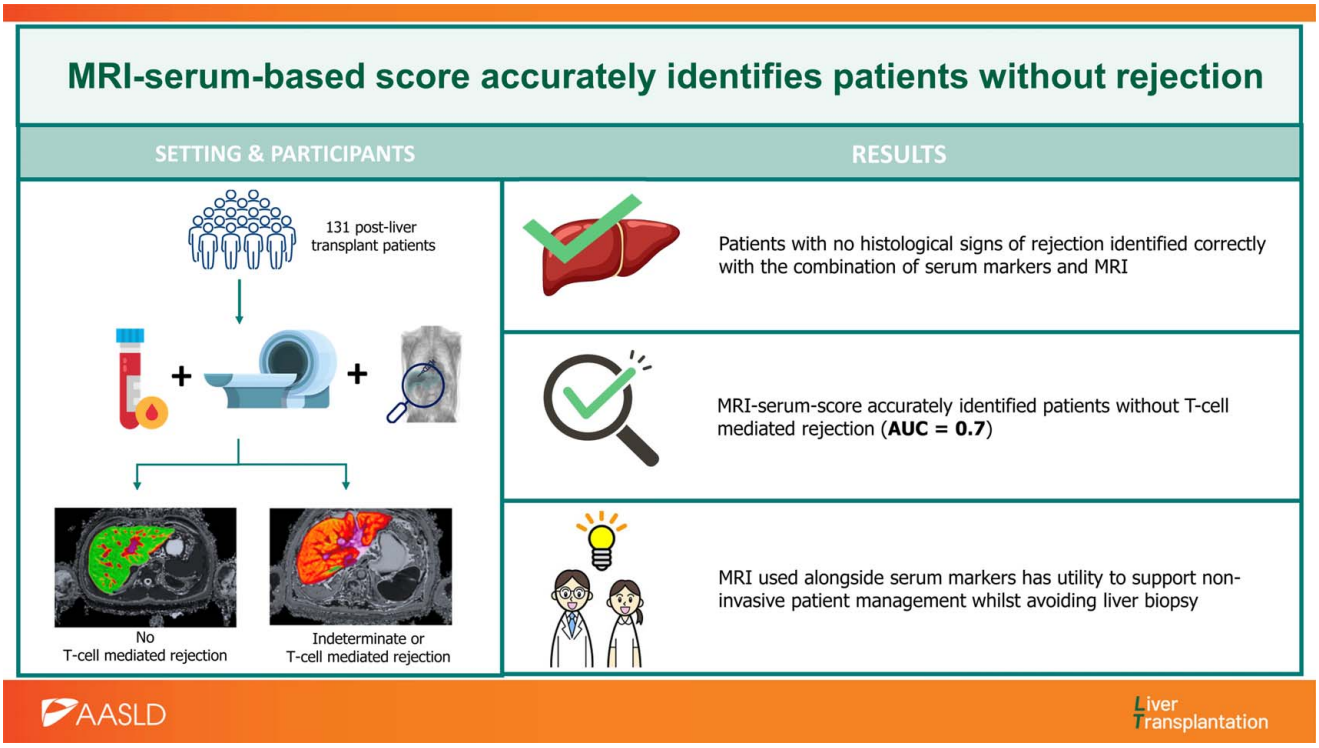
VISUAL ABSTRACT



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ORIGINAL ARTICLE

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MRI-serum–based score accurately identifies patients undergoing liver transplant without rejection avoiding the need for liver biopsy: A multisite European study

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Abstract

Serum liver tests (serum tests) and histological assessment for T-cell–mediated rejection are essential for post-liver transplant monitoring. Liver biopsy carries a risk of complications that are preferably avoided in low-risk patients. Multiparametric magnetic resonance imaging (mpMRI) is a reliable noninvasive diagnostic method that quantifies liver disease activity and has prognostic utility. Our aim was to determine whether using mpMRI in combination with serum tests could noninvasively identify low-risk patients who underwent liver transplants who are eligible to avoid invasive liver biopsies. In a multicenter prospective study (RADIcAL2), including 131 adult and pediatric (children and adolescent) patients with previous liver transplants from the Netherlands, Portugal, and the United Kingdom, concomitant mpMRI and liver biopsies were performed. Biopsies were centrally read by 2 expert pathologists. T-cell–mediated rejection was assessed using the BANFF global assessment. Diagnostic accuracy to discriminate no rejection versus indeterminate or T-cell–mediated liver transplant rejection was performed using the area under the receiver operating characteristic curve. In this study, 52% of patients received a routine (protocol) biopsy, while 48% had a biopsy for suspicion of pathology. Thirty-eight percent of patients had no rejection, while 62% had either indeterminate (21%) or T-cell–mediated

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BANFF-GA, BANFF global assessment; cT1, corrected T1; IS, immunosuppression; GGT, gamma-glutamyl transferase; mpMRI, multiparametric magnetic resonance imaging; NPV, negative predictive value; NIT, noninvasive technology; PDFF, proton density fat fraction; RADIcAL2, Rapid Assessment of Patients with Liver Transplants Using Magnetic Resonance Imaging with LiverMultiScan; SoC, standard of care; ULN, upper limit of normal.

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rejection (41%). However, there was a high interobserver variability (0 < Cohen's Kappa < 0.85) across all histology scores. The combined score of mpMRI and serum tests had area under the receiver operating characteristic curve 0.7 (negative predictive value 0.8) to identify those without either indeterminate or T-cell–mediated rejection. Combining both imaging and serum biomarkers into a composite biomarker (imaging and serum biomarkers) has the potential to monitor the liver graft to effectively risk stratify patients and identify those most likely to benefit from a noninvasive diagnostic approach, reducing the need for liver biopsy.

INTRODUCTION

Long-term survival after solid organ transplantation has increased during the last few decades due to improvements in surgical techniques, perioperative care, and more efficient immunosuppression (IS) for rejection.[1] However, despite these advances, due to increased susceptibility to adverse outcomes related to comorbidities and the chronic use of IS when compared to the general population, recipients of transplants still exhibit higher morbidity and mortality.[1]

In current guidelines, liver biopsies and blood tests (serum biochemistry) are considered essential for long-term monitoring of recipients of liver transplants to evaluate graft health and exclude graft rejection.[2,3] Currently, there are no noninvasive tests that are recommended and considered both sensitive and specific for identifying or excluding T-cell–mediated rejection.[3] Hence, despite well-documented limitations (including the risk of complications, patient discomfort, reader variability, and cost), liver biopsy remains the recommended assessment tool to exclude rejection.[3] Although current noninvasive technologies (NITs) have shown equivalent utility as liver biopsy in predicting outcomes from liver disease and can stage disease activity and fibrosis,[4,5] there is very little evidence showing their utility in ruling out those having T-cell–mediated rejection.[3] This paucity has resulted in a need for objective, quantitative, and reproducible noninvasive methods of excluding acute T-cell–mediated rejection of the liver graft in this population. The identification of NITs that can characterize key aspects of acute T-cell–mediated rejection would, therefore, substantially benefit the liver transplant population, and potentially actively support clinical decision-making and patient management, which may support IS titration.[3]

Liver multiparametric magnetic resonance imaging (mpMRI) is a reliable noninvasive diagnostic method that quantifies liver disease activity and has shown prognostic utility in management and risk stratification of numerous chronic liver diseases.[6–9] For instance, iron corrected T1 (cT1), an mpMRI marker assessing disease activity, is a reliable marker predictive of clinical outcome,[10,11] with low inter-observer variability and good correlation with liver histology.[12,13] By providing a panoramic view of the liver, cT1 has shown potential to support pivotal steps including informing risk stratification and positively impacting clinical decision-making.[14,15] This has proven especially beneficial as cT1 IQR has shown utility in assessing heterogeneity in the liver[11] as well as being a predictor of fibrosis stage.[16]

Currently, it is unknown if the use of mpMRI techniques to support routine noninvasive management of the posttransplant population could provide a similar yield as shown in chronic liver diseases. Clinical guidelines have noted the need for investigation and evaluation of potential noninvasive tools to detect or exclude allograft dysfunction and T-cell–mediated rejection.[2,3] Therefore, this study aims to determine whether using mpMRI alongside commonly used liver serum tests could noninvasively identify recipients of liver transplants who are eligible to avoid invasive liver biopsy to diagnose or exclude T-cell–mediated rejection. By identifying the most ideal combination of mpMRI and serum tests, we seek to investigate the utility of this approach to decrease the need for liver biopsies in recipients of liver transplants with a low risk of T-cell–mediated rejection.

METHODS

Patients and methods

This study, titled *Rapid Assessment of Patients with Liver Transplants Using Magnetic Resonance Imaging with LiverMultiScan* (RADIcAL2), was a real-world prospective, multinational, biomarker trial that recruited patients from 3 clinical centers in Leiden (the Netherlands), Coimbra (Portugal), and London (UK). As part of their standard of care (SoC), alongside their clinical history assessment, examination, and serum (biochemical blood) panel analysis, patients also received a research noncontrast MRI scan. Patients included in RADIcAL2

received a liver biopsy that was indicated as per local protocol at the recruitment site.

Eligibility criteria

This study was designed in accordance with the STARD criteria (Figure 1) and included patients aged over 6 years old who had undergone a liver transplant at least 6 months before inclusion and were due to undergo liver biopsy either for routine (protocol) assessment or suspected pathology.[3] If the patient had any contraindication to MRI, had any contraindication to liver biopsy, or could not tolerate MRI without sedation or general anesthetic, they were excluded from the study (Figure 1).

Histopathology assessment

Liver tissue samples were centrally read at a tertiary referral center with the largest transplantation program in Europe. Central reads were performed by 2 experienced pathologists (with over 15 years of experience each), with each pathologist conducting 2 rounds of blinded reads for each patient. The liver tissue samples were examined using several scoring systems, including the modified Ishak score, METAVIR score, Non-Alcoholic Steatohepatitis Clinical Research Network score, and liver allograft fibrosis score to evaluate fibrosis, inflammation, and steatosis. The BANFF global assessment score was used to assess rejection, while the siderosis hepatocellular score was used to evaluate iron.

Quantitative MR acquisition protocol and image analysis

The mpMR scanning protocols were installed, calibrated, and phantom tested on a Philips Ingenia 3T scanner (Philips) at the Leiden University Medical Center in Leiden, a Siemens Trio 3T scanner (Siemens Healthcare GMBH) at Instituto de Ciencias Nucleares Aplicades a Saude in Coimbra, and a Siemens Skyra 3T scanner (Siemens Healthcare GMBH) at Guys and St Thomas' NHS Foundation Trust, London. All scans were performed using the LiverMultiScan protocol, which has been standardized across scanners (GE, Phillips, and Siemens) and field strength (1.5 T and 3 T).[17] During a 15-minute noncontrast MRI scan, 4 transverse slices centered at the porta hepatis location in the liver were acquired for each participant using a shortened modified look-locker inversion and a multiecho spoiled gradient-echo sequence to quantify T1, iron (T2*), and fat (proton density fat fraction [PDFF]).[17] All images were analyzed by trained analysts blinded to the clinical data (Figure 1), and no additional incidental findings were identified following the addition of the MRI scan.

Study design and variation between study centers

This study was a real-world evidence prospective study. It is possible that there was variation across patient management from the different centers; however, to enable evaluation of the true benefit that inclusion of new technology would have on SoC, care across centers was not homogenized (mandated to a specific hypothetical pathway), but rather reflected real-life clinical practice. Clinical evaluation reflected the variable treatment and management pattern, which depended on clinician discretion and the SoC at each included site.[18]

Ethics and registration

This study was sponsored by the European Union's Horizon 2020 SME Instrument Phase-2 Program grant (719445) and received ethical approval in Ulm (Germany; 198/17), Coimbra (Portugal; CE-030/2017), Leiden (the Netherlands; P17.076), and London (UK; 18/SC/0725). The study was registered as a clinical investigation (NCT03165201), and principles of Good Clinical Practice and those of the 1975 Declaration of Helsinki were observed. All adults gave written informed consent, and for pediatric patients, parents or legal guardians gave informed consent/assent. All patient-identifiable information was kept securely and encrypted within the servers at the study sites.

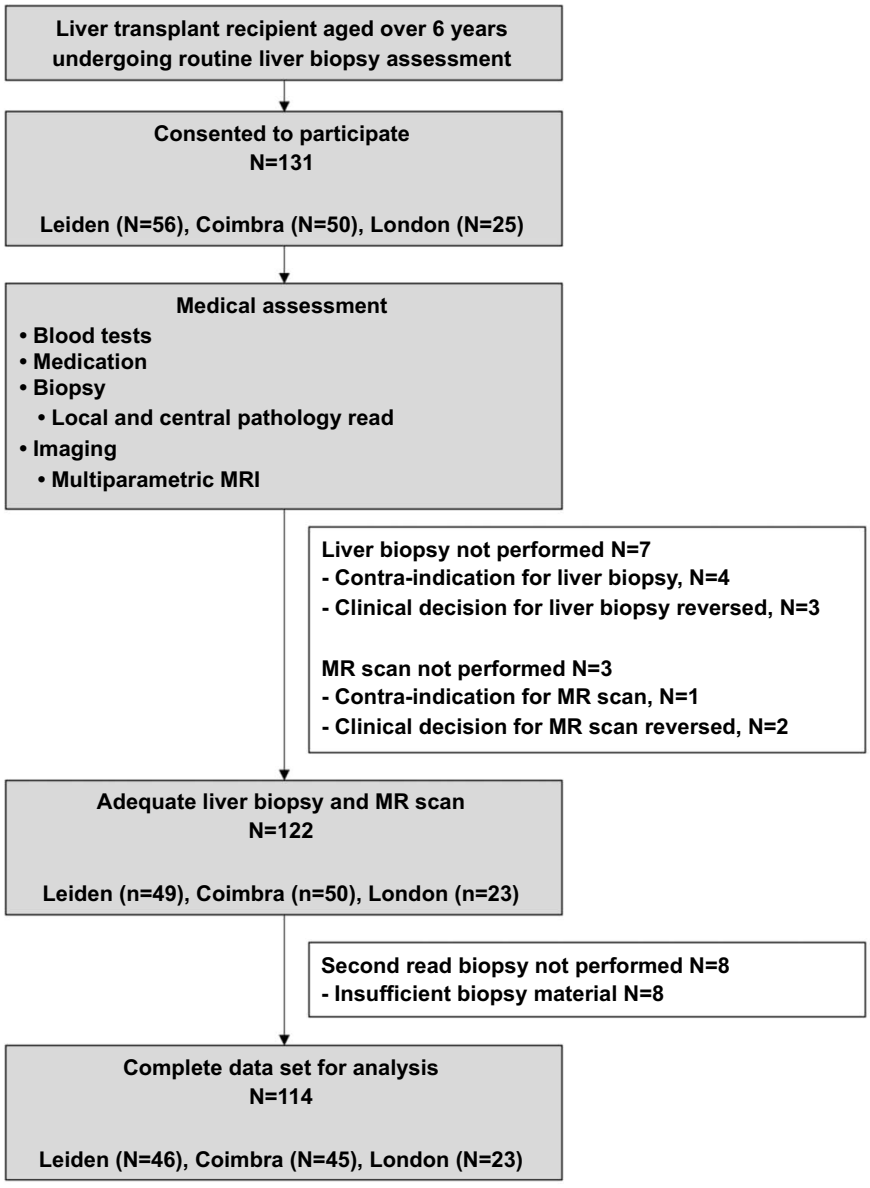
Sample size calculation

The aim of this study was to determine if the inclusion of mpMRI biomarkers can support the identification of those without histological rejection, that is, if the inclusion of mpMRI biomarkers can match biopsy results in a cohort following liver transplantation. The sample size was determined by a statistical power analysis.[19] Considering the mean rejection rate following liver transplantation is 20% as reported in the literature,[20] using an acceptable level of significance of 5% ($\alpha = 0.05$), 90% power, and assuming a 25% dropout rate, a minimum of $N = 111$ are required to meet the primary endpoint.

Statistical analysis

Patients were ranked based on their BANFF global assessment (BANFF-GA) of T-cell–mediated rejection, no rejection, indeterminate, and rejection (mild, moderate, or severe). Inter-reader and intra-reader variability

(A)



(B)

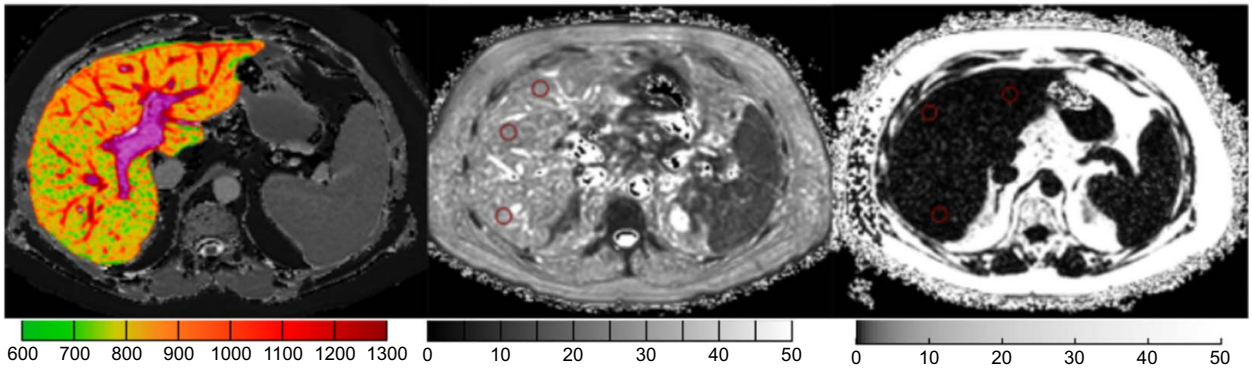


FIGURE 1 (A) Study design showing patient enrollment/exclusions, study visits, as well as collected clinical, laboratory, and imaging data. (B) Illustration of multiparametric MRI metrics of the liver (cT1, PDFF, and T2*). Abbreviations: cT1, corrected T1; PDFF, proton density fat fraction.

between the central readers were assessed using linearly weighted Cohen's Kappa (Kappa, K). All scores used in the analyses were from the first read of the pathologist with the highest intra-reader agreement between the 2 histology reads.

Diagnostic accuracy of noninvasive markers (both serum and imaging), both independently and in combination, to identify those without histological T-cell–mediated rejection, was assessed by means of bidirectional stepwise regression using the area under the receiver operating characteristic curve (AUC). Multivariate stepwise regression was used to identify the best combination of markers to identify those patients without T-cell–mediated rejection who could avoid a liver biopsy. For this analysis, patients were dichotomized into 2 groups based on their BANFF-GA: no rejection (low risk) versus those with indeterminate or any degree of T-cell–mediated rejection (high risk). The Youden Index for the combination marker was used to obtain the best cutoff and related sensitivity, specificity, NPV, and positive predictive value.

Planned subgroup analyses evaluating the differences between the adult patients (recruited from Leiden and Coimbra) and pediatric patients (recruited from London) were performed. Categorical data were compared using the chi-squared test and continuous data were compared using the Wilcoxon rank-sum test.

In RADIcAL2, the upper limit of normal (ULN) for serum tests was considered as 40 IU/L for alanine transaminase (ALT) and aspartate aminotransferase (AST), and 147 IU/L for alkaline phosphatase (ALP) and 48 U/L for gamma-glutamyl transferase (GGT). All statistical analyses were performed using R version 4.0.0 (2020-04-24).

Role of funders

The funding source had no role in the study design, data collection, data analyses, data interpretation, writing of the report, and the decision to submit the manuscript for publication. All authors had access to the data and jointly took the decision to submit the paper for publication.

RESULTS

Patient demographics and histological assessment

A total of 131 participants (70% male, aged 46 ± 20 y [range: 10–74]) were enrolled in the study. Of these, 106 (81%) were adults and 25 (19%) were children and adolescents (Figure 1). All participants were on a combination of therapies with the majority taking tacrolimus (67%), prednisolone (63%), and mycophenolate mofetil (36%) (Supplemental Table S1, http://links.lww.com/LVT/A635). The etiology of primary liver disease resulting in transplantation in this cohort was of cholestatic origin (28.1%), alcoholic liver disease (19.3%), hepatitis (16.7%), and other (36%) (including autoimmune hepatitis and nonalcoholic steatohepatitis). There were no patients included with cancer or active infections. The time between procedures (biopsy, MRI scan, and serum tests) was comparable across sites (Supplemental Table S1, http://links.lww.com/LVT/A635). The median time between MRI and biopsy in this study was 1 day (IQR: 1–4). Moreover, the median time between transplant and enrollment was 4.0 (0.5–10.7) years. Patient demographics for those included in the study are summarized in Table 1.

In this cohort, the majority of patients (52%) underwent liver biopsy for routine (protocol) assessment, while 48% had a biopsy for suspicion of pathology (Table 2). Furthermore, 70% of patients had at least 1 elevated liver-related enzyme (26% with AST > ULN, 35% with ALT > ULN, 47% ALP > ULN, and 48% with GGT > ULN), with 16% having all 4 serum tests elevated above the ULN (Supplemental Table S1, http://links.lww.com/LVT/A635). Although there were no significant differences in patient demographics between the subgroups, those who had a suspicion of pathology had significantly higher biochemical markers (AST, ALT, ALP, and GGT) compared to those having routine (protocol) assessments (Table 2).

Concordance and variability in histological assessment

According to the central histological assessment, 38% of patients had no T-cell–mediated rejection, 21% had indeterminate T-cell–mediated rejection, and 41% had histologically confirmed T-cell–mediated rejection (Table 1). We observed a wide range of inter-reader variability between pathologists (Table 3 and Supplemental Table S2, http://links.lww.com/LVT/A636). Although agreement between pathologists was comparable for both the BANFF-GA (K: 0.493 for the first read and 0.570 for the second read) and the BANFF rejection activity index score (K: 0.449 for the first read and 0.513 for the second read) (Table 3), agreement was moderate-to-fair ($0.4 < K < 0.58$). Similarly, there was moderate-to-fair agreement ($0.5 < K < 0.6$) across all fibrosis scoring systems, with modified Ishak fibrosis grading having the highest agreement (K: 0.66) and Non-Alcoholic Steatohepatitis Clinical Research Network having the lowest (K: 0.502) (Table 3). Agreement between pathologists for inflammation was more variable, ranging from no agreement to moderate-to-fair agreement (0.126–0.577 for the first read and 0.098–0.595 for the second read) depending on the scoring system used (Table 3). Steatosis scoring had strong agreement ($0.8 < K < 0.85$), while siderosis scoring showed moderate-to-strong agreement ($0.65 < K < 0.8$) between pathologists and reads.

TABLE 1 Summary of patient demographics, biochemical markers, and mpMRI metrics

		Histological classification		
	Total cohort (N = 114)	No histological rejection (n = 43)	Indeterminate (n = 24)	Histological rejection (n = 47)
Demographics				
Female (n, %)	35 (30.7)	13 (30.2)	7 (29.2)	15 (31.9)
Age (y)	51 (36)	50 (36)	51 (45)	51 (28)
Height (cm)	170 (20)	173 (17)	169 (22)	167 (12)
Weight (kg)	70.7 (19.6)	74.2 (22.3)	72.1 (19.1)	66.1 (16.2)
BMI (kg/m^2)	24.3 (6.4)	24.5 (8.7)	25.9 (6.9)	23.7 (5.0)
Biochemical markers				
AST	31 (22)	28 (18)	29 (18)	35 (26)
ALT	32 (47)	25 (29)	24 (39)	47 (55)
ALP	157 (167)	124 (126)	129 (151)	208 (190)
GGT	65 (142)	38 (91)	30 (128)	124 (198)
mpMRI metrics				
cT1 (ms)	799 (79)	800 (85)	787 (62)	803 (81)
cT1 IQR (ms)	130 (61)	131 (80)	124 (37)	132 (52)
PDFF (%)	3.4 (6.0)	5.1 (9.4)	2.8 (2.0)	2.2 (1.1)
T2* (ms)	26 (7)	27 (8)	26 (7)	26 (7)
Iron (mg Fe/g)	0.6 (0.3)	0.6 (0.3)	0.7 (0.3)	0.6 (0.3)
Patient subgroup				
Adult (n, %)	91 (79.8)	35 (81.4)	16 (66.7)	40 (85.1)
Pediatric (n, %)	23 (20.2)	8 (18.6)	8 (33.3)	7 (14.9)

Note: All normally distributed continuous variables are reported as mean (SD), and non-normally distributed continuous variables as median (IQR).
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; cT1, corrected T1; GGT, gamma-glutamyl transferase; mpMRI, multiparametric MRI; PDFF, proton density fat fraction.

Correlation between serum-based and imaging markers with histology

Correlations between biochemical markers with histology were moderate ($-0.18 \leq R \leq 0.37$), while those between mpMRI markers and histology were moderate-to-strong ($-0.51 \leq R \leq 0.33$) (Supplemental Figure S1, http://links.lww.com/LVT/A637). The relationship between biochemical and imaging markers was similar to those between histology and imaging markers and ranged from moderate to strong ($-0.51 \leq R \leq 0.36$).

Identifying the absence of histological T-cell–mediated rejection

For the identification of those without histological T-cell–mediated rejection, bivariate logistic regression showed a combination of cT1 and PDFF to have an AUC of 0.66 (95% CI: 0.56–0.77), while a combination of ALT and AST had an AUC of 0.64 (95% CI: 0.52–0.75). The combination of these best-performing markers into a multivariate marker showed improved performance with an AUC of 0.7 (95% CI: 0.59–0.81) to discriminate between those without T-cell–mediated rejection from those with either indeterminate or T-cell–mediated rejection (Figures 2 and 3). Using the Youden threshold (0.34), this combination of markers had sensitivity: 0.69, specificity: 0.69, positive predictive value: 0.56, and NPV: 0.8 for identifying those without histological T-cell–mediated rejection.

Noninvasive assessment, rejection, and patient management

To understand the clinical utility and relationship between noninvasive markers and histological rejection, similar to that proposed in other liver diseases, we evaluated the effectiveness of using this combination of NITs (serum and imaging) to identify high-risk patients. In a population with mild disease, having ALT and AST $< 2\times$ ULN (N = 80), 32 (40%) were classed as having "low risk" of histological rejection. Of these 32, 22 (69%) had no histological rejection, 4 (12%) had indeterminate rejection, and 6 (19%) had mild T-cell–mediated rejection. For the patients classed as having "increased risk" of rejection (N = 48, 60%), 12 (25%) had no histological rejection, 12 (25%) had indeterminate rejection, and 24 (50%) had T-cell–mediated rejection (mild: 17 [35%], moderate: 6 [12.5%], and severe: 1 [2%]) (Figure 2). Despite the considerable overlap between the scoring of intermediate and mild rejection, and having mildly elevated serum markers, all patients with moderate and severe T-cell–mediated rejection were classified correctly using this combination of markers. It is

TABLE 2 Summary of patient demographics, biochemical markers, and mpMRI metrics for those who had liver biopsy for either routine (protocol) assessment of suspicion of pathology

	Full cohort (N = 114)	Routine (protocol) biopsy (N = 59)	Suspicion of pathology (N = 55)	*P*
Demographics				
Female (n, %)	35 (30.7)	19 (32.2)	16 (29.1)	0.72
Age (y)	51 (36)	52 (50)	50 (25)	0.19
Height (cm)	170 (20)	172 (23)	167 (11)	0.64
Weight (kg)	70.7 (19.6)	68.9 (20.9)	72.8 (18.0)	0.40
BMI (kg/m^2)	24.3 (6.4)	24.2 (5.2)	24.9 (8.9)	0.45
Biochemical markers				
AST	31 (22)	28 (14)	43 (37)	**< 0.001**
ALT	32 (47)	23 (20)	54 (90)	**< 0.001**
ALP	157 (167)	124 (139)	180 (188)	**0.01**
GGT	65 (142)	26 (54)	153 (166)	**< 0.001**
Multiparametric MRI metrics				
cT1 (ms)	785 (89)	783 (103)	797 (79)	0.42
cT1 IQR (ms)	118 (46)	118 (42)	119 (50)	0.93
PDFF (%)	2.3 (1.4)	2.4 (1.5)	2.1 (1.4)	0.14
T2* (ms)	26 (9)	31 (9)	23 (7)	**< 0.001**
Iron (mg Fe/g)	0.6 (0.2)	0.5 (0.2)	0.6 (0.2)	**0.001**
Patient subgroup				
Adult (n, %)	91	39	52	**< 0.001**
Leiden	46	39	7	
Coimbra	45	0	45	
Pediatric (n, %)	23	20	3	**< 0.001**

Bold vaules are statistically significant comparisons $P < 0.05$.
Note: All normally distributed continuous variables are reported as mean (SD).
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; cT1, corrected T1; GGT, gamma-glutamyl transferase; PDFF, proton density fat fraction.

worth noting that all patients with T-cell–mediated rejection (mild/moderate/severe) had all liver serum markers < 2× ULN and, therefore, were classified as having mild disease using serum markers alone.

There was a high proportion of patients with indeterminate rejection with 1 in 5 (20%) classified into this group following central histology reading. Only body mass index and PDFF were significantly different between those with indeterminate T-cell–mediated rejection classed as high-risk or low-risk using the composite marker (Supplemental Table S3, http://links.lww.com/LVT/A638). Patients with all serum markers (AST, ALT, ALP, and GGT) < ULN (11%) had normal cT1 (758ms ± 31 ms).

Utility of noninvasive markers to support patient management

In Figure 4A, we show the current patient pathway for risk assessment of patients after liver transplantation as well as a proposed algorithm using mpMRI (Figure 4B).[2] In addition to the current SoC, patients who are asymptomatic with no signs of active fibro-inflammatory disease (cT1 < 800 ms) or elevated liver fat (PDFF < 5%) can be considered low risk and can be considered to avoid liver biopsy. These low-risk patients can continue post-liver transplant routine follow-up (Figure 4).

Differences between adult and pediatric patients after transplant

Although the management of patients who underwent liver transplants (adults and pediatrics) is similar, it is possible that this subgroup of patients may present differently when compared to the adult population. The biochemical and imaging markers evaluated (apart from AST) were significantly different between the 2 age groups. In addition, mpMRI imaging markers were significantly higher in the adult group compared to the pediatric group (Table 4). Although no significant differences in the frequency of rejection type were observed, the adult group had more patients with moderate/severe rejection (Table 4).

DISCUSSION

In this study investigating liver transplant patient monitoring to support the identification of those without

TABLE 3 Intra-observer variability between both pathologists (1 and 2) for both central reads (read 1 and read 2), as well as inter-observer variability between central read 1 and 2 for both pathologists (1 and 2)

	Intra-observer variability						Inter-observer variability					
	Pathologist 1			Pathologist 2			Read 1			Read 2		
Histology marker	Cohen's Kappa	SE	*P* (R1 vs. R2)	Cohen's Kappa	SE	*P* (R1 vs. R2)	Cohen's Kappa	SE	*P* (P1 vs. P2)	Cohen's Kappa	SE	*P* (P1 vs. P2)
Fibrosis												
Modified Ishak	0.930	0.022	< 0.001	0.773	0.042	< 0.001	0.585	0.056	< 0.001	0.660	0.049	< 0.001
Metavir	0.863	0.037	< 0.001	0.814	0.049	< 0.001	0.584	0.065	< 0.001	0.652	0.061	< 0.001
CRN	0.908	0.029	< 0.001	0.762	0.048	< 0.001	0.592	0.056	< 0.001	0.502	0.061	< 0.001
LAFSc	0.923	0.019	< 0.001	0.588	0.046	< 0.001	0.579	0.046	< 0.001	0.599	0.044	< 0.001
Inflammation												
Modified Ishak	0.880	0.026	< 0.001	0.732	0.041	< 0.001	0.577	0.058	< 0.001	0.595	0.055	< 0.001
Metavir	0.985	0.015	< 0.001	0.740	0.052	< 0.001	0.310	0.078	< 0.001	0.405	0.077	< 0.001
CRN	0.893	0.036	< 0.001	0.858	0.039	< 0.001	0.126	0.050	0.01	0.098	0.053	0.06
BANFF												
Global Assessment	0.963	0.022	< 0.001	0.676	0.052	< 0.001	0.493	0.060	< 0.001	0.570	0.061	< 0.001
RAI	0.961	0.016	< 0.001	0.670	0.046	< 0.001	0.449	0.053	< 0.001	0.513	0.052	< 0.001
Steatosis												
CRN	0.984	0.016	< 0.001	0.936	0.033	< 0.001	0.840	0.048	< 0.001	0.827	0.050	< 0.001
Siderosis												
Hepatocellular	0.924	0.032	< 0.001	0.932	0.030	< 0.001	0.685	0.047	< 0.001	0.758	0.056	< 0.001

Note: P1: pathologist one, P2: pathologist 2, R1: read one, R2: read two.
Abbreviations: CRN, Clinical Research Network; LAFSc, liver allograft fibrosis score; RAI, rejection activity index.

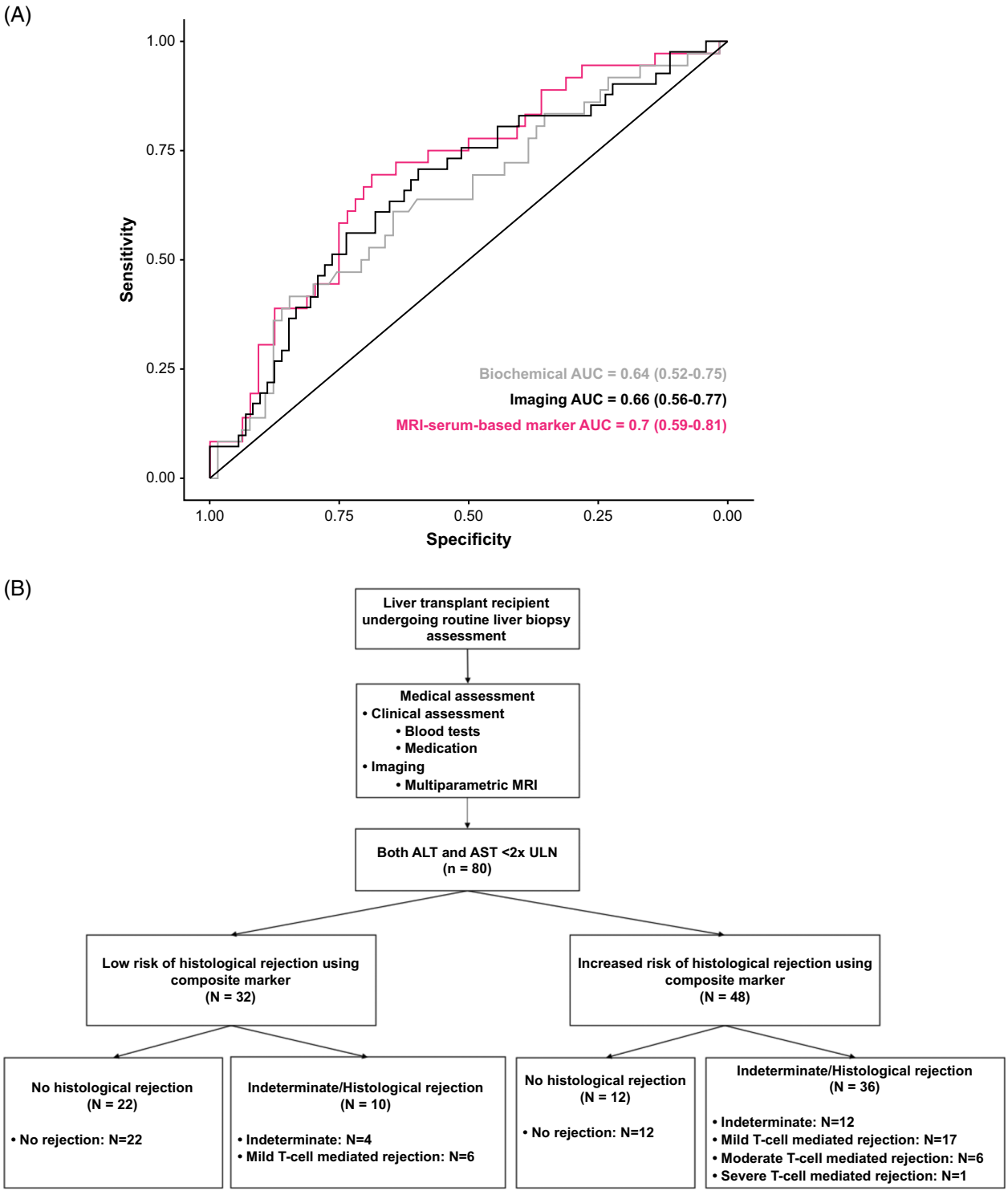
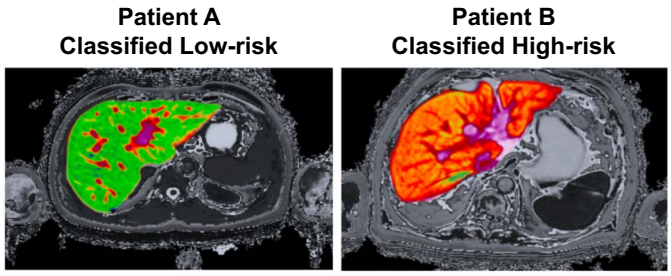


FIGURE 2 (A) Diagnostic accuracy of the best combination of biochemical markers (AST and ALT), imaging markers (cT1 and PDFF), and an MRI-serum–based score (combining imaging and biochemical markers) to identify those without histological rejection scored using the BANFF global assessment score. (B) Classification of patients using a composite marker (cT1, PDFF, ALT, and AST) in patients without significantly raised biochemical markers (serum markers of liver function and serum markers ($<2\times$ ULN). Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; cT1, corrected T1; PDFF, proton density fat fraction; ULN, upper limit of normal.

histological rejection who might benefit from a non-invasive diagnostic approach, we identified 3 main findings. First, we highlighted the added benefit imaging markers have in the management of both adult and pediatric patients following liver transplants. By combining serum and imaging-based markers, the diagnostic performance of currently used serum-based markers was improved in discriminating patients without



ALT (IU/L)	37	10
AST (IU/L)	25	12
BANFF GA	No rejection	Mild rejection
BANFF RAI	1	5
cT1 (ms)	749	904
PDFF (%)	2.0	6.8

FIGURE 3 cT1 maps for 2 patients after transplant with low blood markers (AST and ALT). Following biopsy, patient A was found to have no signs of rejection and patient B had mild rejection. These patients were correctly classified as either high-risk or low-risk using the noninvasive composite marker made up of blood (ALT and AST) and imaging (cT1 and PDFF) markers. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; cT1, corrected T1; PDFF, proton density fat fraction; RAI, rejection activity index.

rejection from those with either indeterminate or T-cell–mediated rejection who may not require a liver biopsy. Second, there was a wide range of inter-reader and intra-reader variability with moderate-to-fair agreement observed for the rejection assessments. Poor agreement between pathologists is not novel and is accepted in clinical practice where there are no central reads to support daily management. Third, there were significant groupwise differences in both biochemical and imaging markers between adults and pediatrics.

Identification and stratification (ruling-out) of patients who are not likely to have histological T-cell–mediated rejection is an aspect of patient management that NITs can significantly support.[3] This is especially evident in pediatric patients who underwent liver biopsy despite having more stable disease (significantly lower biochemical and imaging metrics) when compared to adults. Liver enzymes do not always necessarily indicate the absence of hepatic disease activity as they can be normal in the presence of disease, as was shown in a large UK Autoimmune Hepatitis Audit[21] where ALT was not an accurate longer-term outcome marker of response to treatment and disease remission. The concept of deep remission (normalization of both biochemistry and imaging markers), which has been suggested as a potential management target in inflammatory-driven diseases,[14,15] could have a place in posttransplant management as the lack of liver-specificity in existing serum-based biomarkers presents a challenge for accurately quantifying disease activity within the liver. For instance, when used in conjunction with SoC tests to provide a comprehensive assessment of liver health after transplant, NITs can be used to guide treatment decisions, such as adjusting immunosuppressive therapy[15] or initiating additional interventions to prevent graft loss.

mpMRI represents a promising noninvasive addition that can characterize the extent of disease activity throughout the liver, as evidenced in multiple chronic liver diseases.[12,15,22,23] Furthermore, mpMRI markers can predict outcomes in metabolic-associated steatohepatitis,[4] hepatitis C,[6] clonal hematopoiesis,[24] autoimmune hepatitis,[10] Fontan-associated liver disease,[25] as well as identifying the presence of radiologic portal hypertension[26–28] and predicting cardiovascular outcomes in large population studies.[29] In RADIcAL2, the combination of serum and imaging markers improved the diagnostic accuracy of ruling-out rejection with good performance (AUC: 0.70, NPV: 0.8) considering the moderate-to-fair agreement between pathologists (Kappa < 0.58).

Despite having well-defined drawbacks, liver biopsy is the reference standard for detecting early histological signs of rejection following transplantation and forms a crucial aspect of posttransplantation monitoring. In RADIcAL2, 40% of patients were classified as being at low risk of having T-cell–mediated rejection (using a combination of serum and imaging markers) and, thus, could have avoided having a liver biopsy. Alongside having a significant impact on patient satisfaction and overall quality of life, minimization of unnecessary liver biopsies can have health economic benefits. For

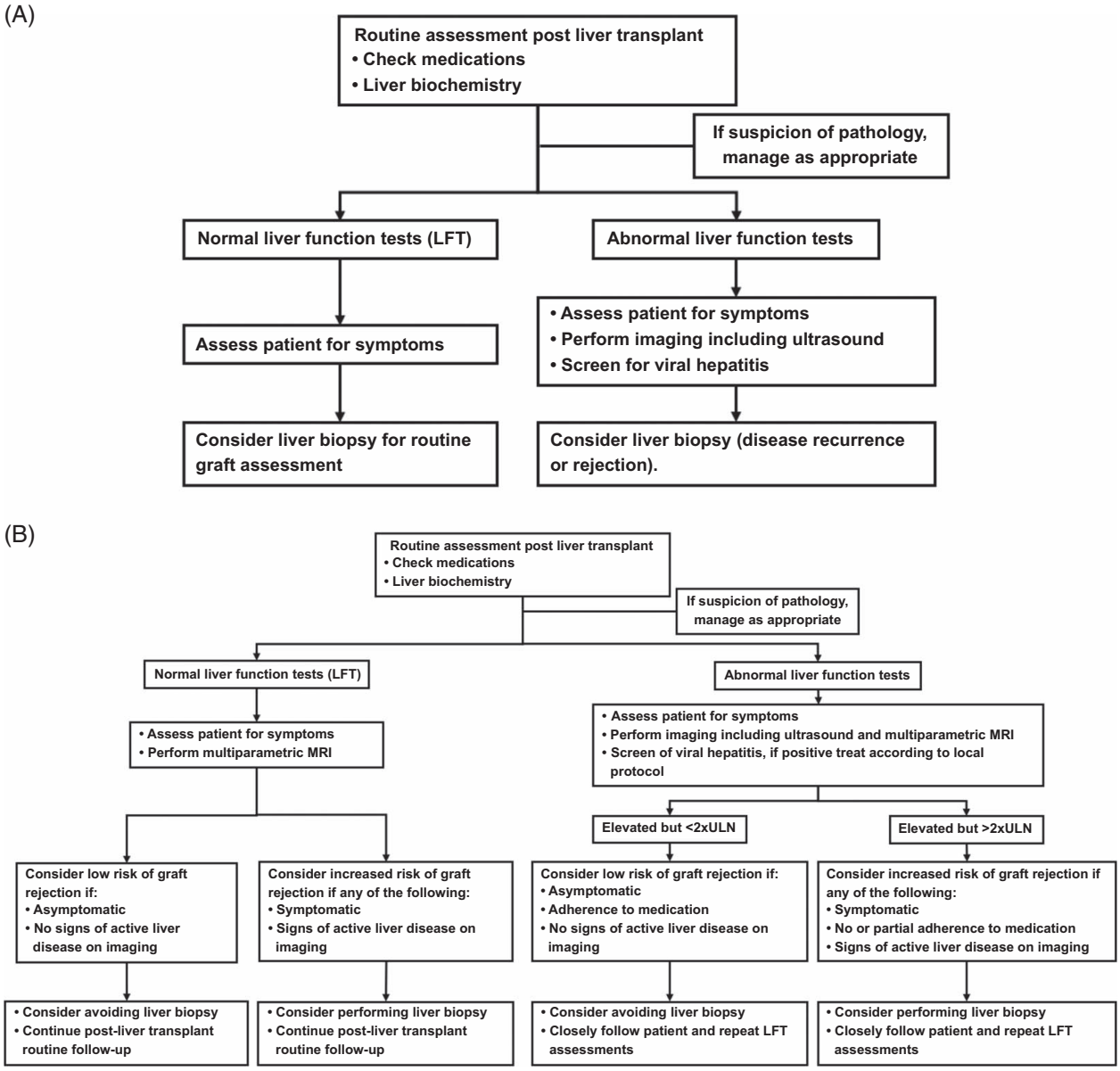


FIGURE 4 Schematic diagram of clinical algorithm. (A) The current management of patients after liver transplant in the clinic reported in clinical guidelines[2] and (B) proposed algorithm based on expert opinion showing patient work-up using multiparametric MRI.

instance, in Europe, ~6000 liver transplants are performed,[1] with most patients having indefinite IS prescribed to them. Excluding the cost of specialist management and care, and patient out-of-pocket costs, annual per-patient therapeutic management costs in Europe (in 2017) ranged from €4200 to €7000.[30] In addition to this cost, management of complications such as major bleeding following biopsy (affecting ~4.5%) can cost up to €6000 per patient.[31] In both the United States and Europe, the implementation of mpMRI ahead of and, in many cases, instead of biopsy in allografts has been cost-effective in recipients of cardiac transplants.[32] This is particularly relevant as mpMRI has specifically been shown to be cost-saving when included in the patient management pathways for liver diseases such as metabolic-associated steatohepatitis[33] and autoimmune hepatitis.[31] Thus, future work should investigate the costs associated with the inclusion of this technology to support post-liver transplant patient management. Therefore, the avoidance of this invasive procedure in low-risk patients could impact health care costs in this population in a positive way by supporting better patient triaging and ensuring only patients classified as being at high risk of T-cell–mediated rejection receive a liver biopsy.

This study had clear strengths and some limitations. First, we included a blinded central reading by 2 expert hepatopathologists. The inclusion of a diverse patient population, including both children and adult patients from different European centers, and the real-world study design increases the generalizability of the

TABLE 4 Groupwise comparison between adults and pediatrics (children and young adults)

	Full cohort (n = 114)	Adult cohort (n = 91)	Pediatric cohort (n = 23)	*P*
Demographics				
Female (n, %)	35 (30.7)	22 (24.2)	13 (56.5)	**0.003**
Age (y)	45 (21)	56 (19)	11 (2)	< **0.001**
BMI (kg/m^2)	27.2 (14.3)	28.9 (15.4)	20.4 (4.2)	< **0.001**
Biochemical markers				
AST (IU/L)	31 (22)	31 (28)	31 (11)	0.84
ALT (IU/L)	32 (47)	39 (53)	21 (13)	**0.004**
ALP (IU/L)	157 (167)	127 (139)	271 (148)	< **0.001**
GGT (U/L)	65 (142)	93 (170)	17 (17)	< **0.001**
Multiparametric MRI metrics				
cT1 (ms)	799 (79)	815 (76)	737 (58)	< **0.001**
cT1 IQR (ms)	130 (61)	140 (65)	91 (13)	< **0.001**
PDFF (%)	3.4 (6.0)	3.7 (6.6)	2.2 (1.5)	**0.04**
T2* (ms)	26 (7)	25 (7)	32 (3)	< **0.001**
Iron (mg Fe/g)	0.6 (0.3)	0.6 (0.3)	0.7 (0.1)	< **0.001**
BANFF global assessment				
No rejection	43 (37.7%)	35	8	0.51
Indeterminate	24 (21.1%)	16	8	
Mild rejection	36 (31.6%)	30	6	
Moderate rejection	10 (8.8%)	9	1	
Severe rejection	1 (0.9%)	1	0	

Note: All continuous variables are reported as mean (SD), and non-normally distributed continuous variables as median (IQR).
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; cT1, corrected T1; GGT, gamma-glutamyl transferase; PDFF, proton density fat fraction.

findings for clinical practice. The use of NITs can potentially provide health economic benefits in ruling out patients who have a low risk of liver rejection and can avoid liver biopsy and subsequent hospitalization. This is especially evident as 1 in 5 patients was classified as having indeterminate rejection and may warrant further investigation. Moreover, patients with T-cell–mediated rejection had serum markers that were either normal or mildly elevated. This underestimation of disease severity, for those with moderate/severe T-cell–mediated rejection, could potentially result in further downstream complications. Therefore, specific cost-effectiveness analyses and studies evaluating the benefits of using imaging to support patient stratification in different health care settings are necessary. Our study was cross-sectional. Longitudinal evaluation might improve better understanding of the changes associated with rejection, tracking of responses to treatment, and the prognostic value NITs may have in this population. We used liver biopsy as our reference standard despite these being shown to significantly impact the perceived performance of NITs. As shown in this study, although poor agreement between pathologists is not novel, it can have a significant effect on study findings, especially in the context of evaluating clinical utility. Future studies should include clinical outcomes to assess the performance of the NIT. For that, detailed prospective data on clinical outcomes, including changes in immunosuppressive therapy and the clinical effect of this change, should be recorded. Biliary complications constitute a high proportion of liver-related complications following liver transplantation. Future studies should evaluate the use of quantitative tools such as quantitative magnetic resonance cholangiopancreatography, which has been recognized in clinical guidelines as having prognostic utility[34] to evaluate the biliary tree health and inform risk. We also included a pediatric population in this study. As only 3% of FDA-approved AI imaging solutions are indicated in pediatrics,[35] it is important to not only investigate but also show the utility of NITs in pediatric populations. Therefore, the inclusion of pediatric patients in this study enriches the findings from this investigation. Lastly, although access to MRI can limit uptake in clinical practice in some geographies, our findings highlight the probable benefits of using NITs in the clinical setting where similar models have been successfully introduced to improve patient management.[34]

In summary, this study aimed to determine if the inclusion of mpMRI biomarkers can support the identification of those without histological rejection and

thus support the development of a noninvasive diagnostic approach that can decrease the need for invasive liver biopsies in patients following liver transplantation. Such an approach could minimize the risks, inconvenience, and costs associated with invasive monitoring of this population. Our results highlight the utility of imaging biomarkers in conjunction with serum biomarkers in monitoring the liver after transplant to effectively risk stratify patients and identify those most likely to benefit from a noninvasive diagnostic approach, thereby reducing the need for liver biopsies.

DATA AVAILABILITY STATEMENT

The data and analytic methods used in this study remain the property of the study sponsors. All deidentified participant data may be made available to other researchers upon request following permission, investigator support, and a signed data access agreement.

AUTHOR CONTRIBUTIONS

Conceptualization: Rajarshi Banerjee, Hildo Lamb, and Minneke Coenraad. Funding acquisition: Rajarshi Banerjee, Hildo Lamb, and Minneke Coenraad. Recruitment, data collection, and data entry: Jelte Schaapman, Miguel Castelo-Branco, Filipe Caseiro Alves, Tania Costa, Emer Fitzpatrick, Hein Verspaget, and Minneke Coenraad. Data quality reports and review: Marika French and Elizabeth Shumbayawonda. Data verification: Elizabeth Shumbayawonda, Marika French, Jelte Schaapman, and Cayden Beyer. Data curation: Elizabeth Shumbayawonda, Jelte Schaapman, and Cayden Beyer. Data analysis: Jelte Schaapman, Elizabeth Shumbayawonda, and Cayden Beyer. Writing original draft: Jelte Schaapman, Elizabeth Shumbayawonda, Cayden Beyer, and Minneke Coenraad. Writing—review and editing: Jelte Schaapman, Elizabeth Shumbayawonda, Miguel Castelo-Branco, Filipe Caseiro Alves, Tania Costa, Emer Fitzpatrick, Katie Tupper, Anil Dhawan, Maesha Deheragoda, Eva Sticova, Marika French, Cayden Beyer, Soubera Rymell, Dimitar Tonev, Hein Verspaget, Stefan Neubauer, Rajarshi Banerjee, Hildo Lamb, and Minneke Coenraad. All authors reviewed, discussed, and agreed with the manuscript.

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CONFLICTS OF INTEREST

Cayden Beyer is employed by Perspectum Ltd. Dimitar Tonev consults for Perspectum Ltd. Elizabeth Shumbayawonda has stock in and is employed by Perspectum Ltd. Marika French is employed by Perspectum Ltd. Soubera Rymell has stock in and is employed by Perspectum Ltd. Stefan Neubauer has stock in and owns intellectual property rights in Perspectum. Rajarshi Banerjee has stock in and is employed by Perspectum Ltd. Hildo Lamb consults for Royal Philips. The remaining authors have no conflicts to report.

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