LIBRA (Liver Breath Analysis) assessment of liver dysfunction for cirrhosis detection in at-risk populations

Giuseppe Ferrandino[1], Federico Ricciardi[1], Antonio Murgia[1], Edward Jackson[1], Liz Thompson[1], Olga Gandelman[1], Chelo Cuquerella[1], Alice Smith[1], Daniela Fonseca[1], Huw Davies[1], Max Allsworth[1], Billy Boyle[1], Claudia Asenjo[2], Francisca Bascur[2], Daniela Riquelme[2], Shahid Farooq[6], Alexandra Ginesta[3-4], Mary Ninkovic[8], Luis Méndez[3-4], Arun Shankar[5], Tapas Das[6], Naim Alkhouri[7]

[1]Owlstone Medical, 183 Cambridge Science Park, Milton Road, Cambridge, UK., [2]Centro de Estudios Clínicos, Instituto de Ciencias e Innovación en Medicina (ICIM), Facultad de Medicina, Clínica Alemana Universidad de Desarrollo, Santiago, Chile., [3]Servicio de Gastroenterología y Endoscopía digestiva, Clínica Alemana, Facultad de Medicina Clínica Alemana Universidad de Desarrollo, Santiago, Chile., [4]Servicio de Gastroenterología y Endoscopía Digestiva, Hospital Padre Hurtado, Santiago, Chile., [5]Department of Gastroenterology, Norfolk and Norwich University Hospital NHS Trust, Norwich, UK., [6]Peterborough City Hospital, Peterborough, UK., [7]Summit Clinica Research, San Antonio, TX, USA., [8]North West Anglia NHS Foundation Trust

1. Introduction

The Lancet Liver Commission has reported that approximately 75% of individuals with liver cirrhosis are first diagnosed during emergency hospitalisation for hepatic decompensation, a clinical stage at which therapeutic interventions are less effective in preserving hepatic function and quality of life[1]. This statistic underscores a critical deficiency in current diagnostic pathways: non-invasive assessments available in primary care settings lack sufficient sensitivity and specificity to detect compensated cirrhosis, while advanced diagnostic modalities such as transient elastography or serum fibrosis panels are typically restricted to secondary care and accessed only following specialist referral. Consequently, a substantial proportion of patients remain undiagnosed until the disease has progressed to an irreversible stage.

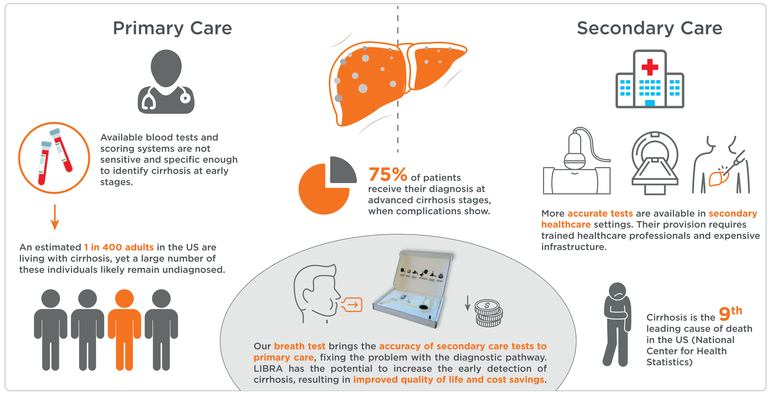


Figure 1: Infographic summarizing the current limitations of the diagnostic pathway for cirrhosis

LIBRA is a non-invasive breath test that evaluates hepatic function by measuring the metabolic processing of orally administered Exogenous Volatile Organic Compounds (EVOCs). These compounds undergo hepatic metabolism and are subsequently quantified in exhaled breath. This study aims to evaluate the diagnostic performance of LIBRA in identifying cirrhosis-associated hepatic dysfunction among individuals with relevant clinical indicators and risk factors, and to determine its suitability as a non-invasive tool to address current limitations in early-stage cirrhosis detection.

LIBRA Test Principle

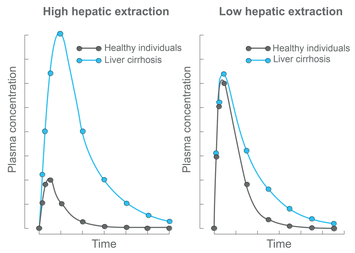


Figure 2: PK Profile Alterations in Cirrhosis. Liver cirrhosis changes the pharmacokinetic (PK) profile of drugs[2]:
- Compounds with high hepatic extraction, known as flow limited, show increased C_{max}
- Compounds with low hepatic extraction, known as enzyme limited, show a delayed decay

Volatile compounds metabolized in the liver and exhaled in the breath allow us to establish their pharmacokinetic profile using breath analysis.

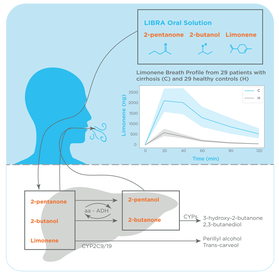
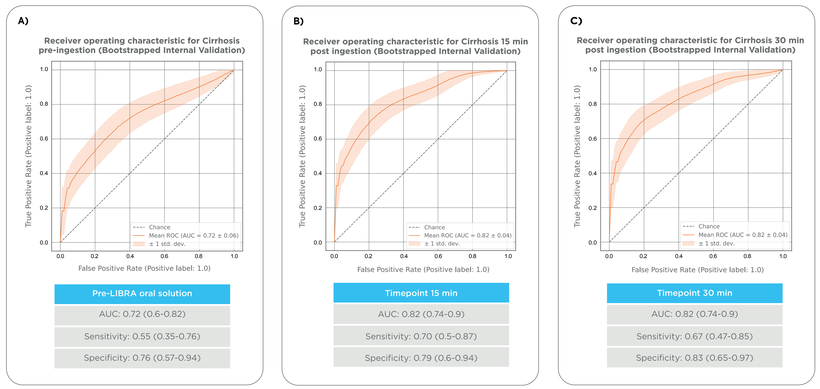


Figure 3: Hepatic Metabolic Pathways Assessed by the LIBRA test. The LIBRA Oral Solution shown in figure 3 is made up of FDA approved GRAS volatile compounds, which are commonly used as flavoring agents. These compounds are metabolized in the liver and exhaled in the breath.

2. Methods

Study type: This is a multi-site case control study
Cohort: We recruited 176 patients with risk factors and symptoms of cirrhosis, of which 85 with early compensated cirrhosis.
Diagnostic Assessment: The diagnosis of cirrhosis was established using the current standard of care consisting of multidisciplinary team (MDT) assessment based on physical examination, laboratory findings, and imaging modalities.
Probe Administration: Participants ingested the LIBRA oral solution containing limonene, 2-butanol, and 2-pentanone, volatile compounds that are metabolized in the liver and exhaled in breath
Breath Collection: Breath was collected using the ReCIVA® Breath Sampler before, 15, and 30 minutes post LIBRA oral solution ingestion.
Breath Analysis: Breath volatile compound concentration was determined using gas chromatography mass spectrometry
Statistical Analysis: Classification models for each timepoint were built using LASSO logistic regression coupled with 2000 iterations of bootstrap resampling with out of bag (OOB) cross-validation.

3. Results



Pre-LIBRA oral solution	Timepoint 15 min	Timepoint 30 min
AUC: 0.72 (0.6-0.82)	AUC: 0.82 (0.74-0.9)	AUC: 0.82 (0.74-0.9)
Sensitivity: 0.55 (0.35-0.76)	Sensitivity: 0.70 (0.5-0.87)	Sensitivity: 0.67 (0.47-0.85)
Specificity: 0.76 (0.57-0.94)	Specificity: 0.79 (0.6-0.94)	Specificity: 0.83 (0.65-0.97)

Figure 4 (A-C): ROC plots comparing positive and false positive rates at A) pre-ingestion of the LIBRA oral solution, B) 15 minutes post-ingestion of the LIBRA oral solution and C) 30 minutes post-ingestion of the LIBRA oral solution. The LIBRA test identified patients with early compensated cirrhosis, against patients with chronic liver disease at a pre-cirrhotic stage with an AUC of 0.82 at both 15 and 30 minutes post ingestion of the LIBRA oral solution. The corresponding tables illustrate the classification parameters for each timepoint as mean of the iteration, with a 95% confidence interval. Sensitivity and specificity have been measured using the Youden index.

	Cirrhosis	Control	p-values
Number of patients	85	91	-
Age median [IQR] years	61 [54-67]	60 [49-67]	2.00
Weight median [IQR] Kg	93 [79-111]	83 [71-109]	.027
Height median [IQR] cm	170 [161-176]	163 [157-170]	< .001
BMI median [IQR]	32 [28-37]	32 [28-37]	.650
AST (IU/L) median [IQR]	33 [24-43]	24 [19-29]	< .001
ALT (IU/L) median [IQR]	25 [19-35]	24 [16-34]	0.333
Bilirubin (mg/dL) median (IQR)	0.8 [0.5-1.2]	0.5 [0.4-0.6]	< 0.001
Albumin (g/dL) median [IQR]	4.2 [3.9-4.4]	4.4 [4.2-4.5]	< .001
Creatine (mg/dL) median [IQR]	0.9 [0.7-1.0]	0.8 [0.7-0.9]	596
Sodium (mmol/L, median [IQR]	140 [138-142]	140 [138-142]	.875
Platelets median [IQR] *10e9/L	150 [102-199]	260 [212-309]	< .001
INR median [IQR]	1.1 [1.0-1.2]	1.0 [0.9-1.2]	< .001
MELD median [IQR]	6.0 [5.0-9.0]	6.0 [6.0 – 6.0]	< .001
FIB4 median [IQR]	2.6 [1.9-4.5]	1.1 [0.7-1.6]	< .001
Fibroscan (kPa) median [IQR]	23 [14-32]	6.3 [4.7-8.6]	< .001
CAP (dB/m) median [IQR]	279 [246-317]	275 [226-8.7]	.768

Table 1: Subject characterisitics.

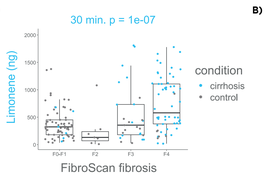


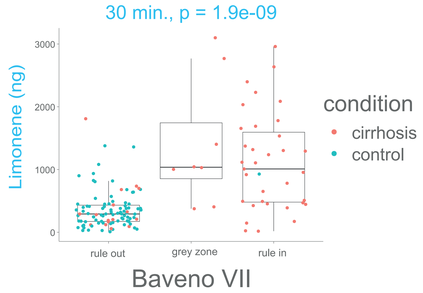
Figure 5: Comparison of LIBRA volatile compounds with Fibroscan. Limonene (A) and 2-butanone to 2-butanol ratio (B) show a significant association with the spectrum of liver fibrosis estimated using Fibroscan. Only plots at 30 minutes are shown.

Figure 6: Association of LIBRA test with clinical suspicion of portal hypertension.

Thrombocytopenia is used as a sign of portal hypertension in subjects with cirrhosis. We observed increased levels of limonene in subjects with thrombocytopenia post ingestion of the LIBRA oral solution.

The patients were stratified in the following groups:
- FibroScan <= 15 kPa and platelets >= 150 G/L = Rule out
- FibroScan >= 25 kPa = Rule in
- Fibroscan between 15 and 25 kPa, and Platelets < 150 = "Grey zone"

Subjects in the Baveno VII grey zone or rule in showed increased levels of limonene compared to subjects in the rule out group. This results further support that LIBRA could be used to monitor the onset of portal hypertension in subjects with cirrhosis.



Parameter (% of population)	LIBRA	FIB4 < 1.3 rule out > 2.67 rule in
True positives	54 (32%)	39 (23%)
True negatives	75 (44%)	50 (29%)
False positives	14 (8%)	6 (3.5%)
False negatives	27 (16)	12 (7%)
Grey zone	Non applicable	63 (37.5)
Accuracy	129 (76%)	89 (52%)

Figure 7: Subject allocation across the overall population as determined by LIBRA and FIB-4.

The LIBRA Test

For more information about the LIBRA test, head over to our website via the QR code, or email us for more information:

breathbiopsy@owlstone.co.uk

4. Conclusions and Next Steps

- LIBRA identified subjects with cirrhosis with an AUC of 0.82, indicating that it is suitable to improve the diagnostic pathway for liver cirrhosis.
- Breath levels of volatile compounds showed association with fibrosis stages estimated using Fibroscan.
- Breath levels of limonene and 2-butanol association with thrombocytopenia and Baveno VII classification indicate that LIBRA could be used to detect and monitor the onset of portal hypertension in subjects with cirrhosis.

5. References

1. Pimpin L, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, et al. Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. Journal of Hepatology. 2018 Sept 1;69(3):718–35. doi: 10.1016/j.jhep.2018.05.011
2. Krähenbühl S, Reichen J. Pharmacokinetics and Pharmacodynamics in Cirrhosis. Medicine. 2002 Nov 1;30(11):24–7. doi: 10.1383/medc.30.11.24.28446

Conflict of Interest: Authors are affiliated with Owlstone Medical.