Editorial

Biomarkers in Liver Disease: Emerging Methods and Potential Applications

Guruprasad P. Aithal,1 Neil Guha,1 Jonathan Fallowfield,2 Laurent Castera,3 and Andrew P. Jackson1

1 NIHR Biomedical Research Unit in Gastrointestinal and Liver Diseases at Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham NG7 2UH, UK
2 MRC/Centre for Inflammation Research, QMRI, University of Edinburgh, Edinburgh EH16 4TJ, UK
3 Department of Hepatology, CRB3 INSERM U 773, Hôpital Beaujon, Assistance Publique Hôpitaux de Paris, Université Paris-7, 75205 Paris Cedex, France

Correspondence should be addressed to Guruprasad P. Aithal, guru.aithal@nuh.nhs.uk

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1. Introduction

Biomarker research represents an evolving area within hepatology. The growing burden of global liver disease, the absence of symptoms until late in the natural history of a disease which may take decades to manifest, the presence of an invasive reference test (liver biopsy) to assess disease severity, and the lack of robust tools to assess the efficacy of therapeutic interventions are some of the key drivers for this research.

The National Institute of Health defines a biomarker as “A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [1]. Moreover, biomarkers can be classified into hierarchical systems based on their ability to assess natural history (type 0: prognosis), biological activity (type 1: response to therapy), and therapeutic efficacy (type 2: surrogate for clinical efficacy) [2].

The spectrum of pathological injury that occurs in liver disease including steatosis, necroinflammation, apoptosis, and fibrosis enhances the pool of potential biomarkers. Furthermore, advances in technology platforms have created an exponential rise in the discovery of putative mediators of pathophysiological injury. This has been countered by the growing need to align surrogate markers of injury with clinical consequences of injury in order to achieve diagnostic, prognostic, and therapeutic effectiveness. This timely special edition comprises original articles and reviews in the subject areas of biomarker discovery, biomarkers of liver injury, and biomarkers to assess the consequences of liver injury.

2. Methods of Biomarker Discovery

Advances in instrumentation design have driven biomarker discovery. The advent of modern biological mass spectroscopic techniques in the 1990s and the evolution of 2-dimensional polyacrylamide gel electrophoresis (2D SDS PAGE) from a highly specialist technique to one that could be carried out in most laboratories around the world drove the development of large-scale ‘omics biomarker discovery projects. Advances in microlitre flow rate HPLC, that could be coupled directly to mass spectrometers (nano-LC/MS), and computing to analyse the data gave further impetus to this work. It became possible to quantify and identify many thousands of proteins from diseased and healthy tissue in a single experiment. Biomarker discovery projects ([3] metabonomics; [4] lipidomics; [5] proteomics; [6] SELDI and transcriptomics) demonstrate the ability to identify novel markers of liver disease. Proteomics, transcriptomics, lipidomics, and metabonomics offer the ability to discover completely novel markers of disease and its progression. This de novo approach to biomarker discovery leads to a great challenge of marker validation. There may be little or no obvious mechanistic connection between the putative marker and disease, demonstrating that a link can be very time and resource intensive.
Mechanism-focussed biomarker discovery has also ben-
efited from advances in instrumental design and technology. 
These projects are based on prior disease knowledge and 
are much more limited in scope but, if successful, are 
more likely to identify a disease-relevant marker. Standard 
ELISA assays methodologies have been developed to use 
valuable patient samples more efficiently by allowing many 
analyses to be quantified simultaneously. In array or planar 
assays, a series of primary antibodies are bound to a surface 
in discrete spots, sample, and secondary antibody, and 
detection reagents are passed over the array and the location 
of the signal is determined using imaging technology. Bead-
based technologies rely on a mixture of antibody-labelled 
beads which are then quantified using flow cytometers or 
dedicated analysers. From 30 to 50 proteins can be analysed 
per experiment using panels of antibodies that have been 
optimised to minimise cross-reactivity. Miniaturisation of 
liquid handling and high-density microplates, currently up 
to 1536 samples per plate, reduces reagent and patient sample 
usage when carrying out enzyme activity-based biomarker 
discovery. A typical 96-well microplate will require 100 μL 
reaction mix per well, the high density; 1536-well plates 
require only 5 μL per well, a reduction of 20-fold in sample 
consumption. Unfortunately, the additional costs that are 
incurred to ensure accurate reagent dispensing and reaction 
monitoring are not trivial. S. K. Hartwell, in this issue, 
describes an alternative approach using flow injection to 
minimise reagent consumption where sample numbers and 
volumes may be limited. The use of commonly available 
laboratory equipment aims to minimise costs and to open 
up the technology to laboratories with limited resources.

3. Biomarkers of Liver Injury

The pathological processes of steatosis, necroinflammation, 
oxidative stress, apoptosis, and fibrosis are common to 
a number of diverse liver diseases. The ability to define 
these individual entities is advantageous for determining 
the mechanistic evidence of efficacy, using biomarkers, for 
proposed treatment strategies. A difficulty remains that the 
pathological processes are often interdependent or cocorre-
lated, and thus, delineating biomarkers specific to one mode 
of injury can be challenging. This is illustrated by the article 
in this special edition by N. Mousa and coworkers describing 
the association of alpha fetoprotein (AFP) and liver steatosis 
genotype 4 infection in chronic viral hepatitis. The authors 
postulate that the elevation in AFP is secondary to increased 
production from hepatic progenitor cells as a response to 
regeneration following injury. In this study, steatosis was 
also associated with the presence of necroinflammation 
and fibrosis, and thus, it is not clear whether it is the 
extent of liver injury or steatosis per se that leads to the 
elevation in AFP. There exists a wider debate in the literature 
on whether benign steatosis (in the absence of significant 
steatohepatitis or fibrosis) has clinical significance. In viral 
hepatitis, steatosis is most commonly seen in genotype 3 
infection and improves following successful viral eradication 
[7]. In long-term studies based on pathological features at 
baseline biopsy, steatosis has not been shown to adversely 
affect outcome in nonalcoholic fatty liver disease [8, 9].

Natural history studies have shown that the presence and 
stage of fibrosis at the index liver biopsy provide prognostic 
information about the subsequent rate of fibrosis progres-
sion ([10–12] and the development of liver-related outcomes 
[9, 13]). It is therefore no surprise that over the last decade 
much of the focus has been to define novel biomarkers based 
on the pathological presence of fibrosis. The success and 
limitations of this strategy have been outlined elsewhere [14]. 
Defining surrogates of pathological entities other than liver 
fibrosis is both necessary and advantageous for a number 
of reasons. Liver fibrosis is essentially a generic wound-
healing response and final common pathway resulting from 
a spectrum of hepatic insults. Moreover, particular charac-
teristics of the hepatic scar including the composition and 
physical/biochemical attributes that limit remodelling and 
angioarchitectural changes have hitherto made the delivery 
of effective antifibrotic therapy challenging. The ability to 
intervene “upstream” in the injury process may yield a larger 
repertoire of therapies with the allure of enhanced targeting 
and superior drug profiles. Apoptosis in the liver may be one 
such example. Whilst the engulfment of apoptotic bodies by 
avtivated hepatic stellate cells (HSCs) may induce TGFβ and 
collagen-α1 synthesis and promote fibrosis, paradoxically, 
in preclinical models, resolution of fibrosis depends on the 
removal of activated HSCs via apoptosis. Thus, the detailed 
characterisation of apoptosis may provide critical insights 
into both fibrogenesis and fibrinolysis. J. B. Chakraborty 
and colleagues provide a comprehensive review in this 
special edition of the mechanisms of apoptosis in the liver, 
candidate apoptosis-related biomarkers, and the potential for 
clinical translation (e.g., assessing treatment response and/or 
monitoring the regression of fibrosis).

4. Biomarkers Assessing the Consequences 
of Liver Injury

Following long-term liver injury, the evolution of liver 
fibrosis to cirrhosis is associated with (1) architectural 
disturbance; (2) angiogenesis and haemodynamic changes 
(intra- and extrahepatic) resulting in portal hypertension; 
(3) a propensity for carcinogenesis. In the event of the injury 
not being removed, a proportion of affected individuals will 
have complications of liver failure, bleeding, hepatocellular 
carcinoma, and death. The ability of biomarkers (at baseline 
and/or changing over time) to predict these events directly 
has the potential to improve prognosis and provide a mean-
ful assessment of clinical effectiveness (as opposed to 
therapeutic efficacy indicators such as reduction in fibrosis). 
In hepatology, the limitations of liver biopsy and rather 
restrictive pathological scoring systems have encouraged 
the extrapolation of biomarkers (originally based upon 
pathological end points) to hard clinical end points. There 
are a number of studies demonstrating that noninvasive 
biomarkers (including serum analytes and transient elastog-
raphy) measured at baseline predict liver-related outcomes 
between 5 and 8 years [15–17].
In this special edition, original research presented by N. Palaniyappan and colleagues has investigated the prognostic accuracy of validated scoring systems for detecting long-term outcomes in alcoholic hepatitis. These scoring systems showed a uniformly poor prognostic performance in detecting mortality at one year (AUC ranges from 0.5 to 0.66), in contrast to abstinence from alcohol within three to six months of initial diagnosis which was associated with an AUC of 0.83. This not only highlights the importance of abstinence but also that dynamic measurement, in this case of behaviour, can have a significant influence on prognosis in the context of liver disease.

Portal hypertension underpins the major complications of liver disease including variceal bleeding, ascites, and renal failure. Both existing and emerging therapeutic strategies in the context of established cirrhosis are directed towards lowering portal hypertension. The gold standard for its assessment remains the hepatic venous pressure gradient (HVPG). Whilst a wealth of evidence supports its prognostic value and utility in directing management [18, 19], it remains an invasive test that is only available in specialist centres. Thus, the search for robust biomarkers that offer a noninvasive alternative to HVPG is important if portal hypertension is to be assessed in routine clinical practice. The review by V. K. Snowdon and colleagues succinctly outlines the pathophysiological basis of portal hypertension and, in particular, uses examples of recent advances in endothelial cell biology/fibrosis and angiogenesis research to support the rationale for emerging biomarkers in this area.

Hepatocellular carcinoma (HCC) is the fifth leading cause of death from cancer in men, the seventh leading cause of death from cancer in women, and the fastest rising cause of cancer mortality worldwide. The majority of patients present at an advanced stage when treatment options are very limited and, consequently, HCC carries a dismal prognosis (overall median survival of 14 weeks, 1-year survival of 13%). Current screening strategies that rely on AFP and ultrasound are widely accepted but have only modest diagnostic accuracy with sensitivity rates between 25% and 65% [20]. There is an urgent need to discover and implement better diagnostic tools for this malignancy that may permit earlier and more accurate detection and the review by T. Behne and M. S. Copur outlines emerging biomarkers that have potential clinical utility.

To provide stratified care for patients with liver disease, we urgently need noninvasive tools that can effectively phenotype patients based on their degree of liver injury, natural history, and clinical outcomes. It is unthinkable that the choice of intervention in an individual patient still remains, in many circumstances, an empirical exercise involving "trial and error." Biomarker research and its dissemination should aim to overcome these barriers to individualising care.

Guruprasad P. Aithal
Neil Guha
Jonathan Fallowfield
Laurent Castéra
Andrew P. Jackson

References


