

# Target biomarker profile for the clinical management of paracetamol overdose

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Paracetamol (acetaminophen) overdose is one of the most common causes of acute liver injury in the Western world. To improve patient care and reduce pressure on already stretched health care providers new biomarkers are needed that identify or exclude liver injury soon after an overdose of paracetamol is ingested. This review highlights the current state of paracetamol poisoning management and how novel biomarkers could improve patient care and save healthcare providers money. Based on the widely used concept of defining a target product profile, a target biomarker profile is proposed that identifies *desirable* and *acceptable* key properties for a biomarker in development to enable the improved treatment of this patient population. The current biomarker candidates, with improved hepatic specificity and based on the fundamental mechanistic basis of paracetamol-induced liver injury, are reviewed and their performance compared with our target profile.

## Introduction

Paracetamol (acetaminophen - APAP) is used by millions of people worldwide as a safe analgesic drug at therapeutic doses. In overdose, it is well known to be toxic to the liver. Indeed, in the Western world, paracetamol is the commonest cause of acute liver injury (ALI) and overdose is a very common reason for hospital attendance (around 100 000 UK patients each year) [1]. In terms of UK hospital admission, the number of patients per year (around 50 000) is comparable with other 'giants' of emergency medicine such as heart failure and hip fracture.

To improve patient care and reduce pressure on already stretched health care providers, new biomarkers are needed that identify or exclude liver toxicity soon after the drug is ingested. This review highlights the current state of paracetamol poisoning management and how novel biomarkers could improve patient care and save healthcare providers money.

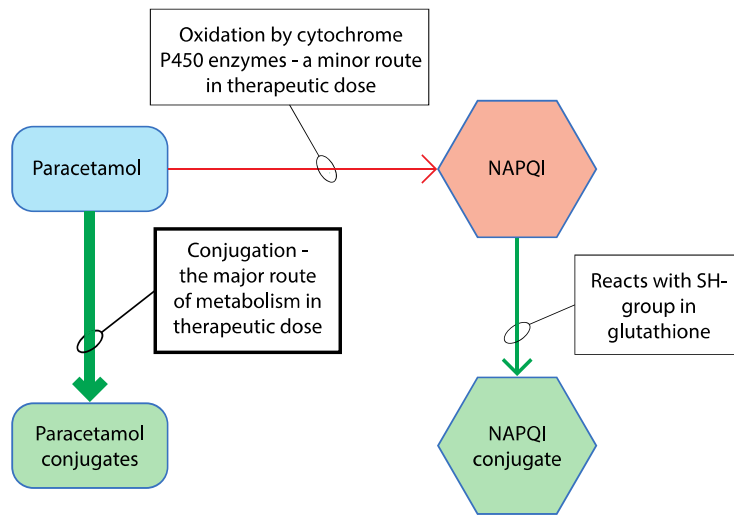
## Mechanism of paracetamol-induced acute liver injury

Adverse drug reactions (ADRs) have been traditionally classified into six types, labelled A through F [2]. However, recent

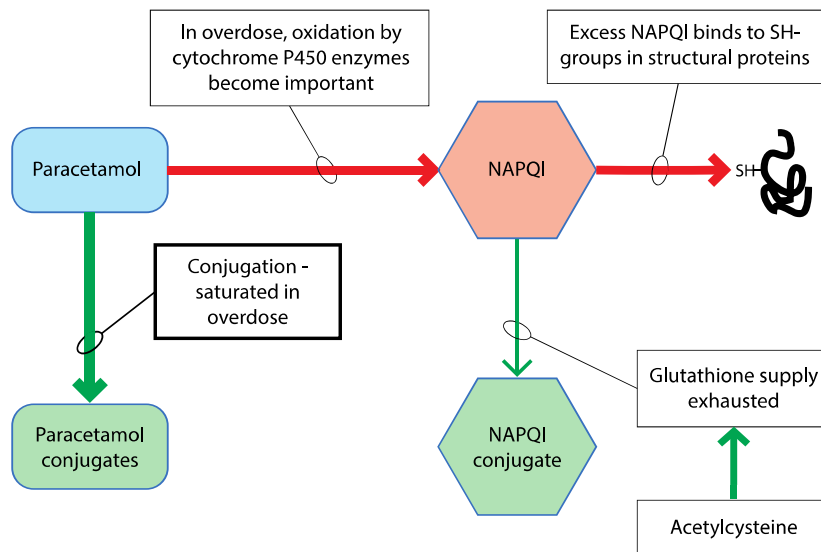
proposals have classified ADRs as either on-target (often predictable from primary or secondary pharmacology, clear dose-response relationship) or off-target (idiosyncratic, complex dose-response relationship). Paracetamol-induced ALI is classified as type A, being predictable and dose-dependent. This has resulted in the pathophysiology being studied widely, especially in rodents, for over 40 years [3]

At therapeutic doses, the major route of paracetamol metabolism is through conjugation. Cellular injury is due to the reactive metabolite N-acetyl-*p*-benzoquinone imine (NAPQI) that is produced by the cytochrome P450 enzymes CYP2E1, CYP1A2, CYP3A4 and CYP2D6 [4]. At therapeutic paracetamol doses, only low concentrations of NAPQI are formed and this metabolite is efficiently detoxified by conjugation with glutathione. At toxic doses, the paracetamol conjugation reaction becomes saturated and more paracetamol becomes oxidized by cytochrome P450 into NAPQI. The cellular stores of glutathione become exhausted, which results in NAPQI covalently binding to sulfhydryl (SH-) groups in structural proteins, forming protein adducts leading to oxidative stress, mitochondrial injury, hepatocyte cell death by either apoptosis (minor pathway) or necrosis (major pathway), multi-organ failure and potentially patient death (Figure 1) [5]. Antidote treatment with acetylcysteine (NAC) restores cellular glutathione concentrations. When administered soon after drug

### At therapeutic dose



### In overdose



**Figure 1**

At therapeutic doses conjugation is the major route for paracetamol metabolism. Oxidation of paracetamol by cytochrome P450 is a minor route at therapeutic doses of paracetamol, forming N-acetyl-*p*-benzoquinone imine (NAPQI) that quickly reacts with glutathione. When an overdose of paracetamol is taken, conjugation becomes saturated and more NAPQI is formed by oxidation. When the glutathione supply is exhausted, NAPQI binds to sulfhydryl (SH-) groups in structural proteins, resulting in cell injury

overdose (within about 8 h), NAC is highly effective in preventing liver injury [6, 7]. However, when NAC treatment is delayed, its efficacy is substantially reduced [6].

### Current risk assessment

Serum alanine aminotransferase (ALT) activity is the current, widely used, biomarker for hepatocyte injury after paracetamol overdose (and in many other settings).

Although ALT has never been formally qualified against liver histology as a biomarker for drug-induced liver injury in humans, its utility has been qualified by decades of clinical experience [8]. However, the majority of patients present to the emergency department soon after overdose with only around 10% presenting later than 12 h post drug ingestion [9]. To exclude the development of liver injury confidently, patients require an ALT measurement at least 24 h after the overdose was ingested. This limits patient stratification in emergency care settings

and potentially increases length of hospital stay. Apart from these time/kinetic issues, there are other important limitations of using ALT as a biomarker of liver injury. Changes in ALT activity do not only occur in paracetamol-induced ALI, but with a wide range of acute and chronic liver pathologies such as fatty liver disease, viral hepatitis and liver cancer which decreases the confidence in its utility for causality assessment of paracetamol-induced liver injury [10]. Increases in serum ALT activity can also be a result of myocardial damage or extreme exercise [11], which may generate false positive results. Because paracetamol-induced ALI cannot be confidently confirmed or excluded by using serum ALT activity as a biomarker at the hospital 'front door', the decision to treat with NAC following an overdose is primarily based on the blood paracetamol concentration [12]. To stratify patients as being 'at risk' for hepatotoxicity after a single overdose (total ingestion of paracetamol taking less than around 1–2 h) a nomogram is used that plots blood paracetamol concentration against time after overdose. This nomogram can only be confidently applied 4 h after overdose ingestion, when absorption is believed to be complete [13]. The utilization of the blood paracetamol nomogram after overdose depends on the correct reporting of the time of overdose. Small errors in timing can result in an incorrect treatment [5]. When the paracetamol overdose is ingested over a longer time period (staggered overdose) or if the patient was exposed to a modified release or intravenous formulation [14] the nomogram cannot be used and the treatment decision is based on the reported dose of paracetamol and the serum ALT activity. There is an unmet clinical need for new biomarkers that can guide treatment to patients at high risk of paracetamol-induced ALI and identify patients with low risk of liver injury who may require shorter,

lower doses of NAC or even no treatment at all. To decrease the risk of developing ALI, the MHRA altered the guidelines associated with NAC and lowered its utility threshold. Recent health economic calculations have estimated that this change resulted in an increase of around £8M in the annual spend by UK healthcare providers [1], which further strengthens the case for new biomarkers that improve stratification of patients with paracetamol overdose.

## Target biomarker profile

In order to identify new biomarkers that could add real value to the management of paracetamol poisoning we propose *desirable* and *acceptable* properties, our target biomarker profile (TBP, Table 1). This approach is widely used in biomarker development to set criteria that will be used to define future success. For further background the US Food and Drug Administration have produced guidance [15]. Our suggested biomarker properties specifically relate to the clinical management of paracetamol overdose. What is desirable or acceptable in other settings may be different. For all biomarkers their diagnostic performance may change when they are measured on different validated assay platforms (for example, a new point-of-care platform in contrast with the laboratory gold standard), so our TBP would need to be re-assessed when new clinical assays become available and validated. It is also important to note that defined *desirable* and *acceptable* properties can assist with the development of prospective qualification studies in man to define the context of use for a putative biomarker [16].

A biomarker specific for paracetamol toxicity is *desired*, as this test could not be misinterpreted due a signal produced

**Table 1**

Desired and acceptable biomarker attributes

Attribute	Desired	Acceptable
Specific for paracetamol overdose	Exclusively elevated by paracetamol-induced injury	Liver injury
Sensitivity for ruling out injury	ROC-AUC 1	AUC $\geq$ 0.90
Rapidly assayed	At point of care	<60 min turn around time
Feasibility of assay	Feasible in settings where resources are sparse (developing countries)	Feasible in standard clinical laboratories (developed countries)
Invasiveness / sample preparation time	Whole blood	Plasma/serum
Conserved (translational) across <i>in vitro</i> models, <i>in vivo</i> models and humans	Fully conserved between <i>in vitro</i> models, <i>in vivo</i> models and humans	Conserved between rodent models and humans
Time after overdose at which it is able to predict the onset of liver injury	4 h	8 h
Signal to noise	Single measure required to differentiate between healthy reference value and disease	Requires measurement at two time points
Quantitative relationship with disease severity	Quantitative	Qualitative
Distinguish benign and clinical relevant increase in ALT	Predicts liver failure	Predicts ALT rise
Mediator of liver injury	Has existing therapeutic intervention	Potential drug target

by other causes of liver injury. A biomarker diagnostic for paracetamol toxicity would be valuable when the aetiology of liver injury cannot be identified, reported to be the case in 17% of patients with ALI [17]. However, a marker that reports liver injury due to any cause is *acceptable*, since in most cases it is known that the patient has ingested an overdose (proven by blood paracetamol measurement).

Ideally, a new biomarker would differentiate disease from non-disease with 100% sensitivity and specificity (area under the receiver operator curve (ROC-AUC) of 1), but we propose an *acceptable* performance as a ROC-AUC of 0.90. This is a comparable accuracy to troponin T assays when they were first introduced into clinical medicine for acute myocardial infarction stratification [18]. In real clinical practice the biomarker's context of use, derived from prognostic qualification studies in man, may prioritize sensitivity or specificity and our *acceptable* criterion is a starting point for development.

If the biomarker could be assayed rapidly at point-of-care it could be used outside of standard hospital laboratories, for example, in an ambulance, phase 1 clinical trial unit or in the developing world. An *acceptable* level of performance would be a turnaround time of 60 min in a standard hospital laboratory, as per the guidance of the clinical biochemists/chemical pathologists and clinical biochemistry services regarding the measurement of commonly requested routine clinical biochemistry and haematology tests in emergency departments [19]. Ideally, the assay would be performed on a drop of whole blood obtained from the fingertip, resulting in a minimally invasive test with short sample preparation time. *Acceptable* would be measuring the biomarker in plasma or serum. We would *desire* the biomarker assay to be measurable in settings where resources are sparse, such as in developing countries, but *acceptable* would be the ability to perform the assay in standard hospital laboratories. Recently, data suggest that ALT can be measured using a robust point-of-care, finger stick test with a rapid turn around time [20]. Despite the drawbacks of ALT, this technology might provide the clinician with a signal that triggers an improved sensitive and specific liver safety assessment. Furthermore, advances in these technologies also point to a pathway of development and validation of such methodologies to assess potential biomarkers with improved characteristics at the point-of-care.

If the biomarker can report liver injury as early as 4 h after paracetamol ingestion, it would complement the paracetamol blood concentration nomogram for making treatment decisions in patients presenting early after overdose. *Acceptable* would be the ability to predict liver injury at 8 h after paracetamol ingestion, the time point after which NAC treatment loses efficacy [6, 21].

A biomarker that is fully translational between *in vitro* models, animals and humans would aid the detection of hepatotoxic compounds in drug development. However, a biomarker being translational between rodents and humans would be *acceptable*.

A biomarker with a high signal-to-noise ratio that only requires a single measurement is *desirable*. Serial measurement would be *acceptable*, as is the case with troponin for acute myocardial infarction [22].

Ideally, the biomarker could distinguish between a benign ALT rise, the development of serious liver injury or potential Hy's Law cases and imminent liver failure. *Acceptable* would be the prediction of an ALT rise. The ability of a biomarker to have a quantitative relationship with the severity of the paracetamol toxicity would allow for different treatment strategies in patients with different severities of liver injury. *Acceptable* would be a biomarker that qualitatively discriminates injury from non-injury.

Should a biomarker also be a mediator of liver injury then the marker could represent a companion diagnostic that identifies patients for a novel therapeutic. A *desirable* criterion would be a marker that has a drug already approved for human use, *acceptable* would be a marker that mediates the disease process and has potential therapeutics in development.

## New biomarker candidates for paracetamol-induced liver injury and their performance compared with the target biomarker profile

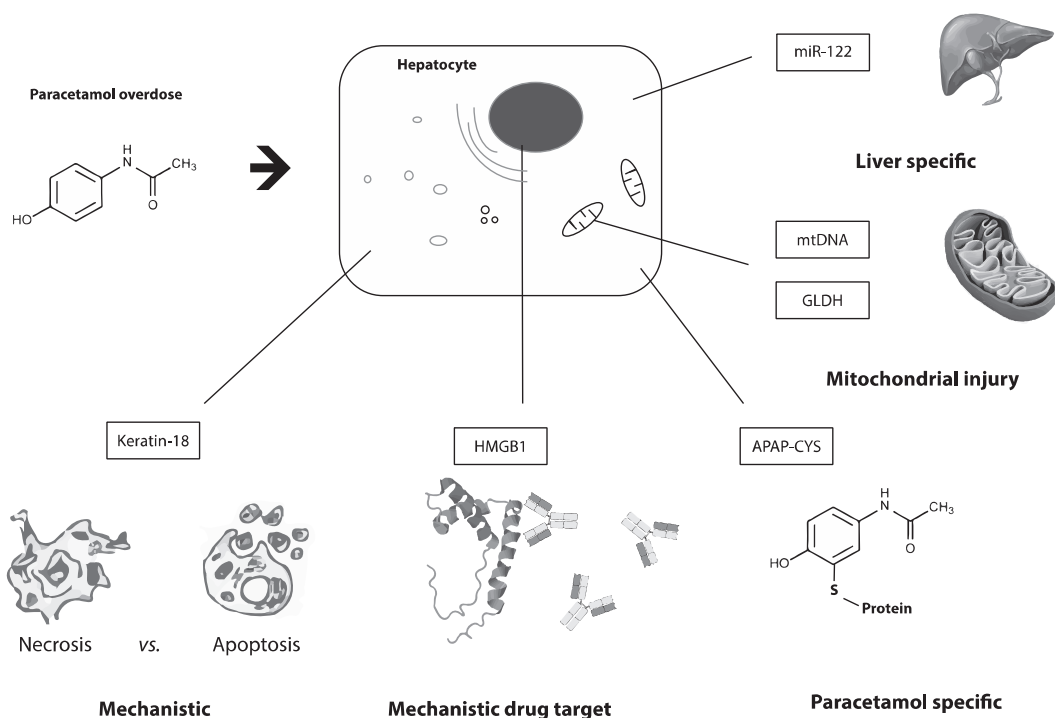
Currently there is huge investment into the development and qualification of novel biomarkers to improve the prediction and monitoring of drug-induced liver injury in preclinical species and in man (Figure 2). A number of public-private consortia exist such as the IMI funded SAFE-T (Safer And Faster Evidence-based Translation) project and the Critical Path institute supported PSTC (Predictive Safety Testing Consortium) with these objectives as specific goals. Although the efforts and achievements of these consortia have been previously reported, here we present the case for our TBP to improve treatment and to aid the understanding of the mechanistic basis of paracetamol overdose.

### Paracetamol protein adducts

Key properties:

- Conserved between rodent models and humans.
- Exclusively selective for paracetamol overdose
- Reflective of the initial molecular initiating event (MIE)

When NAPQI is formed during paracetamol metabolism it covalently binds with proteins forming paracetamol-protein adducts, of which cysteine adducts are the most common [23]. After the binding of NAPQI with cysteine, the structure of NAPQI reverts to that of paracetamol resulting in paracetamol-cysteine (APAP-CYS). In mouse models of paracetamol-induced ALI, immunohistochemical methods report that APAP-CYS is formed in the liver in a temporally progressive, central to peripheral pattern [24].

**Figure 2**

Key properties of novel biomarkers for paracetamol-induced liver injury

Experiments in mice also report APAP-CYS in serum, suggesting that injured liver cells release APAP-CYS into the circulation. Notably, serum APAP-CYS was only detectable after toxic doses of paracetamol [25].

More recently, a high performance liquid chromatography with electrochemical detection (HPLC-ECD) assay has been developed. This assay has been used to detect APAP-CYS in liver and serum after hepatotoxic dosing of paracetamol to mice [26] and in both adult and paediatric human serum samples with paracetamol-induced ALI [27–29]. Due to the relatively long plasma half time of  $1.7 \pm 0.3$  days in adults [30] and  $1.5 \pm 0.3$  days in children and adolescents [31], APAP-CYS can be detected up to 7 days after a large overdose [27]. By contrast, the plasma half-life for paracetamol is 1.5–2.5h [32]. The longer half-life of APAP-CYS could potentially allow risk assessment/diagnosis in patients who present when paracetamol has been cleared from the circulation. When the aetiology of ALI is known, APAP-CYS has no defined advantage over ALT because kinetic changes in APAP-CYS track ALT activity [27]. A point-of-care test is currently in development [33].

### microRNA-122 (miR-122)

Key properties:

- Specific for liver injury
- Fully conserved (translational) across *in vitro* models, *in vivo* models and humans
- Early marker for ALI with a ROC-AUC of  $>0.90$
- Predicts ALT rise

MicroRNAs (miRNAs) are small (~22 nucleotides long) non-protein coding RNA species involved in post-transcriptional gene product regulation. miRNAs are involved in a wide range of biological processes and the biogenesis and function of miRNAs have been comprehensively reviewed elsewhere [34, 35]. Outside the cell, miRNAs are very stable due to protection from degradation by RNases by microvesicles [36] and RNA binding protein complexes [37]. Although a number of miRNAs are widely expressed, certain miRNAs appear to be highly organ specific [38]. Liver tissue expresses a number of distinct miRNAs, especially miR-122, the most abundant hepatic miRNA that has very low to no expression in other healthy tissues, which makes this marker highly liver specific [38]. miR-122 is a multifunctional RNA species that modulates multiple pathways involved in stress response [39], fatty acid metabolism [40], cholesterol synthesis [41] and hepatocellular carcinoma [42].

The potential of circulating miRNAs to serve as biomarkers for ALI was first reported in mice treated with a toxic dose of paracetamol [43]. miR-122 was the miRNA species that had the largest fold change between control and paracetamol treated mice [43]. Subsequently circulating miR-122 has been reported as a marker for ALI in rats [44], dogs [45], zebrafish [46] and pigs [47].

*In patients with established ALI* circulating miR-122 is around 100-fold higher compared with healthy controls and overdose patients without ALI [48]. Furthermore, miR-122 has also been shown to provide utility at reporting liver injury in paediatric populations of

paracetamol overdose. *At first presentation to hospital*, in a UK cohort of 129 patients from Edinburgh and Newcastle-upon-Tyne, miR-122 was measured at a median of 8 h post-overdose in patients requiring subsequent NAC therapy (Edinburgh and Newcastle study) [49]. In this first sample miR-122 correlated significantly with peak hospital stay ALT activity and INR. miR-122 was significantly higher in those patients who developed subsequent ALI. ROC analysis revealed that miR-122 had an AUC value (sensitivity at 90% specificity) of 0.93 (0.83, 95% CI 0.86, 1.0,  $P < 0.0001$ ) suggesting that miR-122 could accurately separate patients with and without ALI at an early time when ALT activity was still normal.

### Keratin-18

Key properties:

- Conserved between rodent models and humans.
- Mechanism-based (apoptosis vs. necrosis)
- Early marker for ALI with a ROC-AUC of  $>0.90$
- Predicts ALT rise
- Prognostic marker

Keratins are intermediate filament proteins, expressed by epithelial cells, that are responsible for cell structure, differentiation, mitosis and apoptosis [50, 51]. Keratin 18 (K18) is abundantly expressed in the liver and other digestive epithelia cells [52]. During apoptosis, phosphorylation and cleavage of K18 results in cellular rearrangement. Full length K18 is passively released from necrotic cells whereas cleaved K18 fragments are released from apoptotic cells once membrane integrity is lost [53]. Circulating cleaved K18 (apoptosis) and full length K18 (necrosis) can be measured with ELISA to report apoptosis and necrosis [54, 55].

Cleaved K18 and full length K18 have been reported to be circulating mechanistic biomarkers for apoptosis and necrosis in mouse models of paracetamol-induced ALI [56, 57]. Full length and cleaved K18 were measured in a mixed UK and US cohort of paracetamol-induced ALI patients. They were increased in the circulation of paracetamol-induced ALI patients compared with paracetamol overdose patients without ALI and healthy controls.

*In patients with established ALI* full length and cleaved K18 were significantly higher in patients who subsequently reached the King's College Criteria (KCC) for liver transplantation. Further analysis revealed that the percentage of total circulating K18 derived from cleaved K18 (from apoptotic cells) was relatively lower in patients who reached the KCC compared with those who did not [57]. ROC analysis confirmed that full length K18 had a higher AUC than ALT for predicting patients who met KCC and for the outcome of liver transplant/death [57]. Another research group has recently confirmed the ability of circulating cleaved and full length K18 to report liver injury after paracetamol overdose [58]. In this study

both cleaved and full length K18 correlated with poor outcome (death or liver transplant).

*At first presentation to hospital*, in the Edinburgh and Newcastle first presentation study, cleaved and full length K18, measured in the first sample, correlated with peak ALT activity and INR during hospital stay. ROC analysis revealed that full length K18 had an AUC value (sensitivity at 90% specificity) of 0.94 (0.9, 95% CI 0.87, 1.0,  $P < 0.0001$ ) suggesting that this form of K18 could accurately separate patients with and without ALI at an early time when ALT activity was still normal. The performance of the cleaved form of K18 was less accurate with regard to reporting ALI at first presentation to hospital (AUC 0.77; sensitivity at 90% specificity 0.21) [49]. This is consistent with necrosis being more prominent than apoptosis in the pathophysiology of paracetamol-induced acute liver injury.

### High-mobility group box-1

Key properties:

- Prognostic marker
- Mechanism-based (DAMP – inflammatory mediator)
- Early marker for ALI with a ROC-AUC of  $>0.90$
- Predicts ALT rise
- Conserved between rodent models and humans
- Potential drug target

High-mobility group box-1 (HMGB1) is an evolutionary conserved chromatin-binding protein expressed in the nucleus of virtually all cells. HMGB1 is passively released into the extracellular space by cells that are undergoing necrosis and plays a key role in alerting the immune system to dying cells and thus works as a damage-associated molecular pattern (DAMP) molecule [59–61]. HMGB1 stimulates an immune response by activating toll-like receptors (TLR) and the receptor for advanced glycation end products (RAGE) [62–64]. Besides being passively released, HMGB1 is actively released as a cytokine in a hyper-acetylated form by various immune cells such as monocytes and macrophages after activation by inflammatory stimuli [65]. Whether the extracellular cytokine activity of HMGB1 functions as a chemo attractant or pro-inflammatory mediator depends on the redox state of three key cysteine residues [64, 66]. HMGB1 can be measured in the circulation and increased levels are related to increased disease activity in sepsis [67], pancreatitis [68] and rheumatoid arthritis [69]. In a mouse model of paracetamol toxicity circulating total and acetylated forms of HMGB1 displayed temporal kinetics that correlated with the onset of necrosis and inflammation respectively, confirming the potential of HMGB1 as an indicator of cell death processes [70]. HMGB1 is a potential mediator of paracetamol-induced hepatotoxicity. Anti-HMGB1 antibodies and knocking out HMGB1 in the liver reduced hepatic inflammation

and liver injury in mouse models of paracetamol poisoning [59, 71, 72]. Anti-HMGB1 antibodies are in development for the treatment of human disease.

*In patients with established ALI* total and acetylated HMGB1 was increased in the circulation compared with paracetamol overdose patients without ALI and healthy controls [57]. Acetylated HMGB1 was significantly increased in patients who reached KCC compared with patients who did not. ROC analysis demonstrated that HMGB1 (both total and acetylated) had a higher AUC than ALT for predicting patients who will reach KCC and predicting liver transplant/death [57].

*At first presentation to hospital* HMGB1 had an AUC value (sensitivity at 90% specificity) of 0.97 (0.91, 95% CI 0.91, 1.0,  $P < 0.0001$ ), suggesting that HMGB1 could accurately identify patients with ALI at the time when ALT activity was still normal [49]. Comparing ROC curves suggests that HMGB1 may be the most accurate biomarker at first presentation, but this needs to be tested in larger studies.

### Glutamate dehydrogenase

Key properties:

- Conserved between rodent models and humans.
- Predicts ALT rise

Glutamate dehydrogenase (GLDH) is a mitochondrial enzyme that catalyzes the reversible deamination of glutamate to  $\alpha$ -ketoglutarate plus free ammonia by using NAD or NADP as a co-factor [73]. Circulating GLDH has been suggested to be a specific mechanistic marker for mitochondrial damage. It was reported that mice treated with furosemide, a loop diuretic deemed to cause centrilobular liver necrosis without affecting mitochondrial function [74], produced a significant increase in serum ALT activity with only a non-significant increase in serum GLDH activity

[75]. By contrast, paracetamol treated mice had a substantial increase in GLDH along with ALT suggesting that the increase of circulating GLDH is indicative of paracetamol-induced mitochondrial damage and not simply caused by leakage of the enzyme from necrotic cells [75].

*In patients with established ALI* circulating GLDH is elevated. *At first presentation to hospital* GLDH is less accurate than miR-122, K18 and HMGB1 with regard to identifying patients with subsequent ALI despite NAC with an AUC value (sensitivity at 90% specificity) of 0.80 (0.19, 95% CI 0.68, 0.93,  $P = 0.0003$ ) [49].

### Mitochondrial DNA fragments

Circulating mitochondrial DNA (mtDNA) has been reported to act as a DAMP molecule via TLR mediated activation of inflammatory cells [76, 77]. Increased concentrations of circulating mtDNA have been associated with the systemic inflammatory response syndrome, multiple organ dysfunction syndrome and mortality in patients admitted to intensive care [78, 79].

*In patients with established ALI* circulating mtDNA was increased as measured by absolute quantification of mtDNA encoding NADH dehydrogenase and cytochrome c oxidase. The plasma concentration of mtDNA in patients with abnormal liver function increased over time and peak levels correlated with peak ALT [75]. As is the case with GLDH, circulating mtDNA may be a mechanistic marker for paracetamol-induced mitochondrial injury [75].

### Kidney injury molecule-1

Key properties:

- Prognostic
- Translational between humans and rodents

**Table 2**

Comparative biomarker profiles

Attribute	Desired	Acceptable
Specific for paracetamol overdose	APAP-CYS	miR-122, GLDH
Sensitivity for ruling out injury	–	miR-122, Keratin-18, HMGB1,
Rapidly assayed	–	GLDH, KIM-1
Feasibility of assay	GLDH?	GLDH
Invasiveness/sample preparation time	–	miR-122, Keratin-18, HMGB1, GLDH, mtDNA
Conserved (translational) across <i>in vitro</i> models, <i>in vivo</i> models and humans	miR-122	Keratin-18, HMGB1, GLDH, mtDNA, KIM-1
Time after overdose at which it is able to predict the onset of liver injury	–	miR-122, Keratin-18, HMGB1, GLDH
Signal to noise	miR-122, Keratin-18, APAP-CYS, HMGB1, GLDH, KIM-1	–
Quantitative relationship with disease severity	–	miR-122, HMGB1
Distinguish benign and clinical relevant increase in ALT	Keratin-18, HMGB1	miR-122, GLDH
Mediator of liver injury		HMGB1

- Point of care tested developed with rapid turn around
- Formally qualified by regulatory authorities for the investigation of drug-induced renal injury in preclinical drug development

Kidney injury molecule-1 (KIM-1) is a transmembrane glycoprotein that confers phagocytic activity on the proximal tubule cells of the kidney. During AKI, KIM-1 is rapidly up-regulated and its ecto-domain is shed into urine and blood where it is a sensitive and specific biomarker of acute kidney injury. Furthermore, KIM-1 has been formally qualified by regulatory authorities for its use to monitor acute kidney injury in the preclinical setting [80]. In patients with paracetamol overdose, secondary injury to the kidney and specifically the proximal tubule epithelia is a major determinate of mortality. Indeed, biomarkers such as serum creatinine are often incorporated into prognostic algorithms. However, serum creatinine is delayed in its onset and data from animal models and in humans have repeatedly demonstrated the ability of KIM-1 to increase earlier following acute kidney injury [81, 82]. *In patients with established ALI* circulating KIM-1 has been recently reported to be elevated, particularly in those patients who subsequently died or required a liver transplant compared with spontaneous survivors [83]. The fold change in KIM-1 in this poor prognostic group was higher than creatinine and KIM-1 outperformed creatinine in a ROC analysis. Furthermore, circulating KIM-1 was an independent predictor of outcome in a logistic regression model [83].

## Current biomarker performances and future challenges

Table 2 gives an overview of the characteristics of each marker. Each of our characteristics has at least one biomarker that meets the *acceptable* or *desired* specifications. However, all these biomarkers have only been measured in relatively small numbers of patients and large multicentre trials are required to qualify current findings and explore and confirm further the attributes of these biomarkers. These studies should confirm at which time after paracetamol overdose the biomarker is able to predict liver injury and the sensitivity for ruling out injury. These studies should also determine the signal-to-noise ratio of each marker, identify which markers (if any) can distinguish between benign and clinically relevant increases in ALT, and establish if there is a quantitative relationship between biomarker level and outcome.

There is considerable scope for improvement in the 'rapidly assayed' and 'feasibility of assay' characteristics in our TBP. At the time of writing, only the calorimetric assay for GLDH has been validated with these performance characteristics in mind. It can be automated in modern

clinical chemistry laboratories with a turnaround time of less than 1 h. All other biomarkers are typically measured manually in research laboratories with time consuming and expensive kits. There is an urgent need for standardized and validated commercial assays that can be used at point-of-care to stratify paracetamol overdose patients for entry into trials of new therapeutic approaches.

In order to have the driver for introducing one or more of these biomarkers into clinical practice their measurement must add value to patient care [84]. In the setting of paracetamol overdose, a normal test result might add value by giving the treating clinician more confidence in discharging a patient. Given the large number of patients, this could reduce the pressure on acute hospital beds and save the health provider money. Conversely, an abnormal test result might trigger entry into a stratified clinical trial, indicate need for different treatment with a new therapy or referral for specialist care.

In conclusion, our target biomarker profile could be useful for biomarker research and development in the settings of drug overdose. Studies to date demonstrate the utility of a number of mechanistic biomarkers that should be evaluated against our TBP in prospective studies. These attributes can be used to define the strengths and weaknesses of a new marker in development and can help in identifying potential clinical benefit.

## Competing Interests

All authors have completed the Unified Competing Interest form at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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