Biomarkers of paracetamol toxicity

Dr James Dear

Edinburgh University

Hospital Episode Statistics 2010-11

Poison	Emergency admissions (England)
Paracetamol	**38,464**
Antidepressants	16,180
NSAIDs	9,429
Opiates	9.135
Benzodiazepines	8,952

Hospital Episode Statistics 2010-11

Reason for admission	Emergency admissions (England)
Paracetamol	**38,464**
Fracture of neck of femur	42,616
Congestive heart failure	37,148
Acute myocardial infarction	26,967
Acute exacerbation of COPD	44,969

National Poisons Information Service UK

Toxbase use

UK		Republic of Ireland		Overseas	
Agent	Count (% of total)	Agent	Count (% of total)	Agent	Count (% of total)
Paracetamol•	88,846 (6.9%)	Paracetamol•	1,595 (6.2%)	Paracetamol•	3,168 (3.6%)
Ibuprofen	44,933 (3.5%)	Zopiclone	677 (2.6%)	Ibuprofen	1,314 (1.5%)
Citalopram	27,091 (2.1%)	Diazepam	666 (2.5%)	Diazepam	1,057 (1.2%)
Diazepam	24,779 (1.9%)	Ibuprofen	591 (2.3%)	Quetiapine	1,000 (1.1%)
Salicylates‡	23,625 (1.8%)	Escitalopram	474 (1.85)	Salicylates‡	976 (1.1%)
Compound analgesics†	21,114 (1.6%)	Quetiapine	462 (1.8%)	Zopiclone	932 (1.1%)
Zopiclone	19,867 (1.5%)	Venlafaxine	458 (1.8%)	Venlafaxine	829 (0.9%)
Tramadol	17,396 (1.4%)	Salicylates‡	444 (1.7%)	Amitriptyline	794 (0.9%)
Codeine	16,062 (1.3%)	Alprazolam	434 (1.7%)	Escitalopram	764 (0.9%)
Fluoxetine	15,935 (1.3%)	Compound analgesics†	400 (1.5%)	Alprazolam	686 (0.8%)

• Does not include compound analgesics

† Containing paracetamol and codeine only

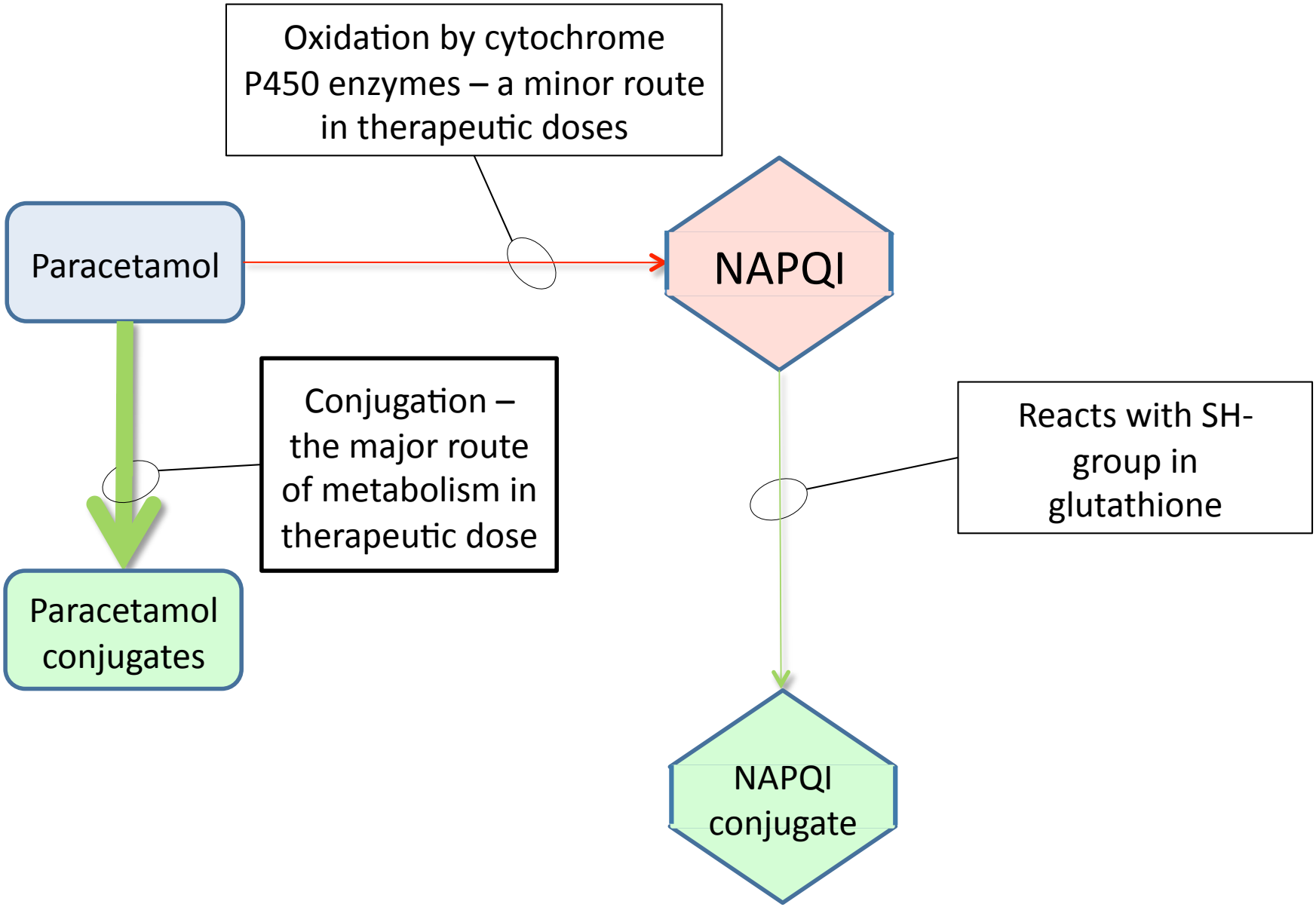
‡ Includes aspirin

National Poisons Information Service UK

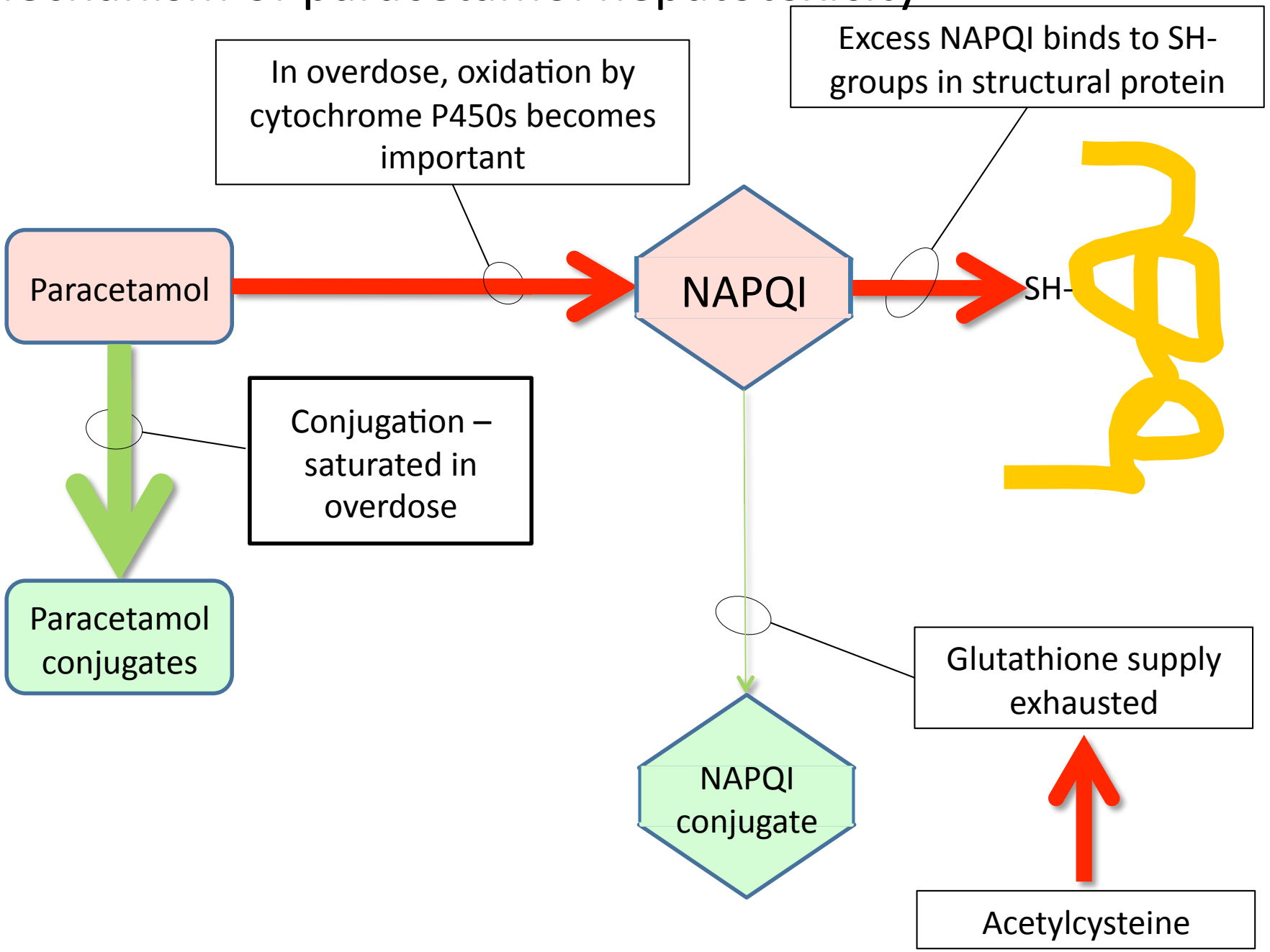
Telephone enquiries

Telephone enquiries	
Agent	**Number of enquiries**
Paracetamol•	5,422
Ibuprofen	2,297
Cocodamol†	1,368
Citalopram	1,062
Diazepam	930
Zopiclone	911
Quetiapine	641
Fluoxetine	639
Tramadol	623
Salicylates‡	591
• Does not include compound analgesics	
† Containing paracetamol and codeine only	
‡ Includes aspirin	

Mechanism of paracetamol hepatotoxicity

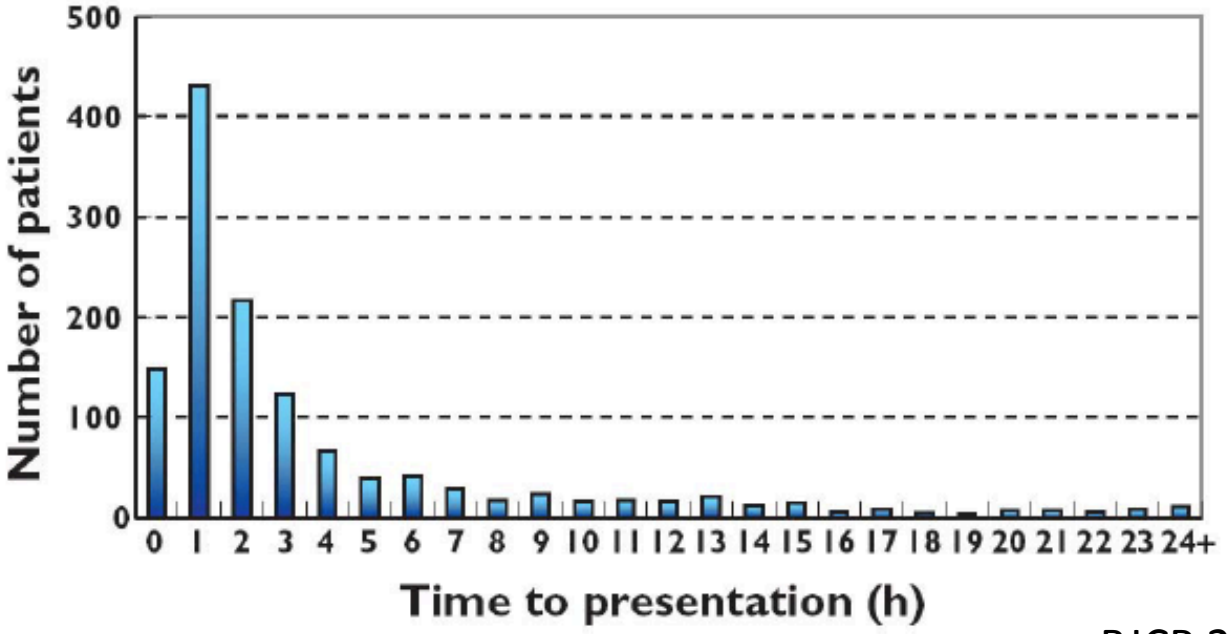


Mechanism of paracetamol hepatotoxicity



Risk assessment

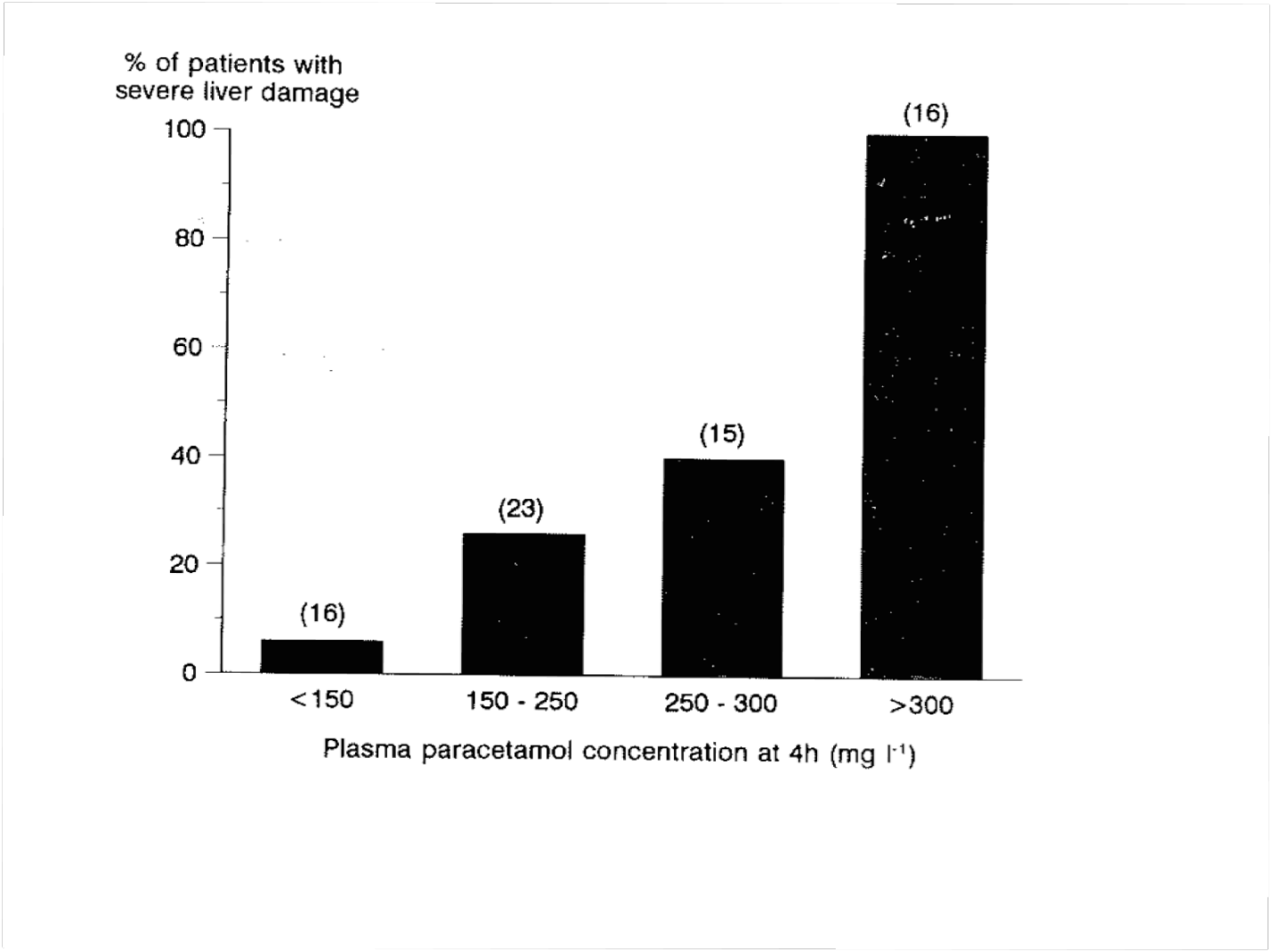
- Majority of patients present soon after OD before liver injury can be diagnosed using current tests such as ALT



BJCP 2009 68 260 - 268

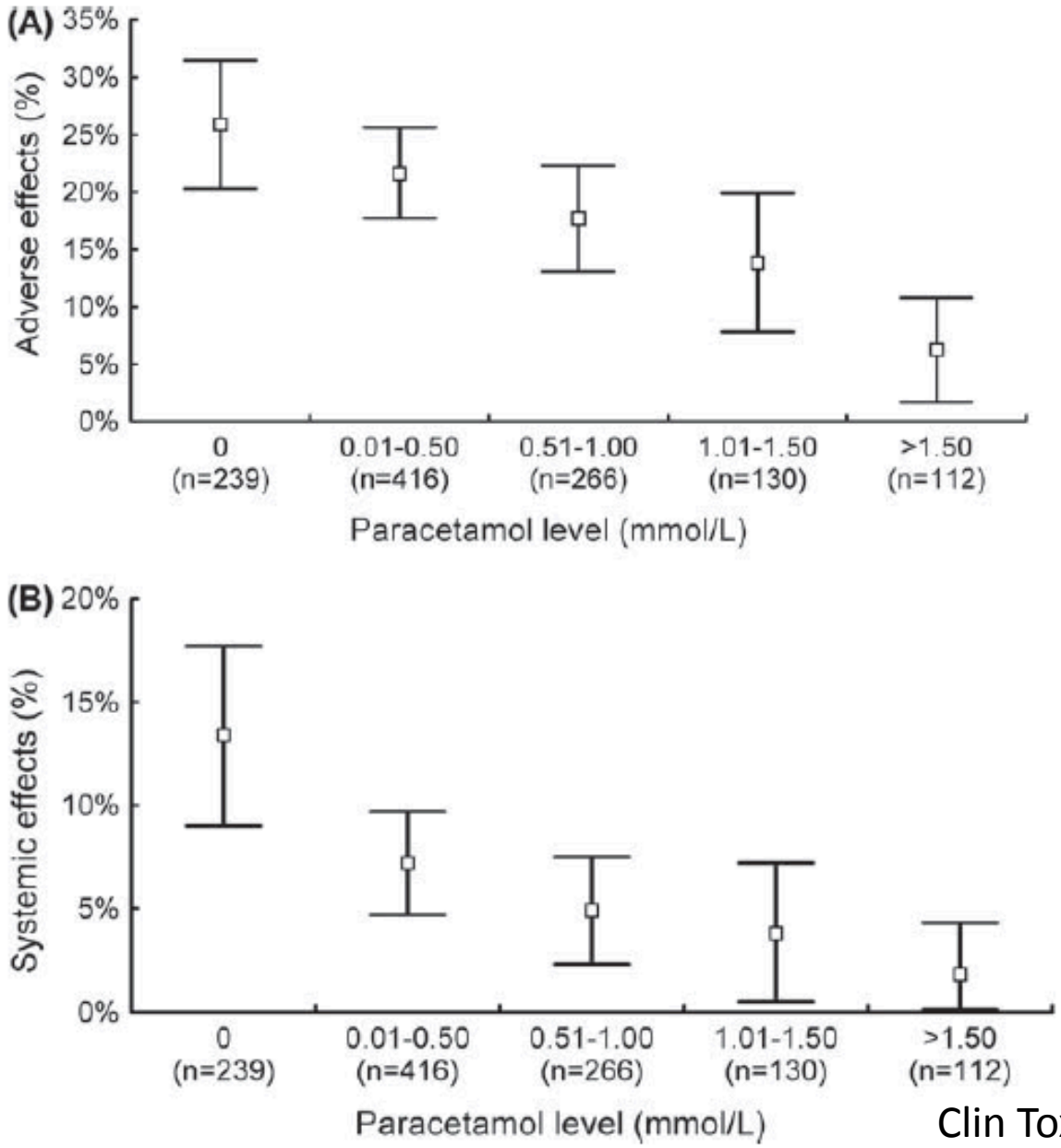
- Therefore, use surrogate marker
- **Blood paracetamol concentration**

Risk assessment – paracetamol concentration



Prescott LF, Health Bulletin 1978, 204-212

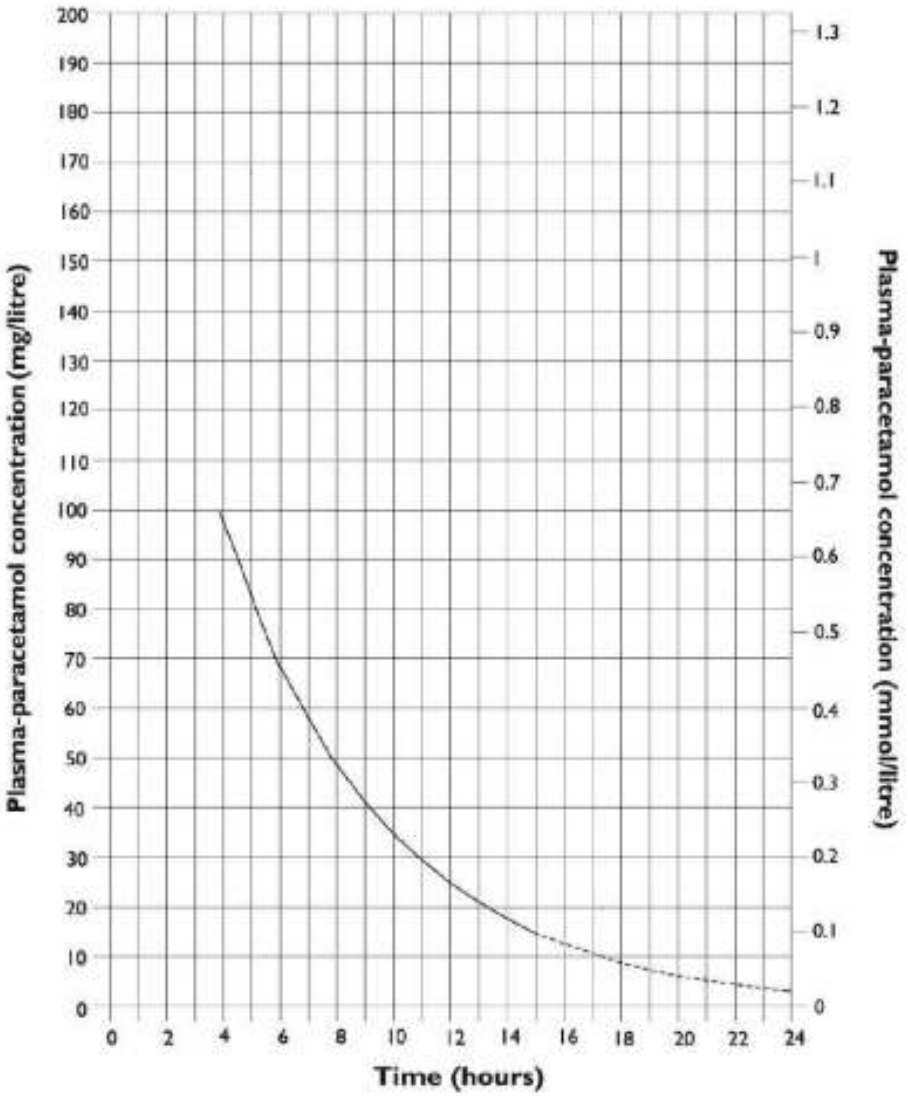
ADRs to acetylcysteine and paracetamol level at presentation



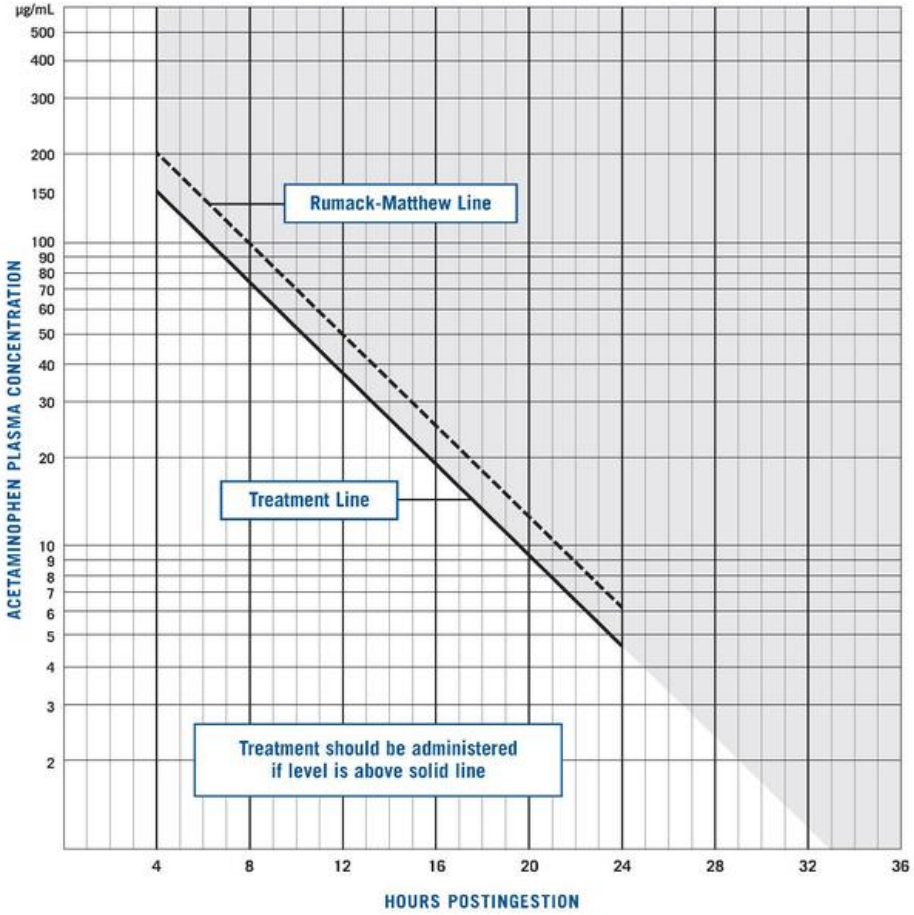
Clin Tox 2013 51 467-472

Risk assessment – paracetamol concentration

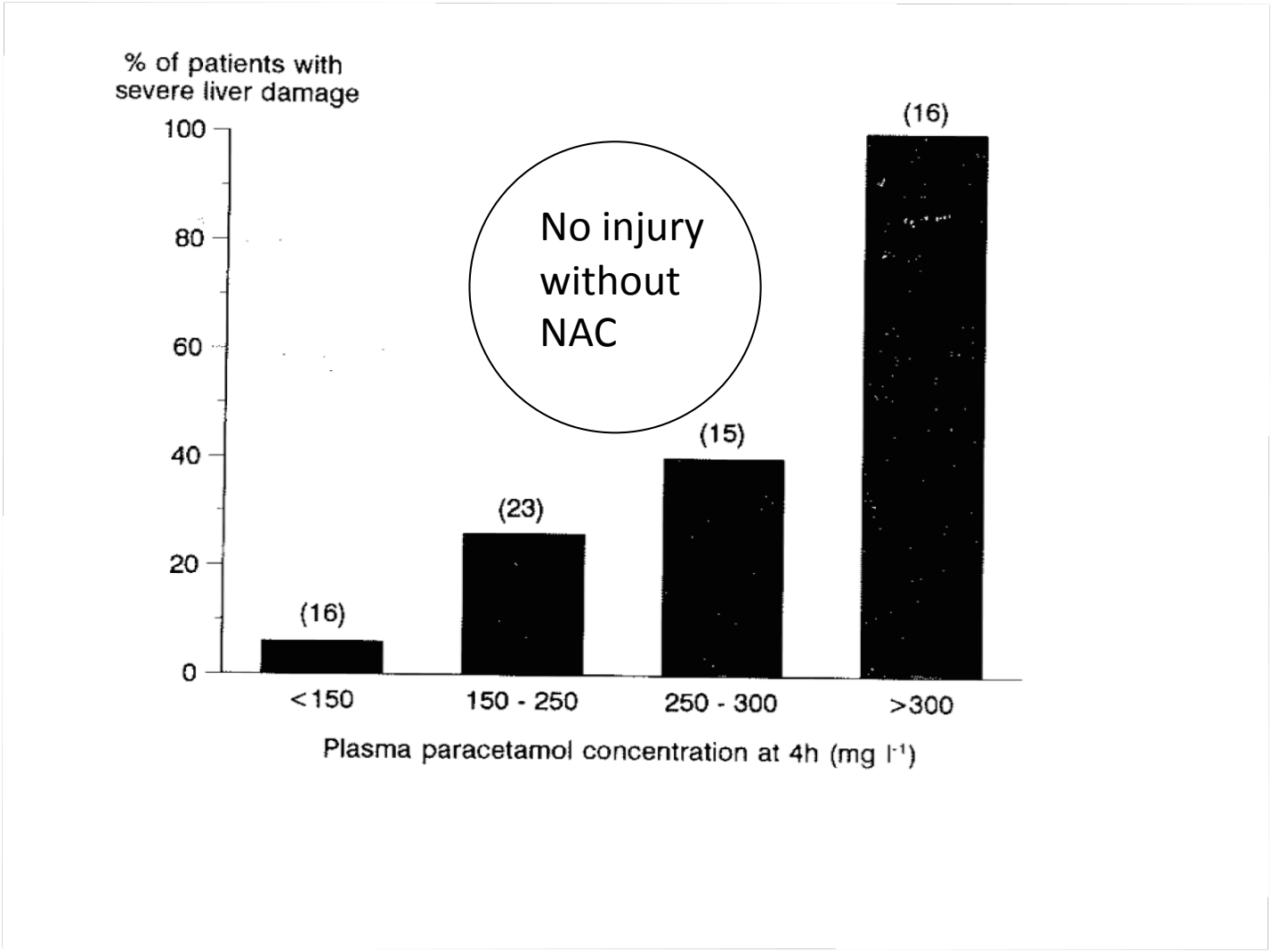
UK



USA



Risk assessment – paracetamol concentration



Prescott LF, Health Bulletin 1978, 204-212

What is a biomarker?

- Biomarker. A biomarker is a biological characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic response to therapeutic intervention.

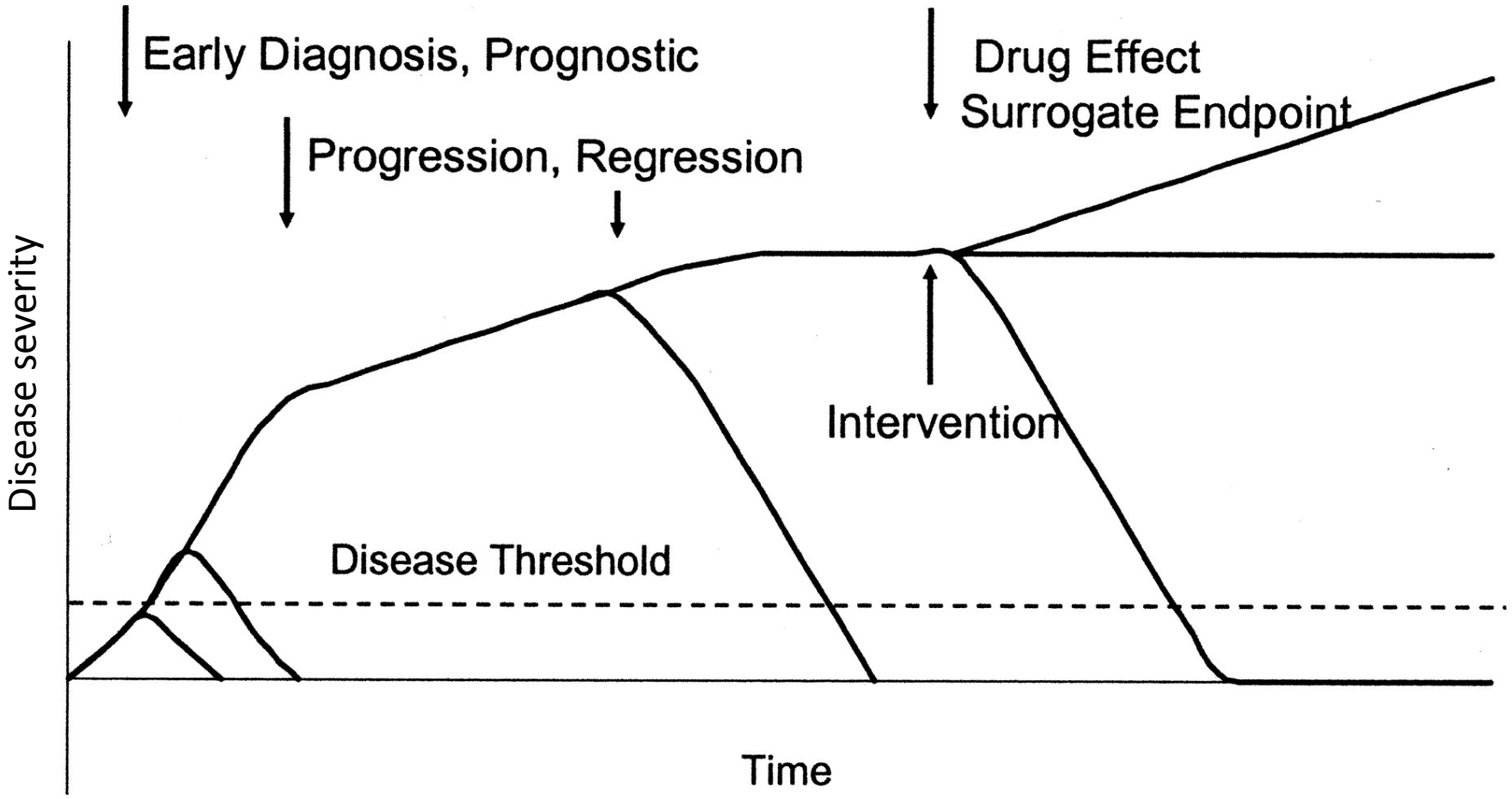
Pregnancy test

- Perfect biomarker!
- Positive result has a significant impact – adds real value
- Highly sensitive/specific for early diagnosis – accurate (in context)
- Detects a urine protein not normally expressed – accessible tissue
- Urine concentration not an issue – no normalization needed
- Simple accurate test – measurable
- Rapid result – appropriate turnaround time
- Widely available – used by those who need it

Paracetamol poisoning

- Good model for biomarker discovery in humans as:
- Young patients
- Often no co-morbidity
- Clearly defined insult
- No symptoms (at first)
- Loads of patients
- Neglected from research viewpoint

Uses of biomarkers



JASN 2004;15:1677-1689

Early, diagnostic markers…….

Heart attacks…..

Presenting complaint

Risk stratify

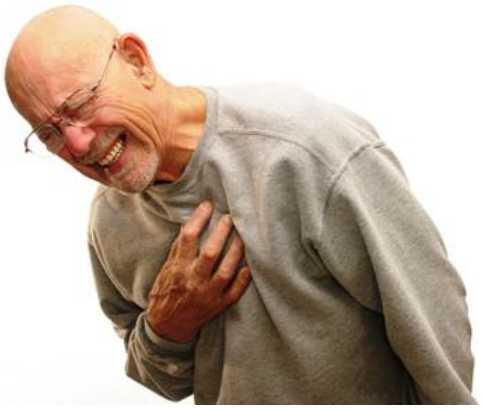
Detect cell death

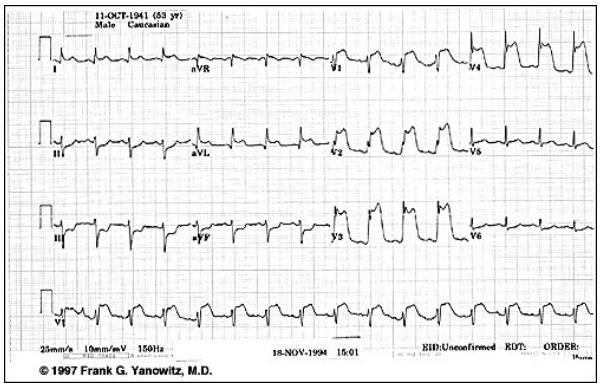
time

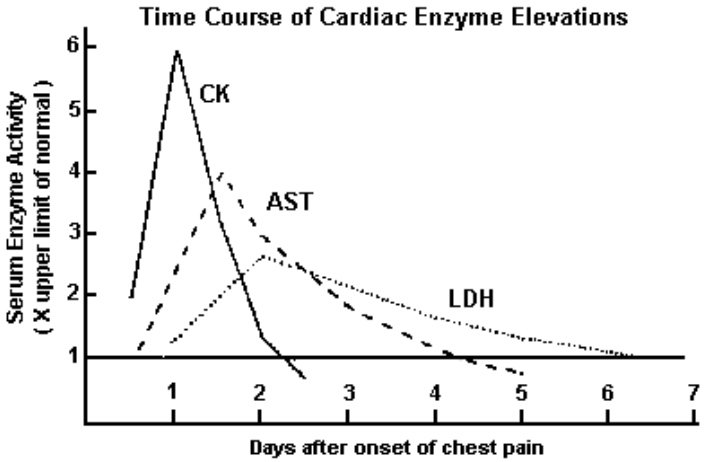
Chest pain

ECG

Cardiac enzymes
CK,AST,LDH

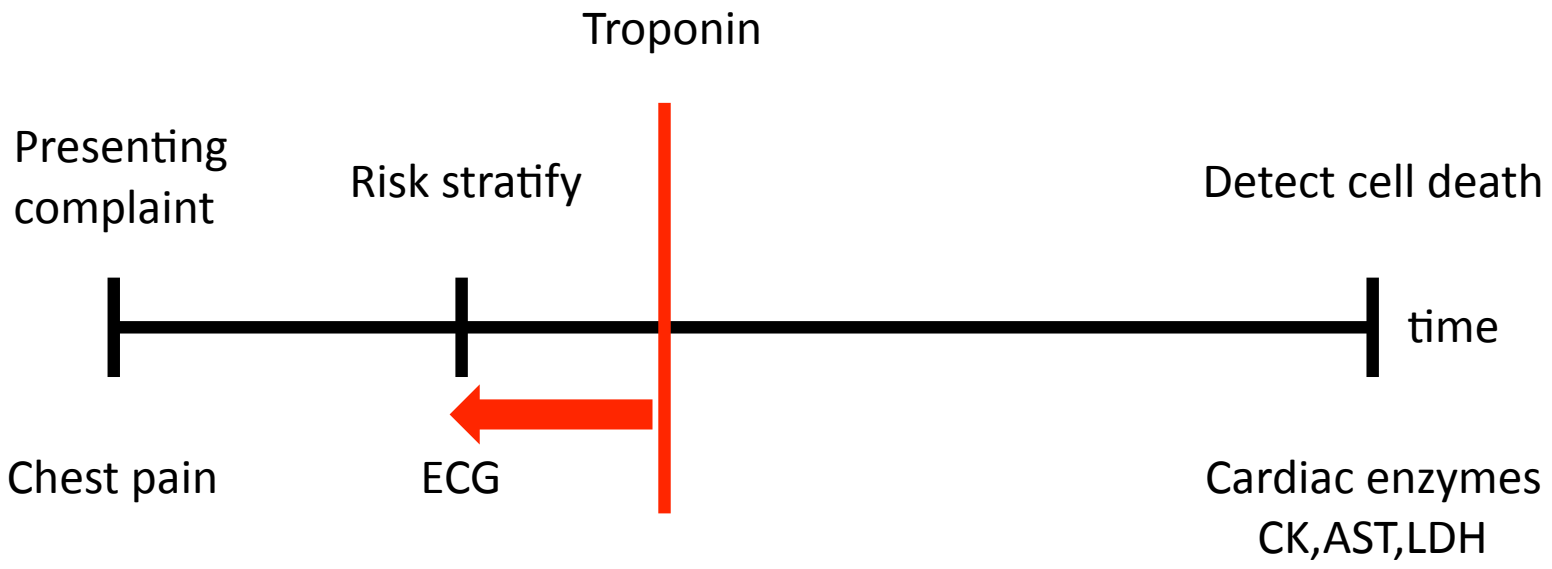


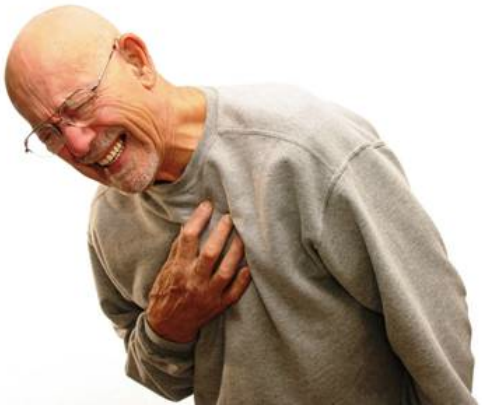


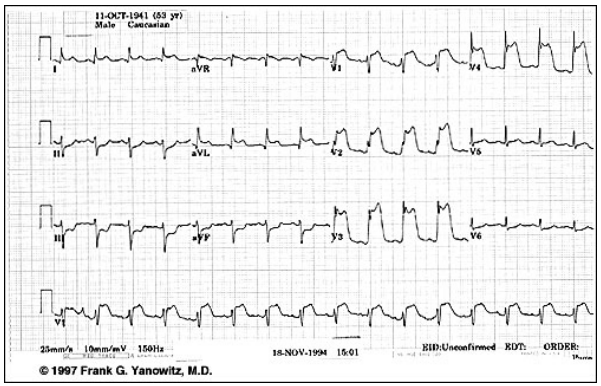


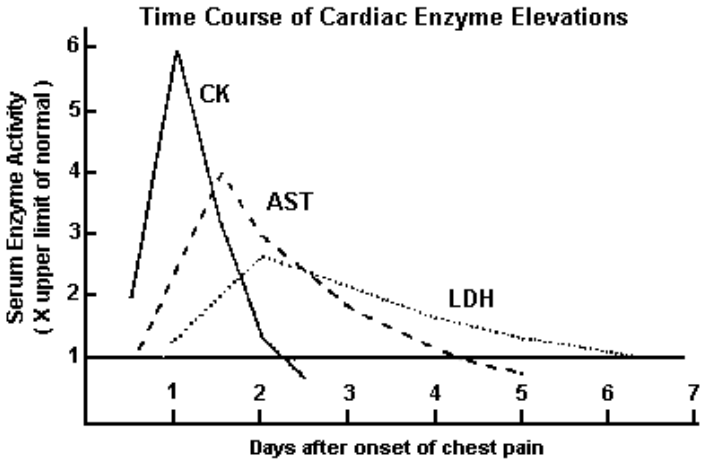
Early, diagnostic markers…….

Heart attacks…..









Early, diagnostic markers…….

Paracetamol OD…..

Presenting complaint

Risk stratify

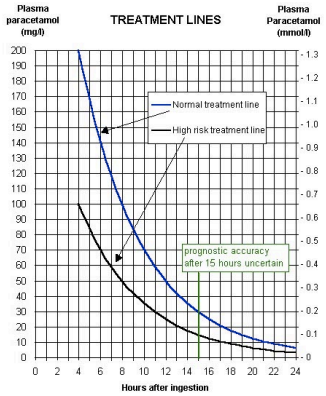
Detect cell death

time

History of overdose

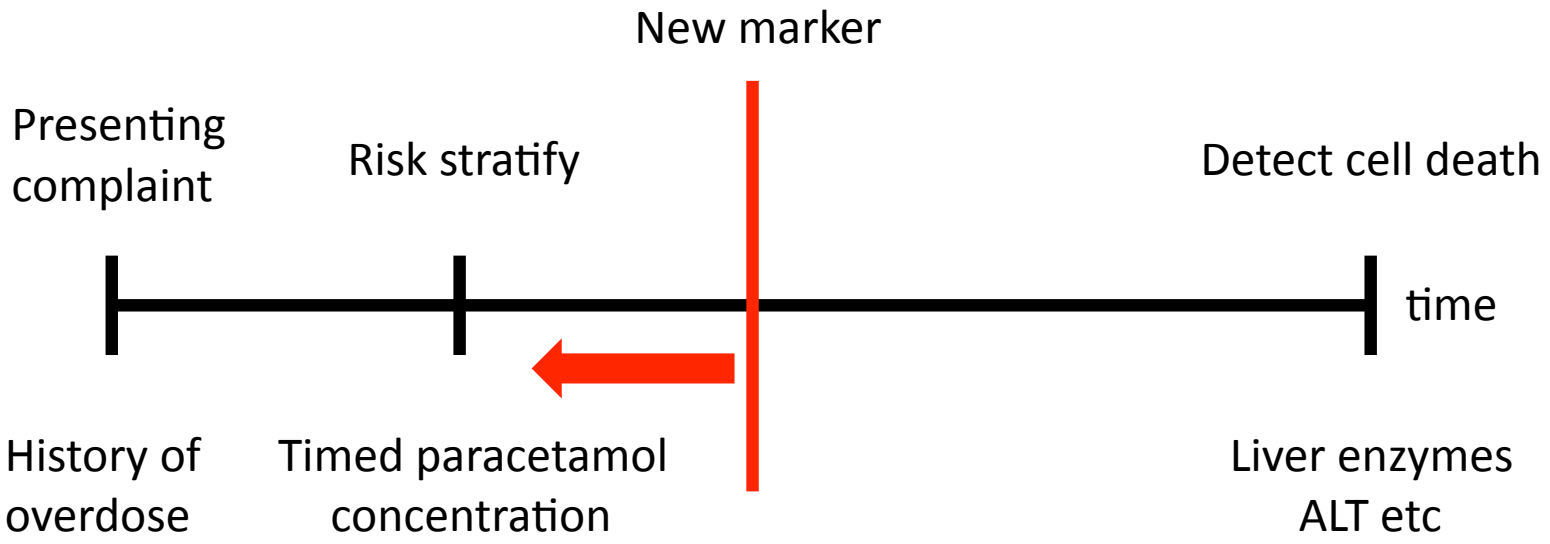
Timed paracetamol concentration

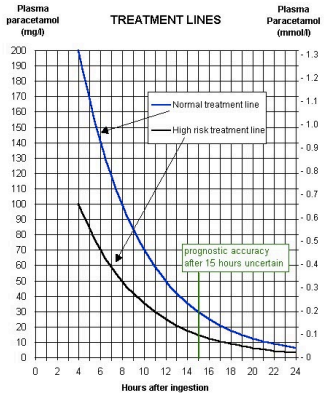
Liver enzymes ALT etc



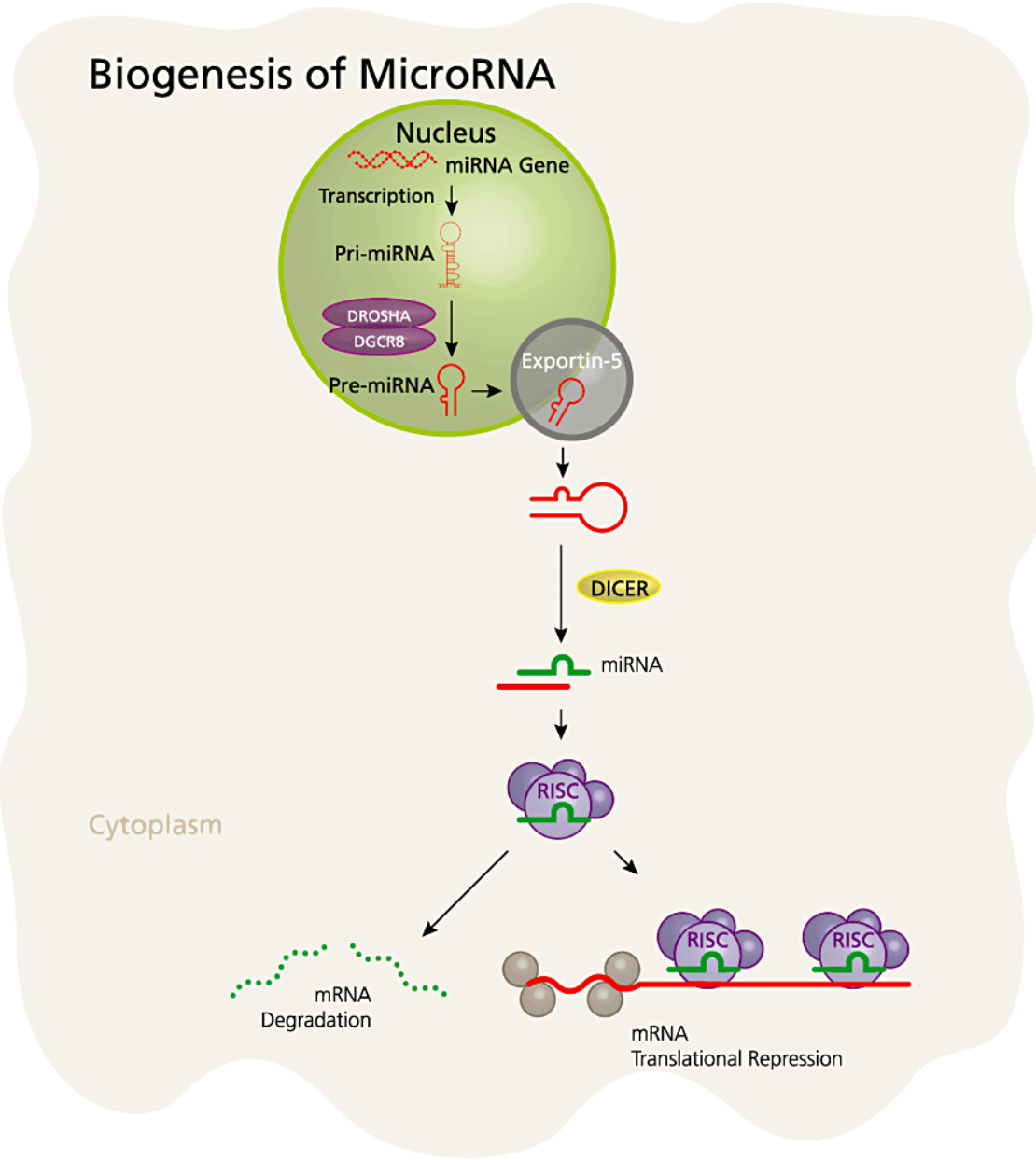
Early, diagnostic markers…….

Paracetamol OD…..

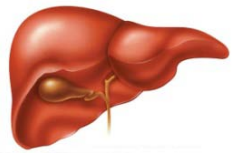




MicroRNA - 122

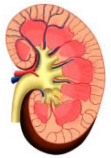

Biogenesis of MicroRNA
Nucleus
miRNA Gene
Transcription
Pri-miRNA
DROSHA
DGCR8
Pre-miRNA
Exportin-5
DICER
miRNA
RISC
Cytoplasm
mRNA
Degradation
RISC
RISC
mRNA
Translational Repression

Circulating microRNAs as translational biomarkers



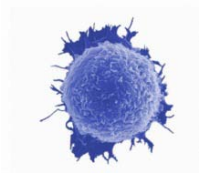
miR-122
miR-192

miR-1
miR-21
miR-133a



miR-218
miR-146a
miR-155

miR-218
miR-219
miR-709



Prostate – miR-629,650

HCC – miR-15b, 130b

Kidney – miR-378 (↑), 451 (↓)

- Tissue specific
- Sensitive
- Early indicator of outcome
- Translational

Circulating microRNAs, potential biomarkers for drug-induced liver injury

PNAS

Kai Wang[1], Shile Zhang, Bruz Marzolf, Pamela Troisch, Amy Brightman, Zhiyuan Hu, Leroy E. Hood[1], and David J. Galas

Circulating MicroRNAs as Potential Markers of Human Drug-Induced Liver Injury

Philip J. Starkey Lewis,[1*] James Dear,[2*] Vivien Platt,[1] Kenneth J. Simpson,[3] Darren G.N. Craig,[3] Daniel J. Antoine,[1] Neil S. French,[1] Neeraj Dhaun,[4] David J. Webb,[4] Eithne M. Costello,[5] John P. Neoptolemos,[5] Jonathan Moggs,[6†] Chris E. Goldring,[1†] and B. Kevin Park[1†]

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Wang *et al*, 2009, Mitchell et al, 2007, Redova *et al,* 2012, Liu *et al*,

Circulating microRNAs, potential biomarkers for drug-induced liver injury

Kai Wang[1], Shile Zhang, Bruz Marzolf, Pamela Troisch, Amy Brightman, Zhiyuan Hu, Leroy E. Hood[1], and David J. Galas

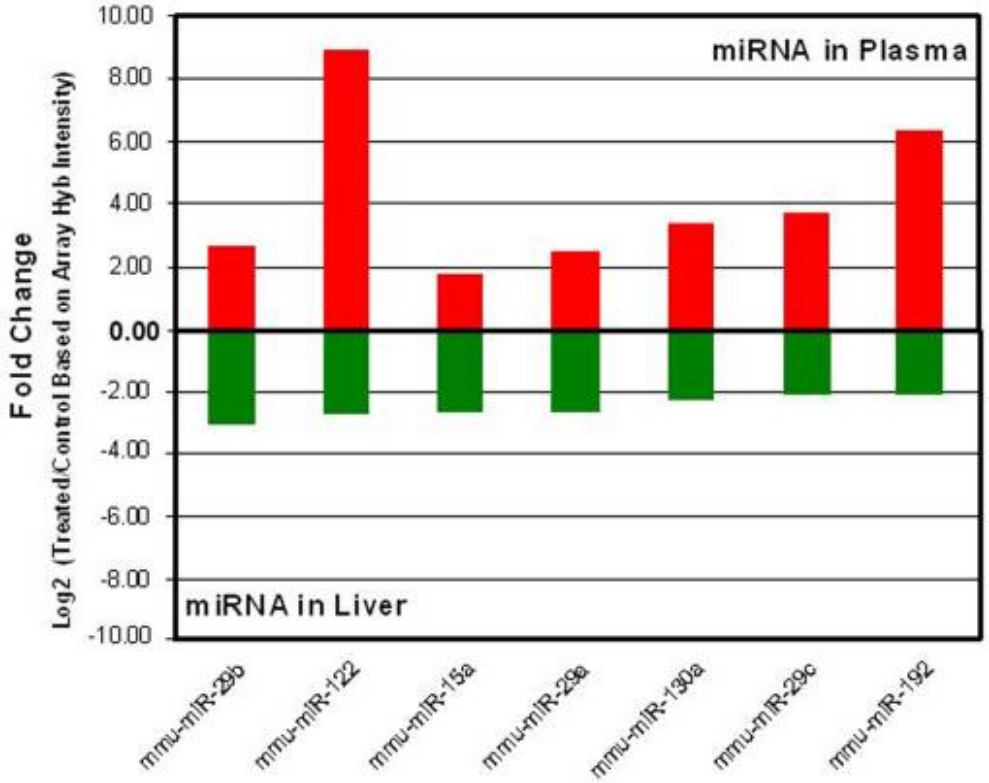
Institute for Systems Biology, 1441 North 34th Street, Seattle, WA 98103

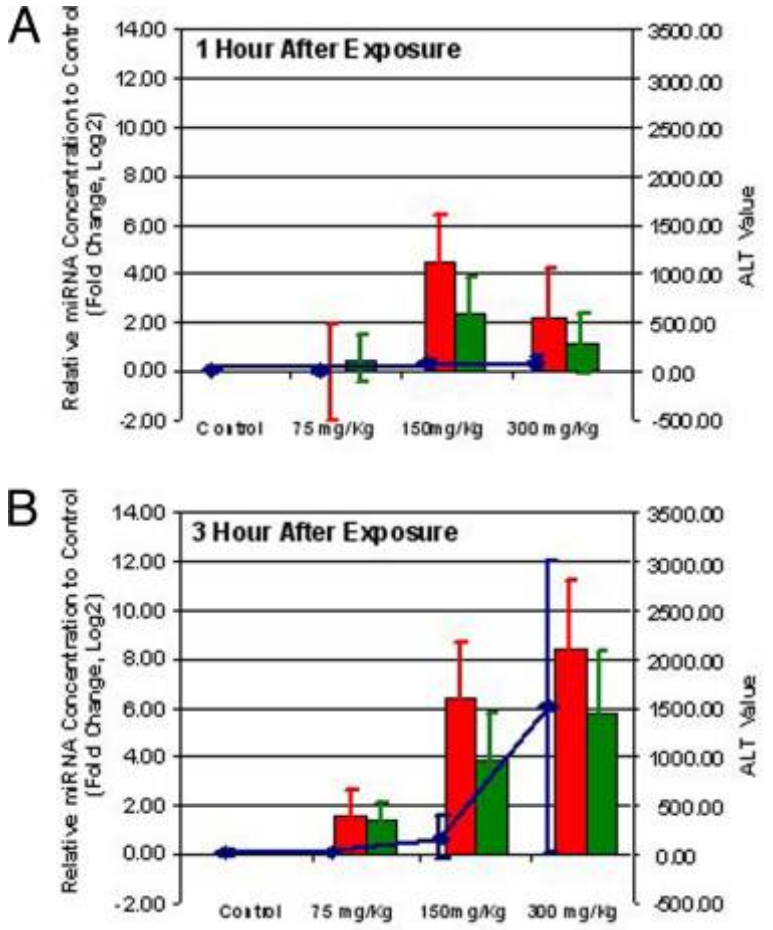
Contributed by Leroy E. Hood, January 16, 2009 (sent for review December 9, 2008)

Drug-induced liver injury is a frequent side effect of many drugs, constitutes a significant threat to patient health and has an enormous economic impact on health care expenditures. Numerous efforts have been made to identify reliable and predictive markers to detect the early signs of drug-induced injury to the liver, one of the most vulnerable organs in the body. These studies have, however, not delivered any more informative candidates than the serum aminotransferase markers that have been available for ~30 years. Using acetaminophen overdose-induced liver injury in the mouse as a model system, we have observed highly significant differences in the spectrum and levels of microRNAs in both liver

injury if it is promptly diagnosed and NAC is administered within 8–10 h after the initial ingestion (11). However, acetaminophen overdose usually produces either no immediate symptoms or nonspecific intestinal irritation during the first 24 h after ingestion, followed by the onset of liver failure. Thus, the need for early and accurate blood-based diagnosis for acetaminophen overdose is acute.

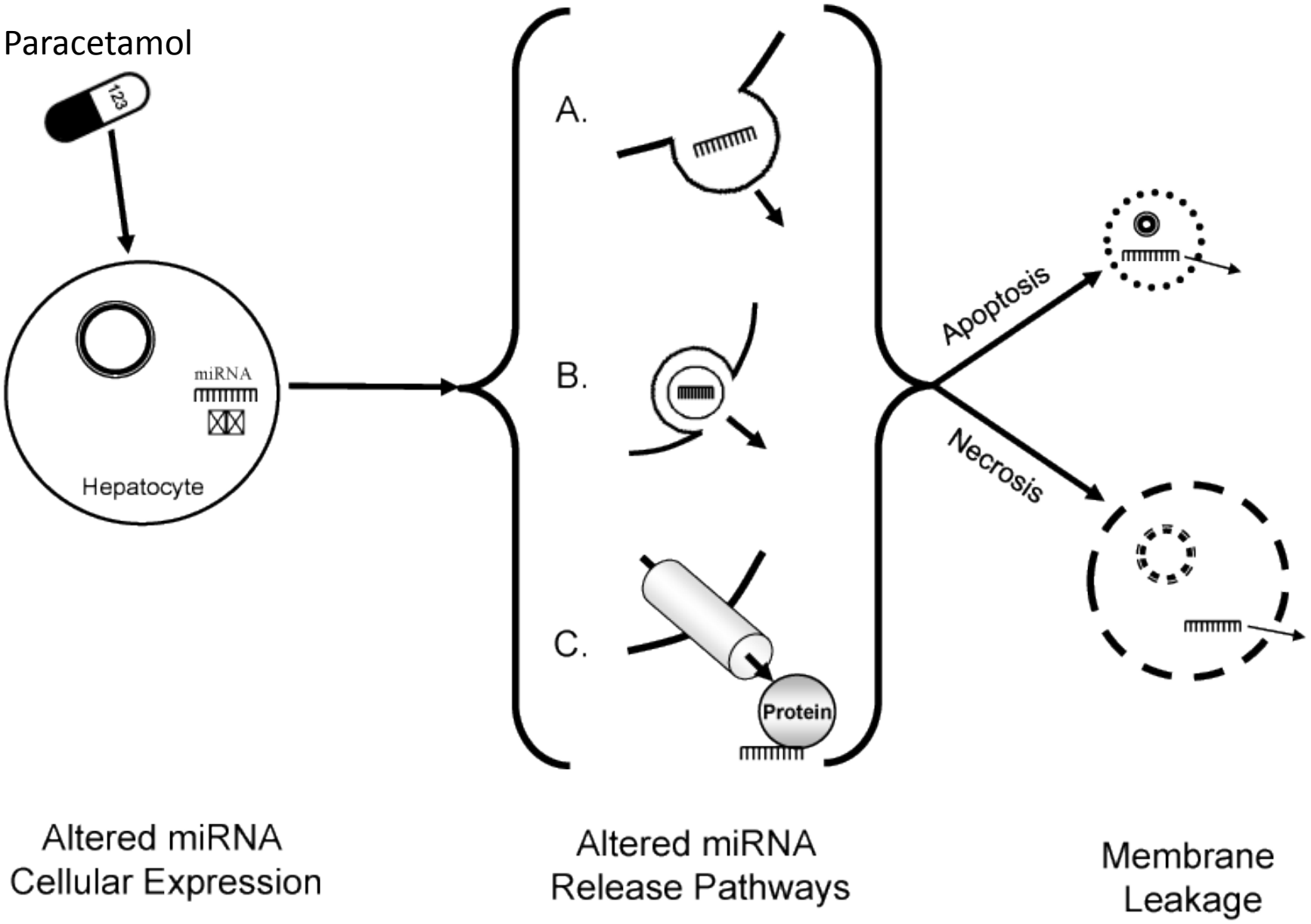
The most commonly used diagnostic test for acetaminophen overdose is to determine the activity of certain hepatocellular enzymes, aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT), in the blood (12).





Proc Natl Acad Sci U S A. 2009 106:4402-7.

How paracetamol could increase microRNA in circulation



Human Studies

Study 1. Patient groups

1. APAP – induced acute liver injury. Defined as a sudden deterioration in liver function with associated coagulopathy in the absence of a history of chronic liver disease. Clear history of excess APAP ingestion. n=53.

2. APAP – no liver injury. Single APAP ingestion in overdose that required treatment with acetylcysteine. Absence of liver injury was confirmed by a normal serum ALT activity (defined as ≤3 x ULN). n=6.

3. Non APAP acute liver injury. Causes: HBV, HCV, AFLP, AIH, DILI, Ischemia, Malignancy. n=11.

4. Healthy controls. Age and sex matched with APAP-ALI group. n=25.

5. Chronic kidney disease. Mean urinary protein excretion rate was 1570 ± 371 µg/min. Mean GFR of 43 ± 5 ml/min/1.73m^2. n=22.

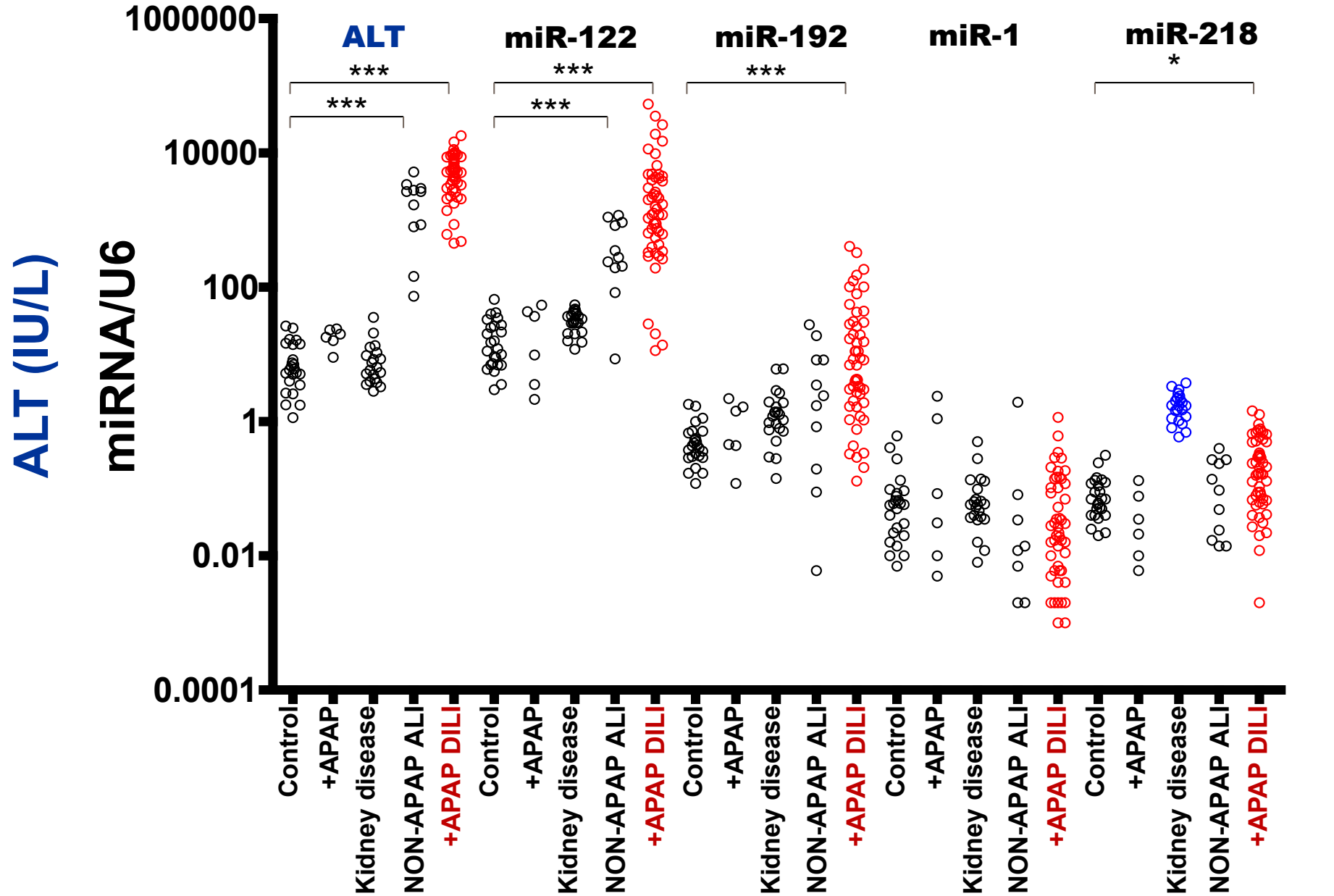
MicroRNA species

- miR-122 Liver enriched *

* Increased in mice

- miR-192 Liver enriched (also kidney) *
- miR-1 Heart enriched
- miR-218 Brain enriched
- All normalized to U6 snRNA
- Day 1 = day of entry into study NOT day of ingestion

miRNAs in paracetamol-overdose patients



Hepatology. 2011 54:1767-76

Study 2. Method

- Patients (total N=129) were recruited from the Royal Infirmary of Edinburgh (N=107) and the Royal Victoria Infirmary, Newcastle-Upon-Tyne (N=22).

- Inclusion criteria were: adults with a clear history of a single excess paracetamol ingestion and a timed blood paracetamol concentration that was judged to necessitate hospital admission for intravenous acetylcysteine therapy, as per UK guidelines at the time of study

- Exclusion criteria were: patients detained under the Mental Health Act; patients with permanent cognitive impairment; patients with a life-threatening illness; unreliable history of paracetamol overdose; patients who take anticoagulants therapeutically or have taken an overdose of anticoagulants; and patients who, in the opinion of the responsible clinician/nurse, were unlikely to complete the full course of acetylcysteine.

- All patients completed the full course of acetylcysteine.

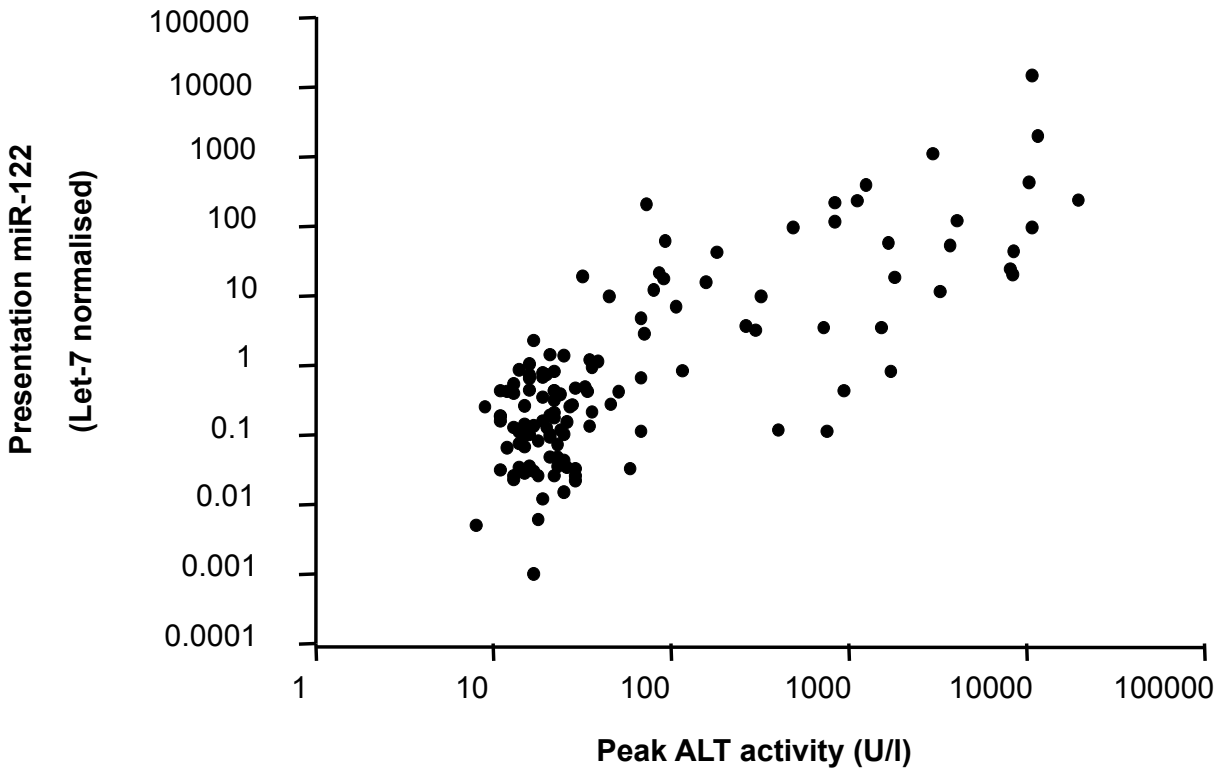
Method

miR-122 was measured in plasma at first presentation to hospital before acetylcysteine started

Primary outcome:

Acute liver injury - peak serum ALT activity greater than 3x the upper limit of normal (>150IU/L)

Presentation biomarkers vs **peak** ALT

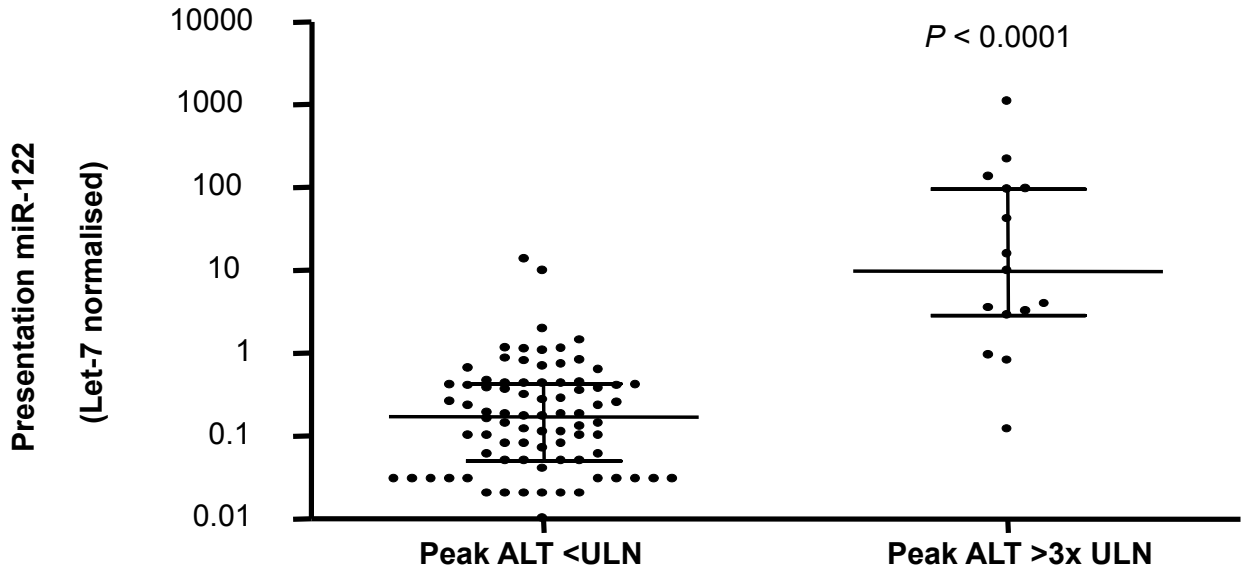


Biomarker	R^2	Pearson R (95% CI)	*P*
miR-122	0.14	0.37 (0.21-0.52)	<0.0001

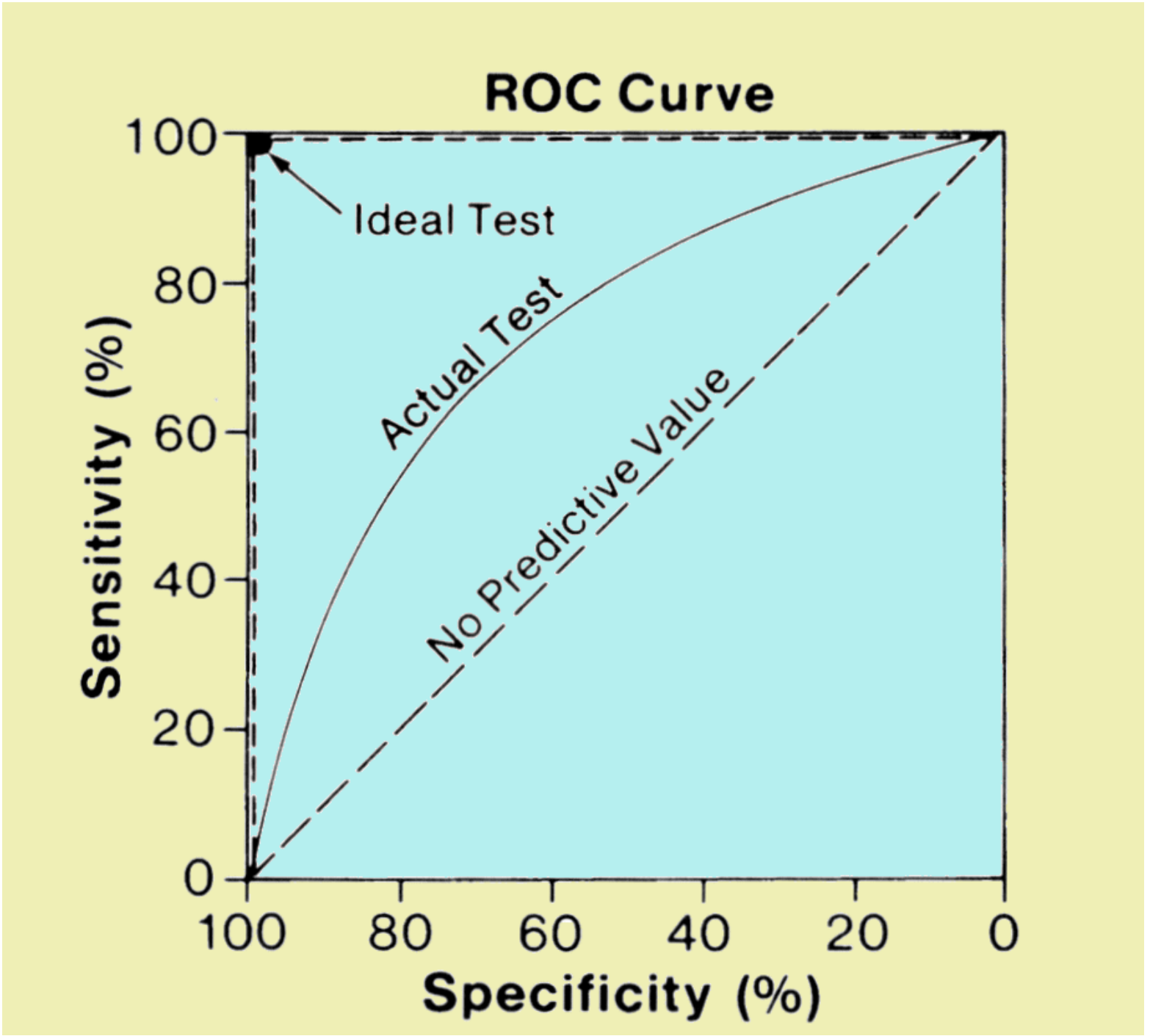
Hepatology. 2013 58:777-787

miR-122 at presentation was elevated in patients who develop ALI

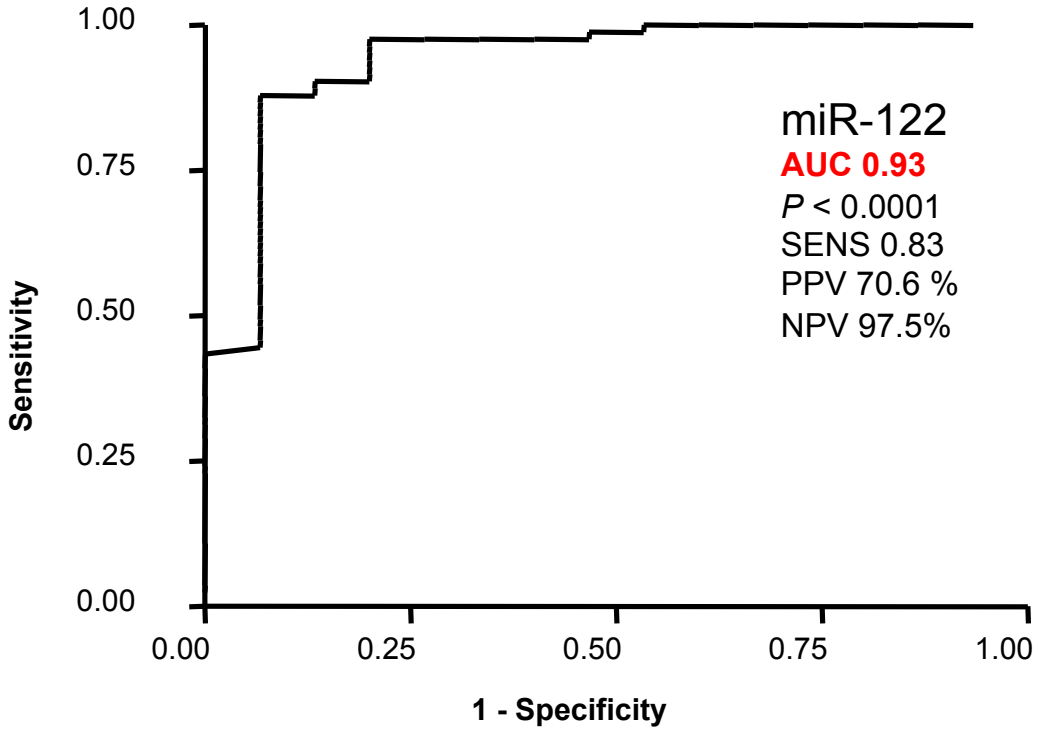
In patients with a **normal ALT**



Hepatology. 2013 58:777-787

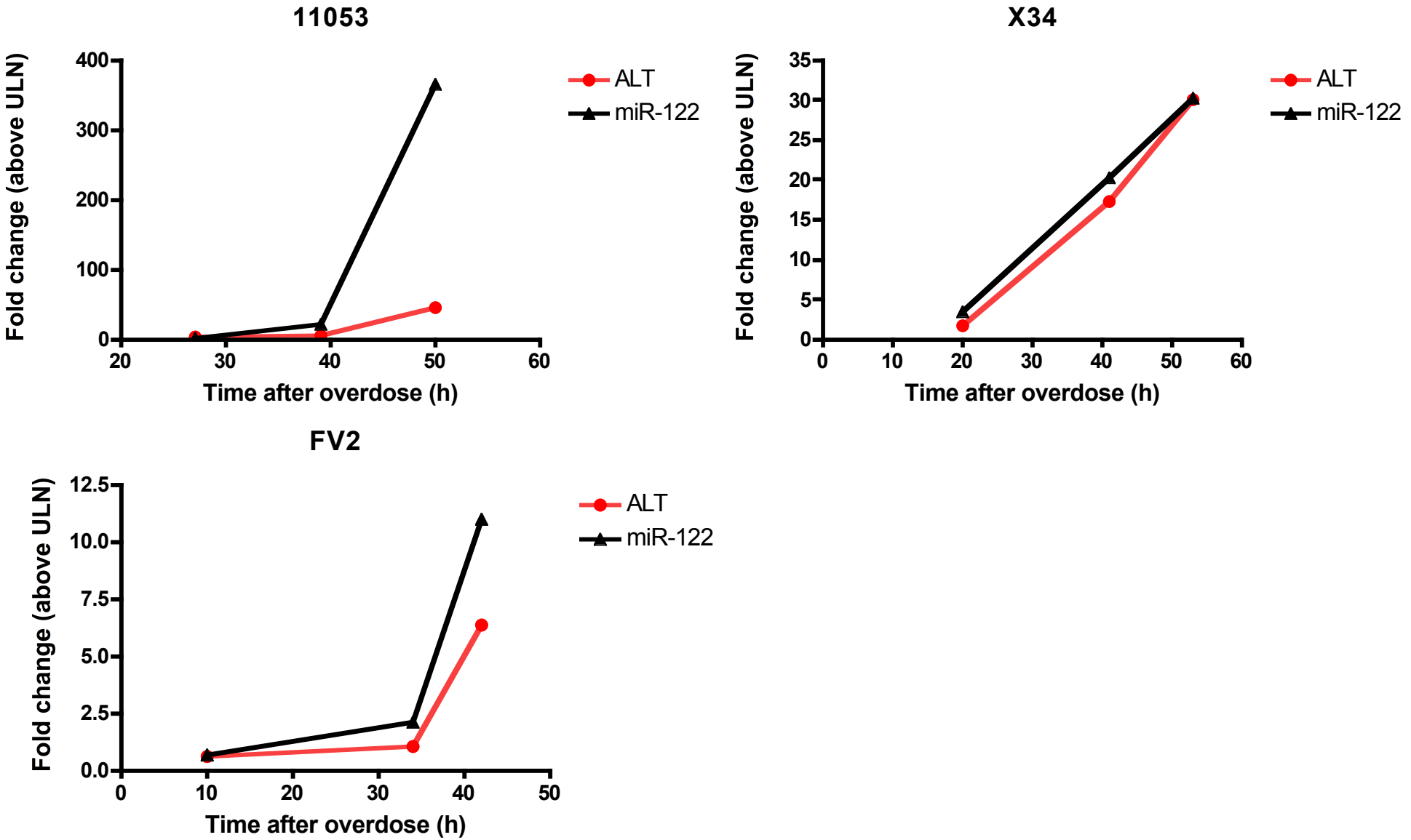

ROC Curve
100
80
60
40
20
0
Sensitivity (%)
Ideal Test
Actual Test
No Predictive Value
100
80
60
40
20
0
Specificity (%)

Performance of first presentation miR-122 at predicting acute liver injury
In patients with a **normal ALT**

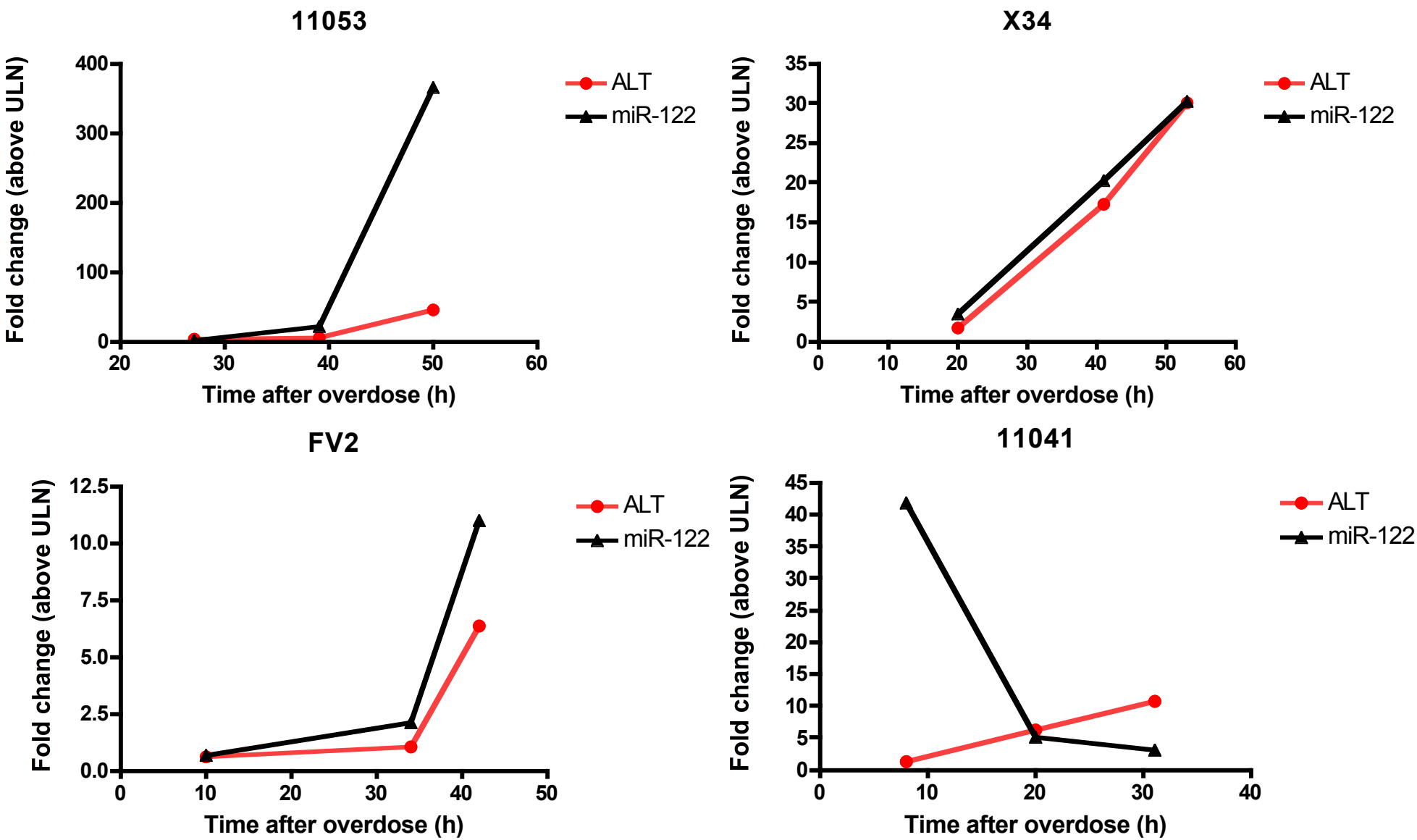


Hepatology. 2013 58:777-787

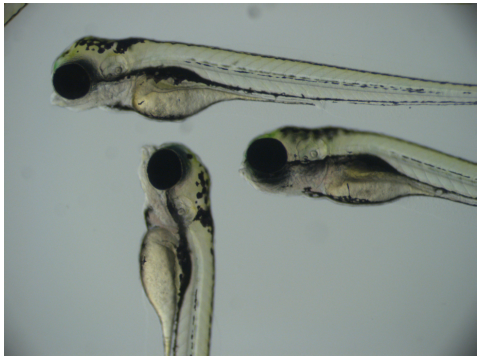
Time course of miR-122

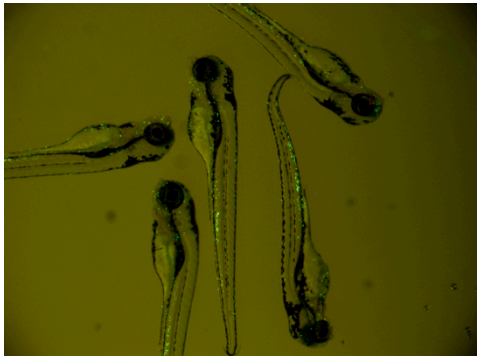


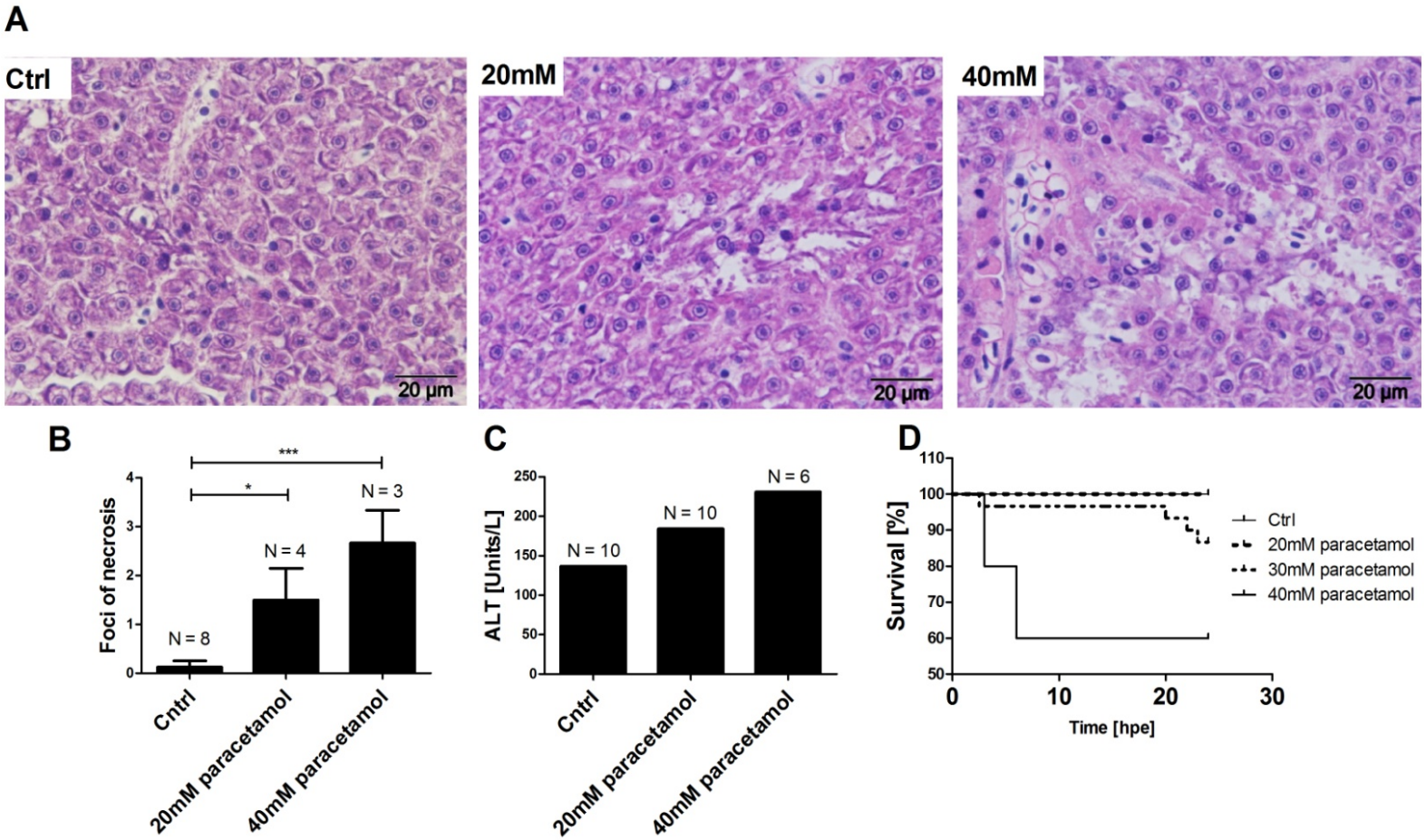
Time course of miR-122



Zebrafish

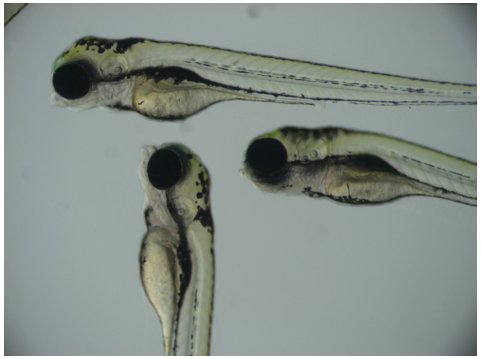


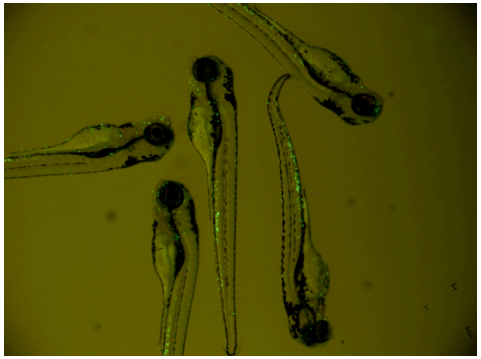


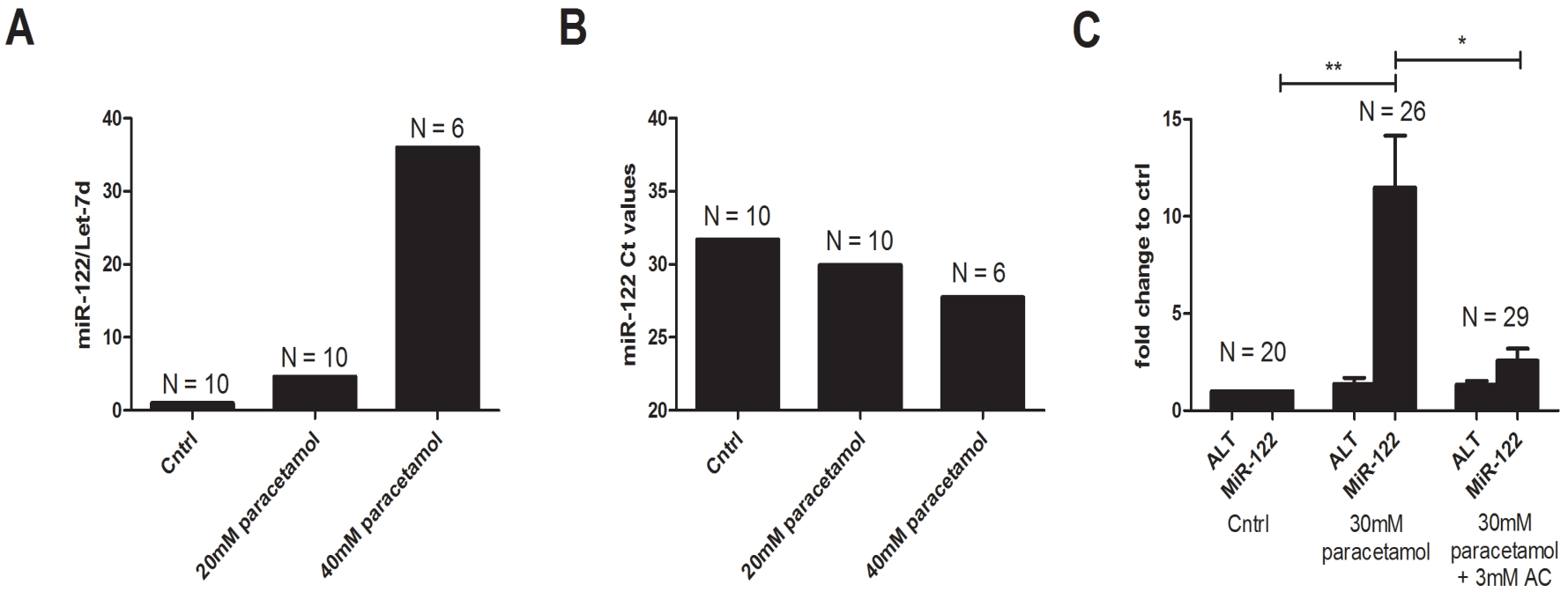


Zebrafish in press

Zebrafish



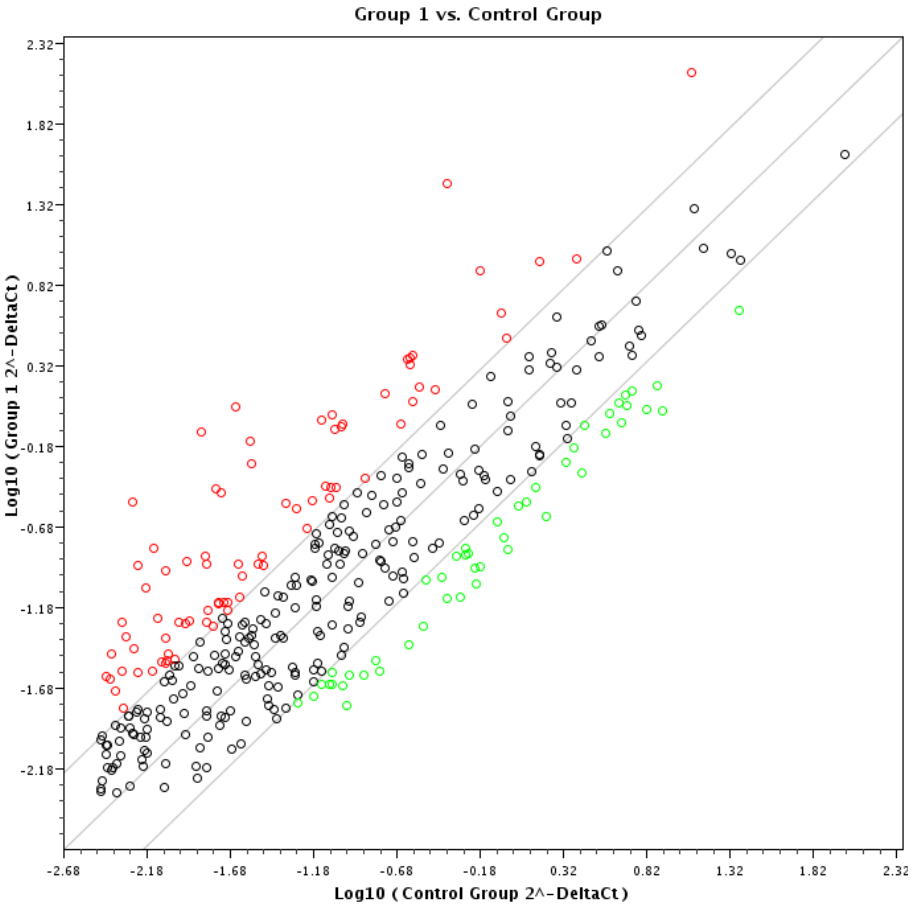


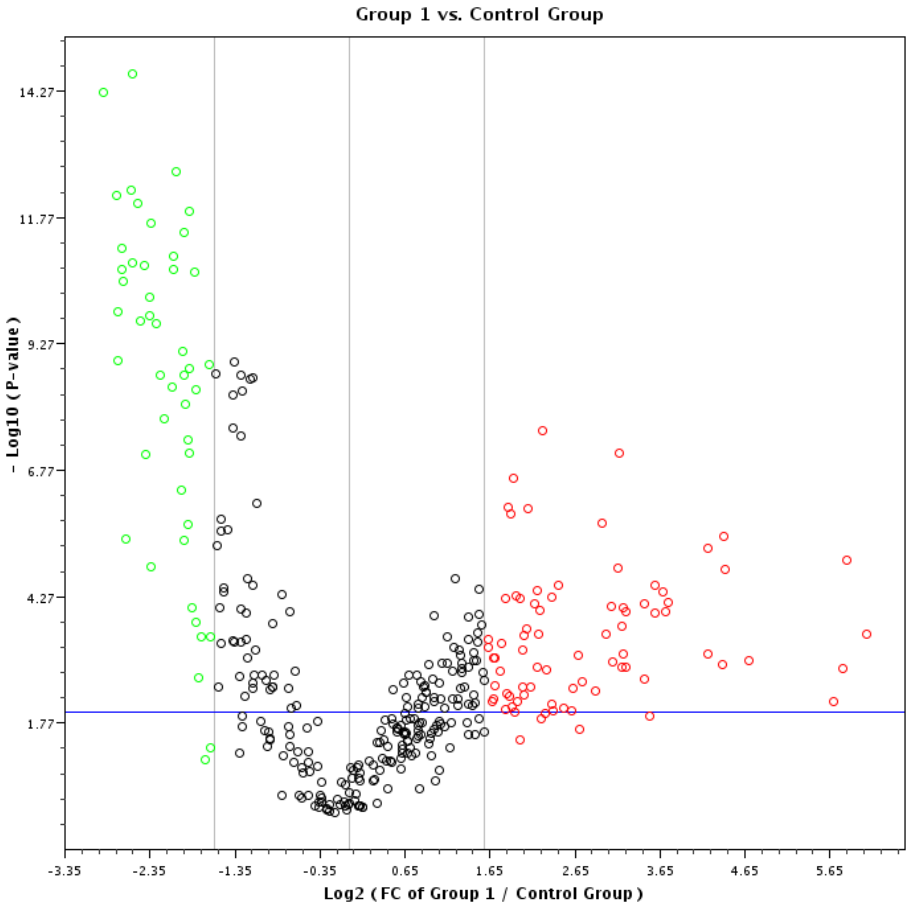


miR-122 is translational across species

Zebrafish in press

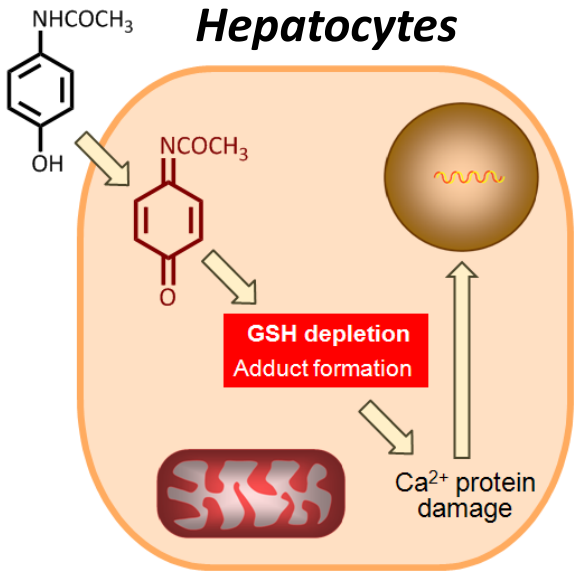
Other microRNA?





Mechanism markers

Mechanism of Cell Death - Biomarkers

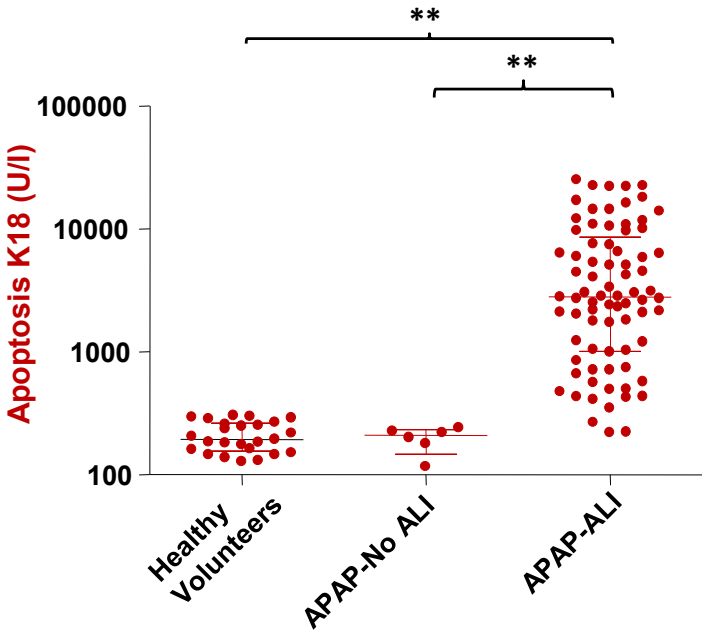


- **Cytokeratin 18 – abundant in hepatocytes**
- **Is cleared by caspases**
- **Fragment released into plasma**

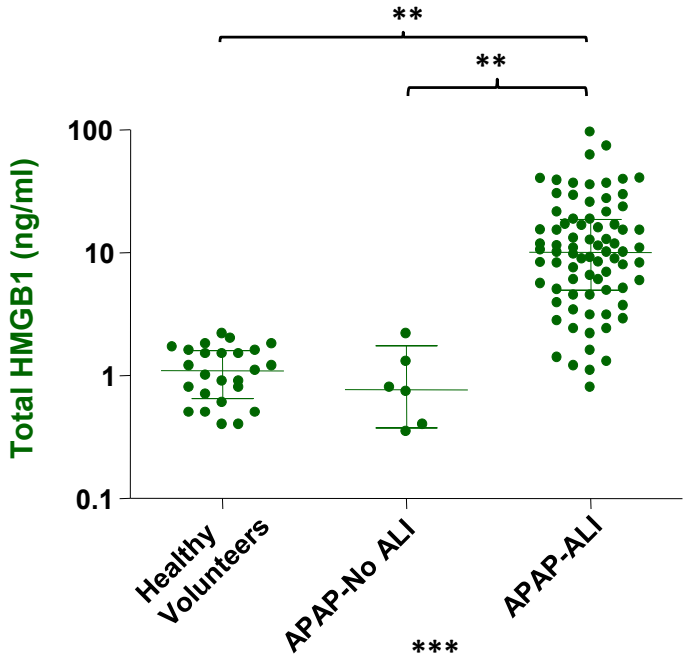
- **HMGB1 - released by necrotic cells**
- **But NOT by apoptotic cells**

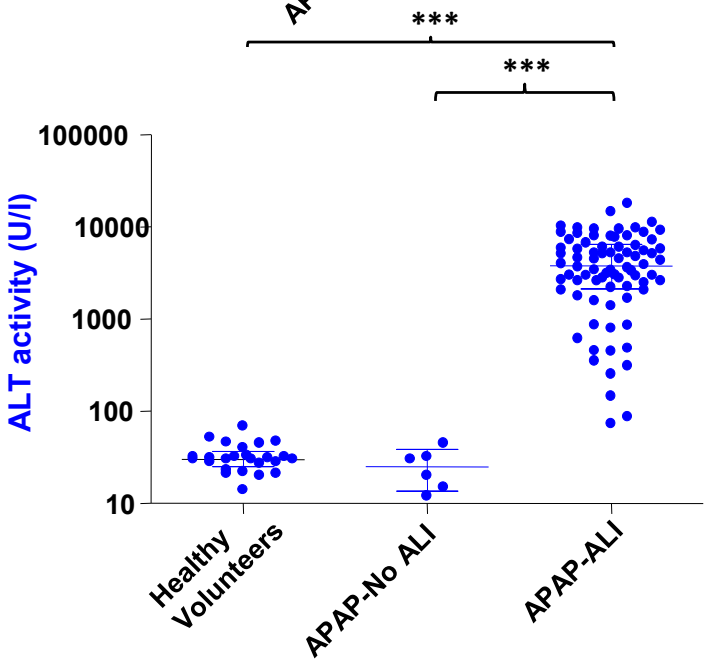
- **Mitochondrial DNA and Enzymes**
- **Reflect mitochondrial damage**

Paracetamol – biomarker bridging



n = 31, 6, 78





Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity

Daniel J. Antoine[1,*], Rosalind E. Jenkins[1], James W. Dear[2], Dominic P. Williams[1], Mitchell R. McGill[3], Matthew R. Sharpe[4], Darren G. Craig[5], Kenneth J. Simpson[5], Hartmut Jaeschke[3], B. Kevin Park[1]

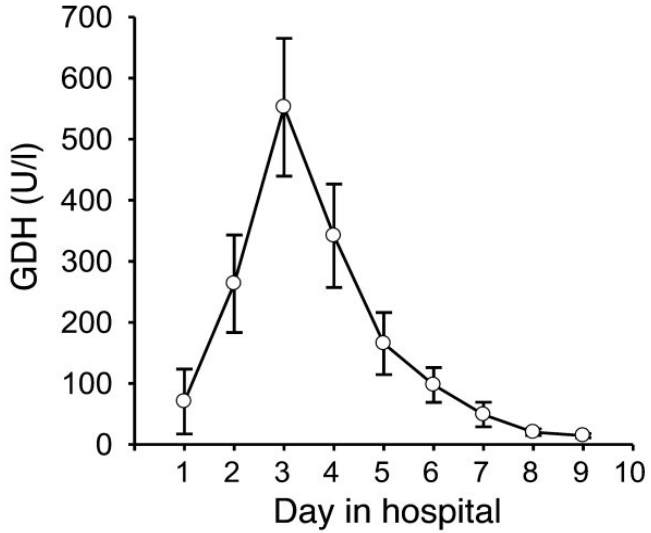
J Hepatol 2012 56:1070-9

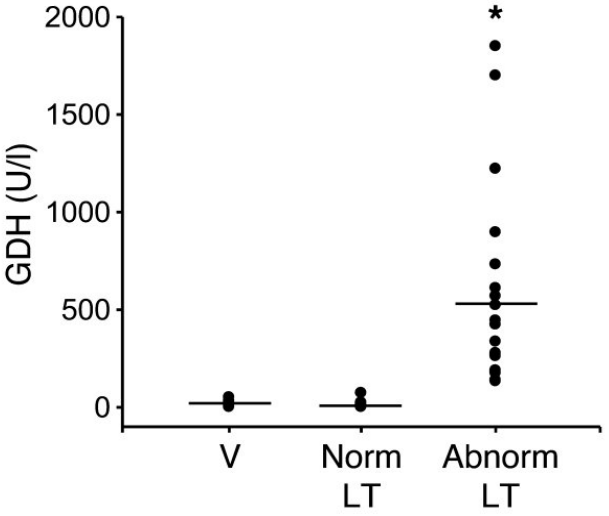
Research article

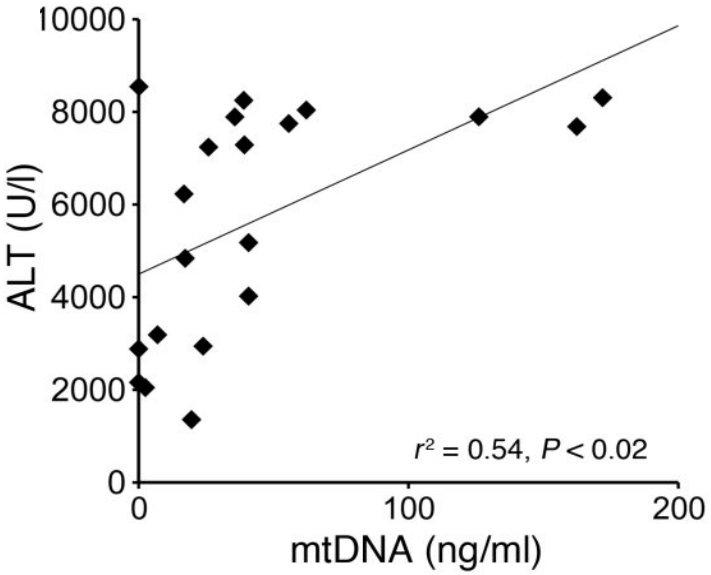
The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation

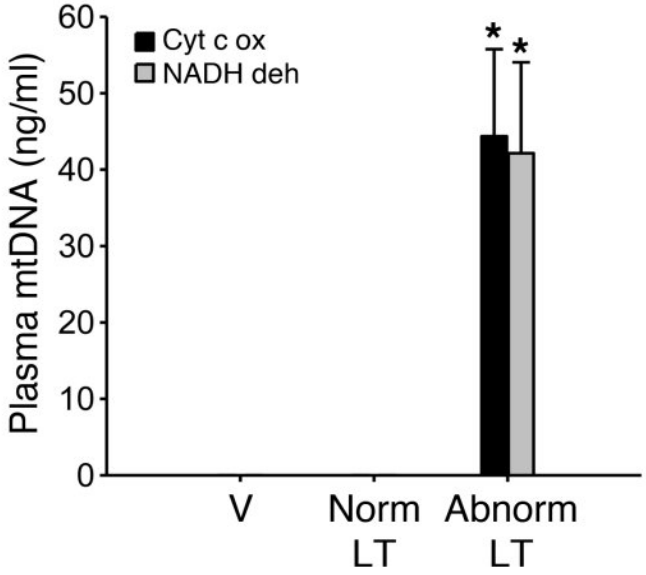
Mitchell R. McGill,[1] Matthew R. Sharpe,[2] C. David Williams,[1] Mohammad Taha,[2] Steven C. Curry,[3] and Hartmut Jaeschke[1]

[1]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas, USA. [2]Department of Internal Medicine, University of Kansas Hospital, Kansas City, Kansas, USA. [3]Department of Medical Toxicology, Banner Good Samaritan Medical Center, Department of Medicine, University of Arizona College of Medicine, Phoenix, Arizona, USA.









J Clin Invest. 2012;122:1574-83

Method

- Patients (total N=129) were recruited from the Royal Infirmary of Edinburgh (N=107) and the Royal Victoria Infirmary, Newcastle-Upon-Tyne (N=22).

- Inclusion criteria were: adults with a clear history of a single excess paracetamol ingestion and a timed blood paracetamol concentration that was judged to necessitate hospital admission for intravenous acetylcysteine therapy, as per UK guidelines at the time of study

- Exclusion criteria were: patients detained under the Mental Health Act; patients with permanent cognitive impairment; patients with a life-threatening illness; unreliable history of paracetamol overdose; patients who take anticoagulants therapeutically or have taken an overdose of anticoagulants; and patients who, in the opinion of the responsible clinician/nurse, were unlikely to complete the full course of acetylcysteine.

- All patients completed the full course of acetylcysteine.

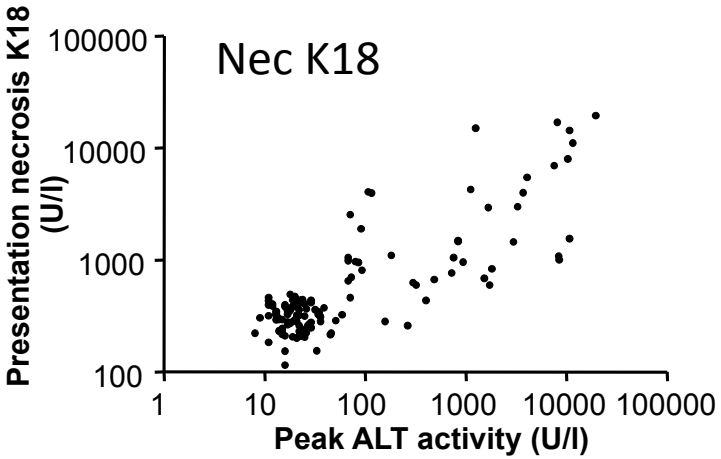
Method

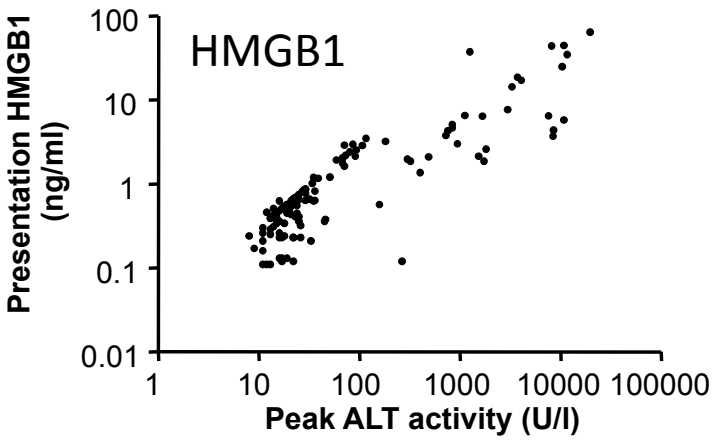
New biomarkers were measured in plasma at first presentation to hospital before acetylcysteine started

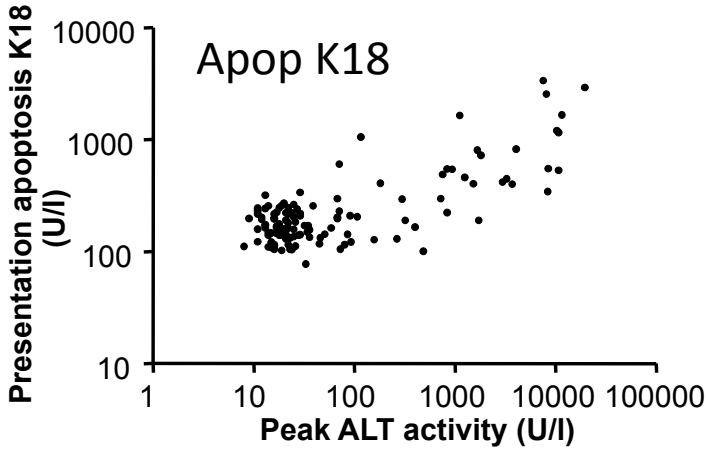
Primary outcome:

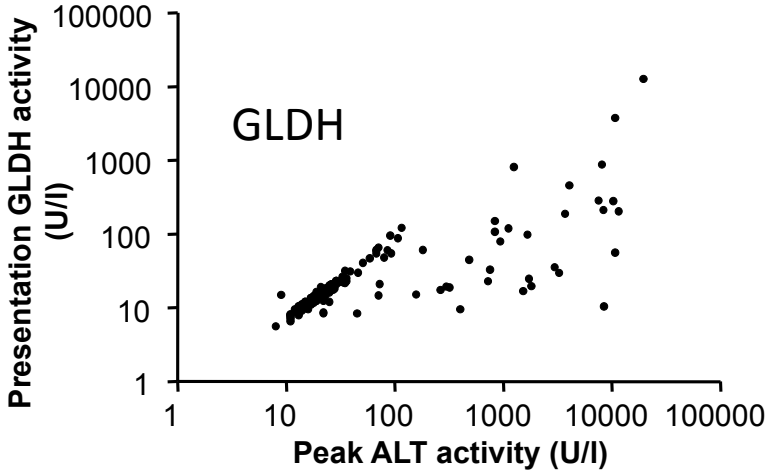
Acute liver injury - peak serum ALT activity greater than 3x the upper limit of normal (>150IU/L)

Presentation biomarkers vs peak ALT





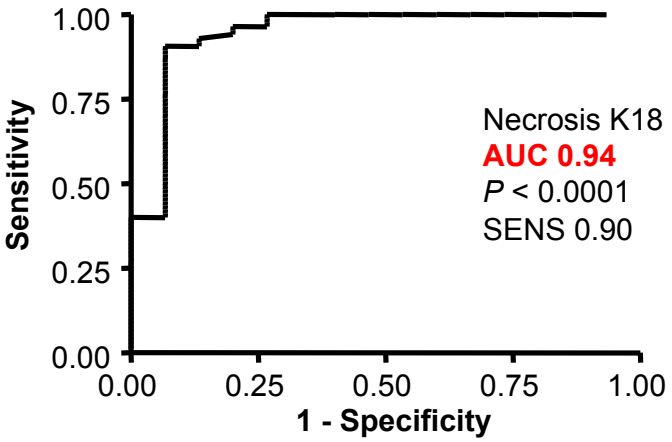


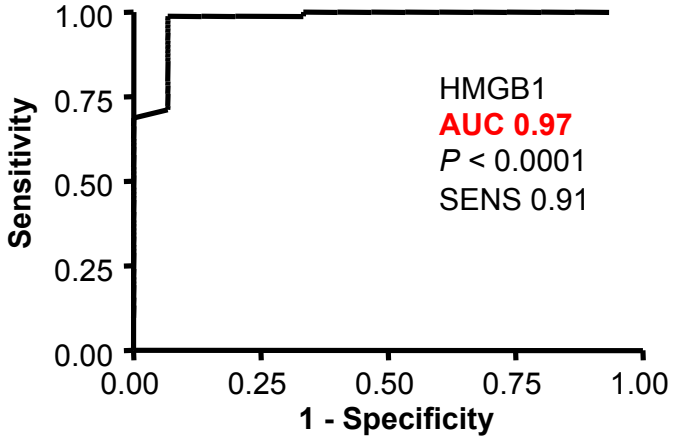


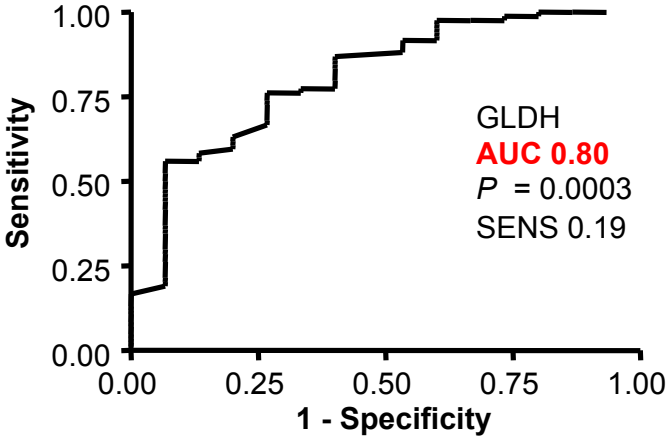
Biomarker	R^2	Pearson R (95% CI)	*P*
GLDH	0.45	0.67 (0.56-0.76)	<0.0001
HMGB1	0.67	0.82 (0.75-0.87)	<0.0001
Apop K18	0.57	0.75 (0.67-0.82)	<0.0001
Necrosis K18	0.59	0.77 (0.69-0.83)	<0.0001

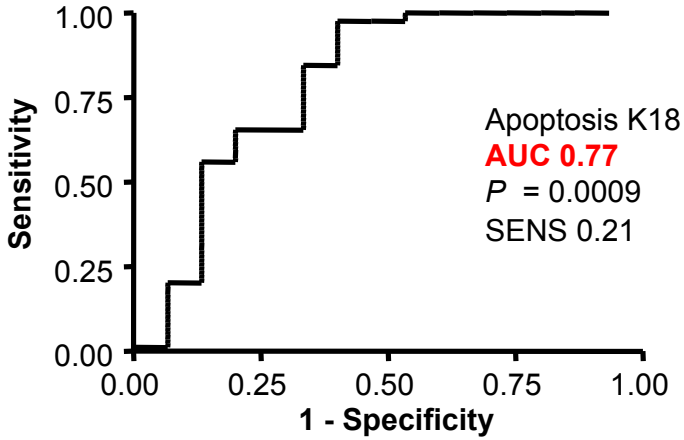
Hepatology 2013 58:777-787

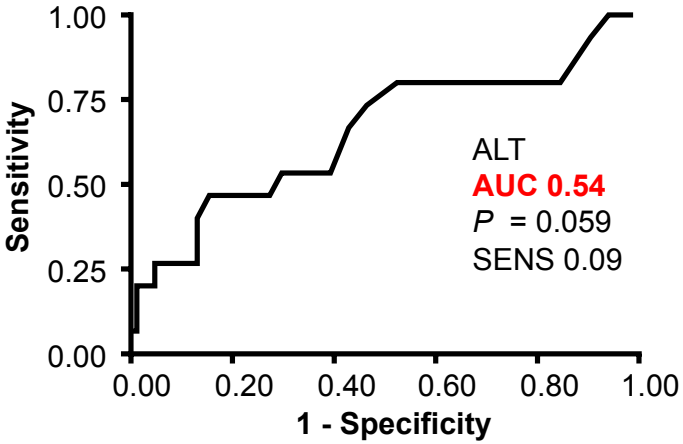
Performance of first presentation biomarkers at predicting acute liver injury









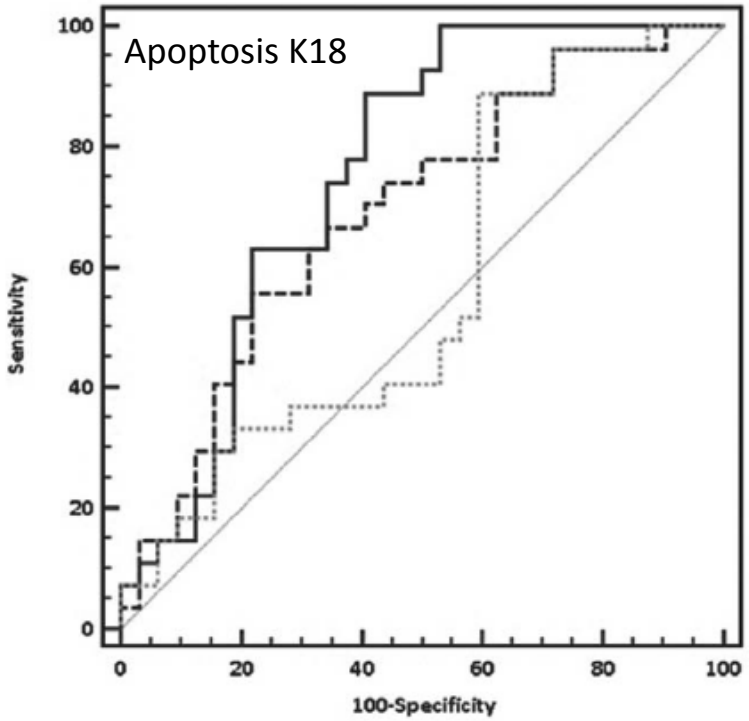


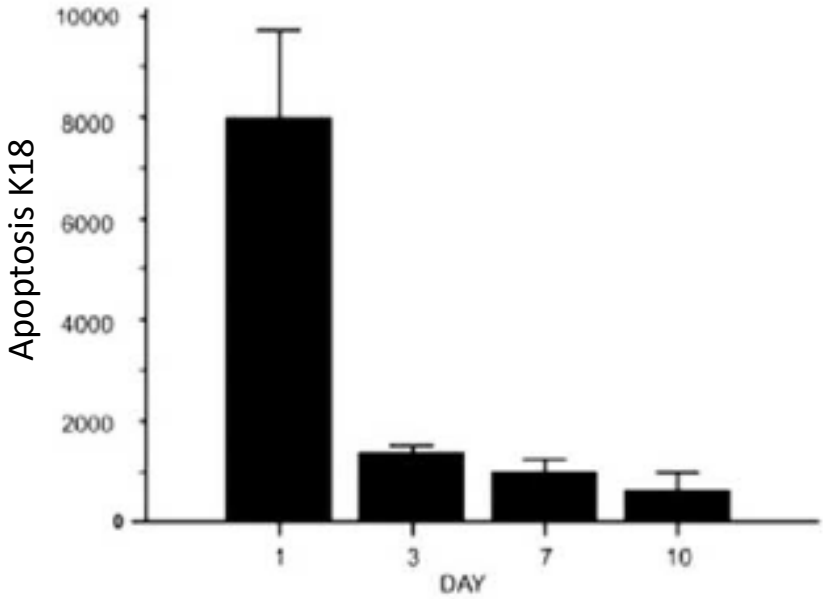
Progression biomarkers

Character and Temporal Evolution of Apoptosis in Acetaminophen-Induced Acute Liver Failure

Lucia A. Possamai, MBChB[1]; Mark J. W. McPhail, PhD, MRCP[1,2]; Alberto Quaglia, PhD[2]; Valentina Zingarelli, BSc[2]; R. Daniel Abeles, MBBS[2]; Robert Tidswell, BSc, MBBS[1]; Zudin Puthucheary, MBBS[3,4]; Jakirty Rawal, MBBS[3]; Constantine J. Karvellas, MD[5]; Elaine M. Leslie, MD[5]; Robin D. Hughes, PhD[2]; Yun Ma, MD, PhD[2]; Wayel Jassem, MD, PhD[2]; Debbie L. Shawcross, PhD[2]; William Bernal, FRCP[2]; Anil Dharwan, MD, FRCPCH, MBBS[2]; Nigel D. Heaton, FRCS[2]; Mark Thursz, MD[1]; Julia A. Wendon, FRCP[2]; Ragai R. Mitry, PhD[2]; Charalambos G. Antoniades, MBBS, MD, MRCP[1]

Day 1 single liver unit
Predicting Death/LT





(AUROC, 0.755 [0.639–0.885, p < 0.001]
sensitivity, 89%; specificity, 61%; cutoff, 2,718)

Crit Care Med 2013

Research Article

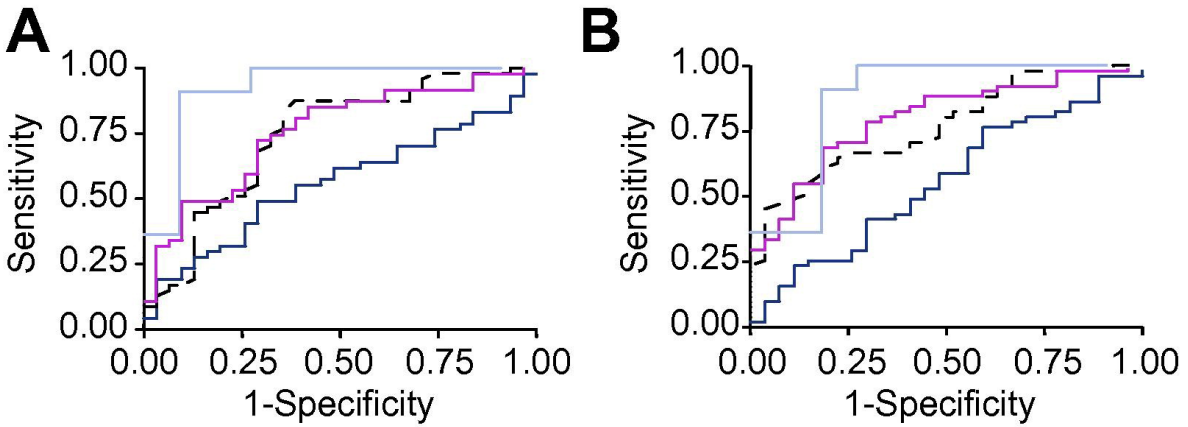
EASL JOURNAL OF HEPATOLOGY

Molecular forms of HMGB1 and keratin-18 as mechanistic biomarkers for mode of cell death and prognosis during clinical acetaminophen hepatotoxicity

Daniel J. Antoine[1,*], Rosalind E. Jenkins[1], James W. Dear[2], Dominic P. Williams[1], Mitchell R. McGill[3], Matthew R. Sharpe[4], Darren G. Craig[5], Kenneth J. Simpson[5], Hartmut Jaeschke[3], B. Kevin Park[1]

[1]MRC Centre for Drug Safety Science, Department of Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK; [2]University/BHF Centre for Cardiovascular Science, Edinburgh University & NPIS Edinburgh, Scottish Poisons Information Bureau, Edinburgh, UK; [3]Department of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160, USA; [4]Department of Pulmonary and Critical Care Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA; [5]Scottish Liver Transplantation Unit, Royal Infirmary of Edinburgh, Edinburgh, UK

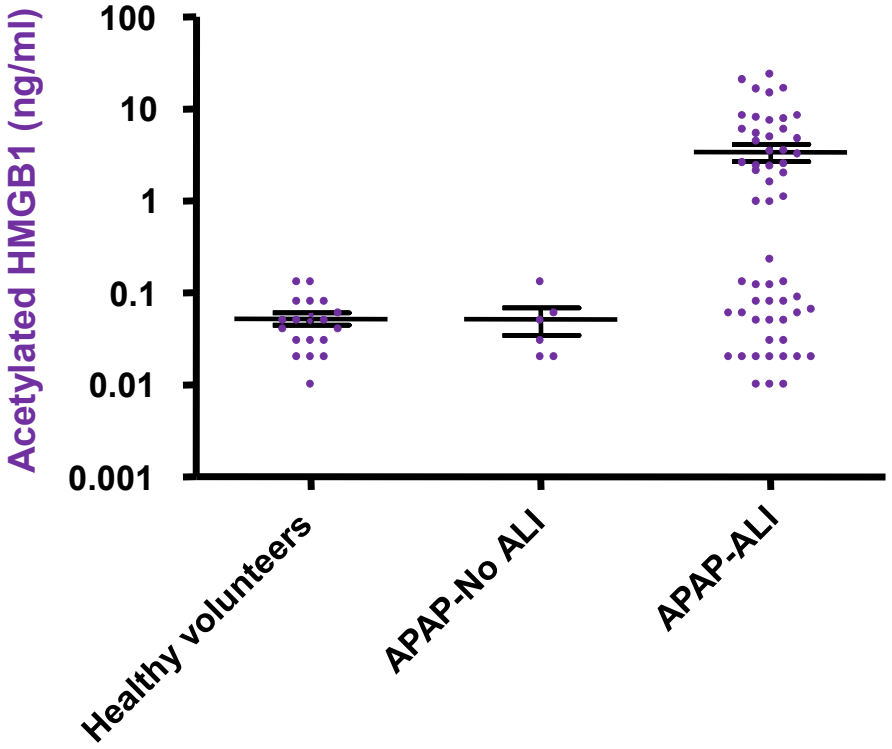
Day 1 UK and USA liver unit
Predicting Death/LT



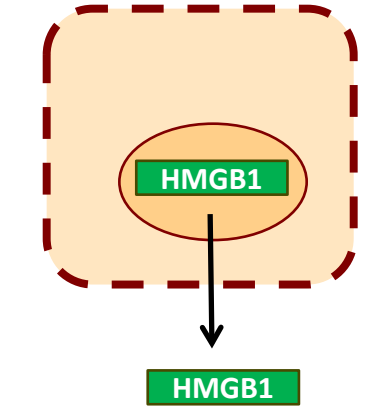
J Hepatol. 2012 56:1070-79

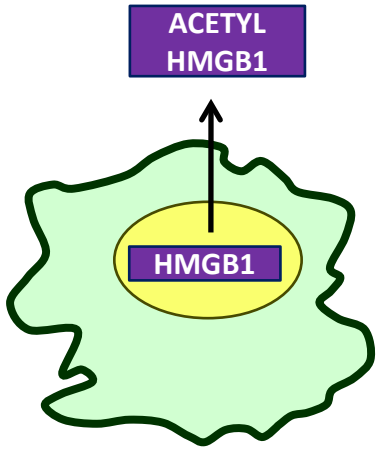
Acetylated HMGB1 – a biomarker of immune cell activation

- Clinical APAP overdose



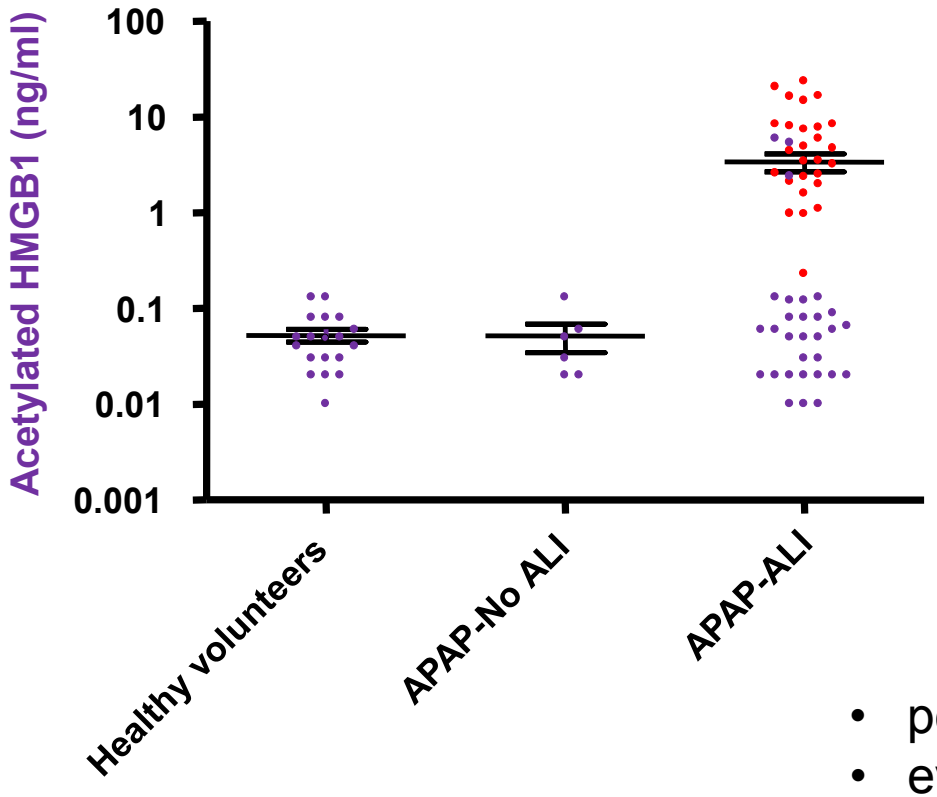
All patients





Acetylated HMGB1 – outcome prediction

- Clinical APAP overdose



Died / Required Liver transplant

Spontaneous survivors

- potential novel biomarker
- evidence in man for innate immune system
- need for multi cellular systems for prediction of <u>human</u> DILI

CASE REPORT:

25 year old male

Single overdose of 35g paracetamol at 02:30 (timing supported by Facebook message)

Assessed 4.5h after OD

No risk factors for hepatotoxicity. Paracetamol level 107 mg/L (below nomogram)

Normal biochemical evidence of liver injury

Assessed by senior doctor and not treated

Discharged after psychiatry review

Time from OD (h)	4.5
Paracetamol (mg/L)	107
ALT (U/L) (ULN 50)	34
INR	1.0

CASE REPORT:

25 year old male

Single overdose of 35g paracetamol at 02:30 (timing supported by Facebook message)

Assessed 4.5h after OD

No risk factors for hepatotoxicity. Paracetamol level 107 mg/L (below nomogram)

Normal biochemical evidence of liver injury

Assessed by senior doctor and not treated

Discharged after psychiatry review

Represented to hospital 43h after OD

Lethargic and vomiting

Tender abdomen

Time from OD (h)	4.5	43
Paracetamol (mg/L)	107	9
ALT (U/L) (ULN 50)	34	11314
INR	1.0	2.1

CASE REPORT:
25 year old male
Single overdose of 35g paracetamol at 02:30 (timing supported by Facebook message)
Assessed 4.5h after OD
No risk factors for hepatotoxicity. Paracetamol level 107 mg/L (below nomogram)
Normal biochemical evidence of liver injury
Assessed by senior doctor and not treated
Discharged after psychiatry review

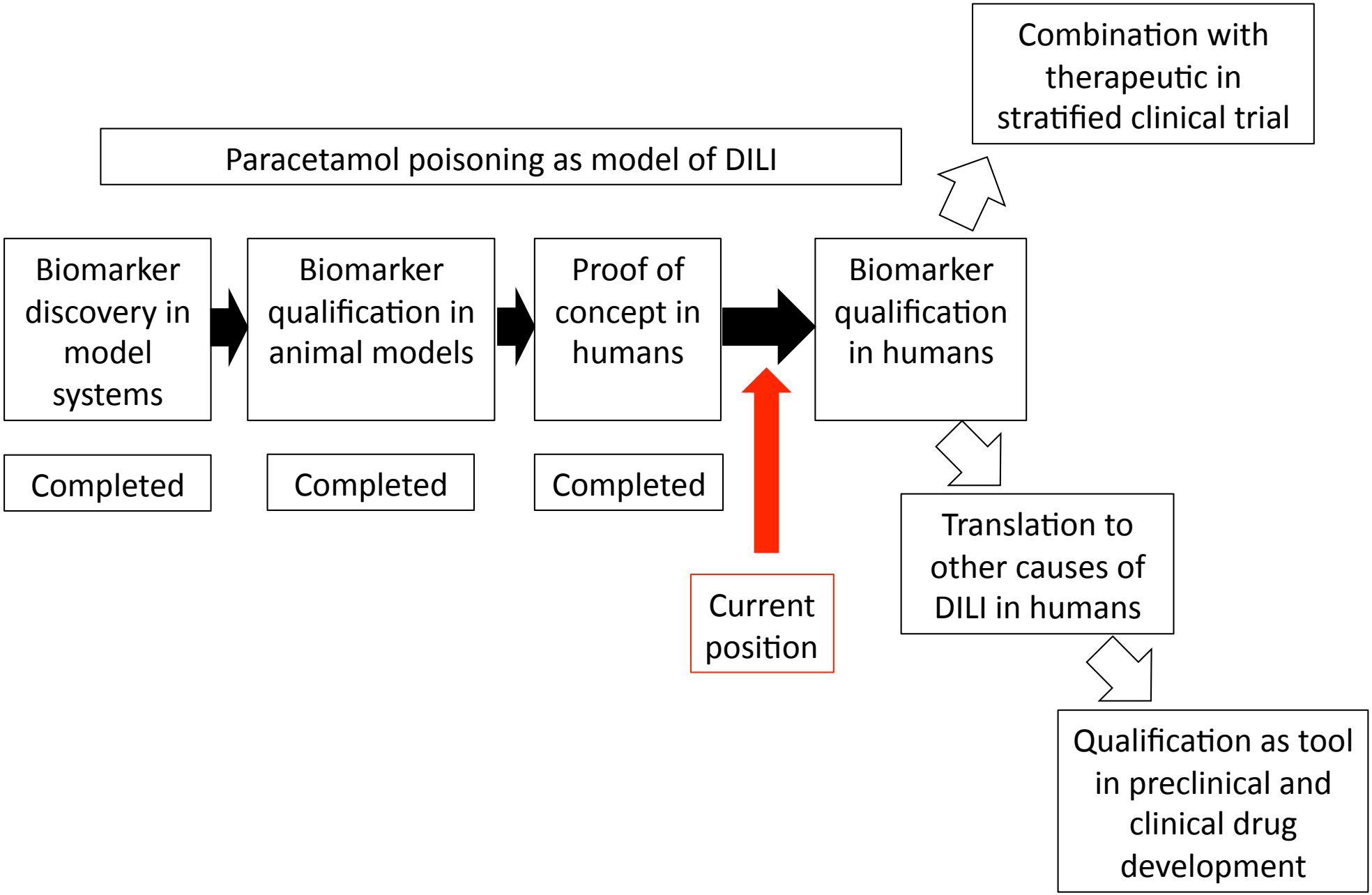
Represented to hospital 43h after OD
Lethargic and vomiting
Tender abdomen

NEW MARKERS CORRECTLY IDENTIFIED
LIFE THREATENING HEPATOTOXICITY MISSED BY CURRENT TESTS

BJCP 2013 Published online

Time from OD (h)	4.5	43
Paracetamol (mg/L)	107	9
ALT (U/L) (ULN 50)	34	11314
INR	1.0	2.1
miR-122 (/ let-7d) (ULN 5.2*)	**261**	**(x50)**
HMGB1 (ng/ml) (ULN 0.9*)	**7.2**	**(x8)**
Necrosis K18 (U/L) (ULN 480*)	**4018**	**(x8)**

*95% prediction interval – no liver injury after overdose n=82 *Hepatology* 2013


Paracetamol poisoning as model of DILI
Biomarker discovery in model systems
Biomarker qualification in animal models
Proof of concept in humans
Biomarker qualification in humans
Combination with therapeutic in stratified clinical trial
Translation to other causes of DILI in humans
Qualification as tool in preclinical and clinical drug development
Completed
Completed
Completed
Current position

Markers and Paracetamol Poisoning Study (MAPP)

Biomarker validation study

Recruiting across UK

Target 1000 patients

Recruited around 400 so far

Will validate the markers performance at hospital front door

Acknowledgements

Edinburgh

Wilna Oosthuyzen
Bastiaan Vliegenthart
Jonathan Street
Matthew Bailey
Professor Nick Bateman
Judy Coyle
Professor Alasdair Gray
Moyra Masson
Professor David J Webb
Professor Chris Gregory
Carl Tucker

CDSS Liverpool

Professor Kevin Park
Dr Dan Antoine
Dr Chris Goldring
Phillip Starkey-Lewis
Vivien Platt

Newcastle
Ruben Thanacoody
Professor Simon Thomas

Novartis
Dr Jonathan Moggs

British Heart Foundation

NHS SCOTLAND
NHS RESEARCH SCOTLAND

NC 3Rs
National Centre for the Replacement Refinement & Reduction of Animals in Research

THE UNIVERSITY OF EDINBURGH

NPIS
National Poisons Information Service

UNIVERSITY OF LIVERPOOL

MRC Medical Research Council

CENTRE FOR DRUG SAFETY SCIENCE