Hepatotoxicity remains a major challenge in drug development. Although alanine aminotransferase (ALT) remains the gold standard biomarker of liver injury, alternative biomarker strategies to better predict the potential for severe drug-induced liver injury (DILI) are essential. In this study, we evaluated the utility of glutamate dehydrogenase (GLDH), purine nucleoside phosphorylase (PNP), malate dehydrogenase (MDH), and paraxonase 1 (PON1) as indicators of liver injury in cohorts of human subjects, including healthy subjects across age and gender, subjects with a variety of liver impairments, and several cases of acetaminophen poisoning. In the healthy subjects, levels of GLDH and MDH were not affected by age or gender. Reference ranges for GLDH and MDH in healthy subjects were 1–10 and 79–176 U/L, respectively. In contrast, the levels of PON1 and PNP were not consistent across cohorts of healthy subjects. Furthermore, GLDH and MDH had a strong correlation with elevated ALT levels and possessed a high predictive power for liver injury, as determined by ROC analysis. In contrast, PON1 and PNP did not detect liver injury in our study. Finally, evaluation of patients with acetaminophen-induced liver injury provided evidence that both GLDH and MDH might have utility as biomarkers of DILI in humans. This study is the first to evaluate GLDH, MDH, PON1, and PNP in a large number of human subjects and, it provides an impetus for prospective clinical studies to fully evaluate the diagnostic value of GLDH and MDH for detection of liver injury.

Key Words: human; biomarker; hepatotoxicity; glutamate dehydrogenase; purine nucleoside phosphorylase; malate dehydrogenase; paraxonase 1.

Drug-induced liver injury (DILI) is the single greatest cause for termination of development of drug candidates and withdrawal of approved drugs from the market (Kaplowitz, 2005). In the clinic, it accounts for more than 50% of acute liver failure cases (Lee, 2003). Serum alanine aminotransferase (ALT) activity is a widely used clinical biomarker (Ozer et al., 2008) of liver damage that is commonly used to assess the risk of liver injury during drug development and for drug approvals by regulatory agencies. However, increases in ALT may also indicate a transient hepatocellular injury that does not progress to severe DILI (e.g., aspirin, heparin) (FDA, 2007). Thus, the differentiation of transient ALT increases from ALT elevations that progress to severe DILI provides a challenge. This is of paramount importance in early clinical trials and highlights the need for the development of alternative biomarker approaches.

As elevated ALT alone is not sufficient to diagnose severe DILI, Hy’s Law is often utilized to assess a drug’s risk of inducing hepatotoxicity (Temple, 2006). This is based on detection of serum ALT elevations greater than threefold of upper limit of normal (ULN) observed in combination with greater than twofold ULN of total bilirubin (Tbil) in absence of hepatobiliary injury assessed using alkaline phosphatase (ALP) (Temple, 2006). Meeting the criteria of Hy’s Law is considered a more specific indication of severe DILI than elevated serum ALT alone (FDA, 2007). Recently, a DILI Working Group of clinicians and scientists recommended a modification of the current DILI criteria. The threshold level of ALT elevation was raised to greater than fivefold ULN in order to exclude clinically irrelevant drug-related events and low-level non-DILI elevations of serum ALT. Furthermore, an increase of ALP greater than twofold indicating hepatobiliary injury was included. These two parameters in combination with the Hy’s Law criteria that indicate functional hepatocellular damage comprise a new biochemical criterion for DILI assessment (Aithal et al., 2011). Although Hy’s law and the DILI Working Group’s definition of the clinical threshold for DILI are useful in differentiating DILI in humans, drug development continues to be slowed or halted due to unexplained ALT elevations in clinical trials. Therefore, development of alternative biomarker approaches is of paramount importance (Lee and Senior, 2005).

To further the development of alternative biomarkers of liver injury, international research projects such as Innovative Medicines Initiative and Critical Path’s Predictive Safety Testing Consortium have been initiated (Goodsaid et al., 2007; Sistare et al., 2010; Vaidya, 2010). Several hepatic biomarkers identified by evidence from peer-reviewed literature and internal datasets at various institutions (Amacher et al., 2005; Vaidya, 2010) are being...
evaluated as viable alternatives by these groups. These include glutamate dehydrogenase (GLDH), purine nucleoside phosphorylase (PNP), malate dehydrogenase (MDH), and paroxonase 1 (PON1). GLDH, a mitochondrial enzyme located primarily in the centrilobular region of the liver, plays a role in amino acid oxidation and urea production (O’Brien et al., 2002). GLDH is primarily found in the liver and to a lesser degree in the kidney with trace amounts in skeletal muscle (Mastorodemos et al., 2005). PNP, a key enzyme in the purine salvage pathway, reversibly catalyzes the phosphorolysis of nucleosides to their respective bases and corresponding 1-(deoxy)-ribose-phosphate. PNP is located primarily in the cytoplasm of endothelial cells, Kupffer cells, and hepatocytes and is released into hepatic sinusoids during necrosis (Ohuchi et al., 1995). PON1 is a high-density lipoprotein (HDL)–associated esterase secreted mainly by the liver, which detoxifies organophosphates and protects low-density lipoproteins from oxidative modifications. PON1 bound to HDL is constitutively released into circulation, and a decrease in serum PON1 is indicative of liver damage (Feingold et al., 1998; Ferré et al., 2002). MDH catalyzes the reversible conversion of malate into oxaloacetate utilizing NAD+ and is a constitutive enzyme in the citric acid cycle. MDH is a perportal enzyme whose release into the serum indicates tissue damage (Osyiw et al., 1994; Ozer et al., 2008).

Although GLDH, PNP, MDH, and PON1 show promise as alternative biomarkers of liver injury, systematic characterization in humans has not been undertaken. In this study, we set out to characterize these markers by assessing the effects of age and gender in the healthy subject population, as defined by the absence of clinical manifestation of liver disease. Next, we evaluated the ability of the biomarker to detect liver injury in a broad range of clinically demonstrated liver injuries. Finally, we evaluated the performance of these markers in a cohort of patients with acetaminophen-induced liver injury. Our study is the first to evaluate these biomarkers in a large cohort of subjects, including the assessment of biomarker performance in cases of incidental acetaminophen (APAP) toxicity.

MATERIALS AND METHODS

Acquisition of Samples From Human Subjects

Healthy volunteers. Samples were collected from the Pfizer Clinical Research Unit (CRU) under an approved IRB from healthy male or female subjects between the ages of 18 and 55. Healthy was defined as an absence of clinically relevant abnormalities detected by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, 12-lead ECG, and lack of positive findings in routine clinical laboratory tests.

Healthy subjects. Samples from healthy subjects were collected from the University of Michigan health care system (UM) under an approved IRB (2000–005) and defined as healthy based on the normal levels of ALT, aspar- tate aminotransferase (AST), ALP, Tbil, glucose, blood urea nitrogen, serum creatinine, and creatine kinase. Subjects whose values for one or more of the above criteria exceeded the normal reference range were not used in this study. In addition, any healthy subject who had an ongoing health problem or immunological flare was omitted from the cohort. Most samples were collected from subjects who were in for routine health examinations.

Hepatic injury subjects. Samples from subjects with abnormal hepatic enzyme profiles were collected from the University of Michigan health care system (UM). Subjects with both AST and ALT levels greater than two times normal healthy levels and with a diagnosed disease resulting in impaired liver function were grouped and loosely categorized as liver transplant (liver transplant within the last 3 years), hepatic carcinoma (diagnosed by biopsy or resec- tion), coronary artery disease (CAD)—coronary heart disease (CHD) (aortic aneurism, myocarditis, atrial mass, aortic valve replacement, or heart catheteri- zation), cirrhosis and liver injury (Hepatitis B or C, hepatic graft vs. host disease, ethanol cirrhosis, drug abuse, or transaminitis/hepatic congestion), pulmonary (Influenza A, H1N1 Influenza, acute respiratory distress syndrome, or latent tuberculosis), and acetaminophen toxicity (APAP-induced liver failure).

Sample Preparation and Analysis

Serum samples were recovered from serum-separator tubes following centrifugation of whole blood at 3000 x g for 10 min at room temperature. Serum samples were kept at 4°C for up to 72 h before aliquots were frozen at -80°C and stored until shipped for biomarker analysis. A stability assessment of GLDH, MDH, PNP, and PON1 confirmed acceptable stability at 4°C for up to 96 h.

All analytes were measured in serum on the Siemens ADVIA 2400 Automated Chemistry System. ALT and AST (Siemens) and GLDH (Randox Labs Ltd, Roche) were measured using commercially available kits. MDH, PNP, and PON1 were determined according to Bergmeyer and Bernt (1983), Chu et al. (1989), and Li et al. (1995), respectively.

Statistical Analysis

Ninety-five percent of reference intervals were calculated for each marker using EP Evaluator software Release 7 (Data innovation, South Burlington, VT). To measure the strength of the relationship between pairs of individual markers, Spearman’s rank correlation coefficient was used. To evaluate the relationship between individual markers and liver injury, receiver operator characteristic (ROC) curves were constructed. The area under the ROC curve was used as an overall assessment of a marker’s predictive ability. Comparisons of healthy subjects from UM with healthy volunteers from CRU within ethnic categories were conducted using a two-sample t-test on log-transformed values.

RESULTS

Characterization of Emerging Biomarkers of Liver Injury in Healthy Human Subjects

To evaluate levels of ALT, GLDH, MDH, and PON1 in human subjects, we analyzed serum samples collected from 550 healthy volunteers participating in clinical trials at the CRU and from healthy subjects at the UM with normal liver function. Selection of UM subjects was based on a retrospec- tive evaluation of clinical chemistry profiles and diagnosis. Samples consisted of serum that was to be discarded, following prescribed medical testing. CRU healthy volunteers (n = 186) were mostly male, aged 20–40 and 41–60 years, reflecting the recruitment criteria for inclusion on phase 1 trials. UM healthy subjects (n = 364) were distributed between both sexes in all age groups above 20 years of age. There was a lack of subjects in the age category below 20 years of age (females only, N = 8). UM healthy subjects were 87% Caucasian and 6% African American, whereas the CRU healthy volunteers were 18% Caucasian, 57% African American, and 25% other ethnicities. Table 1 outlines ALT, GLDH, MDH, and PON1 lev- els by age and gender. There were no differences in biomarker
levels between age groups and genders among subjects within UM and CRU cohorts. Comparing data across UM and CRU cohorts, there was no significant difference in GLDH and MDH levels irrespective of age and genders. On the other hand, PON1 and PNP levels differed between the UM and CRU cohorts. Although neither PON1 nor PNP levels varied in respect to age or gender, PON1 was generally lower and PNP higher in the UM subjects compared with subjects from the CRU. Although the differences for PNP were statistically significant, they were very small (< 1 U/L) and were not considered biologically relevant. On the other hand, PON1 levels were consistently higher in African Americans compared with Caucasians ($p$ value < 0.0001) regardless of the cohort, CRU or UM, reflecting an ethnic difference (Fig. 1). Therefore, we could establish reference ranges spanning 95% of the healthy population only for GLDH and MDH. The reference ranges for GLDH and MDH were 1–10 and 79–176 U/L, respectively.

**Capacity of Emerging Biomarkers to Detect Liver Injury**

Biomarker (GLDH, MDH, PNP, and PON1) levels were evaluated in subjects from UM with a variety of liver impairments and compared with healthy UM subjects and healthy clinical trial volunteers. A total of 843 (364 healthy subjects and 479 subjects with varying degrees of liver damage) samples were used in this evaluation. As serum ALT activity is currently the primary biomarker of liver injury and was used for selection of subjects in our study, we first established the correlation of ALT levels with GLDH, MDH, PNP, and PON1 activity levels. Spearman’s rank correlation analysis between the four markers (GLDH, MDH, PNP, and PON1)  

![FIG. 1. Influence of ethnicity on PON1 levels in healthy human subjects.](https://academic.oup.com/toxsci/article-abstract/132/2/276/1671502)

**TABLE 1**

<table>
<thead>
<tr>
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<th>Age &lt; 20</th>
<th>Age 20–40</th>
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| **UM**           | $n = 0$ | $n = 87$  | $n = 23$  | $n = 74$ | $n = 53$ | $n = 35$
| A. Male          |         |           |           |          |         |        |
| ALT              |         |           |           |          |         |        |
| UM               | 22.8±9.3| 24.4±6.2  | 23.9±6.1  | 23.2±7.2 |          |        |
| CRU              | 3.2±2.4 | 2.9±1.3   | 4.1±3.9   | 3.1±2.2  | 2.9±1.7 |        |
| MDH              | 118.0±28.1| 119.6±23.6| 115.3±22.4| 113.8±22.7| 114.0±23.8|        |
| PNP              | 1.7±0.9 | 3.0±1.5   | 1.8±1.3   | 2.4±1.5  | 2.3±1.1 |        |
| PON1             | 286.6±114.1| 147.2±87.0| 237.2±134.7| 169.0±89.9| 145.7±76.3|        |
| **UM**           | $n = 8$ | $n = 18$  | $n = 77$  | $n = 7$  | $n = 108$| $n = 60$
| B. Female        |         |           |           |          |         |        |
| ALT              |         |           |           |          |         |        |
| UM               | 21.4±7.9| 17.4±6.6  | 19.4±5.4  | 13.9±3.3 | 21.7±5.8 | 21.0±5.7|
| CRU              | 2.6±1.7 | 3.1±4.4   | 2.4±2.2   | 1.9±1.2  | 3.5±2.7 | 3.0±1.9 |
| MDH              | 123.4±35.7| 106.8±15.3| 115.5±30.0| 109.6±25.3| 118.7±28.3| 121.4±21.2|
| PNP              | 2.6±1.7 | 1.4±0.8   | 2.0±1.8   | 1.0±0.0  | 2.4±1.6 | 2.1±1.1 |
| PON1             | 211.1±118.2| 279.9±155.9| 202.1±128.6| 310.1±71.2| 178.8±94.1| 179.8±96.5|

**Notes.** Data represent mean ± SD.
and ALT yielded correlation coefficients of $r_s = 0.88$, 0.74, 0.26, and -0.33, respectively (Fig. 2). GLDH and MDH were found to be highly correlated with ALT, whereas little or no correlation was observed between ALT and either PNP or PON1.

Evaluation of the sensitivity and specificity of markers to detect liver injury was performed via the area under the ROC curve method. For this analysis, we used a clinical chemistry criterion of DILI as a diagnostic measure of liver damage. The criterion includes the manifestation of one or more of the following: ≥ 5× ALT or ≥ 2× ALP or (≥ 3× ALT and ≥ 2× TBil) (Aithal et al., 2011). We recognize that the majority of the liver injury in the patient population defined as hepatic injury subjects might not be a result of DILI but likely due to a disease resulting in impaired liver function. Nevertheless, the definition of liver injury classified by the clinical chemistry criteria described above enabled the evaluation of the emerging biomarkers in the absence of histopathologic evaluation that was not feasible due to ethical and practical reasons. We assessed biomarker levels against the clinical chemistry criterion of liver injury in 843 subjects to determine the diagnostic performance of each of the biomarkers. GLDH had the highest diagnostic power with an area under the curve (AUC) of 0.98, followed by MDH (AUC = 0.91), PON1 (AUC = 0.70), and PNP (AUC = 0.62) (Fig. 3). As expected, ALT had a high diagnostic power (AUC = 0.99) as this was a primary component of the definition of liver injury. 

FIG. 2. Correlation of ALT with GLDH, MDH, PNP, and PON1. Data points represent individual samples from healthy subjects and subjects with various liver impairments. Healthy subjects are shown in red, subjects with ALT levels above the upper limit of normal (35 U/L) are shown in green or blue, and subjects with laboratory values that triggered the Expert Working group’s definition of liver injury (≥ 5× ALT or ≥ 2× ALP or [≥ 3× ALT and ≥ 2× TBil]) are shown in blue.

FIG. 3. ROC curve analysis of emerging biomarkers and liver injury. ROC curve analyses for 843 subjects demonstrating sensitivity and specificity of GLDH, MDH, PNP, and PON1 with respect to liver injury defined using the Expert Working group’s definition (≥ 5× ALT or ≥ 2× ALP or [≥ 3× ALT and ≥ 2× TBil]). AUC values range from 0.5 to 1.0 and are a measure of the biomarker’s diagnostic performance.
Furthermore, we investigated whether the performance of emerging biomarkers of liver injury could be affected by the underlying disease or diagnosis and thus uncover advantages and/or limitations of their use in comparison to ALT. In this analysis, we examined the correlation between the emerging biomarkers and ALT for subjects divided into groups based on primary diagnosis. The results of the Spearman’s rank correlation analysis between each biomarker and ALT levels in individual diagnosis groups are listed in Table 2. As expected, there was little correlation between ALT and PNP or PON1 for subjects in most diagnosis groups as indicated by correlation coefficients below 0.6 in absolute value. The only exception was observed in the pulmonary diagnosis group with correlations of $r_s = 0.68$ and 0.80 in absolute values for PNP and PON1, respectively. In contrast, GLDH and MDH correlated well with ALT in the majority of the diagnosis groups represented by correlation coefficients over 0.6. Interestingly, the correlation coefficient was lower for samples from subjects with acetaminophen poisoning ($r_s = 0.39$), warranting a detailed evaluation of these cases.

### GLDH and MDH Detect Acute Liver Damage in Subjects With APAP Toxicity

Subjects with acetaminophen-induced injury (grouped as APAP Toxicity) were identified following admission to UM with elevated AST/ALT levels and APAP levels exceeding 10 µg/ml of serum. Several subjects had levels of AST/ALT exceeding 13,000 and 5000 IU/ml, respectively, and levels of APAP that exceeded 200 µg/ml of serum. Following admission and subsequent treatment, subjects were discharged when AST/ALT levels demonstrated sequential decrease over consecutive blood draws. Figure 4 compares the activities of ALT, AST, MDH, and GLDH from consecutive blood draws for six subjects identified as having acetaminophen-induced injury upon admission. All subjects (subjects 1–6) showed reductions in ALT, AST, MDH, and GLDH, indicating recovery from the acetaminophen toxicity. Although reductions in levels were observed for all markers indicating a recovery of liver injury, two response patterns were discerned among these subjects. The biomarker response to acetaminophen overdose of subjects 1–3 showed a similar time course–dependent decrease (slope) of ALT, AST, GLDH, and MDH levels. All markers remained $> 5\times$ the upper limit of the reference range (ULN) for subjects 1 and 2 at the end of the 3-day observation period. Because the observation period for subject 3 was 8 days, we were able to observe almost complete recovery of GLDH, AST, and MDH levels. In contrast, subjects 4–6, displayed a rapid time course–dependent decrease of GLDH and MDH, followed by AST with ALT levels decreasing only slightly over the observation period. In the case of subject 5, the GLDH and MDH returned to normal levels by the end of the observation period, whereas ALT and AST showed 82- and 6-fold increase, respectively.

### DISCUSSION

In this study, we evaluated four emerging biomarkers, GLDH, PNP, MDH, and PON1, as indicators of liver injury in the human population. We assessed the performance of these markers in healthy subjects and subjects with liver impairment. In addition, we evaluated the emerging biomarkers (GLDH and MDH) in six cases of acetaminophen toxicity.

Levels of GLDH and MDH were stable across healthy volunteers participating in clinical trials (CRU) and healthy subjects (UM) and were not impacted by gender or age. The reference ranges for both GLDH and MDH levels in healthy subjects detected in our studies (GLDH, 1–10 U/L; MDH, 79–176 U/L) were similar to levels cited in the literature (Kawai and Hosaki, 1990; Misra et al., 1991; Schmidt and Schmidt, 1988; Van Waes and Lieber, 1977). On the other hand, both PNP and PON1 exhibited a larger variability. Although the range of PON1 established in this study showed the largest variability among the four markers, it was similar to published ranges (Ferré et al., 2002; Mackness et al., 1998). The interindividual variability may be explained by genetic determinants and to some extent by nutrition and lifestyle factors (Camps et al., 2009; Ferré et al., 2002; Mackness et al., 1998). In our study, PON1 levels were not affected by age of subjects. This is inconsistent with a published study that included 129 healthy Caucasian subjects (aged 22–89) in which PON1 activity significantly decreased in the age groups 46–65 and > 65 years compared with subjects < 45 years (Seres et al., 2004). In our study, a statistically significant difference in PON1 levels was observed between the CRU healthy volunteers and the UM healthy subjects. This was possibly due to the ethnic differences between the two groups. UM healthy subjects were primarily Caucasian (87%), whereas the CRU healthy volunteers were predominantly African American (57%). Indeed, similar ethnic-based differences in PON1 levels have been reported previously (Thyagarajan et al., 2008). Although serum PNP levels are often reported in rodents, limited data are available.
on serum PNP levels in healthy humans. Although, in our study, the serum PNP activity was measured by an automated clinical chemistry analyzer (0–6 U/L), the results were in agreement with levels measured by HPLC in previous studies (~3.0 ± 1.5 U/L) (Roberts et al., 2004). The fact that measurable levels of all four markers (GLDH, PNP, MDH, and PON1) in normal healthy populations were comparable to those reported in the literature indicates that enzymatic activity–based assays for GLDH, PNP, MDH, and PON1 provide a suitable method for their detection.

In our studies, GLDH had the strongest correlation with elevated ALT levels and exhibited the greatest predictive power for liver injury, as determined by ROC analysis. Because GLDH is not abundant in muscle tissue (Mastorodemos et al., 2005) and not directly linked to any specific disease or injury, the GLDH increases are considered a clinically informative indicator of liver disease (Schmidt and Schmidt, 1988; Van Waes and Lieber, 1977) and a more liver-specific biomarker than ALT (O’Brien et al., 2002). Similar to ALT, elevations of GLDH can be a result of enzyme release from cellular membranes due to hepatocellular necrosis. In a recent publication (Harrill et al., 2012), elevations in GLDH, similar to those observed with ALT, were reported in healthy volunteers after treatment with heparins and were attributed to mild hepatocyte necrosis. Much like ALT, serum elevations of GLDH may be due to hepatic induction of the enzyme in response to certain drugs (Shimizu et al., 1997) and glucocorticoids (Hardin-Pouzet et al., 1996; Timmerman et al., 2003). Therefore, the potential added value of GLDH as a DILI biomarker needs to be further evaluated. In our study, with the exception of acetaminophen toxicity, the underlying disease or diagnosis did not alter the correlation of GLDH with ALT. The lower correlation of GLDH with ALT in subjects with APAP toxicity could reflect the time course kinetics of GLDH (Fig. 3). Further research is needed to elucidate whether GLDH would add predictive value or provide a better prognostic measure for DILI outcomes as part of a liver panel in the clinic.

Although MDH had a high correlation with ALT levels and a high predictive ability in our study, only a few reports evaluating MDH as clinical biomarker of hepatotoxicity have been published. MDH has been used to distinguish subjects with liver disease (cirrhosis, hepatitis, and hepatocellular carcinoma) from healthy subjects (Kawai and Hosaki, 1990; Misra et al., 1991) and has been used as a biomarker for heart disease and hypertension (Goldberg et al., 1983; Misra et al., 1991). Interestingly, MDH showed no correlation with the CAD-CHD (r = 0.48) subjects in this study. MDH was strongly correlated with ALT and had positive predictive power for liver injury, as per ROC analysis. Although MDH levels in the serum are influenced by
injury and disease in other organs, this work offers impetus to further evaluate MDH as a useful biomarker of liver injury.

Our data demonstrate that PNP is not a suitable marker for the detection of liver injury, increasing less than twofold in over 80% of the subjects who met the liver injury criteria in our analysis. Although there are numerous publications detailing the value of PNP as a marker of liver damage in rodents (Öz et al., 2008; Vaidya, 2010), very little literature is available to support its use as a hepatic marker in humans. Our data show that PNP does not discriminate between healthy subjects and those with liver injury and thus is not a reliable marker of hepatic injury in humans.

Unlike PNP, there is literature weighing the relative value of PON1 as a biomarker of liver injury, demonstrating that PON1 has a diagnostic value in chronic liver disease and that a decrease in PON1 activity is independent of PON1 genotype (Ferré et al., 2002; Keskin et al., 2009). However, it has also been shown that serum PON1 activity is not liver specific, as indicated by varying levels in subjects with insulin-dependent diabetes, heart disease, and vasculitis (Mackness et al., 2001, 2002; Rozek et al., 2005). The lack of predictivity of PON1 as a marker of liver injury in our study may be partially due to the variability seen in the control populations. This large variation is well documented in the literature, with as much as a ~40-fold difference among individuals (Ferré et al., 2003). Even though these variations can be explained by both genetic and environmental factors, such wide variability in the healthy population complicates the interpretation and as such limits the marker’s value as a clinical indicator of hepatic injury.

Monitoring GLDH and MDH levels in subjects with acetaminophen toxicity provides the first example of GLDH and MDH as biomarkers of DILI in humans. In a set of six subjects, we observed two patterns in the GLDH and MDH response. In the first group (subjects 1–3), activity levels of GLDH and MDH showed a similar rate of decline as ALT and AST. In contrast, levels of GLDH and MDH in subjects 4–6 decreased at a faster rate, whereas the level of ALT decreased only slightly. The observed levels of ALT, AST, MDH, and GLDH in serum are dependent on their rate of release from liver tissue, subsequent distribution, and final elimination from circulation (Ramaiah, 2001). Even though this decrease of serum activities of these two markers. The faster rate of decline observed with GLDH and MDH may, however, better reflect the liver’s recovery from injury and thus show added value in comparison to ALT. The clinical importance of this observation could not be fully explored in this retrospective study design and remains to be fully examined in subsequent studies.

To our knowledge, our study is the first to systematically evaluate emerging biomarkers (GLDH, MDH, PON1, and PNP) of liver injury in a large cohort of human subjects. We have established reference ranges for GLDH and MDH. Both GLDH and MDH showed promise as biomarkers of hepatotoxicity. In contrast, PON1 and PNP did not detect liver injury in human subjects. The retrospective design of our study did not allow for fully assessing the added value of GLDH and MDH as biomarkers of liver injury. Nevertheless, our data provide crucial impetus for further prospective clinical studies to fully evaluate the diagnostic value of GLDH and MDH for detection of liver injury.

**FUNDING**

This study was funded by Pfizer, Inc.

**REFERENCES**


Kawai, M., and Hosaki, S. (1990). Clinical usefulness of malate dehydrogenase and its mitochondrial isoenzyme in comparison with aspartate...


