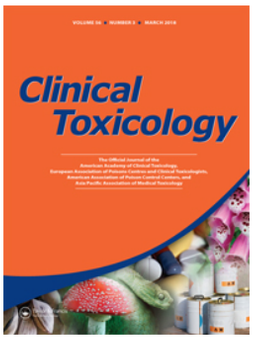

Clinical Toxicology

ISSN: 1556-3650 (Print) 1556-9519 (Online) Journal homepage: www.tandfonline.com/journals/ictx20

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To cite this article: Nicholas Nacca, Michael J. Hodgman, Kirselle Lao, Matthew Elkins & Michael G. Holland (2018) Can elevated lactate and LDH produce a false positive enzymatic ethanol result in live patients presenting to the emergency department?, Clinical Toxicology, 56:3, 189-192, DOI: 10.1080/15563650.2017.1357825

To link to this article: https://doi.org/10.1080/15563650.2017.1357825

Published online: 16 Aug 2017.

CLINICAL TOXICOLOGY, 2018
VOL. 56, NO. 3, 189–192
https://doi.org/10.1080/15563650.2017.1357825

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CLINICAL RESEARCH

Can elevated lactate and LDH produce a false positive enzymatic ethanol result in live patients presenting to the emergency department?

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ABSTRACT

Background: There have been allegations in the courtroom that elevated serum lactic acid in trauma victims can yield a falsely elevated serum ethanol assay. Most hospitals utilize an indirect method of ethanol measurement where a serum sample is added to a mix of alcohol dehydrogenase and oxidized nicotinamide adenine dinucleotide (NAD+). This allows any ethanol in the patient's serum to be metabolized to acetaldehyde, and in the process results in the reduction of NAD + to NADH. NADH is then measured using spectrophotometry. The courtroom allegation stems from the concept that oxidation of lactate to pyruvate by lactate dehydrogenase (LDH) results in the same molar-for-molar reduction of NAD + to NADH, and could therefore theoretically cause patients with elevated lactate and LDH to have a falsely elevated ethanol concentration.

Methods: Patients with elevated lactic acid and LDH concentrations who presented to a university hospital from 20 April 2015 to 13 December 2015 were identified to provide possible test specimens. If a sufficient amount of serum was available, the sample was used to re-run the lactate and LDH concentration simultaneously with an enzymatic ethanol assay. Any samples that had elevated lactic acid and LDH concentrations on this retesting, and also yielded a positive ethanol concentration, were sent for confirmatory gas chromatography testing of ethanol concentrations. A control group of 20 samples with normal lactate and LDH were included.

Results: A total of 37 samples were included in the final analysis. Only 4 patients had an elevated enzymatic ethanol concentration, and all 4 also had a measurable GC ethanol concentration. The lactate in this dataset ranged from 2.4 to 24.2 mmol/L, with a mean of 6.53 mmol/L (normal value 0.5–2.2). The LDH ranged from 242 to 8838 U/L with a mean of 1695 U/L (normal value 122–225 U/L). Twenty control samples were run on patients with normal lactate and LDH, none of which yielded a positive enzymatic ethanol result.

Conclusions: This data does not support the contention that an elevated LDH and lactate can yield a false positive serum ethanol result as run by enzymatic ethanol assay in live patients presenting to the emergency department.

ARTICLE HISTORY
Received 22 April 2017
Revised 18 June 2017
Accepted 6 July 2017
Published online 16 August 2017

KEYWORDS
Ethanol assay; alcohol assay; false positive ethanol; false positive alcohol; lactate interference; lactate; lactic acid; ethanol; lactate dehydrogenase

Introduction

There have been allegations in the courtroom that simultaneously elevated serum lactic acid (lactate) and lactate dehydrogenase (LDH) in trauma victims can result in a falsely elevated serum ethyl alcohol (ethanol) assay result [1]. Most hospitals utilize an indirect method of ethanol measurement where a serum sample is added to a mix of alcohol dehydrogenase (ADH) and oxidized nicotinamide adenine dinucleotide (NAD+). This allows any ethanol in the patient's serum to be metabolized to acetaldehyde, and in the process results in the reduction of NAD + to NADH. NADH is then measured using spectrophotometry, and the concentration is correlated to a serum ethanol concentration.

The courtroom allegation stems from the concept that oxidation of lactate to pyruvate by LDH results at the same molar-for-molar reduction of NAD + to NADH, and can therefore cause patients with elevated lactate and LDH to have a falsely-elevated ethanol concentration (Figure 1). The possibility of a false positive ethanol result by this mechanism is also commonly taught by toxicology educators in toxicology fellowships and conferences. Though this notion has led to *in-vitro* measurement and analysis which suggests the possibility of a false positive result, to the authors' knowledge, it has never been prospectively investigated in live patients.

$$\text{Ethyl alcohol} + \text{NAD+} \xrightarrow{\text{ADH}} \text{Acetaldehyde} + \text{NADH}$$

$$\text{Lactate} + \text{NAD+} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

Figure 1. Chemical reactions forming the basis of suggestion that false positive ethanol may result from patients with elevated lactate and LDH. ADH: alcohol dehydrogenase; LDH: lactate dehydrogenase.

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Methods

The institutional review board approved this study with waiver of consent. We sought to determine if a false positive ethanol result could be obtained from patients with elevated lactate and LDH, but negative ethanol by gas chromatography. Patients presenting to a single university hospital emergency department between 20 April 2015 and 13 December 2015 who had both a lactic acid and LDH concentration ordered as standard of care were eligible for inclusion. Patients with concomitant elevations of both lactic acid and LDH concentrations were identified by an automatically generated secure email system that notified the research staff of results on a daily basis. Research staff then identified and located samples which had extra serum available after all patient care oriented assays had been run. Samples were stored in a freezer regulated between 2 and 8 °C. LDH and ethanol were drawn in plastic serum separator tubes (BD vacutainer 367983), lactic acid was drawn in plastic sodium fluoride/potassium oxalate tubes 10mg/8mg (BD vacutainer 367922). If a sufficient amount of serum was available, the sample was used to re-run the lactate and LDH concentration and ethanol concentration by enzymatic assay for study purposes. These repeat tests were performed in order to control for degradation that may have occurred during sample storage resulting in an unmeasured confounding variable. All samples were run on a P800 Roche Modular Analyzer with commercially available kits (ethyl alcohol: Roche Diagnostics, reference number 11776312 190; LDH: Roche Diagnostics, reference number 03002209 122; lactate: Pointe Scientific Inc, reference number L7596-50). Any samples that had elevated lactic acid and LDH concentrations on retesting and yielded a measurable ethanol concentration were sent for confirmatory gas chromatography-flame ionization detector testing (GC-FID: Agilent 6890 series; headspace: G1888, column screen: Agilent DB-ALC2; quantitation on Agilent DB-ALC1). No study results were reported to the medical treatment team. A control group of 20 samples with normal lactate and LDH were identified and repeat lactate and LDH were performed simultaneously with an enzymatic ethanol assay. Patient age, sex, and admission diagnosis were recorded and all data were entered into an excel spread sheet.

Results

A total of 46 patients were identified as having concomitantly elevated serum lactate and LDH during the study period. For two patients there were no samples associated with the identified accession number. Two patients were incorrectly identified and did not have elevated lactate or LDH. In four patients repeat testing yielded a normal lactate result. In one other patient, there was an insufficient amount

Table 1. Results of serum ethanol, lactate, and LDH from patients included in analysis.

Age and gender	Admission diagnosis	Lactate (mmol/L)	LDH (U/L)	Enzymatic ethanol (g/dL)	GC ethanol (g/dL)
59 M	Smoke inhalation	7.1	335	0.29	0.25
62 M	Cellulitis	4.2	303	0.01	0.007
59 M	Ethanol intoxication	3.3	242	0.02	0.01
23 M	Gastrointestinal hemorrhage, NSTEMI	11.9	8119	0.07	0.05
84 F	Sepsis	5.48	5751	0	NP
55 M	Sepsis	2.9	258	0	NP
68 M	Sepsis	8.0	330	0	NP
81 F	Sepsis	6.1	526	0	NP
53 M	Sepsis	6.2	310	0	NP
72 M	Sepsis	4.5	590	0	NP
58 M	Sepsis	10	676	0	NP
30 M	Sepsis	4.0	3623	0	NP
85 M	Sepsis	9.0	2018	0	NP
62 F	Sepsis	9.5	554	0	NP
60 M	Metastatic prostate cancer	10	8160	0	NP
54 M	Metastatic lung cancer	4.6	1165	0	NP
67 M	Metastatic lung cancer	3.0	3405	0	NP
54 M	Metastatic unspecified cancer	5.6	1746	0	NP
54 F	Metastatic pancreatic cancer	3.6	297	0	NP
51 M	Acute myocardial infarction	10.7	8838	0	NP
20 M	Cardiac arrest	3.1	581	0	NP
67 F	Acute myocardial infarction	24.2	1071	0	NP
44 F	Acetaminophen toxicity	4.5	599	0	NP
85 F	Altered mental status	5.9	462	0	NP
67 F	Acute respiratory failure	4.3	313	0	NP
81 M	Autoimmune hemolytic anemia	3.7	903	0	NP
45 F	COPD exacerbation	7.4	289	0	NP
52 F	Gangrene	4.0	383	0	NP
26 M	Hemophagocytic syndrome	5.2	791	0	NP
92 F	Idiopathic thrombocytopenic purpura	3.1	615	0	NP
64 M	Ischemic colitis	4.1	496	0	NP
26 M	Neutropenic fever	20	1293	0	NP
70 F	Neutropenic typhlitis	8.6	260	0	NP
81 F	Peritonitis	4.9	323	0	NP
40 M	Pyelonephritis	2.4	1853	0	NP
66 F	Rhabdomyolysis	3.2	4866	0	NP
55 M	Spontaneous bacterial peritonitis	3.4	373	0	NP

LDH: lactate dehydrogenase; GC: gas chromatography; NP: not performed.

of blood available for analysis. This resulted in a total of 37 samples remaining to be included in the final analysis.

The mean age was 59 years, and 62% of the patients included were male. The most common admission diagnosis was sepsis, followed by metastatic cancer. Only 4 of the 37 patients had an elevated enzymatic ethanol concentration, and all 4 also had a measurable GC ethanol level (Table 1). Of those patients included in the analysis, the lactate ranged from 2.4 to 24.2 mmol/L, with a mean of 6.53 mmol/L (normal value 0.5–2.2). The LDH ranged from 242 to 8838 U/L with a mean of 1695 U/L (normal 122–225 U/L). Twenty control samples were run on patients with normal lactate and LDH, none of which yielded a positive enzymatic ethanol result. Of note, one patient included in the analysis was admitted with a diagnosis of ethanol intoxication. The initial ethanol level was 0.2 g/dL, however, the repeat assay as per study protocol was 0.02 g/dL, likely owing to volatilization in the time period in between assays.

Discussion

In 1992, while looking for biomarkers in Sudden Infant Death Syndrome, Australian researchers noted that a commercially available homogenous enzyme linked immunoassay for ethanol was positive in several postmortem infant plasma and vitreous humor samples. They noted that gas chromatography was negative for ethanol in these same samples, and concluded that use of this enzymatic assay is unreliable postmortem, presumably due to elevated LDH and lactate levels [2]. In 1994, Thompson et al. reported two cases of critically ill patients with severe lactic acidosis that were found to have profoundly elevated ethanol concentrations by enzyme linked assay testing. Rerunning these samples after protein free ultrafiltration (i.e., removal of LDH) resulted in normalization of the false positive ethanol [3]. The survival of these patients was not reported, and the enzymatic assay used to measure serum ethanol is not likely to be in use any longer. The observation that two post mortem pediatric cardiopulmonary arrest patients had positive ethanol concentrations by enzyme linked assay led another group to investigate this phenomenon. In 1995, Nine et al. used postmortem blood samples to test three commercially available enzymatic screening assays that utilize the enzymatic process of ethanol to acetalydehyde by the enzyme alcohol dehydrogenase. The conclusion was that one commercially available test, in particular, (Syva) led to more false positive ethanol concentrations than did other assays (Roche and Abott). Two critically ill patients were identified as having false positive ethanol results on the Syva, but not Roche or Abott. The survival of these patients was not reported. The same study confirmed that all three assays were subject to false positive results when the samples were spiked with exogenous lactate and LDH. The authors suggested that due to the possibility of false positive results, testing should be interpreted carefully [4]. A letter to the editor on this article pointed out that the conclusions are based on postmortem blood samples, and such an extrapolation to a live patient population is irresponsible given the potential medico-legal ramifications [5].

These observations showed potential clinical relevance in a case such as that presented by Powers and Dean in 2009. They described a case where a patient in a motor vehicle collision was found to have mild liver injury and an elevated ethanol concentration by enzyme linked testing. The defendant in the case claimed that minor liver injury and subsequent lactic acidosis (which was not measured by the medical team) resulted in false positive ethanol. The authors were able to indirectly refute this claim based on extrapolation from the available laboratory investigations which did not suggest an elevated LDH or anion gap metabolic acidosis sufficient to result in a lactic acid high enough to cause a false positive ethanol concentration.

To the authors' knowledge, no studies using unadulterated blood specimens from living adults hospitalized with an acute illness using modern assays have been performed to evaluate whether this phenomenon is clinically relevant. The only available cases in the literature suggesting the possibility of false positive were perimortem, postmortem, or performed on antiquated equipment.

In the present study, no false positive ethanol concentrations were identified in living patients with elevated LDH and lactate. Of note, the ethanol concentrations from GC/FID in the 4 patients with positive enzymatic ethanol results were slightly, though consistently, lower than the initial assay. It is possible, that the lactate and LDH in these samples contributed to the slightly higher result on the enzymatic assay. Interpreted in the context of the rest of the data set, it seems more likely the some ethanol volatilized between assays. There were several limitations to this study. There was no a priori power analysis performed due to the extremely low predicted false positive rate. The relatively small sample size of the study is a significant limitation. During the study period there were no trauma victims meeting inclusion criteria. This is likely the result of the rarity in which a treatment team would order a lactate and LDH concentration in a trauma victim. Trauma victims are known to have elevations in both LDH and Lactate that are thought to correlate with severity of illness. In a retrospective study of 75 abdominal trauma victims with known liver injury, the range of LDH was 106–2,577 IU/L [6]. Another retrospective study of 5995 general trauma patients identified a range of Lactate to be 0–40 mmol/L [7]. In the authors' opinion, it is unlikely that the mechanism leading to elevation of LDH and lactate has influence over the result of the enzymatic ethanol assay given that false positives have been demonstrated in vitro spiked blood samples. While this study only applies to the Roche chemistry analyzer used by our lab, it likely applies to the other available products. Data from the College of American Pathologists (CAP) lactate dehydrogenase proficiency testing statistical evaluation indicates that 21% of 3000 labs use the Roche analyzer, but there are 4 other analyzers in common use (Beckman, Siemen's, Vitros, Abbott). The CAP's serum ethanol proficiency testing event treats this assay similarly regardless of the analyzer manufacturer, because the assay is so similar across all platforms. Therefore, it is likely these results would be generalizable to the other chemistry analyzers. It is known that samples spiked with LDH and lactate, run on the same equipment used in this

study can result in false positive ethanol assay, and the minimum LDH required is 26,366 IU/L which must be associated with a lactate of at least 14mM. The minimum lactate required is 4mM which must be associated with an LDH of 43,991 IU/L [4]. No patients in this study had serum concentrations that matched those spiked samples. This suggests that obtaining these concentrations in a live patient is extremely unlikely, and when lactate and LDH concentrations are this high, it is usually a perimortem or postmortem finding. Repeat testing with GC/MS or GC/FID, or repeat enzymatic testing after ultrafiltration to remove LDH can accurately confirm or refute the presence of ethanol. The present study does not support the contention that an elevated LDH and lactate can yield a false positive serum ethanol result as run by enzymatic ethanol assay in live patients presenting to the emergency department.

Acknowledgements

The authors would like to thank the Onondaga County Medical Examiner's Office for their assistance in provision of advanced laboratory testing.

Disclosure statement

The authors of this manuscript have no conflicts of interest.

Disclaimer: The views expressed in the submitted article are his or her own and not an official position of the institution or funding source.

Funding

This study was funded by the 2015 AACT Research Award.

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