

Appraisal of Analytes in the Laboratory Panels for Liver Function Tests: Do we Need Aspartate Aminotransferase and Direct Bilirubin in the Panels? Running Head: AST and Direct Bilirubin in Lab Panels

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Abstract

Background: The Centers for Medicaid and Medicare Services approved a number of panels as screening tests including those for liver functions. The utility of routinely testing for Aspartate aminotransferase (AST) and direct bilirubin was questioned and empirical data analyzed to suggest alternatives.

Methods: Tests done over six months were examined for AST, ALT, total and direct bilirubin values. Panels in which AST exceeded the ALT value by 40 units or more and the AST/ALT ratio was 2.0 or greater, were subjected to review of medical records. The incidence of elevated direct bilirubin at various levels of total bilirubin was assessed.

Results: 2.9% of the AST and ALT tests involving 379 patients met the criteria of $AST \geq ALT$ by 40 units and $AST/ALT \geq 2.0$. Alcohol use/abuse was the primary reason for the AST-ALT abnormality in only 49.3% of the patients. AST-ALT levels and ratio were useful in revealing alcohol abuse in perhaps one patient. Bilirubin results revealed that direct bilirubin was elevated in only 0.4% of the samples if the total bilirubin was <0.9 mg/dL.

Conclusions: AST determinations do not add meaningful information about liver function and removing this analyte from the panels will not materially affect the usefulness of results. Testing for direct bilirubin should be changed to reflex testing only if total bilirubin is ≥ 0.9 mg/dL. Not performing AST and direct bilirubin routinely has the potential of saving $> \$92$ MM/year.

Keywords: CMS Panels; Liver Panel; AST; Bilirubin; Direct bilirubin; ALT/AST ratio

Introduction

The Centers for Medicare and Medicaid Services (CMS) approved a number of panels for screening tests, such as basic metabolic panel, liver function panel, renal panel, comprehensive metabolic panel and lipid panel etc [1]. The ostensible purpose of approving these panels was to facilitate billing and avoiding overbilling by billing for the analytes in these panels individually. Even though providers may not need all of the items in the panel, as long as some items are needed, the whole panel is performed with consequent over-utilization of some tests. However; given the nature of the *CMS rules and regulations* and lack of local choice in modifying the panels, we all continue to perform these panels, without a critical appraisal of the medical need, utility and advisability of the practice. While the clinicians are free to order the tests individually, this is not the usual practice. Most hospital laboratories limit the individual tests that are part of a panel so as to not run afoul of the unbundling regulation of CMS.

The liver function panel consists of seven analytes; namely, total serum proteins, albumin, total bilirubin, direct bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [1]. All of these analytes are measured even if results of only some are needed. AST and ALT are also included in the

14 analyte comprehensive metabolic panel (CMP), though this panel has only total bilirubin but not direct bilirubin [1]. An example of uncritically following the panel is performing direct bilirubin even when the total bilirubin is well within the normal limits. Arguably, no useful information is gathered by performing the assay for direct bilirubin when total bilirubin is normal; however, laboratories do not have the option to deviate from the CMS panels by creating a reflex rule to not do direct bilirubin if the total bilirubin is at less than a given concentration.

Another likely example of less than useful practice of adherence to the liver panel is performing AST testing in all cases. Informal consultation with the clinical colleagues revealed that they use the AST values in general and AST/ALT ratio in particular to ascertain effects of alcohol intake [2]. The De Ritis ratio apparently is still in use despite there being easier and better ways to determine alcohol intake, especially taking history, as well as other markers of alcohol use, at least in the usual healthcare settings [3]. The increase in AST, more than that in ALT, generally suggests hepatocyte mitochondrial injury and/or necrosis and is the basis for using AST/ALT ratio for alcoholic liver injury [4,5]. Alcohol can increase AST through mitochondrial injury without causing hepatocyte necrosis, though alcohol is an important cause of hepatocellular necrosis. The ratio of mitochondrial AST to total AST is better for this purpose; however, few laboratories measure mitochondrial AST [6,7].

Both AST and ALT are liver enzymes and are elevated in circumstances of hepatocyte injury. ALT is present predominantly in the cytoplasm whereas AST is present in cytoplasm and hepatocyte mitochondria as well. ALT is present mainly in liver and kidney, whereas AST is present in many other tissues, including heart, skeletal muscle, kidney, pancreas, spleen, lungs, and red blood cells. AST content of heart is higher than that of liver. The relative concentration of AST and ALT in various tissues is presented in (Table 1) [8]. ALT is present in highest concentration in the liver, followed by decreasing amounts in kidney, heart, skeletal muscle, pancreas, spleen, lungs and red blood cells. The ALT content is more than twice as high in the liver as in the kidney, which the second richest organ in ALT. The half-life of ALT in serum is about 47 hours and that of AST is much shorter 17 hours; however, mitochondrial AST has a much longer half-life of 87 hours. There is greater circadian variability in ALT levels than for AST. AST levels increase modestly with age whereas ALT is not affected by aging. ALT levels are higher in men than in women and may be further depressed by female sex hormones, including birth control pills [6,7]. The usual reference ranges for ALT are 34 and 45 in women and men, respectively and corresponding values for AST are 31 and 35, however, informally 40 units is used as the upper limit for both analytes [6,7,9].

Tissue	AST	ALT
Heart	7800	450
Liver	7100	2850
Skeletal muscle	5000	300
Kidney	4500	1200
Pancreas	1400	130
Spleen	700	80
Lungs	500	45
Red Blood Cells	15	7

Table 1: Relative AST and ALT content of various tissues: The enzyme concentration of serum is taken to be unity or one [8]. Relative tissue content of AST and ALT with serum concentration taken as one.

The upper limit of normal for total bilirubin in adults is generally accepted to be 1.2 mg/ dL, though some sources list the upper limit to be as high as 2.0 mg/dL [10]. The normal levels of direct bilirubin are considered to be ≤ 0.3 mg/dL [7,9,10]. These values refer to methods using the diazo reagents that measure direct and indirect bilirubin, but not conjugated and unconjugated bilirubin specifically [11]. If conjugated bilirubin is measured specifically, most healthy individuals have virtually undetectable levels of conjugated bilirubin [12].

The usefulness of routine AST testing in the liver function panel and comprehensive metabolic panel; and direct bilirubin, as part of the liver panel, was conducted by retrospective review of all tests done over a six month period and the findings are reported here.

Methods

The study was conducted at a two-campus medical center in the Mid-west, United States. One campus is located in downtown, in a medically underserved area, has 242 approved beds. It is the primary teaching hospital for the affiliated medical school, is a level one trauma

center and serves a largely indigent, multi-ethnic, uninsured population. The second campus is in a suburban location and has 333 approved beds, including 188 long term care (nursing home) beds. This campus provides primarily family medicine services. Both campuses serve as the safety-net hospitals for the county. The assays for AST, ALT, total and direct bilirubin's were performed in a CLIA-88 certified laboratory by using Beckman Coulter UniCel Dx C 880i analyzers. Similar equipment and reagents were used at both institutions and both are CLIA certified laboratories.

The medical centers use CERNER for electronic medical record system; both hospitals have electronic order entry and remote access to the records.

The study was approved by the Privacy Board of the medical center and the Adult Health section of the Institutional Review Board of the University.

All laboratory analyses with AST and ALT results from the liver function and comprehensive metabolic panels were gathered for a period of six months in 2013.

The initial review and analysis was done for results from each month and the data were then pooled for the final summary. The process for selecting appropriate medical records for review was as follows: The AST and ALT results for each testing episode for each patient were aligned in spreadsheets. ALT and AST values were expressed in Units/Liter. ALT values were subtracted from AST values and episodes of testing in which AST minus ALT was ≥ 40 were selected. This selected population of test results was further subjected to mathematical manipulation to select episodes in which AST/ALT ratio was ≥ 2.0 . Results from adult patients only were subjected to further review and analysis. The number of newborns excluded was six and four subjects were excluded due to lack of clinical data in the medical records.

The medical records for the patients meeting these selection criteria were reviewed to ascertain the cause of disproportionate elevation of AST, in particular to ascertain the usefulness of such finding in detecting alcohol use/abuse. In addition to reviewing the clinical notes, the following laboratory parameters were recorded: serum creatinine, albumin, total bilirubin, alkaline phosphate, AST, ALT, gamma glutamyl transferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), hemoglobin, mean corpuscular volume (MCV), hepatitis C status, and blood alcohol level. Additional items collected in the data sheet were age, sex, BMI, presence of diabetes mellitus, and items in clinical history relevant to liver functions and serum levels of AST, e.g., hemolysis, hematoma, heart and skeletal muscle pathology, alcohol and substance use/abuse, tissue necrosis usually in association with malignancy, pancreatitis, gall stones, blood transfusions, seizures, trauma, HIV infection, sepsis and septic shock, pre-eclampsia, hypo- or hyper-thyroidism etc. An attempt was made to ascertain the primary cause of AST elevation. A particular note was made if there were any records of comments on the AST levels or AST/ALT ratio in the clinical notes.

The testing episodes meeting the above stated criteria for AST minus ALT value and AST/ALT ratio were subjected to medical record review for each month. Only one set of data were created for a given patient who had been tested multiple times. The data collected for each of the six months were pooled and duplications were eliminated but the clinical data from each patient for each month was retained in the pooled file.

Results for total and direct bilirubin testing done over six months were also extracted. The results for direct bilirubin, for instances in which total bilirubin ranged from 0 to 0.8 mg/dL, 0.9 mg/dL, 1.0 mg/dL, 1.1 mg/dL and greater than 1.1 mg/dL, were stratified. The number of direct bilirubin values at <0.3 mg/dL, 0.3 mg/dL, 0.4 mg/dL and >0.4 mg/dL were identified for each of the subgroups of total bilirubin results.

Yr-2013	Number of tests Meeting criteria	Number of test episodes meeting criteria for chart review	Pooled unique Patient/month
April	4742	142	89
May	4539	104	69
June	4331	146	69
July	4676	112	77
August	4615	134	78
September	4220	145	80
Total	27123	783	462
Meeting criteria=2.9%		Unique Patients in data set=379	

Table 2: The numbers of AST and ALT tests done in each month are listed. The total number of tests was 27,123 for an estimated yearly volume of 54,246. The number of episodes of testing meeting the criteria of AST-ALT ≥ 40 and AST/ALT ≥ 2.0 are given in column three. The number of unique patients accounting for these tests, in each month is given in column 4. Unique patient lists from each of the months were pooled and duplicates were eliminated, leaving a set of 379 patients who met the selection criteria. The clinical information about the patients, in the various months, was retained during the pooling process.

Results

The total number of instances in which AST and ALT were measured in six months was 27123 for an annual volume of 54246. The frequency of Liver panels and CMPs was 17186 and 9937, respectively. In fewer than 3% of the episodes, AST value was ≥ 40 units higher than ALT and the AST/ALT ratio was ≥ 2.0 . The numbers of patients meeting this criterion for each month are shown in table 2. When the results for six months were pooled there were 379 patients who met the established criteria suggestive of alcohol use/abuse, i.e., AST>ALT by 40 or more units and AST/ALT ratio ≥ 2.0 . There were only three patients in whom the AST value was < 60 and the main reason for an AST/ALT ratio ≥ 2.0 was low ALT. Alcohol use/abuse was the commonest primary cause for this abnormality and accounted for about half the patients. The results showing the primary cause of enzyme abnormalities are presented in (Table 3).

Likely primary explanations for AST>ALT by 40 units or more and AST/ALT ≥ 2.0		
Cause	Number of patients	% of total patients
Alcohol	187	49.3
Hepatitis C	23	6.1

Heart	31	8.2
Skeletal muscle disease	28	7.4
Trauma	17	4.7
Malignancies	18	4.7
NASH	14	3.7
Hb SS	13	3.4
Medication effects	7	1.8
Gall Stones	6	1.6
Seizures	5	1.3
HIV/AIDS	5	1.3
Sepsis	4	1.1
Pre-eclampsia	3	0.8
Hemolysis	2	0.5
Hematoma	2	0.5
Malnutrition	2	0.5
Myxedema	1	0.3
Primary sclerosing cholangitis	1	0.3
Unresolved	10	2.6
Total =379		

Table 3: Most of the patients had multiple pathologies and many had more than one potential explanation for the altered AST-ALT enzyme pattern. The likely dominant etiologic factor was determined from the laboratory data and clinical history. Further breakdown of the various malignancies is presented in table 4. The pathologies present in the ten cases in which the primary cause could not be ascertained with confidence are given in table 5

The average age of the patients was 49 years. There were 215 men and 164 women. This is notable because the general ratio of men to women served by the medical centers is about 2:3 and probably reflects the higher rate of alcohol and substance abuse in men. The average BMI of the 371 patients for whom this information was available was 27.2. Diabetes mellitus was one of the diagnoses in 69 of the 379 patients and hepatitis C was noted in 80 patients.

It is worth noting that in all 187 cases, in which alcohol use/abuse was determined to be the primary cause of liver enzyme abnormality, there was clearly documented history of alcohol abuse and the altered AST-ALT enzyme results were not a revelation in any of the patients. The one possible exception may have been the single patient who was supposed to be under treatment with Acamprosate and supposedly not taking alcohol but still had altered liver enzymes suggesting that she was not abstinent. In only four of the patients was the altered AST/ALT ratio commented upon in the medical records.

Hepatitis C was judged to the primary cause of AST-ALT enzyme alteration in 23 cases or 6.1% of the patients. However, hepatitis C infection was noted in 80 patients. As expected, alcohol abuse and hepatitis C infection frequently co-existed. Alcohol abuse was judged

to be the primary cause of liver function abnormalities when elevated blood alcohol levels were part of the laboratory test results or there were other stigmata of alcohol use, e.g., elevated mean corpuscular volume (MCV) and or elevated HDL. Information in the clinical notes was also taken in consideration in ascertaining the primary cause of pathology between hepatitis C and alcohol.

Next to alcohol abuse, cardiac injury, mostly acute coronary syndrome, was the commonest primary explanation for elevation of AST more than ALT and was noted in 31 patients or 8.2% of the cases. Cardiac disease, in the form of acute coronary syndrome, cardiomyopathy, ischemic heart disease, and trauma, was noted in a total of 59 patients. Rhabdomyolysis accounted for 28 cases or about 7.4% of the patients with AST-ALT enzyme abnormalities. The issues causing rhabdomyolysis were generally substance abuse and often included alcohol intake; however, these patients had marked elevations of creatine kinase that was not seen in patients for whom alcohol was assigned as the primary cause of liver disease and AST-ALT alterations. The next common cause of altered AST-ALT enzymes was trauma affecting skeletal muscles and other organs and was the primary cause of enzyme abnormalities in 17 cases comprising 4.7% of this population. Skeletal muscle pathology of one form or another was present in 74 of the cases. In many of the trauma patients, alcohol was an underlying factor in prompting the falls, motor vehicles accidents and assaults. In all, 233 of the 379 patients had recorded history of alcohol intake.

Advanced malignancy with tissue necrosis, usually due to chemotherapy and/or radiation, was suspected to be the primary cause of AST-ALT enzyme anomalies in 18 patients or 4.7% cases. Metastatic breast carcinoma was the tumor responsible for nearly half the patients, accounting for 8 of the 18 cases with malignancy. A breakdown of the various malignancies is shown in (Table 4).

Primary Cancer	Number of patients
Breast	8
Lung	2
Colon	2
Cervix	1
Carcinomatosis	1
Bile duct	1
Lymphoma	1
Kidney	1
Mesothelioma	1

Table 4: Advanced malignancy was judged to be the primary cause of AST and ALT enzyme alterations in 18 cases. Most of the patients had disseminated tumors and were treated with chemotherapy and/or radiation. Breast carcinoma accounted for nearly half of the 18 cases

Non-alcoholic steatohepatitis (NASH) was the likely primary cause of enzyme abnormalities in 14 cases (3.7%). The diagnosis of NASH was mostly presumptive in patients with obesity and other markers of metabolic syndrome and without history or other evidence of alcohol abuse. The diagnosis had to be presumptive in part due to the fact that

care for many of these patients was limited to visits to the Emergency Department (ED).

Hemoglobin S disease and resultant hemolytic anemia was the primary cause of elevated AST in 13 patients (3.4%). Other primary causes affecting fewer than 10 patients each were: Medication toxicity; gall stones, often with obstruction and/or pancreatitis; seizures, usually due to substance abuse and withdrawal; HIV-AIDS with related medications and infections; sepsis and septic shock leading to tissue anoxia; pre-eclampsia; hemolytic anemia, other than hemoglobin S disease; large hematomas, one each in the retro-peritoneum and thigh with fracture. Severe malnutrition; advanced myxedema; and primary sclerosing cholangitis accounted for one case each.

In ten cases a definitive primary cause for the AST-ALT anomaly could not be ascertained with confidence. The pathologic processes in each of these cases are listed in (Table 5). Many of these patients had visits to Emergency Department only and were not fully investigated.

1	Renal stone, hepatomegaly, history of cholecystectomy
2	BMI, 11.7, died on admission to ED
3	Hypothyroid, BMI 35
4	Deep vein thrombosis, suspected pulmonary embolism, thrombo-embolic Cerebro-vascular accident, atrial fibrillation, heroin use
5	BMI 29.6, Diabetes mellitus, PCP use, only one ED visit
6	History of gastric bypass, single reading of altered enzymes, Alcohol 3-5 drinks/week
7	Diverticulosis, splenic rupture without explanation, low ALT
8	BMI 30.8, Lamisil for nail fungus
9	History of cholecystectomy, hepatic duct to jejunum anastomosis, intrahepatic bile duct pneumobilia, history of renal stones
10	BMI 34.5, gall stones, probable NASH, only one visit to ED

Table 5: Pathologic processes noted in patients, in whom the primary cause of AST and ALT alterations could not be determined with certainty.

One item of note was the low frequency of testing for GGT. In only 11 patients was this enzyme measured and in no instance was there any comment on the results of GGT analysis. During informal consultation with clinicians, the lack of specificity of GGT was cited as the cause for minimal use of this the analyte.

Alcohol as primary cause of AST and ALT anomaly		
Total	MCV data available in	MCV ≥ 100 fL in
187	184	101(54.9%)
	HDL data available in	HDL ≥ 80 mg/dL in
	77	31 (40.3%)
Both MCV and HDL available in	Either or both elevated in	Both elevated in

78	56 (71.8%)	16 (20.5%)
Alcohol not the primary cause of AST and ALT anomaly		
Total	MCV data available in	MCV \geq 100 fL in
191	188	29* (15.4%)
	HDL data available in	HDL \geq 80 mg/dL in
	86	4** (4.7%)
16 of the 29 cases had history of alcohol intake even though alcohol was judged to be not the primary cause of AST-ALT anomalies. ** All four cases had history of alcohol intake even though alcohol was judged to be not the primary cause of AST-ALT anomalies		

Table 6: Prevalence of MCV \geq 100 fL and HDL \geq 80 mg/dL

Other laboratory parameters affected by alcohol use/abuse are MCV and HDL. Alcohol intake is known to result in macrocytosis and increase in HDL levels. The results for these laboratory parameters with reference to alcohol are presented in table 6. In about 55% of the cases when alcohol was judged to be the primary cause of AST-ALT anomalies, MCV was \geq 100 fL. The corresponding figure for cases where alcohol was not judged to be the primary cause, the prevalence

of MCV \geq 100 fL was 15% though 16 of the 29 relevant patients had history of alcohol intake even though alcohol was not determined to be the cause of AST-ALT anomalies. When data for both MCV and HDL were available, about 72% of the patients with alcohol as the primary cause of AST-ALT anomalies, had elevation of either MCV or HDL or both. HDL was \geq 80 mg/dL in 31 of 77 cases (40%), where data were available, in patients with alcohol as the primary cause of liver enzyme abnormalities. The corresponding figure for patients in whom alcohol abuse was judged to be not the primary cause was 4 out of 86 (4.7%), and all four cases had history of alcohol intake.

Bilirubin levels were analyzed in 16042 episodes of paired testing for total and direct bilirubin. The episodes of paired testing for total and direct bilirubin were fewer than those for AST and ALT as the latter were done on both liver function panel and comprehensive metabolic panel, whereas the paired results for total and direct bilirubin were available in the liver function panel only, as comprehensive metabolic panel has only total bilirubin.

The results of direct bilirubin levels in various sub-groups of total bilirubin levels are shown in Table 7. In instances in which total bilirubin was up to 0.8 mg/dL (i.e., less than 0.9 mg/dL) 97.9% of the samples had direct bilirubin level of less than 0.3 mg/dL and only 0.4% had direct bilirubin in excess of 0.3 mg/dL. In samples with total bilirubin of 0.9 mg/dL, 7.1% had direct bilirubin levels $>$ 0.3 mg/dL (Table 7 and 8).

Total	16042	D-Bil		D-Bil		D-Bil		D-Bil		D-Bil	
		$<$ 0.3	%	0.3	%	0.4	%	$>$ 0.4	%	$>$ 0.3	%
T-Bilirubin 0 to 0.8	11861	11607	97.9	202	1.7	39	0.3	13	0.1	52	0.4
T-Bilirubin 0.9	762	617	81	91	11.9	38	5	16	2.1	54	7.1
T-Bilirubin 1.0	586	452	77.1	66	11.3	47	8	21	3.6	68	11.6
T-Bilirubin 1.1	399	252	63.2	73	18.3	44	11	30	7.5	74	18.5
T-Bilirubin $>$ 1.1	2434	627	25.8	368	15.1	231	9.5	1208	49.6	1439	59.1

Table 7: The paired values of total and direct bilirubin were segregated according to the total bilirubin levels into the subgroups with total bilirubin up to 0.8 mg/dL (i.e. $<$ 0.9 mg/dL), total bilirubin of 0.9 mg/dL, total bilirubin of 1.0 mg/dL, total bilirubin of 1.1 mg/dL and total bilirubin of $>$ 1.1 mg/dL (i.e. total bilirubin of 1.2 mg/dL or greater). Each group was then sorted in ascending order of direct bilirubin and segregated into subgroups with direct bilirubin $<$ 0.3 mg/dL, direct bilirubin of 0.3 mg/dL, direct bilirubin of 0.4 mg/dL, and direct bilirubin of $>$ 0.4 mg/dL. A separate subgroup of direct bilirubin greater than 0.3 mg/dL was also created. The raw numbers and the numbers as a percentage of the total in each group are presented. The data support not doing direct bilirubin assay if the total bilirubin is less than 0.9 mg/DL. T-Bil=Total bilirubin in mg/dL; D-Bil=Direct bilirubin in mg/dL

	Observations	D-Bil	$>$ 0.3 %
T-Bil0 to 0.8	11861	52	0.4
T-Bil0 to 0.9	12623	106	0.8
T-Bil0 to 1.0	13209	174	1.3
T-Bil0 to 1.1	13608	248	1.8
T-Bil $>$ 1.1	2434	1439	59.1
T-Bil=Total bilirubin in mg/dL; D-Bil=direct bilirubin in mg/dL			

Table 8: The numbers presented in table 7 are a conservative case for not performing direct bilirubin when total bilirubin is $<$ 0.9 mg/dL as

the percentage of observations with direct bilirubin $>$ 0.3 mg/dL is only 0.4% when total bilirubin is $<$ 0.9 mg/dL. If we examine the percentage of patients with total bilirubin up to 1.1 mg/dL, only 1.8% had direct bilirubin exceeding 0.3 mg/dL. However the rate of direct bilirubin exceeding 0.3 mg/dL in patients with total bilirubin of 0.9 mg/dL is a clinically meaningful 7.1%, and in those with total bilirubin of 1.0 mg/dL the rate is, as expected, even higher at 11.6%. Therefore, despite the low prevalence of direct bilirubin $>$ 0.3 mg/dL in patients with total bilirubin of up to 1.1 mg/dL, it would be prudent to perform reflex testing for direct bilirubin when total bilirubin is \geq 0.9 mg/dL

Discussion

As stated in the introduction, rules from the Centers for Medicare and Medicaid Services have led the laboratories in the US to test for seven analytes in the CMS approved liver panel, even if results of all the analytes are not clinically needed [1]. The inclusion of AST in this and comprehensive metabolic panel is being questioned as the enzyme is not liver specific [6]. One ostensible reason for including AST in these panels is to allow detection of hepatocyte injury due to alcohol use/abuse [2-6]. For a screening test to yield about 3% positive detection rate, as seen in this study, would be considered good, if the screening test were to reveal information not otherwise available. However; in the experience at this institution, in none of the 379 patients did the elevated AST level and AST/ALT ratio contribute meaningful information. There was a readily available and well documented history of alcohol use/abuse in each of the 187 patients in whom alcohol was the primary cause of liver enzyme abnormalities, with one possible exception. There were a total of 233 patients with history of alcohol use but only in 187 patients was the alcohol abuse thought to be the primary etiologic agent of liver enzyme abnormalities due to hepatocyte damage. Therefore, it is doubtful that any clinically useful information was gleaned from testing AST. While the laboratory results are not always commented upon in the clinical records, a record of AST abnormality was noted in at best four of the 379 patients further pointing out the less than cardinal importance or salience of this assay.

AST values are used in some, but not all, indicators of hepatic fibrosis such as Fib-4 and APRI. However, imaging can provide a better non-invasive test for hepatic fibrosis. These indices were developed to detect fibrosis in viral hepatitis but have been applied to other disease states, including non-alcoholic fatty liver disease (NAFLD) [13-16]. Another reason for testing for AST in the liver panel may be the abnormally low levels of ALT in some patients with advanced cirrhosis [6]. However, continuing liver cells damage that may be highlighted by elevated AST would hardly be a revelation in these patients.

The rationale for selecting cases in which AST was ≥ 40 units than ALT and the AST/ALT ratio was ≥ 2.0 for further examination was somewhat arbitrary. The AST/ALT ratio of two is the basis for the De Ritis ratio therefore this value for the ratio was chosen [3]. However, it was observed that in some cases, an AST value in the normal range could be more than twice the ALT in patients with low ALT levels; therefore the first cut included the requirement that AST be greater than ALT by 40 or more units [6]. Even though AST/ALT ratio has been cited as a marker of alcohol use/abuse, it is by no means specific to alcohol intake and is seen in other liver disorders, such as ischemic hepatitis, viral hepatitis, NASH, and advanced cirrhosis from any cause [6,17-20].

In addition to the clinical history, other markers of alcohol use were also available in the laboratory tests done routinely [2]. For example MCV was greater than 100 fL in about 55% of the 187 patients with alcohol as the primary etiologic agent. When both MCV and HDL level were available, one or both of these parameters were elevated in about 72% of the patients. It was apparent that the MCV values were negatively affected by the repeated bleeding episodes in some of the patients. The hemorrhages lowered the MCV by inducing iron deficiency and the parameter was also affected by the transfusions given to patients with hemorrhage [7, 21-23].

The role of routine testing for AST in a healthcare setting may differ from one in which substance abuse treatment is the primary aim and subjects may have reasons to be less than truthful about their history of imbibing and testing for AST, as a routine, may be appropriate to detect recidivism [21]. To reiterate, serum AST levels provide minimal clinically relevant information in the usual adult healthcare setting.

The usefulness of AST in patients without alcohol abuse or alcohol abuse as not the primary cause of altered AST-ALT levels and ratio was likely negligible. There were no comments about the ratio in such patients. Clinical findings and other laboratory parameters were often far more useful in establishing the pathology and AST level was not a determining factor in any of the patients.

The case for direct bilirubin not adding any meaningful value to the liver function panel is even simpler. In nearly three quarter of the samples, total bilirubin was less than 0.9 mg/dL and only 0.4% of these samples had a direct bilirubin level of >0.3 mg/dL. Thus the laboratories could safely avoid performing direct bilirubin assay in all samples with total bilirubin of <0.9 mg/dL. This process can be easily implemented by writing a rule for reflex testing thus limiting the direct bilirubin testing to samples with total bilirubin of 0.9 mg/dL or more.

The institution of panels, by CMS, was apparently implemented to control the “unbundling” of laboratory tests and to regulate the billing for laboratory tests. The reimbursement for a panel is much lower than the sum of reimbursements for each of the tests in a panel. In one of the cases under study, the reimbursement for the liver panel of seven analytes is \$11.23 whereas the reimbursement for AST alone is \$7.11.

If the laboratories were allowed to customize the panels and say, offer a conventional liver panel with seven analytes and allow the providers to choose an alternative panel of five analytes, with the latter being the default panel, and one with AST and direct bilirubin requiring an order as a miscellaneous test, the utilization of these often un-needed tests could be reduced. Direct bilirubin test would be done reflexly on samples with total bilirubin ≥ 0.9 mg/mL. A similar change would be made in the CMP panel in that AST would not be included in the routine panel and the conventional CMP panel would require a miscellaneous test order. The CMS may offer a lower reimbursement for the smaller panels.

The cost implications of reducing utilization of laboratory tests, while not large, are not trivial either. Laboratory costs account for less 3% of the healthcare costs nationally. In the case of AST, the marginal cost of performing an AST determination is, on average, about \$1.0 with a range of \$0.1 to \$2.7, depending on the volume of testing in a given laboratory. At this institution, eliminating AST testing would reduce the cost to the laboratory by about \$54,246.00 per year. This does not include the cost of quality control, and proficiency testing as the laboratory would maintain the ability to perform AST analysis for the occasional patient for whom it may be requested. Also, not performing AST will not alter any of the fixed costs nor would it affect personnel or any indirect costs.

The marginal cost of a direct bilirubin test ranges from \$0.1 to 0.15. The savings in marginal costs, at an average institution, for limiting direct bilirubin assays to only samples with total bilirubin ≥ 0.9 mg/dL would be about \$0.12/sample not tested for direct bilirubin. For the 11,861 direct bilirubin tests not done for samples with total bilirubin levels of <0.9 mg/mL about \$2,846.64 would be avoided/year.

This institution has 575 approved beds and the national number of approved beds is 924,333. If extrapolated to the national level removal

of routine AST and direct bilirubin testing, as proposed, this has the potential of saving about \$92 MM in laboratory costs. This is likely a conservative estimate as the test volume is based on hospital testing only and does not include the volume at the various non-hospital based laboratories and commercial reference laboratories.

This retrospective, observational study has the usual shortcomings of such studies. An unselected population without a defined protocol for testing, treatment, and follow-up forms the basis of the conclusions. Such studies obviously do not provide complete and uniform data for all participants and often require guessing the thought process of the treating physician. Alcohol as the primary cause of altered liver enzymes was determined based on the clinical history, blood alcohol levels, and laboratory test results affected by chronic alcoholic intake and in part as judgment of the author and could be considered a weakness of the study. Many of the patients were seen only in the ED and thus had less than complete investigation in the face of acute issues that were addressed and patients advised to attend primary care clinics. The patients often were less than compliant. However, it is also highly unlikely that such a study could be conducted as a prospective one due to the expense of carrying out prospective controlled trials.

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