Guidelines for Performance of Laboratory Tests of Liver Function and Injury

Performance Specifications for Laboratory Tests

Laboratory tests are used by clinicians for diagnosis, monitoring, and prognosis in patients with liver disease. A number of factors, primarily preanalytical and analytical, affect the accuracy of test results. The key characteristics of any test are its bias and imprecision. Bias is primarily an analytical characteristic, in which reported results differ from the actual value. Imprecision, or lack of reproducibility, is due to both physiological and analytical factors. In the baseline state, tests results fluctuate in an individual due to random and predictable variation; this is termed intra-individual variation. The degree of variation can be increased under certain conditions, such as food ingestion, time of day, exercise, acute illness, or other forms of stress. In general, for many tests, there is also significant differences from one person to the next, termed inter-individual variation. Intra-individual, inter-individual, and analytical causes of variation must be considered in interpreting the results of laboratory tests as indicating a change in an individual's health status.

Performance specifications serve as a guide to the laboratory as to the degree of analytical variation that will allow the clinician to accurately determine the physiologic state of an individual. Performance specifications can be established by different methods, including (in decreasing order of importance) medical outcome studies, data on biological variation, opinions of clinicians or professional societies, or data from proficiency testing or government directives. (5) Performance goals should specify acceptable imprecision, bias, and total error (bias + 1.65 ∗ imprecision). When goals are derived from biological data, the target for imprecision is less than half of the intraindividual variation for the test, while the target for bias is less than one-fourth of the average intraindividual (cv_I) and interindividual (cv_g) variation, calculated as ¼ $(cv_i^2 + cv_g^2)^{1/2}$. (6) Table 2 summarizes published data on performance specifications and within-laboratory precision for liver related tests.

Table 2 – Performance Specifications and Precision for Liver Tests (Percent)

Source	Type	ALT	AST	ALP	GGT	Albumin	Bilirubin
Performance Specifications							
CLIA	Mandate	TE 20	TE 20	TE 30		TE 10	TE 20 or 0.4 mg/dL
Eurpoean (7)	Biological variation	I 13.6 B 13.6 TE 36	I 7.2 B 6.2 TE 18	I 3.4 B 6.4 TE 12	N/S	I 1.4 B 1.1 TE 3.4	I 11.3 B 9.8 TE 28
Ricos (8)	Biological variation	I 12.2 B 12.2 TE 32	I 6.0 B 5.4 TE 15	I 3.2 B 6.4 TE 12	I 6.9 B 10.8 TE 22	I 1.6 B 1.3 TE 3.9	I 12.8 B 10 TE 31
Skendzel (9)	Clinician opinion	N/S	TE 26	N/S	N/S	N/S	TE 23
Within Laboratory Precision (Percent)							
Lott (10)	Proficiency tests	8	9	5	6	N/S	N/S
Ross (11)	Proficiency tests	N/S	N/S	N/S	N/S	4.4	8.9

TE – total error; I – imprecision; B – bias; N/S – not specified

Reference Intervals

In order to determine the likelihood that disease is present, test results are typically compared to values obtained from health individuals; the range of such results is termed the reference interval, while the high and low ends of the interval are termed the upper and lower reference limits, respectively. Most laboratories publish a single reference interval for most laboratory tests, defined as the central 95% of results obtained from healthy persons. In many cases, there are recognized factors that can affect the results of tests without indicating the presence of disease, particularly when only a single reference interval is used. For each chemical laboratory test listed, factors that affect results are summarized in tables and figures.

For some tests, reference limits are defined by health outcomes; examples include currently used reference limits for cholesterol and fasting glucose. Use of outcome-based reference limits also requires a high degree of standardization of measurement between laboratories to assure that results from all laboratories have a similar relationship to the upper reference limit. While data from studies on the likelihood of transmission of infection after transfusion suggest that an outcome based upper reference limit may be appropriate for ALT, there is not sufficient standardization of ALT measurements between laboratories to allow use of such an approach at the current time. There is no data on outcome-based reference limits for other tests of hepatic injury and function.

Aminotransferases

Aspartate aminotransferase (AST, also sometimes termed SGOT) and alanine aminotransferase (ALT, also sometimes termed SGPT) are widely distributed in cells throughout the body. AST is found primarily in heart, liver, skeletal muscle, and kidney, while ALT is found primarily in liver and kidney, with lesser amounts in heart and skeletal muscle. AST and ALT activity in liver are about 7,000 and 3,000 times serum activities, respectively. (12) ALT is exclusively cytoplasmic; both mitochondrial and cytoplasmic forms of AST are found in all cells. (13) The half-life of total AST is 17 ± 5 hours, while that of ALT is 47 ± 10 hours. (14). The half-life of mitochondrial AST averages 87 hours. (15) In adults, AST and ALT activities are significantly higher in males than in females, and reference intervals vary with age (Figures 1 and 2). Until about age 15, AST activity is slightly higher than that of ALT, with the pattern reversing by age 15 in males but persisting till age 20 in females. (17) In adults, AST activity tends to be lower than that of ALT until approximately age 60, when they become roughly equal. Because upper reference limits vary little between the ages of 25 and 60, age-adjusted reference limits need not be used for this population, which comprises most persons with chronic liver injury. Separate reference limits are needed for children and older adults; these may require national efforts to obtain enough samples from healthy individuals to accurately determine reference limits.

Table 3 – Factors Affecting AST and ALT Activity Besides Liver Injury

Factor	AST	ALT	Reference	Comments

Table 3 – Factors Affecting AST and ALT Activity Besides Liver Injury				
Factor	AST	ALT	Reference	Comments
Time of day		45% variation during day; highest in afternoon, lowest at night	18	No significant difference between 9 am and 9 pm; similar in liver disease and health
Day to day	5-10% variation from one day to next	10-30% variation from one day to next	19	Similar in liver disease and health, and in elderly and young
Race/gender	15% higher in African-American men		21	No significant difference between African-American, other women
Body mass index (BMI)	40-50% higher with high BMI	40-50% higher with high BMI	17, 22, 23	Direct relationship between weight and AST, ALT
Meals	No effect	No effect	17	
Exercise	3-fold increase with strenuous exercise	20% lower in those who exercise at usual levels than in those who do not exercise or exercise more strenuously than usual	24, 25	Effect of exercise seen predominantly in men; minimal difference in women (< 10%). Enzymes increase more with strength training
Specimen storage	Stable at room temp for 3 d, in refrigerator for 3 wks (< 10% decrease); stable for years frozen (10-15% decrease)	Stable at room temp for 3 d, in refrigerator for 3 wks (10-15% decrease). Marked decrease with freezing/thawing	26, 27, 28	Stability based on serum separated from cells; stable for 24 h in whole blood, marked increase after 24 h
Hemolysis, hemolytic anemia	Significant increase	Moderate increase		Dependent on degree of hemolysis; usually several-fold lower than LDH elevation
Muscle injury	Significant increase	Moderate increase		Related to degree of elevation in CK
Other	Macroenzymes	Macroenzymes	29, 30	Typically stable elevation, affects only AST or ALT

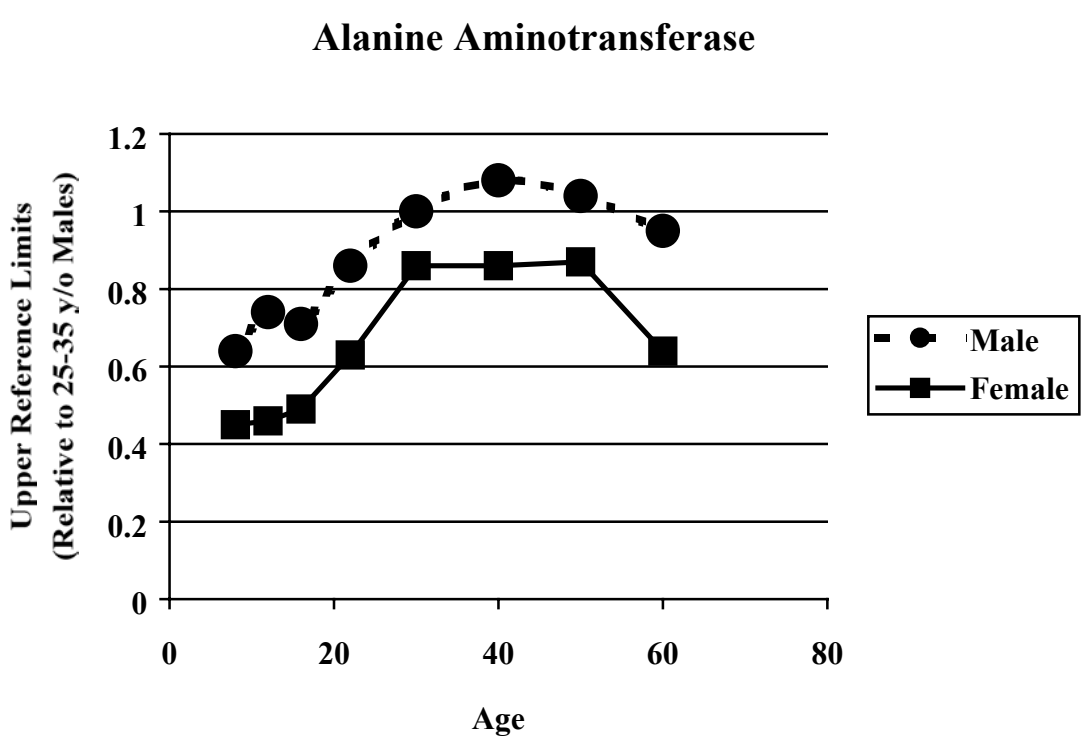

Figure 1 – Age and Gender Effects on Upper Reference Limits for ALT. The upper reference limit for 25-35 year old males is set at 1.0 relative value units. ALT upper reference limits increase from childhood to about age 40, with greater increases seen in men than in women; upper reference limits are approximately 30% higher in 40 year old men than in those 25 years old. After age 40, ALT upper reference limits again decline, with the decline more pronounced in men than in women. Data from reference 16.

Liver disease is the most important cause of increased ALT activity and a common cause of increased AST activity. A number of factors other than liver disease affect AST and ALT activities; these are summarized in Table 3. Unexpectedly abnormal results are often normal on repeat testing. (c49, c50) In most types of liver disease, ALT activity is higher than that of AST; an exception is in alcoholic hepatitis. The reasons for the higher AST activity in alcoholic hepatitis appear to be multiple. Alcohol increases mitochondrial AST activity in plasma, while other forms of hepatitis do not. (31) Most forms of liver injury decrease hepatocyte activity of both cytosolic and mitochondrial AST, but alcohol leads to decrease only in cytosolic AST activity. (32) Pyridoxine deficiency, common in alcoholics, decreases hepatic ALT activity (33); and alcohol induces release of mitochondrial AST from cells without visible cell damage. (34)

AST and ALT are typically measured by catalytic activity (35); both require pyridoxal-5'-phosphate (P-5'-P) for maximum activity, although the effect of deficient P-5'-P on ALT is greater than that on AST. (36) In renal failure, AST and ALT are significantly lower than in healthy individuals, perhaps due to serum binders of P-5'-P, as total P-5'-P is elevated. (37) Because of marked differences between laboratories,

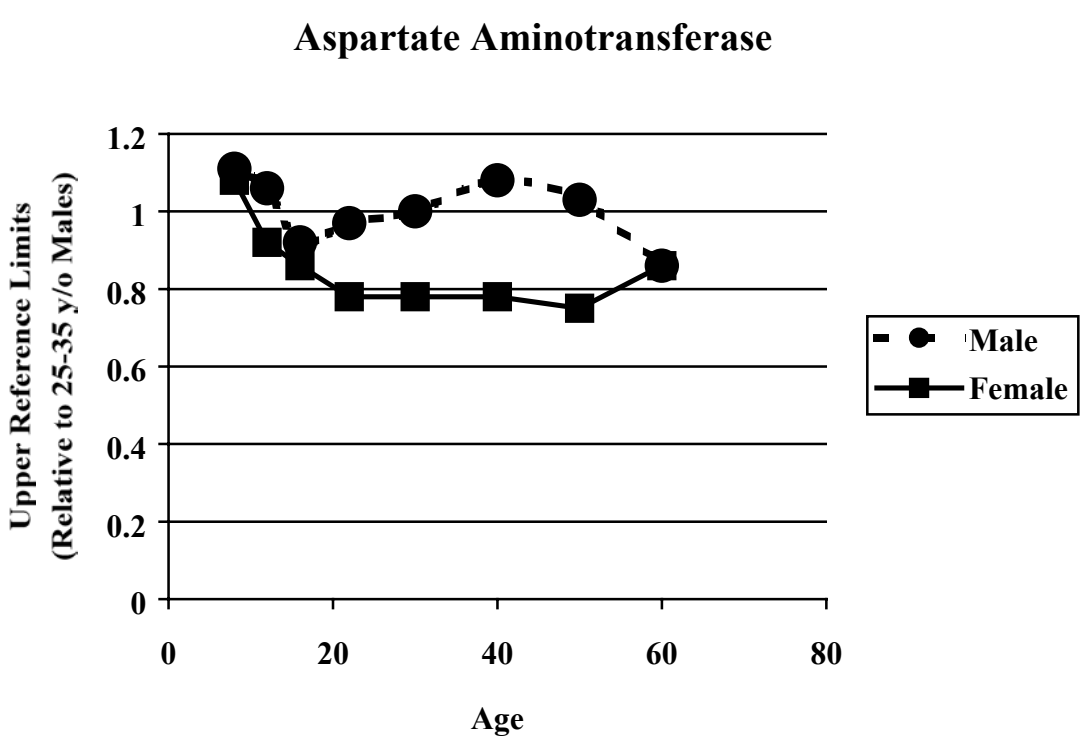

Figure 2. Age and Gender Effects on Upper Reference Limits for AST. The upper reference limit for 25-35 year old males is set at 1.0 relative value units. AST upper reference limits increase from childhood to young adult years, but change relatively little with increasing age in adults until after age 60. At all ages except childhood and old age, AST upper reference limits are approximately 25-30% higher in men than in women. Data from reference 16.

standardization of methods is a priority. In the interim, alternative methods to minimize differences between laboratories, such as expressing results as multiples of the reference limit (38), have been shown to minimize between-laboratory variation. (39)

Current target values for performance goals for total error in ALT activity measurements are 20% (CLIA) are not available for most laboratory tests for liver evaluation, with the exception of ALT. Little data exist on the biological variation of ALT in chronic hepatitis, particularly hepatitis C, although it is commonly stated that ALT results are highly variable. In a study of 151 patients with confirmed HCV infection, the average intraindividual coefficient of variation was 38%, although in a smaller group of patients with relatively stable ALT, it was 23%. (Dufour, unpublished observations) Several studies have shown that treatment of chronic HCV infection is not indicated if ALT is within the reference range. Thus, accurate determination of ALT at the reference limit is critical for correct treatment of patients with HCV infection. The consensus of the authors and the AASLD Practice Guidelines committee is that performance criteria for ALT should be defined at the upper reference limits, and that current performance goals are inadequate for clinical use. The data in patients with stable ALT suggest that total error of < 10% is required at the upper reference limits for

accurate detection of patients who may benefit from treatment for HCV. Current data on within-lab precision (Table 2) suggest that this target cannot be met by current methods. It will likely be necessary to develop a standardization program for ALT measurements, similar to that used for CK-MB. This may require use of other methods, such as immunoassay, to achieve the necessary total error target for management of patients with chronic hepatitis.

Performance goals for total error in AST activity measurement are 15-20%, both by CLIA requirements and based on biological variation. These meet the perceived needs of clinicians for diagnosis and management of liver disease (9). Performance goals are not as critical for AST as for ALT; a lower percentage of AST results are abnormal in chronic HCV compared to ALT (33% vs 71%). AST is seldom (6%) abnormal when ALT is normal, except in cirrhosis or alcohol abuse. (Dufour, unpublished observations)

Recommendations

Assays for ALT activity should have total analytical error of ≤ 10% at the upper reference limit (IIB). Current published performance goals for AST, with total error of 15-20%, are adequate for clinical use. (IIIB)

Standardization of ALT values between methods and across laboratories is a priority need for patient care. Until standardization is accomplished, use of normalized results should be considered. (IIIB).

At a minimum, laboratories should have separate upper reference limits for adult males and females; reference limits should also be established for children and adults over age 60 by cooperative efforts (IIB).

Unexpectedly elevated ALT and/or AST should be evaluated by repeat testing; in individuals engaging in strenuous exercise, repeat should be performed after a period of abstinence from exercise. Research is needed to determine the appropriate time interval required (IIB, E).

Alkaline Phosphatase

Alkaline phosphatase (ALP), involved in metabolite transport across cell membranes, is found, in decreasing order of abundance, in placenta, ileal mucosa, kidney, bone, and liver. Bone, liver, and kidney alkaline phosphatase share a common protein structure, coded for by the same gene (40, 41); they differ in carbohydrate content. The half-life of the liver isoenzyme is three days. (42) Age and gender related changes in alkaline phosphatase upper reference limits are illustrated in Figure 3. Interpretation of alkaline phosphatase results using appropriate reference populations is particularly important in children; reference limits differ little in adult males and females between the ages of 25 and 60. After age 60, reference limits increase in women, although studies have not consistently evaluated for the presence of osteoporosis, which can increase alkaline phosphatase activity in serum. Separate reference ranges are required for children and pregnant women.

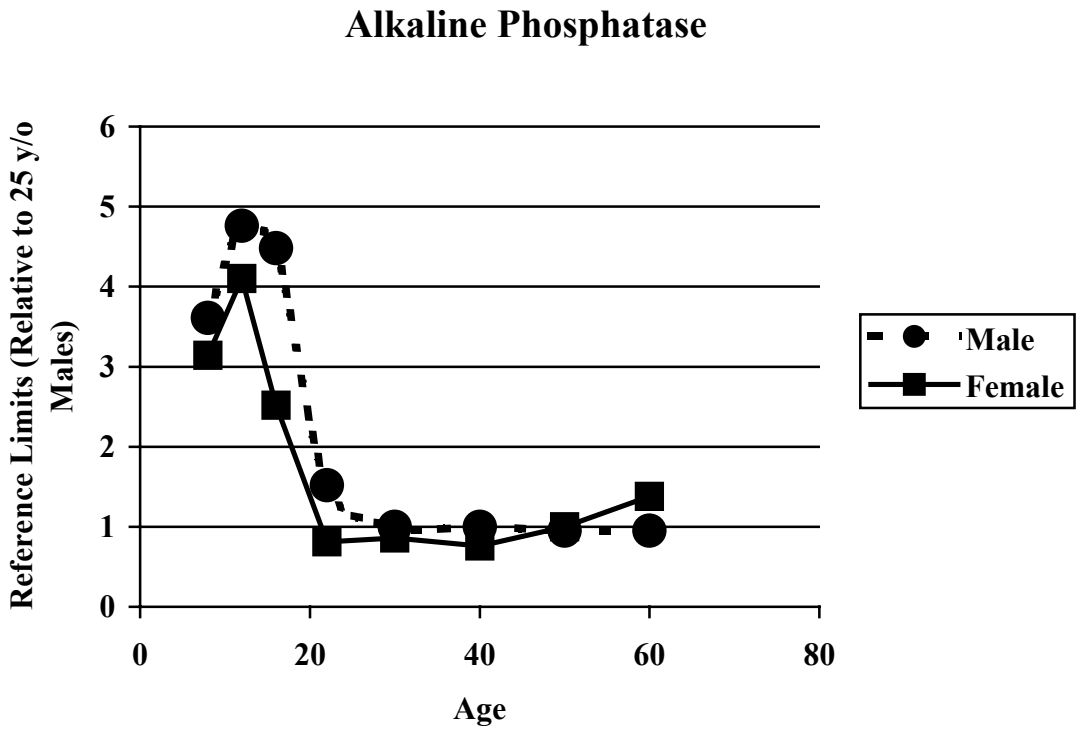

Figure 3. Age and Gender Effects on Upper Reference Limits for Alkaline Phosphatase. The upper reference limit for 25-35 year old males is set at 1.0 relative value units. Alkaline phosphatase is many fold higher in children and adolescents, reaching adult activities by about age 25. Values are slightly higher in men than in women until late in life. In adult men, upper reference limits do not change with age, while in women upper reference limits increase after menopause. Data from reference 16.

Cholestasis stimulates synthesis of ALP by hepatocytes; bile salts, detergents or other surface-active agents facilitate release of ALP from cell membranes. (43, 44) Other factors affecting alkaline phosphatase are summarized in Table 4.

The method for total ALP in widest use is the p-nitrophenylphosphate method of Bowers, McComb and Kelly. (50) Complexing agents such as citrate, oxalate, or EDTA bind cations such as zinc and magnesium, necessary cofactors for ALP activity measurement, causing falsely decreased values, as low as zero. Blood transfusion (containing citrate) causes transient decrease in ALP through a similar mechanism.

Table 4 – Factors Affecting Alkaline Phosphatase Activity Besides Liver Injury

Factor	Change	Reference	Comments
Day to day	5-10%	19	Similar in liver disease and health, and in elderly and young
Food ingestion	Increases as much as 30 U/L	45, 46	In types B and O; remains elevated up to 12 hours; due to intestinal isoenzyme
Race/gender	15% higher in African-American men, 10% higher in African-American women	21	
Body mass index	25% higher with increased BMI	46	

(BMI)			
Exercise	No significant effect	25	
Specimen storage	Stable for up to 7 d in refrigerator, months in freezer	27	
Hemolysis	Hemoglobin inhibits enzyme activity	47	
Pregnancy	Increases up to 2-3 fold in third trimester	48	Due to placental isoenzyme
Smoking	10% higher	21, 46	
Oral contraceptives	20% lower	49	
Other	High in bone disease, tumors producing alkaline phosphatase Low after severe enteritis (in children) and in hypophosphataasia	47	Can be separated from liver causes by alkaline phosphatase isoenzymes and/or normal GGT

Separation of tissue nonspecific ALP forms (bone, liver, and kidney) is difficult owing to structural similarity; high resolution electrophoresis and isoelectric focusing are the most useful techniques. Bone-specific ALP can be measured by heat inactivation (a poor method), immunologically and by electrophoretic methods. Immunoassays of bone ALP are now available from several sources (51), and can be used to monitor patients with bone disease. Because there is good agreement between increases in alkaline phosphatase of liver origin and an increase in the activity of other canalicular enzymes such as γ-glutamyl transferase (GGT) is a good indication of a liver source, but does not rule out coexisting bone disease. (52)

In contrast to most enzymes, intraindividual variation in ALP is low, averaging slightly over 3% (Table 2). The current average within-laboratory imprecision of 5% is close to recommended performance specifications; a total error of 10-15% would meet health based target values of 12%. The CLIA specified total error range of 30% appears too wide for clinical use and should be narrowed.

Recommendations

Assays for alkaline phosphatase activity should have total analytical error of ≤ 10-15% at the upper reference limit. (IIIB).

Separate reference limits should be provided for children, based on age and gender, and for pregnant women. A single reference range is adequate for adults over age 25 (IIB).

Specimens for alkaline phosphatase activity should be obtained in the fasting state; if not, mildly elevated patient values should be re-evaluated in the fasting state before further evaluation (IIB, E).

Assays for alkaline phosphatase isoenzymes or measurement of other associated enzymes (such as GGT) are needed only when the source of an elevated alkaline phosphatase activity is not obvious from clinical and laboratory features (IIIB, E)

Gamma-Glutamyl Transferase

Gamma-glutamyltransferase (GGT), a membrane bound enzyme, is present in decreasing order of abundance in proximal renal tubule, liver, pancreas (ductules and acinar cells), and intestine. GGT activity in serum comes primarily from liver. The half-life of GGT in humans is about seven to 10 days; in alcohol-associated liver injury, the half-life increases to as much as 28 days, suggesting impaired clearance. Age- and gender-related differences in GGT are summarized in Figure 4. In adult men, a single reference range is adequate between the ages of 25 and 80. Although upper reference limits are approximately 2 fold higher in those of African ancestry, information on racial characteristics is not commonly provided to laboratories; it would thus be difficult for laboratories to report values with the appropriate race-based reference interval. In women and children, GGT upper reference limits increase gradually with age, and are considerably lower than those in adult men. Separate reference limits should be established for men and women, and for different age ranges in women and children. In

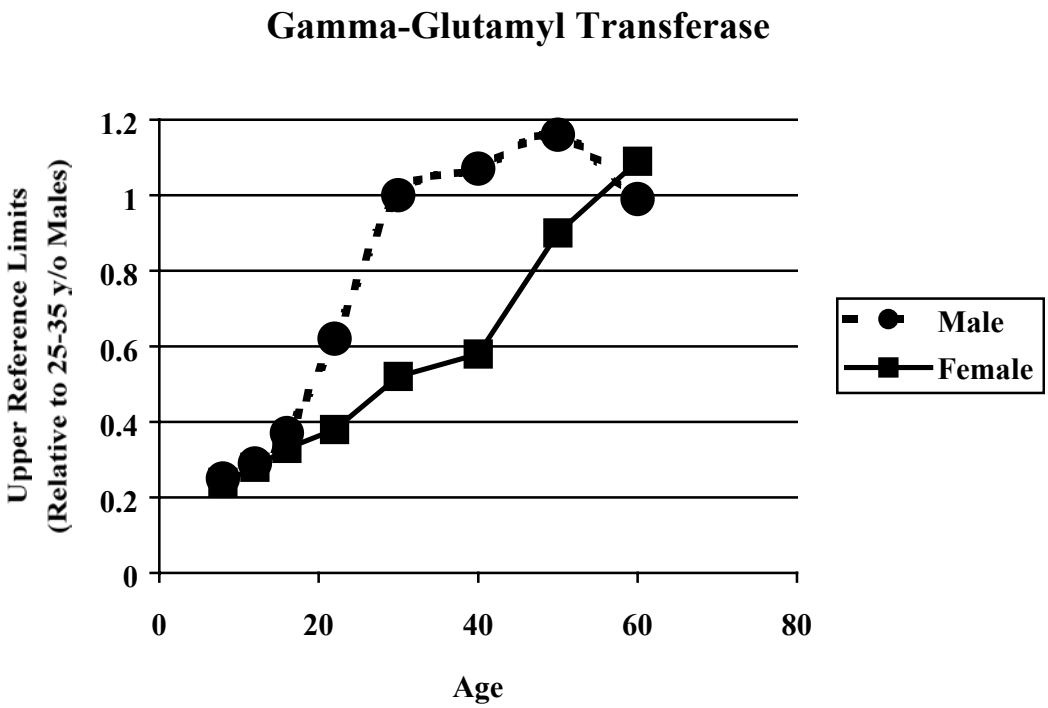

Figure 4. Age and Gender Effects on Upper Reference Limits for γ-Glutamyl Transferase (GGT). The upper reference limit for 25-35 year old males is set at 1.0 relative value units. GGT upper reference limits increase throughout life, with the effect more marked in women than in men. Before age 50, upper reference limits in men are approximately 25-40% higher than those in women, but the differences decrease with increasing age. Data from reference 16.

children, this will probably require a cooperative effort of laboratories to obtain adequate numbers of specimens from healthy children.

GGT is slightly more sensitive than ALP in obstructive liver disease. GGT is increased an average of 12 times the upper reference limit in 93-100% of those with

cholestasis, while ALP is increased an average of 3 times the upper reference limit in 91% of the same group. (52, 53, 54) GGT appears to increase in cholestasis by the same mechanisms as does ALP. (54, 55) GGT is increased in 80-95% of patients with any form of acute hepatitis. (55, 56) Other factors that affect GGT activity are summarized in Table 5. Patients with diabetes, hyperthyroidism, rheumatoid arthritis and obstructive pulmonary disease often have an increased GGT; the reasons for these findings are largely obscure. After acute myocardial infarction, GGT may remain abnormal for weeks. (62) These other factors cause a low predictive value of GGT (32%) for liver disease. (63)

Table 5 – Factors Affecting GGT Besides Liver Injury

Factor	Change	Reference	Comments
Day to day	10-15%	19	Similar in liver disease and health, and in elderly and young
Race	Approximately double in African-Americans	21	Similar differences in men, women
Body mass index (BMI)	25% higher with mild increase in BMI, 50% higher with BMI > 30	22	Effect similar in men, women
Food ingestion	Decreases after meals; increases with increasing time since food ingestion	57	
Exercise	No significant effect	57	
Specimen storage	Stable for up to 7 d in refrigerator, for months in freezer	47	
Pregnancy	25% lower during early pregnancy	58, 59	
Drugs	Increased by carbamazepine, cimetidine, furosemide, heparin, isotretinoin, methotrexate, oral contraceptives, phenobarbital, phenytoin, valproic acid	60	Values up to 2 times reference limits commonly, may be up to 5 times reference limits, especially with phenytoin
Smoking	10% higher with 1 pack/d; approximately double with heavier smoking	57	
Alcohol consumption	Direct relation between alcohol intake and GGT	57, 61	May remain elevated for weeks after cessation of chronic alcohol intake

The International Federation of Clinical Chemistry method described by Shaw (64) is used by most laboratories. Precision with activities less than one-half the upper reference limit is about 10%; at about twice the upper reference limit, it is closer to 5%. Performance goals for GGT are primarily based on biological variation, with total error tolerance limits of approximately 20%. These are adequate for clinical purposes, given the limited clinical utility of GGT measurements.

Recommendations

Assays for gamma-glutamyl transferase activity should have total analytical error of ≤ 20% at the upper reference limit (IIIB).

Use of fasting morning specimens is recommended (IIB).

While a single upper reference limit is appropriate for adult men, separate reference limits (based on age) are needed for children and adult women (IIB).

Because of lack of specificity, GGT should be reserved for specific indications such as determining the source of an increased alkaline phosphatase (IIIB, E).

Bilirubin

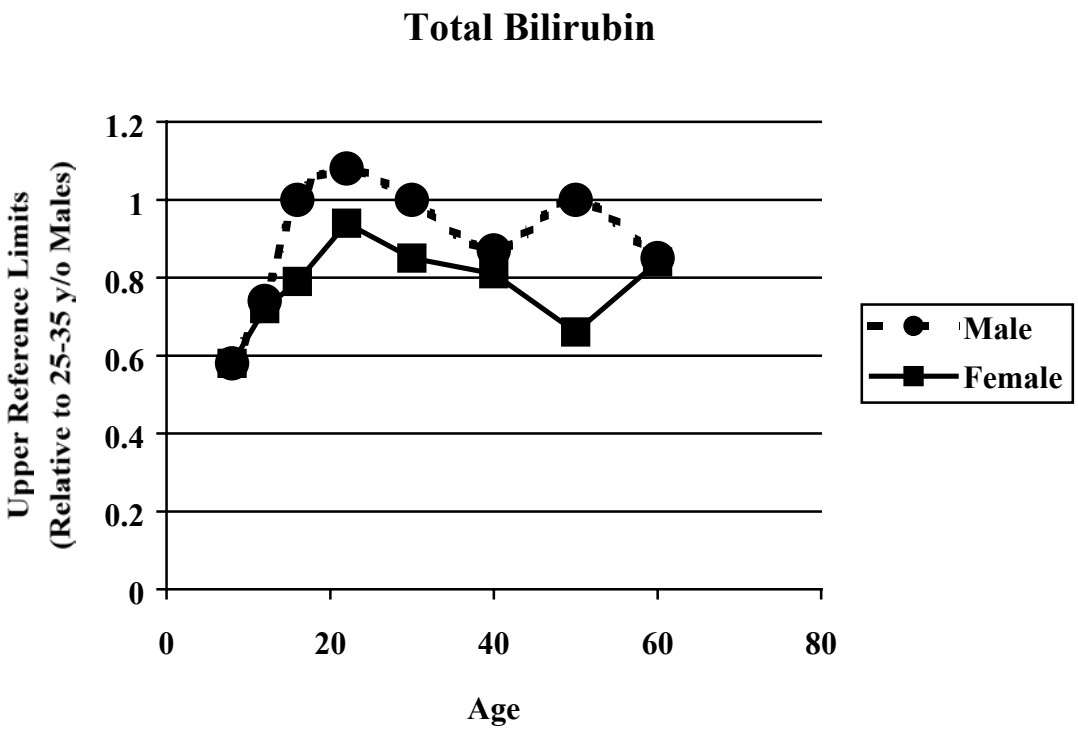

Figure 5. Age and Gender Effects on Reference Limits for Total Bilirubin. The upper reference limit for 25-35 year old males is set at 1.0 relative value units. Upper reference limits increase throughout childhood and adolescence, reaching peak values at approximately age 20; following this, values gradually decrease with increasing age. At all ages, upper reference limits are higher in men than in women, although the differences are minimal at the extremes of life. Data from reference 16.

Daily production of unconjugated bilirubin is 250 to 350 mg, mainly from senescent erythrocytes. (65) Clearance at normal values is 5 mg/kg/day, or about 400 mg/day in adults; the rate does not increase significantly with hemolysis. (66) The half-life of unconjugated bilirubin is <5 minutes. (67) UDP-glucuronyltransferase catalyzes rapid conjugation of bilirubin in the liver; conjugated bilirubin is excreted into bile and is essentially absent from blood in normal individuals. Delta bilirubin (δ-bilirubin, also sometimes termed biliprotein) is produced by reaction of conjugated bilirubin with albumin (68); it has a half-life of about 17-20 days (the same as albumin), accounting for prolonged jaundice in patients recovering from hepatitis or obstruction. (69) Age and gender related changes in bilirubin reference limits are illustrated in Figure 5.

Increases in conjugated bilirubin are highly specific for disease of the liver or bile ducts. (70). Increased conjugated bilirubin may also occur with impaired energy-dependent bilirubin excretion in sepsis, total parenteral nutrition, and following surgery. (71) With recovery from hepatitis or obstruction, conjugated bilirubin falls quickly, while δ-bilirubin declines more slowly. (72) Gilbert's syndrome, found in about 5% of the population, causes mild unconjugated hyperbilirubinemia due to impaired UDP-glucuronyltransferase activity along with decreased organic ion uptake. (73, 74) Total bilirubin rarely exceeds 68-85 μmol/L (4-5 mg/dL), even during prolonged fasting, unless other factors increasing bilirubin are also present. (75) Other factors affecting bilirubin are summarized in Table 6

Table 6 – Factors Affecting Bilirubin Besides Liver Injury

Factor	Change	Reference	Comments
Day to day	15-30%	19	
Food Ingestion	Bilirubin increases an average of 1-2 fold with fasting up to 48 h	76, 77	Averages 20-25% higher after overnight fast than after meals
Race	33% lower in African-American men, 15% lower in African-American women	21, 78	Compared to values in other racial/ethnic groups
Exercise	30% higher in men	25	No significant effect in women
Light exposure	Up to 50% decrease in one hour	79	Affects unconjugated bilirubin more than direct reacting bilirubin
Pregnancy	Decreases 33% by second trimester	48	Similar in second, third trimester
Hemolysis	Cross-reacts in some assays	47	Hemoglobin absorbs light at the same wavelength as bilirubin
Oral contraceptives	15% lower	49	
Hemolytic anemia	Increases in unconjugated bilirubin	47	

Bilirubin is typically measured using two assays for total and “direct reacting” or direct bilirubin; subtracting direct from total gives “indirect bilirubin”. The direct bilirubin assay measures the majority of δ-bilirubin and conjugated bilirubin, and a variable but small percentage of unconjugated bilirubin. (79, 80) High pH or the presence of a wetting agent promotes reaction of unconjugated bilirubin in the “direct” assay; the reagent for "direct" bilirubin should have at least 50 mmol/L HCl to prevent measurement of unconjugated bilirubin. (81) Light can convert unconjugated bilirubin to a photoisomer that reacts directly (79); it also causes total bilirubin to decrease by 0.34 μmol/L/hour (0.02 mg/dL/hour). Direct spectrophotometry (dry film methods) measures conjugated and unconjugated bilirubin individually, and then calculates δ-bilirubin as the difference between the sum of these and total bilirubin. Some have suggested conjugated bilirubin is better than “direct” bilirubin to measure recovery from liver disease. (82)

Performance goals for bilirubin measurement allow 20% (CLIA) to 30% (biological variation) total error. Clinicians felt that a 23% change in bilirubin at the upper reference limits indicates a significant change in condition. (9) Thus, CLIA performance goals appear to meet clinical performance needs. At elevated concentrations, a change of 2 mg/dL (5%) was considered clinically significant. Target values for total error should thus specify the concentration of bilirubin.

Recommendations

Assays for bilirubin should have total analytical error of ≤ 20% (or 6.8 μmol/L [0.4 mg/dL]) at the upper reference limit (IIIB).

Separate upper reference limits should be used for men and women. While bilirubin upper reference limits decline with age in adults, there is little significance to slight elevations in bilirubin and separate adult age-adjusted upper reference limits are not needed. In children, separate reference ranges should be used. (IIIB).

Albumin

Albumin is the most abundant plasma protein produced by hepatocytes. Rate of production is dependent on several factors, including supply of amino acids, plasma oncotic pressure, levels of inhibitory cytokines (particularly IL-6), and number of functioning hepatocytes. (83) The half-life of plasma albumin is normally about 19-21 days. Plasma albumin concentrations are low in neonates, typically 28 to 44 g/L (2.8-4.4 g/dL). By the first week of life, adult values of 37 to 50 g/L (3.7-5.0 g/dL) are reached, rising to 45-54 g/L (4.5-5.4 g/dL) by age 6 and remaining at these concentrations through young adulthood before declining to typical adult values. There is no significant difference in reference limits between males and females. (84) Increased albumin is typically due to hemoconcentration, caused either by dehydration, prolonged tourniquet use during collection, or specimen evaporation. The main causes for decreased albumin include protein loss (nephrotic syndrome, burns, protein losing enteropathy), increased albumin turnover (catabolic states, glucocorticoids), decreased protein intake (malnutrition, very low protein diets), and liver disease. Plasma albumin is seldom decreased in acute hepatitis, due to its long half-life, but in chronic hepatitis albumin gradually falls with progression to cirrhosis. Albumin concentrations are a marker of decompensation and prognosis in cirrhosis.

Albumin is most commonly measured by dye binding methods, particularly bromcresol green and bromcresol purple; currently, about 50% of laboratories use each method. Bromcresol green methods may overestimate albumin (85), although differences between the two methods are small. (83) Bromcresol purple underestimates albumin in renal failure (86) and in patients with increased δ-bilirubin (87), making this method unsuitable for patients with jaundice. Estimation of albumin from protein electrophoresis is not recommended due to significant overestimation of albumin based on higher dye binding (83). Immunoassays for albumin are available but not widely used in plasma. (88)

Performance goals for albumin measurement based on biological variation are typically around 4%, while CLIA allows an error of 10%. The clinical use of albumin measurements for liver disease is primarily in recognition of cirrhosis, and in determining its severity; these require significant changes from reference limits. Data from CAP surveys indicate that only 2% of laboratories can meet the error limits based on biological variation. The opinion of the committee is that the CLIA performance goals are adequate for clinical purposes.

Recommendations

Total error of < 10% at the lower reference limit is adequate for clinical purposes; performance goals based on biological variation, while an ideal goal for measurement, cannot be met by most laboratories (IIB)

Assays for albumin in patients with liver disease should use bromcresol green. Bromcresol purple and electrophoresis determinations of albumin may be inaccurate in patients with liver disease (IIB).

Prothrombin Time

Prothrombin time (PT) measures the time required for plasma to clot after addition of Tissue Factor and phospholipid; it is affected by changes in the activity of factors X, VII, V, II (prothrombin) and I (fibrinogen). All of these factors are synthesized in the liver, and three (II, VII, and X) are activated by a vitamin K-dependent enzyme through addition of a second, γ-carboxyl group on glutamic acid residues. Warfarin, a vitamin K antagonist, causes anticoagulation by inhibition of γ-carboxylation, rendering the factors unable to bind calcium and reducing their activity. Individuals on warfarin or with vitamin K deficiency synthesize normal amounts of the clotting factor precursors, but in an inactive form termed "proteins induced by vitamin K antagonists (PIVKA)". Immunoassays are available to measure the most important PIVKA, des-γ-carboxy prothrombin. PT is relatively insensitive to deficiency of any single clotting factor; there is no significant increase until concentrations fall below 10% of normal. (89)

PT is commonly reported in seconds and compared to patient reference values. The time required for a specimen to clot is inversely related to the amount of Tissue Factor present in the reagents. To minimize variation in PT between reagents with different amounts of Tissue Factor, each is assigned an International Sensitivity Index (ISI); the lower the amount of Tissue Factor, the lower the ISI value and the longer the prothrombin time. To adjust for differences in the ISI of reagents, the international normalized ratio (INR): is used; the value is calculated as: $INR = (\frac{PT_{patient}}{PT_{control\ mean}})^{ISI}$. Use of reagents with low ISI improves the reproducibility of INR measurement, making use of low ISI reagents ideal for monitoring anticoagulant therapy. (90)

The effect of ISI is much greater on PT in warfarin use than in liver disease, so that INR does not accurately reflect inhibition of coagulation in liver disease. (89, 91, 92) A sample from a patient receiving warfarin has a PT of 20 s with high ISI reagents and a PT of 40 s when tested with low ISI reagents, but INR is essentially identical with both reagents. (89) INR thus normalizes results in a patient on warfarin, despite differences in the ISI of reagents used. In liver disease, decreasing the ISI of reagents used causes only a slight increase in PT. For example, a sample from a patient with liver

disease has a PT of 20 s with high ISI reagents but a PT of 23.6 s with low ISI reagents. In contrast to the patient on warfarin, where INR is virtually identical when differing ISI reagents are used, INR varied 2.90 with high ISI reagents and 1.86 with low ISI reagents. (89) If reagents with low ISI are used, INR thus markedly underestimates degree of coagulation impairment in liver disease. A possible cause for the discerpancy in INR utility between warfarin use and liver disease is the marked difference in the relative amounts of native prothrombin versus des-γ-carboxy prothrombin present in the two conditions. Patients on warfarin or with vitamin K deficiency have marked elevation of des-γ-carboxy prothrombin and decrease in native prothrombin, while patients with acute hepatitis or cirrhosis have decreased native prothrombin but only slight elevation of des-γ-carboxy prothrombin. (93) Some preparations of Tissue Factor are inhibited by des-γ-carboxy prothrombin. (93)

PT is reproducibly increased, usually at least 3 sec beyond the population mean, in acute ischemic (94, 95) and toxic (96) hepatitis, but is rarely elevated more than three seconds in viral (97) or alcoholic (98, 99) hepatitis. PT is often elevated in obstructive jaundice, and may respond to parenteral vitamin K administration. In chronic hepatitis, PT is typically within reference limits, but increases as progression to cirrhosis occurs, and is elevated in cirrhotic patients. (100) Other factors affecting prothrombin time are summarized in Table 7.

Table 7 – Factors Affecting Prothrombin Time

Factor	Change	Reference
Specimen storage	No change at room temperature up to 3 d; refrigeration falsely shortens PT	101
Citrate concentration	3.2% citrate minimizes problems compared to other concentrations	102
Inadequate tube filling	Falsely increases PT	102
High hematocrit	Falsely increases PT	102
Other factors	Warfarin, malabsorption, vitamin K deficiency, drugs that decrease vitamin K production (especially antibiotics, fibric acid derivatives), consumptive coagulopathy increase PT	

Reagents with the same ISI typically give different results on different instruments, even of the same model. (103) In addition, when using different manufacturer's reagents with same ISI, the same specimen can give different INR's. (104, c158, c159) The reproducibility of PT results in laboratories using the same instrument and reagents is from 3-8% when prothrombin times are prolonged; variation is greater for INR than it is for the prothrombin time itself. Within a single laboratory, average variation in INR is estimated to be ±10%. (105) The difference in PT between laboratories using different reagents may be marked; in one study, the average difference was 20%. (104) Recently, use of calibrant plasmas to determine ISI in each laboratory for its own reagents and instrument has been shown to significantly improve reproducibility of INR. (104, 106, 107)

Recommendations

PT (in seconds) rather than INR should be used to express results of prothrombin time in patients with liver disease; however, this does not standardize results between laboratories (IIB).

Additional research into standardization of reagents and use of derived indices (percent activity, INR) in liver disease is needed (IVB).

Ammonia (NH_3)

Ammonia is a product of amino acid metabolism; it is cleared primarily by urea synthesis in the liver. *Helicobacter pylori* in the stomach appears to be an important source of ammonia in patients with cirrhosis. (108) In liver disease, increased NH_3 is typically a sign of hepatic failure. High concentrations are seen with deficiency of urea cycle enzymes (110), in Reye's syndrome (111), and with acute or chronic hepatic encephalopathy. (112, 113) Mild increases in plasma NH_3 are seen in patients with chronic hepatitis, in proportion to the extent of disease. (114) Use of NH_3 for monitoring of patients with encephalopathy is controversial; some studies have shown good correlation of NH_3 concentrations with degree of encephalopathy (111, 113), while others have not. (115) NH_3 appears to enhance the effects of γ-aminobutyric acid (GABA) (116) and increases benzodiazepine receptors (117); both GABA and benzodiazepines have been implicated in the pathogenesis of hepatic encephalopathy. On the other hand, clinical features seen in persons with isolated hyperammonemia are not identical to those of hepatic encephalopathy. (118) Other factors affecting NH_3 are summarized in Table 8. Specimens should have plasma separated from cells within one hour of collection maximum; in patients with liver disease, separation within 15 minutes is ideal. (120, 122)

Table 8 – Factors Affecting Ammonia Besides Liver Injury

Factor	Change	Reference	Comments
Age	4-8 fold higher in neonates; 2-3 fold higher in children < 3 yr; reach adult concentrations by adolescence	119	
Specimen source	Arterial higher than venous; difference greater in renal, hepatic disease. Capillary blood falsely increased due to NH_3 in sweat if skin inadequately cleaned.	112, 120	Only arterial ammonia correlates with change in liver function. Tourniquet use, clenching fist increase venous ammonia.
Exercise	Increases up to 3 fold after exercise	121	Increase greater in males than in females.
Smoking	Increases 10 μmol/L after 1 cigarette	120	
Delay in analysis	Ammonia increases due to cellular metabolism; 20% in 1 hour and by 100% by 2 hours.	122	Use of ice water, rapid centrifugation and separation of plasma minimize increases. Rate of increase higher in liver disease due to high GGT activity in specimens
Other factors	Increased in acute leukemia, blood transfusion, bone marrow transplantation, portal-systemic	123, 124	

	shunts, GI bleeding or high protein intake		
Medications	Valproic acid, glycine (in irrigation fluids used in prostate, endometrial resection) increase ammonia production	125, 126	

Several methods have been used to measure ammonia (120), with enzymatic assays currently the most widely employed. One manufacturer uses slide technology with alkaline pH to convert ammonium to ammonia and measurement of ammonia with bromphenol blue. Reproducibility within laboratories using the same method averages 10 to 20%, with mean values using different methods differing by less than 10% on average. (127)

Recommendations

Measurement of plasma ammonia for diagnosis or monitoring of hepatic encephalopathy is not routinely recommended in patients with acute or chronic liver disease; it may be useful in patients with encephalopathy of uncertain etiology (IIIB).

For most accurate measurement, arterial, rather than venous, specimens should be used (IIB).

Plasma should be separated from cells within 15 minutes of collection to prevent artifactual increases in ammonia (IIB).