blood and measures factors 1, 2, 5, 7, 9, and 10. Because factors 2, 7, 9, and 10 are vitamin K dependent, the presence of cholestasis, where vitamin K is not absorbed, will prolong the prothrombin time. Also, significant hepatocellular dysfunction can result in prolongation of the prothrombin time. This does not typically occur until concentrations of clotting factors fall below 10% of normal. As a general rule, in the absence of liver disease, a prothrombin time that is prolonged is due to vitamin K deficiency and/or steatorrhea. It should be noted that prothrombin time can also be elevated with warfarin, heparin bolus, disseminated intravascular coagulation (DIC), and hypothermia.

This practice guideline will discuss the interpretation and evaluation of those with elevation of the major chemistries including ALT, AST, alkaline phosphatase, and bilirubin. Other liver tests (including GGT, albumin and prothrombin time) will be incorporated into the evaluation of these major liver chemistries but will not be discussed separately.

WHAT ARE TRULY NORMAL LIVER CHEMISTRY TESTS?

Summary statements:

1. A true healthy normal ALT level in prospectively studied populations without identifiable risk factors for liver disease ranges from 29 to 33 IU/l for males and 19 to 25 IU/l for females, and levels above this should be assessed by physicians.
2. Elevated ALT or AST above the upper limit of normal (ULN) in a population without identifiable risk factors is associated with increased liver-related mortality.
3. There is a linear relationship between ALT level and body mass index (BMI) that should be assessed by physicians.
4. A normal ALT level may not exclude significant liver disease.
5. ALT levels are higher in males than females.
6. AST and ALT ULN ranges can vary between different labs.
7. Clinicians may rely on local lab ULN ranges for alkaline phosphatase and bilirubin.

Normal lab values are generally defined as the mean value of a healthy population±2 s.d.'s. This incorporates 95% of subjects. By definition, 2.5% of the population will be greater than the ULN of the reference population. For alkaline phosphatase and bilirubin levels, establishing normal liver enzyme levels that can be replicated across different reference labs has not been reported as problematic, which differs from the wide variations in ranges reported as normal for ALT levels. However, establishing normal ranges for ALT and AST levels have been problematic due to differences in the definition of healthy control populations that are used to establish the normal reference ranges. One report examined the local reference laboratory ranges for ALT used by the non-alcoholic steatohepatitis (NASH) Clinical Research Network and demonstrated significant differences in the defined ALT ULN (range 35–79 IU/l for men and 31–55 IU/l for women) (13). These wide ranges appeared to be due to the use of different reference populations utilized by the different laboratories with local populations used to establish the normal range of ALT, apparently without consideration of factors such as BMI. Another study found that 67 reference laboratories within one state used different ALT ULN levels ranging from 31 to 72 U/l (ref. 14). In this report, the majority of the labs used equipment from one of four manufacturers, but used different methods to define the ULN, with 40% utilizing manufacturer's recommendations and local healthy control testing, 38.5% only using the manufacturer's recommendations, 17% only using local healthy controls, and 8% using published normal levels from textbooks. However, inter-laboratory differences for ALT levels have not been reported to differ significantly (13,15). When defining a normal population to be used for the establishment of a reference range, the possible presence of underlying liver disease must be considered. Conditions such as non-alcoholic fatty liver disease (NAFLD), viral hepatitis, alcoholic liver disease and the use of medications and herbal agents or supplements need to be factored into the development of these normal ranges. Most importantly, multiple studies have demonstrated that ALT levels correlate with increasing BMI (16–18).

Determining an ALT level that is normal is clinically relevant to practicing clinicians and patients as there is substantial clinical significance to these different reference ranges for ALT levels between labs. Both practice guidelines and diagnostic and therapeutic studies base clinical decisions including evaluation of abnormal liver tests, therapy for hepatitis B, and evaluation of potential drug-induced liver injury on multiples of the ULN of ALT (19–23).

Several studies and guidelines have proposed a standardized ULN for ALT based on prospectively acquired data using various methodologies (**Table 2**). These studies defined normal reference populations by excluding subjects with viral hepatitis, high-risk behavior and NAFLD risk factors (elevated BMI, triglycerides, glucose, and cholesterol). These proposed normal ALT values are lower than commonly reported reference ranges and differ by gender. Of the major liver chemistries, there is sufficient data on the measurement of ALT levels. The true ULN for ALT was proposed in a large study of 6,835 blood donors with normal viral serologies, and BMI under 24.9 kg/m^2 to be 30 IU/l for men, and 19 IU/l for women (24). In a Korean study of 1,105 potential liver donors with normal liver biopsies, they reported that age, BMI, and metabolic factors significantly affected ALT levels (25). They proposed ULN for ALT to be 33 IU/l for men and 25 IU/l for women. In an examination of the National Health and Nutrition Examination Survey (NHANES) 1999–2002 and 2005–2008 databases, after eliminating subjects with viral hepatitis, significant alcohol use, diabetes, BMI>25, or enlarged waist circumference, and using statistical analysis, the calculated "maximum correct classification" for ULN of ALT was found to be 29 IU/l for men and 22 IU/l for women (26).

ELEVATED AMINOTRANSFERASE LEVELS AND THE EFFECT ON MORBIDITY AND MORTALITY

There is an accumulating set of data demonstrating that AST and ALT elevations correlate with morbidity and mortality (**Table 3**). An initial report from Germany noted that those with AST>18 U/l had a 3X increased risk of all-cause mortality (27). A Korean