Regulatory Toxicology and Pharmacology 73 (2015) 349–355

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Children's liver chemistries vary with age and gender and require customized pediatric reference ranges

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ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form
14 July 2015
Accepted 15 July 2015
Available online 17 July 2015

Keywords:
Alanine aminotransferase
Alkaline phosphatase
Laboratory reference ranges
Liver chemistries
Pediatric clinical trials

ABSTRACT

Used to detect liver disease and injury, baseline liver chemistry distributions were evaluated by age and gender in children without known liver disease. Baseline liver chemistries [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBIL)] were analyzed from 24 randomized controlled pediatric clinical trials. Using quantile regression, liver chemistry distributions were examined by age and gender; upper limit normal (ULN) ranges were compared to the 97.5th percentiles of the distributions for the specified ages and genders. 5410 subjects without known liver disease (0–18 years; 60% male) were studied. The median ALT varied little with age. In males age 5–18, the ALT 97.5th percentile increased from 34 to 63 IU/L. In both genders, the median and 97.5th percentile AST decreased with age. After age 9, ALP decreased. TBIL increased with age. Despite most liver chemistry 97.5th percentiles changing substantively with age and gender, the reference lab ULN generally changed minimally and did not correlate with the 97.5th percentile. Gender and age specific 97.5th percentile data should therefore be considered for the reference laboratory ULN in children to more accurately detect liver injury and disease.

1. Introduction

Alanine aminotransferase (ALT) is the single most important marker of acute and chronic liver injury (Green and Flamm, 2002; Dufour et al., 2000). In children, acute and chronic liver injury results from viral hepatitis, nonalcoholic fatty liver disease (NAFLD), drug-induced hepatotoxicity and other factors (Green and Flamm, 2002; Patton et al., 2008, Schwimmer et al., 2006). While drug-induced liver injury generally resolves with drug cessation, it may rarely progress to fatal acute liver failure in children (Mindikoglu et al., 2009). Relative to adults, children less than age 8 exhibit age-related changes in drug absorption, distribution, metabolism and excretion (Kearns et al., 2003), which could put them at higher risk of adverse drug events.

Since the FDA's pediatric exclusivity provision in 1997, the number of clinical trials in children has greatly increased (FDA website). In drug trials, liver injury is a key safety issue, monitored with ALT, aspartate aminotransaminase (AST), alkaline phosphatase (ALP), and total bilirubin (TBIL) (Aithal et al., 2011, Stirnadel-Farrant et al., 2012).

Despite its routine use in clinical trials, there is very limited data on the normal range of liver chemistries in healthy children (Stirnadel-Farrant et al., 2012, Dufour et al., 2000, England et al., 2009, Schwimmer et al., 2010, Southcott et al., 2010, Turan et al., 2011). Recent studies have raised concerns that current high ALT reference ranges may not sensitively detect chronic liver disease in infants and adolescents and support use of a statistically defined ULN from population-based ALT measurements (England et al., 2009, Schwimmer et al., 2010). Furthermore, despite marked growth-related ALP changes, most hospital assays use adult reference ranges in children (Turan et al., 2011). As analytic variability is modest (Dufour et al., 2000, Schwimmer et al., 2010), liver

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransaminase; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Survey; TBIL, total bilirubin; ULN, upper limit of normal.

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http://dx.doi.org/10.1016/j.yrtph.2015.07.013
0273-2300/

chemistry reference ranges are developed using subject panels, of as few as 100 subjects, and may include subjects with undiagnosed nonalcoholic fatty liver disease, elevating the liver chemistry ULN (Schwimmer et al., 2010). Hence, most reference laboratories use an inappropriately high ULN which can affect liver disease diagnosis (Schwimmer et al., 2010).

To examine liver chemistries and reference ranges across all ages, pediatric clinical trial data provide a valuable controlled dataset to develop the 2.5th and 97.5th percentiles for the lower and upper reference limits (Heiduk et al., 2009, Southcott et al., 2010), using quantile regression. From 24 controlled clinical studies of 5410 children, age 0 to 18, without liver disease, baseline pediatric liver chemistry data were analyzed to assess age and gender-specific liver chemistry variability (ALT, ALP, AST and TBIL), and to compare to the upper limit of normal (ULN) reference ranges. While targeted liver chemistries in select age ranges have been analyzed in population- or hospital-based studies (England et al., 2009, Heiduk et al., 2009, Schwimmer et al., 2010, Turan et al., 2011, Southcott et al., 2010), the current study is the first to examine age and gender threshold variation across all liver chemistries in children from age 0 to 18, of whom more than 4000 are under age 12, an age range with a paucity of data (England et al., 2009, Heiduk et al., 2009, Schwimmer et al., 2010, Turan et al., 2011, Southcott et al., 2010). In this large controlled dataset, liver chemistry variation by age and gender will be examined in children without liver disease to provide a comparator for other pediatric liver chemistry datasets. We hypothesize that these children without liver disease will exhibit liver chemistries well below the reference laboratory upper limits of normal. If confirmed, this data would affirm the value of revising pediatric lab reference ranges to better assess liver injury and liver disease in children.

2. Materials and methods

2.1. Study population

From 24 pediatric Phase II-IV clinical trials (1995–2009), the study subjects were included if they were age $\leq$ 18 years, had no underlying liver disease, and had baseline (pretreatment) ALT measured, as earlier reported (Stirnadel-Farrant et al., 2012). The selection of clinical trials without liver disease was based on (1) the disease indication was not liver disease and not a condition related to liver disease; (2) the active or comparator treatment had no apparent relationship to liver disease, and (3) liver disease was generally excluded in these trials. The laboratory upper limit of normal (ULN) range was recorded for each study. All studies were reviewed and approved by institutional review committees, all subjects or their guardians completed written informed consent prior to study participation, and all study protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Eligible subjects were pooled from 24 pediatric clinical trials; these trials included asthma, impetigo, rhinitis, influenza and migraine studies. Subjects were categorized by gender, race/ethnicity, weight categories, and age group: 0–1, 2–4, 5–8, 9–11, 12–15, and 16–18 years. The weight categories were defined as described previously (Stirnadel-Farrant et al., 2012).

2.2. Laboratory analysis

Liver chemistries were quantified by autoanalyzers, which exhibit $\approx$5–10% between-laboratory measurement variation using the same methodology (Dufour et al., 2000). However, cross-laboratory methods and values may exhibit higher variability, which was not explicitly evaluated in this study and suggests the value of global method standardization (Dufour et al., 2000). Specifically, ALT and AST catalytic activity with pyridoxal-5′-phosphate was measured, while total alkaline phosphatase was quantified using the *p*-nitrophenylphosphate method (Dufour et al., 2000). Bilirubin was measured by two assays to quantify total and "direct reacting" bilirubin. In some assays, hemolysis can increase bilirubin measurements through cross-reacting. In contrast, light exposure can decrease bilirubin measurement, with unconjugated bilirubin affected more than total bilirubin (Dufour et al., 2000).

2.3. Data analysis

Liver chemistry, age and gender data were analyzed. The age- and gender-specific 1st, 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, 97.5th and 99th percentiles, and the 2.5th, 50th, and 97.5th percentiles obtained from quantile regression, of ALT, ALP, AST and TBIL were calculated. Baseline variability and threshold variation were evaluated by age and gender using quantile regression. The regression model was fit utilizing the model: log(response) ~gender + age + age^2+gender*age + gender*age^2. For analysis with quantile regression, the same regression model was fit to estimate each of a set of quantiles (the median, 2.5% and 97.5%). The clinical trial upper limit normal ranges were compared to the 97.5th percentiles over the range of ages and genders. Statistical analysis was performed using SAS PROC QUANTREG.

A 'gentle introduction' to quantile regression, in the context of ecology, is given by Cade and Noon (2003), and the implementation of this method in SAS's PROC QUANTREG is described elsewhere (Chen, 2005).

3. Results

The study population included 5410 subjects from 24 randomized controlled clinical studies, of whom 60% were male and age ranged from 0 to 18 years including 574 under age 2, 3814 age 2–11, and 1022 age 12–18 as described previously (Stirnadel-Farrant et al., 2012). Briefly, most (71%) subjects were Caucasian, 11% were Black, 7% Hispanic and 1% Asian, and 10% were of unknown ethnicity. The majority (57%) of subjects were of healthy weight, 6% were underweight, 12% overweight, 11% obese, and 14% had missing weight data. Most subjects participated in asthma clinical trials (N = 3417, 63%), followed by impetigo (N = 824, 15%), rhinitis, influenza, or migraine trials.

3.1. Alanine aminotransferase

In males and females, the median (or 50th percentile) ALT changed little from age 0–18. However, in males, the ALT 97.5th percentile decreased from age 0 to 8 (44–34 IU/L) and increased from age 8–18 years, with the most notable increase from age 12–19 (34–63 IU/L respectively, Fig. 1), while the median ALT varied little (15–18 IU/L respectively) (Table 1). In females, the ALT 97.5th percentile decreased from birth to age 12 (46–30 IU/L respectively, Fig. 1) and increased back to 35 IU/L by age 19; the median ALT also varied minimally (18–15 IU/L).

Comparing the ALT 97.5th percentile to the ULN range utilized by the clinical trials, the reference laboratory ULN values were generally higher and did not always correlate with the 97.5th percentiles of the study population in males and females (Table 1). The 16–18 year old males were an exception, exhibiting a higher 97.5th percentile value (63 IU/L) than the reference laboratory ULN of 50 IU/L (range 40–56 IU/L) (Fig. 1, Table 1). Interestingly, the ALT 97.5th percentiles were very similar in males and females up to the age of 8.