Variability of Laboratory Markers of Liver Injury in Patients with Biopsy Confirmed Non-alcoholic Steatohepatitis (NASH) and Fibrosis

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Background

Interventional clinical trials require close safety monitoring for drug-induced liver injuries (DILIs). Specific DILI biomarkers are still missing. The current regulatory and clinical guidelines define criteria for safety signals related to liver toxicity which incorporate clinical and laboratory features such as elevations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBil) or alkaline phosphatase (AP)[1]. Reference to normal ranges of these standard laboratory markers are used in monitoring for DILI in patients with unaffected livers at baseline, while abnormally elevated baseline liver marker values serve as reference for safety monitoring in patients with background liver disease. Due to intrinsic laboratory factors or natural history of liver conditions, liver enzymes fluctuate around their usual values in individual subjects. Such fluctuations or variability have been recognized by clinical researchers and authorities as affecting the ability of liver markers to signal potential DILI and resulted in requirements towards establishing a maximum level of laboratory markers' baseline variability.

There has been a significant increase in number of clinical trials in NASH and NAFLD, which have become by far the most frequently studied non-viral hepatic indication in interventional clinical trials. Variability in liver markers of injury have been recognized. They may represent fluctuating course of disease activity e.g., pointing to progressive or regressive course of disease (e.g., less active hepatic inflammation with development of significant fibrosis) or an effect of intrinsic or environmental factors such as diet or therapeutic intervention. Fluctuations of these biomarkers may lead to incorrect decisions related to patients' disposition in clinical trials at study entry and during experimental interventions.

We aimed at establishing and analyzing the natural, i.e., unaffected by a therapeutic or toxic intervention, variability of liver enzymes regularly used in monitoring of liver injury in clinical trials (AST, ALT, TBil, AP) in patients with NASH fibrosis and its implications on trial eligibility.

Methods

We pooled retrospective data from baseline (prior to any therapeutic intervention) assessments of AST, ALT, AP, TBil in patients who were successfully randomized into trials with NASH fibrosis (NASH CRN Stage F1 through F3) confirmed with a biopsy. Each patient contributed two values (the trial protocols required a screening assessment and an assessment performed on the day of the first drug administration but prior to it). The minimum time span between the 2 assessments was 7 days, which minimized the occurrence of situations when an assessment was repeated due to a perceived lab error. The maximum time span between the 2 assessments was restricted by the duration of the screening period as designed in the trial protocol. If more than 2 assessments of any of the liver parameters were available, we chose the 2 assessments closest to dosing observing the minimum 7 days requirement.

We provided descriptive statistics for each of the two values of the liver enzymes for each patient and calculated the following within patient measures of variability:

- coefficient of variation (CV),
- absolute difference divided by mean of the 2 values,
- absolute difference divided by the lower of the 2 values,
- absolute difference divided by the higher of the 2 values, and
- absolute difference divided by the more recent of the 2 values.

We analyzed how application of different ranges of variability could affect eligibility of patients into clinical trials using 10% point intervals for each measure of variability. The coefficients of variation were referenced to published healthy and chronic liver disease cohorts.

Results

The sample comprised 874 patients with histologically confirmed NASH. The demographic and NASH characteristics of patients is provided in Tables 1 and 2.

Of the 4 liver enzymes, AP demonstrated numerically the lowest variability, while variability of ALT, AST, or TBil was similarly high (Figure 1).

Of the 5 measures of variability, the coefficient of variation resulted in the lowest measure of variability for all 4 liver enzymes. It translated into a substantial effect on patient eligibility into clinical trials where application of 20% variability threshold would result in patient eligibility failure rate of 30% for ALT, 33% for AST, 2.7% for AP, and 34% for TBil (Table 3, red boxes).

The coefficients of variation (CV) for ALT, AST, TBil and AP in the NASH population studied were numerically similar to referenced healthy population (Table 4).

Table 1: Patient demographics

Parameter	Statistics	Total (n=874)
Age (years)	Mean (SD)	51.9 (11.54)
	Median	53.0
	Min-Max	18-81
Sex	Female	58.8%
Race	Am. Indian/Alaskan Native	6 (0.7%)
	Asian	38 (4.3%)
	African American	21(2.4%)
	Caucasian	795 (91.0%)
	Nat.Hawaiian/Pac. Islander	4 (0.5%)
	Multiple/Other	10 (1.1%)
Ethnicity	Hispanic or Latino	221 (25.3%)
	Non-Hispanic or Latino	650 (74.4%)
	Not Reported/Unknown	3 (0.3%)
BMI (kg/m^2) (n=873)	Mean (SD)	34.91 (6.463)
	Median	33.90
	Min-Max	17.6-65.9
Diabetic Status	Yes	45.9%

Table 2: Histological characteristics of NASH

Parameter	Statistic	Total (n=862)
Fibrosis Stage	0	32 (3.7%)
	1	317 (36.8%)
	2	254 (29.5%)
	3	259 (30.0%)
Steatosis Grade	0	1 (0.1%)
	1	364 (42.2%)
	2	340 (39.4%)
	3	157 (18.2%)
Hepatocellular Ballooning	0	11 (1.3%)
	1	441 (51.2%)
	2	410 (47.6%)
Lobular Inflammation	0	0 (0%)
	1	221 (25.6%)
	2	498 (57.8%)
	3	143 (16.6%)
NAFLD Activity Score	0	0 (0%)
	1	0 (0%)
	2	0 (0%)
	3	4 (0.5%)
	4	269 (31.2%)
	5	299 (34.7%)
	6	198 (23.0%)
	7	85 (9.9%)
	8	7 (0.8%)
	Mean (SD)	5.1 (1.01)
	Median	5.0

Figure 1: Mean liver enzyme variability measures

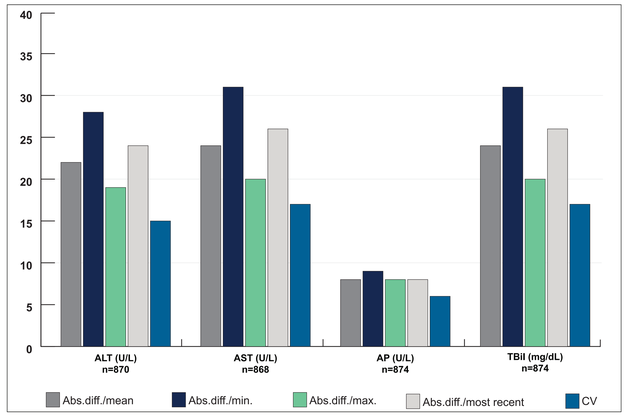

Table 3: Distribution (%) of patients in different variability stratum by all 5 measures of variability for each liver enzyme

ALT (%)	≤10	>10 - ≤20	>20 - ≤30	>30 - ≤40	>40 - ≤50	>50
Abs. diff./mean	31	24	18	12	7	8
Abs. diff./min.	30	21	15	12	7	15
Abs.diff./max.	32	26	22	12	6	2
Abs. diff./most recent	31	23	17	13	6	10
CV	41	29	18	8	3	1

AST (%)	≤10	>10 - ≤20	>20 - ≤30	>30 - ≤40	>40 - ≤50	>50
Abs. diff./mean	30	23	17	12	7	11
Abs. diff./min.	28	21	15	11	7	18
Abs.diff./max.	31	26	19	13	8	3
Abs. diff./most recent	29	23	16	12	8	12
CV	39	28	16	10	4	3

AP (%)	≤10	>10 - ≤20	>20 - ≤30	>30 - ≤40	>40 - ≤50	>50
Abs. diff./mean	69	24	5	1	<1	<1
Abs. diff./min.	67	25	5	2	<1	<1
Abs.diff./max.	71	24	4	1	<1	<1
Abs. diff./most recent	68	24	5	2	<1	<1
CV	84	13	2	<1	<1	0

Tbil (%)	≤10	>10 - ≤20	>20 - ≤30	>30 - ≤40	>40 - ≤50	>50
Abs. diff./mean	25	23	21	15	8	8
Abs. diff./min.	24	21	17	13	9	16
Abs.diff./max.	26	28	23	15	5	3
Abs. diff./most recent	25	24	20	13	7	11
CV	36	30	20	8	4	2

Table 4: CVs for liver enzymes from healthy and chronic liver disease patients and NASH (from current study)

Liver Enzyme	Healthy[2] (mean)	Healthy[3]	Healthy[4] (median)	Healthy[5]	Chronic Liver Disease[2]	Current Study (mean)
ALT	15.1	18.0	9.3	19.4	11.1	15.5
AST	10.1	11.9	9.5	12.3	10.6	16.9
AP	6.9	--	5.3	10.0	6.6	6.0
TBil	--	--	--	21.8	--	17.0

Conclusions

We confirmed and quantified natural variability of liver enzymes used for safety monitoring in a well characterized population of NASH fibrosis patients. The variability is substantial, and thus indiscriminate application of a variability threshold negatively affects eligibility and enrollment in clinical trials in NASH. On the other hand, the variability seen in patients with NASH is not numerically different to the variability experienced in subjects with unaffected livers in whom variability thresholds are not considered necessary for clinical trial eligibility or safety monitoring. With multiples of baseline values used for safety signal detection, we postulate that the use of a liver enzyme variability threshold for assessment of eligibility in NASH fibrosis trials does not yield any additional benefit and should be abandoned.

References

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