

Clinical meaning of elevated aminotransferase activity

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DR. WATKINS: Our first session is really focused on when an investigational drug should be stopped, particularly based on ALT elevations. As you know, the Guidance document says a number of things about ALT, including continuing use of the investigated drug until ALT rises up to eight times the upper limit of normal in a Phase III clinical trial. If the ALT is abnormal before starting, there's some mention of two times the baseline serum ALT. These are the issues that I think we're going to want to explore in greater detail in discussion. As a way of background, we have three talks that really center on serum ALT levels. The first speaker is Naga Chalasani from Indiana University, and here's Naga.

DR. CHALASANI: Thank you, Paul. A big thanks to Dr. Senior for asking me to speak here. It's a very big auditorium and a lot of very distinguished faces. It's really my pleasure and privilege to be the leadoff speaker.

The topic given to me is to discuss about the fundamentals of aminotransferase, what exactly it means in a clinic. Some of you I'm sure will find it like a medical student lecture, but that's really what I have been asked to do.

Outline

- Properties
- Variability
- Clinical value of different patterns
- Clinical value of different levels
- Reference range
- ALT as a population screening test

So the outline, some discussion about the properties of AST and ALT, The variability in their measurements, whether it is physiological or different disease stage and some discussion about the clinical value of different patterns and different levels, and we should discuss some about the reference range, how it is established, what is the variability, some evolving data, and lastly AASLD has suggested and is proposing ALT as a population screening test, some discussion about that as well.

AT: Not specific to liver

AST

Liver (9000:1)

Muscle (5200:1)

Heart

Kidney

Red cells

Brain

ALT

Liver (7600:1)

Muscle (750:1)

Kidney

And I think most of you know, although AST and ALT are called liver enzymes and are also sometimes called liver function test which is a mistake, it is not necessarily specific to the liver. In case of AST, it is predominantly concentrated in liver but also you see in muscle, heart, kidneys, red cells and brain, small bowel. ALT is not as widely spread. You see it in the liver, muscle and the kidney.

What you see in the parenthesis is the concentration per unit of tissue. For example, for 1 unit of liver tissue, you have 9,000 units of AST and 7600 units of ALT. And if you just look at the concentration, I think liver has the most concentration of the AST and ALT but if you just look at the total activity per organ, I think muscle obviously has more AST and ALT just because it has more muscle mass compared to the liver.

AT: Properties

- Source of normally circulating AT unclear
- AST and ALT activity in liver is 7000 and 3000 times higher than in serum
- AT are released either due to cell destruction or leaky cell membrane
- ? Increased synthesis (Increased ALT in MCD-NASH model was due to increased transcription)

So what is the source of normally circulating aminotransferase? It's not clear and I think it may be partly liver and partly the muscle, and compared to the circulating, the serum activity of aminotransferase is, the activity of AST and ALT in liver is far higher by 7,000 fold. For example, in the case of AST, compared to serum in liver, it's about 7,000 fold higher. In case of ALT, it's about 3,000 fold higher.

The circulating serum AST and ALT activities, when you see elevated AST and ALT, it could either be traditionally simply described either from cell destruction or just leaky cell membrane. And I think on and off you've heard some discussion whether it presents increased synthesis, and whether people have looked at increased message for AST and ALT in different types of cell injury or liver injury models and have proposed not only there is destruction and leak across the cell membrane, but actually increased synthesis.

I have come across an abstract that was presented last liver meeting by Peter Whittington's group in Chicago, where they looked at ALT synthesis in a cell injury model or a liver injury model from -- it's a NASH model. Next slide.

ALT in experimental NASH

- MCD fed mice develop NASH and have elevated ALT at 12 weeks (383 ± 5.6 U/L)
- ALT protein expression in liver is 7-10 fold higher than in serum
- Hepatic protein expression correlated with mRNA expression
- Hepatocyte necrosis and apoptosis were minimal
- In vitro release and protein/mRNA expression was completely blocked with PI-kinase inhibitor

Liu R, Pan X, Whittington P. AASLD 2007 abstract 1174

This group has induced nonalcoholic steatohepatitis in mice using methionine–choline deficient diet and also they had a control group. At week 12, these animals in a reproducible fashion get fatty liver. They get significant steatosis, hepatitis, fibrosis and compared to the control animals which have some age related changes but certainly not steatohepatitis. One problem with this NASH model is this is a weight loss model and they're non-insulin resistant, but this is probably the most widely used NASH model.

At week 12, the ALT in MCD fed mice is about 400 IU/l compared to the control mice of about 80 to 90 IU/l. They looked at the ALT expression in the hepatocytes both by protein expression as well as mRNA. They have shown that the protein expression correlated nicely with the mRNA expression and also have shown that there is a good correlation between the serum levels and the hepatic expression. On microscopy, there was very minimal necrosis and apoptosis, and interestingly when they looked at the relationship between the serum ALT and the liver ALT there was 7 to 10 fold higher expression of ALT in the liver tissue compared to the serum. This I think validates that at least in some instances elevated serum ALT may represent increased synthesis

I think this was pretty interesting because this is the first time I have seen that elevated ALT is not necessarily about apoptosis or necrosis but increased synthesis that is driving this elevation. .

AT: Properties

- ALT is exclusively in cytoplasm whereas AST is both cytoplasm and mitochondrial
- Half life of total AST 17 ± 5 hours; ALT 47 ± 10 hrs
- AST/ALT ratio depends on gender and age

More about the AST and ALT. ALT is exclusively in cytoplasm, cytosol. AST is in both mitochondria as well as cytoplasm. The two isoforms of AST, one in mitochondria, the other one in cytosol, are immunologically distinct and they are two different isoforms.

About the half life of AST is about 17 hours whereas the half life of ALT is 47 hours, and I think it makes sense when you take care of patients, the way the enzymes improve after liver injury, whether it is ischemia or some other etiology, fits in quite nicely with what's been described.

The AST and ALT are not excreted in the urine. They're predominantly metabolized in the reticuloendothelial system. The AST and ALT ratio depends on gender and age. In general, men have higher levels of AST and ALT compared to women. I haven't seen any data but my opinion is that it is related to the muscle mass and in most of the adult life, the ALT levels are higher than AST except in children up to 15, 16, you may see a higher AST compare to ALT. That may once again reflect the evolving muscle mass I think, and I'm not so sure but there's a lot written about the effect of age and the ratios within so-called normal range.

Variability

	AST	ALT
Time of day	-	45% variability; highest in afternoon
Day to day	5-10%	10-30%
Race	AfroAmericans 15% higher	-
Strenuous exercise	3 fold increase	20% lower

So when you evaluate aminotransferase or whether it's in clinical trials or whether in clinical practice, I think it is very important to know that there are some physiological factors that lead to variability. It could be as simple as time of the day, although it doesn't change the AST. It seems like it's been written that the ALT values are the highest in the afternoon. They increase by 45 percent. They're lowest at nighttime. I think most people are tested fasting early in the morning. So that should not be an issue.

There is a day-to-day variability, anywhere from 5 to 10 percent in AST and 10 to 30 percent in ALT. There was a recent paper by Jeannie Clark that was published in Annals of Internal Medicine showing that there is a great deal of variability in, even those with abnormal liver tests based on the NHANES3 study. So it is not advised to jump the gun based on one isolated abnormality, but look for abnormal values on repeat testing.

Afro-Americans, especially African-American men have higher, about 15 percent ST but not ALT. I suspect this is once again related to the muscle mass. The effect of exercise seems to be somewhat different on AST and ALT. Strenuous exercise leads to, for example, there are reports of AST measurements after marathon running and their levels go up. A good workout may increase AST levels by threefold.

But with the ALT, it doesn't seem to do that. In a conditioned athlete, the ALT levels are lower. For example, for a set level of exercise, if you do more vigorous exercise, it apparently goes down as well. There seems to be some difference in how exercise influences AST and the ALT levels.

AT: Variability

	AST	ALT
Specimen Storage	<ul style="list-style-type: none">■ Stable at room temp for 3 days■ Stable in refrigerator for 3 weeks (<10%)■ Stable for years frozen (<15%)	<ul style="list-style-type: none">■ Stable at room temp for 3 days■ Stable in refrigerator for 3 weeks (<10%)■ Marked decrease with freezing and thawing■ Whole blood is unstable after 24 hours

Specimen storage is probably very important. It doesn't seem to have a whole lot of effect on AST activity in serum, but in ALT it is important. For example, for ALT, it is stable in the serum at room temperature for 3 days, in the refrigerator at minus 20 for about 3 weeks. However, when you freeze and thaw it, there's marked loss of serum activity, and this is very important for some of us doing the freezer studies. This has to be taken into account.

If you're using whole blood, I think you can only use that for ALT measurements or for that matter, AST within the first 24 hours. After 24 hours, there is a great (off mic). So I think it's after 24 hours there is significant increase in the AST and ALT activity in whole blood. So I think a word of caution if you're freezing or using whole blood to measure these tests.

AT elevation: Non-Hepatic

- Hemolysis
- Muscle injury
- Macroenzymes

Non-hepatic causes, the list is long. You see hemolysis, muscle injury, macroenzymes. I have some slides. You have small bowel ischemia, renal injury, a number of non-hepatic causes, heart attack, myocardial infarction, that can lead to elevated AST and ALT. So in other words, when you see in a clinical trial elevated AST and ALT, you have to take the whole situation under evaluation rather than just being focused on liver.

Muscle Injury

- 83 yr old male with heart disease
- On a statin for many years but recently was switched at the VA to generic simvastatin 80 mg one half tablet every day
- Started to receive sporanox 100 mg once daily for histoplasmosis
- Developed severe muscle pain

Just to highlight the muscle injury, this is a patient that we have seen in the last two months or so in Southern Indiana, an 83-year-old male with heart disease, has been on a statin for many years, recently goes to VA. They switch the statin to generic simvastatin. Now he's on half a tablet of 80 milligrams, and that's about 6 weeks prior to the event. Subsequently he was diagnosed with pulmonary histoplasmosis and he was placed on itraconazole. And there was not even a discussion between the physician who was giving statin and ID physician who was giving itraconazole. Obviously there was a drug to drug interaction. About two weeks after he was started on Sporanox or the itraconazole, he developed severe muscle pain and was hospitalized and it was seen that the AST and the ALT are quite high but this is muscle injury because you see very high CPK. During the course of the next several days, with hydration and stopping both compounds, he has improved.

Muscle Injury

	2/21	2/22	2/23	2/25	3/5
AST	817	797	682	324	24
ALT	548	599	637	496	118
CPK	23115	17400	10708	3146	94
Cr	2.09	2.06	1.98	1.80	1.9

What I would like to point out though, this patient had come either late in the course or if he did not have a striking muscle injury, and the CPK is not part of the panels that you do, so you may just be keep thinking this is a liver injury, in case. And actually the way this patient came to us, the patient's son was told, he lives in Arkansas, and he was told that the dad had liver injury from a Statin and so he was referred to our hepatotoxicity study but if you look through the records, actually it is muscle injury-- . There was very little to suggest this is liver injury.

So unless you get the CPK, unless you get the history of muscle pain, you may be just getting the liver profile and may be focusing on liver actually when it's the muscle. And also if somebody comes late in the course, for example, if you look at the profile on the March 5th, the CPK is completely normal but your ALT is still high. You may be completely missing the muscle as the source. So only thing you have to broader suspicion that the elevated ALT can be more than just simply the muscle. .

Macro AST

- 43 year old female with prior multiple myeloma
- AST 448 U/L but normal ALT and AP
- Usual work up negative except for ANA 1:80 and monoclonal gamma spike
- Immunoprecipitation and electrophoresis confirmed enzyme complex
- Normal enzymes complexed with Ig G and Ig A
- Can be seen in liver disease, malignancy, IBD

Macroenzymes have been written in the literature. In the review articles, you can see macro ALT but I have not seen any reports of macro ALT but this is a case report that we have reported macro AST. This is for real. It looks like a lab anomaly. It's a 43-year-old female with prior multiple myeloma in remission and when the hematologist noticed the AST remained very high, around 400, was sent to our clinic but the patient's ALT, alkaline phosphatase and the remaining liver tests were completely normal, and this was quite striking, just one of the liver tests. And the work up was negative except for a monoclonal spike which is from her multiple myeloma.

The macro AST is just a normal enzyme but it is complexed with immunoglobulins. This can be diagnosed by immunoprecipitation or electrophoresis in a gel, which just has a different gel pattern. This is something you must keep in mind. If it looks odd that you only had AST high with normal ALT and normal alkaline phosphatase. You can see macro liver enzymes in malignancy and inflammatory bowel disease, ulcerative colitis. It's rare. I think in my 10 years I probably have seen three, four cases. .

Clinical Value of Different Patterns

- In almost all liver diseases, ALT is higher than AST except in alcoholic liver disease and in advanced fibrosis
- In alcoholic hepatitis, AST is greater than ALT
 - Alcohol increases mitochondrial AST and decreases cytoplasmic ALT
 - ALT is also low due to pyridoxine deficiency
- AST and ALT are significantly lower in patients with renal failure

And what is the clinical value of pattern of elevation? I'm talking from a clinic setting. I think you can make something out of the pattern of elevation, you know, how much is the AST higher compared to the ALT or the ratio of the AST and ALT. One must recognize in most types of liver disease, ALT is higher than AST. That's one, except alcoholic liver disease, especially alcoholic hepatitis. This is one where AST is higher than ALT. This is once again medical students thing, but nonetheless it's important to remind ourselves.

In alcohol liver injury, why AST is higher? It could be related to increase in mitochondrial AST or decrease in cytosolic AST or excuse me, cytosolic ALT and also there's been talk about ALT's lower in alcoholics, it's because of pyridoxine deficiency. So regardless why it is, it is very clear in clinic when you evaluate patients with alcoholic liver injury, your AST is higher than the ALT.

One other thing. When you, from time to time, you're evaluating ALT in clinical trials and you tend to attribute it to alcohol, be very cautious. I think pure alcoholic liver injury rarely gets ALT above 150, and even that I think is unusual. If you see an ALT of 300 in any clinical setting, it's hard to say this is purely from alcohol injury alone.

Another thing very striking is ALT and AST activity in serum is lower in renal disease patients especially there are advancement of the -- and dialysis patients. This actually could be artifactual because when you do measurements of AST and ALT, it is by catalytic activity and you need to pyridoxal phosphate. In renal failure, there could be an accumulation of binders. That may be the reason why you see low levels. .

Clinical value of different values

- < 8 fold elevations are non-specific
- Fluctuating levels are not uncommon
- Normal AT in patients with HCV and NAFLD may still be associated abnormal hepatic histology
- Levels < 300 IU/L in chronic HCV/HBV, NAFLD, and hemochromatosis

What about different values? I think based on the magnitude of abnormality, you can just use some value. If the value is less than 8 to 10 fold, it is pretty non-specific and you also must recognize that certain diseases, the ALT and AST can be fluctuating. For example, hepatitis C, nonalcoholic fatty liver disease, chronic hepatitis B, can have fluctuations but you don't really see fluctuations in autoimmune liver disease or some other types of liver diseases.

In certain types of liver diseases, for example, hepatitis C and nonalcoholic fatty liver disease, so-called normal ALT doesn't preclude or doesn't exclude significant abnormality when you do a liver biopsy. So that's another paradox one needs to keep in mind. And as I said, typically the levels of ALT are lower than 300 in chronic Hepatitis C, Hepatitis B, NAFLD, hemochromatosis. So if somebody comes in very high ALT, it's not prudent to be hemochromatosis. You just don't see that type of value in that type of liver disease.

Clinical value of different values

■ Very high values in thousands

Ischemic injury

Drug or toxin injury

Viral Hepatitis

Autoimmune

Budd-Chiari

Stones

When you see very high values of aminotransferase, it's not we're talking about several thousands, there are only handful of conditions that can do that. For example, hepatic ischemia, whether it is hypertension or heart failure, drugs and toxins, Acetaminophen can certainly do this, viral hepatitis, acute hepatitis, autoimmune liver disease, like Budd-Chiari and also you don't realize but CBD stones can do very high transaminases.

AT elevation in the context of other abnormalities

- Elevated bilirubin: severity of injury, hemolysis, obstruction
- Very high levels with hemolysis: Wilson's
- Very high levels with new onset ascites: cardiac etiology or Budd-Chiari
- Very high LDH: ischemia
- High CPK: Rhabdo
- APAP phenotype: High AT and INR but not as high bilirubin

But in reality, you rarely evaluate the ALT in isolation. You have a clinical scenario. You have other blood tests available. So ALT elevation's clinical meaning should be taken in the context of other pertinent positives. For example, you have somebody with elevated ALT or AST with elevated bilirubin. We all know it could mean significant liver injury such as high -- or hemolysis or obstruction. If you have very high levels with the hemolysis, -- disease is a consideration. The list is included.

Dr. Lee has described the acetaminophen phenotype which you see in acetaminophen toxicity but you can also see other types of drug injuries as well. We have very high aminotransferase and high INR but no bilirubin, and also the pattern of recovery is quite dramatic. You see improvement pretty quickly.

Pattern of recovery

- Dramatically improving AT indicate ischemic or toxic injury
- Fluctuating at high levels – congestive heart failure, surreptitious APAP intake, arrhythmias

This is pattern of recovery, you have dramatically improving AST This is pattern of recovery, you have dramatically improving AST and ALT. It is ischemia or some types of toxic insult, for example, being paracetamol or acetaminophen.

As I said, fluctuating low levels you can see in certain types of liver disease, but the fluctuating very high levels, 800, 1,000, especially in hospitalized patient, you tend to see that in patients with heart disease, with congestive heart failure, arrhythmia, and you also should consider hypotension or shock .

Reference Range/Limit

- Central 95% of results obtained from healthy persons (AT, hemoglobin)
- Outcome based – fasting glucose or LDL
- Outcome based threshold for ALT to prevent post-transfusion hepatitis
- Newly proposed normal limits of AT are also somewhat outcome based

Last few slides, so much is written about the reference range, and liver injury is, at least when you use aminotransferase, or ALT as a marker for injury, you use times upper limit of normal but as you know, each lab has different upper limit of normal, the reference range.

So looking at how is the reference range established, in case of aminotransferase and hemoglobin, it seems like you take a cohort of normal people and you take the central 95 percent of the people and you describe them as the normal range. However, in the case of some other tests, it can be outcome based. For example, the LDL, the total cholesterol, the triglycerides or for example, fasting glucose, it is not based on normal but it is based on the outcome. In case of diabetes or glucose, we know anything over 125 is considered abnormal. Anything over 100 is considered glucose intolerant.

There's been some attempt to use outcome based on limits for ALT as well, Dr. Seeff-- knows better than I do. There's been an attempt to use ALT thresholds to link and prevent post-transfusion NANB-- hepatitis. And the newly proposed limits of ALT, for example, in men 31 and women 19, are also somewhat outcome based because if you adopt those limits, then you can exclude or you can identify most of the occult cases of Hepatitis C or fatty liver disease, and I think there is some attempt to adopt the limits of ALT more linked to the outcomes rather than just normal distribution. ALT more linked to the outcomes rather than just normal distribution.

NASH CRN study

- 11 clinical laboratories
- ALT values for five CAP samples
- Make and model of analyzer used
- Criteria for reference population

Neuschwander-Tetri B, et al. Arch Intern Med 2008; 168:663-666

I want to show you the data from a paper that was just published in this week, maybe last week's of Archives of Internal Medicine, really highlights how messy the normal range is at least in this country.

NASH CRN study

- Wide range for ULN
- Intra-laboratory variability was minimal
- Inter-laboratory variability was minimal when same analyzer was used
- Maximum variability attributable to different analyzers was 18 U/L
- Most of variability in ULN can be explained by reference population

This was done by the NASH CRN which is funded by the NIDDK and I am PI for one of the centers. Dr. Brent Tetri from SLU was the first author on this. When we were setting up our studies, we realized each of our labs have completely different normal range. So there were 11 clinical labs, part of this network. So each lab director was asked to fill a survey questionnaire about the make and model of analyzer used and what are the criteria that shows the reference population and as part of their certification, they have to test cap samples and what are the results of five cap samples, and you'll was surprised with the results.

One not a surprise, there's the upper limit of normal was quite variable across different labs. These are university labs and they were all over the place, men, women, children and adults, it's quite variable.

Within the lab, the variability was quite minimal and given this, on one hand, the different labs, if they used the same analyzer, the variability for a cap sample was quite minimal. Okay. Even if the labs use different analyzers, for a given sample, cap sample, the variability was not as high. So most of the variability is not from the machine. It is actually coming from how the reference population is described.

Reference Population

1) Established long time ago	7) Excluded those with medical conditions and those taking any medicines (n=94)
2) Selected blood donors (n=100)	8) Unchanged for 25 years (n=?)
3) Excluded those with medical conditions and those taking any medicines and IVDA	
4) None (n=222)	
5) Excluded those with medical conditions and those taking any medicines and IVDA (>120)	11) Excluded those with medical conditions (n=1029)
6) Selected people with normal AST, AP and bilirubin (n=1354)	

And this table looks busy, but ask how the reference population was chosen. What you see is that some labs didn't have any reference population. Some just got from textbooks, and some just excluded some medical conditions. There was no consideration of obesity at all and the only thing I thought was interesting from this is one lab selected people with normal AST, ALT, normal AST, alkaline phosphatase and bilirubin. So I thought that was interesting, had a large enough sample size.

So really, you know, we have done a larger study and in Indiana, about 100 labs and it's the same thing. It's just all over the place and some of the lab directors written to us saying they used the textbook to establish the reference population. So I think there needs to be some standardization of how we establish the reference range.

Serum ALT as an indicator of health and disease

- Serum ALT activity correlates with mortality rate
- Of 46,923 Olmsted county residents, 6,792 had ALT measured in 1995 and 907 ALT values were abnormal
- Standardized mortality rate for ALT
 - > 1-2 ULN: 1.22
 - > 2 ULN: 1.63
 - < ULN: 0.61
- In a large population-based Korean study, ALT activity correlated with all cause mortality and cardiovascular mortality

Finally, serum ALT as an indicator of health and disease, there are now data that in addition to the ALT as a liver biomarker, liver disease can predict future death. There are data from Mayo Clinic and from Korea to ALT levels can predict overall and cardiovascular mortality. I think this lends credit to the AASLD's suggestion that serum ALT should be used not just as a marker for liver injury but all health and disease.

Summary

- Serum AT measurements are clinically relevant laboratory tests
- ALT is more specific than AST to liver
- When evaluating elevated AT activity, one needs to keep extrahepatic sources as well as accompanying abnormalities in context
- ALT measurements deteriorate rapidly with freezing and thawing

To summarize, and I think these are AST and ALT are very old tests but they remain to be widely used in routine clinical practice, and when we evaluate elevated aminotransferase in patients, we need to take into consideration the extrahepatic sources and other tests in the context. And ALT measurements can deteriorate rapidly with the freeze/thaw cycle.

Summary

- Wide variability in reported reference range is largely due to inconsistent/poor choice of reference population
- As ALT activity is indicator of overall health, one might consider accepting outcome-based reference range

The wide variability in the reference range across different labs is largely due to the poor choice or inconsistent reference population selection. And as the ALT is an indicator of overall health, very similar to cholesterol or glucose, one could make an argument that we should think about setting ALT limits more outcome based, not necessarily what's the distribution or so-called normal but we should just say this would capture most of fatty liver rather than the reference population. I think that may avoid some of the difficulties with the normal range. That's my last slide.

Thank you. Thanks for your attention.

(Applause.)