NON-INVASIVE STAGING OF HEPATITIS C PATIENTS

A CAPSTONE
SUBMITTED TO THE DAOM PROGRAM
AND THE COMMITTEE ON DOCTORAL STUDIES
OF FIVE BRANCHES UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTORATE IN ACUPUNCUTURE AND ORIENTAL MEDICINE

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Abstract

Background: The current standard of staging Hepatitis C patients is through liver biopsies. There are many issues with liver biopsies including accuracy, cost, injury, and how often they can be performed. There is tremendous need for new non-invasive methods for staging liver disease. One system called APRI (AST to platelet ratio index) uses inexpensive blood tests, commonly performed with a freely shared mathematical formula. This paper will present the author's new system, called AL after my company Alchemist Lab, which also shares that same criteria of inexpensive and routinely performed blood tests, with a non-proprietary formula.

Methods: Three patient cohorts who have had a liver biopsy and blood tests within 60 days of that biopsy are grouped by biopsy stage. The Mild Cohort is stage 0 -1.5. The Moderate Cohort is stage 2 & 2.5. The Advanced cohort is stage 3 – 4. An additional group of patients with at least one of the symptoms of decompensated cirrhosis are grouped together. The last available blood test is used for this group. In total there are 72 patients in the study.

For each patient their case file was examined for ALT, AST, the ALT/AST Ratio, platelets, albumin, creatinine, INR, prothrombin, alpha-fetoprotein, total bilirubin, age, and gender. Both the APRI and AL scores were calculated.

Results: APRI, AL AST, AST/ALT Ratio, and platelets were all scored. There were 45 patients who had all the markers to calculate both the AL and APRI. These patients were ranked from stage 0 to the decompensated cirrhosis group numbered 1 to 45. Each test subject like AST was judged as to whether they placed each patient in the correct sequence +/- 5.

Both AST and APRI scored 64%. The AST/ALT ratio was 71%. Platelets scored 84%, and AL was 89%.

In the second test model AL alone was scored in reference to how well it matched up with the Metavir Biopsy Scale. Stage 0 & 0.5, Stage 1, 1.5, 2, 2.5, 3, 3.5, 4, and the decompensated group was assigned stage 5. (Different variations of the Metavir assign this group as 4B, or 4B & 4C, for clarity sake I chose stage 5). In all nine different categories were formed.

The average deviation of the 45 patients was 0.611 of a stage.

Conclusion: AL is a more accurate system than APRI for two reasons. The first is that AL uses AST/ALT Ratio divided by platelets as its core. APRI utilizes AST divided by platelets as its formula. AST/ALT Ratio is a more accurate measure of the progression of liver disease than AST alone. Secondarily AL uses albumin, bilirubin, age, and either AST or ALT to refine its formula. The Italians have a modified APRI using many other markers as well to improve on the performance of APRI, however they use a proprietary formula.

Introduction: ***A Small Moving Target:*** Hepatitis C is a very tiny, mutable RNA virus that infects 3% of the world's population and approximately 5 million Americans. It is widely considered to be the most dangerous of the ABC's of liver viruses. Hepatitis C is responsible for up to half of all American cases of end-stage liver disease.

HCV is spread primarily through blood contact. IV drug use accounts for the majority of cases of transmission, including approximately 70% of the patients seen in our clinic. Blood transfusions and blood products used before the advent of a screening test in 1992 in America is another major pathway of infection. These cases account for about 15% percent of our patient population. In developing countries the re-use of syringes and lack of proper sterilization of medical equipment is a primary route of transmission. In the Vietnam War era new recruits were lined up in alphabetical order and were vaccinated en masse with the same vaccination gun. This was also a significant spread of the infection.

Current pharmaceutical treatment of HCV is experiencing rapid improvement as a new generation of drugs are being studied that are both far more effective as well as being less toxic than interferon and ribavirin. PSI-7977 made by Pharmasset has cleared all our patients enrolled in trials within the first 2 weeks of therapy. This is a nucleoside analog. Nucleosides are the building blocks of RNA and DNA chains. When the virus goes to replicate and it grabs the PSI-7977 and incorporates it into the RNA strand that is used for viral replication, that strand is dead-ended.

Ribavirin is an oral broad-spectrum antiviral that in combination with the PSI-7977 increases that rate of sustaining viral clearance.

Diagnosis of hepatitis C is made through a blood test. Staging liver disease involves determining the extent of liver scarring. The most definitive method of staging hepatitis C is made through liver biopsies.

Blood Tests and Staging Hepatitis C

The overall biopsy rate of HCV patients in America runs around 50%. Of those who do get liver biopsies, they are generally performed once every five years. A wide array of blood and other tests have been created to fill that long vacuum. One such test is transient elastography, or the FibroScan which measures the stiffness of the liver. Healthy livers are soft and pliable, as they get scarred they harden.

A recent study from Germany concluded that serum or blood tests are more accurate than transient elastography for staging liver fibrosis[1]. The fact that transient elastography costs around $3000 per patient and was less accurate than the blood panels it was compared to, helped focus my research on blood tests.

Many of the newer blood panels marketed for assessing progression of hepatitis C cases include secret algorithms, newly developed tests, and are also expensive. The author's own criteria focused on inexpensive and commonly performed blood tests. These similar

goals are shared by third world researchers as the needs of their societies propel them in that direction. For example the Turkish researcher R Vardar[2] studied AST, ALT, GGT, and Platelets. Then with also including the patient's age his team looked at these markers plus Age/Platelet Index, AST/Platelet Index (APRI), GGT/Platelet Ratio, AST/GGT Ratio, and AST/ALT Ratio. These are all routine, inexpensive tests and simple mathematical formulas can be derived from them.

Inability of liver enzymes and viral load to reflect progression of disease. The Turkish study by Vardar showed no statistical significance for both AST and ALT between the cohorts stage 0 -1 and the cohort stage 3 -4. As patients first come to our clinic or phone in, most often they offer their current liver enzymes (ALT and AST) and/or their viral load as the central evidence of how they are doing. However these primary focal points of Hepatitis C lab work have no bearing on the stage of fibrotic liver damage. Liver enzymes measure inflammation, not scarring.

Likewise, the viral load gives us little information on the condition of the liver. The author has seen a patient with a HCV (Hepatitis C Virus) viral load of over 50 million out running triathlons, who felt like Superman. Our clinic has also seen a number of patients with very low HCV counts on their deathbeds.

Inaccuracy of the HCV Fibrosure Test: This highlights the question of how best to utilize blood tests to be able to assess HCV patients. One of the first attempts to answer this question was the HCV Fibrosure Panel. This test measures both inflammation as well as fibrotic scarring as do liver biopsies themselves. Unfortunately our clinic has found the Fibrosure Panel woefully inaccurate with our patients.

One of the author's patients had a mark on his HCV Fibrosure Panel which showed him in a state of cirrhosis. He was in an extremely high state of panic, convinced that death was lurking around the corner. After reviewing his case, he was informed that he was clearly not cirrhotic and to go get a biopsy to establish his true condition and to not panic. He did just that and when the biopsy report came back, he was between Stage 1 & 2 (1.5 in my jargon). The Metavir scale for liver biopies runs from 0 which is no damage to cirrhosis which is a 4.

This same pattern happened three more times in the next few months as the Fibrosure Panel became more widely used. With all three patients the author went through the same routine, suggested that they did not have cirrhosis and that the test could not be right. The author recommended they get biopsies to allay their fear and to achieve some measure of certainty. The three patients ended up being Stage 2, 2.5, and 3 in terms of fibrotic damage on their respective liver biopsies.

One study of the accuracy of the Fibrosure Panel showed a 29% rate of error by 2 stages or more.[3] Essentially, it is worse than useless, in fact, it scares people causing unnecessary suffering, as it tends to score high compared to liver biopsy. The author surmised that he could get a much more accurate sense of a patient's stage of liver

disease by correlating simple blood tests, symptoms, and palpating the liver. This paper is largely about substantiating that assertion, and backing it up with research.

As inaccurate as the Fibrosure Panel is it has still been very useful as a forerunner leading to more precise means of assessment via blood tests. Listed below are the components of the HCV Fibrosure as well as other systems that are already in place to assess the progression of liver damage of hepatitis C patients.

The **HCV Fibrosure Panel** uses the markers:

- Alpha 2-Macroglobulin
- Haptoglobin
- GGT
- Total Bilirubin
- Apolipoprotein A1
- ALT
- Age
- Gender

The **MELD system** is the Model for End-Stage Liver Disease. It is a calculation that is used to allocate liver transplants, originally formulated by the Mayo Clinic. MELD relies on just three markers:

- Bilirubin
- INR
- Creatinine

If patients are receiving dialysis their creatinine level is set to 4.0 mg/dl.

The **PELD system** is the Pediatric End-Stage Liver Disease calculator, replaces Creatinine with Albumin, and adds in age and growth failure calculated by weight, height, and gender. It is used for children under the age of 12.

FibroSpect II uses different components in the fibrogenic cascade:

- Hyaluronic Acid
- TIMP-1
- Alpha 2-Macroglobulin

The **Enhanced Liver Fibrosis Panel** was designed to test for Non-Alcoholic Fatty Liver Disease. It is included here even though it is not a panel used to assess HCV as it is intelligently designed with applicability to Hepatitis C cases:

- Age
- Body Mass Index
- Fasting Glucose
- Presence of Diabetes
- ALT/AST ratio
- Platelets
- Albumin

Forn's Index also uses commonly performed tests with a non proprietary formula.

Platelets
GGT
Age
Cholesterol

Case Study Project of Hepatitis C

Purpose

Many patients choose to not get liver biopsies for many reasons; from the financial to a visceral disinclination to having a large needle jabbed into their liver, as well as fear of the dangers involved. Patients die during liver biopsies at the rate of 1 in every 9,000 to 12,000 performed, depending on the study. Hospitalization rates also vary, but can be as high as 5.4%.[4] Both guided ultrasound and in-patient care make liver biopsy safer. Blood panels that assess the stage of liver disease use a wide array of blood markers and are increasingly looked to as alternatives to liver biopsy.

Methodology

The methodology of this study consisted of studying the records of more than 400 cases to select the 72 included in this project.

Criteria for selection of the Decompensated Cirrhosis Cohort: The author chose one grouping of 18 extremely advanced patients, the Decompensated Cirrhosis Cohort (DCC) to study. The criteria for this group was any patient meeting the definition of decompensated cirrhosis. These patients do not get biopsied as often. The staging of their disease is very clear from their symptoms. Biopsies are significantly more dangerous in this patient population due to uncontrolled bleeding. This group gave a clear image of where numbers culminate in end-stage HCV. The most recent available bloodwork was used for this cohort.

Definition of decompensated cirrhosis: Decompensated cirrhosis is simply when the liver becomes so damaged that primary functions fail. For example, the liver clears ammonia from the body, when it can no longer do that it leads to hepatic encephalopathy as the brain is poisoned by high ammonia levels. It also leads to muscle wasting as the muscles have an inefficient mechanism of clearing ammonia that creates wasting. The advanced symptoms come directly from a failing liver. The symptoms that give a clear indication of decompensated cirrhosis are ascites, variceal hemorrhage, encephalopathy, muscle wasting and jaundice. Of these, jaundice can appear in acute hepatitis as well. I would also include HCC (hepato-cellular carcinoma or liver cancer) because in Hep C patients it almost always occurs in end-stage patients. The image of death in end-stage HCV is stick-like arms and legs with a swollen belly, very similar to the distended bellies of starving children.

Variations in the Metavir Biopsy Scale in numbering decompensated cirrhosis patients: In the most utilized biopsy system all cirrhosis cases are labeled a 4. Often people will use 4A to designate cirrhosis and 4B to label decompensated cirrhosis (DCC). Some use a system of 4A for cirrhosis, 4B for cirrhosis with advanced symptoms that are controllable with medicines. For example, someone has ascites and it is controlled with diuretics effectively. In this system they designate 4C for those patients with advanced symptoms that cannot be controlled with meds. In the system that the author utilizes the decompensated cirrhosis cohort is designated as 5. This is largely done as there is an enormous range of health and disease within the label of cirrhosis and the author believes it does a disservice to patients to group end-stage patients with patients who often still feel quite well. The liver has incredible capacity to remain functioning even after severe damage has taken place.

Criteria for the Mild, Moderate, and Advanced Cohorts: For the other 54 patients in the study group the author chose cases with liver biopsies, that also had labs within 60 days of the biopsy date. The great majority of the cases had bloodwork within 30 days of the biopsy date. If there were multiple labs within the 60 day framework, the labs were chosen that were closest to the biopsy date for that marker. For example if there was a blood test taken on the same day as the biopsy that had a comprehensive metabolic panel which includes ALT and AST. Then 30 days later there were more blood tests taken with another comprehensive metabolic panel as well as a CBC (Complete Blood Count) with platelets taken as well. The author would use the platelet reading from the later date, but the liver enzymes from the same date as the biopsy.

Metavir Biopsy Scale: The groups were divided into roughly 4 cohorts with between 17 and 19 patients in each grouping. Each group is sorted according to their biopsy stage using the Metavir Liver Biopsy Scale. This is the most commonly used scale for staging liver biopsies. 0 indicates no fibrotic liver damage, 1 is mild fibrosis, 2 moderate, 3 more advanced, with 4 being cirrhosis of the liver.

Results

Immediately following are the charts that were made from the research in the order of **Platelets, Albumin, Alpha-Fetoprotein, Bilirubin, ALT & AST, INR & PT, Creatinine,** and **Age.** Definitions of the markers as well as interpretations of the results are presented together.

The **four cohorts, Mild, Moderate, Advanced,** and **Decompensated Cirrhosis** with all the markers that were studied are laid out **Appendix A.**

Appendix B consists of head-to-head match-ups of **AST** and the **AST/ALT Ratio**. Then **APRI, Platelets,** and my system **AL** are directly compared to each other.

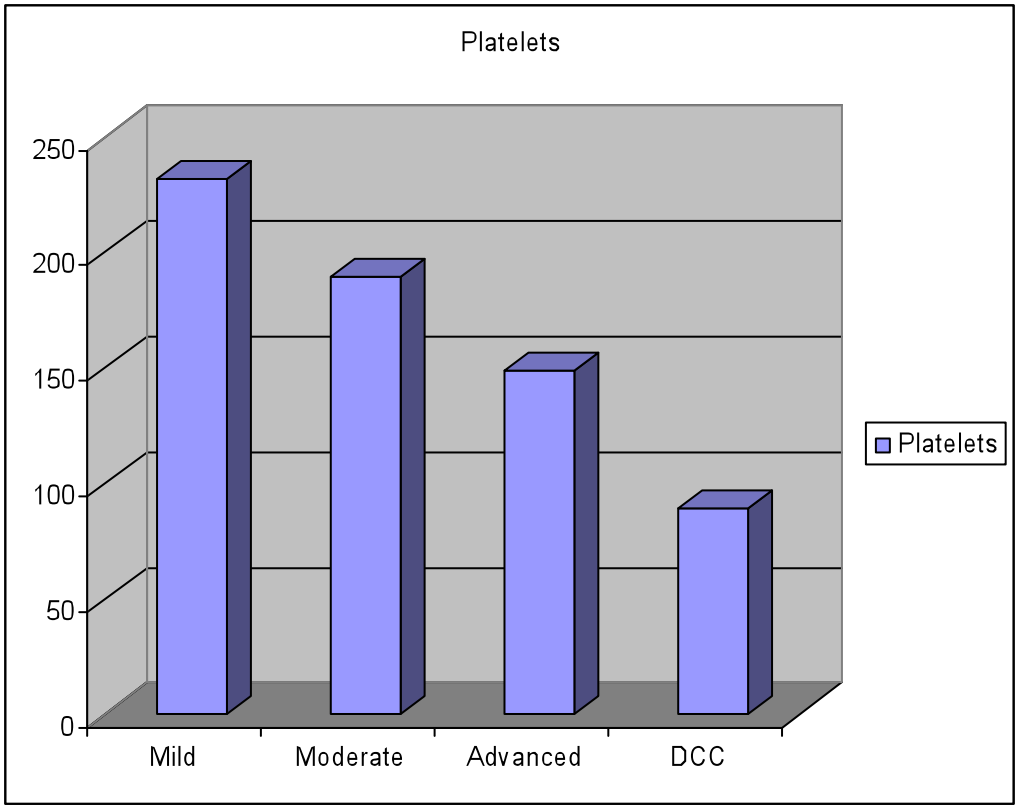
Platelets are blood cells that help in the clotting of the blood. They are the most important of the blood markers in terms of accurately reflecting the progression of liver damage in Hepatitis C. Normal reference ranges vary, typically they can be 140 – $400x10^3$ per microliter. The spleen both filters and stores platelets and can become damaged in Hepatitis C.

Platelets were the only marker to exhibit a predictable drop in a step-like pattern through each grouping, as well as no group overlap between the Mild and the DCC Group. The lowest platelet is the Mild Group was 160, the highest in the DCC Group was 146.

Platelet Scale for HCV Patients

250 – 400	Strong
200 – 249	Good
140 – 199	Normal-Lower End
100 – 139	Low
70 – 99	Of Concern Low
50 – 69	Critically Low
49 and under	Danger Zone for Hemorrhage

Platelets staircase downwards as the cohorts progress.



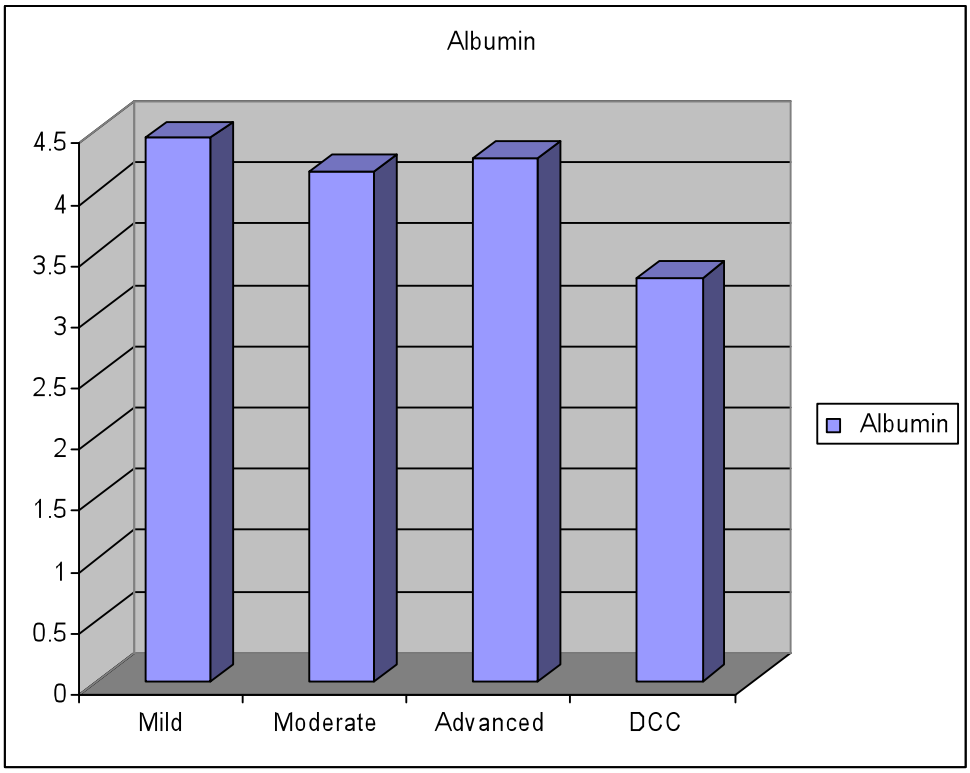
Albumin is produced by the liver. Albumin is both a transport protein and it maintains the osmotic pressure in blood vessels. When albumin levels drop too low, fluid leaks out of blood vessels causing edema and/or ascites. It is measured in g/dl (grams per deciliter), with reference (normal) range 3.5 to 5.1. Note that cases of protein malnutrition also results in low albumin with the ascites. The image of a starving African child with swollen belly and stick-like arms and legs is also the image of advanced Hepatitis C.

Creating an accurate scale for albumin is more challenging as we have seen edema and ascites in patients with greater variation, including albumin levels well into the normal range. Here is a rough guide for you.

Albumin Scale for HCV Patients

4.5 to 5.0 Strong
4.0 to 4.4 Good
3.5 to 3.9 Low Normal
3.0 to 3.4 Low
Under 3 Critically low

Albumin levels are maintained well until end- stage hepatitis as the liver preserves function very well.



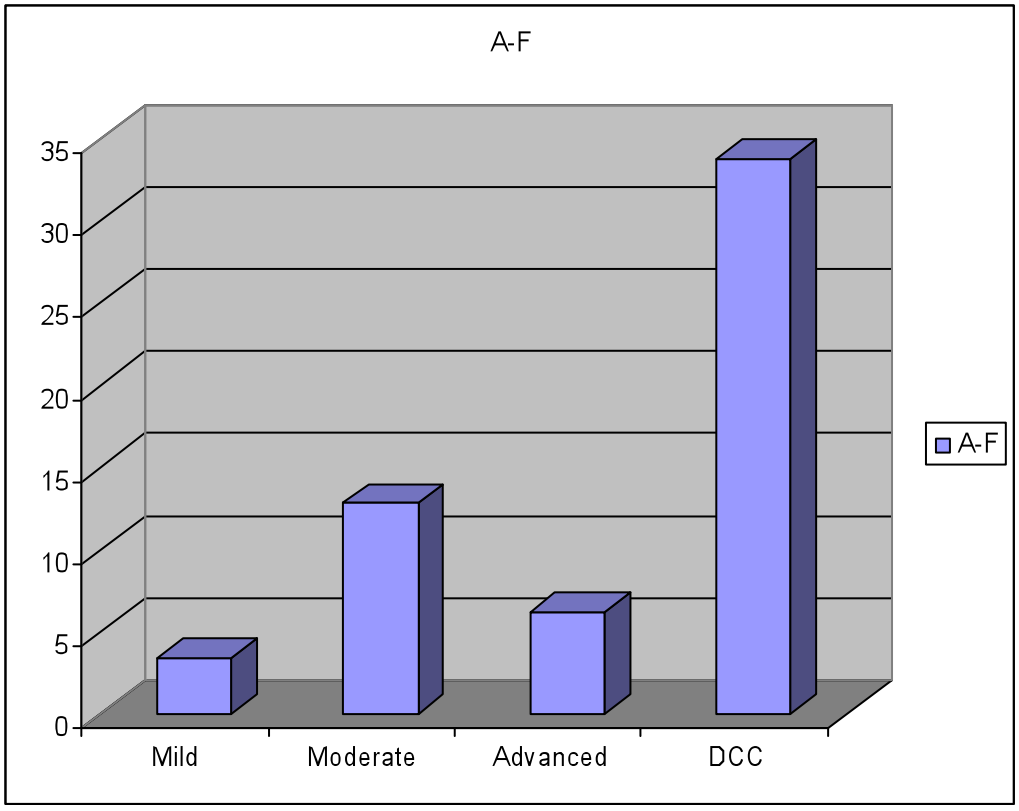
Alpha-Fetoprotein is produced by the fetal yolk sac as well as the liver. In rat studies it was found to prevent the masculinization of female fetuses. By 8 to 12 months after being born A-F levels drop to a very low level, as it has no known function in healthy humans after birth. A-F can rise to very high levels in primary liver cancer.[5] AFP is measured by ng/ml (nanograms per milliliter). Lab values differ tremendously, with anywhere from over 6 ng/ml thought of as out of range to over 20 ng/ml.

The Alchemist Lab Scale for Alpha-Fetoprotein:

Normal under 10
Elevated 10 to 200
Beginning Stage of Danger Zone 200 to 400
Danger Zone for liver cancer over 400

In all these years of practicing the author has only seen one person who actually had liver cancer whose AFP value was under 200 (161). The majority of cases of frank liver cancer we have seen the Alpha Fetoprotein value was over 1000. Yet we routinely see patients who have been told that they are dying when their levels are 25 by uninformed doctors..

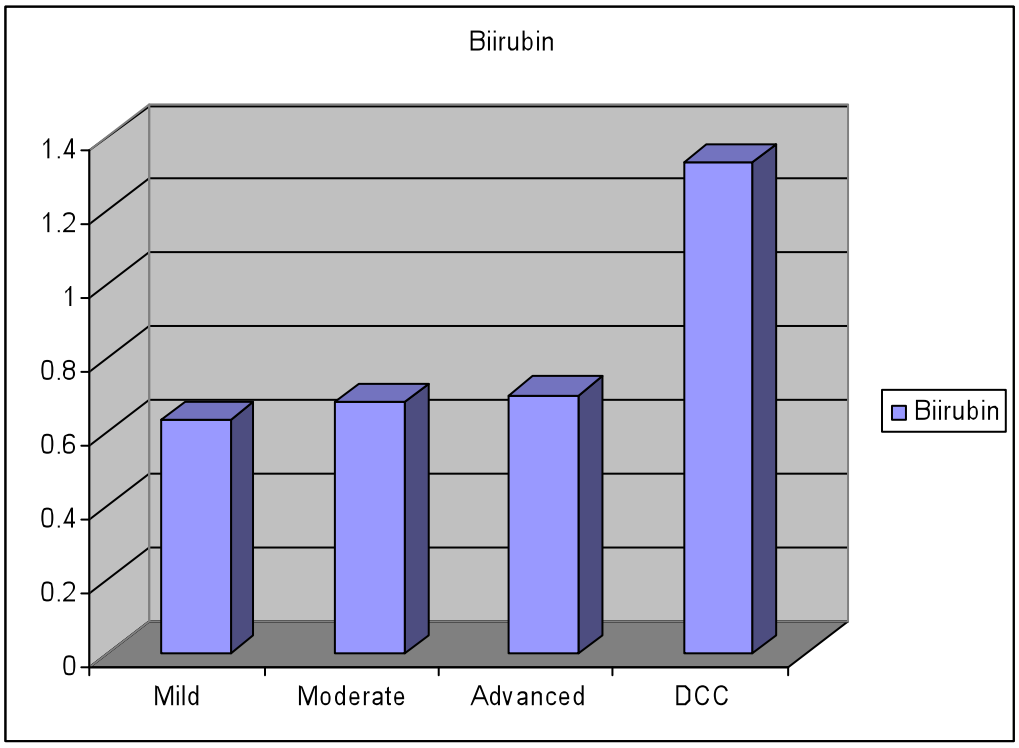
Alpha-Fetoprotein, which prior to this study, the author had thought would only rise when the liver was heavily damaged, flared up as early as Stage 2.



Bilirubin: This is the yellow colored pigment that the liver produces when it recycles the hemoglobin from worn out red blood cells. It is excreted in the bile and the urine. In cases of jaundice it is what gives the skin and the eyes the yellow color. It is also what colors the urine yellow as well as gives some bruises their yellowish hue. High levels make the skin extremely itchy. Bilirubin is measured by mg/dl (milligram per deciliter). The reference range varies from lab to lab, but normal bilirubin is generally under 1.2 mg/dl.

Bilirubin is not as critical of a sign as extremely low platelets, albumin, or high alpha-fetoprotein in assessing the level of liver damage. This is because bilirubin can be high for different conditions as in cases of acute hepatitis or in conditions where bile is blocked. Also it is not life threatening like the other three markers, for example low platelets can result in traumatic hemorrages. High alpha-fetoprotein can signify liver cancer, which is a very difficult cancer to treat successfully.

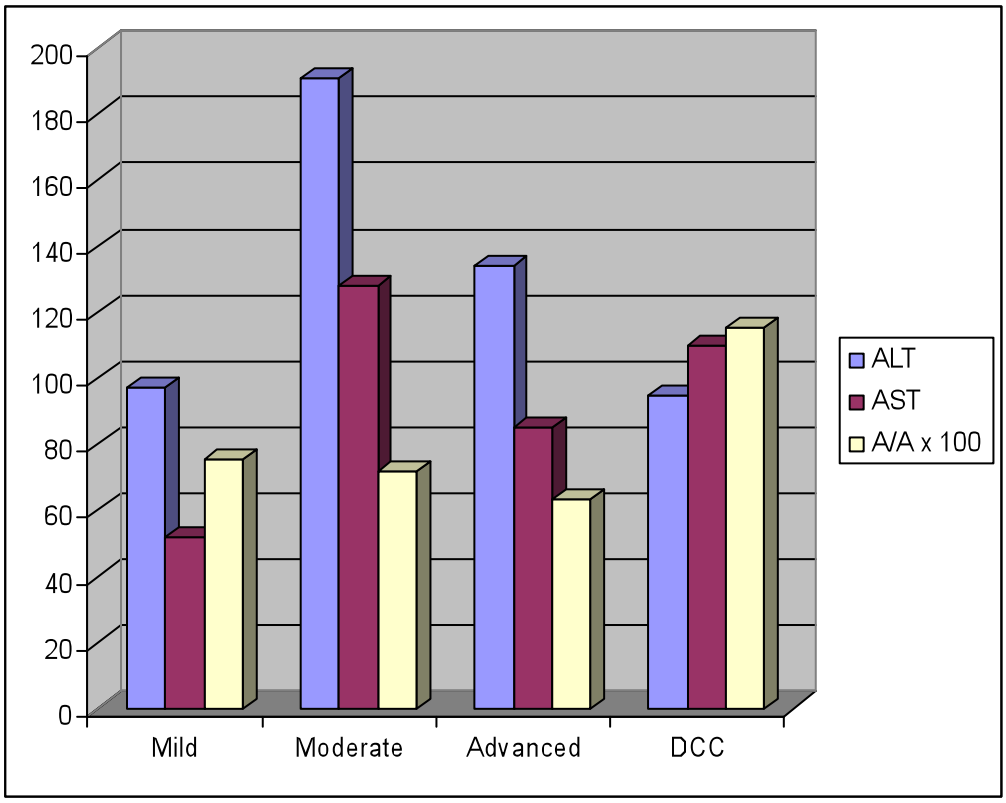
Bilirubin like albumin shoots up in end-stage HCV patients, illustrating how the liver preserves function well until it is very heavily damaged.



ALT & AST: Collectively they are called transaminases. They help to make the energy storage protein glycogen. ALT or alanine aminotransferase is only produced in the liver, whereby AST or aspartate aminotransferase is found in other organs as well. High levels of AST for example, can reflect a heart attack. The transaminases leak out when there is cellular injury or inflammation.[6] They are measured in international units per liter. The normal range of AST is 0 – 45iu/l, that of ALT is higher 0 – 55 iu/l. Again there is enormous variability in the reference ranges from lab to lab.

The Decompensated Cirrhosis Group is the only one to have higher AST values than ALT, with a ratio of **1.157**. The AST/ALT ratio, whereby the AST was higher than the ALT happened in **76%** of the **Decompensated Group**, but in only slightly over **5%** in the other 3 cohorts. **This is a very important marker for progressed liver damage.**

The two main liver enzymes and their ratio to one another.

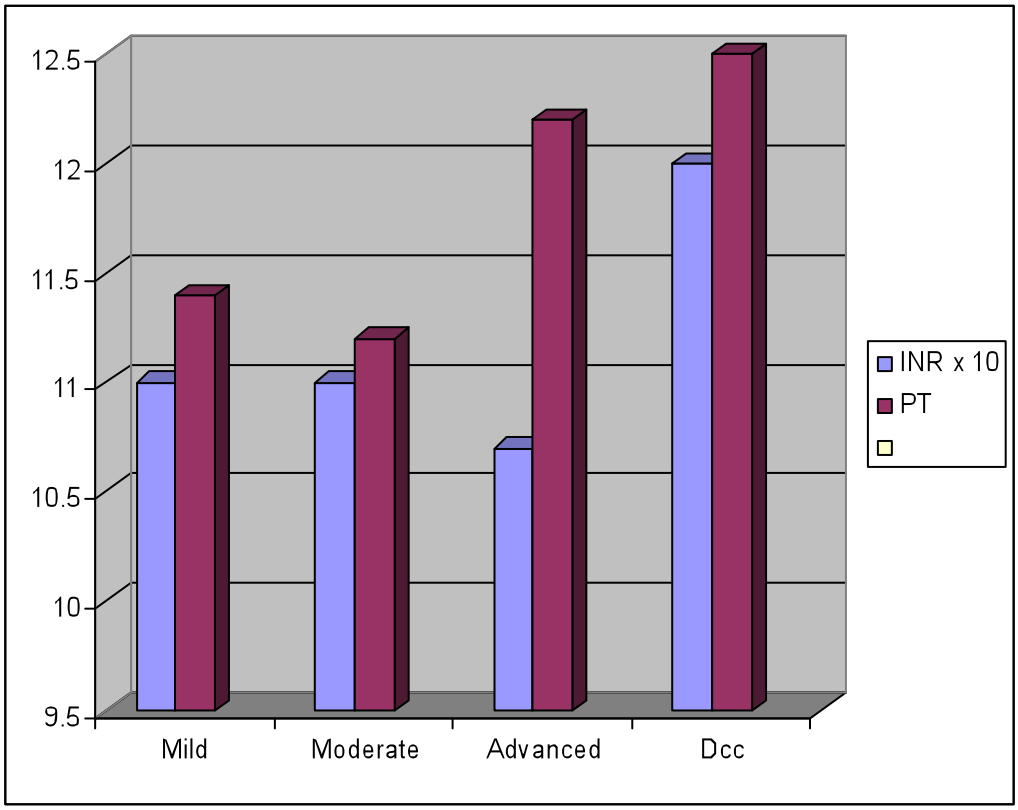


INR/PT: PT or Prothrombin is the time (it is measured in seconds as is INR) that it takes plasma to clot after the addition of tissue factor. PT, or Prothrombin Time, measures blood clotting factors II, V, VII, and fibrogen. The liver produces all the blood clotting factors that are tested by PT.

INR or Internalized Norm Ratio is a method of standardizing results for PT. This is because different batches of tissue factor vary. Each manufacturer assigns a sensitivity index for each batch.

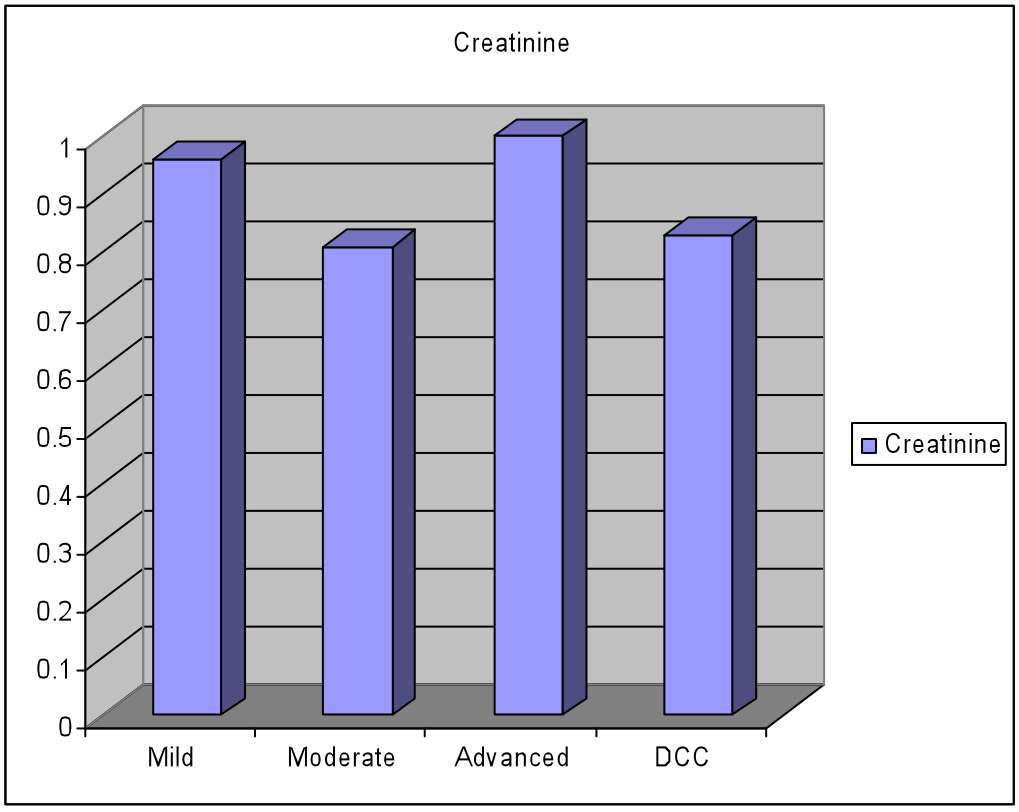
Both INR and PT measure the extrinsic pathway of coagulation which is the most common one.

Coagulation markers INR and PT generally rose as the cases progressed, but were not performed on a routine basis as often as some of the other tests.



Creatinine: This is a waste product produced in muscle metabolism from creatine, which is made and excreted into the blood at a constant rate. It is eliminated primarily through the kidneys and indicates the glomerular filtration rate (GFR). Since it has a stable value in the blood, raised levels show poor kidney function. Creatinine is measured in mg/dl (milligram per deciliter). Range varies in terms of how muscular the individual is, with the reference range for men being higher than for women, with blacks higher than whites.

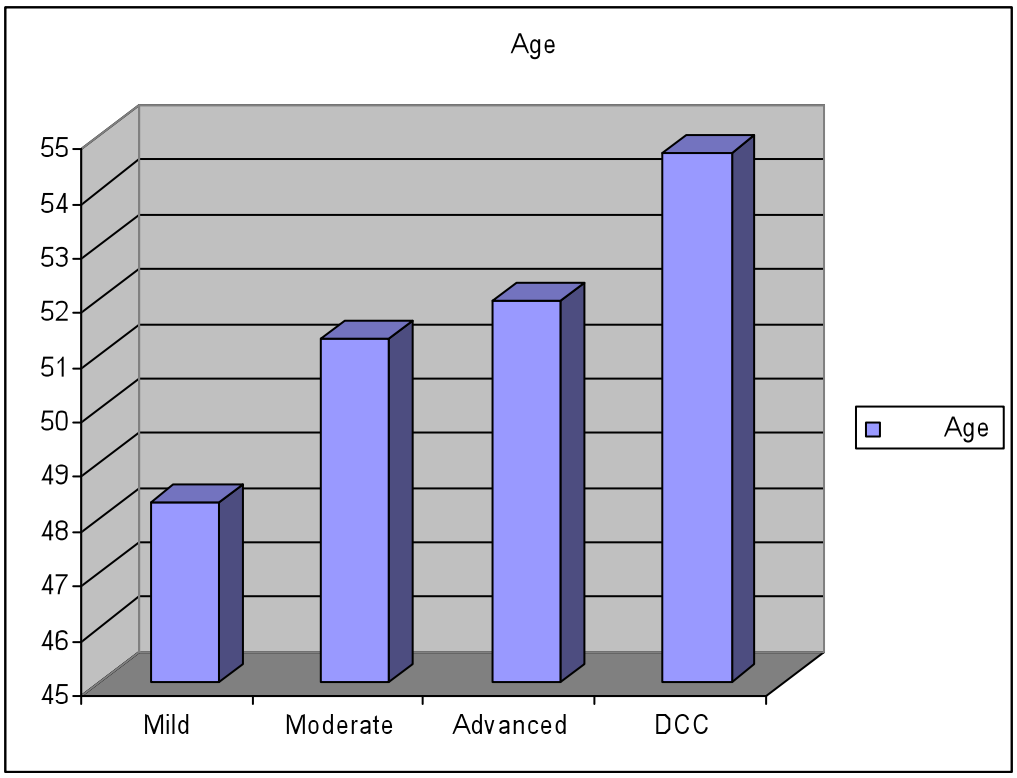
Creatinine was not a reliable predictor of staging, and it is telling that in the PELD Model it was replaced with Albumin, showing that others have come to a similar conclusion - at least with children.



Age

Because it is often difficult to pinpoint time of infection, it is hard to ascertain whether the more damaged groups simply had the disease longer, or whether aging speeds up the disease process itself, but clearly there is a strong correlation between disease state and age.

Age has a congruent rise between groups.



Discussion

Biopsy Accuracy: **Liver biopsies have two main problems in terms of accuracy. The first is sampling error.** The liver is a large organ and damage is not consistent throughout. Tissue that is extracted in one area might not represent the liver as a whole. One study took samples from the different lobes of the liver and then examined each lobe's tissue slides separately. They then compared the two separate biopsy reports from the same patient and found a surprisingly high rate of different stage classification.

The second big issue is error of interpretation or reading. Researchers from UCSF[7] studied this issue closely and came to the conclusion that some readers staged biopsies incorrectly. They found that these pathologists were consistent, but consistently higher or lower than the correct staging.

Questions about biopsy accuracy in this study, in terms of a few patients in the Moderate Cohort with more progressed numbers as well as symptoms. Both albumin and the APRI value were higher in the Moderate Group than the Advanced. The Alpha-Fetoprotein was also significantly raised in three patients and several of the patients involved went on to exhibit more advanced symptoms faster than moderately progressed patients normally do. Perhaps these discrepancies would disappear in larger studies or perhaps some liver biopsies are tarnished by subjective pathologists or unrepresentative samples.

The APRI & Other Non-Invasive Systems Re-Visited

An expended discussion on APRI as it also uses commonly performed blood tests, with no proprietary algorithm. This allows us to compare APRI head-to-head with AL, the system the author has created.

APRI is a very simple formula using AST divided by its reference range, times 100, then divided by the platelet count. Every lab has a different reference range of what is normal. With AST it often varies between 40 and 45.

A study from Saudi Arabia[8] **concluded** that the **APRI was more accurate than platelet levels, followed by the AST/ALT ratio** in predicting significant fibrosis when compared with liver biopsy. Another study from Mexico[9] concluded that APRI is a useful noninvasive alternative for the diagnosis of significant fibrosis and cirrhosis in hepatitis C patients.

The promise of the APRI is that it uses simple markers, both routinely performed in HCV cases and inexpensive. This is one reason that it is frequently used in developing countries around the world like Brazil and Mexico. It can also give a historical perspective as these tests have been performed all along with most hepatitis C patients.

The Italians[10]combined the APRI, with the **FibroTest** (which is bilirubin, GGT, apolipoprotein A1, alfa-2-macroglobulin, and haptoglobin), and the **Forn's Index** (platelets, GGT, Age, and cholesterol in a non-proprietary formula) into an algorithm With this system they achieved a 94% rate of correlating significant fibrosis. Here again, badly damaged livers are easier to identify with precision than portraying the progression of mild fibrotic cases. Nevertheless these are excellent results compared to all other models that have been developed. Significant fibrosis is usually defined as Stage 2 or higher.

The **Enhanced Liver Fibrosis Panel** used for assessing fatty liver utilizes body mass index, fasting glucose, presence of diabetes, ALT/AST ratio, platelets, albumin, and age. The central problem of the **ELF** is that it is more accurate in cases of severe fibrosis – 98%, 93% in moderate fibrosis, and 84% for no fibrosis.[11] **This pattern of more accurately identifying significant fibrosis is similarly characteristic of all non-invasive systems for staging HCV, including AL.** The other issue inherent in this discussion is that the very advanced patients can be staged from their symptoms, like the

author did in choosing the Decompensated Cirrhosis Cohort. Meaning the patients that are the easiest to stage using blood tests are the ones that need it least.

Weaknesses and strenths of APRI: The author recognizes the success of the Italian APRI, which achieves a higher level of accuracy than other models. However there is a downside of using a proprietary algorithm with the addition of less common tests. Both issues affect cost and availability. The Alchemist Lab starting point was to use tests that are both universal to hepatitis C patients along with reflecting progression of liver damage. The author's other critique of the Italian model is its use of APRI as its base. In this study of the Alchemist Lab patients the APRI did not perform well and in looking at its make-up the author reasoned that a system based on ALT/AST ratio (A/A) divided by platelets would be more accurate than one based on AST divided by platelets. In the Saudi[10] studies A/A followed platelets for single marker accuracy in predicting significant fibrotic damage.

The Alchemist Lab Formula

Utilizing **platelets and the A/A ratio as the core**, the author created a formula selecting **albumin, bilirubin, age, and a system for weighing AST and ALT.** This was compared to just using the A/A ratio divided by platelets, and the enhanced version was more accurate.

For AST and ALT the endpoint of the reference range was employed. In researching five different lab reference ranges, the AST was spread from 35 to 57, the average being close to 45, so 45 is used for the AST and likewise 60 for ALT. If the person had an **AST under 45 the amount under 45 is subtracted** If it was **over 45**, **the amount over 45 is added** to the score.

Balancing out cases with very high ALT scores that might skew the formula: Some subjects had a very low A/A range because their ALT was so elevated. For a subject with an **A/A under 0.5**, and their **AST over 45** the AL Formula takes the **ALT number and adds in anything above 90**. For instance, one subject had an AST of 65 and an ALT of 263, this gave an A/A of 0.247 - a very low number. Instead of adding in 20 points for an AST over 45, 173 was added in for an ALT over 90. This is a balancing feature for data that can be skewed by extremely high ALT readings.

For age **simply add in the age**. For both albumin and bilirubin the midpoints of the averages of reference ranges are used; for albumin 4.3 and bilirubin 0.7. **Albumin** is a more important marker and **10 points plus or minus for each .1 deviation from 4.3** is utilized in the Al Formula**.** For **bilirubin 5 points for each .1 deviation from 0.7** is used. For albumin scores **add in the number for under 4.3, subtract for any albumin score over 4.3.** For **bilirubin over 0.7 add in that score, for bilirubin under 0.7 subtract** from the AL total score.

In this study women were more advanced by .20 of a stage. Because of conflicting evidence with some studies showing that men advancing more quickly the author chose

to leave out gender as a factor in the AL Formula. Men have a higher incidence of HCV infection attributed to higher rates of IV drug use. One fascinating study[12] showed that women were actually less able to fight off the virus when exposed due to higher levels of interleukin 10, which makes for less efficient viral clearance.

An example of using the formula on an individual patient: Choosing the first subject in the Mild Group we take the A/A ratio of 0.411. We actually multiply it by 100,000 or for simplicity, calculate 411 and punch in two more zeros. 41,100 divided by 237 equals 173.4. Add in age, which is 56. Albumin is 4.5, 0.2 over the midpoint so we subtract 20. Bilirubin is 0.6, 0.1 below the midpoint and we subtract 5 points. The A/A ratio is under .5, but AST is below 45, so we use AST as the marker. Subtract 45-23=22, so we take another 22 off. 173+56-5-20-22=182.

The lower the number the less fibrotic damage of the patient. Tthe markers age, bilirubin, AST and ALT the lower the number the better for the patient. Only platelets and albumin are the higher the number the more favorable for the patients and in this formula platelets are divided and albumin over 4.3 is subtracted.

Comparing AST to the AST/ALT Ratio, and APRI, AL, and Platelets

To assess how well each single marker predicted the stage, the author took the **45** subjects that each had all the markers that were needed for comparison. These were **AST**, the **ALT/AST ratio, platelets, APRI**, and **AL**, which relies on platelets, A/A, albumin, age, bilirubin, AST, and ALT. Of these 45 subjects 9 were from the mild group, 12 from the moderate, 9 from the advanced, and 15 from the DCC. The cases were judged to be in range with a +/- 5. In this matrix a Mild Group member within the first 14 numbers was classified as in the target range. The Moderate Group was counted as in by being 5 – 26. Advanced Group was included by numbering 17 – 35. DCC hit the mark from 26 – 45.

AL scores much more accurately than APRI: Both APRI and AST scored a 64%. APRI is bound by the weaker correlation of using AST as half of its formula. The **AST/ALT ratio scored a 71%** giving AL a stronger core to go along with division by platelets and a more thorough approach using age, albumin, bilirubin as well as AST or ALT to balance data. **Platelets were at 84% and AL at 89%.** See Appendix A for the charts.

AL Staging Model

AL is extremely accurate in predicting end-stage HCV patients: With AL there are some definitive guideposts for staging HCV cases. The strongest is that no patient not in the Decompensated Cirrhosis Group scored over a 1000 and just 1 patient out of 15 in the DCC Cohort scored under it. Another is all the Mild Group patients except 1 scored 375 or lower.

APRI is used to determine if significant fibrosis is present or not, sometimes at two different thresholds. It is often then used to trigger pharmaceutical treatment if that mark has been met. Especially in third world countries this is a rational policy to allocate finite resource. By this measure **AL has over a 93% rate of predicting significant fibrosis in the binary model.**

The staging model at use here is a far more difficult endeavor than predicting the binary significant fibrosis or no significant fibrosis used to judge APRI in countries around the world. Here AL is correlated with the Metavir Scoring System for Biopsy, with the modification of including the Decompensated Cirrhosis Cohort in its own stage of 5. Rather than 2 or in some cases 3 categories of patients, this staging model has 9 categories primarily to closely scrutinize the accuracy of this system. It starts with 0 rather than .5 as biopsies scored as .5 are very unusual. In this study we had 1 out of 75 total patients with a .5 biopsy stage.

AL Staging Model

Stage 0	**0 – 125**
Stage 1	**126 – 250**
Stage 1.5	**251 – 375**
Stage 2	**376 – 500**
Stage 2.5	**501 – 625**
Stage 3	**626 – 750**
Stage 3.5	**751 – 875**
Stage 4	**876- 1000**
Stage 5	**1001 and up**

Of the 45 cases 29 were on target or .5 a stage away, this is 64%. **The total average was a 0.611 deviation.** This number is helped in that this study by a larger contingent of DCC patients who are much easier to place by this method. There were only 3 patients who were off by 2 stages or more. This is a 6.67% rate of being grossly inaccurate. One study of the HCV Fibrosure found a 29% incidence of being off by 2 stages or more.

This study had only slightly more deviation above the scale than below. 52.7% of the total deviation was high with the remaining running too low.

This research could also make a strong argument for a few inaccurate biopsies. For example one patient in the Moderate Cohort had both high alpha-fetoprotein and low albumin readings on a stage 2.5 biopsy and who progressed to life threatening symptoms less than 2 years after that biopsy. HCV tends to be a very slow illness and this case was almost certainly more progressed at that juncture.

Conclusion

In sum the AL formula provides an important tool in giving patients feedback on the fibrotic state of their livers. It utilizes commonly performed and inexpensive blood tests that most all HCV patients are already routinely given. It also provides a historical context for the progression of their cases as the same tests have been given for many years. It is a boon to TCM practitioners as it gives them a valuable tool within their scope of practice.

AL scores more accurate in this study than the APRI System which is used in a number of countries, mostly in the developing world. APRI also does not rely on new tests and secret algorithms, which enables us to readily compare the two systems. It has a more accurate core equation than APRI using the AST/ALT ratio rather than AST alone divided by platelets. The Fibrosure as currently formulated is woefully inaccurate. The MELD system for rating liver transplant patients is antiquated. Creatinine, one of only 3 markers used in the MELD system, did not even correlate with liver damage in this study.

Future studies hopefully will refine the AL Staging Model. At this point the deviation between the AL Staging Model and biopsy results is skewed slightly high. 52.7% of deviation was too high, with 47.3% low. This shows that the model itself can be more accurately tuned. Areas of research improvement could be a larger study with more than 45 patients. In this larger study gender and GGT can be examined in much more detail to see if those markers show a predictive significance. Another area could be what they did in the APRI Saudi Arabian studies where the biopsies themselves are examined more carefully to screen for their accuracy. This can be done through multiple readers and an increased number of biopsy samples.

The study the author would most like to see done next, is one done for those awaiting liver transplants. It would examine the patients who died while awaiting transplant with how their MELD score rated the urgency of their cases, compared with how the AL Model and where that would place them in the line-up. My belief is that the AL Staging Model would be a much more accurate predictor of end-stage liver disease than the MELD Score.

This model is freely shared with any clinician or patient. Any feedback, comment, or question email is steven@alchemistlab.com.

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April 21, 2011

Appendix A

The Mild Fibrosis Cohort is for all the patients with biopsies that were Stage 0 to Stage 1.5, with the data of the respective blood tests that they had taken that were within 60 days of the biopsy date.

#	Stage	Plate	Alb	TBil	INR	PT	AF	Creat	ALT	AST	A/A	APRI	Age	AL
1	0	237	4.5	0.6					56	23	0.411	0.228	56	182
2	0	280						0.96					55	
3	0	172	4.5	1.28	1	11.7			77	39	0.564	0.533	46	375
4	0	240	4.3	0.49					71	44	0.62	0.431	48	300
5	0.5			0.6	1.1	11				31				
6	1	273	4.8	0.4									37	
7	1	160	4	0.5		10.4								
8	1				1	10.2	4.6						54	
9	1	239	4.6	0.3					65	36	0.553	0.354	47	199
10	1	250	5	0.45	1				58	52	0.897	0.489	43	232
11	1	340	4.4	0.48	1				18	18	1	0.124	45	291
12	1		4.8	0.6	1.1				483	198	0.409			
13	1	236	3.9	0.5			3	0.9	35	66	1.886	0.658	46	896
14	1	233	4.5	0.5		11.9		1.1	98	46	0.469	0.464	51	230
15	1	238	3.9	1.6		11.9		0.9						
16	1.5	177	4.4	0.6					74	45	0.608	0.598	51	374
17	1.5	172	4.5	1	1.2	12.6	2.7		33	30	0.909	0.41		
Ave		232	4.44	0.66	1.1	11.4	3.4	0.96	97	52	0.757	0.429	48.3	342

The Moderate Fibrosis Cohort is for the patients who were biopsy stage 2 & 2.5

#	Stage	Plate	Alb	T.Bil	INR	PT	A-F	Creat	ALT	AST	A/A	APRI	Age	AL
1	2	159	4.1	0.9					310	203	0.655	3.004	52	651
2	2	160	4.8	1	1	10.7	14.1	0.8	95	62	0.653	0.911	47	437
3	2	225	3.7	0.6				0.9	47	40	0.851	0.418	54	482
4	2			0.5					105	77	0.733			
5	2	198	4.4	0.8	1.1	11.5			250	247	0.988	2.935	52	747
6	2		4.2	0.7		11.2							41	
7	2	133	4.4	0.4				0.71	395	350	0.886	6.191	46	992
8	2	225	4.1	0.4				0.8		67		0.729		
9	2	177				11.1	3.8						52	
10	2	216	4.4	0.4		10.1			148	66	0.445	0.718	55	294
11	2	255				10.1							51	
12	2	157	4.4	0.5				0.8	263	65	0.247	0.974	48	359

13	2	213	4.1					1	148	114	0.77	1.259	55	511
14	2	139	4	0.6			17.5		139	119	0.856	2.014		
15	2.5	166	3.6	0.6	1.1	14.6	26	0.7	265	227	0.857	3.217	58	821
16	2.5	170	3.7	0.7		10.6			337	158	0.468	2.187	52	634
17	2.5		3.5	1.1					235	142	0.604			
18	2.5	233	4.5	0.8					60	57	0.95	0.575	57	460
19	2.5	204	4.8	0.5			2.4		63	52	0.825	0.6	50	401
Ave		189	565	565	565	565	565	565	565	565	565	565	565	565

The Advanced Cohort for patients with biopsy stage 3 – 4.

#	Stage	Plate	Alb	T.Bil	INR	PT	AF	Creat	ALT	AST	A/A	APRI	Age	AL
1	3	130	4.3	1.3	1.1	10.9			67	70	1.045	1.266	57	916
2	3	169			1	13							47	
3	3	233	4.6	0.4				0.8	83	42	0.506	0.424	52	221
4	3		4	0.7	1		5.4	1	391	188	0.481		57	
5	3				1	11.5	7						67	
6	3	153	4.3	0.67					84	51	0.607	0.784		
7	3		4.6	0.6										
8	3	192	4.5	0.7		11.1			220	95	0.432	1.164	35	388
9	3		4	1				0.9	38	29	0.763		51	
10	3.5	100	3.4	0.9	1.1	12.7			165	126	0.764	2.964	44	989
11	3.5	157	4.4	0.4										
12	3.5	127	4.9	0.3	1.1	12.2			135	80	0.593	1.482	56	478
13	4	140	4.4	0.5	1.1	11.6		1.1	188	110	0.585	1.848	74	537
14	4	146	3.9	0.9				0.9	64	51	0.898	1.368	51	722
15	4	85				13.1							47	
16	4		3.9	0.9	1.15	13.5			124	111	0.895		50	
17	4	111	4.1	0.9				1.3	70	41	0.586	0.869	43	603
18	4	196	4.1	0.5		12.7			127	114	0.898	1.368	49	576
Ave		149	4.28	0.71	1.07	12.2	6.2	1	134	85	0.634	1.354	52	603

The Decompensate Cirrhosis Cohort is for those patients who had one of the symptoms of ascites, varices, hepatic encephalopathy, wasting of limbs. The last available blood test was used in this group.

#	Stage	Plate	Alb	T.Bil	INR	PT	A-F	Creat	ALT	AST	A/A	APRI	Age	AL
1	5	93	3.1	0.7	1.1	11.4		0.8	34	54	1.588	1.366	58	1894
2	5	43	3.2	3.7	1.5	13.9	2.8	0.9	29	51	1.759	2.79	44	4400
3	5	145	2.8	1.9	1.2				46	69	1.5	1.119	50	1055
4	5	142	2.7	1.1			12	0.9	78	86	1.103	1.425	55	1053
5	5		2.4		1.5	16.4	19	0.7	101	146	1.446		58	
6	5	90	3.9	0.3					68	73	1.074	1.908		
7	5	146	4	0.5	1.2				31	56	1.806	0.902	52	1320
8	5	54	4.1	1.1	1.1	10.8	5.2	0.7	47	46	0.979	2.004	53	1907
9	5	64	3.3		1.5		18	0.81	251	256	1.02	9.412	60	1970
10	5	117	3.5	0.5	0.9	11.5	11	0.9	125	103	0.824	2.071	52	884
11	5	116	2.7	1.4	1.2	11.4		0.6	29	51	1.758	1.034	72	1789
12	5	32	3.2	2.2	1.1	11.4		0.83	137	204	1.489	15.001	50	5047
13	5	131	3.8	1.6	1.3			0.58	180	198	1.1	3.556	54	1142
14	5	51	3.6	1.3			131.8	1	142	171	1.204	7.889	58	2645
15	5	54	3.5	1.6	1.2	12.2	108	0.8	111	108	0.973	4.705	53	2043
16	5	83	3				2.6	0.9	51	70	1.373	1.984	51	1860
17	5	79	3.4	1.1	1.1	12.2	28	1.1		11		3.306	49	
18	5	74	3.4	0.7				0.9	100	129	1.29	4.101	61	1973
	Ave	89	3.3	1.31	1.2	12.5	33.8	0.83	95	110	1.157	3.798	55	2065

Appendix B

Total correlation for AST was 64%, and for the A/A ratio 71%.

Number	Stage	AST		A/A	Stage	
1	1	18		0.247	2	Out
2	0	23		0.411	0	
3	1	36		0.432	3	Out
4	0	39		0.445	2	Out
5	2	40		0.468	2.5	
6	4	41	Out	0.469	1	
7	3	42	Out	0.506	3	Out
8	0	44		0.553	1	
9	1.5	45		0.585	4	Out
10	1	46		0.586	0	
11	5	46	Out	0.586	4	Out
12	4	51	Out	0.593	3.5	Out
13	5	51	Out	0.608	1.5	
14	5	51	Out	0.62	0	
15	5	51	Out	0.653	2	
16	1	52	Out	0.655	2	
17	2.5	52		0.764	3.5	
18	2	54		0.77	2	
19	5	56	Out	0.824	5	Out
20	2.5	57		0.825	2.5	
21	2	62		0.851	2	
22	2	65		0.857	2.5	
23	1	66	Out	0.886	2	
24	2	66		0.897	1	Out
25	5	69	Out	0.898	4	
26	3	70		0.898	4	
27	5	70		0.95	2.5	Out
28	3.5	80		0.973	5	
29	5	86		0.979	5	
30	3	95		0.988	2	Out
31	5	103		1	1	Out
32	5	108		1.02	5	
33	4	110		1.045	3	
34	4	114		1.1	5	
35	3.5	126		1.103	5	
36	5	129		1.204	5	
37	2.5	158	Out	1.29	5	
38	5	171		1.373	5	
39	5	198		1.489	5	
40	2	203	Out	1.5	5	
41	5	204		1.588	5	
42	2.5	227	Out	1.758	5	
43	2	247	Out	1.759	5	
44	5	256		1.806	5	
45	2	350	Out	1.886	1	Out

In this guide APRI matched group sequence 64%, platelets 84%, and AL 89%.

Number	Stage	AL		Stage	Platelets		APRI	Stage	
1	0	182		1	340		0.228	0	
2	1	195		1	250		0.354	1	
3	3	221	Out	0	240		0.418	2	Out
4	1	230		1	239		0.424	3	Out
5	1	232		0	237		0.431	0	
6	1	291		1	236		0.432	3	Out
7	2	294		1	233		0.464	1	
8	0	300		2.5	233		0.489	1	
9	2	359		3	233	Out	0.533	0	
10	1.5	374		2	225		0.575	2.5	
11	0	375		2	225		0.598	1.5	
12	3	388	Out	2	216		0.6	2.5	
13	2.5	401		2	213		0.658	1	
14	2	437		2.5	204		0.718	2	
15	2.5	460		2	198		0.819	4	Out
16	3.5	478	Out	4	196	Out	0.869	4	Out
17	2	482		3	192		0.902	5	Out
18	2	511		1.5	177	Out	0.911	2	
19	4	537		1.5	172	Out	0.974	2	
20	4	576		2.5	170		1.034	5	Out
21	4	603		2.5	166		1.103	5	Out
22	2.5	634		2	160		1.119	5	Out
23	2	651		2	159		1.124	1	Out
24	4	722		2	157		1.259	2	
25	2	747		5	146	Out	1.266	3	
26	2.5	821		5	145		1.366	5	
27	5	884		5	142		1.368	4	
28	1	896	Out	4	140		1.482	3.5	
29	3	916		2	133	Out	1.848	4	
30	3.5	989		5	131		1.984	5	
31	2	992	Out	3	130		2.004	5	
32	5	1053		3.5	127		2.071	5	
33	5	1055		5	117		2.187	2.5	Out
34	5	1142		5	116		2.79	5	
35	5	1320		4	111		2.935	2	Out
36	5	1789		3.5	100	Out	2.964	3.5	Out
37	5	1860		5	93		3.004	2	Out
38	5	1894		5	83		3.217	2.5	Out
39	5	1907		5	74		3.556	5	
40	5	1970		5	64		4.101	5	
41	5	1973		5	54		4.705	5	
42	5	2043		5	54		6.191	2	Out
43	5	2645		5	51		7.889	5	
44	5	4400		5	43		9.412	5	
45	5	5047		5	32		15.001	5	

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