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AST to ALT Ratio is elevated in disseminated histoplasmosis as compared to localized pulmonary disease and other endemic mycoses

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Abstract

Severe pulmonary or disseminated histoplasmosis often necessitates presumptive antifungal treatment while awaiting definitive diagnosis. Histoplasma antigen assays have improved sensitivity but results may lag up to 7 days. In order to increase diagnostic certainty, "soft clues" may be looked for in laboratory and radiologic data, such as elevated alkaline phosphatase or ferritin levels and findings of mediastinal adenopathy or hepatosplenomegaly. To determine if elevated aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio is specific to histoplasmosis or a non-specific marker for disseminated fungal infection or sepsis in general, we retrospectively examined records of all patients diagnosed with an endemic fungal infection (EFI) at Rush University Medical Center from January of 1997 to October of 2012, and a cohort of septic patients with elevated liver enzymes. We identified 90 cases of EFIs during the study period that met all inclusion criteria (Histoplasma 21, Blastomyces 56, Coccidioides 12, Paracoccidioides 1). We also evaluated 10 control patients with bacterial sepsis. The mean ratio of AST to ALT in patients with disseminated histoplasmosis was 2.69 (95% CI:1.22, 4.16) while for other EFIs, the mean ratio ranged from 0.38 to 1.14 with disseminated coccidioidomycosis and blastomycosis respectively ($P < 0.0001$). The ratio in patients with bacterial sepsis was 0.84. We propose the use of the AST/ALT ratio as a clinical "soft clue" suggestive of disseminated histoplasmosis in the appropriate host, and to possibly distinguish cross reactivity of the Histoplasma antigen assay with other EFIs.

Key words: Endemic Fungi, Histoplasmosis, Fungal Infection, Diagnosis.

Introduction

Histoplasmosis remains the most common endemic mycosis in the United States.[1] Dissemination occurs predominantly in immunocompromised patients, and may be acute and rapidly fatal.[1] Time to diagnosis and effective treatment is an important predictor of mortality. However, the mean

time to diagnosis has been reported to be as high as 21.2 days.[2]

The enzyme immunoassay (EIA) for detecting *Histoplasma* antigen in serum or urine has improved the ability to diagnose disseminated histoplasmosis. However, the results may be delayed by up to a week due to specimen processing and shipping to specialized labs. Clinicians must rely on clinical suspicion prior to the availability of test results.

Laboratory clues such as pancytopenia, elevated alkaline phosphatase and markedly elevated lactate dehydrogenase (>600)[2] and ferritin,[3] are nonspecific markers of disseminated histoplasmosis and may aid in guiding empiric antifungal therapy while awaiting results of diagnostic testing.

This is a retrospective study aimed at determining whether elevated aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio is specific to disseminated histoplasmosis, and may be used as a clinical 'soft clue' to aid in presumptive diagnosis, or a non-specific marker for disseminated fungal infection or sepsis in general.

Materials and methods

Rush University Medical Center is a 676 bed tertiary care hospital in Chicago, Illinois. A retrospective cohort of all patients diagnosed with an endemic fungal infection (EFI) from January 1, 1997 to October 1, 2012, was identified through review of clinical microbiology records.

Medical records were reviewed retrospectively for the following data: demographics, underlying immune status and comorbidities, characteristics of EFI, laboratory values and microbiology including method of diagnosis. In the case of multiple values of AST and ALT, only the data from the day of admission was used.

From this cohort, patients diagnosed with EFI by EIA alone were excluded to minimize uncertainty due to antigen cross reactivity with EFIs other than histoplasmosis. Only patients with positive cultures or histopathology were included in the final analysis. Patients that had missing crucial data (transaminase levels, clinical history, etc.) were excluded from the analysis.

Patients were then further divided into those with localized pulmonary disease or disseminated EFI. Disseminated disease was defined by the presence of radiographic, microbiologic, or histologic confirmation of infection in more than one organ or extra-pulmonary site. Thoracic lymphadenopathy was not considered dissemination for the purposes of classification.

In addition, data were collected on each patient's underlying immune status. Immunocompromise was defined a priori as presence of one of seven criteria: 1. neutropenia, 2. solid organ or hematopoietic stem cell transplantation, 3. immunosuppressive medications, 4. diabetes, 5. cirrhosis, 6. human immunodeficiency virus (HIV), and 7. advanced cancer. Immunosuppressive medications were defined as chronic glucocorticoids, anti-rejection medications, and anti-neoplastics. Patients who had no identifiable immunosuppression were classified as immunocompetent.

In order to determine whether transaminitis was due to sepsis in general, ten consecutive patients with sepsis and abnormal liver enzymes, but no *a priori* liver disease, were included in this analysis. For the purposes of the study, sepsis was defined as three or more positive systemic inflammatory response syndrome (SIRS) criteria[4] and a culture proven bacterial infection.

All data were analyzed in SAS 9.2 with a one-way ANOVA and Fishers Exact Test. Normality of the data was assessed visually using histograms.

Results

167 patients with EFI were identified during the study period. Of those, 121 were diagnosed by histopathology and/or culture. Of those, 31 were missing significant data and were excluded from the final analysis. Ninety patients, meeting all selection criteria, were diagnosed with EFI caused by *Blastomyces dermatitidis* (56), *Histoplasma capsulatum* (21), *Coccidioides spp.* (12), and *Paracoccidioides brasiliensis* (1).

Twenty patients with localized pulmonary blastomycosis were diagnosed from lung specimens; disseminated cases of blastomycosis were diagnosed from the following sources: musculoskeletal (14/36), pulmonary (6/36), both pulmonary and musculoskeletal (4/36), lymph node (4/36), CNS (4/36), and skin (4/36).

Eight patients with localized pulmonary coccidioidomycosis were diagnosed from a lung source (7/8), and mediastinal lymph node (1/8). Disseminated cases of coccidioidomycosis were diagnosed from CNS specimens (3/4), and skin (1/4).

Four cases of localized pulmonary histoplasmosis were diagnosed from lung specimens (4/4). Disseminated cases of histoplasmosis were diagnosed from blood (7/17), lung (6/17), both blood and lung (1/17), lymph node (1/17), bone marrow (1/17) and a musculoskeletal source (1/17).

The single case of *Paracoccidioides* infection in the cohort was diagnosed by cardiac biopsy.

For patients included in the sepsis control group, bacteria were isolated from lung (4/10), urine (3/10), blood (2/10), and peritoneal specimens (1/10).

Histoplasma did not appear to disseminate more frequently than other EFIs ($P = .45$) (Table 1).

Table 1. Underlying characteristics of patients by diagnosis.

Species	Number of cases	Localized disease (%)	Disseminated disease (%)	Male (%)	Mean age (±SD)
Blastomyces	56	20 (36)	36 (64)	38 (68)	45.1 (17.6)
Histoplasma	21	4 (19)	17 (81)	17 (81)	48.5 (14.1)
Coccidioides	12	8 (67)	4 (33)	3 (25)	46.6 (14.6)
Paracoccidioides	1	0 (0)	1 (100)	1 (100)	47.0 (*)
Bacterial sepsis	10	N/A	N/A	6 (60)	62.3 (12.5)

Note. N/A, not applicable; SD, standard deviation; *, could not be calculated.

Table 2. Immune status of patients by diagnosis.

Fungus	Extent of disease	Immunocompromised (%)	Underlying condition
Blastomyces (n = 56)	L	2 (10)	1 RTX 1 Neutropenic
	D	7 (19)	3 Diabetes 2 RTX 1 CP 1 HIV (CD4 867)
Histoplasma (n = 21)	L	4 (100)	1 CP 2 RTX 1 LTX
	D	16 (94)	6 RTX 4 LTX 6 HIV (median CD4 = 9)
Coccidioides (n = 12)	L	1 (12)	1 Diabetes
	D	0 (0)	N/A
Paracoccidioides (N = 1)	D	1 (100)	CP
Bacterial sepsis (N = 10)	N/A	4 (40)	2 AC 1 RTX 1 Neutropenia

Note. L, localized; D-disseminated; N/A, not applicable; LTX, liver transplant; RTX, renal transplant; CP, chronic prednisone; AC, advanced cancer; HIV, Human Immunodeficiency Virus.

Patients with histoplasmosis were almost exclusively immunocompromised (20/21), in contrast to patients with blastomycosis (9/56) and coccidioidomycosis (1/12) ($P < .0001$) (Table 2).

The mean ratio of AST to ALT in patients with disseminated histoplasmosis was 2.69 (95% CI:1.22, 4.16), which was significantly higher than the ratios observed with other EFIs (mean ratio range 0.38 and 1.14 respectively, $P < .0001$, Table 3).

Specifically, all AST/ALT ratios above 2.5 were seen exclusively in patients with disseminated histoplasmosis (see Fig. 1). Using a cutoff of 2.0, the odds ratio of a patient having disseminated histoplasmosis compared to other EFIs or bacterial sepsis is 14 (95% CI 3.5 to 56.4).

Discussion

The diagnosis of histoplasmosis remains elusive in some cases, even in the setting of extrapulmonary dissemination with multiorgan involvement. The mean time between admission and diagnosis may exceed 20 days and the associated mortality can be high.[2] Even when histoplasmosis is suspected, there may be delays in obtaining diagnostic confirmation. Given this and the potential severity of disseminated disease, the recognition of early clues may hasten the initiation of empiric antifungal therapy while awaiting definitive diagnosis.

This study shows that a ratio of AST to ALT of 2.5 or greater occurred only in cases of disseminated histoplasmosis (not in bacterial sepsis or other EFI), and a ratio of 2.0 or higher had an odds ratio of 14 (95% CI 3.5 to 56.4).

This difference in the mean ratio was statistically significant when compared to the ratio of AST to ALT seen in patients with other EFIs and in bacterial sepsis. It was also significantly higher than the ratio observed in patients with *B. dermatitidis* isolated from skeletal muscle, a tissue known to produce high amounts of AST.

Table 3. AST/ALT ratio of patients by diagnosis.

Species	Extent of disease	Number of cases	Mean AST U/l(±SD)	Mean ALT U/l(±SD)	Mean AST/ALT ratio (±SD)
Blastomyces	L	20	30 (25)	29 (23)	1.05 (0.34)
	D	36	27 (23)	33 (34)	1.14 (0.68)
Histoplasma	L	4	28 (15)	38 (29)	0.86 (0.32)
	D	17	157 (187)	54 (59)	2.69 (0.75)
Coccidioides	L	8	26 (9)	33 (15)	0.90 (0.48)
	D	4	28 (9)	152 (167)	0.38 (0.33)
Paracoccioides	D	1	977 (*)	1,506 (*)	0.65 (*)
Bacterial Sepsis	N/A	10	103 (131)	163 (218)	0.84 (0.47)

Note. L, localized; D, disseminated; AST, aspartate aminotransferase; ALT, alanine aminotransferase; U/l, units per liter; N/A, not applicable; SD, standardized deviation; *, could not be calculated.

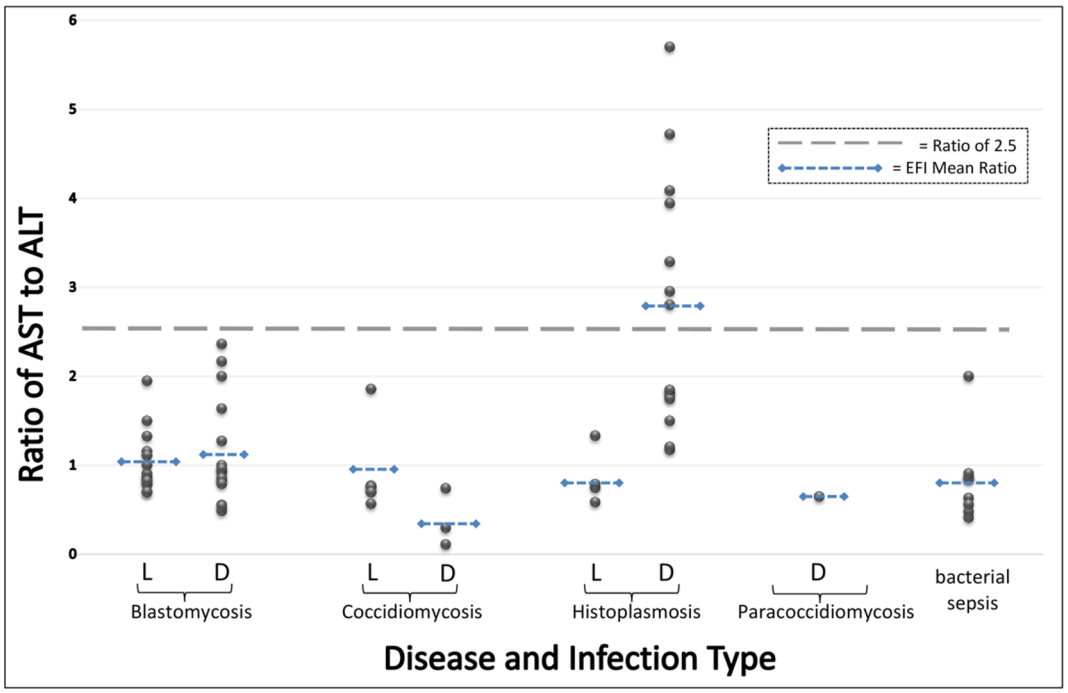

Figure 1. AST/ALT ratio by microbiologic diagnosis and dissemination status. A line at a ratio of 2.5 demonstrates that a higher ratio was seen exclusively in disseminated histoplasmosis. Note: AST, aspartate aminotransferase; ALT, alanine aminotransferase; EFI, endemic fungal infection; L, localized; D, disseminated.

This could be very useful, as AST and ALT are tests obtained ubiquitously in hospitalized patients. This is not the case with the other markers of histoplasmosis, such as LDH and ferritin.

Due to these subtle but important differences, changes in the AST to ALT ratio have long been used as clues to specific conditions, such as heavy ethanol consumption, acute rhabdomyolysis and ingestion of certain toxins. Transaminases are often thought of interchangeably, but distinct and clinically useful differences exist. ALT is produced almost exclusively in the liver, and all but the lowest elevations are associated with hepatocyte injury and cell death.[5] However, even though AST is also predominantly associated with liver,[6] it is produced by other cell types such as cardiac and skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes.[5,6] In addition, ALT requires B6 as cofactor for its biosynthesis, while AST does not.[7]

The cause of this elevated ratio in disseminated histoplasmosis is unclear, but there are multiple possible causes. In alcoholism, the most common disease associated with AST/ALT ratio elevation, three possible causes have been proposed: (1) deficiency of pyridoxal 5′-phosphate (the active metabolite of B6 which is required for creation of ALT, but not AST),[7] (2) mitochondrial damage leading to increased mitochondrial AST in the serum,[8] and (3) decreased production of ALT from a chronically damaged liver.[9]

In addition, damage to organs that produce AST but not ALT, is possible in patients with disseminated histoplasmosis. This would include the reticuloendothelial system, including the spleen, muscle, intestine and kidneys.

The absence of laboratory evidence of vitamin deficiency and recovery of liver function in surviving patients with histoplasmosis argue against causes 1 and 3, respectively. Ultimately, further laboratory work would be necessary to determine the cause.

These results are not without important limitations. This ratio is not diagnostic but instead suggestive of disseminated histoplasmosis in the appropriate patient population. If used in settings with low pretest probability for this infection, it is likely to be limited in its value or misleading in its results.

In addition, attempts to obtain CPK and LDH levels were unsuccessful, as they are not routinely collected in our hospital. Less than 5% of patients in our sample had both (data not included). Thus, we were not able to correlate this elevated ratio with other known features of disseminated histoplasmosis, such as markedly elevated LDH (>600) and ferritin.

In summary, this study shows that patients with disseminated histoplasmosis had significantly elevated AST to ALT ratios when compared to patients with other EFIs and bacterial sepsis. This may be a diagnostic clue available in commonly collected laboratory data and such a clue has the potential to expedite disease treatment while awaiting definitive diagnosis in the appropriate host.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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