Serum Arginase, a biomarker of treatment efficacy in human African trypanosomiasis

Running title: Increased arginase in human African trypanosomiasis

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Abstract

Arginase serum levels were increased in human African trypanosomiasis patients and resumed control values after treatment. Arginase hydrolyzes L-arginine to L-ornithine, essential for parasite growth. Moreover, L-arginine depletion impairs immune functions. Arginase may be considered as a biomarker for treatment efficacy.

Key words Trypanosoma brucei gambiense; arginase; serum; human African trypanosomiasis; L-ornithine
Trypanosoma brucei gambiense (T. b. gambiense) and T. b. rhodesiense, the causative agents of human African trypanosomiasis (HAT) or sleeping sickness, are tsetse fly transmitted protozoan parasites (1). They multiply extracellularly in the bloodstream, lymph, and interstitial fluids during the disease first stage (stage 1). The second stage (stage 2) begins when they cross the blood-brain barrier. Major immune system impairments are observed (2). Medications are toxic and difficult to administer, in particular in stage 2. Diagnosis, treatment, and post-treatment follow-up remain complex due to poorly-equipped health facilities and political unrest in most HAT foci, therefore new biomarkers are needed.

The inducible metabolism of L-arginine plays an important role in infections (3). Arginase (two isoforms) hydrolyzes L-arginine to L-ornithine and urea. L-arginine is the common substrate of arginases and nitric oxide (NO) synthases (NOS). Its depletion represents a limiting factor of NO production. The balance of inducible NOS/arginase is regulated by the patient's cytokine profile. L-ornithine is the precursor for the synthesis of L-glutamine, L-proline and polyamines via the ornithine decarboxylase pathway (4). In trypanosomes, polyamines facilitate DNA and trypanothione synthesis, essential for maintaining the intracellular redox system and representing a defense against oxidative stress (5). Moreover, L-arginine depletion, caused by arginase, leads to the loss of T cell receptor-associated CD3 ζ chain and stops T cell proliferation (3). Thus, increased arginase activity is likely to damage the host immune response and favor parasite growth.

Arginase activity and expression were measured in sera and cerebrospinal fluid (CSF) from HAT patients before treatment and six months later, and healthy controls' sera. Patients (n=32) were recruited from a previously described group (6). The field survey took place in the “Couloir” focus in Congo (Brazzaville). The scientific protocol was approved by the WHO Research Ethic Review Committee and authorized by the Congo Ministry of Health. Informed consent was obtained from the patients. Screening tests on whole blood used the serological card agglutination trypanosomiasis test. Parasites were searched in aspirates from cervical lymph glands, and/or blood after parasite concentration by capillary
tube centrifugation and/or the mini anion-exchange centrifugation technique (6). Inclusion criteria stipulated that patients were parasitologically confirmed. HAT patients were classified as being in stage 1 (n=25) or stage 2 (n=7), according to patient CSF white blood cell counts and/or absence or presence of CSF trypanosomes. Persons tested negative for HAT were enrolled as controls (n=23) and matched for age and sex. HIV serology was performed to exclude HIV positive people.

Two independent measures of arginase were performed. A sandwich enzyme immunoassay was performed to quantify human Arginase I (Liver-Type), according to the manufacturer’s instructions (BioVendor, Heidelberg, Germany), as described (7). It was confirmed by the measurement of arginase activity (8). Arginase activity indicates a potential role of this enzyme in ornithine production.

Arginase values are shown in figure 1. Inter-group comparisons were made using a nonparametric test (Mann-Whitney U test). Tests of significance were two-tailed. All P values less than 0.01 were considered significant. Arginase activity was higher in HAT patients before treatment compared to controls (medians 4.82 U/L vs. 2.65 U/L, P<0.001). The sample of stage 2 HAT patients was not statistically significant, but their arginase activity and expression levels were in the same range as those of stage 1 patients. Higher serum arginase I expression was measured in HAT patients compared to controls (medians 37 ng/mL vs. 17 ng/mL, P<0.001).

A high correlation was found between arginase activity and arginase expression (Spearman coefficient correlation r = 0.84). Arginase I expression and activity were not detected in patient CSF.

No differences were found between controls and HAT patients in terms of serum markers for hepatic injury and hemolysis. Values, expressed as median (interquartile range), were for patients versus controls respectively: Alanine Amino Transferase, 32.1 (22.6-50.3) vs. 31.6 (22.5-48.1) IU/L; Aspartate Amino Transferase 26.7 (11.2-35.5) vs. 25.9 (11.4-36.2) IU/L; total bilirubin 13.3 (8.2-17.6) vs. 12.8 (8.3-16.4) µmol/L; indirect bilirubin 6.8 (5.4-8.5) vs
6.4 (5.5-8.3) µmol/L. Treatment was performed as described (6): stage 1 patients received pentamidine and stage 2 patients difluoro methyl ornithine.

Patient follow-up was performed 6 months later for comparison of pre- and post-treatment sera. Full comparisons were obtained for 14 patients who were present for the arginase follow-up. They were all parasitologically negative after treatment. Two patients maintained normal arginase values. In the 12 patients with higher pre-treatment serum arginase I expression (median 43 ng/mL), values decreased after treatment (18.5 ng/mL, P<0.005), to resume healthy control values (17 ng/mL). Treatment had a similar effect on arginase activity: median 5.54 U/L before treatment vs. 2.72 U/L afterwards (P<0.001). Post-treatment values were similar to healthy control values (2.65 U/L).

High serum arginase levels, confirmed by two independent techniques, were found in HAT patients. Values resumed healthy control levels after treatment. The origin of this increase has not yet been elucidated. Arginase increase did not reflect hemolysis, as hemolysis serum markers were not found, and did not result from liver injury, as HAT patients did not exhibit any increase in Alanine Amino Transferase and Aspartate Amino Transferase levels compared to healthy controls, consistent with previous studies (9, 10). In trypanosome-infected mice, arginase activity increases in macrophages, the main producers of arginase (11). Macrophages from trypanosome-susceptible mice exhibit a greater increase in arginase expression than those from resistant animals (12). In humans, various leukocyte subpopulations synthesizing arginase may be involved (13). High arginase blood levels have been measured in sera from acute hepatitis B, resulting in T cell function suppression, and in various cancers (14, 15, 16). High arginase activity has been measured in cutaneous leishmaniasis lesions (17). High IL-10 levels have been measured in HAT (18). Interestingly, a correlation has been reported between increased arginase activity and increased circulating IL-10 levels in trauma (19).

These findings support the hypothesis that arginase plays an essential role in HAT, in terms of parasite proliferation and/or impairment of immune responses. The assessment of
NO production and, under suboptimal arginine concentrations due to arginase activity, the generation of various reactive species by inducible NOS, represents a next step to investigate this impairment. The best times to perform arginase measurements in the follow up of stage 1 and stage 2 HAT patients, in addition to standard protocols, deserves further investigations.

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REFERENCES


LEGENDS

Figure 1
Determination of arginase I expression (A) and arginase activity (B) in sera from controls (n=23) and patients with human African trypanosomiasis (n=32) from the Couloir focus (Republic of Congo). Round points represent controls, diamond points stage 1 patients and square points stage 2 patients. Black lines represent the median and the quartiles for each group. Arginase activity was higher in HAT patients compared to controls (median 4.82 U/L vs 2.65 U/L, P<0.001). Serum arginase I expression was higher in HAT patients compared to controls (median 37 ng/mL vs. 17 ng/mL, P<0.001).

Figure 2
Determination of arginase I expression (A) and arginase activity (B) in sera from patients with human African trypanosomiasis before treatment and 6 months later (n=12). Open circles represent the median for each group. Values for each patient are joined by a black line and medians by a dashed line. Arginase I expression values (median 43 ng/mL before treatment) decreased after treatment (median 18.5 ng/mL, P<0.005) to resume healthy control values (17 ng/mL). Arginase activity (median 5.54 U/L before treatment) decreased after treatment (median 2.72 U/L, P<0.001) to resume healthy control values (2.65 U/L).
Arginase I expression

Arginase I (ng/mL)

Before After Treatment