

---

## ORIGINAL RESEARCH

Courtney L. Bryan BS<sup>1,2</sup>

K. Scott Beard MS<sup>1,2</sup>

Gregory B. Pott PhD<sup>1,2</sup>

Jeremy Rahkola BS<sup>1,2,4</sup>

Edward M. Gardner MD<sup>2,3</sup>

Edward N. Janoff MD<sup>1,2,4</sup>

Leland Shapiro MD<sup>1,2</sup>

<sup>1</sup> Department of Medicine, Denver Veterans Affairs Medical Center, Denver, CO, USA.

<sup>2</sup> Department of Medicine, University of Colorado Denver, Aurora, CO, USA.

<sup>3</sup> Department of Public Health, Denver Public Health, Denver, CO, USA.

<sup>4</sup> Department of Medicine, Mucosal and Vaccine Research Program Colorado (MAVRC), Denver, CO, USA.

# HIV infection is associated with reduced serum alpha-1-antitrypsin concentrations

---

## Abstract

**Purpose:** Several observations suggest the presence of HIV-suppressive factors in the fluid phase of blood. Alpha-1-antitrypsin (AAT), the most abundant serine protease inhibitor in the circulation, has potent anti-HIV activity *in vitro*, and may function as an endogenous HIV suppressor. Therefore, we assessed serum AAT concentrations for association with HIV infection.

**Methods:** In this cross-sectional study, serum AAT concentrations were measured in 66 persons with HIV infection and in 45 healthy persons (Controls). In the HIV-infected group, antiretroviral therapy (ART) use was assessed and CD4<sup>+</sup> T cell levels and plasma HIV RNA were quantified.

**Results:** Median AAT concentration was significantly lower in the HIV-infected group (1.64 mg/mL) in comparison with Controls (1.94 mg/mL;  $p=0.001$ ). AAT reduction was most pronounced in the HIV-infected subgroup with CD4<sup>+</sup> T cell levels  $>200$  cells/ $\mu$ L in comparison with Controls ( $p<0.01$ ). Serum AAT concentrations  $<1.0$  mg/mL are clinically significant, and concentrations below this level were identified in 4.5% of the HIV-infected group and in no Control subjects. No association between AAT levels and viral load or use of ART was observed in HIV-infected subjects.

**Conclusion:** The association between reduced serum AAT concentration and HIV infection is consistent with a role for AAT as an endogenous HIV suppressor.

---

Manuscript submitted 17th June, 2010

Manuscript accepted 20th October, 2010

*Clin Invest Med* 2010; 33 (6): E384-E389.



## Correspondence to:

Leland Shapiro, MD,  
Denver Veterans Affairs Medical Center,  
1055 Clermont Street, Box 111L,  
Denver, CO 80220  
Email: leland.shapiro@ucdenver.edu

HIV can infect and replicate in human peripheral blood mononuclear cells (PBMC) when separated from blood and incubated in tissue culture medium; however, HIV has not been observed to replicate in exogenously-infected whole blood from healthy persons. Furthermore, no report has demonstrated HIV replication in whole blood obtained from HIV-infected persons. These observations suggest endogenous HIV-suppressive factors reside in the fluid phase of blood. Attempts to isolate antiretroviral factors in blood revealed potent HIV-antagonist activity of alpha-1-antitrypsin (AAT), the most abundant serine protease inhibitor in the circulation [1,2]. AAT has been shown to inhibit HIV production in a monocytic cell line and suppress HIV replication in infected PBMC. In a novel demonstration of endogenous AAT antiretroviral activity, HIV was shown to propagate in exogenously infected whole blood from individuals with hereditary AAT deficiency but not in whole blood from persons with normal AAT levels [1].

AAT is a 52 kD glycoprotein that circulates at relatively high serum concentrations (1.5-3.5 mg/mL) [3], and levels can increase several-fold during inflammation [4]. AAT is synthesized primarily by hepatocytes [3] with smaller amounts originating from intestinal epithelial cells, neutrophils, pulmonary alveolar cells, and macrophages [5,6]. AAT is thought to function primarily as an inhibitor of host-derived serine proteases with human neutrophil elastase as the prototype target. Possible mechanisms by which AAT inhibits HIV include blockade

of viral gp160 and p55 proteolytic processing [7], suppression of NF $\kappa$ B activation<sup>1</sup>, and binding to the gp41 envelope fusion protein [2].

AAT quantification in HIV-infected patients has received little attention. A single preliminary abstract describes reduced serum AAT concentrations in HIV-infected persons [8]. One publication, which showed no AAT concentration difference in 36 subjects with acquired immunodeficiency syndrome (AIDS) compared to 30 healthy controls, did not report AAT levels in all HIV-infected study subjects [9]. A case report documented rapid CD4<sup>+</sup> T cell decline in an HIV-infected person with known AAT deficiency, suggesting antiretroviral AAT function *in vivo* [10]. Since AAT may function as an endogenous HIV suppressor, serum AAT concentrations were assessed and evaluated for association with HIV infection.

## Materials and Methods

### Study subjects

Written informed consent was obtained from each study subject and protocols were approved by the institutional review boards at the Minneapolis and Denver Veterans Affairs Medical Centers and the Universities of Minnesota and Colorado. HIV-infected persons, already enrolled in studies of B cell function between 1995 and 2007, were examined (66 subjects with complete data, as shown in Table 1). Inclusion criteria included age 18-50 years and HIV infection documented by

TABLE 1. Selected characteristics of Control and HIV-infected subjects.

	Control (N=45)	HIV-infected (N=66)
Median age (interquartile range, IQR)	33 (28-42)	40 (35-48)
Sex (male/female)		
Male	18	59
Female	27	7
Median CD4 <sup>+</sup> T cell levels in cells/ $\mu$ L blood (IQR)	NA	281 (130-395)
CD4 <sup>+</sup> T cell levels in cells/ $\mu$ L blood		
>200	NA	41
$\leq$ 200		25
ART		
Yes	NA	35 (HAART=22)
No		31
Median HIV viral load in copies/mL plasma (IQR)	NA	19,848 (1,892-94,579)
Ethnicity:		
White	39	44
Black	2	18
Hispanic	1	1
Asian	1	1
Native American	0	1
Other	2	1

ART=antiretroviral therapy, HAART=highly-active antiretroviral therapy, N=number of subjects with available data, NA=not applicable

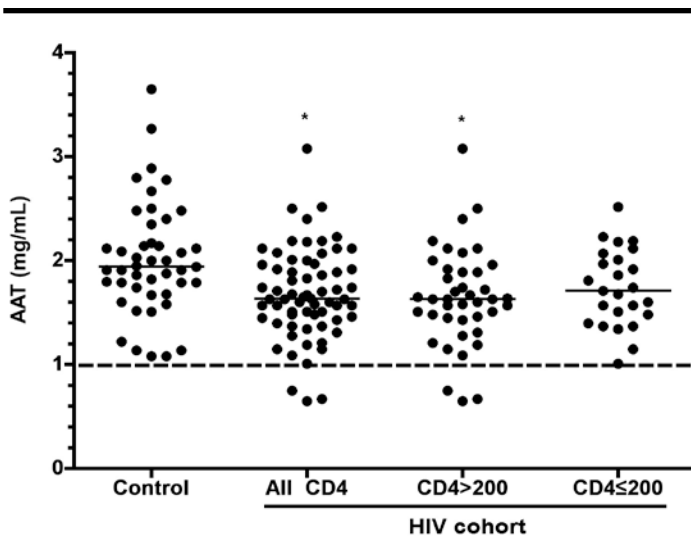


FIGURE 1. AAT concentrations in Control subjects and in all HIV-infected subjects. Serum AAT concentrations are given for the Control group (N=45), the total HIV-infected group (All CD4, N=66), and the HIV-infected subgroups with CD4<sup>+</sup> T cell levels >200 cells/ $\mu$ L (CD4>200, N=41) or  $\leq$ 200 cells/ $\mu$ L (CD4 $\leq$ 200, N=25). Dashed line indicates AAT=1.0 mg/mL. Horizontal bars within groups depict median concentrations. \*p=0.001 in comparison with Control group.

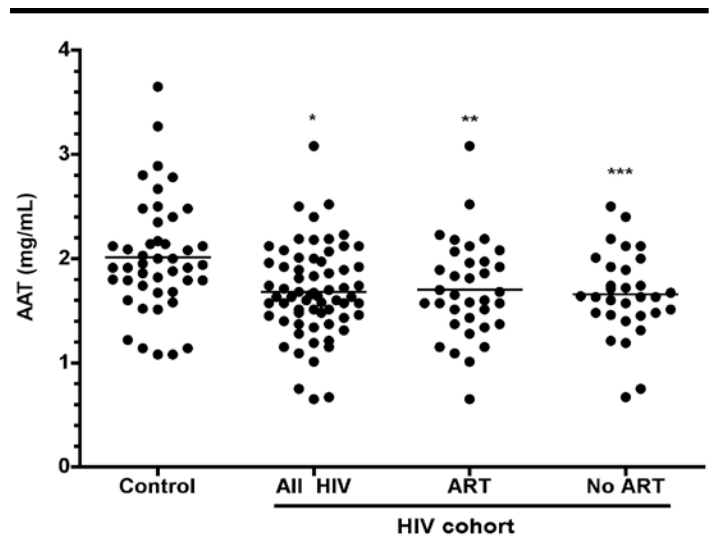


FIGURE 2. AAT concentrations in Control subjects and in HIV-infected subjects grouped by use of ART. AAT concentrations are shown in the Control group (N=45), the total HIV-infected group (All HIV, N=66), and HIV-infected subgroups on ART (ART, N=35) or not on ART (No ART, N=31). \*p=0.001, \*\*p=0.01 and \*\*\*p=0.003 in comparison with the Control group.

both enzyme-linked immunosorbent assay (ELISA) and immunoblot. Exclusion criteria included acute infectious disease, chronic heart, liver, lung or kidney disease, cancer other than skin cancer, and pharmacologic immunosuppression. CD4<sup>+</sup> T cell levels were determined by flow cytometry, and plasma HIV RNA was quantified using RT-PCR (Roche Amplicor 1.0 and 1.5; Roche, Indianapolis, IN, USA). Serum was isolated and stored at -70°C. Controls consisted of 45 healthy persons at low risk for HIV infection and HIV-negative by ELISA. Exclusion criteria and serum isolation were the same as described for persons with HIV infection.

#### AAT ELISA

AAT was quantified in duplicate in serum samples using a commercially available ELISA as instructed by the manufacturer (GenWay, San Diego, CA, USA). Assays were performed without identifiers to limit bias. Prior to AAT quantification, Triton-X-100 was added to each HIV-infected and Control serum sample (1% vol/vol final concentration) to inactivate HIV in the infected samples. Triton-X-100 did not affect AAT measurements in serum samples or in standard AAT protein aliquots. Sample AAT concentrations were calculated as the mean of the duplicate measurements.

#### Statistical analysis

For continuous variables, median values with an interquartile range (IQR) are presented. For comparison of AAT concentrations (Fig. 1) and ART use (Fig. 2) the Wilcoxon rank-sum test was used. Since current CDC guidelines define a CD4<sup>+</sup> T cell level of <200 cells/ $\mu$ L as an AIDS criterion [11], the HIV group was divided into those with CD4<sup>+</sup> T cell levels >200 or  $\leq$ 200 cells/ $\mu$ L. For multiple group AAT comparisons (Figs. 1 and 2), the Kruskal-Wallis test was used. To adjust for multiple comparisons an ANOVA with Duncan's test and  $\alpha=0.01$  was used. The proportion of individuals in each group with AAT concentrations <1.0 mg/mL was compared using the Chi square test. To adjust for baseline demographic differences between Control and HIV-infected groups, multiple linear regression was used to examine the association between HIV infection status and demographic factors (AAT concentration assessed as a continuous variable). Correlation between AAT concentrations and CD4<sup>+</sup> T cell levels in the HIV-infected subjects was performed using the Spearman  $r$  statistic. SAS statistical software (SAS Inc., Cary, NC, USA) version 9.1 was used for all analyses.

## Results

### *Study group characteristics*

As shown in Table 1, the 66 HIV-infected subjects were comprised of 59 men and 7 women, with median age 40 years (IQR 35-48 years). At the time of AAT analysis, the median CD4<sup>+</sup> T cell level was 281 cells/ $\mu$ L (IQR 130-395). Forty-one HIV-infected subjects had CD4<sup>+</sup> T cell levels >200 cells/ $\mu$ L and 25 had levels  $\leq$ 200 cells/ $\mu$ L. The median plasma viral load in the HIV group was 19,848 copies/mL (IQR 1,892-94,579), and the viral load was undetectable in 12 (18%) of the 66 subjects. Thirty-one HIV-infected subjects received no ART and 35 received any ART (including 22 on HAART, defined as  $\geq$ 3 antiretroviral medications). The Control group comprised 27 females and 18 males with median age 33 years (IQR 28-42 years).

### *Decreased serum AAT concentrations in HIV-infected subjects*

AAT serum concentrations were normally distributed with similar variances in Control and HIV-infected subjects. AAT concentrations in Control and HIV-infected groups are shown in Fig. 1. The Control group median AAT concentration was 1.94 mg/mL (IQR 1.74-2.17), and the total HIV group (All CD4) median AAT concentration was 1.64 mg/mL (IQR 1.45-1.96). This difference in AAT concentrations was significant ( $p=0.001$ ). AAT concentrations <1.0 mg/mL are considered clinically significant and suggest pathological AAT deficiency [12,13]. The dashed horizontal line in Fig. 1 indicates 1.0 mg/mL AAT, and the prevalence of concentrations below this was 0 (0%) of 45 in the Control group and 3 (4.5%) of 66 in the HIV-infected group. This difference in proportions was not statistically significant ( $p=0.15$ ).

Median AAT concentrations were 1.63 (IQR 1.45-1.89) and 1.71 mg/mL (1.48-2.01) in HIV-infected persons with CD4<sup>+</sup> T cell levels >200 cells/ $\mu$ L or  $\leq$ 200 cells/ $\mu$ L, respectively (Fig. 1). After adjusting for multiple comparisons, median AAT concentration in the HIV-infected subgroup with CD4<sup>+</sup> T cell levels >200 cells/ $\mu$ L was significantly lower than median AAT concentration in the Control group ( $p<0.01$ ). No significant difference was detected in median AAT concentration in the HIV-infected subgroup with  $\leq$ 200 CD4<sup>+</sup> T cells/ $\mu$ L compared to the Control group. No significant difference in median AAT concentrations between HIV-infected patients with lower vs higher CD4<sup>+</sup> T cell levels was observed. All persons with AAT measurements <1.0 mg/mL were identified among HIV-infected patients with >200 CD4<sup>+</sup> T cells/ $\mu$ L. In multivariate analysis, HIV infection status associated with lower AAT concentrations, and increasing age was significantly asso-

ciated with elevated AAT concentration. In contrast, gender and race were not associated with AAT concentration in these models.

### *Association between AAT concentrations and ART use in HIV-infected subjects*

The relationship between ART use and AAT concentration is unexplored. The high proportion of subjects in our HIV-infected cohort not on ART (31 of 66 subjects=47%) provided an opportunity to assess the association between AAT concentration and ART use. As shown in Fig. 2, median AAT concentration was significantly higher in Controls in comparison with all HIV-infected subjects ( $p=0.001$ ), with HIV-infected subjects on ART ( $p=0.01$ ), and with HIV-infected subjects not on ART ( $p=0.003$ ). There was no significant difference in AAT concentration between subjects on ART and subjects not on ART ( $p=0.90$ ).

## Discussion

Several endogenous HIV antagonists have been characterized. In addition to AAT, alpha defensins [14], the serine protease inhibitors antithrombin 3 [15] and secretory leukocyte protease inhibitor (SLPI) [16] can suppress HIV synthesis *in vitro*. Endogenous chemokines, which bind the HIV co-receptors CCR5 and CXCR4, inhibit HIV entry into cells and suppress viral replication [17]. Maraviroc is an approved antiretroviral drug that suppresses HIV replication by blocking HIV engagement of the CCR5 co-receptor [18], emphasizing the potential clinical benefit of investigating endogenous HIV antagonism.

In this cross-sectional study, significantly lower serum AAT concentrations were seen in HIV-infected persons in comparison with HIV-seronegative Control subjects. Clinically-significant AAT reductions (<1.0 mg/mL) [12,13] were observed exclusively in the HIV-infected group. Although no studies define the prevalence of this magnitude of AAT reduction in the U.S. population, De Serres, *et al.* project a prevalence of all AAT deficiency-associated alleles that approaches 0.08% (a liberal estimate of deficiency) [19,20]. As a conservative estimate of AAT deficiency, approximately 0.033% of persons in the U.S. are thought to have the classic form of AAT deficiency characterized by exclusive expression of Z-form AAT protein [12]. Therefore, the 4.5% prevalence of AAT <1.0 mg/mL in our HIV-infected group represents a likely increase of 56 to 136-fold in comparison with the rest of the U.S. population.

It was unexpected that all persons with AAT concentration  $<1.0$  mg/mL had CD4<sup>+</sup> T cell levels  $>200$  cells/ $\mu$ L, and none was observed in the HIV-infected subgroup with CD4<sup>+</sup> T cell levels  $\leq 200$  cells/ $\mu$ L (Fig. 1). The lack of association between very low AAT concentrations ( $<1.0$  mg/mL) and CD4<sup>+</sup> T cell levels  $\leq 200$  cells/ $\mu$ L may relate to the role of AAT as an acute phase reactant [3,4]. In HIV-infected patients, low CD4<sup>+</sup> T cell levels associate with elevated levels of the acute-phase reactant C-reactive protein (CRP) [21]. Since AAT and CRP are both acute-phase reactants, it is likely that low CD4<sup>+</sup> T cell levels also associate with increased AAT. In fact, in our HIV-infected subjects the relationship between AAT concentrations and CD4<sup>+</sup> T cell levels showed a negative (inverse) correlation that approached, but did not achieve, statistical significance ( $p=0.095$ ). A larger sample size may have shown a significant correlation between low CD4<sup>+</sup> T cell levels and increased AAT concentrations.

Our study has several limitations. The high prevalence of detectable viral load in our HIV-infected subjects (82% of 66 subjects) indicates a lack of effective ART in our study subjects. Therefore, it is likely that our subjects were under less viral control in comparison with contemporary infected persons. Since AAT concentration did not correlate with viral load in our HIV-infected subjects (data not presented), elevated viral load in our subjects may not limit extension of results to contemporary infected persons.

The imbalance between the Control and HIV-infected groups in gender, race, and age presents the possibility of confounding. A multivariate model derived from a linear regression analysis that examined HIV infection status, gender, race, and age showed HIV infection status (decreased AAT in the HIV-infected group) and age (elevated AAT with advancing age) related to AAT levels. As shown in Table 1, the median age of the HIV-infected subjects was greater than the median age of the Control subjects. Therefore, the older median age in the HIV-infected group (associated with increased AAT) is expected to blunt the association between HIV infection and low AAT (Fig. 1). For this reason, the association between decreased AAT with HIV infection in our study subjects likely underestimates the true magnitude of AAT difference between Control and HIV-infected persons. Finally, our study design does not allow us to determine if low AAT concentrations preceded HIV infection, or if HIV infection preceded low AAT concentrations. Consequently, we cannot discern if reduced AAT concentrations predisposed individuals to HIV infection, or if HIV infection caused AAT reduction.

In summary, AAT concentrations were determined in adults without and with HIV infection. AAT concentrations

were significantly lower among persons with HIV, and the frequency of clinically-significant AAT reduction was increased in the presence of HIV infection. AAT concentration did not associate with use of ART, viral load, or with CD4<sup>+</sup> T cell levels. Broader analyses of larger groups and longitudinal studies will facilitate understanding of the role AAT plays in HIV infection. Further study may determine the feasibility of using AAT or AAT-like molecules to inhibit HIV replication and control disease.

## Acknowledgments

We thank the clinical research staff and patients for their participation.

## Sources of support

Campbell Foundation and Baxter Catalyst awards to Leland Shapiro, MD, and Veterans Affairs Research Service and Mucosal and Vaccine Research Program Colorado (MAVRC) to Edward N. Janoff, MD

## References

1. Shapiro L, Pott GB, Ralston AH. Alpha-1-antitrypsin inhibits human immunodeficiency virus type-1. *FASEB J* 2001;15:115-122.
2. Münch J, Ständker L, Adermann K et al. Discovery and optimization of a natural HIV-1 entry inhibitor targeting the gp41 fusion peptide. *Cell* 2007;129:263-75.
3. Blank CA, Brantly M. Clinical features and molecular characteristics of  $\alpha$ -antitrypsin deficiency. *Annals Allergy* 1994;72:105-121.
4. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448-54.
5. Aldonyte R, Jansson L, Ljungberg O et al. Polymerized alpha-antitrypsin is present on lung vascular endothelium. New insights into the biological significance of alpha-antitrypsin polymerization. *Histopathology* 2004;45:587-92.
6. Dabbagh K, Laurent GJ, Shock A et al. Alpha-1-antitrypsin stimulates fibroblast proliferation and procollagen production and activates classical MAP kinase signalling pathways. *J Cell Physiol* 2001;186:73-81.
7. Cordelier P, Strayer DS. Mechanisms of alpha1-antitrypsin inhibition of cellular serine proteases and HIV-1 protease that are essential for HIV-1 morphogenesis. *Biochim Biophys Acta* 2003;1638:197-207.
8. Papuashvili MN, Savitskaya J, Kostrikin D et al. Low level alpha-1-antitrypsin in serum of HIV-infected patients demonstrates progression of disease. International Conference on AIDS, July 7-14 2002; Abstract number A10113:

9. Bristow CL, Patel H, Arnold RR. Self antigen prognostic for human immunodeficiency virus disease progression. *Clin Diagn Lab Immunol* 2001;8:937-42.
10. Potthoff AV, Münch J, Kirchhoff F et al. HIV infection in a patient with alpha-1 antitrypsin deficiency: a detrimental combination? *AIDS* 2007;21:2115-6.
11. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992;41:1-19.
12. Stoller JK, Fromer L, Brantly M et al. Primary care diagnosis of alpha-1 antitrypsin deficiency: issues and opportunities. *Cleve Clin J Med* 2007;74:869-74.
13. American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. *Am J Respir Crit Care Med* 2003;168:818-900.
14. Nakashima H, Yamamoto N, Masuda M et al. Defensins inhibit HIV replication *in vitro*. *AIDS* 1993;7:1129.
15. Elmaleh DR, Brown NV, Geiben-Lynn R. Anti-viral activity of human antithrombin III. *Int J Mol Med* 2005;16:191-200.
16. McNeely TB, Dealy M, Dripps DJ et al. Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. *J Clin Invest* 1995;96:456-64.
17. D'Souza MP, Harden VA. Chemokines and HIV-1 second receptors. *Nature Medicine* 1996;2:1293-1300.
18. Gulick RM, Lalezari J, Goodrich J et al. Maraviroc for previously treated patients with R5 HIV-1 infection. *N Engl J Med* 2008;359:1429-41.
19. de Serres FJ. Worldwide racial and ethnic distribution of  $\alpha$ -antitrypsin deficiency: Summary of an analysis of published genetic epidemiologic surveys. *Chest* 2002;122:1818-1829.
20. de Serres FJ, Blanco I, Fernandez-Bustillo E. Genetic epidemiology of alpha-1 antitrypsin deficiency in North America and Australia/New Zealand: Australia, Canada, New Zealand, and the United States of America. *Clin Genet* 2003;64:382-397.
21. Lau B, Sharrett AR, Kingsley LA et al. C-reactive protein is a marker for human immunodeficiency virus disease progression. *Arch Intern Med* 2006;166:64-70.