Antithrombin III (AT III) is an alpha$_2$-globulin with a molecular weight of 58,000 to 65,000(1,2). The normal concentration in human plasma is 29 mg/100 ml, with a narrow range of variation(3). Synthesis occurs primarily in hepatocytes, and to a lesser extent, in endothelial cells(3). AT III is a serine protease inhibitor which protects against thrombus formation. In addition to its major function of inactivating thrombin, AT III also inactivates Factors IXa, Xa, XIa, XIIa, plasmin, and kallikrein.(1) In the presence of heparin, the activity of AT III is instantaneously enhanced 100 fold(4). AT III, alone, accounts for 70% to 90% of the total anticoagulant capacity of human plasma(1).

Not surprisingly, deficiency of AT III has significant clinical relevance. AT III deficiency is associated with hypercoagulability and venous thromboembolism in unusual anatomical sites, at early ages(1,2,3,5,6,7). Arterial thromboembolism also occurs, but much less frequently(2,3,7,8,9). Resistance to heparin therapy is another significant clinical concern in AT III deficiency(3). This observation, however, should not be initially made during a thrombotic episode or during IV heparin therapy, since resistance to heparin is acquired under these circumstances, and is therefore not specific for inherited AT III deficiency(1).

The prevalence of congenital deficiency in the general population is 1/2000 to 1/5000(1,2). The gene is transmitted as an autosomal co-dominant trait(2). Affected individuals are heterozygous, with AT III levels or activity decreased to about 55% of normal(3). The AT III level or activity in congenital deficiency, however, may range widely, from 20% to 60%(4). Inherited AT III deficiencies manifest in two major types, decreased synthesis of AT III, or synthesis of dysfunctional AT III. These types are further divided into subtypes, which have been correlated with different risks of thromboembolic
complications(3,5). The relative risks range from 50% to 70%, although type IIic (an abnormality only of the heparin binding site) may have a risk of less than 10%(5). Typing and subtyping is little more than an academic exercise, however, since all patients with congenital AT III deficiency are managed the same way: they are not treated unless they manifest with thromboembolism, at which time lifelong anticoagulation is initiated.

About 10% of patients with congenital AT III deficiency suffer from venous thromboembolism while still in the mid-teens. The rest continue to present into middle age, usually prior to age 55(3). Among patients who suffer thrombotic events, the prevalence of hereditary AT III deficiency appears to be around 3%(1). The prevalence will increase further in a patient population screened by clinicians for other risk factors, such as a positive family history for thromboembolism and presentation at an early age.

When considering the diagnosis of congenital AT III deficiency, it is important realize that AT III deficiency can also be acquired in several ways. Renal disease with proteinuria causes loss of AT III in the urine along with other plasma proteins. Chronic liver disease, or acute hepatitis may result in reduced synthesis of AT III. Use of oral contraceptives has been associated with reduced AT III activity. Chronic DIC and acute venous thrombosis result in decreased AT III due to increased consumption, and can drop AT III activity to 60%(1,3). Intravenous heparin therapy will reduce AT III activity by 10% to 15%, attributable to rapid turnover and decreased half life(1,3). The clinical relevance of acquired AT III deficiency with regard to risk of thromboembolism remains obscure(3).

Studies have been published suggesting efficacy of AT III replacement therapy, using FFP or heat-treated AT III concentrates to prevent or alleviate thromboembolism or DIC(1,10,11,12). Because the experimental trials have included neither controls, nor double-blind protocols, the results remain controversial. Nevertheless, AT III concentrates have been used therapeutically in countries outside the United States for greater than 10 years(10). Patients with congenital AT III deficiency are given supplements prior to surgery, after trauma, during bedrest of greater than 24 hours, and during pregnancy,
but are not maintained on lifelong prophylaxis(10). A larger use of AT III concentrate is to prevent or control thrombosis or DIC in patients with severe acquired AT III deficiencies due to cirrhosis or nephrotic syndrome, when AT III activity drops beyond 70%(10). DIC is also treated with AT III, in conjunction with heparin, when AT III falls below 75%(10).

AT III concentrates, however, have not been approved for medical use in the United States, and are not available to clinicians. Nor do blood banks readily relinquish fresh frozen plasma for its meager content of AT III (1 unit/ml)(4), especially since effects have been reported to be minimal and short lived(11). Because clinicians do not have access to replacement therapy in the United States, documenting AT III levels in acquired deficiencies has no impact on patient management, and is not necessary.

**AT III Assays:**

There are two types of AT III assays, quantitative and functional. Both assays require citrated plasma, centrifuged and separated from cells as soon as possible, and frozen.

The quantitative assay is an immunologic determination of antigenicity. As discussed above, AT III concentration may be normal, while the molecule is dysfunctional. Because the quantitative assay cannot rule out functional AT III deficiency, a functional assay should be ordered instead.

The functional assay has become quite sophisticated, and is now automated in many laboratories(13,14). The specimen is flooded with heparin, to activate all endogenous AT III. Subsequently excess thrombin is added. The AT III, activated by heparin, interacts with thrombin stoichiometrically, inactivating it in a 1:1 ratio. The remaining thrombin activity is proportional to the original plasma concentration/activity of AT III. Activity is measured by adding a synthetic, thrombin-specific substrate that yields a chromogenic compound which can be measured by absorbance(13,14). The reference range is generally 80% to 115% compared to a standard control.

Sensitivity, specificity, and predictive value of the AT III assay are difficult to evaluate, due to the multitude of clinical
factors which affect the probability of identifying a true hereditary AT III deficiency. Sensitivity should approach 100% (using a threshold of 60% to diagnose congenital deficiency). Use of oral anticoagulants may raise AT III activity(11), however, which might cause false negative results. Specificity depends heavily on careful screening of patients to be tested. As discussed above, numerous clinical conditions result in acquired AT III deficiency, which could depress AT III activity into the range of false positive.

**Suggested Screening Protocol**

In order to obtain meaningful results from the AT III assay and to avoid extraneous laboratory testing, the following points are useful to keep in mind:

1. Does the patient in question fit the high prevalence profile?
   - Positive family history for thromboembolism (the trait is autosomal dominant, so must be expressed in each generation).
   - Presentation with thromboembolism at a young age (the younger the patient, the higher the probability of inherited AT III deficiency).
   - Is the thrombus in an unusual site, and/or without an obvious precipitating cause (i.e. trauma, anatomic abnormalities, neoplasia, etc.).

2. Is the patient resistant to heparin therapy?
   - Reports estimate that AT III levels of 60% to 75% allow for an adequate response to heparin (elevation of the PTT)(1). Therefore, response to heparin effectively rules out congenital AT III deficiency (threshold of 60%).
   - During episodes of thromboembolism and heparin treatment, resistance to heparin develops as acquired AT III deficiency progresses. This should not be mistaken as indication of congenital AT III deficiency, and will
usually respond to increased dosing.

3. Does the patient have any risk factors for acquired AT III deficiency?
   - Nephrotic syndrome.
   - Cirrhosis or acute hepatitis.
   - Active thromboembolism and IV heparin.
   - Active DIC.

   If so, interpretation of the result will be complicated. The AT III assay will be more meaningful when the patient is stable, and interfering disease states have resolved. (It is not useful to document acquired AT III deficiency since replacement therapy is not recommended or available in the United States).

4. Is the patient receiving oral anticoagulant therapy?
   - Oral anticoagulants may increase AT III activity and mask a true congenital deficiency.
Footnotes and References


