

# Antithrombin: anti-inflammatory properties and clinical applications

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## Summary

Many humoral and cellular components participate in bidirectional communication between the coagulation and inflammation pathways. Natural anticoagulant proteins, including antithrombin (AT), tissue factor pathway inhibitor, and protein C, suppress proinflammatory mediators. Conversely, inflammation blunts anticoagulant activity and, when uncontrolled, promotes systemic inflammation-induced coagulation, such as those that occur in disseminated intravascular coagulation and severe sepsis. This review discusses the mechanisms of action and clinical use of AT concentrate in critically ill patients and in the settings of perioperative anticoagulation management for surgery and obstetrics. AT is a serine protease inhibitor with broad anticoagulant activity and potent anti-inflammatory properties. In clinical conditions associated with hereditary or acquired AT deficiency, administration of AT concentrate has been shown to restore proper haemostasis and attenuate inflammation. Of

note, AT modulates inflammatory responses not only by inhibiting thrombin and other clotting factors that induce cytokine activity and leukocyte-endothelial cell interaction, but also by coagulation-independent effects, including direct interaction with cellular mediators of inflammation. An increasing body of evidence suggests that AT concentrate may be a potential therapeutic agent in certain clinical settings associated with inflammation. In addition to the well-known anticoagulation properties of AT for the treatment of hereditary AT deficiency, AT also possesses noteworthy anti-inflammatory properties that could be valuable in treating acquired AT deficiency, which often result in thrombotic states associated with an inflammatory component.

## Keywords

Antithrombin, cardiac surgery, coagulation, disseminated intravascular coagulation, inflammation, pregnancy

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## Introduction

An extensive network of humoral and cellular components participates in bidirectional communication between the coagulation and inflammation pathways (1, 2). Inflammatory mediators, such as cytokines, promote the expression of coagulation initiating factors, like tissue factor (TF), on cell surfaces, thereby causing downstream activation of coagulation (3). At the same time, natural anticoagulant mechanisms, including antithrombin (AT), tissue factor pathway inhibitor (TFPI), and the protein C system, are suppressed. This suppression may propagate an uncontrolled cycle of coagulation and inflammation, leading to disseminated intravascular coagulation (DIC) and contributing to multiple organ dysfunctions, particularly in septic patients (3). Interactions between the inflammatory and coagulation systems become even more complex in light of growing evidence that anticoagulant pathways possess coagulation-independent anti-inflammatory properties. AT plays a central role in mediating both anticoagulation and anti-inflammatory effects.

AT is a 58-kDa plasma glycoprotein synthesised in the liver and belongs to the family of serine protease inhibitors (serpins) (4). AT is the predominant naturally occurring inhibitor of coagulation which mainly, but not exclusively, targets activated factor II (factor

Ila; thrombin) and factor Xa. The normal concentration of AT in human plasma is approximately 2.57  $\mu$ M, or 0.125 mg/ml to 0.160 mg/ml which equates to 80%–120% AT activity (5–7). AT deficiency can be either hereditary or acquired and is associated with inadequate anticoagulation. Hereditary AT deficiency, inherited as an autosomal dominant trait, reduces functional AT levels to 40% to 60% of normal and increases the risk of venous thromboembolism (VTE) (8–10). A recent prospective study (11) of 823 VTE patients reported that even mild AT deficiency (AT levels of 70% to 80%) increases the risk of VTE recurrence significantly higher than that in patients with normal AT levels (>80%). The true prevalence of hereditary AT deficiency is unknown, but estimates suggest that 1 in 500 to 5,000 individuals are affected (12). Acquired AT deficiency is much more common in clinical practice and is the result of decreased protein synthesis (e.g. liver failure) and/or increased consumption (e.g. prolonged heparin infusions, DIC) (4). The benefits of AT use without acute adverse events were documented in critically ill children on extracorporeal life support (ECLS) with extracorporeal membrane oxygenation (ECMO), who often do not achieve adequate anticoagulation effects with unfractionated heparin alone (13). In the United States, the use of AT has been rising, particularly in acquired AT deficiency. A large retrospective, multicentre study (n=4,040) found that off-label AT

use (i.e. cardiovascular and congenital/genetic abnormalities, sepsis, venous thrombosis, arterial thrombosis, chylothorax, and liver transplantation) increased by five-fold between 2002 and 2011 (0.04% to 0.19%, respectively), with ECMO being the most common procedure associated with AT concentrate use (14). Moreover, a recent retrospective cohort study of 51 paediatric patients (median age of 3 months) demonstrated that a single dose of human plasma-derived AT concentrate given during unfractionated heparin therapy resulted in statistically significant increases in AT and antifactor Xa activity levels ( $p < 0.001$ ), despite no change in the dose of unfractionated heparin (15).

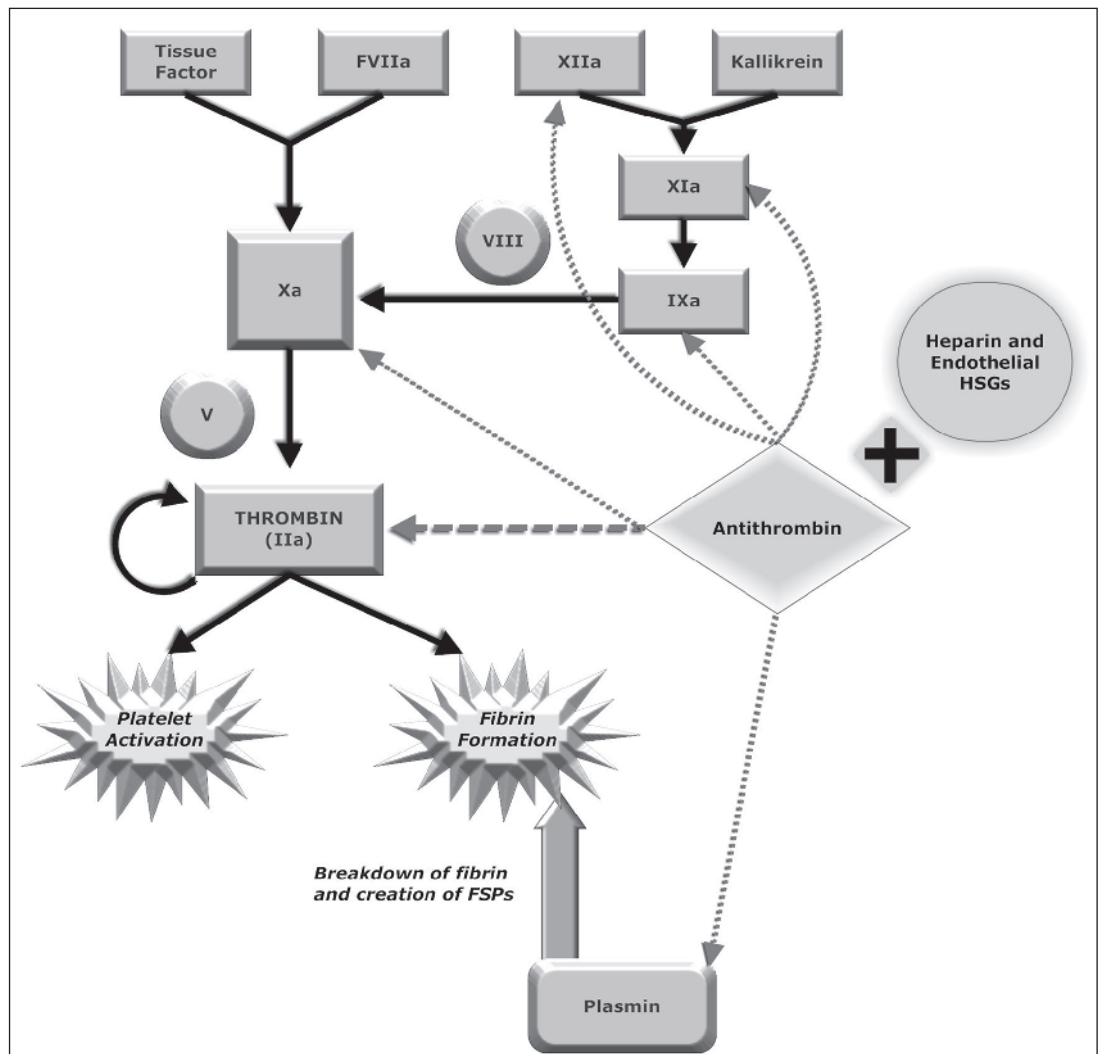
AT has also been implicated in angiogenesis; its *in vivo* anti-angiogenic and antitumour activity provides evidence that the clotting and fibrinolytic pathways are directly related to the regulation of angiogenesis (16). Several studies suggest that it may target the endothelial cell at multiple levels, resulting in a profound blockade of the angiogenic cascade (16). However, the exact mechanism has not yet been defined. This review, therefore, will focus on the anticoagulation-dependent and independent anti-inflammatory actions of AT and the potential clinical applications of AT

concentrates, with emphasis on obstetrical and surgical settings associated with inflammation. We performed an extensive literature search of Embase, Medline, and Pubmed for the terms, “antithrombin,” “antithrombin,” “acquired,” “deficiency,” “mechanism,” “anti-inflammatory,” and “anti-inflammatory properties.”

## Mechanism of action of AT

### Coagulation and haemostasis

AT neutralises many coagulation enzymes, including thrombin, plasmin, and factors IXa, Xa, XIa, XIIa (► Figure 1) (1, 4, 17). AT interacts with thrombin in a manner that incapacitates both molecules; thrombin cleaves the reactive centre of AT, which in turn traps the thrombin molecule and forms an enzyme-inhibitor complex that is rapidly removed from the circulation (17). Of note, the AT-mediated, irreversible inhibition of thrombin differs from that of “direct thrombin inhibitors,” which target thrombin in a reversible fashion and, therefore, only transiently block thrombin activity (18).



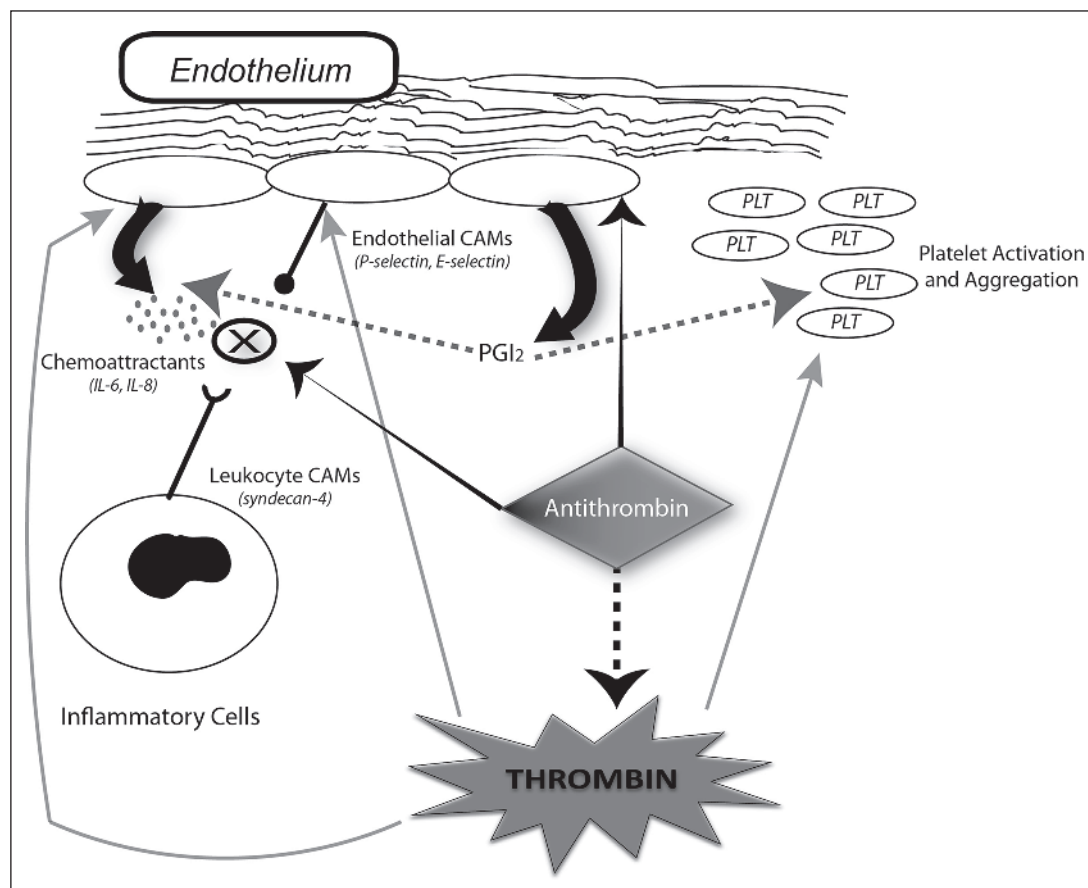
**Figure 1: Overview of the anticoagulant effects of antithrombin.** Dashed/dotted lines indicate an inhibitory function. HSGs, heparan sulfate groups; FSPs, fibrin split products.

Without heparin, AT neutralises coagulation enzymes in a slow, progressive manner. Heparin induces conformational changes in AT that result in  $\geq 1,000$ -fold enhancement of AT activity (17, 18). Thus, the clinical efficacy of heparin is attributed to its interaction with AT. Endogenous glycosaminoglycans (e.g. heparan sulfates) on the vessel wall also promote AT-mediated inhibition of thrombin and other coagulation enzymes (2).

### Anti-inflammation

#### Coagulation-dependent anti-inflammatory effects of AT

Some anti-inflammatory properties of AT are mediated by its anti-coagulation actions, such as thrombin inhibition (► Figure 2, ► Table 1) (1, 2). Thrombin activates platelets and endothelial



**Figure 2: Illustration of some of the anti-inflammatory mechanisms of antithrombin.** See Table 1 for details. Dotted/dashed lines indicate an inhibitory effect. CAM, cellular adhesion molecules; PLT, platelets; PGI<sub>2</sub>, prostacyclin.

Coagulation-dependent effects	Physiologic effect
Thrombin inhibition	<ul style="list-style-type: none"> <li>Prevents activation of platelets and endothelial cells</li> <li>Suppresses factors that promote neutrophil–endothelial cell interactions (e.g. IL-1, IL-6, IL-8, MCP-1, P-selectin)</li> </ul>
Factor Xa inhibition	<ul style="list-style-type: none"> <li>Suppresses factors that promote neutrophil–endothelial cell interactions (e.g. IL-6, IL-8, E-selectin)</li> </ul>
Factor VIIa inhibition	<ul style="list-style-type: none"> <li>Prevents complex formation with tissue factor and subsequent upregulation of cytokines (e.g. IL-6, IL-8)</li> </ul>
Coagulation-independent effects	Physiologic effect
Increased prostacyclin synthesis/secretion	<ul style="list-style-type: none"> <li>Suppresses platelet activity (aggregation and attachment)</li> <li>Inhibits attachment of neutrophils to endothelial cells</li> <li>Decreases release of IL-6, IL-8, and TNF by endothelial cells</li> </ul>
Leukocyte inactivation	<ul style="list-style-type: none"> <li>Prevents neutrophil rolling and adhesion and subsequent tissue damage</li> </ul>

AT, antithrombin; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumour necrosis factor.

**Table 1: Anti-inflammatory effects of AT (1, 2).**

cells, which in turn contribute to local inflammation; activated platelets secrete cytokines that stimulate leukocyte activity. Recruitment and adhesion of neutrophils and monocytes to blood vessels within the microcirculation promote inflammation and provide a procoagulant surface for further thrombin generation. In response to thrombin, platelets and endothelial cells express P-selectin, which further promotes interaction with neutrophils, and upregulates TF expression, leading to accelerated thrombin generation (19). Activated neutrophils release enzymes that inhibit AT and other anticoagulant mechanisms, exacerbating coagulation-dependent inflammation (1, 2). When uncontrolled, these cellular events can adversely impact the microcirculation, leading to capillary leakage, tissue damage, and eventually organ failure (20).

In addition to blocking thrombin-induced inflammatory pathways, AT inhibits other proinflammatory coagulation enzymes (1, 2). For example, AT prevents factor Xa-induced production of interleukin (IL)-6, IL-8, E-selectin, and other molecules involved in monocyte recruitment and adhesion to endothelial cells. AT may also interfere in TF and factor VIIa complex formation. Like factor Xa, the TF/VIIa complex promotes synthesis of inflammatory cytokines and chemokines. Thus, AT may suppress inflammation through its central inhibitory role in many coagulation processes.

### Coagulation-independent anti-inflammatory effects of AT

There is an increasing amount of evidence indicating AT possesses potent anti-inflammatory properties that is independent of its anticoagulation activity (► Figure 2, ► Table 1) (1, 2). The majority of these effects have been demonstrated at high AT concentrations in *in vitro* or *in vivo* studies. These mechanisms may be important in clinical conditions driven by the activation of both coagulation and inflammation. Most importantly, AT induces endothelial cell release of prostacyclin (2, 21–26), which inhibits aggregation and activation of platelets, prohibits adhesion of neutrophil to blood vessel wall, and reduces various cytokine and chemokine production by endothelial cells (2, 21, 27, 28).

In addition, direct interaction of AT with leukocytes and lymphocytes contribute to the anti-inflammatory actions of AT. AT binds to receptors (i.e. syndecan-4) on neutrophils, monocytes and lymphocytes and inhibits their interaction with endothelial cells (2, 23, 25). The inhibition of leukocyte-endothelial cell interactions may be attributable to the ability of AT to induce prostacyclin release, downregulate P-selectin activity, or prevent leukocyte activation (23, 24). Therefore, it is hypothesised that AT directly interferes with leukocyte migration and adhesion to endothelial cells, which may alleviate the severity of capillary leakage and subsequent organ damage (2). In the setting of microcirculatory perfusion failure due to soft-tissue injury and systemic endotoxaemia, AT prevents inflammatory leukocyte adherence to endothelial cells, microvascular leakage, and leukocyte-dependent secondary tissue injury (20). Additionally, AT has been shown to prevent apoptosis and ischaemic tissue damage in a liver transplant model and exhibit cardioprotective effects through anti-inflammatory cellular signalling (via upregulation of prostacyclin and downregulation

**Table 2: Summary of potential clinical applications of AT concentrate.**

Clinical condition	Effect of AT concentrate treatment
Pregnancy	• Thromboprophylaxis in women with severe AT deficiency
Cardiac surgery	• Successful resolution of heparin resistance • Reduction in CPB-induced inflammation (thrombin generation, activation of platelets and leukocytes)
Severe sepsis	• Lower mortality in subgroups of patients with DIC not receiving heparin (needs prospective confirmation)
Transplantation	• Decrease in risk of early graft thrombosis • Better outcome in patients with multiple organ dysfunction • Potential reduction in ischaemia/reperfusion injury

AT, antithrombin; CPB, cardiopulmonary bypass; DIC, disseminated intravascular coagulation.

of TNF- $\alpha$  and IL-6) in a myocardial ischaemia/reperfusion injury model (29, 30).

Because of the broad effects of AT on both coagulation and inflammation, potential clinical applications of AT concentrate comprise thrombotic conditions generally associated with inflammation (e.g. pregnancy) and coagulation-related disease states with strong proinflammatory elements (e.g. sepsis, DIC) (► Table 2).

## Clinical applications

### AT administration

Two forms of AT are available for patient use to maintain adequate levels of anticoagulation during hypercoagulable states. A human plasma-derived AT (pdAT) concentrate is produced from pooled human plasma, heat-treated, nanofiltered, and then reconstituted with sterile water for administration. It is indicated for treating patients with hereditary AT deficiency in connection with surgical or obstetrical procedures, or when they suffer from thromboembolism (31). Although hepatitis B seroconversion has been reported with plasma-derived AT, these patients were also receiving multiple blood products at the time (32). A recombinant form of human AT concentrate (rhAT) produced from goat milk is also available for the prevention, but not treatment, of perioperative and peripartum thrombotic events in patients with hereditary AT deficiency (33). Both forms of AT concentrates have been used for acquired AT deficiency as well.

AT concentrate is usually given slowly to allay the risk of anaphylaxis. The current recommendation is an infusion rate of 100 IU/minute (min). However, in a recent study of 40 patients undergoing elective heart surgery, Lund et al. found no difference in haemodynamics or reactions with regard to the rate of administration when comparing infusions at 100 IU/min vs 250 IU/min (34). Additional guidance for AT replacement and monitoring will be discussed in a later section.

## Pregnancy and preeclampsia

The body of evidence supporting the need for anticoagulation therapy during pregnancy and/or child birth is growing. Although AT levels have been generally believed to remain stable during pregnancy in women without thrombophilia (35), several studies suggest that AT consumption during pregnancy and child birth warrants even more careful considerations for patients with either AT deficiency or an AT-deficient disease state. One study of 153 healthy pregnant women reported diminished AT levels in the third trimester and postpartum (36). Another study (n=172) confirmed that AT levels are reduced by ~20% below baseline throughout gestation, decreased further by 30% at delivery, and reached the lowest levels around 12 hours (h) postpartum before returning to baseline after 72 h (37). A recent study compared haemostatic abnormalities between 18 pregnant women with thrombosis and 35 patients with deep-vein thrombosis (DVT) undergoing major orthopaedic surgery (38). Fourteen of the 18 women (~56%) had thrombosis at the first pregnancy, and 17 women had pregnancy-related VTE, with AT deficiency being the cause of thrombosis in eight cases (44%). AT activity levels were significantly lower in pregnant women at the onset of thrombosis (62%) than after delivery and anticoagulation therapy (89%,  $p < 0.005$ ) and in DVT patients undergoing orthopaedic surgery (86%,  $p < 0.001$ ). In those with congenital AT deficiency, AT activity, which was already low at stable state, became even lower at thrombosis onset. Even though it is difficult to ascertain AT deficiency status during acute thrombosis due to AT consumption in the clot, the culminating evidence from these reports confirm that AT deficiency is an important factor in pregnancy-related thrombosis.

Despite conflicting reports regarding AT levels during normal pregnancy, the haemostatic balance clearly shifts toward hypercoagulability. One potential complication of pregnancy is preeclampsia, a vasospasmodic condition associated with decreased AT levels (39, 40) and increased expression of inflammatory cytokines (41). In a randomised clinical trial, patients with severe preeclampsia received 3,000 IU/day AT concentrate (n=66) or placebo (n=67) daily for seven days (39). Treatment with AT concentrate significantly prolonged gestation by 6.5 days ( $p = 0.004$ ) and reduced the frequency of low-birth-weight infants ( $p = 0.011$ ). In addition, no adverse events related to AT concentrate were observed. A subgroup re-analysis of the data from 84 of the original 147 women (57%) from this trial was performed to investigate the effects of AT concentrate on premature infants (42). AT concentrate significantly improved the foetal biophysical profile score or foetal heart rate monitoring (relative risk [RR] 0.24, 95% confidence interval [CI] 0.07–0.8), and prolonged gestational age to  $\geq 34$  weeks (RR 3.6, 95% CI 1.05–12.6). However, infant mortality rates did not differ between the two groups (42). In another study of 23 women with preeclampsia, a similar high-dose AT concentrate treatment regimen (n=10, 3000 IU/day over 5 days, activity level ~130%) vs standard-dose AT concentrate therapy (n=13, sufficient dose to maintain activity level  $\geq 80\%$ ) prolonged gestation by 2.5 days ( $p = 0.03$ ) (40). There was also a trend towards improvement in the incidence of maternal bleeding following caesarean

section in the high-dose group, although results were not statistically significant. Thus, AT concentrate may be beneficial in complex, critical obstetrical settings. Findings from the on-going Prospective Randomised Evaluation of the Safety and Efficacy of Recombinant Antithrombin in Very Preterm Preeclampsia (PRESERVE-1) study should shed more light into the pharmacokinetics and safety of AT therapy in preeclampsia (43).

Hereditary thrombophilia is linked to maternal thromboembolism and adverse pregnancy outcomes (44, 45). The incidence of thrombotic complications during pregnancy may be as high as 70% in women with hereditary AT deficiency (44). Thromboprophylaxis with heparin during pregnancy is associated with a 15-fold reduction in foetal loss rate in women with hereditary thrombophilia, including women with AT deficiency (46). The use of rhAT has been reported as an effective perioperative and peripartum prophylaxis against VTE in patients with hereditary AT deficiency (47). Thromboprophylaxis may also improve clinical outcomes in women with hereditary thrombophilia with recurrent pregnancy loss (RPL) and without other causes of RPL (48). In a study of women with RPL, a strong association was found between one or more thrombophilic defects and RPL if other common causes of miscarriage (i.e. uterine malformation, anovulation, tube patency, endocrine diseases, chronic and/or infectious diseases, and karyotype alterations) had been excluded. The study advocated that women affected by RPL should be tested for thrombophilia (48). Although there are reports of the potential usefulness of AT and other thrombophilia testing, this topic is still very controversial.

Yamada et al. reviewed 12 reports of 25 women with hereditary AT deficiency who received treatment with AT concentrate for managing pregnancy, either alone or in combination with heparin or warfarin (49). Seven women (28%) who received thromboprophylaxis that included AT concentrate experienced antepartum thrombosis, and one woman (4%) experienced postpartum thrombosis. Most pregnancies had favourable outcomes, with 15 vaginal deliveries (60%), four caesarean sections (16%), and six cases of unknown mode of delivery (24%). Treatment with AT concentrate was not associated with reported adverse effects on the foetus.

Therapy with low-molecular-weight heparin (LMWH) and AT concentrates has also demonstrated improvements in maternal and foetal outcomes in a study by Sabadell et al. (50). They performed a descriptive retrospective study of 18 pregnancies in nine women with AT deficiency between 1991 and 2005. LMWH was started upon pregnancy confirmation in 12 of the 18 cases. In six of the cases, AT deficiency was not known at the time. In the known cases of AT deficiency, AT concentrate was administered during labour and delivery to achieve a normal activity level. Although 10 of the 18 pregnancies suffered an adverse outcome, no women who were treated with LMWH and AT concentrate suffered a thromboembolic event, while three women in the non-treatment group did. There was also a lower incidence of stillbirth and miscarriage in the treatment group, although intrauterine growth retardation and foetal distress still occurred. Similar findings were also reported in a retrospective, multicentre analysis of

18 pregnancies with hereditary AT deficiency between 2006 and 2012 (51). Rogenhofer et al. observed that administration of heparin/LMWH and AT concentrate resulted in the most optimal neonatal health and the least maternal complications during pregnancy, delivery and postpartum recovery.

### Transplantation and ischaemia/reperfusion

Transplantation operations are often conducted in an unfavourable haemostatic environment due to consumption of coagulation factors and perioperative haemodilution, resulting in bleeding and transfusion of blood products (4). Low AT levels may predispose patients to thrombotic complications (e.g. veno-occlusive disease) and coagulation-associated inflammatory states (e.g. multiple organ dysfunction syndrome) (52, 53). Primary graft failure resulting from ischaemia/reperfusion injury is estimated to cause nearly one-third of perioperative deaths in patients undergoing lung transplantation (54). Thus, controlling the inflammatory response to transplantation and ischaemia/reperfusion is a major clinical goal.

Preclinical evidence supports a therapeutic role for AT concentrate in minimising graft rejection and ischaemia/reperfusion injury associated with organ transplantation. AT concentrate reduced organ injury in settings of transplantation and ischaemia/reperfusion through prostacyclin-mediated inhibition of leukocyte-endothelial cell interactions in animal models (24, 25, 55). Selective thrombin inhibitors failed to elevate levels of prostacyclin, suggesting that AT administration prevented tissue injury via coagulation-independent actions. AT concentrate also prevented acute graft rejection in a study of cardiac transplantation in mice; untreated mice rejected cardiac grafts acutely (mean survival time [MST], 9 days), whereas mice treated with 50 IU/kg AT concentrate on the day of transplantation showed significantly prolonged MST (25 days;  $p=0.008$ ) (56).

A limited number of studies have evaluated the effect of AT administration on transplantation outcomes in humans. Patel et al. (57) reported a case of successful perioperative management with AT concentrate for kidney transplantation in a patient with hereditary AT deficiency. Treatment with AT concentrate avoided the necessity for aggressive early postoperative anticoagulation. Other trials evaluated the value of AT administration for transplantation in patients without thrombophilia. In a retrospective study, Fertmann et al. (58) analysed the effect of AT concentrate on coagulation parameters and graft thrombosis in patients undergoing simultaneous pancreas-kidney transplantation. Patients received either a single intravenous bolus of AT concentrate (3,000 IU) before pancreatic reperfusion ( $n=24$ ) or standard therapy, which included postoperative AT supplementation ( $n=29$ ). Treatment with AT concentrate did not affect coagulation parameters or bleeding incidence but significantly decreased the rate of early graft thrombosis (17%) compared with standard therapy (24%), representing a 30% risk reduction ( $p<0.05$ ). In addition, AT concentrate treatment was associated with a trend towards reduced leukocyte counts and a significant decrease in lipase activity ( $p<0.05$ ), possibly reflecting an AT-mediated decrease in reperfusion pancreati-

tis. In another retrospective study of graft protection from ischaemia/reperfusion, Fertmann et al. compared standard control therapy ( $n=13$ ) to prophylactic high-dose AT treatment ( $n=18$ ) in patients receiving solitary pancreas transplantation, which is typically associated with complications that may lead to early graft loss and poor outcomes (59). While AT did not alter coagulatory parameters nor the incidence of blood transfusion, AT treatment did significantly reduce serum amylase ( $p<0.01$ ) and lipase ( $p<0.01$ ) release from the grafts during the first three postoperative days, as well as reduced C-reactive protein levels in half ( $p<0.01$ ).

A pilot study was performed by Kaneko et al. (60) to clarify the feasibility of using AT concentrate in an anticoagulant protocol in the early stages after liver transplantation. The first 15 consecutive cases received 1,500 IU/day AT concentrate on postoperative days 1 through 3 and the subsequent 10 cases served as control cases. In the AT concentrate group, AT activity was maintained at levels  $>80\%$  for five days after transplantation, whereas in the control group, AT activity did not return to normal during the first two weeks after the operation. Fibrin degradation product D-dimer (FDP-DD) levels were significantly higher in the control group than in the AT concentrate group ( $p<0.05$ ). Six patients in the control group and three patients in the treatment group required transfusions with platelet concentrate ( $p<0.5$ ) (60). Therefore, AT concentrate might reduce FDP-DD levels and prevent decreased platelet counts in the early stages after liver transplantation.

Other studies reported clinical improvement with AT concentrate in patients with severe organ dysfunction following bone marrow transplantation or haematopoietic stem cell transplantation (52, 53, 61). A retrospective study of the largest series of patients ( $n=48$ ) that has received AT concentrate for the treatment of veno-occlusive disease (VOD) following haematopoietic stem cell transplantation showed that AT concentrate should be further considered in patients with severe VOD (61). AT administration reduced the overall mortality from VOD, while the 100-day mortality for the severe VOD group was only 31% compared to  $>95\%$  in other studies (62). There was no significant treatment-related morbidity (61).

Although large prospective trials are lacking, these studies suggest a potential therapeutic role for AT concentrate in the setting of transplantation.

### Cardiac surgery with cardiopulmonary bypass

Case reports have described successful anticoagulation of individuals with hereditary AT deficiency using heparin and AT concentrate (63, 64) or rhAT (65) during cardiopulmonary bypass (CPB). However, acquired AT deficiency in the setting of cardiac surgery is far more common. Reductions in AT activity level of 31% to 50% occur commonly in patients undergoing CPB; preoperative heparin treatment, haemodilution, and AT consumption related to haemostatic activation are all thought to contribute (66–68).

A study of 405 patients who underwent cardiac operations, including CPB, showed that low AT levels after cardiac surgery were associated with higher incidences of perioperative complications and worse mid-term survival (69). Patients were assessed on

arrival at the intensive care unit (ICU) for AT levels either greater than or less than 63.7% (high and low AT groups, respectively), and the predictive role on in-hospital mortality and morbidity along with 18-month follow-up was evaluated. Low AT activity upon ICU arrival was not associated with increased in-hospital mortality, but it was an independent risk factor for longer mechanical ventilation, need of inotropic support, excessive bleeding and blood products transfusion. Low AT activity upon ICU arrival also was associated with lower survival during follow-up at 18 months

(92.3% and 85.4% in the high AT and low AT groups, respectively;  $p=0.05$ ).

Garvin et al. examined the relationship between AT activity level and major cardiac adverse events (MACE) in 1,403 patients undergoing primary CABG surgery (70). Postoperative, but not preoperative, AT activity levels were associated with a higher incidence of MACE. However, there was no temporal correlation between the two, with most MACE occurring at or before postoperative day 1 and AT activity levels not independently predictive of MACE until postoperative day 2. This calls into question the

**Table 3: Definitions of heparin resistance used in studies of AT concentrate treatment during CPB.**

HR definition	Study design	Activator	AT activity level	ACT levels post-Rx	Rx	Reference
< 480 s after > 450 IU/kg heparin in nonaprotinin-treated pts; < 600 s in aprotinin-treated patients	Prospective, crossover RCT	Celite	Baseline: 56% $\pm$ 25%. Improvement in patients given AT concentrate vs heparin (75% $\pm$ 31% vs 50% $\pm$ 23%; $p<0.0005$ )	Therapeutic levels achieved in 42/44 (96%) patients given AT concentrate vs 28/41 (68%) given heparin ( $p=0.001$ )	1,000 IU pdAT (n=44) or additional heparin (n=41)	Williams et al. 2000 (79)
< 480 s after 600–800 IU/kg heparin	Retrospective	Celite or Kaolin	NS	Average increase of 176 s ( $p<0.001$ ) following AT concentrate Rx	500 IU pdAT (n=44)	Brown et al. 2000 (76)
< 600 s after 600 IU/kg in aprotinin-treated patients	Prospective	Kaolin	Mean at baseline: 67%. Median at baseline: 67% (range, 33%–102%; SD, 15%)	Prolongation of mean ACT from 492 s to 789 s following AT concentrate Rx	500 IU (n=45) or 1,000 IU pdAT (n=8)	Lemmer et al. 2002 (78)
< 480 s after 300 IU/kg heparin	Prospective	NS	AT-dependent HR patients (n=68 [65%]) had AT activity of 79.6% $\pm$ 13.5%; AT-independent HR patients (n=36 [35%]) had AT activity of 111.3% $\pm$ 8.2%	NS	Additional heparin, then 1,000 IU pdAT on Rx failure	Ranucci et al. 2002 (84)
< 480 s after 500 IU/kg heparin	Prospective RCT	NS	Significant increase in heparin + AT concentrate group compared with the heparin group (99.8% $\pm$ 13.7% vs 40.1% $\pm$ 12.6%; $p<0.05$ )	NS	Additional heparin (n=20) or heparin + 50 IU/kg pdAT (n=20)	Koster et al. 2003 (77)
< 480 s after 400 IU/kg heparin	Prospective RCT, DB, PC	Kaolin	Significant increase in rhAT group to within normal range. Decrease in placebo group below baseline levels	Significant increase in ACT levels in the rhAT group vs placebo group ( $p=0.001$ )	75 IU/kg rhAT (n=28) or placebo (n=24) 2 U FFP upon Rx failure: n=6 (21%) of rhAT group vs n=22 (92%) of placebo group ( $p<0.001$ )	Avidan et al. 2005 (83)
< 480 s after 400 IU/kg heparin	Prospective RCT, DB, PC	Kaolin	Significant increase in rhAT group from 78% to 122% 30 min after CPB initiation. Decrease in placebo group from 74% to 52% 30 min after CPB initiation	Higher mean ACT value in rhAT group vs placebo (601 s vs 442 s; $p<0.001$ ), compared with 415 s vs 424 s at baseline ( $p=0.472$ )	75 rhAT IU/kg (n=27) or placebo (n=27) 2 U FFP upon Rx failure: n=5 (19%) of rhAT group vs n=22 (81%) of placebo group ( $p=0.009$ )	Avidan et al. 2005 (82)

ACT, activated clotting time; AT, antithrombin; CPB, cardiopulmonary bypass; DB, double-blind; FFP, fresh frozen plasma; HR, heparin resistance; IU, international unit; NS, not specified; PC, placebo-controlled; pdAT, plasma-derived AT concentrate; RCT, randomised controlled trial; rhAT, recombinant human AT concentrate; Rx, treatment; s, seconds; SD, standard deviation.

clinical value of AT activity levels as a predictor of MACE, although low activity levels may still be a contributing factor.

AT activity levels  $\leq 60\%$  are associated with a high risk of developing heparin resistance (71). In 1994, Staples et al. (72) defined heparin resistance as a failure of 500 IU/kg heparin to prolong the activated clotting time (ACT) to 480 seconds (s). Since then, various definitions of heparin resistance have been used in studies of AT concentrate for heparin resistance during CPB (► Table 3). Garvin et al. (73) surmised that reduced AT activity would be associated with heparin response. However, they found that AT activity is not associated with impaired response to heparin when using target ACTs of 300 to 350 s. Despite this finding, multiple case reports (74, 75), a retrospective study (76), and prospective clinical trials (77–79) have reported that AT treatment (range, 500–3,400 IU) for heparin resistance during CPB increased ACT to values acceptable for CPB. In a dose-finding study to identify an AT dosage that would result in  $>100\%$  AT activity at the end of cardiac surgery, Dietrich et al. found that the dosage needed to be quite high (approximately 50 IU/kg) to restore normal physiologic AT values (80).

In clinical practice, the question of whether to use fresh frozen plasma (FFP) or AT concentrate in heparin resistant patients during cardiac surgery often arises, because AT concentrate is more expensive than FFP and could put patients at risk for heparin rebound immediately after surgery. A review by Beattie and Jeffrey (81) seems to support the use of AT concentrate over FFP to treat heparin resistance in cardiac surgery. Although FFP may be cheaper, AT concentrate is more efficient at restoring ACT to therapeutic levels with adequate heparinisation and has lower risk for transfusion-related acute lung injury and infections, as well as less intraoperative time delay. Two double-blind, placebo-controlled, randomised trials of rhAT treatment (75 IU/kg) in patients with heparin resistance ( $n=55$ ) reported prolonged ACT values, increased AT activity, and a decreased requirement for administration of FFP or additional heparin compared with placebo-treated patients ( $n=51$ ) (82, 83). An important consideration is that heparin resistance may develop independently of AT deficiency in a substantial subset of patients (35%) (84). Thus, proper management of heparin resistance during CPB must take into account its underlying mechanism (e.g. AT deficiency, hyperactivity of coagulation factors or natural heparin inhibitors). Further studies are needed to determine whether the benefit of AT concentrate or rhAT treatment in resolving heparin resistance translates into positive survival outcomes.

Despite heparin use, microemboli formation still poses a problem during and after CPB surgery (85). Transcranial Doppler monitoring has been used to detect microemboli as high-intensity transient signals (HITS) during CPB. HITS occur despite administration of unfractionated heparin; however, an AT-heparin covalent complex (ATH) – a more potent anticoagulant than heparin – has been shown to reduce HITS during CPB in pigs in a dose-dependent manner. This work supports the hypothesis that ATH reduces emboli formation during CPB and might have the potential for improved clinical care.

Besides decreasing haemostatic activation during CPB, AT concentrate may limit inflammatory conditions related to CPB, such as systemic inflammation and DIC (66). In a prospective study examining the effect of AT concentrate administration on haemostatic and leukocyte activation during CPB, Koster et al. (86) randomised 80 patients to receive pre-CPB anticoagulation with heparin ( $n=40$ ) or heparin plus 50 IU/kg AT concentrate ( $n=40$ ). Blood samples were collected 10 min after anticoagulation and after termination of CPB. Markers of haemostatic activation, including prothrombin activation fragments 1 and 2 (F1+2) and soluble fibrin, and markers of leukocyte activation, including neutrophil elastase and IL-6, were evaluated. Blood samples collected after CPB revealed persistently increased AT activity in patients treated with heparin plus AT concentrate vs heparin alone (98% vs 37%;  $p<0.01$ ), and reduced concentrations of F1+2 (1.4 nmol/l vs 2.6 nmol/l;  $p<0.01$ ), soluble fibrin (11 ng/ml vs 16 ng/ml;  $p=0.02$ ), IL-6 (37 ng/ml vs 198 ng/ml;  $p<0.01$ ) and neutrophil elastase (509 ng/ml vs 756 ng/ml;  $p=0.03$ ).

Similarly, Rinder et al. (87) assessed the effect of AT supplementation on activation of coagulation factors, platelets, and leukocytes during simulated CPB (sCPB). Blood was drawn from healthy volunteers, collected in a transfer pack containing 2 IU/ml heparin (final concentration), and processed in control sCPB runs without AT supplementation ( $n=8$  runs) or with AT supplemented at 1 IU/ml ( $n=5$  runs) or 5 IU/ml ( $n=6$  runs). Low and high doses of AT concentrate significantly increased AT activity ( $p<0.001$ ) and decreased thrombin activity ( $p<0.02$ ) during sCPB vs control runs. High-dose but not low-dose AT supplementation also significantly inhibited thrombin generation ( $p=0.04$ ), reduced platelet activation ( $p<0.01$ ), and blunted CD11b upregulation by monocytes and neutrophils ( $p<0.001$ ). Thus, administration of pharmacologic doses of AT concentrate during sCPB resulted in anti-inflammatory effects on leukocytes in addition to inhibition of platelet activity and coagulation.

Plasma AT levels may severely decrease after CPB with deep hypothermic circulatory arrest (DHCA). This is likely due to increased CPB duration and the greater complexity of surgical procedures performed. In an *in vitro* study, blood samples were obtained from five patients undergoing cardiac surgery with DHCA before heparinisation and the balance between procoagulant and anticoagulant elements was manipulated in the patients' plasma by adding normal donor plasma, AT-deficient plasma, or purified AT (88). Median peak thrombin generation was 56.6 nM (range, 42.1–61.0) in plasma after CPB, and remained at 61.1 nM (range, 54.9–64.5) when donor plasma with normal AT (105%) was added. When AT-deficient plasma was added, peak thrombin generation was increased from 56.6 nM (42.1–61.0) to 117 nM (95.0–188) ( $p<0.05$  vs control). After the addition of purified AT, the peak thrombin generation was reduced to 12.2 nM (9.0–29.3;  $p<0.05$  vs control). These results show that administration of coagulation factor components without AT repletion may lead to excessive thrombin generation which, clinically, may potentially lead to a hypercoagulable state.

## Sepsis and DIC

Sepsis – the leading cause of death in critically ill patients in the United States – is characterised by an uncontrolled systemic inflammatory response (89). In addition, severe sepsis invariably results in pathophysiologic coagulation activity such as DIC, which is characterised by widespread microvascular thrombosis and profuse bleeding (90). The pathophysiology of septic coagulopathy is attributable to immunothrombosis – the process in which thrombi formation and subsequent endothelial cell damage can lead to systemic microcirculatory damage, multiple organ failure, and ultimately death (91). At the onset of infection, proinflammatory cytokines induce endothelial and tissue damage, causing the release of additional cytokines (92). Lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, induces inflammation and coagulation and is a common mediator of sepsis. Souther et al. (92) investigated the *in vitro* ability of treatment with AT concentrate to suppress LPS-induced production of proinflammatory and coagulant agents. Three different cellular systems – whole blood, mononuclear peripheral blood cells, and human umbilical vein endothelial cells – were stimulated with LPS for 4 to 6 h in the presence of AT concentrate (0–40 IU/ml). All three systems demonstrated a dose-dependent reduction in the production of IL-6 and TF. Interestingly, the specific thrombin inhibitor *hirudin* failed to suppress LPS-induced production of IL-6 and TF, suggesting that AT affects other inflammatory and coagulant mechanisms in addition to its inhibitory effect on thrombin.

A mechanism has been suggested by Komura et al. (93) whereby AT might inhibit LPS-induced TNF- $\alpha$  production by inhibiting the increase in Egr-1 expression in monocytes. AT (5 IU/ml) was applied to human peripheral monocytes stimulated with LPS *in vitro*. Chemically modified AT, lacking affinity for heparin, did not inhibit the LPS-induced increase in TNF- $\alpha$  production, suggesting that AT interacts with heparin-like substances expressed on cell surfaces (93). Using intravital fluorescent microscopy to visualise venules in an LPS-induced sepsis model, Iba et al. (91) showed leukocyte-platelet conjugates accumulating on damaged endothelial cells in the presence of components from neutrophil extracellular traps (NETs). It is proposed that immunothrombosis formation involves neutrophils, in collaboration with platelets, secreting TF via neutrophil microparticles and expelling NETs to activate the coagulation cascade. In addition, neutrophil-derived granule proteins (i.e. neutrophil elastase) can inhibit TFPI and anticoagulants (i.e. activated protein C, AT) to drive thrombus formation out of control even further.

AT levels are diminished in patients with sepsis as a result of consumption because of continuing thrombin generation, degradation by enzymes released from neutrophils, removal from the circulation due to capillary leakage, and impaired synthesis because of liver failure (90). AT levels may fall to 30% of normal in patients with severe sepsis, and such low levels may contribute to higher mortality rates in these patients. Multiple clinical studies have been carried out to address this issue and are summarised in ► Table 4.

The theory of AT levels as a predictor of outcome in septic shock patients was first suggested in the 1980s. Consequently, Sakr et al. (94) tested the hypothesis that AT levels may be associated with morbidity and increased mortality rates. In a cohort of 327 consecutive surgical ICU patients, AT levels were below the lower limit of normal in 84.1% (n=275), regardless of sepsis. In those without sepsis, levels increased significantly by 48 h after admission to reach normal values by the seventh ICU day (n=208). However, this increase in AT levels was delayed in patients with sepsis, although associated with the degree of organ dysfunction and the severity of sepsis. Interestingly, there was no independent association between AT levels and worse outcome. Another study by Choi et al. emphasised the importance of anticoagulants in DIC by demonstrating that AT and protein C have significant prognostic value in clinical practice (95). This large study (n=1,846) showed the prognostic values of AT and protein C were higher in patients with sepsis/severe infections than those with other underlying diseases, such as solid tumour, haematologic malignancy and liver disease. Moreover, patients with low AT levels ( $\leq 63\%$ , n=669) exhibited a lower cumulative survival rate compared to those with high AT levels ( $\geq 63\%$ , n=1,177) ( $p < 0.001$ ). A recent study by Hjørleifsson et al. (96), conducted in Iceland, confirmed that reduced AT and protein C activity levels were predictive of upfront mortality risk in critically ill patients (n=1,814) suspected of acute DIC. The lower the AT and PC levels, the higher 28-day and one-year mortality occurred. These results were observed across the spectrum of overt International Society on Thrombosis and Haemostasis (ISTH) score and not only in patients who fulfilled overt DIC diagnostic criteria, suggesting that patients who do not fulfill these criteria should not be dismissed as such action may lead to the failure in identifying many acutely ill patients who may be at substantial risk of death.

A retrospective analysis of patients treated with AT concentrate showed that the severity of sepsis and liver dysfunction were independent predictors for the response to AT activity (97). The study included 42 patients with sepsis, 75 with severe sepsis, and 65 with septic shock. In patients with septic shock, the AT response after supplementation was  $0.37\% \pm 1.21\%$  per IU/kg body weight, which was significantly lower than in patients with severe sepsis ( $1.36 \pm 1.65$ ;  $p < 0.001$ ) and sepsis ( $1.81 \pm 1.75$ ;  $p < 0.001$ ). Patients with liver dysfunction had a significantly lower response to AT supplementation than patients without liver dysfunction ( $p < 0.0001$ ). This study suggested that the response to AT supplementation may be affected by both a systemic inflammation and impaired synthesis in patients with sepsis (97).

In a meta-analysis of 20 clinical trials including 3,458 patients with critical illnesses (e.g. sepsis, septic shock, DIC), Afshari et al. (98) reported no significant effect of treatment with AT concentrate on mortality, with 667 (39%) deaths in the AT concentrate group and 699 (40%) deaths in the control group. Representing a relative weight of 80% of the data included in the meta-analysis, the KyberSept trial (99) randomised 2,314 patients with severe sepsis to receive intravenous high-dose AT concentrate (30,000 IU over 4 days) or placebo and reported no difference between groups in 28-day mortality. This overall finding of no treatment effect of

Table 4: Outcomes of clinical studies of AT concentrate treatment in patients with sepsis.

AT activity level at baseline	Study design	AT concentrate Rx	Outcomes	Mortality	Reference
<60 % of normal levels in >50 % of patients	KyberSept trial: double-blind RCT phase 3 trial in patients (n=2,314) with severe sepsis	30,000 IU pdAT over 4 days (n=1,157, including n=698 not receiving concomitant heparin) vs placebo (n=1,157)	AT concentrate Rx resulted in an increase of mean AT activity levels by 115 % on average to approximately 180 % of normal; AT activity levels were unchanged in placebo group	Significant difference in 90-day mortality between AT concentrate Rx in patients not receiving concomitant heparin vs placebo group (44.9 % vs 52.5 %; p=0.03) No difference in: <ul style="list-style-type: none"> <li>• 28-day all-cause mortality between AT concentrate Rx group vs placebo group (38.9 % vs 38.7 %; p=0.94)</li> <li>• 56- and 90-day mortality between AT concentrate Rx group vs placebo group</li> <li>• 28-day mortality in AT concentrate Rx patients not receiving concomitant heparin vs placebo group (37.8 % vs 43.6 %; p=0.08)</li> </ul>	Warren et al. 2001 (99)
No difference in % patients with baseline AT activity levels <60 % across groups	Subanalysis of KyberSept trial in patients with DIC (n=229) vs no DIC (n=334)	30,000 IU pdAT over 4 days (n=286) vs placebo (n=277)	Major bleeding rates in Rx group were higher in patients with no DIC vs placebo (9.8 % vs 3.1 %, p<0.02)	For 28-day mortality: <ul style="list-style-type: none"> <li>• DIC in placebo group associated with excess risk (40.0 % vs 22.2 %, p&lt;0.01)</li> <li>• Rx group had absolute risk reduction of 14.6 % vs placebo (p=0.02)</li> <li>• No effect in patients with no DIC</li> </ul>	Kienast et al. 2006 (101)
<60 % in all grps	Subanalysis of KyberSept trial in patients with a SAPS II-predicted mortality rate of 30 %–60 %	30,000 IU pdAT over 4 days (n=490) vs placebo (n=518) Subgroups not treated with heparin: AT concentrate (n=140) vs placebo (n=162)	Although AT concentrate Rx was associated with more “any bleeding” than placebo, survival rates were also higher with AT administration in patients with and without bleeding complications	AT concentrate group had higher survival time for up to 90 days compared to placebo group (p=0.04) Treatment effect was even more pronounced without concomitant heparin (AT concentrate vs placebo, respectively): <ul style="list-style-type: none"> <li>• 28-day mortality rate: 35.7 % vs 44.4 %</li> <li>• 56-day mortality rate, 39.9 % vs 52.2 %</li> <li>• 90-day mortality rate, 42.8 % vs 55.1 %</li> </ul>	Weidermann et al. 2006 (102)
-	Meta-analysis of 20 clinical trials (n=3,458)	Single bolus to 14 days of administration	AT concentrate Rx was not associated with incidence of respiratory failure, duration of mechanical ventilation, need for surgical intervention, length of hospital or ICU unit stay. AT concentrate Rx was associated with significantly increased risk of bleeding events	AT concentrate Rx was not associated with reduction in overall mortality. Control group mortality: 599 (40 %) vs AT concentrate group mortality: 667 (39 %)	Afshari et al. 2007 (69, 98)
No sepsis group: 62.6 % ± 17.5 %, sepsis group: 65.7 % ± 20.0 %, severe sepsis group: 53.0 % ± 18.9 %* (*p<0.05 compared with no sepsis group)	327 surgical ICU patients: no sepsis (n=208), sepsis (n=64), severe sepsis (n=55)	NS	AT activity levels were associated with: <ul style="list-style-type: none"> <li>• Sepsis severity: Delayed increase in AT activity levels in sepsis group vs no sepsis group with mean levels staying below LLN. Consistently low levels in severe sepsis group</li> <li>• Degree of organ dysfunction</li> </ul> AT activity levels were not associated with worse outcome	AT levels were not associated with increased mortality. Overall rate: 15.0 % (n=49: 29 with severe sepsis, 5 with sepsis, 15 with no sepsis)	Sakr et al. 2007 (94)
Sepsis group: 59.7 % ± 19.4 %, severe sepsis group: 58.3 % ± 12.7 %, septic shock group: 55.5 % ± 14.7 % (p=0.275 among groups)	Retrospective analysis of patients with sepsis (n=42), severe sepsis (n=75), septic shock (n=65)	pdAT dose administered (IU/kg): <ul style="list-style-type: none"> <li>• Sepsis group: 32.5 ± 12.9</li> <li>• Severe sepsis group: 31.7 ± 9.7</li> <li>• Septic shock group: 29.4 ± 11.3 (p=0.085 among groups)</li> </ul>	AT response after AT concentrate Rx decreased in proportion to sepsis severity (p<0.0001) Patients with liver dysfunction had significantly lower AT responses after Rx than those without (p<0.0001) DIC frequency increased in proportion to sepsis severity (p<0.05) Patients with DIC had significantly lower response to AT Rx than those without (p=0.041)	28-day survival rate associated with sepsis severity; no sepsis group survival was higher than severe sepsis or septic shock groups (p<0.01)	Hayakawa et al. 2008 (97)

Table 4: continued

AT activity level at baseline	Study design	AT concentrate Rx	Outcomes	Mortality	Reference
NS	Post-hoc results of the KyberSept trial (n=81) in patients with severe sepsis treated early after new onset organ failure	30,000 IU pdAT over 4 days (n=40) vs placebo (n=41)	When AT concentrate Rx was given early, absolute risk reduction at 28 days for organ failure was 21 % (p=ns) Significant increased bleeding incidence 8/40 (20 %) for AT concentrate group vs 1/41 (2.4 %) for placebo group (p<0.015)	Absolute risk reduction at 28 days was 14 % (p=ns) when AT concentrate was given early	Eid et al. 2008 (100)
≤ 70 % in all patients; % of patients with initial AT level < 50 % was lower in AT3000 group (69.6 %) than in AT1500 group (48.2 %, p<0.01)	Prospective, nonrandomised multicentre survey of septic DIC patients (n=729)	1,500 IU/day (n=79, AT1500 group) or 3,000 IU/day (n=650, AT3000 group) AT concentrate for 3 consecutive days	Bleeding events occurred in 6.52 % of all patients, with 1.71 % being severe bleeding; there was no difference in bleeding rates with or without heparin AT activities increased to > 80 % in AT3000 group on day 2, but the AT1500 group did not achieve normal range DIC resolution rate (JAAM DIC score < 4) on day 7 was significantly higher in AT3000 group than for AT1500 group (69.6 % vs 55.4 %; p<0.05)	Significant factors for enhanced 28-day survival were higher initial AT activity (p<0.001), higher AT dose (p=0.026), and younger age (p=0.023) Survival was higher in AT3000 group (74.7 %) than AT1500 group (65.2 %); however, there was no difference between patients who received heparin (62.7 %) and those who did not (67.5 %) Survival improved significantly when AT activity recovered to ≥ 70 % on day 4 (OR: 1.034, p<0.001)	Iba et al. 2012 (107)
No difference in % patients with AT levels of 50 %–80 %	Multicentre, open-label, randomised clinical trial in patients with sepsis and JAAM DIC score ≥ 4 (n=60)	30 IU/kg/day AT over 3 days (n=30) vs control group with no intervention (n=30)	AT concentrate group exhibited: <ul style="list-style-type: none"> <li>Higher mean day-3 AT level of 107 ± 24.5 % (p&lt;0.001)</li> <li>Significantly lower day-3 DIC scores, especially ISTH overt DIC scores, vs those in control group (p=0.021)</li> <li>&gt; 2-fold higher DIC recovery rate of 53.3 % compared to 20.0 % in control group (p=0.015)</li> </ul> No difference between the 2 groups in: <ul style="list-style-type: none"> <li>Bleeding complications</li> <li>Day-3 SOFA score or coagulation and fibrinolysis markers</li> </ul>	No difference in 28-day and hospital mortality rates between the 2 groups	Gando et al. 2013 (109)
NS	Retrospective, nationwide database study of patients with sepsis-associated DIC following severe pneumonia (n=9075).	Patients were categorised into AT Rx group (n=2,663) and control group (n=6,412); propensity score matching yielded 2,194 pairs of patients with and without AT use	Significantly shorter ventilation periods found in the AT group than control group, respectively: <ul style="list-style-type: none"> <li>Unmatched groups: 17.9 vs 20.6 days; difference, 2.7 (p=0.001)</li> <li>Propensity score-matched groups: 17.8 vs 20.5 days; difference, 2.7 (p=0.009)</li> <li>Inverse probability-weighted analysis: 18.3 vs 20.4; difference, 2.1 (p&lt;0.001)</li> </ul>	AT Rx was associated with higher survival rate. There were significant differences in mortality rates of AT vs control groups, respectively: <ul style="list-style-type: none"> <li>Unmatched cohort: 40.8 % vs 45.7 % (p&lt;0.001)</li> <li>Propensity-matched: 40.6 % vs 44.2 % (p=0.02)</li> <li>Inverse probability-weighted: 41.1 % vs 45.1 % (p&lt;0.001)</li> <li>In-hospital mortality: 51.3 % vs 60.7 % (p&lt;0.001)</li> </ul> Receipt of AT was associated with reduction in 28-day mortality by 9.9 %	Tagami et al. 2014 (110)
< 40 %	Nonrandomised multicentre survey	1500 IU/day (n=259, AT1500 group) or 3000 IU/day (n=48, AT3000 group) AT concentrate for 3 consecutive days	AT concentrate Rx significantly decreased DIC score and to the enhanced DIC recovery in the AT3000 group compared with the AT1500 group (66.7 % vs 45.2 %, p=0.007) Bleeding events, including severe bleeding, were similar between the 2 groups	AT3000 group had higher survival compared to AT1500 group (77.1 % vs 56.4 %, p=0.010) Significant factors for improved survival were higher AT dose (OR, 2.419; p=0.025), higher initial platelet count (OR, 1.054; p=0.027), and younger age (OR, 0.977; p=0.045)	Iba et al. 2014 (112)

AT, antithrombin; DIC, disseminated intravascular coagulation; ICU, intensive care unit; IU, international unit; ISTH, International Society on Thrombosis and Haemostasis; JAAM, Japanese Association for Acute Medicine; LLN, lower limit of normal; NS, not specified; ns, not significant; OR, odds ratio; pdAT, plasma-derived AT concentrate; RCT, randomised controlled trial; Rx, treatment; SAPS II, Simplified Acute Physiology Score II; SOFA, sequential organ failure assessment.

**Table 5: Clinical situations in which antithrombin administration may be considered.**

Congenital AT deficiency
Correction of hereditary antithrombin deficiency in connection with major surgical or obstetrical procedures or when they suffer from thromboembolism
Acquired AT deficiency (off-label use)
DIC associated with sepsis, trauma, burns, complicated pregnancy (and withhold heparin)
Acquired heparin resistance for cardiopulmonary bypass
Thrombosis with low AT activity levels (< 50 %–60 %) and resistance to heparin
Acute thromboembolism during treatment with L-asparaginase
Extracorporeal membrane oxygenation with low AT activity levels (< 50 %–60 %)
Thrombosis of the hepatic artery following orthotopic liver transplantation
Veno-occlusive disease following stem cell transplantation
AT, antithrombin.

high-dose AT concentrate in severe sepsis was inconsistent for the primary outcome, 28-day mortality, possibly because of patient heterogeneity. Post-hoc results of the KyberSept trial from patients (placebo: n=41; AT concentrate: n=40) with severe sepsis treated at a single centre early after new onset organ failure showed that, when AT concentrate was given early in severe sepsis, absolute risk reductions at 28 days were 21 % and 14 % for organ failure and

mortality, respectively (100). These reductions were not statistically significant, but they may indicate a potential for treatment benefit in selected sepsis patients and could have implications for continuing sepsis trials with AT concentrate that feature reduced patient heterogeneity (100).

A subanalysis of 229 patients with DIC from the KyberSept trial reported a significantly reduced 28-day mortality rate in patients who received AT concentrate without adjuvant heparin (n=114) vs matched controls (n=115; 25 % vs 40 %, respectively; p=0.02) (101). Another subanalysis of the KyberSept trial demonstrated that AT concentrate (n=490), compared to placebo (n=518), had a statistically significant decrease in the absolute risk of all-cause mortality (6.1 %; p=0.04) for up to 90 days after admission in DIC patients who did not receive concomitant heparin treatment and had a high predicted mortality rate (30 % to 60 %) (102). Similarly, the meta-analysis by Afshari et al. (98) reported significantly lower mortality in the subgroup of critically ill patients who received AT concentrate without heparin (n=563) compared with matched controls (n=560; 36 % vs 41 %, respectively; p=0.04). A systematic review of AT administration also showed that AT may improve overall survival in patients with DIC and severe sepsis (103).

Data from subanalyses of the KyberSept trial are supported by experimental evidence from studies by Sorg et al. (104), which investigated AT concentrate as a potential antithrombotic monotherapy for microvascular thrombosis formation under septic conditions. Using an experimental endotoxemia model, they showed that AT concentrate could effectively prevent thrombotic microvascular occlusion when given without heparin. This suggests a promising strategy for AT concentrate therapy, which needs to be

**Table 6: Dosing regimen for AT concentrates.**

	Surgical Dosing	Obstetrical Dosing
Plasma-derived AT concentrate (31)		
Initial dose (in IU)	$[(\text{desired \% AT activity} - \text{baseline \% AT activity}) \times \text{body weight in kilograms}] \div 1.4$ Infuse intravenously over 10–20 minutes	$[(\text{desired \% AT activity} - \text{baseline \% AT activity}) \times \text{body weight in kilograms}] \div 1.4$ Infuse intravenously over 10–20 minutes
Maintenance dose (in IU)	Approximately 60 % of the initial dose, given every 24 hours	
Dose adjustments	Adjust dose/interval to maintain AT activity levels of 80 %–120 % Measure plasma AT activity levels 20 minutes postinfusion of initial dose, after 12 hours, and before the next infusion	
Recombinant AT concentrate (33)		
Initial dose (in IU)	$[(100 - \text{baseline \% AT activity}) \div 2.3] \times \text{body weight in kilograms}$ Administer initial dose as a 15-minute intravenous infusion immediately followed by continuous infusion of the maintenance dose	$[(100 - \text{baseline \% AT activity}) \div 1.3] \times \text{body weight in kilograms}$ Administer initial dose as a 15-minute intravenous infusion immediately followed by continuous infusion of the maintenance dose
Maintenance dose (in IU)	$[(100 - \text{baseline \% AT activity}) \div 10.2] \times \text{body weight in kilograms, given per hour}$	$[(100 - \text{baseline \% AT activity}) \div 5.4] \times \text{body weight in kilograms, given per hour}$
Dose adjustments	Adjust based on the % AT activity level 2 hours after initial treatment For AT activity level < 80 %, increase dose 30 % and recheck 2 hours after dose adjustment For AT activity level 80 %–120 %, do not adjust dose and recheck 6 hours after last dose For AT activity level > 120 %, decrease dose 30 % and recheck 2 hours after dose adjustment	
AT, antithrombin; IU, international units.		

tested in clinical conditions. Sun et al. (105) also suggested that AT concentrate should be administered alone, and not with concomitant heparin, to patients with acute lung injury (ALI) and sepsis. Their studies implicated AT in the inhibition of ERK1/2 and P38 MAPK phosphorylation in lung tissues in mouse and rat models, where AT was shown to down-regulate the levels of downstream cytokines TNF- $\alpha$  and IL-6, relieve endothelial permeability, and improve endotoxin-induced ALI. However, use of AT with concomitant heparin yielded no improvement.

Based on the above findings, the 2010 guidelines in Japan recommended AT concentrate for the treatment of sepsis-related DIC, and AT are currently used widely in Japanese clinical practice (106, 107). In efforts to harmonise three guidelines for DIC from Britain, Japan, and Italy, the 2013 guidance of the ISTH recommended AT administration “may be considered” in patients with DIC (108).

Research on DIC in Japan has been robust in recent years. In a small randomised, controlled, multicentre trial of patients (N=60) with sepsis-related DIC and AT levels of 50% to 80%, Gando et al. (109) found that AT concentrate (30 IU/kg per day for 3 days) improved DIC scores, and hence DIC recovery rate, without increasing bleeding risk. Unfortunately, this study was not powered sufficiently to show statistical significance in mortality data. In efforts to demonstrate the efficacy of AT substitution in decreasing the mortality of patient with sepsis-related DIC, Tagami et al. (110) conducted a large, nationwide, retrospective study of 9,075 patients with severe pneumonia and sepsis-related DIC from 1,114 hospitals. Data comparing the the control group vs AT-treated group showed that AT administration significantly reduced 28-day mortality (45.7% vs 40.8%, respectively;  $p < 0.001$ ), in-hospital mortality (60.7% vs 51.3%, respectively;  $p < 0.001$ ), and ventilation periods (20.6 vs 17.9 days, respectively;  $p = 0.001$ ). In addition, Tagami et al. recently reported on a retrospective cohort study of 518 propensity-matched patient pairs with sepsis-associated DIC originating from emergency abdominal laparotomy from the Japanese Diagnosis Procedure Combination inpatient database (111). Their analysis found statistically significant association (odds ratio [OR], 0.65; 95% CI, 0.49–0.87) between AT supplementation and lower 28-day mortality for patients who did not or did receive AT treatment (27.6% vs 19.9%, respectively). Interestingly, they also demonstrated that hospital AT-prescribing rate was associated with a 6.5% reduction in 28-day mortality.

While the potential benefits of treatment with AT concentrate in sepsis and DIC are becoming more favourable, the optimal dosage for enhancing survival without causing excessive bleeding has not been clearly understood. Perhaps similar to the high AT dosage (50 IU/kg body weight) needed to maintain physiologic AT levels during cardiac surgery (80), a high dose of AT may also be required for sepsis and DIC. Two studies by Iba et al. attempted to elucidate this issue (107, 112). The first study published in 2012 showed that septic DIC patients (AT activity  $< 70\%$ ) who were treated with an AT dose equivalent to  $\sim 54$  IU/kg per day (3,000 IU over 3 days for a mean body weight of 55.7 kg) survived better than those treated with  $\sim 28$  IU/kg per day (1,500 IU over 3 days for a mean body weight of 53.1 kg) (74.7% vs 62.5%, respectively).

Stepwise logistic-regression analysis showed an increase of AT activity level on day 4 also contributed to longer survival (OR=1.034;  $p < 0.001$ ). The second study of septic DIC patients with AT activity level  $< 40\%$  further confirmed that an AT dose equivalent to  $\sim 56$  IU/kg per day (3,000 IU over 3 days for a mean body weight of 52.7 kg) exhibited better survival rate than those treated with  $\sim 30$  IU/kg per day (1,500 IU over 3 days for a mean body weight of 52.3 kg) (77.1% vs 56.4%, respectively;  $p = 0.01$ ) without an increased risk of bleeding.

While Seam and Suffredini (113) made valid points that these results are limited by the retrospective and nonrandomised nature of the study, it should also be recognised that AT is potentially effective for treating DIC and improving survival without increasing the risk of bleeding.

Of note, a recent publication by Iba and colleagues (114) further examined clinical characteristics required to determine dosing decisions between 1,500 IU/day and 3,000 IU/day. Results from this retrospective study of 926 patients with sepsis-related DIC suggested that AT supplementation at 1,500 IU/day may be sufficient in some cases, but the AT dosage would need to be increased if baseline AT activity reduction is moderate or severe. Monitoring AT activity level and DIC score during AT supplementation therapy was helpful in predicting patient outcomes. The authors concluded that achieving a target AT activity of  $> 70\%$  provided sufficient AT efficacy to patients regardless of whether they had received 1,500 IU/day or 3,000 IU/day. Furthermore, patient outcomes were statistically significantly associated with patient age, baseline AT activity, change in AT activity, baseline DIC score, and change in DIC score, as the cut-off values of AT activities for death were 41.3% for baseline AT activity, 72.9% for posttreatment AT activity, and 37% for change in AT activity.

Even though the cumulative body of evidence on AT efficacy and safety profile has been more favourable in recent years, optimal AT dosing and target patient selection for AT therapy remain controversial topics and need further investigation.

## Guidance for AT replacement and monitoring

The approach to AT administration depends on the disease context of each patient. In ► Table 5, we outline certain clinical situations in which AT administration may be considered. It is important to emphasise that our guidance is not data-driven, as there is a paucity of information above and beyond what is available for congenital AT deficiency. However, certain recommendations have been made more specifically for patients undergoing ECMO as follows.

For paediatric patients, the recent Extracorporeal Life Support Organisation (ELSO) Anticoagulation Guideline for the management of anticoagulation in ECMO patients recommended on-demand measurement of AT concentration in case of heparin resistance (115). These high-risk and critically ill patients usually receive large volumes of plasma in the ECLS prime, which may increase plasma AT concentration, and they frequently develop AT deficiency likely as a result of exposure to long-term anticoagu-

lation with heparin. Most ECLS programs that routinely administer AT replacement as part of their anticoagulation protocols target AT activity levels of 50% to 100%; while others recommend an AT level of >80% for neonates and >100% for infants and children (115).

For the rest of the patient populations mentioned in this review, the goals of AT replacement are to elevate and maintain the AT activity of an individual patient at 80% to 120% of the normal AT activity level (approximately 2.57  $\mu$ M or 0.125 mg/ml to 0.160 mg/ml) (5–7, 31, 33). The dosing regimens and monitoring of AT concentrates are summarised in ► Table 6. It is important to recognise that pdAT and rhAT concentrates have distinct pharmacokinetics and require different protocols for administration and monitoring. Plasma-derived AT concentrate is typically given as an intravenous infusion over 10 to 20 min followed by initial monitoring at least every 12 h after the initial dose, as well as before the next maintenance dose (approximately 60% of the initial dose) (31). Due to its long elimination half-life (2.6 to 3.8 days), the maintenance dose of pdAT is administered every 24 h (10). In contrast, the half-life of rhAT is nine-fold shorter (11.6 to 17.7 h) than that of pdAT; its clearance is also seven-fold faster (33). Thus, rhAT needs to be given intravenously as a 15-min initial infusion immediately followed by continuous infusion of the maintenance dose, which is calculated based on a different formula from the initial dose and given hourly. To avoid excessive or inadequate effectiveness of rhAT, coagulation tests should be performed at regular, close intervals, especially in the first hour of treatment start or termination. Regardless of the type of AT concentration used, it may be necessary to monitor patients more frequently in certain situations, such as concomitant heparin administration, acute thrombosis, or haemorrhage, as the typical half-life of AT may be reduced by these events. Physicians should also keep in mind that the risk of bleeding exists when AT concentrates are used with heparin, especially in the various disease settings (82, 116). All patients should be monitored appropriately.

There are two types of assays to monitor AT levels: functional assays for assessment of activity level and immunoassays for quantification of protein concentration (117). Chromogenic amidolytic methods are the predominant commercially available AT activity assays. To assess AT activity level, functional assays measure the ability of heparin to inhibit thrombin or factor Xa. Assays based on factor Xa inhibition may be more reliable than thrombin-based assays due to the high specificity of factor Xa inhibition for AT deficiency (118). The exception to this preference is when patients have AT Cambridge (AT A384S) mutation, which cannot be detected well by neither factor Xa-based nor thrombin-based assays (119). Typical methods for assessing AT protein concentrations are automated immuno-turbidimetric methods and enzyme-linked immunosorbent assays (ELISAs). If one wishes to determine whether the AT functional defect is caused by an abnormality at the AT-thrombin binding site or at the AT-heparin binding site, then additional testing can be done with progressive AT activity assays, which measures the extent of thrombin neutralisation by AT in the absence of heparin. As acquired causes of low AT are more common than congenital de-

fects, it is important to measure AT activity levels with functional assays first and exclude aetiological causes of AT deficiency before pursuing the congenital AT deficiency diagnosis. Laboratory tests (i.e. liver function tests, DIC screen, urinalysis) can also help determine some of the acquired causes of low AT activity levels. Other methods, such as DNA testing, are not available outside of research settings.

## Future research

Although results are promising for AT, further studies are needed to better assess the cost-effectiveness of AT supplementation in different clinical settings. AT monitoring and supplementation might certainly be useful and cost-effective in some specific situations described by this narrative. AT has recently been found to have broad-spectrum antiviral activity against HIV-1, HCV and HSV (120). Multiple host-cell signal transduction pathways have been shown to be activated by AT in cells infected with HIV-1, with the most up-regulated transcript being prostaglandin synthetase-2. These findings suggest a central role for AT in the host innate antiviral response (120). This provides a potential new application for AT and, along with its previously mentioned antiangiogenic and antitumor activities, new avenues for further research.

## Conclusions

Hereditary AT deficiency is associated with hypercoagulable states for which anticoagulation is the primary treatment goal; AT supplementation has already been demonstrated to improve patient outcomes, particularly in the obstetrical and surgical settings. Acquired AT deficiency, being more common and more complex, often results in thrombotic states associated with an inflammatory component as well. Growing evidence that AT possesses noteworthy anti-inflammatory properties in addition to its anticoagulation activity suggests that AT concentrate may have potential therapeutic applications in certain clinical settings associated with inflammation.

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## Conflicts of interest

JHL serves on steering committees for Boehringer Ingelheim, CSL Behring, Grifols, Instrumentation Labs, Janssen. RMS serves on an advisory board for Grifols and has received research funding from Grifols and Shire Viropharma. IJW has received funding for investigator-initiated studies from Terumo BCT and CSL Behring. ML declares no conflicts of interest.

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