Thrombosis and Cardiopulmonary Bypass
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"Ideas do not have to be correct in order to be good; it’s only necessary that, if they do fail, they do so in an interesting way.”  Robert Rosen

We face a profound and irreconcilable tension in the OR that is as old as the first use of heparin for cardiopulmonary bypass (CPB), 56 years ago.  Our patients are treated with antiplatelet and anticoagulant drugs prior to surgery, with markedly improved short-term survival after MI and overall long-term survival.  The “Wood” and “This Old House” magazines I receive every month contain 2 page ads for Plavix. Yet when I step into the OR in the morning, I am constantly reminded by my colleagues that the patient had Plavix yesterday and so I better order some platelets.  By contrast, I am constantly reminded how we should not be transfusing patients, as blood products are bad.  Within a few days after surgery the patient is back on Plavix for prevention of thrombosis of his coated stents.  In essence, we crave perfect platelet and coagulation function for about 24 hours after completion of surgery, in the face of deliberate anticoagulation, platelet inhibition and depletion of all components of the coagulation system by surgery and CPB.

Endogenous Causes of Perioperative Thrombosis

Being an aspiring geneticist, I will concentrate on some genetic causes of thrombosis.  It may be the larger at-risk group overall, compared to iatrogenic causes. Even if it is not, it is more interesting to me. However, that comparison of group size is somewhat fallacious, as Virchow implied, well before anyone had thought of the environment modifying genetic effects. Thrombosis is somewhat analogous to the Swiss cheese theory of adverse events. Many holes have to line up before bad things happen. One of those holes may be a genetic propensity to thrombosis.

In the Caucasian population, the rate of deficiencies of the endogenous anticoagulants (antithrombin, Protein C, Protein S) and ligands of these anticoagulants (Factor V Leiden) is moderate. In addition, the immune production of antigenic causes of thrombus formation (heparin-induced thrombocytopenia, cold agglutinins) is also moderate. For the latter, there are no identified genetic causes; however, they have several hallmarks of genetic mechanisms occurring in response to an environmental stimulus; namely, their sporadic nature and variable response.

Antithrombin deficiency

Antithrombin (AT) is a serine protease inhibitor (serpin) and the principal inhibitor of the final common pathway of the coagulation system, by inactivation of circulating thrombin (Factor IIa) as well as Factor Xa, amongst other serine proteases of the contact activation pathway (intrinsic pathway), kallikrein, plasmin and the C1s complement protein.  AT also inactivates the activated form of Factor VII (VIIa) from the tissue factor pathway (extrinsic pathway).  Serpin inactivation results from AT binding, so that the serpin’s protease activity is lost because the substrate can no longer bind. In the presence of heparin, AT activity is increased 2000-4000 fold due to a conformational change in the quaternary structure of AT by heparin binding and also formation of a ternary complex of thrombin, AT and heparin.

Inherited antithrombin deficiency is rare (1:2000) but is usually associated with low AT function (<50% of normal). By contrast acquired AT deficiency is common in hospitalized populations, but is usually not severe. AT levels are decreased after administration of heparin due to degradation of the ternary complex. Additionally, acquired AT deficiency is common in hospitalized patients, principally in neonates, and adult patients with critical illness, severe hepatic dysfunction and after cardiac or other major surgery.(1,2) The magnitude of reduction in AT after cardiac surgery is similar to that in patients with heterozygous AT deficiency, which is
associated with increased risk of thromboembolic events.(3,4) After cardiac surgery, lower levels of AT have been independently associated with prolonged intensive care unit (ICU) stay and a higher incidence of neurologic and thromboembolic events.(5)

Recently, we examined for an association between perioperative AT levels and activity, and the frequency of postoperative major adverse cardiac events (MACE), in patients undergoing coronary artery bypass graft (CABG) surgery. The study cohort (n = 1,403) underwent primary CABG surgery with CPB. Demographic data, past medical history, medications and laboratory tests were recorded. The primary clinical end point was occurrence of MACE, defined as a composite outcome of any one or more of: operative death, reoperation for coronary graft occlusion, myocardial infarction, stroke, pulmonary embolism, or cardiac arrest until first hospital discharge. Plasma AT activity was measured using a colorimetric method (Siemens Healthcare Diagnostics, Tarrytown, NY) prior to induction of general anesthesia, five minutes after post-CPB protamine, and on postoperative days 1-5. Multivariate logistic regression modeling was performed to estimate the independent effect of perioperative AT activity upon MACE.

Table 1. Postoperative changes in Antithrombin activity and content in patients with MACE (n=146) and without MACE (n=1257): Results of univariate analyses at each sampling time

<table>
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<th>Antithrombin III Activity (IU/mL)</th>
<th>Postoperative day</th>
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<tr>
<td></td>
<td>Baseline</td>
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<tr>
<td>MACE</td>
<td>0.91±0.13</td>
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<tr>
<td>No MACE</td>
<td>0.92±0.13</td>
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MACE occurred in 146 patients (10.4%), consisting of postoperative mortality (N=12), MI (N=108), stroke (N=17), pulmonary embolism (N=8), cardiac arrest (N=16) or a subsequent operative or catheter-based treatment for graft occlusion (N=6). AT activity at baseline did not differ between patients with (0.91±0.13 IU/mL; n=146) and without (0.92±0.13 IU/mL; n=1257) (P=0.18) MACE. Similarly, postoperative AT levels did not significantly differ in patients with and without MACE, with AT activity in both groups being markedly reduced immediately post-CPB and recovering over the ensuing 5 postoperative days to baseline values (Figure 2). MACE was associated with AT activity, but only on POD 2 and beyond. So, although there is the statistical appearance of an association between low AT levels and MACE, the temporal sequence of events belies it. I interpret these results as saying no matter how good (or bad) a statistical job I have done, the relationship cannot be assumed to be mediated by AT; rather the effect of more extensive surgery, longer CPB time, greater hemodilution and more “unwellness” results in lower AT levels and, separately, a greater incidence of MACE. Others will argue with that.

**Protein C and Factor V**

Protein C is a vitamin K-dependent anticoagulant, that is activated by thrombin and degrades Factor Va and Factor VIIIa, thus inhibiting further thrombin production.

Protein C deficiency is a rare genetic disorder that predisposes to venous thrombosis and habitual abortion. Activated protein C resistance is the inability of protein C to cleave factors V and/or VIII. This may be hereditary or acquired. The best known and most common hereditary form is Factor V Leiden.
**Factor V Leiden**

Normal Factor Va and its cofactor, Factor Xa, are the first members of the final common pathway or thrombin pathway and combine to form the prothrombinase complex. The prothrombinase complex catalyzes the conversion of prothrombin (Factor II), to thrombin (Factor IIa). To produce thrombin, the prothrombinase complex cleaves two peptide bonds in prothrombin, one after Arg^{271} and the other after Arg^{320}. As previously mentioned, activated protein C (aPC) cleaves and degrades factor Va.

Factor V Leiden is a single nucleotide polymorphism (SNP) (rs6025) in exon 10 that results in a Factor V variant which cannot be as easily degraded by aPC. The nucleotide variant (G1691A) results in conversion of an arginine to a glycine (Arg506Gln). This amino acid is normally the cleavage site for aPC and the protein change markedly reduces the activity of aPC on Factor Va. When factor Va remains active, it facilitates overproduction of thrombin leading to excess fibrin generation and excess clotting. The clotting is almost always venous, resulting in deep vein thrombosis (DVT) or pulmonary embolus (PE).

About 5% of Caucasians in North America have Factor V Leiden. The SNP is less common in Hispanics and African-Americans and is extremely rare in Asian people. About 30% of people who have a DVT or PE, especially in younger patients carry the SNP. Having the SNP and also having other risk factors for DVT including smoking, taking oral contraceptives, pregnancy and recent surgery, markedly increases risk.

My colleagues and I spent a long time trying to show that Factor V Leiden results in less bleeding. Essentially we hypothesized that a prothrombotic variant would result in less bleeding. Using a dataset of >1000 genotyped patients we were unable to show any effect. Thus it appears that there is no payoff for carrying this greater risk of DVT and PE. Unfortunately, we are still underpowered for showing that it is a risk factor for perioperative DVT or PE after cardiac surgery.

**Cold agglutinins**

Cold agglutinin disease is rare (~2/100,000). Some patients have a monoclonal gammopathy due to lymphoma. Other rare causes include renal cell carcinoma or infection. The cause is frequently unidentified. Cold agglutinins are usually IgM autoantibodies that react at cold temperatures with surface antigens on red blood cells. The disease of cold aglutination is one of the classes of the autoimmune hemolytic anemias. The antibody is usually an IgM directed against polysaccharide antigens and reacts either with adult (anti I) or fetal (anti i) red cells. Titres (1:16) of cold agglutinins are often found in the sera of healthy individuals. The presence of high titers of cold agglutinins (titers over 1:1000 at 4°C) can lead to hemagglutination and thrombosis at low temperatures, followed by complement fixation and subsequent hemolysis on rewarming. (6,7). Binding of antibodies to red blood cells activates the classical pathway of the complement system. If the complement response is sufficient, red blood cells are damaged by the membrane attack complex.

In contrast to the warm agglutinins, patients with cold agglutinins do not respond to steroids or splenectomy, but sometimes respond to rituximab (8). Rituximab destroys both normal and malignant B lymphocytes, and is used to treat B-cell lymphomas and rheumatoid arthritis. It specifically targets the CD20 antigen; the function of CD20 is unknown.

The term “cold agglutinin” is somewhat misleading because it implies a clear relationship between cold and agglutination. The term is derived from the immunological properties of the disorder. Agglutination from warm antibody-mediated hemolytic anemia requires incubation of red blood cells, antibody-containing serum and...
antihuman globulin antisera (Coombs’ antibody) at 37°C for 2 hours. The Coombs’ antibody binds IgG on the red blood cell surface and leads to agglutination that is visible to the naked eye. In cold agglutinin disease, however, because the IgM molecule that mediates hemolysis has a molecular weight of 1000 kD, the antibody can span the intercellular distance between red blood cells and cause agglutination at 4°C.

So, what should we do? Should we screen? What do we do when we find one? There are conflicting opinions expressed in the literature (9-12). One author has written .. “Our approach is “don’t screen for them; don’t report them.” We discourage requests for titration and thermal amplitude tests and recommend use of room temperature crystalloid instead of cold cardioplegia.”(13) Do you agree? Is it outdated advice?

**Environmental causes of Thrombosis**

**Recombinant Factor VIIa**

Until recently, we thought we had a partial answer to bleeding – Aprotinin. Aprotinin has gone, to the joy of some and the chagrin of others. Yet the problem has not gone, and is perhaps even more pernicious. So, like most explorers, we search for alternatives, as the solutions we currently have are incomplete. Recombinant Factor VIIa (rFVIIa) (Novo Nordisk, Princeton, New Jersey), is approved by the FDA for treatment of hemophilia. Off-label use of rFVIIa is expanding in surgical populations, but concerns have been raised about the potential safety, efficacy, and cost of rFVIIa use in surgery [Bowman 9 –14], especially for thrombotic events.

Cardiac surgery and CPB increase tissue factor levels both locally in areas of tissue injury and systemically. Because rFVIIa has interaction with tissue factor for thrombin generation, rFVIIa could potentially results in increased intravascular thrombus formation and thrombotic adverse events.

The data for rFVIIa’s safety and efficacy are profoundly limited. Bowman et al retrospective review evaluating 36 patients who received rFVIIa in the absence of a protocol for administration. Five patients received two doses of rFVIIa. After administration of rFVIIa the rate of blood product administration was reduced, however, this is probably not surprising as rFVIIa was not administered alone, or in a fashion that allowed rigorous examination. Four patients had a thrombotic event (Stroke, DVT or PE). A higher incidence of postoperative renal failure (23% vs. 6%), dialysis (12% vs. 1%) and pneumonia (29% vs. 6%) was seen in patients who receiving rFVIIa compared to a set of unmatched controls.

Karkouti compared a composite adverse event (death, stroke, renal failure, myocardial infarction, and major vein thrombosis) rate between 114 consecutive cardiac surgical patients who received rFVIIa for refractory “excessive blood loss” and 541 concurrent patients who developed excessive blood loss but did not receive rFVIIa.(14) Excessive blood loss was defined as evidence of microvascular bleeding after reversal of heparin in the operating room or > 100 mL·hr⁻¹ chest tube drainage in the ICU. Risk adjustment was performed using multivariable logistic regression modeling. In an effort to account for the timing of rFVIIa, patients were dichotomized into early and late groups based on whether or not they had received 8 units of RBC prior to rFVIIa administration. Adverse event rates in the untreated, early and late treated patients were 24%, 30%, and 60%, respectively. The overall unadjusted composite adverse event rate was 2.41 times greater (CI 1.58 – 3.67) in the rFVIIa-treated group vs. the rFVIIa-untreated group. Interestingly, the rFVIIa group, the adjusted OR was lower in the early treated group (OR 0.41; CI 0.18 – 0.92). Importantly, the most frequent adverse event was acute renal failure (dialysis or two-fold increase in serum creatinine) (29% in the rFVIIa treated group vs. 15% in the non-rFVIIa treated group). However, all these findings were negated after adjustment for predictors of adverse events. Variables in the multivariable logistic regression model were (in order of importance): total RBC units transfused during hospitalization, difficulty weaning from CPB, CPB duration, weight, gender, cerebrovascular disease, and age. After adjustment for these variables, rFVIIa use was not associated with an increased adverse event rate (OR 1.04; 95% CI 0.60 – 1.81).

Sellke et al. have reported the only randomized trial of rFVIIa I could find.(15) 172 patients who had undergone cardiac surgery and were bleeding (definition unstated) were randomized to receive placebo (N=68), 40 mcg/kg rFVIIa (N=35), or 80 mcg/kg rFVIIa (N=69) as a single bolus in the ICU. The primary endpoint was the number of patients suffering serious adverse events at 30 days although I am not sure from the abstract report what those
were. Secondary endpoints included rates of re-operation, blood loss volumes and transfusion of allogeneic blood. There were more SAEs in the rFVIIa groups (40 mcg/kg, 14%; 80 mcg/kg; 12%) than in placebo group (7%) but these differences were not significant because of the low sample sizes. Significantly fewer patients in the rFVIIa groups underwent re-operations for bleeding (placebo, 25%; 40 mcg/kg, 14%; 80mcg/kg, 12%) and had smaller allogeneic blood transfusion volumes. Four hours after randomization and drug administration the median drainage rate in the 80mcg/kg group was significantly less (24 mL/hr, 25%–75% IQR: 13–32) than in the placebo (51 mL/hr; IQR 21–83) and 40mcg/kg groups (35 mL/hr, IQR: 27–85). There was an approximately 50% reduction in the drainage volume within the 4 hours after treatment with 80mcg/kg rFVIIa (P < 0.001) compared with placebo. It is likely that the higher dose rFVIIa may be effective, however despite the authors’ conclusions, safety is not proven. rFVIIa may be of use and it may be safe, however I’m afraid we may be about to relearn the soon-forgotten lessons of Aprotinin if we don’t do the efficacy and safety studies correctly.

**rFVIIa has not been systematically examined in the cardiac surgical environment we plan to use it.** rFVIIa will be used in only the most dire cases of bleeding due to its great cost. rFVIIa looks like it is even less likely than Aprotinin to undergo rigorous examination in high-risk bleeding patients. The roadblocks to rational approval of its use in cardiac surgery appear high: the drug is profoundly expensive, the company is not planning trials in non-hemophiliacs undergoing cardiac surgery (Novo Nordisk; Jan ’09) and physicians will much rather use a drug than have a patient randomized to the drug, or placebo. Thus the majority of trials we have seen, and are likely to see, are small single-institution trials that are underpowered for bleeding outcomes and certainly underpowered for rare events such as intravascular thrombosis. Worse still, we are likely to continue to see single-arm or historical-control studies that provide us with no clinically-useful message.

**We are likely to see case reports of thrombosis temporally related to rFVIIa use.** It seems incongruous that bleeding patients have intravascular thrombosis, but they do. Worse still, the bleeding patient often has routine postoperative anticoagulants withheld for fear of further bleeding and thus are at higher risk of delayed postoperative thrombosis that appears to be unrelated to any prior intervention, such as Aprotinin or rFVIIa use. Thus, we cannot deconvolute the consequences of severe bleeding from the consequences of treatment for severe bleeding. Risking ridicule, I’m going to predict that like the reports of higher graft thrombosis rates and intravascular thrombosis after Aprotinin, we will sometime soon see these for rFVIIa. Will we then have a debate in the absence of data; not unlike the Aprotinin debate of the late 1990’s?

**Glues**

There’s nothing more appealing to a surgeon than glue plugging a hole that a suture won’t. There are two overall classes – fibrin glues and topical bovine or human thrombin combined with a thrombotic substrate. Fibrin glue (Tisseel in the US, Tissucol in Europe; Baxter) is the most commonly used fibrin glue. Most fibrin glues are manufactured from human plasma and have been associated with hepatitis B and C transmission, notably in Japan, but not with HIV transmission. A component of its manufacture is the addition of Aprotinin to prevent fibrinolysis. However, they may have a risk of inducing transmural coagulation and perhaps thrombosis of coronary vein grafts.

Two concurrently-published large European studies have shown greater mortality (7% vs. 3% in controls) and MI after use of Tissucol in CABG surgery, even after well-conducted accounting for other mortality risk factors or risk scores such as Euroscore.(16,17) Mortality was related to dose of fibrin glue used. However, these studies were retrospective and the indication for use of fibrin glue is lacking. Use of fibrin glue may be a marker for problem cases, although this was not apparent from the reasonably well matched patient groups and the reasonably well-conducted multi-variable modeling. No matter what the answer is, there is an immediate need for well-conducted randomized clinical trials. It is profoundly disappointing that the FDA did not mandate these trials during the approval process.

![Figure. Cumulative survival. Patients with fibrin glue versus patients without fibrin glue.](Goerler et al., 2007)
Bovine thrombin preparations have been associated with development of increased titers of anti-coagulant factor antibodies. The lifetime risk for the development of anti-bovine thrombin antibodies is increased in those patients who receive multiple exposures. However, the clinical significance of such observations is difficult to assess. In a seemingly well conducted literature review performed at the behest of a company making topical thrombin, the increased risk of antibody production was reported. They probably-correctly reported that teasing out the risk of adverse events causally due to topical thrombin was essentially impossible due to the non-randomized nature of most reports. However, case reports of adverse responses to topical thrombin, notably coagulopathy, are reported and seem severe.(18-20)

References