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Alterations in Coagulation During Transurethral Prostatectomy

Richard C W Bell MBBS FRCS

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Abstract

Introduction Bleeding remains a significant cause of morbidity following transurethral prostatectomy (TURP). Fibrinolysis has been implicated. However good evidence for this is lacking, and surgery is associated with a hypercoagulable state.

Objective To evaluate the coagulation status and the role of fibrinolysis in patients undergoing TURP.

Method Systemic fibrinolysis and global coagulation status was assessed by thrombelastography in a prospective study of 40 patients undergoing TURP. Urinary fibrinolytic activity was measured using the fibrin plate technique. Thrombin-antithrombin III (TAT) and D-dimer levels were measured as markers of systemic activation of the coagulation cascade, and the role of postoperative fibrinolytic `shutdown' established by monitoring tissue plasminogen activator (tPA) and inhibitor (PAI-1) levels. The role of Factor XIII levels on clot fragility, and the effects of endotoxin on the clotting cascade were studied.

Results No evidence of systemic or local fibrinolysis was found in any patient over the perioperative period. Thrombelastographic evidence of a hypercoagulable state was observed perioperatively. Systemic activation of the coagulation cascade was evident from the significant rise in TAT complexes 6 hours postoperatively (ANOVA $p = 0.01$). The significant increase in mean D-dimer levels 24 hours postoperatively (ANOVA

p= 0.015) in the absence of any significant increase in mean tPA levels (ANOVA p= 0.737) indicates a *physiological* fibrinolytic response to the procoagulant state. The absence of any significant increase in PAI-1 antigen perioperatively (ANOVA p= 0.348) suggests that the observed hypercoagulability is not due to fibrinolytic `shutdown' reported in other forms of surgery. Elevated mean thrombelastographic maximum amplitude values and normal Factor XIII levels postoperatively discount bleeding due to clot fragility. Endotoxin does not appear to be a trigger for the observed activation of the coagulation cascade.

Conclusion TURP is associated with a hypercoagulable state comparable with other surgical procedures. No evidence of *pathological* fibrinolysis was observed.

Declaration

The work presented in this dissertation was performed by the author at the Departments of Urology and Intensive Care and The Katherine Dormandy Haemophilia & Haemostasis Unit at the Royal Free Hospital, London. The work was carried out between April 1994 and August 1995.

This study was approved by the Ethical Committee of The Royal Free Hospital NHS Trust. All participants gave full informed consent prior to their inclusion into the study.

Whilst I am indebted to a large number of people for their support and guidance, all the ideas are original to me and all the experimental and investigative procedures were performed by myself except where specifically indicated in the acknowledgements.

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Synopsis

Transurethral prostatectomy (TURP) is one of the most frequently performed routine surgical procedures. Bleeding remains a significant causes of morbidity, re-operation and prolonged hospitalisation following TURP. This is largely seen in the form of clot retention. In the past this has been attributed to both systemic fibrinolysis and local fibrinolytic activity at the prostate bed.

The work described in this thesis considers the null hypothesis that bleeding following transurethral prostatectomy (TURP) cannot be attributed to fibrinolysis.

The objectives of the study were to demonstrate the changes in overall coagulation status, the *in vitro* dynamics of clot formation, and the degree of clinically significant systemic or local fibrinolysis occurring in patients undergoing TURP.

The first chapter describes the factors believed to predispose the patient to haemorrhage following TURP. The evidence in the published literature for the presence of both systemic and local fibrinolysis in patients undergoing TURP is examined. The coagulation changes believed to occur in patients following TURP are critically reviewed and compared with other forms of surgery.

The second chapter describes in detail the overall aim and methodology of the study. Statistical data analysis, inclusion and exclusion criteria, anaesthetic, operative and serial sampling techniques are detailed. The results indicate evidence of irrigant fluid absorption and a procoagulant state.

In the third chapter the overall coagulation status of the patients and the *in vitro* dynamics of clot formation retraction and lysis was assessed by thrombelastography. A critical review of the literature and the validity of the technique is examined. No evidence of any systemic fibrinolysis, but clear thrombelastographic evidence of hypercoagulability was seen over the perioperative period.

The fourth chapter examines the theory of urinary fibrinolysis causing clot lysis and bleeding from the prostate bed. No evidence of any increase in urinary fibrinolytic activity over the perioperative period when compared to controls was demonstrated.

The concept of inhibition of systemic fibrinolysis or 'fibrinolytic shutdown' seen in other forms of surgery as a cause of the observed procoagulant state following TURP is examined and discounted in chapter 5.

Further evidence for the presence of an hypercoagulable state is sought in chapter 6. A significant increase in thrombin-antithrombin III complexes - a marker for activation of the coagulation cascade - is seen postoperatively. A secondary *physiological* fibrinolytic response to the activation of the coagulation cascade is demonstrated.

The role of Factor XIII in clot stability and wound healing following TURP is examined in chapter 7. No significant depletion in factor XIII was evident perioperatively.

The role of sepsis and endotoxaemia in haemostasis has only recently been clarified with activation of the clotting cascade by the release of tumour necrosis factor alpha from damaged endothelial cells, a subsequent inhibition of the fibrinolytic system and the consequent development of a consumptive coagulopathy. Systemic absorption of

irrigant fluid during resection is well recognised and provides a theoretical route for the release of tissue thromboplastins and endotoxin into the systemic circulation, with the subsequent activation of the coagulation system. Evidence for the presence of endotoxaemia during TURP is examined in chapter 8.

The findings of the study are discussed in depth in the conclusion. Confirmation of the null hypothesis, the evidence for an hypercoagulable state and the possible procoagulant triggers are examined. The shortfalls of the study and areas of future research are highlighted in chapter 10.

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CHAPTER 1

INTRODUCTION

Introduction

Approximately 31,000 prostatectomies for bladder outlet obstruction are carried out annually in the United Kingdom (Office of Population and Census Survey 1985), and over 90% of these are performed transurethrally. Whilst the average perioperative mortality from previous published series is less for TURP (1.25 %) than for open prostatectomy (2.28%), extensive studies by Roos and Ramsey (1987), Roos et al (1989), Malenka et al (1990) and Andersen et al (1990) all showed elevated risk of death for up to eight years after a TURP. These studies however were all retrospective, relying on administrative databases and advanced statistical methods, and their findings have not been substantiated by Concato et al (1992) who showed no difference in long term mortality rate between TURP and open prostatectomy in their retrospective analysis when age and severity of co-morbid illness was adjusted for, or by Fuglsig et al (1994) who prospectively showed no surplus mortality over a ten year period when patients undergoing TURP were compared with age-matched controls from the general population. Thus the transurethral technique of prostatectomy for the treatment of bladder outlet obstruction remains the method of choice for prostatectomy (Chilton et al 1978) against which all other innovative methods of relieving bladder outlet obstruction due to benign prostatic hyperplasia (BPH) such as prostatic stenting, thermotherapy, laser prostatectomy α_1 adrenoceptor blockade and androgen antagonism must be compared. Thus far, no alternative method of prostatic ablation has produced results comparable with TURP. For the forseable future TURP will be the most effective

method of relieving significant bladder outlet obstruction due to BPH.

1.1 The History of Transurethral Prostatectomy

The origins of transurethral surgery are derived from early attempts to negotiate difficult strictures of the prostatic urethra. Creation of a false passage by means of specially developed catheters with sharp stylettes or other form of tunneling device at their tips were widely used at the beginning of the nineteenth century. In 1834 Guthrie modified this technique by developing a sound with a concealed blade used to divide the posterior prostate or bladder neck. A factor common to all these techniques was the difficulty in controlling haemorrhage after the procedure, relying on the positioning of a urethral catheter with the subsequent high risk of urethritis, epididymitis and pyelonephritis. This difficulty was not addressed until 1877, when Bottini and Pavia introduced the technique of galvanocautery, using a platinum element designed to burn prostatic tissue. With several modifications the Bottini incisor remained in popular use as a blind technique, until the development of the cystoscope in the late nineteenth century, and its incorporation into the Bottini incisor by Freudenberg in 1900.

Young (1913) developed the punch operation for prostatic obstruction - a partially blind technique with no method for securing haemostasis - which often resulted in severe haemorrhage. This technique was modified by Braasch (1918) who incorporated direct vision cystoscopy, and by Caulk and Bumpus (1927) who added electrocautery in an attempt to achieve haemostasis.

With the development by Stern (1926) of a cutting loop of tungsten that could be moved along the axis of a sheath excising strips of prostatic tissue under direct vision, and the subsequent modification by McCarthy (1931) to improve the optics and the effectiveness of the cutting current, the forerunner of the modern resectoscope was created. Nesbit (1939) refined the Stern-McCarthy instrument to allow three dimensional visualisation of the prostate. Additional improvements by Iglesias (1975) who designed the continuous flow resectoscope, and the development of improved coagulation and cutting currents and isotonic irrigating fluids have further improved the safety of transurethral prostatectomy.

1.2 Factors Affecting TURP Blood Loss

Despite the advances in instrumentation bleeding remains the most significant cause of early morbidity and prolonged hospitalisation following TURP (Doll et al 1992; Mebust et al 1989). Perioperative haemorrhage is traditionally classified into primary, reactionary and secondary - primary haemorrhage occurring at the time of surgery, reactionary haemorrhage occurring within 24 hours of the surgical procedure (but usually within the first four to six hours) on restoration of the patients preoperative blood pressure and refilling of the venous system, and secondary haemorrhage occuring at a time subsequent to this but classically at 7 to 14 days postoperatively (Rains & Ritchie 1984).

Peroperative blood loss and a degree of primary haemorrhage are inevitable consequences of transurethral resection of the prostate, and as such cannot be regarded as a complication of the procedure. At what

point does perioperative blood loss become a complication? There are no internationally accepted criteria for this. To a large extent this is because what constitutes a significant perioperative blood loss varies greatly between individuals, and is dependent on a number of factors including the patients preoperative haemoglobin, circulating blood volume and cardiovascular status. The lack of a uniform standard as to what degree of blood loss constitutes a complication of the procedure in TURP, together with the difficulties in accurately assessing the blood loss may explain the wide variation in the reported incidence of early haemorrhagic complications of between 2.8 and 11% (Melchior et al 1974a; Doll et al 1992), and secondary haemorrhagic complications of between 2.1 and 25% (Chilton et al 1978; Harvey et al 1986) following TURP.

It is an accepted surgical principal that a transfusion of red cells is required after 20% of the blood volume has been lost (Shearer 1988). It can be argued that any blood transfusion is a potential hazard to the recipient (NIH Consensus Panel on Infectious Disease Testing for Blood Transfusions 1995), and therefore a blood loss requiring transfusion of red cells is a complication of the procedure. However there is a well documented and strong correlation between the weight of prostate tissue resected per unit time and peroperative blood loss (Abrams et al 1982; Robertson et al 1993; Kolmert & Norlén 1989). Robertson et al 1993 demonstrated a 58% transfusion rate in patients in whom greater than 60 grams were resected, but a rate of only 5.6% in patients having less than 60 grams resected. Thus criteria for unacceptable perioperative blood loss must take into account the weight of prostate tissue resected. Reasonable criteria for a haemorrhagic complication might therefore be bleeding

requiring re-operation, continued bleeding prolonging hospital stay, blood loss requiring transfusion with less than 60 grams resected and secondary haemorrhage.

The TURP series reported in the literature make no such distinction, but document a blood transfusion rate of between 6.4 - 22%, and a further interventional procedure rate of between 1 - 6.3% (Doll et al 1992; Habib & Luck 1983; Kolmert & Norlen 1989; Mebust et al 1989; Melchior et al 1974a; Singh, Tresidder & Blandy 1973). With the associated risks of a blood transfusion and the added cost to both the patient and the Health Service of prolonged hospital stay, any attempts to limit perioperative haemorrhage are of fundamental importance.

The factors contributing to perioperative blood loss in TURP are not fully understood. A number of premorbid factors are known to increase peroperative blood loss. Experience suggests that increasing age and the presence of poorly controlled hypertension both increase blood loss over the perioperative period. Indeed poorly controlled hypertension is a contra-indication to TURP. However Perkins & Miller (1969) found no correlation between age and blood loss, and more interestingly Abrams et al (1982) did not demonstrate a correlation between systolic blood pressure and peroperative blood loss.

Melchior et al (1974b) found an increased peroperative and postoperative transfusion rate in uraemic patients. Robertson et al (1993) found the need for perioperative transfusion in TURP was significantly associated with presentation in chronic or acute-on-chronic retention. The presence of an indwelling urethral catheter preoperatively appears to increase peroperative blood loss (Faber, Hansen & Genster 1987). The mechanism

for this is unclear, but may be due to the fact that a number of patients with indwelling catheters preoperatively will be uraemic. Uraemia is known to inhibit platelet function by a variety of mechanisms (Remuzzi & Rossi 1996), and this may account for the observed increased blood loss.

The majority of patients undergoing a transurethral prostatectomy are elderly, and a significant proportion of these have co-existent cardiovascular disease and are at risk of sustaining a vascular event (unstable angina, myocardial infarction, transient ischaemic attack or cerebrovascular accident) over the perioperative period. Thirty-nine per cent of reported perioperative urological deaths between 1994 and 1995 occurred in patients undergoing TURP - largely as a result of coronary artery disease (Gallimore et al 1997). Following reports by the Antiplatelet Trialists' Collaboration (1994) that low dose aspirin therapy (75-150 mg daily) can reduce vascular events by up to 25% in these high risk patients, increasing numbers of patients undergoing TURP will be on low dose aspirin therapy. In a retrospective study of patients undergoing TURP, Watson et al (1990) described a significantly increased incidence of haemorrhagic complications in patients on low dose aspirin therapy. These findings were confirmed by Thurston & Bryant (1993) in a further retrospective series. Watson et al (1990) recommended withdrawal of aspirin from 10 days preoperatively until the risk of postoperative bleeding has ceased. These findings however have yet to be confirmed by a prospective study, and in a large retrospective series of patients undergoing TURP Donat, McNeill & Brame (1996) failed to show any relationship between aspirin ingestion and perioperative blood transfusion rate. Bricker, Savage & Hanning (1987) also failed to show any increase in

blood loss in a prospective study of patients given diclofenac, another non-steroidal anti-inflammatory drug, over the perioperative period.

The reported incidence of deep venous thrombosis in patients undergoing TURP is between 6.8 and 10 per cent (van Arsdalen et al 1983; Hedlund 1975). There is a smaller but significant reported incidence of pulmonary embolus of between 0.2 and 2.2 per cent (Melchior et al 1974a; Kass et al 1978). The use of low dose standard heparin prophylactically is widely used in surgery in high risk patients to reduce the incidence of thromboembolic complications (Sharnoff & DeBlasio 1970). Despite this significant incidence of thromboembolic complications, prophylactic subcutaneous standard heparin is not routinely used in patients undergoing TURP. This practice is based on the findings of Halverstadt et al (1977) who found a greater degree of haemorrhagic complications in patients undergoing TURP receiving minidose heparin over the perioperative period. These findings were confirmed by both Allen, Jenkins & Smart (1978) and Sleight (1982). Of these, only that of Allen, Jenkins & Smart (1978) was a randomised prospective study, but their findings are complicated by the use of the antifibrinolytic agent epsilon aminocaproic acid.

In contrast to their findings, prospective randomised studies by Bejjani et al (1983) and Wilson et al (1988) found no significant difference in perioperative blood loss in patients undergoing TURP receiving prophylactic subcutaneous heparin compared to controls. Kakkar et al (1993) showed a 23 per cent reduction in the incidence of bleeding complications in general surgical patients when low molecular weight

heparin was compared with standard heparin. No such comparisons have been made in patients undergoing TURP.

The usual practice for patients on long-term warfarin anticoagulant treatment undergoing TURP is to discontinue treatment over the perioperative period (Mulcahy et al 1975). However this is not without risk, and Tscoll, Straub & Zingg (1980) and more recently Chakravarti & MacDermott (1998) advocate conversion to full intravenous heparin anticoagulation over the perioperative period in these high risk cases. In a retrospective review of twelve TURP's on long-term warfarin therapy, Parr, Loh and Desmond (1989) found no significant haemorrhagic complications despite being maintained on full dose oral anticoagulant therapy if fresh frozen plasma was utilised perioperatively.

The role of anaesthesia in perioperative blood loss during TURP is contentious. TURP is commonly performed under regional anaesthesia (spinal or epidural), general anaesthesia or a combination of the two. Regional anaesthesia acts by producing a pharmacological sympathectomy resulting in reduced venous tone and potentially reducing blood loss in pelvic surgery. A reduction in peroperative blood loss with regional anaesthesia when compared to general anaesthesia has been described in patients undergoing TURP (Slade et al 1964; Abrams et al 1982). However neither of the studies by McGowan & Smith (1982) and Freedman, Van Der Molen & Makings (1985) found any significant difference in blood loss between regional and general anaesthesia. The use of controlled hypotensive anaesthesia (Bruce, Zorab & Still 1960; Madsen & Madsen 1967) to reduce peroperative blood loss has not gained acceptance because of the potential risk of cerebral and

myocardial infarction in the elderly, and a subsequent reactionary haemorrhage postoperatively.

Irrigant temperature has been reported as affecting perioperative blood loss. By cooling the irrigant temperature to 36 degrees Fahrenheit (2.2 degrees Celsius) Robson & Sales (1966) demonstrated an almost two-fold reduction in blood loss per gram resected. They found a 3 to 6 degree Fahrenheit reduction in core temperature with patients under spinal anaesthesia shivering towards the end of the procedure, but encountered no cardiac irregularity or rewarming shock. These findings were confirmed by Serrao, Mallik et al (1976) who found a significant reduction in peroperative and postoperative blood loss using irrigant fluid cooled to 2 degrees Celsius. Kulatilake et al (1981) demonstrated a reduction in volume of irrigant fluid used when cooled to 8 degrees Celsius, thus reducing the risk of absorption of irrigant fluid and consequent hypervolaemia and hyponatraemia. The likely mechanism of action of the cooled irrigant in reducing both blood loss and fluid absoption is local vasoconstriction. However, Madsen, Kaveggia & Atassi (1964) failed to show any reduction in peroperative blood loss using irrigant cooled to between 2 to 4 degrees Celsius, and found a potentially dangerous drop in core temperature in 2 patients. Walton & Rawstron (1981) in a prospective study of 93 patients undergoing TURP also failed to demonstrate a significant reduction in peroperative blood loss using irrigant cooled to 2 degrees celsius.

There appears to be a correlation between the amount of irrigant fluid absorbed into the circulation and blood loss. Hahn & Ekengren (1993) showed a 40% incidence of significant irrigant fluid absorption (greater

than 1,000 millilitres) in transurethral prostatectomies with a blood loss of greater than 500 millilitres.

There is little doubt that careful operative technique with specific attention to haemostasis by electrocoagulation reduces perioperative blood loss (Robertson et al 1993). The most important interoperative factors affecting peroperative blood loss appears to be the weight of prostatic tissue resected with values of 20 to 37 ml per gram reported, and duration of resection (Abrams et al 1982; Freedman, Van Der Molen & Makings 1985; Fujita 1988; Gatenberg & Johansson 1984; Jenkins & Mintz 1981; Levin, Nyrén & Pompeius 1981; Lewi et al 1983; Perkins & Miller 1969). Operative blood loss also appears to correlate with postoperative blood loss (Lewi et al 1983). Common sense suggests the experience of the resectionist would be a significant factor in the amount of blood lost peroperatively. However this has not been confirmed in the literature (Freedman et al 1985; Perkins & Miller 1969).

Although over one half of patients report a degree of secondary haemorrhage following TURP it is usually minor and transient, with only 4 per cent require re-admission for bleeding (Doll et al 1992). The aetiological role of infection in the predisposition to secondary wound haemorrhage is fundamental to the principles of surgical practice (Rains & Ritchie 1984). Blandy (1972) concurred with this view, citing urinary tract infection as the major cause of secondary haemorrhage following TURP. The reported incidence of postoperative urinary infection in TURP varies between 6 and 63 per cent (Lacey, Drach & Cox 1971; Symes et al 1972). Harvey et al (1986) documented a 25 per cent incidence of secondary haemorrhage, and demonstrated a significant correlation between post-

operative urinary infection and secondary haemorrhage. However the use of a prophylactic antibiotic postoperatively failed to reduce the incidence of bleeding. Robertson et al (1993) found a significant correlation between the presence of postoperative - but not preoperative - urinary infection and blood transfusion requirement over the perioperative period. Faber, Hansen & Genster (1987) found a significant correlation between preoperative urethral catheterisation and the need for a perioperative blood transfusion. This however may be due to the increased incidence of urinary tract infection in catheterised patients.

There has been conflicting reports concerning the effect of histological diagnosis of the resected prostatic tissue on blood loss. Abrams et al (1982) and Bejjani et al (1983) found a reduced peroperative blood loss in patients with prostatic carcinoma undergoing TURP when compared to those with benign histology. However de Campos Freire et al (1986) found an increased blood loss in patients with prostatic carcinoma and Fujita (1988) found no difference in blood loss between benign and carcinomatous glands.

1.3 Techniques to Reduce TURP Blood Loss

Careful preoperative assessment of each individual patients risk factors for increased blood loss in advance of their surgery is accepted as good practice. Patients with poorly controlled hypertension should be postponed until their blood pressure is adequately controlled on antihypertensives. Current opinion suggests that patients with very enlarged prostate glands of over 100 grams should undergo an open prostatectomy in preference to TURP in order to reduce operative blood loss and minimise irrigant fluid absorption. In patients with obstructive uropathy due to an obstructive prostate a period of urethral catheterisation and correction of any fluid and electrolyte imbalance and optimalisation of their renal function prior to surgery should be undertaken to reduce the degree of uraemia (Melchior et al 1974b). Withdrawal of aspirin 10 days preoperatively (Watson et al 1990) is not universally practiced, and no consensus on the use of low dose subcutaneous heparin or the management of patients on warfarin anticoagulation has been established. Recently Bolton & Costello (1994) recommended the use of endoscopic laser ablation of the prostate (ELAP) in preference to TURP in patients on warfarin therapy. However even ELAP is not without haemorrhagic complications in these high risk patients (Bellringer, Anson & Schweitzer 1996).

Many different techniques to reduce perioperative blood loss in patients undergoing TURP have been investigated. Electrocautery of visible blood vessels and bleeding points remains the principal and single most effective method of achieving haemostasis during transurethral prostatectomy. The use of compression of the blood vessels of the bladder neck and prostatic bed to reduce venous bleeding immediately

after the procedure is a common technique. Compression is achieved usually by means of a catheter balloon. This technique was first described by Foley (1937) who recommended placement of a haemostatic bag within the prostatic urethra to tamponade prostatic venous sinuses. However there has been some debate as to the correct positioning of the balloon, the volume it is inflated to, and the theoretical risk of urinary incontinence due to sphincter damage. Greene (1971) concluded that provided adequate haemostasis was achieved by electrocoagulation the balloon position was unimportant; however if haemostasis was deemed inadequate at the end of the procedure, a haemostatic bag positioned within the prostatic cavity remained in situ and was more effective in reducing postoperative blood loss than one positioned at the bladder neck or free within the bladder. He detected no increased incidence of urinary incontinence with any particular balloon position.

Traction placed on the urethral catheter immediately postoperatively following a TURP is a common method of reducing venous blood loss. Pengelly (1985) advocates filling of the balloon to at least twice the weight in grams of the resected tissue and positioning it at the bladder neck. Traction using a one kilogram weight should then be applied for no longer than twice the bleeding time (15 minutes) to prevent ischaemic damage. A variety of methods of applying traction to the catheter to minimise postoperative venous blood loss have been described, including securing the catheter to the thigh with adhesive tape (Mitchell 1981) and wrapping gauze tightly around the catheter at the urethral meatus thereby using the glans penis as a means of traction (Blandy 1978). These techniques all have their disadvantages. Oesterling (1992) described the use of an

abdominal catheter holder in 76 patients undergoing TURP. No patients required a blood transfusion, 91 per cent required no postoperative bladder irrigation and in 74 per cent of patients the catheter was removed on the second postoperative day. The proposed advantage of this device is that it produces controlled constant tension, unlike attachment at the thigh where movement at the hip causes variable tension, trauma to the bladder neck and possible dislodgement of the eschar forming at the prostatic bed with the consequent increase in blood loss. Whether this technique reduces blood loss when compared with other techniques remains to be seen.

A variety of different drugs have been investigated as potential haemostatic agents in transurethral prostatectomy. The effectiveness of Premarin (an intravenously administered conjugated oestrogen which acts to reduce capillary bleeding by an unknown mechanism) in reducing perioperative blood loss in TURP were studied by Madsen et al (1964), who detected no reduction in blood loss or duration of operation with the drug.

Yang & Cheng (1989), in a prospective study, investigated the use of preoperative 5-flourouracil (5-FU) in patients with benign prostatic hyperplasia (BPH) undergoing TURP and found a significant reduction in operative blood loss when compared to controls. They found a strong correlation between operative blood loss and the number and calibre of blood vessels in the resected prostatic tissue. They observed a reduction in vasculature in the 5-FU treated prostates and ascribed its haemostatic properties to this effect.

The use of locally administered haemostatic agents to reduce peroperative blood loss during transurethral prostatectomy has also been investigated. Smart (1984) described a technique of peroperative endoscopic injection of the synthetic vasoconstrictor ornithine-8-vasopressin into the prostate prior to resection. In two prospective trials of patients with BPH undergoing TURP either under general or spinal anaesthesia, he demonstrated a significant reduction in blood loss over the first 24 hours in patients receiving the vasoconstrictor. Apart from a mild transient increase in blood pressure no complications were reported. Luke et al (1986) developed a method of locally instilling fibrin glue (fibrinogen concentrate, thrombin and aprotinin) into the prostatic cavity immediately following resection; in a prospective randomised study of 30 patients with presumed BPH undergoing TURP, they demonstrated a significant reduction in postoperative blood loss in patients receiving the fibrin glue. No complications were recorded.

Several studies of the systemically administered haemostatic agent ethamsylate (Dicynene) have been performed with conflicting findings. The mode of action of ethamsylate is not fully understood but it is known to increase platelet adhesiveness and the rate of release of intrinsic thromboplastin thereby reducing capillary bleeding. Symes et al (1975) reported a reduction in both peroperative and postoperative blood loss with Dicynene in patients undergoing TURP. However the mean resected weight of prostate tissue was only 7 grams. Towler & Valerio (1978) in a large randomised double-blind study of 100 patients undergoing TURP failed to show any significant reduction in either peroperative or postoperative blood loss using Dicynene. However anaesthetic and

operative conditions were not standardised. They found no increase in the incidence of lower limb deep venous thrombosis. Their findings were confirmed by Lyth & Booth (1990) who, in a further randomised double-blind trial, failed to show any benefit in patients receiving Dicynene.

In the past a variety of other drugs including Kutapressin a liver extract with vasoconstrictor properties (Weisenthal et al 1961), carbazochrome salicylate an oxidative derivative of adrenalin which retains the vasoconstrictor properties without the other sympathomimetic effects (Frank & Lloyd, 1959), bioflavinoids and Hemostase (a thrombin-like derivative of snake venom) and polyoestradiol phosphate (Madsen et al 1968) have been investigated as possible haemostatic agents in TURP without success.

The use of regional and hypotensive anaesthesia, and local hypothermia to control blood loss have already been discussed. None of these techniques, with the possible exception of spinal anaesthesia and catheter balloon traction, have been accepted into Urological practice.

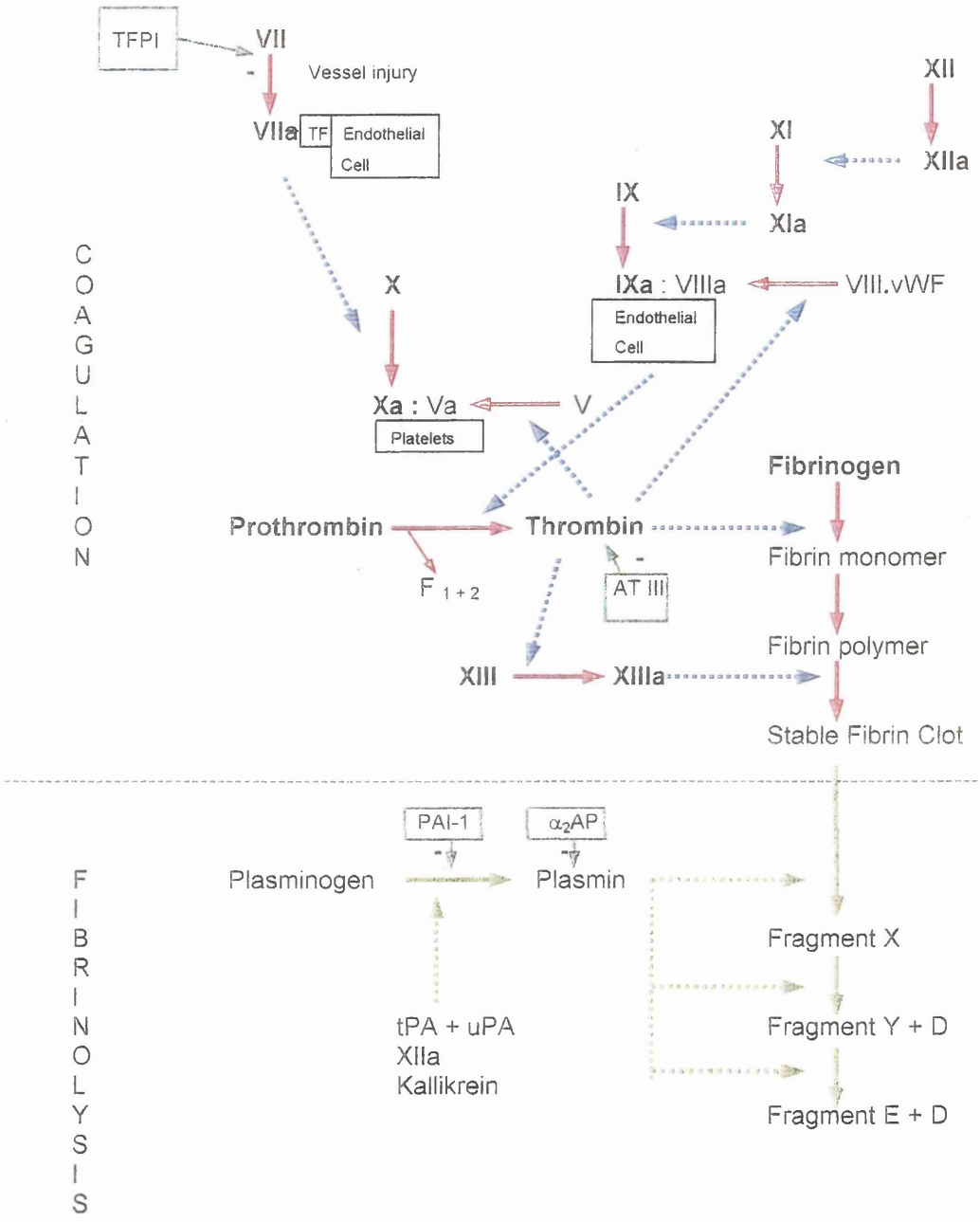


Figure 1.1

The coagulation and fibrinolytic cascade, initiated by tissue factor (TF) released following endothelial cell injury. The two systems are activated concurrently and are intricatly interlinked. The **clotting factors** (serine proteases) and co-factors are depicted in Roman numerals. The antecedent 'a' denotes activated factor. Tissue factor pathway inhibitor (TFPI) and antithrombin III are key inhibitors of the coagulation cascade. Plasminogen activator inhibitor 1 (PAI-1) and alpha-2 antiplasmin (α_2AP) inhibit fibrinolysis.

1.4 Prostatic Disease and Coagulopathy

John Hunter (1794) noted that the blood of dying patients often lost the ability to clot. Later Dastre (1893) observed this phenomenom in dogs after severe bleeding and used the title "La Fibrinolyse" to describe his findings, from which the term fibrinolysis is derived. Fibrinolytic activity in animal tissue was first described by Fleisher & Loeb (1915) who demonstrated the lytic effect of rat and guinea-pig tissue on clotted blood. A further study by Dale & Walpole (1916) showed that the in vitro treatment of blood with chloroform prevented it from clotting. Later Tagnon (1942) showed that the proteolytic enzyme responsible for the inability of blood to clot was fibrinolysin (plasmin), and that chloroform probably acted by eliminating an inhibitor. In 1933 Tillet and Garner demonstrated the fibrinolytic properties of culture fluid from certain strains of haemolytic streptococci.

Milstone (1941) demonstrated that the streptococcal filtrate and chloroform acted on a factor in the blood (now known as plasminogen) converting it to an active enzyme (plasmin). The streptococcal substance is now called streptokinase. Chloroform was later shown to act by inactivating a major inhibitor of plasmin (α2-antiplasmin) in the blood (Christensen & MacLeod 1945). An initiator of the conversion of plasminogen to plasmin found in normal blood was described by Astrup & Permin (1947). They called this initiator fibrinokinase, which is now known as tissue plasminogen activator (tPA). Later the same year, Macfarlane & Pilling (1947) demonstrated fibrinolytic activity in the urine, now known to

be due to a different plasminogen activator called urokinase plasminogen activator (uPA).

The fibrinolytic system is now recognised to be a complex system of pro-enzymes, enzymes and inhibitors intricately linked to the coagulation cascade to maintain vascular haemostasis. Inhibitors of the fibrinolytic system prevent widespread systemic fibrinolysis. Collen (1976) identified a fast-reacting plasmin inhibitor in human plasma (α2-antiplasmin) which complexes with free circulating plasmin to inactivate it thus preventing systemic fibrinolysis. The presence of specific inhibitors (PAI's) of the plasminogen activators tPA and uPA was confirmed by Chmielewska, Rånby & Wiman (1983).

Since the original report by Jurgens & Trautwein (1930) linking excessive bleeding in patients with prostatic carcinoma with hypofibrinogenaemia, many authors have reported alterations in coagulation in prostatic disease. Because of the inability of the patients plasma to lyse a normal blood clot, Jurgens & Trautwein (1930) discounted the possibility of proteolysis of fibrinogen (factor I) and falsely concluded that the hypofibrinogenaemia was secondary to metastatic involvement of the reticuloendothelial system. later Marder et al (1949) and Zeh & Ott (1954) and others also linked the presence of afibrinogenaemia in metastatic prostate cancer with haemorrhagic complications. Tagnon, Whitmore & Schulman (1952) were the first to describe 2 cases of hypofibrinogenaemia associated with primary fibrinolysis in patients with prostate cancer. Later Tagnon et al (1953) documented a 12 per cent incidence of fibrinolysis associated with hypofibrinogenaemia in 48 patients with metastatic cancer of the prostate. Five of the 6 cases of fibrinolysis were associated with haemorrhagic

complications. They found fibrinolytic activity in both the primary and metastatic carcinomatous tissue. Concurring with previous reports by Huggins & Neal (1942) and Huggins & Vail (1943) describing the presence of a fibrinolytic enzyme in human and dog prostatic tissue with similar properties to plasmin, they concluded that their findings were due to leakage of a fibrinolytic enzyme fibrinolysin (plasmin) into the circulation, in quantities sufficient to exceed the capacity of the plasma inhibitors to mask its presence, resulting in primary systemic fibrinolysis (fibrinogenolysis).

Cosgriff & Leifer (1952) reported a prolongation of the prothrombin time associated with factor V (proaccelerin) deficiency in a patient with prostatic carcinoma and haemorrhagic complications. Reports by Seale, Jampolis & Bargen (1951), Rapaport & Chapman (1959) and Gormsen (1961) described bleeding complications in prostatic carcinoma associated with thrombocytopenia, widespread microthrombi and coexistent hypercoagulability without evidence of fibrinolysis. These findings failed to support the concept of primary fibrinolysis as a cause of the observed haemorrhagic manifestations, and in conjunction with the documented hypofibrinogenaemia are strongly suggestive of disseminated intravascular coagulopathy (Colman, Robboy & Minna 1972; Merskey et al 1967; Rodriguez-Erdmann 1974). Straub (1971) described a 25 per cent incidence of prostatic cancer in his series of patients with disseminated intravascular coagulation (DIC).

Further support for this theory came from Straub, Riedler & Frick (1967) who described coexistent hypofibrinogenaemia and systemic fibrinolysis in patients with metastatic carcinoma of the prostate. Suppression of the

systemic fibrinolysis was repeatedly achieved by heparin. From this they concluded that the fibrinolysis was a normal protective physiological response secondary to intravascular coagulation (secondary fibrinolysis). This theory concurred with the findings of other authors (Samaha, Bruns & Ross 1973; Gans 1973) and Oliver et al (1991) found evidence of concommitant activation of coagulation and fibrinolysis in 40 patients with prostate cancer.

However Heinert et al (1988) later demonstrated an increase in urokinase-type plasminogen activator (uPA) levels in the serum of patients with metastatic carcinoma of the prostate, suggesting a possible mechanism for primary fibrinolysis in these cases.

Adamson et al (1994), by measuring D-dimer and fibrinopeptide-A levels, detected activation of the coagulation cascade and reactive fibrinolysis indicating compensated DIC in 40% of patients with untreated prostatic carcinoma and 16% of patients with BPH.

The exact mechanism of activation of coagulation in patients with prostatic carcinoma remains unclear. The overall picture may be further complicated by hormonal manipulation. Oestrogen therapy lowers antithrombin III activity (Büller et al 1982; Varenhorst, Wallentin & Risberg 1981), plasminogen and PAI activity (Aro et al 1990) and increases blood viscosity (Nandi & Knox 1986) producing a tendency towards hypercoagulability. The reduction in plasminogen and PAI activity found by Aro et al (1990) in the presence of activation of the clotting cascade suggests secondary fibrinolysis and low grade DIC. As this was not present in the orchidectomy group,the implication is that the cause is the exogenous oestrogen and not the prostate cancer.

Activation of the extrinsic pathway of the clotting cascade by increased expression of tissue factor on monocytes and malignant cells has been described in malignancy (Niemetz et al 1977; Szczepañski et al 1988). Van Deijk, van Dam-Mieras & Muller (1983) described elevated levels of activated factor VII in both patients with prostatic carcinoma and BPH, consistent with activation of the extrinsic pathway by tissue factor. Factor VII-independent activation of the coagulation system in malignancy has also been described (Gordon, Franks & Lewis 1975). Whether this occurs in prostatic disease is unknown, although the finding of reduced factor X activating procoagulant activity in malignant prostatic tissue (Adamson et al 1994) makes this unlikely.

1.5 Coagulation Changes during TURP

Lombardo (1958) described 5 cases of primary fibrinolysis and hypofibrinogenaemia out of 302 transurethral procedures (1.7%). Four of these cases were in patients with BPH. Using in vitro standard and heat-inactivated fibrin plates he demonstrated both fibrinolytic and proteolytic activity of benign and malignant prostatic tissue, and that the agents toluidine blue (3 amino-7-dimethyl-amino-2-methylphenazatheonium) and Klot (aqueous solution of 7 per cent n-butyl alcohol and 0.002 per cent toluidine blue in saline) inhibited this lysis. In a study of three groups of 30 patients undergoing TURP, he demonstrated a 20.3 per cent reduction in fibrinogen levels in the control group over the perioperative period. The reduction in fibrinogen levels was decreased in the toluidine blue and Klot

groups to 11.0 and 6.3 per cent respectively, supporting the authors theory of fibrinogenolysis as the cause of the hypofibrinogenaemia.

Elliot, McDonald & Fowell (1963) assessed plasma fibrinolytic activity in 48 patients undergoing TURP by means of the euglobulin lysis time. They found a higher fibrinolytic activity immediately after surgery in a significant number of cases, but plasma fibrinolytic activity 24 hours after surgery was significantly reduced. They found no correlation between fibrinolytic activity and peroperative blood loss.

Following these early reports of fibrinolysis several authors investigated the role of antifibrinolytic agents as haemostatic agents in patients undergoing TURP. Epsilon aminocaproic acid (EACA), a synthetic amino acid with a similar structure to lysine, is a potent inhibitor of plasminogen activation and urinary urokinase activity (Alkjaersig, Fletcher & Sherry 1959). McNicol et al (1961b), Andersson (1964), Sack et al (1962) and Vinnicombe & Shuttleworth (1966a) all described a significant reduction in postoperative blood loss after open prostatectomy, with no increase in thrombotic complications (Vinnicombe & Shuttleworth 1966b). However Pearson (1969) found no such benefit with either EACA or the protease inhibitor aprotonin (Trasylol). McNicol et al (1961b) and Madsen & Strauch (1966) showed a similar significant reduction in postoperative blood loss in patients undergoing TURP, but no significant reduction in peroperative blood loss could be demonstrated. Kösters & Wand (1973) could only demonstrate a significant reduction in postoperative bleeding after TURP when the EACA was given in combination with aprotinin.

In a prospective randomised study of 80 patients undergoing TURP, Sharifi et al (1986) compared 0.5% EACA solution with 0.9% saline as a

postoperative bladder irrigant in an attempt to inhibit urinary urokinase locally, thus avoiding any potential systemic side effects. However they found no significant difference in postoperative blood loss between the two groups and concluded that early postoperative bleeding was largely due to technical error and not fibrinolysis.

Tranexamic acid (trans p-aminomethyl cyclohexane carboxylic acid, AMCA) acts in a similar way to EACA, by inhibiting plasminogen activation, but is 8 times more potent than EACA (Andersson et al 1968). Hedlund (1969) described a reduction in postoperative blood loss with AMCA after prostatectomy. However, following the withdrawal of EACA because of instability in solution, Ward & Richards (1979) cautioned against the use of AMCA in the early perioperative period in TURP, following 3 cases out of 6 patients receiving AMCA who developed indissoluble bladder clots requiring surgical evacuation.

Miller et al (1980) investigated the use of oral AMCA in preventing secondary haemorrhage following TURP. They found a significant reduction in patient reported bleeding in the first 4 weeks following discharge from hospital, but no significant increase in the number of re-admissions for bleeding complications.

Several authors have studied coagulation parameters over the perioperative period in patients undergoing TURP in an attempt to predict those patients likely to bleed excessively. Mertens et al (1974) investigated the preoperative fibrinolytic and coagulation activity in 42 patients undergoing TURP by measuring fibrinolytic split products (FSP) and the presence of soluble fibrin intermediates (ethanol gelation test) respectively. Eighty-six per cent of patients with BPH and 85% of those

with carcinoma had elevated FSP values, indicating fibrinolytic activity preoperatively in a significant number of poeple.The ethanol gelation test was strongly positive in 42% (5) of carcinoma patients assessed but only 8% (1) with BPH. However the test was weakly positive in 38% (5) with benign histology. They concluded that a degree of intravascular coagulation and fibrinolysis was occurring preoperatively in these patients, and that bleeding was greater in patients with positive test results. Both tests are usually abnormal in patients with DIC, although the specificity of the ethanol gelation test has been questioned (Kisker & Rush 1971).

Rader (1978) performed a coagulation screen (prothrombin time, partial thromboplastin time, serum fibrinogen and platelet count) preoperatively in 165 patients with operative prostatic disease. He found hyperfibrinogenaemia in 29% of patients and concluded this was a non-specific stress response. He described a local fibrinolytic reaction in one out of 77 patients undergoing TURP on the basis of the patients normal clotting screen and rapid response to EACA. Apart from 2 cases of thrombocytopenia in patients with prostatic carcinoma, no other abnormalities in the coagulation screening tests were recorded, and he concluded that preoperative haematological screening was useful in only a small (3.7 per cent) number of cases.

Betkurer et al (1979) compared the effects of TURP on coagulation in benign and malignant prostates. They performed a full clotting screen (haematocrit, prothrombin time, partial thromboplastin time, fibrinogen, FSP, factors II, V, VII, VIII, IX, X, XI and XII) preoperatively and within 24 hours postoperatively on 11 carcinoma and 10 BPH patients undergoing TURP. They found no statistically significant difference between

preoperative and postoperative values in any of the coagulation parameters in either prostatic carcinoma or BPH groups. There was a decrease in fibrinogen and an elevation in FSP in both groups after TURP suggestive of potential incipient DIC which they felt may be biologically rather than statistically significant. However this remains conjecture.

In a larger prospective study de Campos Freire et al (1986) similarly found no statistically significant difference outside the normal range of the clotting parameters (haematocrit, prothrombin time, thrombin time, partial thromboplastin time, fibrinogen, fibrin split products, recalcification time and platelet count) measured 24 hours postoperatively compared to preoperative values in either carcinoma or BPH groups. However they too found a trend within the normal range towards hypofibrinogenaemia and increased FSP, but only in patients with prostatic carcinoma. They concluded that this showed a tendency to increased fibrinolysis in prostatic carcinoma patients, explaining the increased blood loss per gram of prostate tissue resected in carcinoma patients that they detected. In the BPH group, they detected a prolongation in mean prothrombin time and an increased mean fibrinogen levels (although within normal limits) postoperatively, when compared to preoperative values.

Iwan-Zi:etek et al (1992) prospectively studied the coagulation changes in 40 patients undergoing TURP. They found evidence of activation of the fibrinolytic system preoperatively (increased tPA, reduced PAI-1 and raised fibrin degredation products). Surgery had the effect of increasing the fibrinolytic activity (increase in tPA, reduced PAI-1, increase in fibrin degradation products, a decrease in plasminogen and α2-antiplasmin and a prolonged euglobin clot lysis time), with a gradual return to normalisation

postoperatively. A finding of positive ethanol gelation tests and a significant decrease in mean platelet count over the perioperative period led them to the conclusion that activation of the coagulation system was occuring, with secondary fibrinolysis (subclinical DIC) due to tissue thromboplastins released during surgery. Von Hundelshausen et al (1992) concurred with these findings in a similar study of 11 TURP and 12 retropubic prostatectomies. They found no significant difference between the two groups, and concluded that the routine use of antifibrinolytic agents could not be justified.

Ahsen, Cartner & English (1993) investigated the value of routine coagulation tests (prothrombin time, activated partial thromboplastin time) taken immediately after surgery in predicting excessive blood loss after prostatic resection. They found that 35% (39) of their patients had an abnormal prothrombin time (PT) immediately after surgery, and this had returned to normal in all cases within 24 hours. They found a significant correlation between abnormal PT and excessive blood loss postoperatively independent of the weight of tissue resected. In those patients where both tests were abnormal, all had excessive bleeding.

The concept of absorption of prostatic substances into the systemic circulation during TURP was favoured by O'Donnell (1984), who showed a correlation between symptoms of early transurethral resection (TUR) syndrome and elevated serum acid phosphatase levels. In an elegant study in dogs, O'Donnell (1990) produced a significant reduction in platelet count, fibrinogen level, white cell count and activated clotting time for up to 60 minutes after intravenous infusion of human prostatic tissue extract when compared to dogs infused with normal saline. Serum acid

phosphatase values in the infused prostate tissue extract group were elevated to a level comparable to those seen in theTUR syndrome (O'Donnell 1983). He concluded that tissue thromboplastins released into the circulation during TURP by the absorption of irrigant fluid (Madsen et al 1970) produced a transient disseminated intravascular coagulation.

1.6 The Haemostatic Response To Surgery

The effects of a surgical procedure on the coagulation and fibrinolytic system has been quite extensively studied mainly in the context of postoperative venous thrombosis, in an attempt to predict those patients at highest risk.

Significant but variable alterations in coagulation and fibrinolytic parameters over the perioperative period have been reported. The presence of an hypercoagulable state postoperatively is now accepted (Baker 1995). The cause of this hypercoagulability is less clear.

The stress response initiated by surgery has for many years been known to modulate the coagulation and fibrinolytic systems (Cannon & Gray 1914; Macfarlane 1937). Infusion of physiological levels of adrenaline increases factor VIII and fibrinopeptide A levels indicating activation of the clotting cascade (Ingram & Jones 1966; Swedenborg & Olsson 1978).

High physiological levels of endogenous vasopressin (aVP) are known to occur intra- and postoperatively, and are thought to be responsible for the reduction in urine output and fluid retention commonly seen postoperatively (Le Quesne & Lewis 1953). Exogenous aVP administered to healthy volunteers at the physiological levels found perioperatively,

increases factor VIII (FVIII) and plasminogen activator activity with shortening of the fibrinopeptide A (FPA) generation time suggesting the induction of a procoagulant state with thrombin generation (Grant et al 1985).

Grant et al (1986) described in 7 patients undergoing major abdominal surgery a sharp elevation in endogenous aVP during bowel manipulation perioperatively, with levels remaining elevated for the first postoperative day. Immediately after and in parallel with this rise in aVP, they found a coexistent rise in FVIII activity, von Willibrand antigen, the ristocetin co-factor, plasminogen activator activity and fibrinopeptide A concentrations with shortening of the APTT indicating the presence of a procoagulant state. They concluded that aVP was likely to be responsible for this hypercoagulability. Wilson et al (1988) found a similar increase in endogenous aVP followed closely by a rise in FVIII and fibrinolytic activity in patients undergoing hip surgery. Vasopressin is also known to have proaggregatory effects on platelets in vitro (Haslam & Rosson 1972).

The concept of a 'postoperative fibrinolytic shutdown' following surgery was first proposed by Chakrabarti, Hocking & Fearnley (1969). Mansfield (1972) confirmed this finding and described an earlier and greater inhibition of fibrinolysis in those patients who developed deep vein thrombosis. Several authors have subsequently reported a similar reduction in fibrinolytic activity both preoperatively and postoperatively, although the correlation with the development of deep venous thrombosis remains contentious (Knight, Dawson & Melrose 1977; Rákóczi et al 1980). Reilly, Burden & Fossard (1980) actually found an increase in

fibrinolytic activity postoperatively in those patients developing venous thrombosis.

The mechanism of 'fibrinolytic shutdown' has not been completely clarified. Griffiths, Woodford & Irving (1977) suggested that this was due to either exhaustion or defective synthesis of plasminogen activators by the vascular endothelium postoperatively. However D'Angelo et al (1985) showed that the vascular endothelial response to desamino-D-arginine vasopressin - which stimulates the release of t-PA from endothelium (Mannucci & Rota 1980) - was preserved postoperatively, refuting this theory.

An imbalance between the rapid postoperative increase in plasminogen activator inhibitor (PAI-1) and a corresponding decrease in tissue plasminogen activator (tPA) levels has been postulated (Kluft et al 1985; Aranda, Paramo & Rocha 1988). D'Angelo et al (1985) confirmed the postoperative reduction in euglobin fibrinolytic activity and specifically t-PA activity associated with an increase in PAI-1 without a corresponding reduction in tPA antigen. Eriksson, Eriksson & Risberg (1991) found a significant correlation between increased preoperative PAI-1 activity and the development of deep vein thrombosis postoperatively. Mellbring et al (1984) found elevated levels of tPA antigen in patients with postoperative venous thrombosis.

A fall in platelet count can be anticipated in the early postoperative period (Hawkey et al 1983). Impairment in haemostasis can occur when circulating platelet count levels fall below 100×10^9 litre^{-1} (Murphy, Davies & Eduardo 1993). Following the initial fall in platelet count in the first few days postoperatively, an increase in platelet count above normal levels

can be expected during the second week (Potts & Pearl 1941; Warren 1953; Pepper & Lindsay 1960; Ygge 1970; Feruglio, Sandberg & Bellet 1960). Increased turnover of platelets as well as fibrinogen perioperatively has also been documented and may explain the initial fall in platelet count (Slichter et al 1974).

O'Brien, Etherington & Jamieson (1971) demonstrated a marked reduction in platelet aggregation response to adenosine diphosphate and collagen immediately postoperatively. Platelet aggregation may be inhibited by proteolytic degradation of the GP1b platelet receptor during cardiopulmonary bypass surgery (van Oeveren et al 1990) or by prostacyclin during aortic aneurysm surgery (Krausz et al 1983). O'Brien et al (1974) and Bennett (1967) demonstrated an increase in platelet reactivty and adhesiveness over the perioperative period, and Emmons & Mitchell (1965) described an increase in platelet clumping at 10 days and one month postoperatively compared to preoperatively.

1.7 Coagulopathy in Sepsis

Instrumentation of the urinary tract alone is commonly associated with a gram negative bacteraemia in elderly patients (Vasanthakumar 1990). Previous studies have shown consistently a perioperative bacteraemia in 27 to 36% of patients undergoing TURP (Sullivan et al 1973; Robinson et al 1980; Robinson et al 1982; Harvey et al 1986). Robinson et al (1982) described an 8% incidence of septicaemia in those patients with bacteraemia following TURP. Reported fatality rates for Gram-negative septicaemia range from 13.7% to 30.8% (Bryan & Reynolds 1984a; Bryan & Reynolds 1984b).

Blandy (1972) first documented the link between urinary tract infection and secondary haemorrhage following TURP. Harvey et al (1986) found a significant correlation between secondary haemorrhage and urine infection at catheter removal following TURP, but failed to show any reduction in bleeding with the use of antibiotic prophylaxis (co-trimoxazole for 10 days following catheter removal). Robinson et al (1982) cultured the same organism from the prostate as was found in the blood in 77% of symptomatic bacteraemic patients following TURP. Morris et al (1976) found positive prostatic chip cultures in 64% of patients with sterile urine preoperatively. The European collaborative study of antibiotic prophylaxis for transurethral resection of the prostate (Hargreave et al 1993) demonstrated a highly significant reduction in postoperative urinary tract infection in patients with sterile urine preoperatively with the use of prophylactic antibiotics perioperatively. Forty-seven per cent of the organisms cultured in those patients not receiving antibiotics were Gram-negative organisms.

The presence of a Gram-negative bacteraemia over the perioperative period in patients undergoing TURP implies a coexistent endotoxaemia. Extensive experimental evidence exists in both animals and man that many of the clinical features of Gram-negative septicaemia are due to endotoxaemia (Hinshaw 1983; Hale et al 1986). Endotoxins are the lipopolysaccharide (LPS) constituents of the outer membrane of Gram-negative bacteria. The LPS consists of a polysaccharide and a covalently bound lipid known as Lipid A which has an uniform structure amongst sub-species of Gram-negative organisms. Lipid A is thought to mediate most of the biological effects of endotoxin (Morrison & Ulevitch 1978; Morrison & Ryan 1987).

Amongst its many biological effects, endotoxin is known to activate the extrinsic coagulation pathway (Levy et al 1985), lead to induction of platelet-activating factor (Doebber, Wu & Robbins 1985) and a monocyte and macrophage procoagulant (Osterud & Flaegstad 1983; Maier & Hahnel 1984), producing a procoagulant state. A consumptive coagulopathy is a common sequalae in septic patients (Voss et al 1990). The effects of endotoxin appear to be mediated by endogenous glycoproteins known as cytokines. Of these cytokines, tumour necrosis factor-α (TNF-α, cachectin) appears to have a central role as mediator of endotoxin-induced coagulation activation (van Deventer et al 1990; Bauer et al 1989; Levi et al 1991a).

Activation of the coagulation cascade in sepsis appears to be via the extrinsic route (Levi et al 1991a; van Deventer et al 1990), although high doses of endotoxin can directly activate the intrinsic system via activation of factor XII (Kalter et al 1985); activation of the intrinsic contact system

may play a role in triggering the fibrinolytic system (Levi et al 1991b). The mechanism of activation of factor VII (the extrinsic pathway) maybe by the increased expression of tissue factor on the surface of vascular endothelial cells and blood monocytes induced by endotoxin, TNF-α and interleukin 1 (IL-1) (Rivers, Hathaway & Weston 1975; Colucci et al 1983; Lyberg et al 1983; Nawroth et al 1986; Bevilacqua et al 1986).

Activation of the fibrinolytic system in septic patients has traditionally been considered to be secondary to activation of the coagulation cascade (Marder et al 1987). However activation of fibrinolysis may occur independently of the coagulation cascade at least in the chimpanzee (Levi et al 1992).The endotoxin-induced activation of fibrinolysis also appears to be mediated by TNF-α, acting to increase plasminogen activator activity by the release of t-PA and u-PA from endothelial cells (Suffredini, Harpel & Parillo 1989; Levi et al1991). Voss et al (1990) demonstrated activation of the fibrinolytic system in septic patients, but the overall systemic fibrinolytic capacity was markedly reduced due to an increase in the levels of the inhibitors of fibrinolysis (PAI-1, α_2-antiplasmin). This imbalance between activation of the coagulation system and inhibition of the fibrinolytic system producing a procoagulant state has obvious analogies to the postoperative state of 'fibrinolytic shutdown'.

The procoagulant effects of endotoxin and TNF-α are increased by its inhibitory effects on the anticoagulant protein C protein S system. Tumour necrosis factor alpha acts by inhibiting expression of the endothelial surface protein thrombomodulin, which complexes with thrombin to activate protein C (Nawroth & Stern 1986; Conway & Rosenberg 1988).

Chapter 2

Study Methodology

2.1 Introduction

In order to assess the coagulation changes and the role of fibinolysis in transurethral prostatectomy, a study of patients undergoing TURP was set up with the aim of measuring systemic and local i.e urinary fibrinolytic activity and the effects of uncomplicated transurethral prostatic surgery on coagulation.

The proposed hypothesis for the study was that fibrinolysis has an aetiological role in primary or secondary haemorrhage in patients undergoing TURP for both benign and malignant prostatic disease. The primary end point of the study was taken as the presence of detectable increase in mean systemic fibrinolytic activity over the perioperative period, as measured by thrombelastography. Thrombelastographic systemic fibrinolysis was defined as a percentage clot lysis (Ly_{60}) of greater than 15 percent (Mallett & Cox 1992). In order to detect a difference in mean Ly_{60} over the perioperative period of only one percent, assuming a standard deviation of up to one, an estimated sample size of 36 patients was required for a type 1 error (α) of 0.01 and giving a power for the study of 95 percent (table 2.1).

Local Ethical Committee approval was therefore sought and obtained for a prospective study into the coagulation changes occuring in a group of 40 men undergoing transurethral prostatectomy.

	α (two sided)			
	0.10	0.05	0.02	0.01
β				
0.2	13	16	21	24
0.1	18	22	27	30
0.05	22	27	32	36

Power = 1 - β

Table 2.1: Study sample sizes for varying type I and II errors.

2.2 Methods

After informed consent the study group was selected from male patients admitted routinely under the care of one Consultant Urological Surgeon for a TURP. Exclusion criteria included a known coagulopathy (including a previous history of excessive bleeding following tooth extraction or other surgery and previous venous thrombosis), aspirin ingestion 14 days prior to admission (Watson et al 1990), poorly controlled hypertension, uraemia, abnormal liver function tests, known hyperlipidaemia, heavy smokers (greater than 5 cigarettes per day), significant cardiovascular or respiratory disease and difficult phlebotomy.

Each patient acted as their own control by using their preoperative values as the baseline against which any change over the perioperative period could be compared. Although it can be argued that there may be variable changes in the coagulation status preoperatively in some patients with both benign and malignant prostatic disease (Adamson et al 1994), this in itself was considered to be of significant interest, and it is the change in

coagulation status over the perioperative period that is of importance rather than the value of the indices taken in isolation.

The TURP was performed under a standardised anaesthetic protocol (temazepam 20mg premedication; general anaesthetic with propofol 1-2mg/kg induction, laryngeal mask airway, 1-2% enflurane with nitrous oxide and oxygen maintenance and a caudal block using 15-20 ml of Marcaine 0.25%). All patients received antibiotic prophylaxis perioperatively (gentamicin120 mg intravenously at induction). Body temperature was measured sublingually preoperatively and postoperatively in recovery. Euvolaemic maintainance intravenous fluid replacement with normal saline was initiated peroperatively, and continued until the morning after the operation.

The procedures were performed by one Consultant Urological Surgeon or one of two Urological Higher Surgical Trainees under direct Consultant supervision with a ratio of 1:3¼ respectively.

All resections were performed with a standard Stortz 24Fr or 27Fr resectoscope, using interrupted irrigation with 1.5% glycine at 25^0C peroperatively and normal saline intracystic irrigation at room temperature postoperatively. Duration of the procedure was recorded.

Venous Sampling

Serial venous blood sampling was performed preoperatively, intraoperatively (20 minutes from the start of the resection), 3, 6, 24, 48, 72 hours and 10 to 14 days postoperatively. The preoperative and postoperative (days 1,2,3 and 10 to 14) sampling was performed mid-morning in order to minimise the effect of the circadian variation in

fibrinolyitc activity (Fearnley, Balmforth & Fearnley 1957). To minimise venepuncture artefacts of coagulation, all blood sampling was performed by one operator, using repeated venepuncture and a two syringe technique - the initial sample being used for blood count analysis and other non coagulation tests. Atraumatic cuffed antecubital venous blood samples were taken using sterile 19 guage needles and 20ml syringes. 0.36 ml of venous whole blood was placed in the thrombelastograph analyser cup and the thrombelastgram (TEG) tracing started 6 minutes after venesection, in order to standardise the r time.

Serial clotting screen evaluations were performed on 30 patients throughout the perioperative period. This included the one-stage prothrombin time (PT) (Quick 1937) and International Normalised Ratio (INR), the activated partial thromboplastin time (APTT/PTTK) (Chanarin 1989), platelet count and plasma fibrinogen levels (24 patients).

Nine millilitres of venous whole blood was immediately added to 1.0ml 0.109M tri-sodium citrate, gently mixed and immediately placed in iced water until centrifugation at 2000 G for 20 minutes at 4^0C. The resulting plasma was centrifuged as before and the supernatant subaliquoted into 0.4ml samples and stored at - 40^0C until assayed.

Fibrinogen ELISA Materials and Methods.

The ELISA used a commercial antiserum preparation, sheep anti-human fibrinogen (Serotec , Kidlington, Oxon, UK cat no AHP061). This was split into 2 equal lots, one portion was dialysed into PBS 0.01% sodium azide, and used as the coat antibody. The second portion was biotinylated. This method has been modified from that of Hnatowich, Virzi & Rusckowski

(1987). Purified IgG was dialysed overnight at 4°C against 1L of 0.1M phosphate buffer pH 8.0. Protein concentration of the dialysate was determined by a dye binding protein assay (Bradford 1976) (Biorad, Hemel Hempstead, UK). Standards for the protein assay were made from rabbit IgG (Sigma, Poole, UK). 1.0mg per 1.3 mg IgG of biotin reagent (NHS-LC-Biotin, Pierce and Warriner, Chester, UK) was weighed out and dissolved in 560µl of moisture free N,N-dimethylformamide (DMF, stored under nitrogen, Aldrich, Poole, UK) to give a ratio of biotin to IgG of 20:1. An appropriate volume of the biotin solution was added to the IgG and the solution left to stand at 4°C overnight. It was then dialysed overnight at 4°C against 1L of PBS-0.1% azide pH 7.4. The biotinylated IgG may be stored at 4°C or in aliquots at -20°C. Biotinylated IgG is then used in conjunction with horse-radish peroxidase/streptavidin (HRP-SA, ICN, Thame, UK) as the tracer component in the ELISA assay. Standards were made from the first international standard for fibrinogen, (89-644) (NIBSC, Potters Bar, UK), and a standard curve established with points at 3, 2.4 ,1.8, 1.2, 0.6, 0.3, 0.15 and O (buffer only) mg/mL. Samples were diluted 1:200 and 1:400. Quality assurance (QA) samples made from a 20 normal plasma pool, treated as samples were used in every assay. Microtitre plates ("Maxisorb", Nunc, through Life Technologies, Paisley UK) were coated with 100µl/well of coating antibody diluted in 0.05M carbonate-bicarbonate buffer pH 9.6, and incubated at 4°C overnight. The plates were then washed five times with 0.01M phosphate buffer pH 7.4, containing 0.5M sodium chloride, and 0.1% Tween 20 (wash buffer), using an automatic plate washer. Samples and the detection antibody were diluted in wash buffer containing 3% PEG 8000, which has been shown to facilitate protein binding to immobilised

antibody (Woodhams and Kernoff, 1983). Sample, QA and standards were incubated (100μl/well) for one hour at room temperature on a plate shaker at 400 RPM. After a further five washes, 100μl of working strength detection antibody was added to each well, and incubated for a further hour on the shaker at room temperature. After further washing, the reaction was visualised by the addition of 100μl/well of 0.1M citrate/acetate buffer containing one 10mg tablet of OPD (o-phenylenediamine dihydrochloride, Sigma Chemical Co, Poole, UK) and 7μl 30% hydrogen peroxide per 15ml. The reaction was stopped after approx. 10 minutes with 100μl/well 1.5M sulphuric acid. Plates were read at 492nm on a Titretek MS-2 reader (ICN, Thame, UK), with automatic linear spline curve fitting and data analysis.
Four millilitres of blood was added to heparinised endotoxin free bottles (EndoTube ET® Chromogenix, Sweden) transported and centrifuged as before, but stored in the endotoxin free bottles at - 70^0C until assayed.
The initial blood sample was used to measure serial full blood counts including haematocrit, preoperative and intraoperative serum prostate specific antigen (Hybritech Tandem-R PSA radioimmunoassay) and Prostatic acid phosphatase (L-tartrate enzyme assay; normal range 2-4 u/l). Urea, creatinine and electrolytes were measured preoperatively and at 6 hours postoperatively.
Blood cultures were taken peroperatively for both aerobic and anaerobic culture (BacTAlert, Organon Teknika Corporation; supplemented tryptic soy broth with SPS) using standard techniques, from the antecubital veins after the skin had been prepared with 70% isopropyl alcohol and allowed to dry. Prostatic tissue was washed initially in sterile saline and then incubated in 20ml of Brewers medium for 24 hours and then subcultured

onto blood agar and incubated at 37° C aerobically for 24 hours and anaerobically in 90% hydrogen and 10% carbon dioxide for 48 hours.

Urine sampling

Urine sampling was performed preoperatively, at 48 hours (or on discontinuation of the intracystic irrigation if later) and 10 to 14 days postoperatively. A midstream urine (MSU) sample was obtained preoperatively and at 10 to 14 days postoperatively. On POD 2 a catheter specimen (CSU) sample was taken from the catheter valve. A 1ml aliquot of the urine sample was then immediately stored at - 40^{0}C until assayed. The remainder of the samples were then sent for microbiological microscopy, culture and antibiotic sensitivity using standard techniques.

Blood Loss

The Steri-Drape™ (3M) TUR Urological drape closed system was used peroperatively in each case to minimise irrigant fluid and blood spillage. Perioperative blood loss was measured until removal of the catheter by photometric analysis of the haemoglobin content in the irrigant fluid (Jansen, Berseus & Johansson 1978), complete haemolysis being acheived in this case by the freeze-thaw technique. Photometric analysis was performed at 550nm wavelength using the Titertekplus© analyser and 96 well plates and a 30 second agitation time.

A standard solution of blood was created by diluting 50 ml of bank whole blood (haemoglobin 13.2g/dl) in 1000ml of normal saline, subaliquoted and stored at - 40^{0}C. A standard curve of haemoglobin concentration for each of the 96 well plates was generated using serial dilutions of the

standard bank blood solution plotted against optical density at 550nm to give a linear relationship (mean correlation coefficient $r^2 = 0.998$, range 0.993 - 1.000).

The blood volume in each collect irrigant sample could thus be calculated by the equation (Hahn 1987):

$$\text{Blood volume (ml)} = \frac{\text{concentration} \times 0.132 \times \text{volume of irrigant sample}}{\text{Patients Hb value at the time of sampling} \times 100}$$

Total blood loss for each patient therefore being the sum of the blood volumes of all the collected irrigant samples. Duplicate wells for each sample were analysed in all cases and the mean of the two values used. Assay error was calculated by measuring the coefficient of variability:

$$\text{Coefficient of variation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100\ (\%)$$

The inter-assay and intra-assay coefficients of variability were 3.6 and 4% respectively.

All the irrigant effluent was collected in a 30 litre capacity plastic container and the volume measured on completion of the TURP, at 6 hours postoperatively, and then daily until removal of the catheter. After measurement of the volume, the container was agitated to ensure adequate mixing of blood, and a 5ml sample taken and stored at -20^0C until analysis. The remainder was decanted through a sieve into a one litre

capacity graduated measuring cylinder to accurately measure total irrigant volume, and then discarded. Clot volume collected in the sieve was measured using a 20ml graduated measuring cylinder and recorded. To prevent further clotting within the container during collection 15,000 iu of standard heparin was placed into the container at the start of each collection.

The blood transfusion rate, serial haemoglobin measurement and haematocrit estimation were documented for each patient.

Irrigant Fluid Absorption

The fact that significant quantities of irrigant fluid can be absorbed into the circulation during the course of a TURP is universally accepted (Hahn & Ekengren 1993) and was first described by Creevy (1947). Using radioisotopes Oester & Madsen (1969) showed greater than a litre of irrigant fluid was absorbed in over 40 per cent of men undergoing TURP, and Norlén et al(1986) by measuring serum glycine levels found evidence of absorption in all patients undergoing TURP.

Several different techniques have been used to quantify the amount of irrigant fluid absorption that occurs peroperatively including a significant decrease in serum sodium concentration (Logie et al 1980; Allen et al 1981; Hjertberg, Jorfeldt & Schelin 1991), changes in serum acid phosphatase levels (O'Donnell 1984) and measurement of expired ethanol in patients irrigated with glycine 1.5% and ethanol 1% (Hahn & Ekengren 1993).

A pilot study of irrigant fluid absorption in 22 patients undergoing TURP was carried out using the expired breath ethanol method as described by

Hahn (1988). The procedures were performed under spinal anaesthesia using ethanol glycine irrigant (Baxter Healthcare Ltd, Newbury, UK) and the Lion Alcometer S-D2. Despite repeated evidence in the literature of significant absorption of irrigant fluid in over 40 per cent of cases using this technique (Hahn & Ekengren 1993; Oester & Madsen 1969; Hjertberg, Jorfeldt & Schelin 1991), absorption of irrigant fluid could not be detected in any patient by this method. For this reason and the use of general anaesthesia, this method was not utilised in the study.

Accurate measurement of the patient's weight before and immediately after the procedure as a marker of fluid absorption (Hagstrom 1955) was considered, but found to be impractical, unnecessarily disruptive and unkind to this largely elderly group of patients in the recovery setting.

In this study therefore evaluation of irrigant fluid absorption was performed by serial measurement of serum sodium concentration (n=37 patients). An acute fall in serum sodium of 5 - 8 mmol/L represents the uptake of one litre of irrigant fluid (Hahn 1996). Perioperative changes in haematocrit values (n=38 patients) and acid phosphatase and prostate specific antigen levels (n=25 patients) were chosen as additional confirmatory markers of irrigant fluid absorption.

Statistical Methods

Data collection and statistical analysis was performed using the GraphPad Instat ™ (Copywright © 1990-1993 GraphPad Software) statistical software program. Non-parametric statistical methods were applied. Fluctuations over the sampling period were evaluated by Analysis of Variance (ANOVA) using Freidman's and Kruskal-Wallis analyses.

Differences between median values were calculated using Dunn's multiple comparison test, and the Wilcoxon matched pairs test. A p value of < 0.05 was considered to indicate statistical significance. Correlation was calculated using Spearman's rank-order correlation test. Data is tabulated and presented graphically as the mean $\pm$ the standard error of the mean (SEM).

2.3 Results

A total of 62 patients were assessed for suitability for the study. Eleven patients were excluded from the study preoperatively on initial assessment (4 patients refused consent, 2 had ingested aspirin within 14 days of admission, 3 had raised creatinine levels preoperatively and 2 patients smoked heavily). One patient withdrew consent immediately postoperatively. Two patients underwent spinal anaesthesia. Four patients were withdrawn postoperatively because of difficult venepuncture. Equipment failure (defective thrombelastographic thermostat) prevented serial TEG analysis in 2 patients. One patient developed unstable angina postoperatively and was withdrawn. Thus 41 patients completed the study. One patient underwent only a cystourethroscopy at the time of planned TURP, but agreed to continue with serial sampling. The histology of the prostatic chips in the 40 patients undergoing TURP showed benign prostatic hyperplasia in 30 cases, benign prostatic hyperplasia with prostatic intraepithelial neoplasia (PIN) in 2 cases (one high and one low grade PIN) and prostatic adenocarcinoma in 8 cases. Six of these had documented T3 prostatic adenocarcinoma preoperatively, and 2 patients

were found to have co-existent prostatic carcinoma (one staged T1a and one T1b) on histological analysis of the prostatic chippings.

The mean age of the patients was 71 years (range 57-93). Nine patients had an indwelling urethral catheter preoperatively for at least 72 hours.

No significant change in mean body temperature was recorded over the perioperative period (mean temperature preoperatively and postoperatively in recovery was 36.7 and 36.5 degrees centigrade respectively; Wilcoxon matched matched pairs test $p = 0.2$)

The mean duration of transurethral resection was 37 minutes (range 15 - 72). The mean weight of prostate tissue resected was 27 grams (range 5.5 - 89). Total blood loss and clot volume could not be assessed in two patients, because complete overnight collection of all the irrigant fluid and bladder washout fluid could not be guaranteed. The mean total blood loss for the remaining 38 patients who underwent resection was 693 ml (range 60 - 2554). This gives an overall mean blood loss per gram of prostate resected of 29.0 ml/g (range 5.8 - 126) and mean blood loss per gram per minute of resection time of 0.813 ml/g/min (range 0.006 - 2.614).

A significant correlation between blood loss and weight of prostate tissue resected was found (Spearman's rank correlation, $r_s=0.5497$ $p=0.0004$).

Clot volume was measured in 38 patients with a mean of 14 ml (range 0 - 60). There was a significant correlation between blood loss per gram of prostate tissue resected and clot volume (Spearman's rank correlation, $r_s = 0.5440$, $p = 0.004$; figure 2.1).

Five out of the 40 patients (13 per cent) undergoing resection required a blood transfusion of 3 units or less. All had a blood loss exceding 600 ml.

A significant decrease in mean venous whole blood haemoglobin

concentration occured at all postoperative sampling times when compared to mean preoperative concentration (ANOVA $p<0.0001$, Dunn's multiple comparison test $p<0.01$; figure 2.2).

There was a significant decrease in mean serum haematocrit levels at all postoperative sampling times including 10-14 days, when compared to the mean preoperative value (ANOVA $p<0.0001$, Dunn's multiple comparison post test $p<0.01$; figure 2.3a).

There was a significant fall in mean serum sodium concentration at 24 hours postoperatively compared to the mean preoperative concentration (2.6 mmol/l; Wilcoxon matched pairs test two-tailed p value = 0.0004, figure 2.3b). However in only one case did the plasma sodium fall below 130 mmol/l. No correlation between fall in plasma sodium level and blood loss was seen (Spearman's rank correlation $r_s = -0.002$, $p = 0.99$; figure 2.4).

A marked increase in both mean intraoperative serum prostate specific antigen (PSA) (19 ng/ml; Wilcoxon matched pairs test $p<0.0001$;figure 2.3c) and serum acid phosphatase (27 IU/l; Wilcoxon matched pairs test $p<0.0001$;figure 2.3d) levels when compared to the mean preoperative values was found.

There was a significant fall in mean platelet count from intraoperatively until 48 hours postoperatively, when compared to the preoperative base line mean (ANOVA $p<0.0001$, Dunn's multiple comparison test $p<0.05$). The platelet count rose significantly at 10 - 14 days postoperatively (figure 2.5).

There was no significant variation in prothrombin time (PT) and thus INR throughout the perioperative period in the 30 patients assessed

(figure 2.6). However there was a significant decrease in mean activated partial thromboplastin time (APTT) at 72 hours and 10 - 14 days postoperatively, compared to the preoperative mean APTT (ANOVA $p=0.0062$, Dunn's multiple comparison test $p<0.02$; figure 2.7).

The intra-assay coefficient of variability calculated for the fibrinogen ELISA assay was 16%. There was a significant variation in mean plasma fibrinogen levels over the perioperative period (ANOVA, $p=0.011$; figure 2.8). There was a gradual trend towards increased mean fibrinogen levels from 3 hours postoperatively, reaching statistical significance at 10-14 days postoperatively when compared to mean preoperative fibrinogen (Dunn's multiple comparison test, preop vs 10-14 days $p<0.05$). A fall in mean fibrinogen level was seen at 3 hours postoperatively but this was not statistically significant when compared to mean the preoperative value.

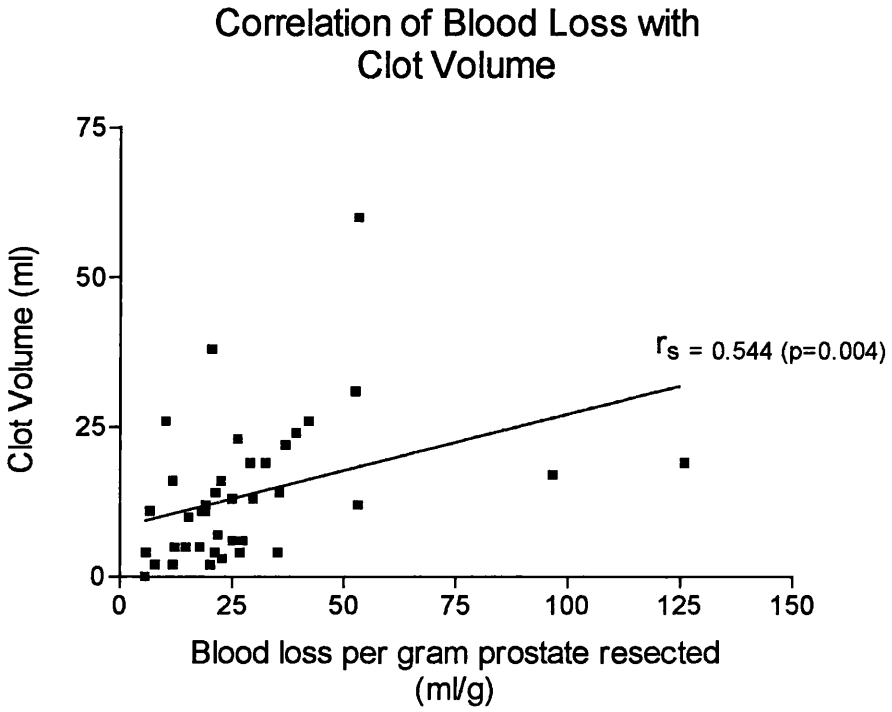
Six patients had a urinary tract infection preoperatively (4 had indwelling urethral catheters). Positive blood cultures peroperatively were found in 4 cases. In three of the positive blood cultures an identical organism was cultured from the prostatic chips, two of whom had catheters preoperatively. A total of six patients had a positive prostatic chips culture. Four patients had persistent urinary infection at 10-14 days (table 2.2).

Preoperative MSU / CSU	Removal of Catheter CSU	10-14 days MSU	Blood Culture	Prostatic Culture	Catheter?
E coli		E coli	E coli	E coli	Yes
			Propriono-bacterium		Yes
Proteus		Coag. -ve staph.		Proteus	yes
				Streptococcus viridans	No
				E coli	No
Coag. -ve staph.		enterococcus	Coag. -ve staph	Coag. -ve staph.	No
Coag. -ve staph.		E coli			Yes
	enterococcus		enterococcus	enterococcus	Yes
Heavy mixed growth					Yes

Table 2.2: Infective organisms cultured from urine, blood and prostatic tissue.

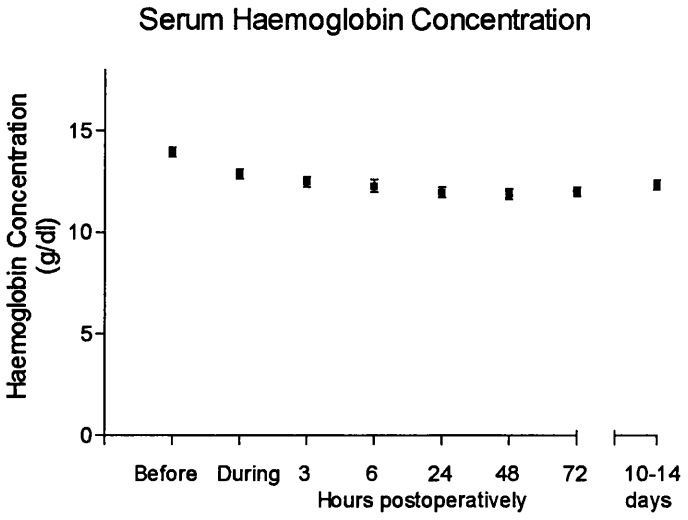
Coag. -ve staph. = Coagulase negative staphylococcus

E coli = Escherichia coli. A gap represents no growth.



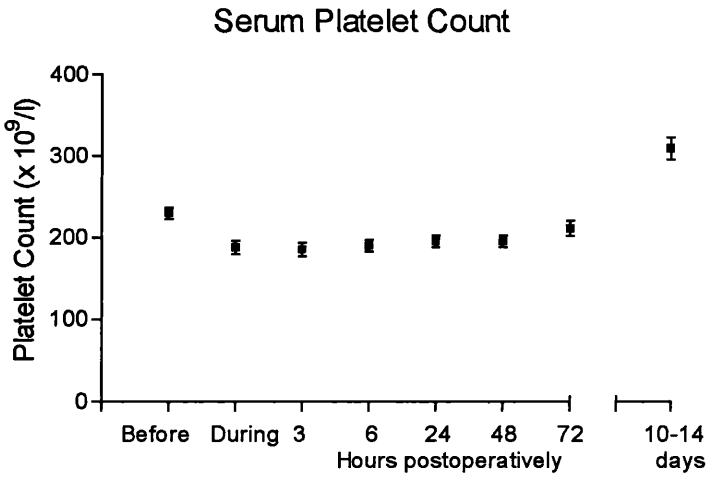
n = 39
Spearman's rank correlation r = 0.544 p = 0.004

Figure 2.1



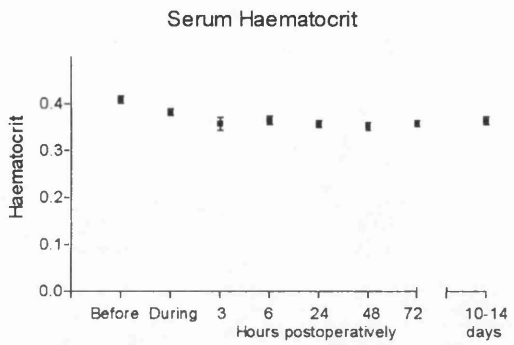
- Mean Haemoglobin Concentration +SEM bar
  Normal range 13.5 - 17.5 g/dl
  n = 38 ANOVA $p < 0.0001$

Figure 2.2



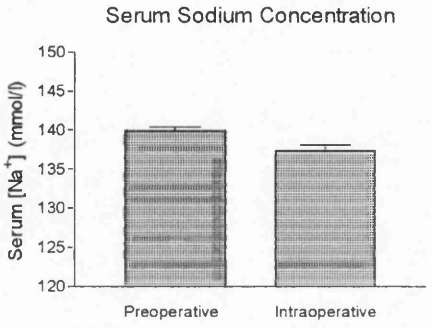
- Mean platelet count + SEM bar
  Normal range 150 - 400 x 10^9/l
  n = 30 ANOVA $p < 0.0001$

Figure 2.5



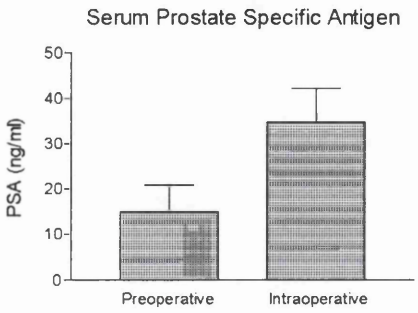
- Mean haematocrit (+SEM bar)
  Normal range 0.4 - 5.2
  n = 38 ANOVA p<0.0001

Figure 2.3a



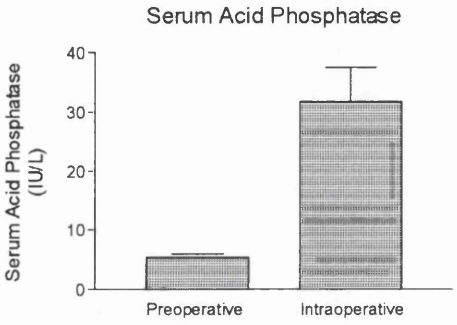
Mean serum sodium concentration + SEM bar
(normal range 133 - 143 mmol/l).
n = 37.
Wilcoxon matched pairs test p = 0.0004

Figure 2.3b



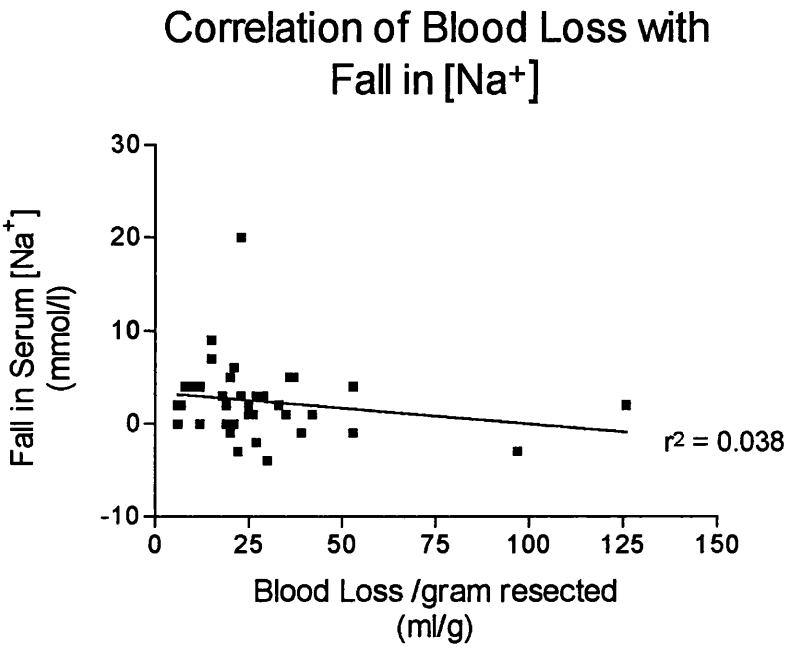
Mean serum prostate specific antigen + SEM bar.
Hybritech Tandem-R PSA Assay (normal range < 4 ng/ml).
n = 25
Wilcoxon matched pairs test p < 0.0001

Figure 2.3c



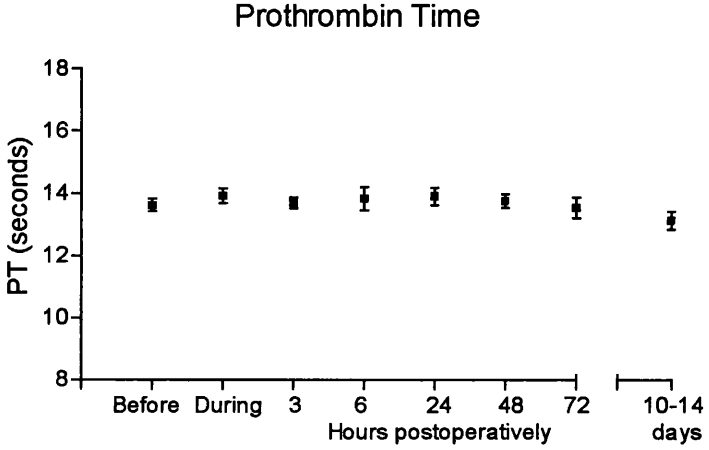
Mean serum acid phosphatase level + SEM bar
L-tartrate enzyme assay
(normal range 2 - 4 International Units per litre).
n = 25
Wilcoxon matched pairs test p < 0.0007.

Figure 2.3d



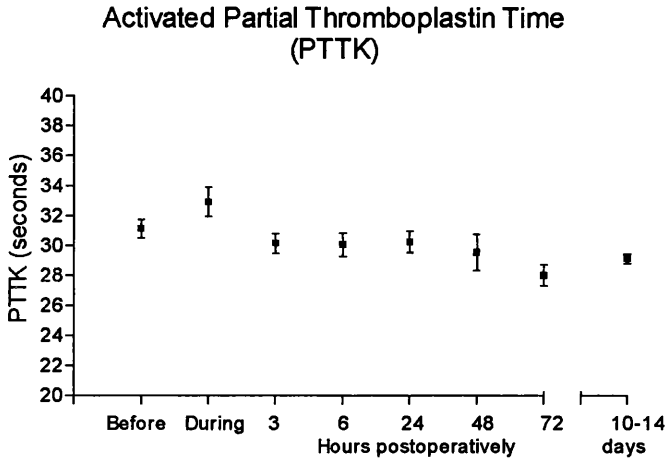
n = 35
Spearman's rank correlation r = -0.002, p = 0.99

Figure 2.4



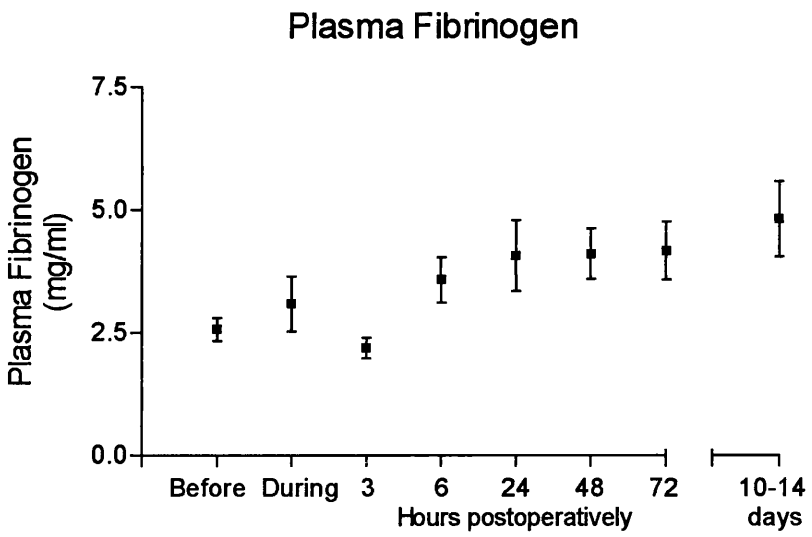
- Mean Prothrombin Time (PT) + SEM bar
  Normal range 12 - 16 seconds
  n = 30 ANOVA p = 0.592

Figure 2.6



- Mean PTTK ± SEM bar
  Normal range 28 - 38 seconds. n = 30
  ANOVA p = 0.0062; Dunn's multiple comparison test
  Preoperative vs 72 hours and 10 - 14 days $p < 0.02$

Figure 2.7



- Mean fibrinogen levels ± SEM bar
  Normal range < 4mg/ml. n = 24
  ANOVA p = 0.011; Dunn's multiple comparison test
  Preoperatively vs 10 - 14 days postoperatively $p < 0.05$.

Figure 2.8

2.5 Discussion

The high proportion of procedures performed by supervised trainees represents standard practice in our Department, and is likely to be consistent with the majority of Urology Departments with trainees in this Country.

Although a fall in body temperature following TURP has been described unless irrigant temperature is kept at body temperature (Allen 1973), no significant fall in mean body temperature was seen perioperatively in this study - using irrigant fluid warmed to 25 degrees centigrade - suggesting that hypothermia was not a contributing factor to the observed coaglation changes in the study (Rohrer & Natale 1992).

The mean total blood loss per gram of prostate tissue resected of 29 ml/g is within the range of 22 to 37 ml/g previously quoted (Perkins & Miller 1969; Lewi et al 1983 Freedman, Van Der Molen & Makings 1985; Wilson et al 1988b). As has been universally described with peroperative blood losses during TURP (Abrams et al 1982; Jansen et al 1978; Perkins & Miller 1969; Levin, Nyrén & Pompeius 1981) we found a similar significant correlation between total blood loss and weight of prostatic tissue resected.

What has not been previously described is the significant correlation between blood loss and clot volume formed intravesically. In theory any significant urinary fibrinolytic activity should limit clot formation or lyse any clots formed. The presence of significant clot formation intracystically and indeed the occurrence of clot retention postoperatively must cast doubt on the role of urinary fibrinolysis in bleeding after TURP.

The significant reduction in APTT at 72 hours and 10-14 days postoperatively - although within the lower limit of the normal range - indicates a tendency towards systemic hypercoagulability postoperatively (Miletich 1995). A postoperative reduction in APTT was similarly found by Hedlund & Blombäck (1979) following transvesical prostatectomy. Our findings of no significant alteration in prothrombin time over the perioperative period concurr with those of Rader (1978), Betkurer et al (1979) and de Campos Freire et al (1986). In contrast to Ahsen, Cartner & English (1993) who found a significant correlation between a raised prothrombin time one hour postoperatively and excessive postoperative blood loss, we found no significant change in mean prothrombin time 3 hours postoperatively compared to mean preoperative values, both of which were within the normal range.

Although homeostatic mechanisms may alter serum sodium concentration making accurate assessment of irrigant fluid volume absorption difficult (Hahn 1988), these mechanisms will attempt to limit the fall in sodium and in so doing underestimate the degree of absorption. The significant fall in mean sodium concentration 24 hours postoperatively together with the significant fall in haematocrit postoperatively are indicative of haemodilution.

Taken in conjunction with the significant increase in mean serum acid phosphatase and mean serum PSA levels peroperatively when compared to mean preoperative levels, there is firm evidence for the haemodilution at least in part being due to absorption of irrigant fluid through open veins in the prostatic fossa into the circulation, over the perioperative period (O'Donnell 1984; O'Donnell et al 1985; Vesey et al 1988).

It is unlikely that the maintainance intravenous fluid replacement with normal saline contributed significantly to the haemodilution (Grathwohl et al 1996). However the lack of any correlation between fall in plasma sodium levels and the degree of blood loss suggests that the absorption of irrigant fluid does not per se cause bleeding.

The fall in platelet count seen in the early postoperative period may simply be due to a haemodilutional effect. However in the presence of accelerated coagulability this reduction may indicate an increase in platelet consumption (O'Donnell 1990). Conversely the accelerated coagulability may be secondary to an increase in platelet aggregability during the postoperative period (Negus, Pinto & Slack 1971).

An acute fall in plasma fibrinogen levels is a marker for coagulation activation and consumption of clotting factors. However fibrinogen levels are reduced in less than half of all patients with disseminated intravascular coagulopathy (Spero, Lewis & Hasiba 1980). Fibrinogen is also an acute phase protein and plasma levels will therefore rise over the perioperative period. The gradual rise in plasma fibrinogen levels over the perioperative period seen in this study suggests that it is acting as an acute phase protein, and may be statistically masking any biological significance of a fall in mean fibrinogen levels seen immediately postoperatively. Such a fall in conjunction with a similar fall in platelet count at 3 hours postoperatively and evidence of a developing hypercoagulable state would be suggestive of a consumptive coagulation process.

CHAPTER 3

Thrombelastographic Assessment of Coagulation during Transurethral Prostatectomy

3.1 Introduction

The thrombelastograph Coagulation Analyser was developed by Hartert (1948) in Heidelberg. It was initially developed as a mechanically operated optical system to provide a continous photokymographic observation of blood or plasma during all phases of coagulation. The resultant recording is termed a thrombelastogram (TEG). Although initially designed as a research tool, it has now become accepted in clinical practice as a reliable method for the rapid empirical assessment of overall haemostatic function (de Nicola 1957) with a valuable role to play in major surgery (Mallett & Cox 1992).

Howland et al (1974) described the use of thrombelastography in detecting and treating hypercoagulability during extensive hepatic resection. Kang et al (1985) opined the use of thrombelastography in monitoring intra-operative changes in blood coagulation during orthotopic liver transplantation. Mallett et al (1991) successfully used thrombelastography to assess and treat hyperfibrinolysis during the anhepatic stage of orthotopic liver transplantation. Kang et al (1989) found similar benefits in monitoring coagulation by thrombelastography during cardiac surgery. Spiess et al (1987) and Essell et al (1993) found thrombelastography to be significantly better predictor of postoperative haemorrhage following cardiopulmonary bypass surgery than conventional screening tests.

Davis & Chandler (1995) compared thrombelastography with routine coagulation screening tests in renal transplant patients, and found it to be a better predictor of haemorrhagic complications following transplant biopsy.

Thrombelastographic evidence of postoperative hypercoagulability was demonstrated by Caprini et al (1995) following laparoscopic cholecystectomy and by Butler (1978) and Gibbs, Crawford & Michalopoulos (1994) following elective abdominal aortic surgery. Hasegawa (1983) and Rodzynek et al (1983) described the use of the TEG in monitoring procoagulant states and consumptive coagulopathies.

3.2 Basic Principles of Thrombelastography

According to Hartert (1948), the TEG measures the modulus of elasticity (m•) of the coagulum which is independent of viscosity over a wide range (Hartert & Schaeder 1962). Hartert described how the absolute elastic shear modulus of the clot can be estimated from the TEG tracing by the equation

$$G = (5000A)/(100-A)$$

Where G represents the elastic shear modulus (dynes per square centimeter) and A is the width of the tracing in millimeters.

However Scott-Blair & Burnett (1968) described the dynamic physical properties of clotting blood in more detail, and suggested that the fibrin clot was analogous to the concept of a Maxwell body, in that it has viscous as well as elastic properties. They concluded therefore that the TEG actually measures the complex modulus of elasticity - or viscoelastic properties - of whole blood or plasma as it clots, retracts and/or lyses.

A disposable plastic cylindrical cup is held firmly in a cuvette within a chamber maintained at 37^0 C (figure 3.1). The cuvette is oscillated through an angle of 4^0 45' around a vertical axis, each rotation lasting 10 seconds

which includes a one second rest period at the end of each excursion to prevent viscosity errors. A disposable plastic pin is suspended from a torsion wire and immersed in the sample. The torque of the rotating cup is transmitted to the pin by the fibrin strands linking the pin to the cup (figure 3.2). While the sample remains fluid the pin will remain motionless. As the tensile strength of the clot increases, there is a proportional increase in transmitted torque. The increasing rotational movements of the pin are converted to an electrical signal by a transducer, which powers the heated stylus motor. The stylus movements are recorded on heat-sensitive paper moving at 2 millimeters per minute, to produce the TEG.

The disadvantage of having to develop the light-sensetive film in the original mechanical-optical system before the TEG could be analysed, led to the development of the direct-writing Computerized Thrombelastograph® Coagulation Analyzer (CTEG ©1990 Haemoscope Corporation, Skokie, Illinois), which provides a continous simultaneous visual and computerised analysis of the TEG (figure 3.1).

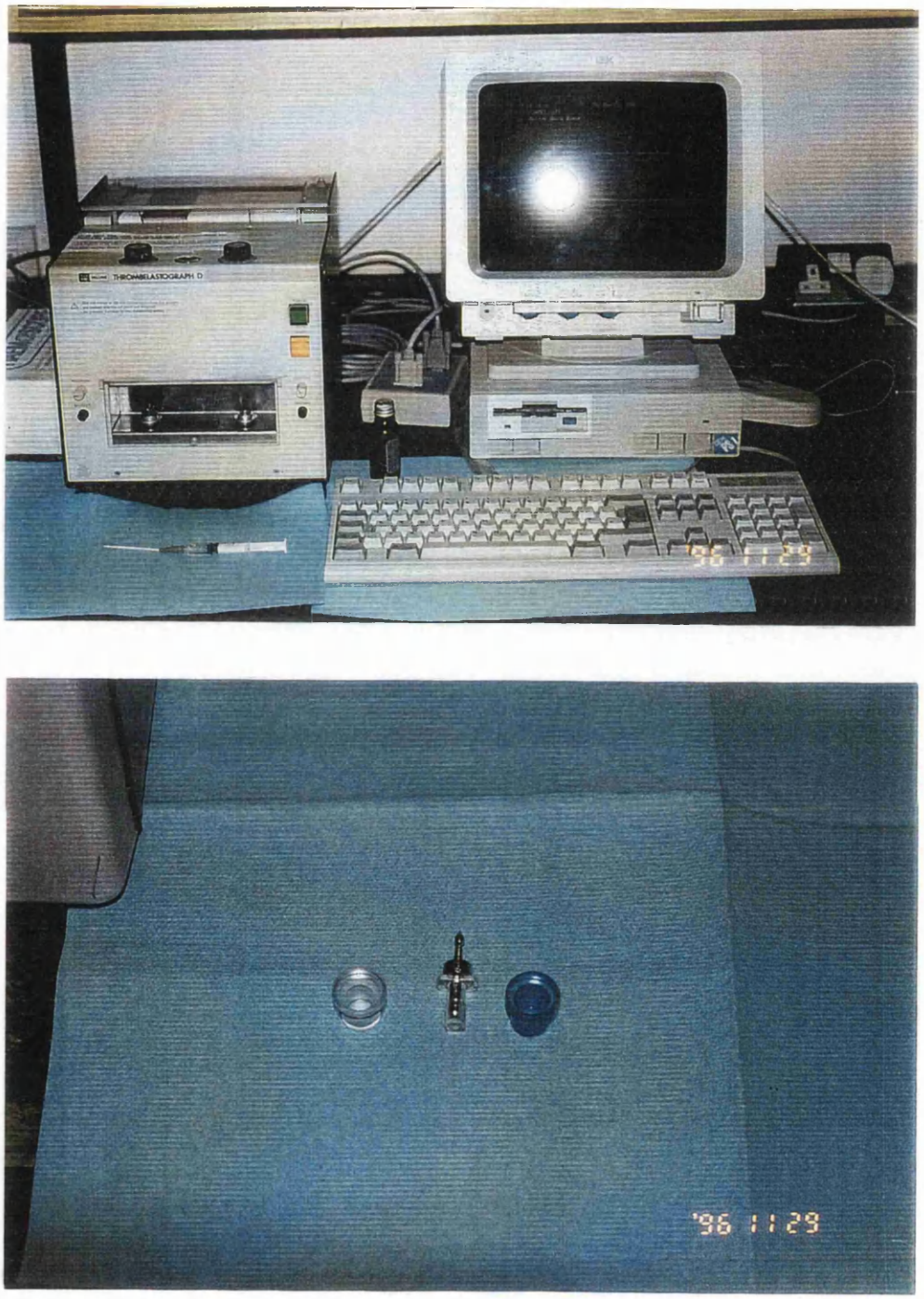


Figure 3.1 Photographs of the Computerised Thrombelastograph® Coagulation Ananyzer (TEG™) Model 30000C (Haemoscope Corporation, Skokie, Illinois) in operation, and the disposable cups and pin.

The Thrombelastographic Technique & Assay System

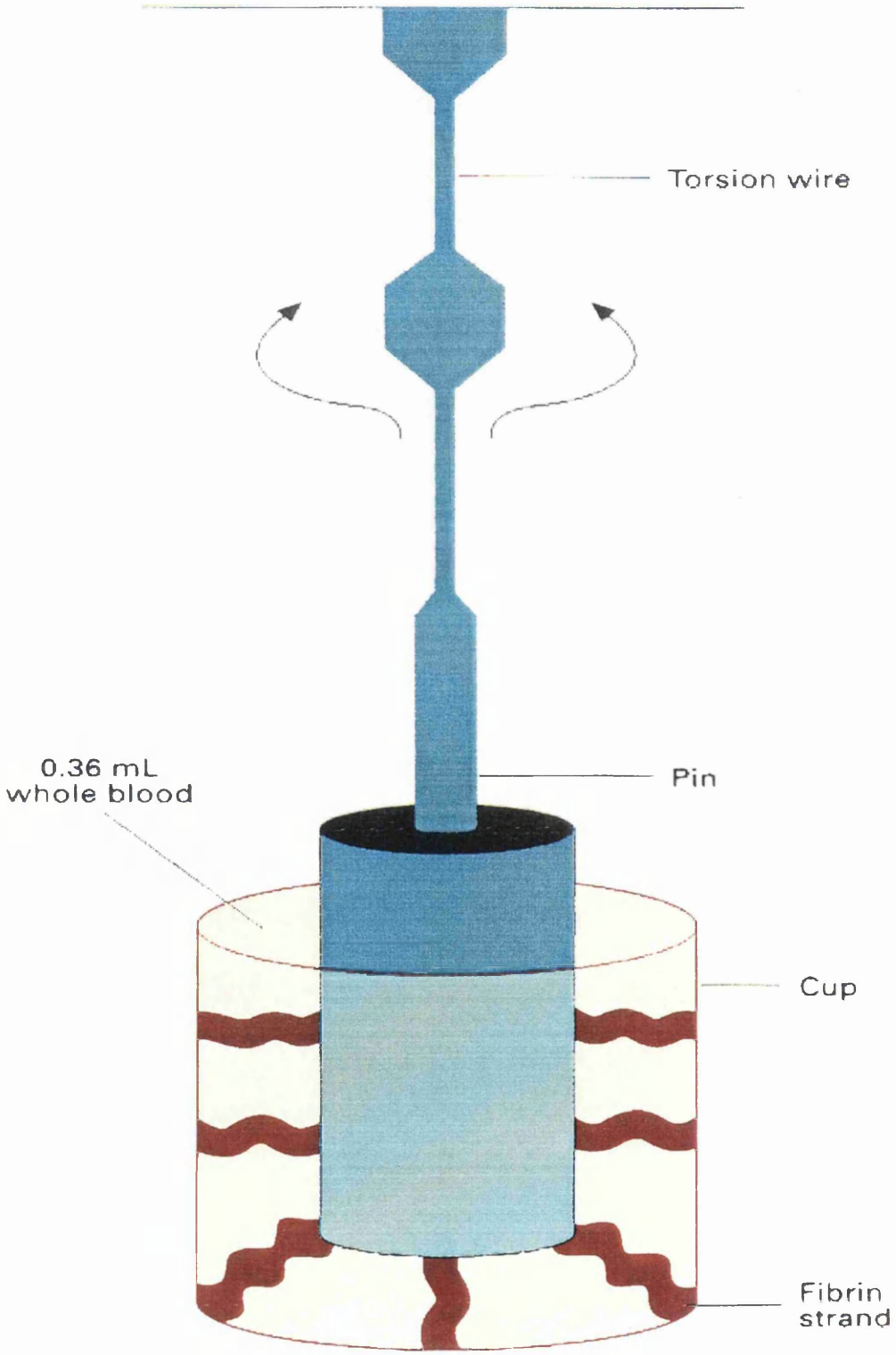


Figure 3.2

3.3 Analysis of the Thrombelastograph

The TEG provides a dynamic qualitative (figure 3.3) and quantitative (figure 3.4) assessment of the whole process of coagulation. The parameters of the TEG (r, rk, ma, Ly_{60}) provide information on various aspects of the coagulation process.

r-value

Hartert (1948) referred to the r-value as the reaction time. This is the initial straight line portion of the TEG and represents the time from the initial venepuncture to the first sign of clot formation (the point where the diameter of the curve reaches 2 millimeters). This is the end point of most coagulation tests. The r-lag time is the time between venepuncture and the starting of the TEG tracing and must be added to the r-value. It was originally described by Marchal, Leroux & Samama (1961) as representing the rate of thrombin generation, and increase linearly with increasing thrombin concentration (Caprini et al 1974). Thus it is affected by the biological determinates that affect the function of the first-stage factors of the classical intrinsic pathway (factors XII, XI, IX and VIII). The r time is analagous to the whole blood clotting time.

Prolongation of the reaction time may occur in inherited defects of thromboplastin generation such as the haemophilioid syndromes - haemophilia A (factor VIII), haemophilia B (factor XI) and Christmas disease (factor IX) (Marchal, Leroux & Samama 1961) - or in acquired defects such as heparin or oral coumarin anticoagulation (Raby 1976). The prolongation of the r-value is proportional to increasing heparin levels (Marchal, Leroux & Samama 1961), and thrombelastography appears to

be a sensitive and reproducible method of monitoring heparin therapy (Sagar & Beckett 1975).

A shortened reaction time is seen in situations of accelerated coagulability (Caprini et al 1976). Examples include the postoperative hypercoagulable state (Sagar & Beckett 1975; Butler 1978) and uraemia (Marchal, Leroux & Samama 1961).

k-value

The k-value is referred to as the clot formation time. It is defined as the time from initiation of the clot (end of the r-time, the point where the diverging lines are 1mm apart) until the TEG reaches a fixed level of clot firmness or viscoelasticity (a shear modulus of 25 represented by a distance between diverging curved lines of 20 millimeters). Hartert (1952) originally interpreted the k-time as representing the rapidity of fibrin formation and cross-linking and therefore clot formation. Marchal, Leroux & Samama (1961) referred to the k-time as the *thrombin constant* which describes both thrombin activity and fibrin formation. It is dependent on the intrinsic clotting factors, fibrinogen levels and platelet function and numbers (Butler 1978; Mallett & Cox 1992).

The arithmetical sum of the r-time and k-time (r + k value) is a frequently used parameter and reflects the coagulation time from its beginning to a predifined clot strength.

Alpha angle (α^0)

This is the angle formed by the slope of the TEG from the r value (the point at which the diameter of the diverging lines reaches 2mm) to the k value, and represents the rate of clot formation. It reflects primarily fibrinogen function (Tuman et al 1987). Reduced values can be seen in thrombocytopenia and hypofibrinogenaemia (Mallett & Cox 1992). An increased alpha angle is seen in a hypercoagulable state (Tuman et al 1987).

Maximum amplitude (ma)

The ma represents the maximal viscoelasticity (shear modulus) or strength of the formed clot, and is measured as the greatest amplitude achieved by the TEG tracing. It is dependent on the haemostatic factors responsible for the stabilisation of the fibrin mesh within the clot, and is therefore highly sensitive to qualitative and quantitative changes in platelets (De Gaetano & Vermylen 1973; Tuman et al 1991; Martin et al 1991) fibrinogen and Thrombin concentrations, fibrin, factors VIII and XIII and haematocrit (Hartert & Schaeder 1962; Caprini et al 1974; Butler 1978; Tuman et al 1987; De Gaetano & Vermylen 1973; Howland et al 1974; Mallett & Cox 1992). Factors influencing platelet adhesiveness such as heparin or dextran therapy or producing a thrombocytopenia significantly reduce the ma.

An increase in ma is usually found in hypercoagulable states (De Nicola 1957; Poller et al 1971; Tuman et al 1987).

The thrombelastographic tracing in excessive systemic fibrinolysis is characterised by a regular smooth decrease in ma (Marchal, Leroux &

Samama 1961; Egeblad 1967). In contrast to this is the stepwise, irregular decline associated with hyperretractility found in hypercoagulable state which is caused by detachment of the clot from the cuvette (Marchal, Leroux & Samama 1961).

The Hypocoagulable State

Various thrombelastographic patterns can be observed in the hypocoagulable state, depending on the clotting defect present. In classic haemophilia (factor VIII deficiency) is characterised by a prolonged r-time and k-time with a normal ma (Marchal, Leroux & Samama 1961). Factor V deficiency produces a prolongation of the r time (De Nicola 1957).

Both quantitative and qualitative platelet disorders will produce a TEG showing a normal r-time, a prolonged k-time and a reduced ma value (Signori, Penner & Kahn 1969).

The effects of low-dose aspirin therapy on platelet function are not detected by thrombelastography (De Gaetano & Vermylen 1973; Orlikowski et al 1992). Mallett & Cox (1992) explain this by the fact that platelet aggregation by thrombin is relatively unaffected by aspirin.

Warfarin therapy within the theraputic range produces a haemophilia-like TEG tracing, with a moderate prolongation in r and k values, and a slight decrease in ma (De Nicola 1957).

Low-dose heparin prophylaxis partly corrects the shortening of the r + k values found after operation (Sagar & Beckett 1975). Full heparin anticoagulation required in procedures such as cardiopulmonary bypass surgery will produce a straight line TEG tracing. Spiess (1990) described the use of celite-activated whole blood thrombelastography to overcome

this problem allowing TEG to be used successfully to monitor coagulopathies in cardiopulmonary bypass surgery.

The Hypercoagulable State

Fisch, Freedman & Pellegrin (1973) defined thrombelastographic hypercoagulability as an accelerated rate of clotting and increased firmness of the formed clot. They emphasised however that an hypercoagulable TEG implies an increased propensity for clotting and not thrombogenesis.

The features of a TEG tracing depicting an hypercoagulable state are a reduction in the r-time, a reduction in the k-time, an increase in ma and increased retractility (de Nicola 1957; von Kualla & Weiner 1955; Fisch et al 1973; Raby 1969; Tuman et al 1987; Ng & Lo 1996).

Raby (1966) described the use of the index of thrombodynamic potential (ITP) as a marker of hypercoagulability, with increased values representing an increased procoagulant state:

$$ITP = Emx / k$$

$$= \frac{100 \times ma / 100\text{-}ma}{k}$$

A coagulation index (CI) obtained from native and celite-activated whole blood has subsequently been described (Caprini et al 1976; Cohen et al 1977):

$$CI\ (native) = -(0.1227)r + (0.0092)k + (0.1655)MA - (0.0241)\alpha - 5.0220$$

$$CI\ (celite) = -(0.3258)R_c - (0.1886)k_c + (0.1224)Ma_c + (0.0759)\alpha_c - 7.7922$$

The normal range is -2 to +2. Values greater than +2 representing hypercoagulability.

Thrombelastographic hypercoagulability has been described in pregnancy and the use of oral contraceptives (Hathaway, Mahasandana & Makowski 1975; Poller et al 1971), cigarette smoking (Howland et al 1974), thermal injury (Zuckerman, Caprini, Lipp & Vagher 1978) and the postoperative state (Sagar & Beckett 1975; Butler1978; Gibbs, Crawford & Michalopoulos 1994; Caprini et al 1995). Hickman (1971) found the increase in thrombelastographic hypercoagulability coincided with a decline in plasma fibrinolytic activity.

The Fibrinolytic State

Fibrinolysis has a characteristic pattern depicted by a continuous regular decrease in maximal amplitude (Egblad 1967). This must be differentiated from the irregular step-like decrease found in the hyperretractility of the hypercoagulable state (Marchal, Leroux & Samama 1961). TEG fibrinolysis is defined as a $\geq$15-20 percent decrement in clot strength measured 60 minutes (A_{60}) after maximum clot strength ma (Kang et al 1987; Tuman et al 1987; Mallet & Cox 1992). Thus the *Whole Blood Clot Lysis Index* (WBCLI) may be calculated by the equation:

$$\text{WBCLI} = A_{60} / \text{MA} \times 100 \ (\%)$$

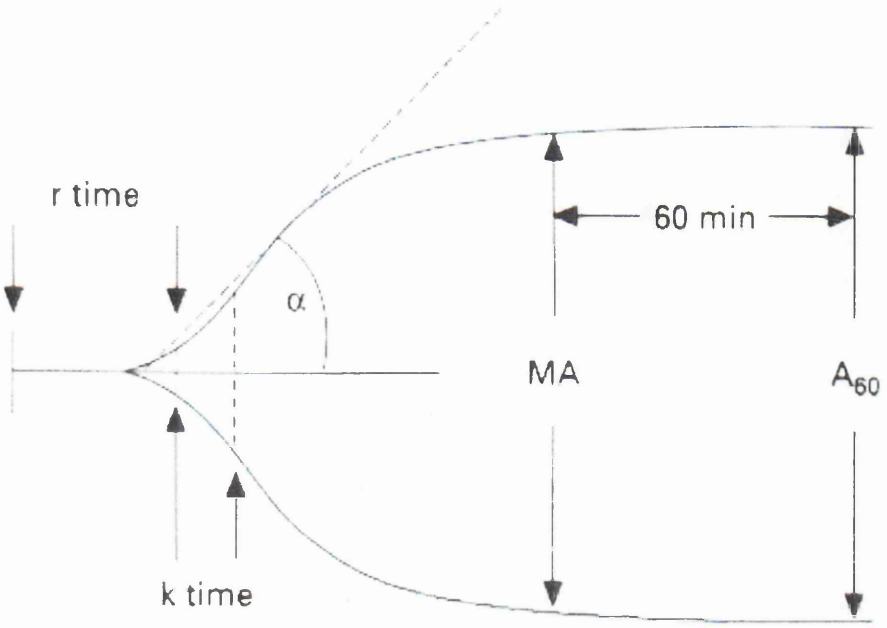
Values of WBCLI < 85% indicating the presence of fibrinolysis.

A more precise method of measuring fibrinolysis is to measure the divergence at four 10mm intervals (A_1 - A_4) after the MA. These intervals can be used to compute the area under the curve and compare it to a

theoretical area that did not converge to give the *Fibrinolytic Index*:

$$= \frac{(1 - [MA + 2A_1 + 2A_2 + 2A_3 + A_4]) \times 100}{8MA}$$

This area under the curve is calculated automatically at 30 and 60 minutes after the MA using the Thrombelastograph® Coagulation Analyzer Model 3000C (Haemoscope Corporation, Skokie IL USA) to give the Ly_{30} and Ly_{60} respectively. Fibrinolysis is indicated when $Ly_{30} > 7.5\%$ or $Ly_{60} > 15\%$.



The Quantitative Analysis of the Thrombelastograph.

1. *r* is the reaction time (normal range= 10.5-15 min) which represents the rate of initial thromboplastin generation and is measured from the time of sampling until the trace amplitude reaches 2 mm. It is related functionally to the clotting factor and circulating inhibitor activity. Values of < 10.5 min (21 mm) indicate hypercoagulability.

2. α is the angle (normal range=30-41°) which indicates the rate of clot formation and is a function of clotting factor activity. Values increase during hypercoagulation and decrease on hypocoagulation.

3. **MA** is the maximum amplitude (normal range= 45-54 mm) which reflects the maximal elasticity of the fibrin clot and is a direct function of the qualitative and quantitative properties of fibrin, factor XIII and other plasma factors, and the platelet count and function. MA is usually increased in hypercoagulable states and decreased with thrombocytopenia, qualitative platelet defects, decreased fibrinogen levels and the presence of fibrin degradation products.

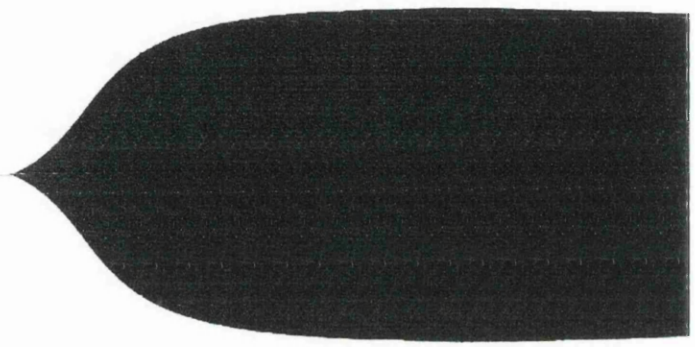
4. $\mathbf{A_{60}}$ is the amplitude of the tracing 60 min after the maximum amplitude has been reached (normal range= MA - 5 mm) and is a measure of clot retraction or lysis.

5. $\mathbf{Ly_{60}}$ is the percentage clot lysis (normal range < 15%) which reflects the loss of clot integrity as a result of fibrinolysis measured 60 min after the MA. It is derived as (MA - A_{60}/ MA) x 100 (%) and gives a quantitative assessment of the degree of clinically sign)ficant systemic fibrinolysis. A thrombelastographic tracing in fibrinolysis is characterized by a sustained regular decrease in MA; a value of < 15% denotes clot retraction only and is seen as a step-wise irregular decline in MA.
(Trace speed=2mm/min)

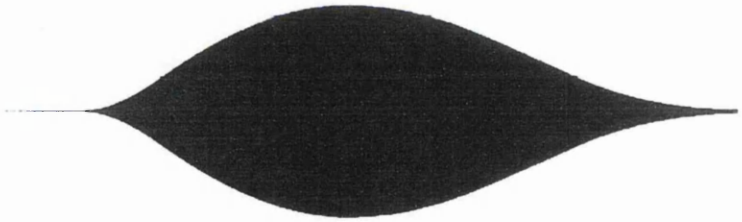
Figure 3.3

The Qualitative Interpretation of the Thrombelastograph in Different Coagulative States

Normal TEG



Fibrinolysis



Hypercoagulation

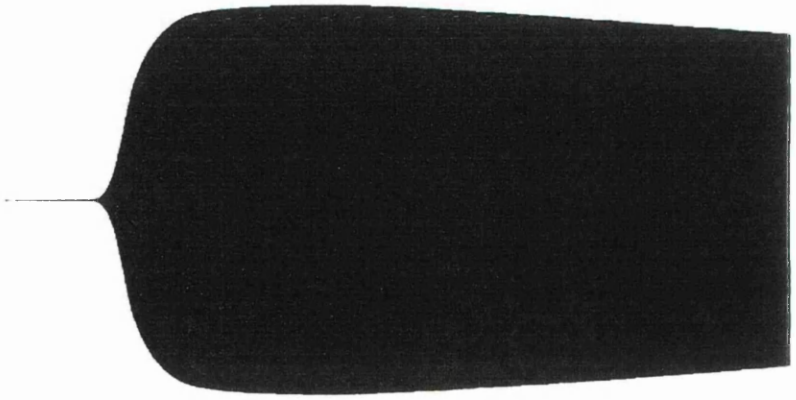


Figure 3.4

3.4 Thrombelastograph Techniques

A variety of different thrombelastographic techniques have been developed to assess coagulation. Thrombelastography can be performed on native venous whole blood, platelet-rich and platelet poor plasma samples and recalcified citrated whole blood and plasma. The advent of disposable plastic cups and pins has increased the safety and acceptability of thrombelastography.

Native Whole Blood (NWB)

A venous whole blood sample is placed directly into a disposable plastic cup and analysed within 4 - 6 minutes of sampling. The time between sampling and starting the TEG represents the r lag time, and is measured with a stop watch. The advantages of this method are that it avoids anticoagulation, centrifugation and its reversal, thus allowing for greater reproducibility. Obviously this method has geographical limitations, and can only be utilised if the TEG analyser is nearby.

The normal range (mean $\pm$ standard deviation derived from n = 97 normal patients from two institutions; Evanston Hospital, Evanston, Illinois and the Mayo Clinic in Rochester, Minnesota) for the CTEG Thrombelastograph® Coagulation Analyzer Model 3000C (Haemoscope Corporation, Skokie, Illinois) using disposable plastic cups and pins and NWB are shown in table 3.1.

	r	k	ma	∝
Mean ± SD	23.6 ± 4.8	10.6 ± 2.8	54.0 ± 5.6	36.0 ± 7.4

Table 3.1 mean ± standard deviation (SD) TEG parameters in n = 97 healthy subjects (Copyright 1990, Haemoscope Corporation; reproduced by permission).

Citrated Whole Blood (CWB)

The venous whole blood sample is immediately placed in a polypropylene tube containing 0.3ml of 0.109 M sodium citrate (pH7.4). It can be stored at 4^0C for up to 4 hours whilst being transferred to the TEG analyzer. Before analysis it must be rewarmed to room temperature and recalcified with 100μl of 0.645% calcium chloride. The increased number of steps in this technique allow for a greater degree of error, thus reducing its reproducibility.

Modified Techniques

Differential centrifugation of CWB can be used to isolate platelet poor plasma, which can be compared with the CWB tracing to assess the effect of platelets on coagulation.

Celite acts as a contact surface (analogous to glass activation) which activates factor XII and platelets. Celite-activated blood (0.030ml of 1% celite suspension) can be used to shorten the coagulation time (reduce the r time) thus speeding up the test. Using celite the r time becomes analogous to the Activated Clotting Time.

Protamine (30μl of 0.48mg/ml solution) can be used to reverse the effects of heparin on clotting. The addition of Epsilon aminocaproic acid (30μl of 12mg/ml Amicar solution) can be used to confirm the presence of reversible hyperfibrinolysis by restoring the TEG tracing to normal in a subsequent sample.

3.5 A Comparison of TEG with Conventional Coagulation Tests

Thrombelastography provides a complete assessment of the interaction between the cellular and humoral components of the coagulation and fibrinolytic systems during the whole process of clot initiation, stabilisation and clot retraction or lysis. It is a dynamic evaluation of the whole clotting process, in direct contrast with conventional laboratory coagulation tests, which examine an isolated part of the coagulation cascade in centrifuged plasma samples thereby missing the importance of cell surfaces in localisation, amplification and modulation of the clotting cascade, and whose end point is the formation of the first fibrin strands (Mann 1984). Zuckerman et al (1981) and Howland, Schweizer & Gould (1974) described a strong correlation between thrombelastography and the routine coagulation tests haematocrit, platelet count, fibrinogen and fibrin split products levels, prothrombin time and activated partial thromboplastin time. However Mallett & Cox (1992) emphasise that there is no direct correlation between TEG and conventional tests as the TEG variables are inter-dependent, measuring the interaction of platelets and clotting factors in whole blood and not isolated end points. Zuckerman et al (1981) stressed that TEG provided additional information to these routine tests.

Several authors (Spies et al 1987; Essell et al 1993; Davis & Chandler 1995) have described the advantages of TEG over routine tests in predicting postoperative haemorrhagic complications. However there conclusions have not been universally accepted (Dorman et al 1993; Wang et al 1992) and extreme caution employed in the use of TEG for this purpose. The literature on the use of routine coagulation tests as a screening procedure to predict perioperative blood loss is also conflicting. Murphy, Davies & Eduardo (1993) showed an impairment in haemostasis with a fall in platelet count below 100×10^9 litre^{-1}. Ahsen, Cartner & English (1993) found a significant correlation between an abnormal prothrombin time immediately postoperatively, and postoperative blood loss following TURP, but no correlation preoperatively. Rader (1978) found routine preoperative screening tests to be unhelpful in predicting perioperative blood loss in TURP. Routine coagulation screening tests in cardiopulmonary bypass surgery have not been shown to be of benefit in predicting perioperative haemorrhagic complications (Spiess et al 1987; and Wang et al 1992).

Tuman et al (1991) demonstrated a significant correlation between TEG variables α angle, ma and A_{60} and both the platelet count and platelet function as assessed by two different methods of platelet aggregometry. Whitten et al (1991) and Summaria et al (1986) demonstrated that TEG was more sensitive a test for the detection of post cardiopulmonary bypass fibrinolysis than both D-dimer levels and euglobin clot lysis time. Tuman et al (1987) found a significant correlation between euglobin clot lysis time (ELT) and thrombelastographic assessment of fibrinolysis, as well as a correlation between the r value and the APTT.

3.6 Study Thrombelastographic Methodology

The primary end-point of the TEG study was taken as thrombelastographic evidence of fibrinolysis. This was defined as a *Fibrinolytic Index* (Ly_{60}) of greater than 15 percent or WBCLI < 15 % (Kang et al 1987; Mallett & Cox 1992). Values of Ly_{60} < 15% indicating clot retraction only and not fibrinolysis. In order to detect a difference in mean Ly_{60} of one percent, assuming a standard deviation of up to one, a sample size of 36 was calculated for a type I error (α) of 0.01 and giving a power for the study of 0.95.

Thus all 41 patients entered into the study had serial thrombelastography performed (40 patients undergoing TURP with a histological diagnosis of benign prostatic hyperplasia BPH in n = 32 and prostatic adenocarcinoma Ca. in n = 8) and included for analysis. Thrombelastography was performed on serial native venous whole blood (NWB) samples. Serial sampling was performed preoperatively, intraoperatively (20 minutes after the start of resection), 3, 6, 24, 48, 72 hours and 10 - 14 days postoperatively.

Cuffed antecubital venous blood samples were obtained using a two-syringe technique and a fresh venepuncture with a 19 guage needle for each sample. The tourniquet was applied immediately prior to venesection. Samples were collected in sterile 20 ml plastic syringes, the initial sample being utilised for additional investigations.

A separate TEG plastic disposable cup and pin was used for each sample, the cup being filled with whole blood to the graduation mark (0.36ml).

The r lag time (the time from venepuncture to the start of the thrombelastograph) was standardised to 6 minutes in order to reduce inter-assay variability. After lowering the pin into the cup, a film of paraffin oil was placed on the surface of each blood sample to prevent it from drying. The thrombelastograph was marked at 6 minutes. All thrombelastographs were run for at least 90 minutes.

Measurement of the TEG indices r, rk, α angle, ma and Ly_{60} for each sample was performed automatically using the Haemoscope Computerized Thrombelastograph Coagulation Analyzer (CTEG) Analytical Software. This allows simultaneous analysis, visual display and data storage of up to 8 TEG channels, comparing results with published normal ranges.

Inter-assay coefficient of variability was measured by running a single sample in 4 simultaneous TEG channels (table 3.2). This was repeated with 5 different venous whole blood samples, using the calculation :

$$\text{coefficient of variation} = \frac{\text{Standard Deviation}}{\text{mean}} \times 100\%$$

		Coefficient of Variation (%)				
		r	rk	α	ma	Ly_{60}
Sample	1	4.4	4.2	1.8	1.9	5.3
	2	3.2	3.7	2.3	1.7	5.1
	3	4.0	3.7	2.5	3.1	3.5
	4	3.1	3.9	2.1	2.9	5.9
	5	3.2	4.3	3.0	2.0	4.7

Table 3.2: The coefficients of variation for the TEG variables from 5 samples analysed simultaneously in 4 TEG channels.

Statistical analysis was performed using Analysis of Variance (ANOVA) and the Dunn's mulitple comparison post test where appropriate, on all 40 patients (BPH = 32, Ca. = 8). Using Bartlett's test for homogeneity of variance, no significant difference in standard deviations was evident ($p > 0.38$).

3.7 Thrombelastograph Results

No evidence of fibrinolysis (TEG *Fibrinolytic Index* $Ly_{60} > 15\%$) could be demonstrated in any patient at any stage over the whole perioperative period (Figure 3.5). The Ly_{60} for all samples were within the normal range, thus representing clot retraction only.

A slight increase in mean TEG parameters rk and ma above the normal range was found preoperatively.

All patients showed thrombelastographic evidence of hypercoagulablility (increased r, rk, α and ma), evidence of clot retraction (as shown by a

Ly_{60} < 15%) and platelet activation over the perioperative period. There was a significant change in mean thrombelastographic parameters towards hypercoagulation intraoperatively and continuing until 10 to 14 days postoperatively, compared to mean preoperative values (table 3.3; figure 3.6 a - d). This was most marked at 3 to 6 hours, but continues up until 10 to 14 days postoperatively. There was a significant increase in mean α and ma values from intraoperatively until 10 to 14 days compared to preoperative mean values, the increase being maximal at 10 to 14 days (ANOVA, $p = 0.0112$ and $p < 0.0001$ respectively; figures 3.6c and d). Maximal increases in mean r and rk compared to preoperative values were seen at 3 hours postoperatively, with both parameters reaching statistical significance at 3 and 6 hours postoperatively (ANOVA $p = 0.0011$ and $p = 0.0083$ respectively; table 3.3). A trend towards a return to normal values can be seen for r and rk from 6 hours postoperatively with mean r values reaching the normal range at 72 hours. No such trend can be seen for either α or ma.

There was a significant increase in mean TEG Coagulation Index ($CI = -0.1227r + 0.0092k + 0.1655MA - 0.0241\alpha - 5.0220$) from intraoperatively until 10-14 days postoperatively compared to mean preoperative levels (ANOVA, $p < 0.0001$; Dunnett multiple comparison test $p < 0.01$ for all sampling times; figure 3.7a). This was maximal at 3 hours postoperatively.

There was a significant increase in the degree of mean clot retraction at 72 hours compared to mean preoperative values (Ly_{60}, ANOVA $p = 0.0002$). No correlation between the degree of haemodilution - as measured by fall in haematocrit - and TEG parameters r, α and ma could

be demonstrated (Spearman's rank correlation r_s = -0.027, 0.062 and 0.159; p = 0.877, 0.721 and 0.353 respectively).

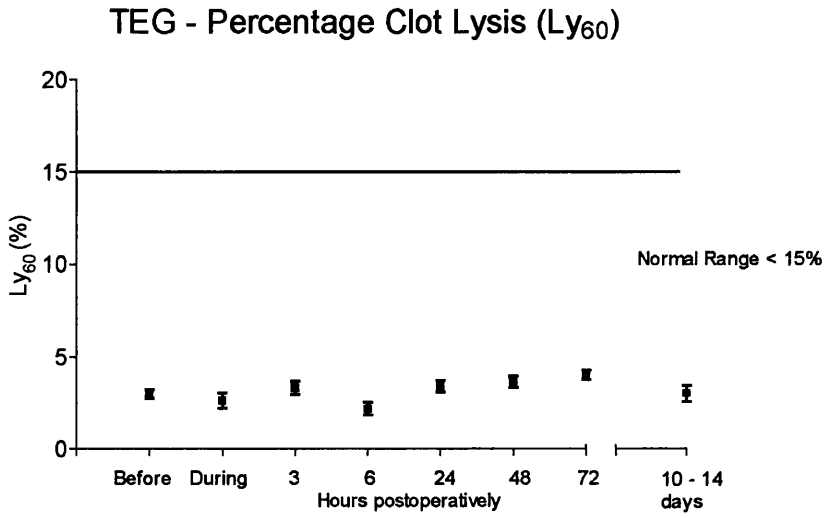
Thrombelastographic evidence of hypercoagulability has been described in patients with cancer (Zuckerman et al 1981). Raina et al (1985) demonstrated the added procoagulant effect of surgery on tumour-induced accelerated coagulation in a rat carcinoma model. To see whether the TEG hypercoagulability seen perioperatively was due to the inclusion of those patients with carcinoma of the prostate, the TEG Coagulation Index of the 30 patients in the study with benign histology were analysed separately. No difference in data variance (figure 3.7b) or statistical analysis was found between the group with benign histology (ANOVA $p < 0.0001$; Dunn's multiple comparison test with preoperative mean, $p < 0.01$ for all sampling times) and the group as a whole.

The patient who underwent cystoscopy alone showed no TEG evidence of hypercoagulability (mean CI -0.18 range -0.68 to 0.26).

TEG Parameter	Sample	Mean ± S.E.M	p Value
Reaction time (r)	preop	23.3 ± 1.5	NA
(mm)	intraop	17.8 ± 1.4	< 0.05
	3 hours	13.8 ± 1.5	< 0.01
	6 hours	18.5 ± 1.7	NS
	24 hours	19.4 ± 1.7	NS
	48 hours	19.0 ± 1.3	NS
	72 hours	21.2 ± 1.8	NS
	10-14 days	22.5 ± 1.7	NS
rk (mm)	preop	40.4 ± 2.0	NA
	intraop	32.4 ± 1.9	< 0.05
	3 hours	27.5 ± 2.2	< 0.01
	6 hours	31.6 ± 2.3	< 0.05
	24 hours	34.0 ± 2.5	NS
	48 hours	32.9 ± 1.7	NS
	72 hours	33.8 ± 2.4	NS
	10-14 days	33.9 ± 2.1	NS
Alpha angle (α^{o})	preop	38.4 ± 1.8	NA
	intraop	49.8 ± 2.7	< 0.01
	3 hours	50.5 ± 2.3	< 0.01
	6 hours	48.8 ± 2.9	< 0.01
	24 hours	48.0 ± 2.7	< 0.01
	48 hours	49.3 ± 2.4	< 0.01
	72 hours	48.4 ± 2.4	< 0.01
	10-14 days	51.6 ± 2.6	< 0.01
Maximum	preop	56.2 ± 1.1	NA
Amplitude (ma)	intraop	63.0 ± 1.5	< 0.01
(mm)	3 hours	63.3 ± 1.6	< 0.01
	6 hours	62.8 ± 1.4	< 0.01
	24 hours	62.4 ± 2.0	< 0.01
	48 hours	64.4 ± 1.5	< 0.01
	72 hours	62.9 ± 1.6	< 0.01
	10-14 days	67.7 ± 1.6	< 0.01
Percentage Clot	preop	2.9 ± 0.2	NA
Lysis (Ly_{60})	intraop	2.8 ± 0.4	NS
(%)	3 hours	3.4 ± 0.3	NS
	6 hours	2.2 ± 0.3	NS
	24 hours	3.5 ± 0.3	NS
	48 hours	3.9 ± 0.2	NS
	72 hours	4.1 ± 0.2	< 0.05
	10-14 days	2.8 ± 0.5	NS

TEG Parameter	Sample	Mean ± S.E.M	p value
Coagulation	**preop**	**-0.37 ± 0.2**	**NA**
Index	**intraop**	**1.15 ± 0.2**	**< 0.01**
	3 hours	**1.83 ± 0.3**	**< 0.01**
	6 hours	**1.47 ± 0.3**	**< 0.01**
	24 hours	**1.31 ± 0.3**	**< 0.01**
	48 hours	**1.35 ± 0.3**	**< 0.01**
	72 hours	**1.45 ± 0.3**	**< 0.01**
	10-14 days	**1.61 ± 0.3**	**< 0.01**
Coagulation	**preop**	**-0.41 ± 0.3**	**< 0.01**
Index (BPH)	**intraop**	**1.20 ± 0.3**	**< 0.01**
	3 hours	**1.80 ± 0.3**	**< 0.01**
	6 hours	**1.42 ± 0.4**	**< 0.01**
	24 hours	**1.41 ± 0.3**	**< 0.01**
	48 hours	**1.46 ± 0.3**	**< 0.01**
	72 hours	**1.44 ± 0.4**	**< 0.01**
	10-14 days	**1.59 ± 0.3**	**< 0.01**

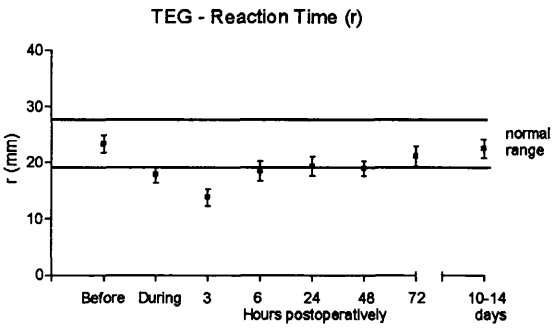
Table 3.3: Results for the thrombelastographic parameters **r**, **rk**, α, **ma**, **Ly_{60}** and **CI** (including BPH subgroup). The p values are derived from the Dunn's multiple comparison post test, using the preoperative values as control.
NA = not applicable. NS = not statistically significant ($p > 0.05$). S.E.M = standard error of the mean.



Mean percentage clot lysis (Ly_{60}) + SEM bar

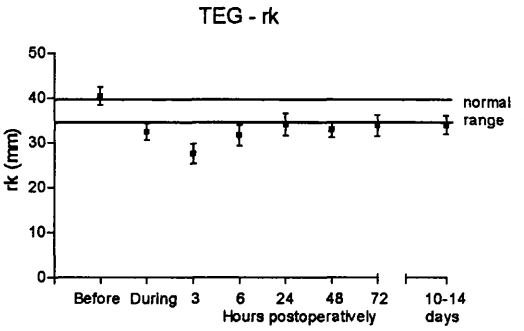
Ly_{60} < 15% indicate clot retraction only

Figure 3.5



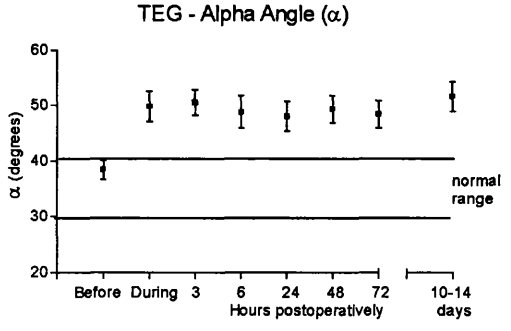
Mean reaction time r + SEM bar.
n = 40 ANOVA p = 0.0011

Figure 3.6a



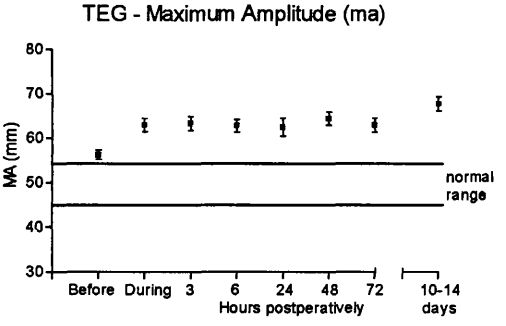
- Mean rk values ± SEM bar
n = 40 ANOVA p = 0.0083

Figure 3.6b



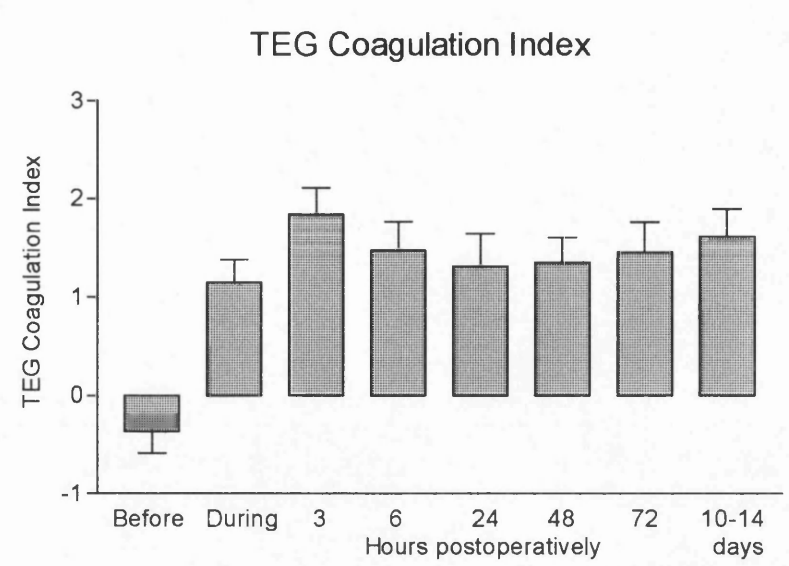
- Mean alpha angle ± SEM bar.
n = 40 ANOVA p = 0.0112

Figure 3.6c



- Mean Maximum Amplitude (ma) ± SEM bar
N = 40 ANOVA p < 0.0001

Figure 3.6d



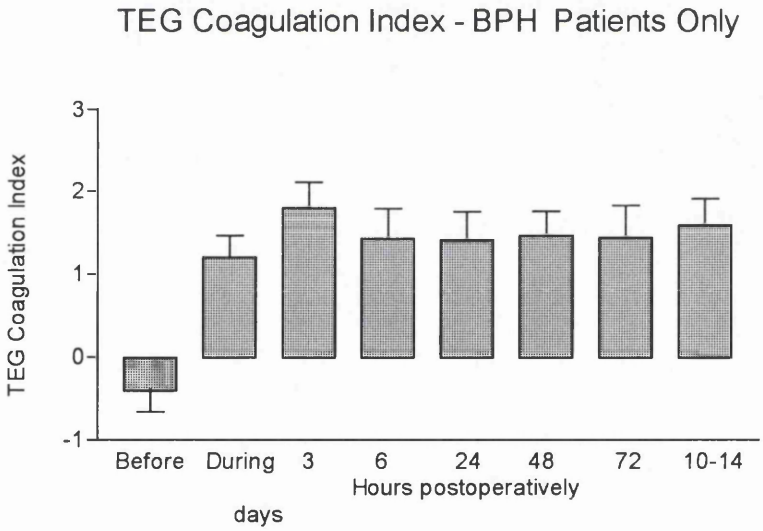
TEG Coagulation Index (CI) mean + SEM bar

CI = -0.1227r + 0.0092k + 0.1655MA - 0.0241a - 5.0220

Normal range = -2 to 2.

n = 40 ANOVA $p < 0.0001$

Figure 3.7a



TEG Coagulation Index (CI) mean + SEM bar

CI = - 0.1227r + 0.0092k - 0.1655MA - 0.0241α - 5.0220

Normal range = -2 to 2

n = 30 (BPH) ANOVA $p < 0.0001$

Figure 3.7b

3.8 Discussion

The finding of an increased mean preoperative ma above the normal range indicates a degree of platelet activation preoperatively. This finding is in keeping with an anxiety stress response to the impending surgery, leading to activation of the clotting cascade (Cannon & Gray 1914; Ingram & Jones 1966; Swedenborg & Olsson 1978). However the mean TEG Coagulation Index preoperatively was at the lower limit of the normal range indicating that the group as a whole were not hypercoagulable preoperatively.

The significant increase in TEG Coagulation Index throughout the perioperative period and the change in mean thrombelastographic parameters r, rk, α and ma towards hypercoagulabilty intraoperatively and at 3 hours postoperatively compared to mean preoperative values is strong evidence for the development of a procoagulant state during TURP (Caprini et al 1991; Blair et al 1986). The persistent elevation in the mean TEG parameter ma above the normal range from preoperatively until 10 to 14 days postoperatively indicates a marked degree of platelet activation throughout the perioperative period. The platelet activation increases peroperatively becoming maximal up to two weeks postoperatively, which is consistent with previous findings (Wright 1942; Bennett 1967). The point at which platelet function returns to normal following TURP is not known.

The significant decrease in both TEG parameters r and rk intraoperatively and at 3 hours postoperatively and the persistent significant increase in α perioperatively indicates an overall increase in the rate of clot formation and is further evidence of accelerated coagulability in the early perioperative period. This concurs with the findings of Britton et al (1974) who described a reduction in thrombelastographic clotting time perioperatively in patients undergoing gastrointestinal surgery, Koh et al (1995) who described a reduction in the reaction time r in the first postoperative day in patients undergoing gynaecological surgery and Caprini et al (1991) who described a significant increase in TEG Coagulation Index and decrease in APPT on the first postoperative day in both open and laparoscopic cholecystectomy.

The increase in mean clot retraction which becomes statistically significant ($p<0.05$) at 72 hours postoperatively when compared to mean preoperative levels gives further support to the thrombelastographic evidence of systemic hypercoagulability over the perioperative period in patients undergoing TURP.

The lack of any correlation between the TEG parameters and haematocrit does not support the proposed theory that haemodilution is the cause of the hypercoagulability postoperatively in patients undergoing TURP.

The similarity in TEG Coagulation Index data between the whole study group and the subgroup with benign histology, suggests that

the procoagulant state is likely to be due to the TURP process itself, and not the presence of carcinoma.

The possibility of the findings of hypercoagulability being due to sampling error was minimised by the use of the two-syringe technique (Gormsen 1961). All blood samples were taken by one person. Traumatic venesection samples were discarded. Patients on whom venesection was cosidered difficult were not included in the study. The mean preoperative TEG Coagulation Index was at the lower limit of the normal range. By using this preoperative CI value for each patient as a control, any change seen over the perioperative period cannot be due sampling error. No evidence of hypercoagulability was demonstrated in the patient undergoing cystoscopy alone.

The complete absence of any thrombelastographic evidence of systemic fibrinolytic activity in any patient over the whole perioperative period studied was an unexpected but significant finding. Thrombelastography appears to be a more sensitive measure of systemic fibrinolytic activity than routine tests of fibrinolysis including D dimer (Whitten et al 1991) and euglobin clot lysis times (Summaria et al 1986). Thus the absence of any demonstratable TEG evidence of a fibrinolytic state must cast considerable doubt upon the hypothesis that systemic fibrinolysis has an aetiological role in bleeding following TURP, and gives further support to the findings of a procoagulant perioperative state in patients undergoing TURP.

CHAPTER 4

Urinary Fibrinolytic Activity in Patients undergoing TURP

4.1 Introduction

The ability of urine to lyse clots was first described by Sahli (1885). Macfarlane & Pilling (1947) demonstrated fibrinolytic activity in urine. This was later ascribed to its content of a potent plasminogen activator known as urokinase (uPA) (Williams 1951; Astrup & Sterndorff 1952). Kester (1969) described the presence of a plasminogen activator in freshly excised human prostate tissue. Approximately 45 per cent of plasminogen activator activity in prostate tissue was found to be due to uPA in both benign and malignant prostatic tissue, but overall plasminogen activator activity was higher in malignant tissue (Camiolo et al 1981). Subsequently Kirchheimer & Binder (1983) localised both uPA and tissue plasminogen activator (tPA) within prostatic tissue. However concentrations of uPA are similar in urine obtained from the renal pelvis and the bladder (Bjerrhuus 1952), suggesting that under normal conditions prostatic secretion does not contribute to urinary uPA activity significantly.

McNicol et al (1961a) described the lysis of haemostatic fibrin clots by physiological levels of urinary uPA causing postoperative impairment of haemostasis within the urinary tract.

Using intravenous epsilon aminocaproic acid (EACA, a potent inhibitor of fibrinolytic activity) peroperatively in a controlled trial of 28 patients undergoing TURP, McNicol et al (1961b) showed a marked reduction in urinary urokinase activity postoperatively together with a reduction in postoperative blood loss, but this was

not statistically significant. Although theoretically there is the potential for a massive release of the plasminogen activators tPA and uPA from the prostate during transurethral resection, McNicol et al (1961b) found no increase in urinary fibrinolytic activity in their control group of patients.

Against a background of systemic hypercoagulability, excessive urinary fibrinolytic activity causing clot lysis at the prostate bed and consequent secondary haemorrhage seems unlikely. However Hong, Pyo & Lee (1990) found an inverse correlation between urine fibrinolytic activity and serum fibrinolytic activity.

4.2 Methods

To investigate further the theory of increased urinary fibrinolytic activity following TURP, the urinary fibrinolytic activity was measured in 20 of the patients with clinically benign prostatic enlargement undergoing TURP in the study.

Urine sampling for fibrinolytic activity was performed preoperatively, at 48 hours postoperatively (after discontinuation of the intracystic irrigation) and at 10 to 14 days postoperatively. A midstream urine specimen was taken preperatively and at 10 to 14 postoperatively. A catheter urine sample was taken from the catheter valve at 48 hours. Peroperative and postoperative urinary sampling prior to this was not performed because variable dilution of the urine with irrigation fluid would have prevented useful comparison of results.

The collected samples were placed in iced water until storage at - 40 ^{o} C in 1 ml aliquots. All samples were subsequently assayed together thereby removing any inter-assay variability.
Urine cultures were performed preoperatively, on removal of the catheter and at 10 to 14 days postoperatively.

4.22 Urinary urokinase activity

Urinary fibrinolytic activity was measured as urinary urokinase activity using a standard fibrin plate assay (Astrup & Mullertz 1952). Standard urokinase solution (International Standard for High Molecular Weight Urokinase [HMW-UK 87/594, 4,300 International Units per ampoule], National Institute for Biological Standards and Control, Potters Bar, UK) was reconstituted in sterile water and six serial dilutions created to give concentrations ranging from 0.0425 IU/ml to 8.5IU/ml, giving a range of lytic zone diameter on the fibrin plate from 3 to 28 mm. A 30 μl aliquot of each sample was pipetted into the central 2mm well of a separate fibrin plate which were then incubated at 37 ^{o}C for 24 hours. Duplicate samples of four of the dilutions were incubated as controls. The maximum and minimum lytic zone diameter on each fibrin plate was measured to the nearest 0.5 mm at 24 hours, and the mean of the lytic zone diameter plotted against $\log_{10}$ urokinase activity giving a linear relationship ($r^2 = 0.947$)(figure 4.1).

All samples were thawed simultaneously in a waterbath to 37° C and 30 μl from each sample was pipetted into the 2mm diameter central well of a separate fibrin plate. All plates were then incubated at 37° C for 24 hours on a flat surface. The mean of the maximum and minimum diameter of the fibrin plate lytic zone was then used to calculate the urokinase activity for each sample from the standard curve. Four age-matched control urine samples were assayed simultaneously and in duplicate to assess intra-assay variability. Five samples were simultaneously assayed on separate fibrin plates preheated to 80° C for 60 minutes to inactivate the plasminogen contained within the fibrin plate, and thus assess what degree of fibrin plate lysis was due to proteolysis rather than fibrinolysis (Marsh & Arocha-Pinango 1972).

4.3 Results

A urine sample from one patient had to be discarded leaving serial samples in 19 patients available for analysis. The intra-assay coefficient of variability was calculated as 2.3 per cent.

Three patients had a clinically significant urinary tract infection (greater than 10^5 organisms per millilitre) preoperatively and at 10 to 14 days postoperatively. All the 48 hour catheter urine specimens showed no significant growth. No patient required re-admission for secondary haemorrhage postoperatively; however 7 patients (37%)

described at least one episode of haematuria following discharge from hospital.

No significant increase in mean urinary urokinase activity was seen at 48 hours or 10 to 14 days postoperatively when compared with mean preoperative activity (ANOVA, p = 0.854). No significant difference between the mean normal urinary urokinase activity and mean preoperative, 48 hour or 10 to 14 day values was found (Mann-Whitey U test, p=0.84, 0.73 and 0.41 respectively) (see table; figure 4.2). No significant increase in urinary urokinase activity was seen in any of the infected urine samples.

The mean percentage of total fibrin plate lysis accountable to proteolysis in the 5 samples assayed on preheated fibrin plates was 62 per cent (range 19 - 88%).

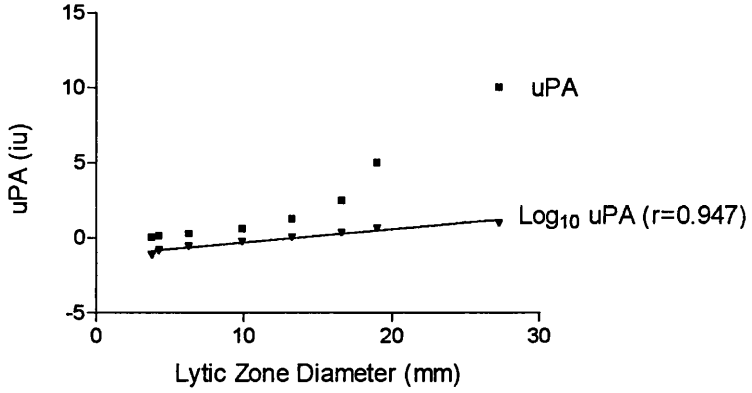
Urinary Urokinase Activity (IU)

	Control	Before	48 hours	10-14 days
Mean	**4.77**	**5.14**	**4.44**	**4.87**
S.E.M	**0.06**	**0.54**	**0.45**	**0.38**
p value	**NA**	**0.84**	**0.73**	**0.41**

Table 4.1: Urinary urokinase activity measured in International Units (IU) for BPH (n=19) and control (n=4) samples.

S.E.M = Standard Error of the Mean. Mann Whitney U test p values indicated.

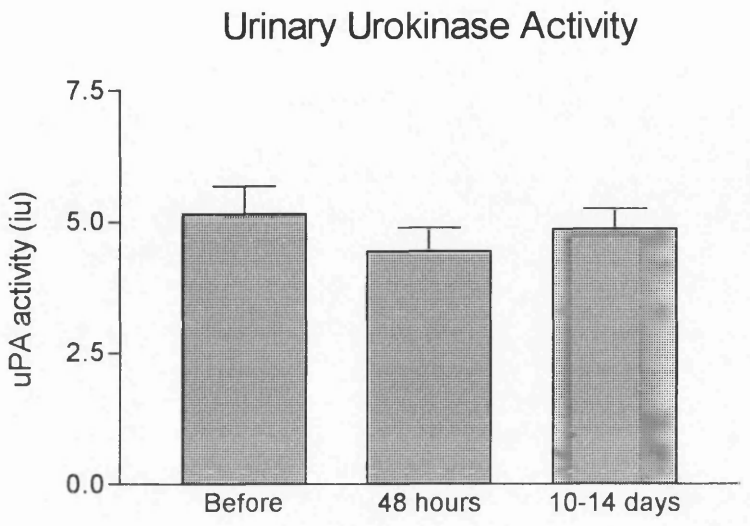
Urinary Urokinase Activity Standard Curve



Standard curve for urinary urokinase activity.

uPA = urinary urokinase activity.
r = correlation coefficient.

Figure 4.1



Mean urinary urokinase (uPA) activity ± SEM bar
n = 19 Normal range 4.55 - 4.99 (mean 4.77 ± 2 Standard Deviations)
ANOVA p = 0.854

Figure 4.2

4.4 Discussion

Secondary haemorrhage most commonly occurs at 10 to 14 days postoperatively, but can occur any time from removal of the catheter. By 10 to 14 days under normal wound healing conditions the prostate bed will no longer be covered by a simple clot but an eschar of granulation tissue consisting of ingrowing capillaries, macrophages, fibroblasts and marginal re-epithelialisation (Silver 1980). The strength of the changing clot becomes less dependent on fibrin for its structural integrity as more collagen is layed down. For physiological levels of urinary fibrinolysis to play an important role in the aetiology of secondary haemorrhage by causing clot lysis at the prostate bed, bleeding would be expected to occur earlier when clot integrity is more dependent on the initial fibrin mesh. In a controlled study comparing EACA with normal saline as a postoperative irrigant fluid in patients undergoing TURP, Sharifi et al (1986) failed to show any significant reduction in early postoperative haemorrhage by inhibiting urinary fibrinolytic activity. This suggests that early blood loss following TURP is dependent upon surgical technique and not on local fibrinolysis in the prostate bed.

The role of urinary fibrinolytic activity in secondary haemorrhage is unclear. Miller et al (1980) studied the use of oral tranexamic acid (a potent antifibrinolytic agent excreted in the urine) for 3 weeks postoperatively in a controlled trial of 100 patients undergoing tranurethral surgery in an attempt to reduce the incidence of secondary haemorrhage by reducing urinary fibrinolytic activity.

Although they found a significant reduction in the incidence of patient-reported haematuria from the second postoperative week in the tranexamic acid group, no significant difference in readmission rate for haemorrhage or clot retention between the two groups was found.

No increase in urinary fibrinolytic activity as measured by urinary urokinase activity was seen over the perioperative period in this study. Indeed there was a trend towards a reduced urinary urokinase activity at 10 to 14 days postoperatively. All patients who reported haematuria following discharge had urinary urokinase levels within the normal range postoperatively. Urinary tract infection has been implicated in secondary haemorrhage following TURP (Blandy 1972; Harvey et al 1986). Certainly it is a well recognised cause of delayed wound healing (Forrester 1988). None of the patients with a urinary tract infection showed any increase in urinary urokinase activity. Urinary fibrinolytic activity does not appear to be increased postoperatively in either sterile or infected urine, and questions the role of local fibrinolysis as a cause of secondary haemorrhage following TURP.

Proteolysis accounted for up to 88 per cent of the lytic activity of the urine samples assayed. Whether proteolysis has a role in eschar dislodgement and secondary haemorrhage following TURP, and the effects of urinary sepsis on urinary proteolytic activity remains unclear.

CHAPTER 5

Fibrinolytic Shutdown

5.1 Introduction

Although thrombelastography is a sensitive measure of the global coagulation status, the presence of activation and coexistent simultaneous inhibition of the fibrinolytic system may be masked against a dominant background of hypercoagulability.

Elevation of the fibrin split products (FSP) both preoperatively and postoperatively following TURP, as evidence of systemic activation of the fibrinolytic system, has previously been described in both benign and malignant prostate disease (Mertens et al 1974; Betkurer et al 1979).

However the absence of any detectable thrombelastographic evidence of systemic fibrinolysis following TURP raises the possibility of 'postoperative fibrinolytic shutdown' previously described following other forms of surgery (Chakrabarti et al1969; Mansfield 1972) due to an imbalance in the activation (tPA mediated) and inhibition (PAI-1 mediated) of fibrinolysis (Kluft et al 1985; Aranda et al 1988; D'Angelo et al 1985).

Native human plasminogen is a pro-enzyme β-globulin with a molecular weight of 88,000 daltons. It appears to be synthesised in the liver and may be produced, stored and transported in eosinophils. Normal plasma concentrations average 20.3 $\pm$ 2.6 mg/dl, with a half-life of 2.2 $\pm$ 0.29 days (Collen & Maeyer 1975). It contains five *kringles* - looplike structures of amino acids containing critical lysine-binding sites - which are competed for by fibrin and α_2-antiplasmin during physiological fibrinolysis (Gaffney 1987).

Large amounts are adsorbed onto the fibrin-platelet plug during haemostasis. It is converted into the proteolytic enzyme plasmin by the action of plasminogen activators, the most important of which being tissue plasminogen activator (tPA).

Native human tissue plasminogen activator (tPA) is a single chain polypeptide serine protease with a molecular weight of 68,000 daltons. It appears to be ubiquitous in the body (Ogston 1978), but is present in high concentrations in vascular endothelium (Todd 1964). Several different stimuli including endotoxin (Suffredini et al 1989) will cause the release of tPA from endothelial cells. On its own tPA is a poor activator of plasminogen, but the presence of fibrin markedly potentiates the process (Rånby 1982).

Hoylaerts et al (1982) provided kinetic data to support the mechanism whereby fibrin provides a surface onto which plasminogen and tPA are adsorbed sequentially to form a ternary complex thereby preventing any significant activation of free plasminogen in the plasma. Binding of tPA to plasminogen at the lysine-binding sites of designated kringles results in an initial slow production of plasmin. This generated plasmin then converts single-chain tPA to two-chain tPA, which more rapidly proceeds to generate plasmin from the bound plasminogen.

The presence of a specific plasminogen activator inhibitor in plasma (PAI-1) was described by Chmielewska, Rånby & Wiman (1983), and was subsequently found to be present in endothelial cells (Loskutoff et al 1983) and platelets (Kruithof et al 1986).

Plasminogen activator inhibitor-1, a 52 kilodalton single chain glycoprotein is the primary inhibitor of both tPA and uPA in plasma. Other plasminogen activator inhibitors have been described. PAI-2 was initially described in human placenta (Astedt et al 1985) and later in the plasma of pregnant women but not normal plasma (Lecander & Astedt 1986). PAI-3 was first isolated in human urine (Stump, Thienpont & Collen 1986) complexed to uPA, and later in plasma in low concentrations where it weakly inhibits tPA (Stump *et al* 1986). Its physiological role is not yet fully understood.

5.2 Method

To investigate the hypothesis of fibrinolytic shutdown following TURP, tPA antigen and PAI-1 antigen levels were measured by enzyme-linked immunosorbant assay (ELISA). Antigen level rather than tPA and PAI-1 activity was measured because the short half-life of tPA (5 minutes) would inevitably lead to artifactual activity results and significant intra-assay sampling errors.

A significant rise in PAI-1 antigen levels above the normal range was used as the primary end-point to prove the hypothesis that fibrinolytic shutdown is present in patients undergoing TURP. In order to achieve a power for the study of 0.95, 21 patients were required to be sampled in order to detect a difference of at least 20 ng/ml with a standard deviation between sampling times of no more than 13.

Commercial tPA and PAI-1 Antigen ELISA kits (Biopool International Inc., Umeå, Sweden) were employed using a standard ELISA technique (see appendix). Twenty-four patients had both tPA and PAI-1 antigen levels assayed on serial plasma samples previously stored at - 40° C and thawed to room temperature in a waterbath. All samples were assayed immediately after thawing. Inter-assay and intra-assay variability were calculated by assaying 20 normal serum in each ELISA, and by repeated sample measurement.

5.3 Results

The normal range for the t(PA) and PAI-1 antigen assays is 3 - 10 ng/ml and 4 - 43 ng/ml respectively. The inter-assay coefficient of variation between the 4 kits using duplicate samples of 20 normal plasma was calculated (table 5.1).

	Sample				Mean	SD	CV
	1	2	3	4			
t(PA)	7.29	6.90	7.00	7.61	7.28	0.62	**8.5**
	8.26	6.35	6.95	7.94			

	Sample				Mean	SD	CV
	1	2	3	4			
PAI-1	27.12	28.31	29.69	30.02	28.69	1.60	**5.6**
	29.90	25.81	30.29	28.37			

Table 5.1: Tissue plasminogen activator and plasminogen activator inhibitor 1 inter-assay coefficients of variation.
SD = Standard Deviation. CV = Coefficient of Variation

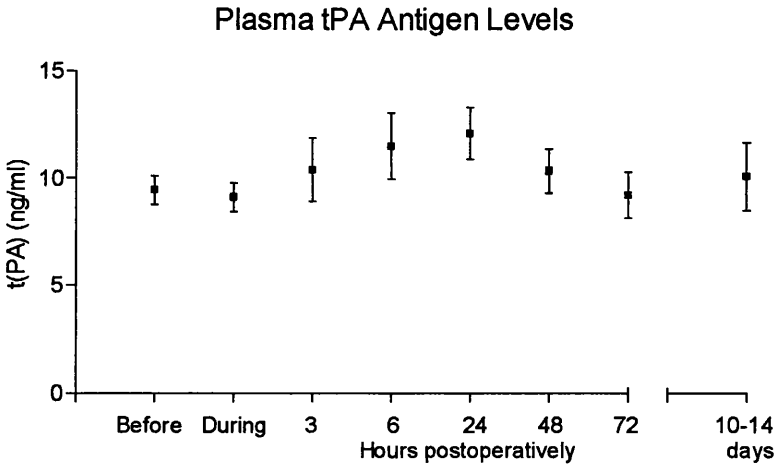
The intra-assay variability was calculated using duplicate wells of at least one sample for each patient. For the t(PA) antigen assay the mean coefficient of variation was 5.6 (range 0.1 - 16.4), and for the PAI-1 antigen assay 4.4 (range 0.8 - 10.1).

Plasma t(PA) antigen levels showed an upward trend above the normal range in the early postoperative period, with mean antigen levels reaching a peak 24 hours postoperatively, before declining towards the normal range (table 5.2; figure 5.1a). However these changes in t(PA) antigen levels were not statistically significant (repeated measures ANOVA, $p = 0.737$).

Mean plasma PAI-1 antigen levels remained within the normal range throughout the perioperative period studied (table 5.2; figure 5.1b), with no significant variation in mean recorded values (repeated measures ANOVA, $p = 0.348$)

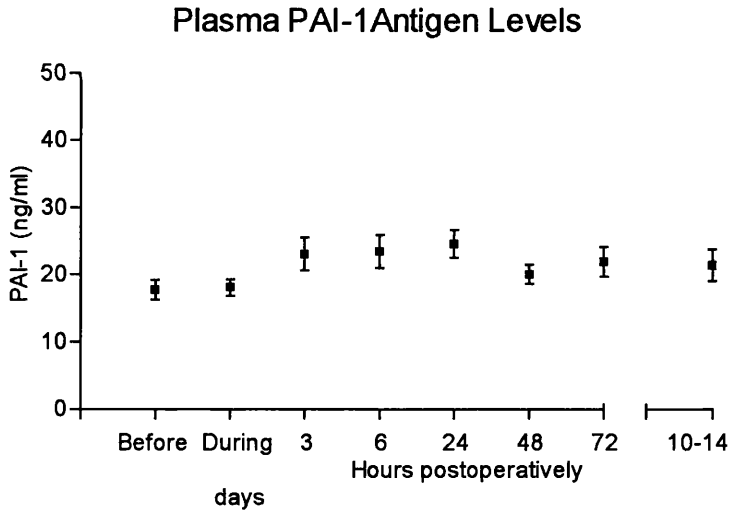
		SAMPLE							
			—— Hours Postoperatively ——						Days
		Before	During	3	6	24	48	72	10-14
tPA	Mean	9.44	9.12	10.39	11.48	12.09	10.33	9.21	10.08
	SD	3.28	3.28	6.88	7.53	5.87	4.98	5.23	6.87
	SEM	0.67	0.67	1.47	1.54	1.20	1.04	1.07	1.58
PAI-1	Mean	17.74	18.11	23.07	23.49	24.61	20.07	21.95	21.39
	SD	7.26	6.09	11.67	12.28	10.10	7.15	10.75	10.29
	SEM	1.48	1.24	2.43	2.51	2.06	1.49	2.24	2.36

Table 5.2: Variation in mean plasma tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1) levels measured perioperatively, with the standard deviations (SD) and standard errors of the mean (SEM) given.



- Mean tissue plasminogen activator (tPA) antigen levels ± SEM bar. Normal range 3 - 10ng/ml
  n = 24 ANOVA p = 0.737

Figure 5.1a



- Mean plasminogen activator inhibitor 1 (PAI-1) levels ± SEM bar. Normal range 4 - 43ng/ml
  n = 24 ANOVA p = 0.348

Figure 5.1b

5.4 Discussion

The absence of any significant increase in mean PAI-1 levels above or even within the normal range over the perioperative period studied, when compared to preoperative values, does not support the hypothesis that fibrinolytic shutdown due to an imbalance in tPA and PAI-1 levels occurs following TURP. A relative excess of PAI-1 with or without a fall in tPA antigen levels therefore cannot account for the absence of any TEG evidence of fibrinolysis, or the observed hypercoagulable state.

The trend towards an increase in mean tPA antigen levels in the early postoperative period gives the graph curves for mean tPA and PAI-1 antigen levels a similar shape. The theory postulated by Kluft et al (1985) and Aranda et al (1988) that a fall in tPA antigen levels may be contributing to an imbalance between activation and inhibition of the fibrinolytic system, therefore, is also discounted in this group of patients studied.

The timing of the rise in mean tPA levels which peaks at 24 hours may indicate a degree of secondary activation of the fibrinolytic system subsequent upon activation of the clotting cascade, seen at 6 hours postoperatively . However this rise is not statistically significant.

CHAPTER 6

Markers of Systemic Activation of The Coagulation Cascade

6.1 Introduction

Using the ethanol gelation test as a marker for activation of the coagulation cascade, Mertens et al (1974) described a preoperative activation of coagulation in 42 per cent of patients with prostatic carcinoma and 8 per cent of men with BPH. Together with their findings of elevated levels of fibrin split products (FSP, FDP, XDP) in 85 per cent of cases preoperatively, they concluded that intravascular coagulation with secondary fibrinolysis was occuring in a significant number of preoperative patients undergoing TURP. Adamson et al (1994) detected raised levels of fibrinopeptide A and D dimer in 16 per cent of patients with BPH and 40 per cent of patients with prostate cancer not undergoing surgical intervention, indicating a degree of independent activation of coagulation in prostatic disease.

Iwan-Zi:etek et al (1992) and von Hundelshausen et al (1992) both found evidence of activation of the clotting cascade over the perioperative period in patients undergoing TURP.

The conversion of prothrombin to thrombin represents a central event within the coagulation cascade. Thrombin is a proteinase acting on different physiological substrates (fibrinogen, factor VIII, V, XIII, protein C, platelets). Its major physiological inhibitor is antithrombin III (ATIII) a plasma α2-glycoprotein. ATIII forms inert irreversible bimolecular complexes with thrombin which is rapidly removed from the circulation by hepatocytes, with a half-life of 15 minutes. Heparin binds to an ϵ-lysine residue in ATIII accelerating

the rate of formation of enzyme-inhibitor complexes thus acting as a cofactor to ATIII enhancing its anticoagulant properties. The inert nature of the thrombin-antithrombin III (TAT) complexes make it an extremely sensitive marker of activation of coagulation (Bauer & Rosenberg 1987); elevated plasma levels of TAT are considered to be a reliable marker of hypercoagulability (Hutten 1992; Lattan & Bradshaw 1995).

The proteolytic action of plasmin on fibrin or fibrinogen leads to the formation of a family of soluble protein fragments known as fibrin degradation or split products (FDP, FSP) (Kwaan & Barlow 1973). Perioperative elevation of FDP's in patients undergoing TURP has already been described (Mertens et al 1974; Betkurer et al 1979). However FDP's (including XDP) are formed from the proteolytic degradation of both fibrin monomers and fibrinogen and cannot therefore distinguish between physiological fibrinolysis that occurs in response to any activation of the coagulation cascade, and pathological hyperfibrinolysis resulting in fibrinogenolysis as well as the lysis of fibrin. Therefore as a marker of pathological fibrinolysis they are unsatisfactory.

For fibrin to be fully cross-linked the presence of both thrombin and factor XIII are required. Thus activation of the coagulation cascade is a prerequisite for this. Only the lysis of cross-linked fibrin by plasmin yields the large degradation product called D-dimer which consists of the C-terminals of two parent molecules with a molecular weight of approximately 180,000 daltons (Marder & Budzynski

1975). With the development of an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against the D-dimer fragment (Rylatt et al 1983), a sensitive specific method of measuring the fibrinolytic response to the presence of cross-linked fibrin was established. The concentration of D-dimer in plasma reflects the degree of ongoing fibrinolytic activity as well as providing an index of intravascular thrombin formation and thus a marker for the systemic activation of the coagulation cascade (Bauer & Rosenberg 1987).

6.2 Methods

D-dimer

Plasma D-dimer levels were measured using a standard commercial plasma D-dimer ELISA assay (Biopool TintElize® Biopool International Inc., Umeå, Sweden) in serial perioperative plasma samples were assayed from 13 patients with BPH in the study. D-dimer levels from all sampling times were measured collectively using 4 kits, thawed from -40°C in a waterbath and assayed immediately. Inter and intra-assay variability was measured using 20 normal serum (see appendix) and duplicate sampling respectively.

TAT

The ELISA used two commercial antibody preparations, a monoclonal coat antibody to TAT (Biogenesis, Poole, Dorset, UK) and a detection antibody (peroxidase conjugated rabbit immunoglobulins to human antithrombin III, Dako Ltd, High Wycombe, UK). Standards were prepared using the technique described by Dawes, James, Micklem, Pepper & Prowse (1984). This was calibrated by reference to a commercial TAT ELISA (Enzygnost TAT micro, Behring, Milton Keynes, UK), and a standard curve established with points at 34, 17, 8, 4, 2, 1. 0.5 and 0 (buffer only) μg/L. Samples were diluted 1:2 and 1:4. Microtitre plates ('Maxisorb', Nunc, through Life Technologies, Paisley, UK) were coated with 100 μl/well of coating antibody diluted in 0.05M carbonate-bicarbonate buffer pH 9.6 and incubated at 4°C overnight. The plates were then washed five times with 0.01M phosphate buffer pH 7.4 containing 0.5M sodium chloride and 0.1% Tween 20 (wash buffer), using an automatic plate washer. Samples and the detection antibody were diluted in wash buffer containing 3% PEG 8000, which has been shown to facilitate protein binding to immobilised antibody (Woodhams & Kernoff 1983). Samples and standards (100μl/well) were incubated for one hour at room temperature on a plate shaker at 400 RPM. After a further five washes, 100μl of working strength detection antibody was added to each well, and incubated for a further hour on the plate shaker at room temperature. After further washing the reaction was visualised

by the addition of 100μl/well of 0.1M citrate/acetate buffer containing one 10mg tablet of OPD (o-phenylenediamine dihydrochloride, Sigma Chemical Co, Poole, UK) and 7μl 30% of hydrogen peroxide per 15ml. The reaction was stopped after 10 minutes with 100μl/well of 1.5M sulphuric acid. Plates were read at 492nm on a Titretek MS-2 reader (ICN, Thame, UK) with automatic linear spline curve fitting and data analysis. The reference range for TAT in normal plasma is quoted for the Behring TAT ELISA is 1.0 - 4.1 μg/ml. Inter and intra assay coefficients of variability were calculated using 100μl/well of 20 normal plasma (see appendix) as controls in each assay. Serial samples were assayed in 24 patients in a total of 13 assays.

6.3 Results

D-Dimer

The upper limit of normal for the D-dimer assay was 531 ng/ml, calculated from the mean of n = 87 healthy blood donors (see appendix) + 2 standard deviations. The inter-assay coefficient of variability between 4 kits was calculated (table 6.1).

	Sample				Mean	SD	CV
	1	2	3	4			
D-dimer	298	367	311	321	329	21.0	**6.4**
	327	341	329	341			

Table 6.1: Inter-assay coefficient of variation for the D-dimer assay.

The intra-assay coefficient of variability was calculated using duplicate wells for samples for each patient. The mean intra-assay coefficient of variability for D-dimer was 4.0 (range 0 - 10.9).

No evidence of preoperative activation of coagulation was seen with the preoperative mean D-dimer level being well below the normal mean.

A progressive rise in mean D-dimer levels to above the normal range was seen over the perioperative period (figure 6.1a), reaching statistical significance at 24 hours postoperatively when compared to the preoperative mean value (ANOVA $p = 0.0154$, Dunn's multiple comparison test $p < 0.05$). The mean D-dimer values

returned to within the normal range by 10 to 14 days.

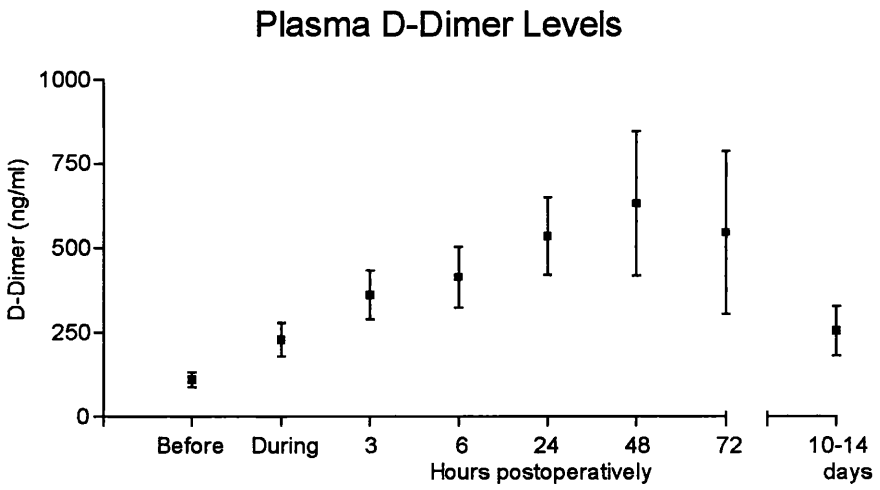
TAT

The precision within and between the four ELISA assays was measured by assaying samples of known TAT concentration in quadruplicate (table 6.2).

	Coeffiecient of Variation			
	assay 1	assay 2	assay 3	assay 4
inter-assay	12.2	11.7	11.3	11.7
intra-assay	7.6	2.0	8.1	6.7

Table 6.2: Inter- and intra-assay coefficients of variation for the thrombin-antithrombin III assay. Linearity of dilution for the four standard curves r^2 = 0.95, 0.96, 0.93 and 0.95.

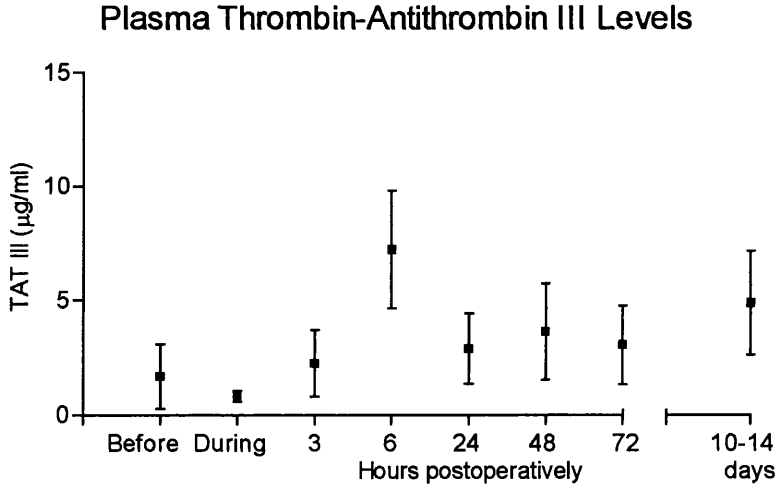
Despite a wide variation in TAT levels within sampling times, there was a significant increase in mean TAT levels 6 hours postoperatively compared to preoperative mean TAT levels (ANOVA p=0.0099, Dunn's multiple comparison test p<0.01; figure 6.1b). There was a trend towards increased levels throughout the postoperative course including 10-14 days. However apart from at 6 hours the increases did not reach statistical significance.



- Mean plasma D-Dimer levels ± SEM bar.
  Normal range (mean of 87 healthy donors + 2 standard deviations [207 + 324] = < 531 ng/ml).n = 13

  ANOVA p = 0.0154. Dunn's multiple comparison test, preoperative vs 24 hours p < 0.05

Figure 6.1a



- Mean plasma thrombin-antithrombin III complex Levels ± SEM bar.
  Normal range = 1.0 - 4.1 µg/ml. n = 24
  ANOVA p = 0.0099. Dunn's multiple comparison test preoperative vs 6 hours p < 0.01

Figure 6.1b

6.4 Discussion

The low levels of D-dimer and TAT preoperatively infers an absence of any significant activation of the coagulation cascade or physiological secondary fibrinolysis preoperatively in this group. This may be due to the small numbers and preponderance of patients with BPH. The low levels preoperatively and the fact that all blood sampling was performed by one person using a standardised technique makes the changes seen over the perioperative period unlikely to be due to artefact.

The significant increase in both TAT and D-dimer levels over the perioperative period even with the small numbers and wide standard deviation provides firm evidence for the perioperative activation of coagulation in patients undergoing TURP, which is appears to be initiated peroperatively becoming maximal over the early postoperative period.

These findings concur with the theory of a postoperative hypercoagulable state (Bithell 1993; Baker 1995). Whether the stress response - with the release of endogenous adrenaline (Ingram & Jones 1966; Swedenberg & Olsson 1978) or vasopressin (Grant et al 1986) - has an aetiological role in the production of the procoagulant state in patients undergoing TURP is uncertain.

The elevated levels of D-dimer postoperatively indicates the presence of fibrinolytic activity. However this appears to be a physiological response to the perioperative activation of the

coagulation cascade with the generation of thrombin and fibrin, a recognised initiator of fibrinolysis (Davis 1987).

CHAPTER 7

Clot Stability and Wound Healing - the Role of Factor XIII

7.1 Introduction

The finding of a significant correlation between blood loss and clot volume following TURP raised the possibility of defective clot formation as a cause of continued bleeding following TURP.

Factor XIII is essential for normal haemostasis. Plasma factor XIII is an α_2-globulin composed of two subunits A and B (FXIIIA, FXIIIB). FXIIIA appears to be the active enzyme, whilst FXIIIB acts purely as a carrier protein (Ichinose et al 1986a; Ichinose et al 1986b).

FXIIIA's most clearly defined physiological role is in the formation of covalent crosslinks between the alpha and gamma chains of adjacent fibrin strands and the incorporation of antiplasmin into the clot, rendering the clot physically stronger with increased fibrinolytic resistance (McDonagh 1987; Beck, Duckert & Ernst 1961).

Factor XIII is also important in wound healing (McDonagh 1987). Abnormal wound healing is seen in patients with factor XIII deficiency (Duckert 1972). FXIIIA encourages wound healing by promoting the binding of fibrin and fibronectin to exposed subendothelial collagen (Mosher, Schad & Vann 1980). The rate of synthesis of factor XIII is slow (McDonagh 1987).

A reduction in factor XIII levels postoperatively has been reported after gynaecological (Kidess et al 1979), thoracic (Yaku et al 1988), and abdominal surgery (Hosenfeld, Dünnweber & Kaiser 1969) where levels can fall to 50 to 60 per cent of normal values between the third and fifth postoperative days. Beck , Duckert & Ernst (1961) described an impairment in wound healing when factor XIII levels

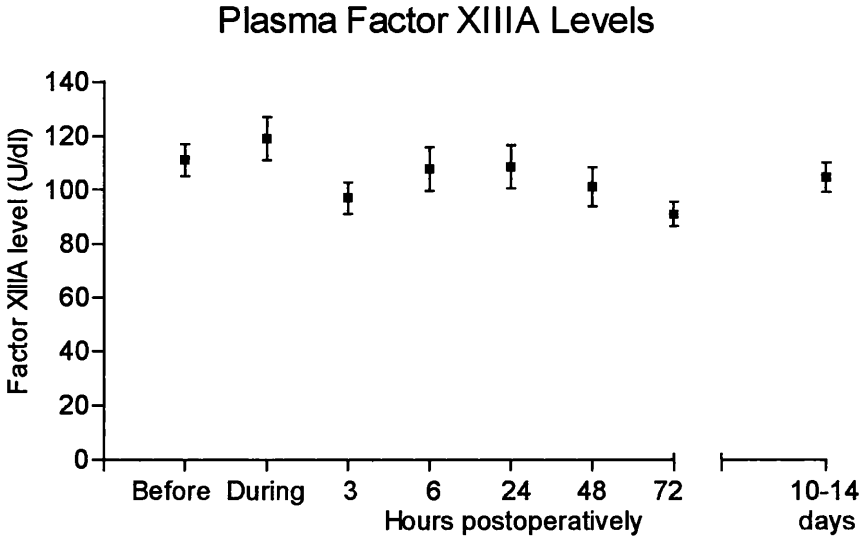
fell below 50 per cent of the normal. A later study by Gierhake et al (1970) described a 10 per cent incidence of poor wound healing in patients with postoperative factor XIII levels of between 66 and 91 per cent of the normal value. No patients with greater than 91 per cent of the normal value demonstrated poor wound healing.
Shainoff et al (1994) described unstable 'soft clot' formation with increased postoperative blood loss following coronary artery bypass surgery in patients with a marked acquired FXIIIA deficiency.
It is not known whether or not there is a significant reduction in factor XIII levels postoperatively in patients undergoing TURP. However it provides an attractive theory to explain the heavy blood loss that occurs postoperatively in some patients. Low levels of FXIIIA would lead to the formation of fragile clots being repeatedly washed off the prostatic bed by the irrigant fluid leading to repeated cycles of bleeding, coagulation (with the formation of a pro-thrombotic state and further consumption of factor XIII) with fragile clot formation, further bleeding and eventually poor wound healing.

7.1 Method

Serial plasma samples prepared as previously described from 20 of the study patients were thawed simultaneously to room temperature and assayed collectively in one assay. FXIIIA and FXIIIB levels were determined by ELISA (Murdock et al 1992). Intra-assay variability was measured by duplicate sampling.

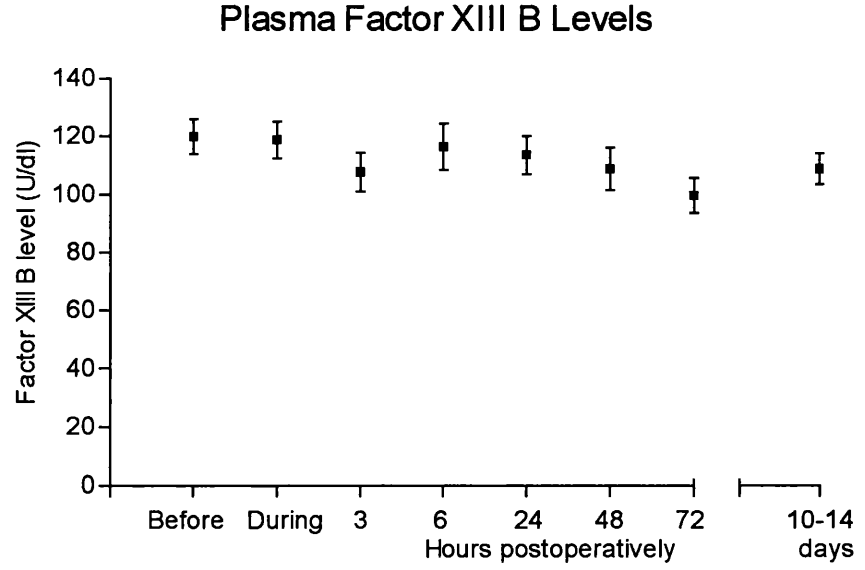
7.3 Results

The mean coefficient of variation for both assays combined was 7.0 (range 3.7 - 9.8). No significant decrease in either mean FXIIIA or mean FXIIIB antigen levels was seen over the perioperative period (ANOVA, $p = 0.134$ and $p = 0.040$ respectively). No fall in mean levels below the normal range was seen for either subunit at any stage throughout the perioperative period. However there was a trend towards a reduction in FXIIIA and FXIIIB levels within the normal range at 3 hours and 72 hours postoperatively (figure 7.1a and 7.1b).



- Mean Factor XIII A antigen levels ± SEM bar.
  Normal range 70 - 130 U/dl
  n = 20 ANOVA p = 0.134

Figure 7.1a



- Mean Factor XIIIB antigen levels ± SEM bar.
  Normal range 70 - 130 U/dl.
  n = 20 ANOVA p = 0.04

Figure 7.1b

7.4 Discussion

Although a small decrease in mean FXIIIA and FXIIIB antigen levels was seen at 3 hours and 10 to 14 days postoperatively, this was within the normal range and certainly not of sufficient magnitude to affect clot stability in the early postoperative period. The normal mean level of FXIIIA at 10 to 14 days postoperatively suggests that dislodgement of the eschar and subsequent secondary haemorrhage seen in some patients at this time is not as a result of poor wound healing consequent upon low FXIIIA levels.

Factor XIII appears to be the key factor in clot stability and wound healing. Levels appear to be normal over the perioperative period and thus it is unlikely that clot fragility and impaired wound healing contribute to bleeding following TURP. A small reduction in FXIIIA levels is indicative of thrombin activation and thus activation of the coagulation cascade (Kolb et al 1991). The finding of a reduction in mean FXIIIA levels postoperatively in patients undergoing TURP provides further evidence for activation of coagulation perioperatively in these patients.

CHAPTER 8

Endotoxaemia - a Procoagulant Trigger during TURP?

8.1 Introduction

The presence of gram negative and gram positive bacteraemia during TURP despite prophylactic antibiotic therapy was seen in 10% of cases in our study (see chapter 2). Bacteraemia following TURP has been previously documented in the literature (Robinson et al 1980; Sullivan et al 1973). As described by Shiina, Himeno & Ishibe (1992) we found positive prostatic cultures in patients with sterile urines. Robinson et al (1982) found a 30% incidence of peroperative bacteraemia in 98 patients undergoing TURP, and an identical organism was cultured from the prostate in approximately 50% of cases. In 40% of cases of bacteraemia, the organism cultured was gram negative. The portal of entry of the organisms is likely to be via irrigant fluid absorption.

Endotoxins are lipopolysaccharides (polysaccharide with a covalently bound lipid A) derived from the outer membrane of gram negative bacterial cell walls. They have been implicated in many pathological features of septicaemia as well as activation of coagulation (Levy et al 1985; Doebber et al 1985; Osterud & Flaegstad 1983; Maier & Hahnel 1984) and fibrinolysis (Levi et al 1992; Suffredini et al 1989; Levi et al 1991; van Deventer et al 1990). The presence of gram negative bacteraemia does not necessarily indicate endotoxaemia per se. However antibiotics can induce endotoxin release from Gram-negative bacteria.

Robinson et al (1975) detected an endotoxaemia in 5 out of 18 patients undergoing cystoscopy or TURP, and Sohn et al (1993)

described an endotoxaemia in 11 out of 41 patients undergoing TURP with antibiotic prophylaxis; 3 of the 4 patients developing signs of TURP Syndrome demonstrated a co-existent increase in tumour necrosis factor alpha (TNF-α). The effects of endotoxins on both the coagulation cascade and fibrinolytic system are thought to be mediated at least in part via the actions of TNF-α. The procoagulant effect of endotoxin appears to be mediated via the increased expression of tissue factor on the surface of vascular endothelial cells and monocytes, triggered by TNF-α and IL-1 release (Rivers, Hathaway & Weston 1975; Colucci et al 1983; Lyberg et al 1983; Nawroth et al 1986; Bevilacqua et al 1986). Activation of the fibrinolytic system by endotoxin also appears to be mediated by TNF-α acting to increase plasminogen activator activity by the release of t-PA and u-PA from endothelial cells (Suffredini et al 1989; levi et al 1991).

8.2 Method

To investigate the role of endotoxin in the coagulation changes in patients undergoing TURP, serial measurements of plasma endotoxin and TNFα levels were performed on samples stored in endotoxin-free heparinised bottles (EndoTube ET®, Chromogenix, Mölndal, Sweden) at -70^{0}C.

Plasma endotoxin levels were measured in 20 patients using a commercial quantitative chromogenic Limulus Amoebocyte Lysate

(LAL) assay (COATEST® Plasma-Endotoxin, Quadratech, Epsom, UK). This assay incorporates the standard techniques of heat inactivation and dilution of plasma to control the interfering factors in plasma (DuBose et al 1980). The chromogenic method utilised by this LAL assay, as opposed to the turbidometric technique, gives a more direct measure of LAL proenzyme activation (Iwanaga et al 1978). The assays were performed simultaneously under aseptic conditions using Costar '96 well cluster' flat bottomed sterile microtitre plates and Rainin 'Finepoint' 250μl sterile micropipette tips (BioWhittaker, Wokingham, UK) and endotoxin-free water (Elgastat Option 4 water purification system, Elga, High Wycombe, UK). Results were calculated by the end-point method (colour reaction run for 10 minutes at 37^0C and then stopped with 20% acetic acid). Plasma TNF-α and IL-6 were measured by ELISA (Biosource International, Camarillo, CA, USA) in serial samples from 19 patients.

8.3 Results

Endotoxin

Precision within and between the two ELISA kits used was measured by assaying samples of known TNF-α concentrations in quadruplicate (table 8.1).

	Coefficient of Variation	
	assay 1	assay 2
inter-assay	6.6	7.2
intra-assay	6.8	8.7

Table 8.1: Inter- and intra-assay coefficients of variation for the endotoxin assays. Linearity of dilution for the standard curves $r^2 = 0.79$ and 0.77 respectively.

No significant increase in mean plasma endotoxin levels was found at any stage over the perioperative period (ANOVA $p = 0.183$; Power ≥ 0.95 for an $\alpha = 0.01$). In fact there was a trend towards reduced mean levels postoperatively when compared to the preoperative mean (figure 8.1).

TNF-

	Coefficient of Variation			
	assay 1	assay 2	assay 3	assay 4
inter-assay	7.1	8.6	5.5	5.4
intra-assay	3.8	0.3	3.3	11.7

Table 8.2: Inter- and intra-assay coefficients of variation for the tumour necrosis factor α assays. Linearity of dilution for the standard curves r^2 = 0.79, 0.79, 0.78 and 0.77.

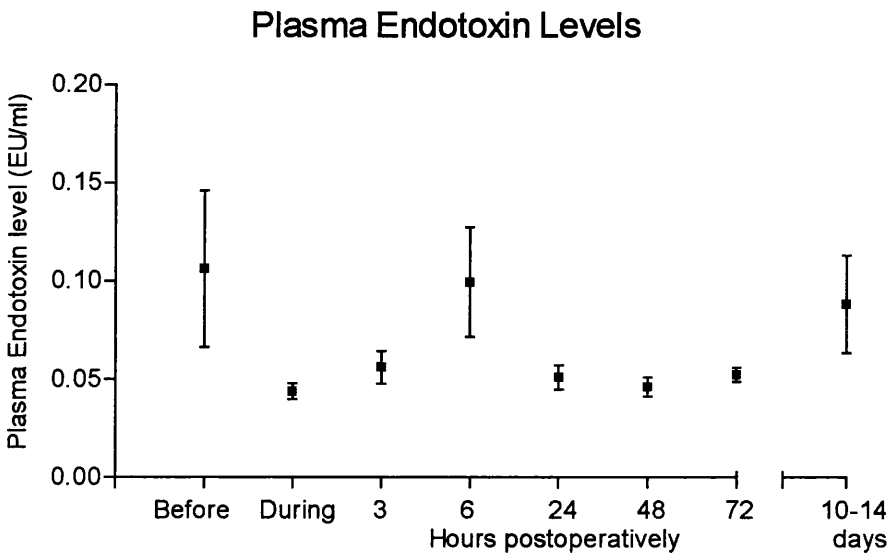
IL-6

Precision within and between the four IL-6 ELISA kits used was measured by assaying samples of known IL-6 concentrations in replicates of 8 (table 8.3).

	Coefficient of Variation			
	assay 1	assay 2	assay 3	assay 4
inter-assay	4.4	5.3	8.8	7.7
intra-assay	2.6	5.1	3.4	4.9

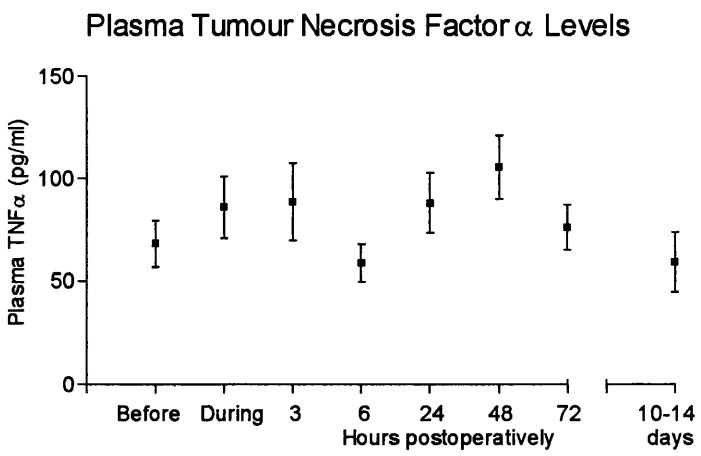
Table 8.3: Inter- and intra-assay coefficients of variation for the interleukin-6 assays.Linearity of dilution for the standard curves r^2 = 0.93, 0.94, 0.89 and 0.92.

No significant change in mean plasma IL-6 was seen at any stage over the perioperative period (ANOVA, p = 0.606; figure 8.2b).



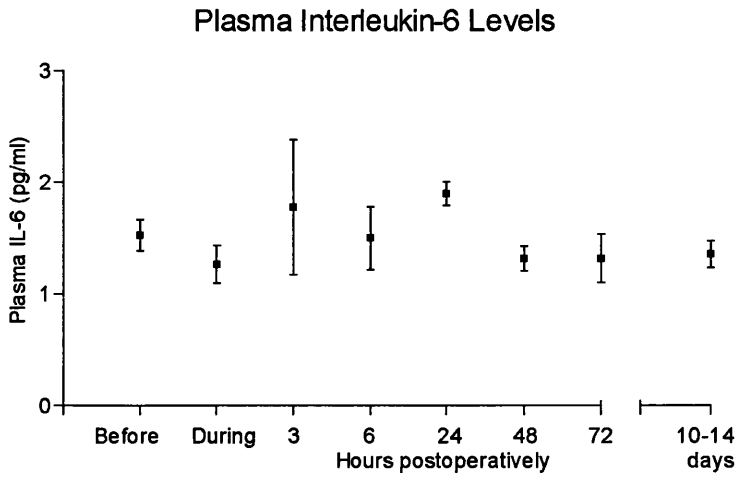
- Mean Plasma Endotoxin Level + SEM bar.
  n = 20 ANOVA, p = 0.1827

Figure 8.1



- Mean plasma tumour necrosis factor α (TNFα) levels ± SEM Bar.
  n = 19 ANOVA, p = 0.240

Figure 8.2a



- Mean plasma interleukin-6 (IL-6) level + SEM bar.
  n = 19 ANOVA, p = 0.606

Figure 8.2b

8.4 Discussion

No significant or sustained increase in serum endotoxin levels were found in any patient perioperatively when compared to preoperative control levels.

Infusion of small amounts of endotoxin into healthy volunteers leads to an increase in serum TNF-α and IL-6 levels followed by a marked increase in circulating markers for the generation of thrombin, suggesting that low circulating levels of endotoxin can induce coagulation activation which is mediated by these cytokines (van Deventer et al 1990). Interleukin 6 appears to be a potent activator of the coagulation cascade (Stouthard et al 1996). The absence of any significant increase in mean plasma TNF-α or IL-6 levels perioperatively supports the absence of endotoxaemia in these study patients, and is strong corroborating evidence discounting the theory of endotoxaemia as the procoagulant trigger producing the observed hypercoagulable state in patients undergoing TURP.

Endotoxins are ubiquitously present in the environment, and therefore any attempts at measurement of serum concentrations of endotoxin may be potentially complicated by spuriously elevated levels. Despite the use of the chromogenic technique, heat inactivation and plasma dilution, significant error in serum endotoxin measurement can occur (Cohen & McConnell 1984). A further significant source of error is the endotoxin contamination of commercially available blood-collection tubes (Redl, Bahrami, Leichtfried & Schlag 1992).

Every precaution to prevent false positive results including the use of endotoxin free blood-collecting bottles was employed in our study. This may explain the absence of any significant endotoxaemia in our study in contrast to previously published findings (Robinson et al 1975; Sohn et al 1993).

CHAPTER 9

Conclusion

The absence of any demonstrable thrombelastographic evidence of systemic fibrinolytic activity in any patient at any stage over the perioperative period was an unexpected finding.The TEG findings are supported by the lack of any significant increase in mean serum tPA antigen levels postoperatively when compared to mean preoperative levels. Any presumed correlation therefore, between blood loss either during or following TURP and serum fibrinolytic activity, has not been substantiated in this study.

These findings are contrary to those of Elliot et al (1963) who found an increase in serum fibrinolytic activity as measured by euglobin lysis time (ELT) immediately postoperatively. However they too found a reduction in systemic fibrinolysis 24 hours postoperatively, and failed to show any correlation between blood loss and fibrinolytic activity. Kang et al (1985) found a significant correlation between euglobin clot lysis time and TEG whole blood clot lysis time.

Our findings concur with those of Nielsen et al (1997a). In a study of 24 men undergoing TURP they found no correlation between systemic markers of fibrinolysis and perioperative blood loss. The mechanism by which EACA acted to reduce peroperative blood loss during TURP (McNicol et al 1961; Madsen & Strauch 1966) is not clear from this study. However our findings agree with those of Smith, Riach & Kaufman (1984) who, in a prospective randomised double-blind study of 61 patients, failed to show any reduction in

blood loss with the use of EACA in the first 72 hours postoperatively.

Increased urinary fibrinolytic activity resulting clot lysis at the prostate bed and subsequent bleeding is an attractive theory. Nielsen et al (1997b) described a significant increase in both urinary tissue plasminogen activator (t-PA) antigen levels at 48 hours and in fibrin degradation products postoperatively in patients undergoing TURP, and this correlated with postoperative blood loss. From these findings they concluded that postoperative blood loss following TURP is related to increased t-PA activity producing a local fibrinolytic response and clot lysis at the prostate bed. Although they did not assess the global fibrinolytic status of urine, they showed clearly that there was no significant increase in postoperative urinary urokinase or t-PA activity, two key markers of fibrinolytic activity. An increase in cross-linked fibrin degradation products is a marker of a fibrinolytic response to the activation of coagulation; a raised level postoperatively in association with increased t-PA antigen levels at 48 hours is likely to reflect the *physiological* fibrinolytic response to activation of the coagulation cascade seen at any wound surface, and not a primary increase in fibrinolytic activity. The absence of any increased urinary fibrinolytic activity perioperatively in patients with sterile and infected urine as shown in this study supports this, and would explain the lack of efficacy of intravesical EACA in reducing intraoperative and early postoperative blood loss (Sharifi et al 1986). Whether increased

proteolytic activity in infected urine plays a role in secondary haemorrhage following TURP (Harvey et al 1986) is unknown. Perioperative blood loss in our study is comparable with previous reports, but no patient in this study was re-admitted with significant secondary haemorrhage. Categorical evidence against the role of fibrinolysis in secondary haemorrhage following TURP would require investigation of urinary and systemic fibrinolytic activity in patients re-admitted with bleeding.

The occurrence of systemic fibrinolytic activity postoperatively in patients undergoing TURP is evident from the significant rise in D-dimer 24 hours postoperatively. However the overall coagulation status is one of hypercoagulability. The observed fibrinolytic response therefore must be a *physiological* response to the observed activation of the coagulation cascade, and not a primary *pathological* entity.

The presence of a hypercoagulable state postoperatively following TURP in both benign and malignant prostatic disease as demonstrated thrombelastographically and by conventional coagulation tests in this study, is less suprising when a comparison with other forms of surgery is considered (Grant et al 1986; Baker 1995).

That systemic activation of coagulation has been triggered in the study patients undergoing TURP is clearly demonstrated by the significant increase in mean TAT levels over the perioperative period. The trigger mechanism for this activation is less clear. The

procoagulant response demonstrated may be simply a primitive physiological response to stress, with catecholamine-induced activation of the coagulation cascade (Ingram & Jones 1966). Haemodilution per se can lead to a hypercoagulable state (Ruttmann, James & Viljoen 1996; Ng & Lo 1996). We have demonstrated evidence of both irrigant fluid absorption and subsequent haemodilution postoperatively in these patients. Seven (18%) of our patients had a fall in serum sodium concentration of 5 mmol/l or more indicating an absorption of at least one litre of irrigant fluid (Hahn 1996). However we found no correlation between change in thrombelastographic coagulation index and fall in serum sodium concentration (Spearman's rank correlation, $r= -0.056$ $p= 0.737$; figure 9.1), suggesting that haemodilution is unlikely to be the major cause of the observed hypercoagulability. Euvolaemic maintainance intravenous fluid replacement with normal saline does not appear to contribute to the effects of haemodilution on haematological parameters (Grathwohl et al 1996).

As with other studies we documented a significant perioperative blood loss in patients undergoing TURP, with blood loss ranging from 5.6 ml to 126 ml per gram of prostate tissue resected. Seventeen patients (43%) in this study had a blood loss of greater than 500ml (approximately 10% of blood volume). Thrombelastographic evidence of hypercoagulability in patients incurring progressive perioperative blood loss has previously been described (Tuman et al 1987). We found no significant correlation

between blood loss per gram of prostate tissue resected and change in thrombelastographic coagulation index perioperatively (Spearman's rank correlation, r= 0.042 p= 0.804; figure 9.2). Thus blood loss per se does not appear to be the major procoagulant trigger in these patients. With a maximum of 3 units of blood being transfused in any one patient, it is unlikely that transfusion itself influenced the coagulation parameters, and would act to prolong rather than reduce the PTTK (Collins & Knudson 1991).

Neither did blood loss during TURP appear to be related to the degree of irrigant fluid absorption in this study - no correlation between blood loss and fall in serum sodium (Spearman's rank correlation, r= -0.002 p= 0.990; figure 2.4) was observed.

A postoperative hypercoagulable state due to an imbalance between tPA and its main inhibitor PAI-1 has been well documented in other forms of surgery (Mansfield 1972; D'Angelo et al 1985; Kluft et al 1985). No significant change or imbalance in mean tPA or PAI-1 antigen levels was seen over the whole perioperative period, indicating that a 'fibrinolytic shutdown' is not the cause of the observed precoagulant state following TURP. Although an increase in tPA or PAI-1 antigen levels does not necessarily indicate increased activity, an increase in activity would by necessity involve an increase in antigen levels.

The combination of an increase in mean TEG ma postoperatively (an *in vitro* marker of clot strength) and normal Factor XIII antigen levels postoperatively, discounts the theory of increased clot fragility

postoperatively as a cause of blood loss.

The 10% incidence of peroperative bacteraemia and 16% incidence of positive prostatic cultures in our study, combined with previous reports of a 45% incidence of endotoxaemia in patients with a sterile preoperative MSU undergoing TURP (Sohn et al 1993), suggested a possible theoretical aetiological link between endotoxaemia and the observed activation of the coagulation cascade. Approximately 10-20% of patients with a gram negative bacteraemia have evidence of disseminated intravascular coagulation (Kreger, Craven & McCabe 1980).

By taking stringent precautions against contamination and false positive results and comparing peroperative and postoperative results with preoperative controls, we found no evidence of any significant endotoxaemia throughout the perioperative period in any of the study patients undergoing TURP.

Tumour necrosis factor-α can stimulate the production of tissue factor by endothelial cells and monocytes whilst reducing the expression of thrombomodulin and thus shifting the haemostatic balance towards coagulation (Esmon 1994). Endotoxin appears to exert its effects on the coagulation cascade through the actions of TNF-α and IL-6 (van Deventer et al 1990). The absence of any significant increase in either of these cytokines over the perioperative period in our study provides strong corroborating evidence against the role of endotoxin in inducing the observed hypercoagulable state.

Is there good evidence in this study for the presence of a low grade disseminated intravascular coagulopathy (DIC) in patients undergoing TURP? The essential laboratory features of DIC are thrombocytopenia, hypofibrinogenaemia, prolonged prothrombin time and activated partial thromboplastin time and elevated levels of fibrin degradation products (Colman, Robboy & Minna 1972; Spero, Lewis & Hasiba 1980). The diagnosis of DIC is basically a clinical one, with many of the laboratory parameters showing variable results. Thrombocytopenia however is the one almost universal finding in acute DIC (Siegal et al 1978; Spero, Lewis & Hasiba 1980). Fibrinogen concentrations are low in less than 50% of cases, and PT and APTT are low in only 70% and 50% of cases respectively (Spero, Lewis & Hasiba 1980). The activated partial thromboplastin time may in fact be reduced due to the procoagulant effect of the activated coagulation factors (Bithell 1993). Other coagulation factors, most commonly factor V and factor XIII, may also be reduced (Bithell 1993). Fibrin degradation products such as D-dimer will be elevated in up to 85% of cases (Carr, McKinney & McDonagh 1989). Fibrinolysis is present in almost all patients, but plays a homeostatic rather than pathological role, in response to endothelial injury or fibrin deposition on endothelial surfaces (Bithell 1993).

O'Donnell (1990) showed a significant decrease in platelet count, fibrinogen level and activated clotting time following infusion of human prostate tissue extract intravenously in dogs, indicating the

presence of a transient DIC.

In this study of patients undergoing TURP, there was certainly no evidence of bleeding from venepuncture sites, spontaneous bruising or petechiae to indicate the development of acute DIC. There was however a significant fall in mean platelet count postoperatively, a rise in mean D-dimer and TAT levels and a decrease in mean APTT postoperatively when compared to mean preoperative values. These findings would be consistent with the diagnosis of an early consumptive and intravascular coagulative process, which have been adequately contained by the natural anticoagulants antithrombin and the thrombomodulin-protein C system. As previously discussed a normal PT, fibrinogen level, tPA and factor XIII antigen levels - as seen in this study - does not exclude the diagnosis of DIC. The presence of prostatic tissue carried into the circulation by absorbed irrigant fluid acting as a tissue thromboplastin to initiate this process, could be inferred by the peroperative rise in mean serum PSA and acid phosphatase levels and postoperative fall in mean sodium levels found in this study. However we found no correlation between rise in serum PSA and acid phosphatase peroperatively and change in thrombelastographic coagulation index (Spearman's rank correlation $r= 0.118$, $p= 0.601$; $r= -0.142$, $p= 0.552$ respectively). If indeed a perioperative rise in serum PSA and acid phosphatase levels are a valid marker for the absorption of prostatic tissue thromboplastin (O'Donnell 1990), its role as a procoagulant trigger

in patients undergoing TURP is questionable.

However taken together our findings are consistent with the presence of a compensated low-grade coagulative process.

A perioperative hypercoagulable state is as evident following TURP as it is in other forms of surgery. Whether the alterations in coagulation parameters are due to the systemic release of prostatic tissue thromboplastins or simply a combined physiological coagulative response to surgical stress, blood loss and haemodilution remains unproven.

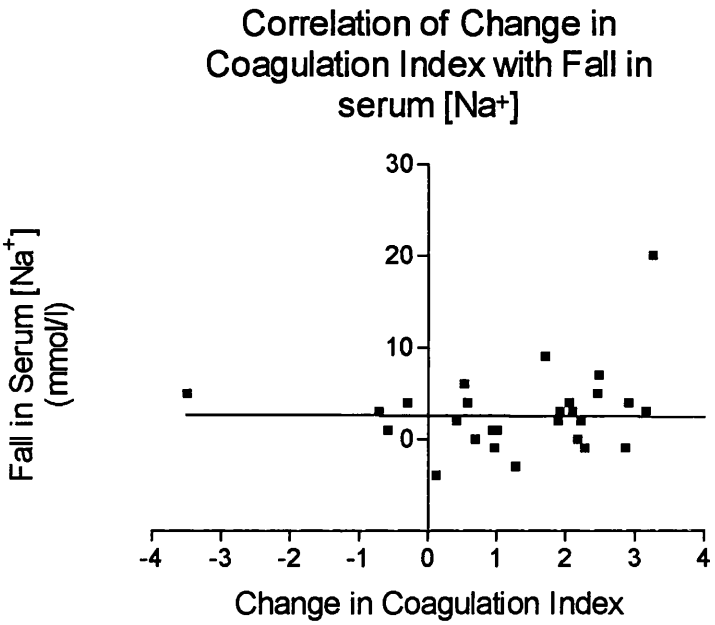
Thirty-nine per cent of reported perioperative urological deaths between 1994 and 1995 occured in patients undergoing TURP - largely as a result of coronary artery disease (Gallimore et al 1997). It is evident that this elderly group of patients is at risk from sustaining a thrombotic vascular event during this perioperative hypercoagulable state. Aspirin withdrawal may further increase this thrombotic tendency. In the absence of any convincing evidence that low dose aspirin increases haemorrhagic complications following TURP, its perioperative withdrawal cannot be recommended. Alternative techniques of prostatic ablation not associated with significant bleeding complications, such as electrovaporization of the prostate (Hammadeh et al 1998) should be considered in these patients.

The assumption that patients undergoing TURP are not at risk of venous thrombosis (DVT) because of peroperative positioning of the lower limbs is erroneous, with a reported incidence of DVT of up

to 10% (Hedlund 1975; Van Arsdalen et al 1993). Although standard heparin prophylaxis is not routinely used, convincing evidence that it increases blood loss following TURP is lacking. Low molecular weight heparin has been shown to reduce bleeding complications in general surgical patients when compared with standard heparin (Kakkar et al 1993). No such comparison has been made in patients undergoing TURP.

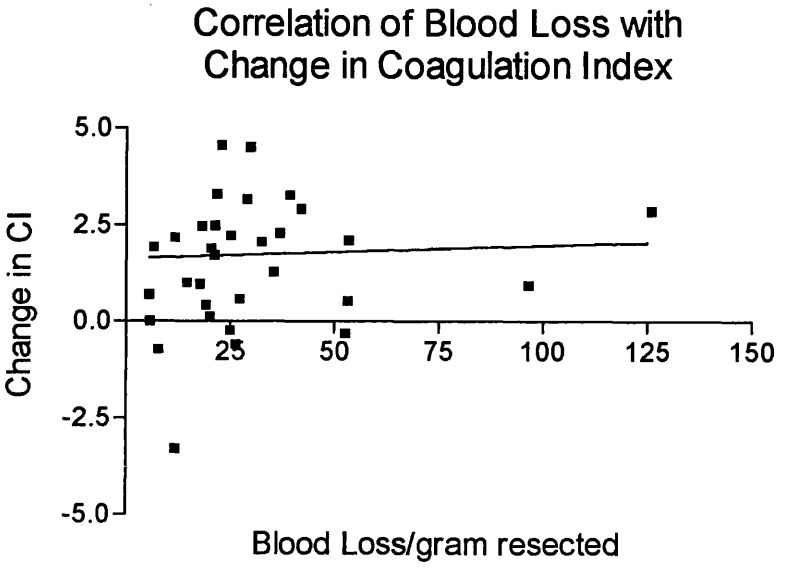
In Summary

This study fails to support the hypothesis that fibrinolysis, either systemically or locally at the prostate bed, has a key role in bleeding following TURP. No evidence of *pathological* systemic or urinary fibrinolysis was seen in any of the studied patients. Clear evidence of a hypercoagulable procoagulant state was seen postoperatively. This does not appear to be a result of 'fibrinolytic shutdown'. A *physiological* fibrinolytic response to the observed activation of the coagulation cascade is evident. The procoagulant trigger in patients undergoing TURP is not immediately apparent. It is likely to be multifactorial - the result of a combination of surgical stresses including catecholamine release, blood loss, and possibly irrigant fluid absorption. It does not appear to be due to circulating endotoxins, and the role of circulating prostatic tissue thromboplastin requires further clarification. Defective clot formation and poor wound healing does not appear to play a role in primary or secondary haemorrhage following TURP.



n = 27
Spearman's rank correlation r = -0.056 p = 0.737

Figure 9.1



n = 32
Spearman's rank correlation r = 0.042, p = 0.804

Figure 9.2

CHAPTER 10

Future Research

Although we have clearly shown the absence of any pathological systemic or local fibrinolytic activity in patients undergoing TURP, the patients studied were largely an uncomplicated group. A degree of primary haemorrhage following TURP is seen in virtually every patient undergoing TURP. The absence of any observed fibrinolytic activity in the early postoperative period and the significant correlation between blood loss and clot formation provides strong corroborating support confirming the null hypothesis that fibrinolysis plays no role in early bleeding following TURP.

Although postoperative bleeding was reported in a significant percentage of patients following discharge, this was self-limiting, and no patient was admitted with secondary haemorrhage and clot retention during the study. To categorically exclude the role of fibrinolysis in significant secondary haemorrhage with or without clot retention, the parameters of urinary and plasma fibrinolytic activity in this group must be studied. The relationship between urinary tract infection and urinary fibrinolytic activity, and the role of proteolysis causing eschar dislodgement with subsequent bleeding in this group needs futher clarification. Self-limiting bleeding after TURP - although a source of anxiety to the patient - does not cause the significant morbidity associated with bleeding with clot retention. Whether the occurence of clot retention is purely dependent on a critical amount of blood loss, or whether there is a difference in the overall coagulative state between patients with bleeding and bleeding with clot retention remains unanswered. Because of the

relative infrequency of clot retention after TURP this would require a prolonged period of observation to include sufficient numbers of patients in a study.

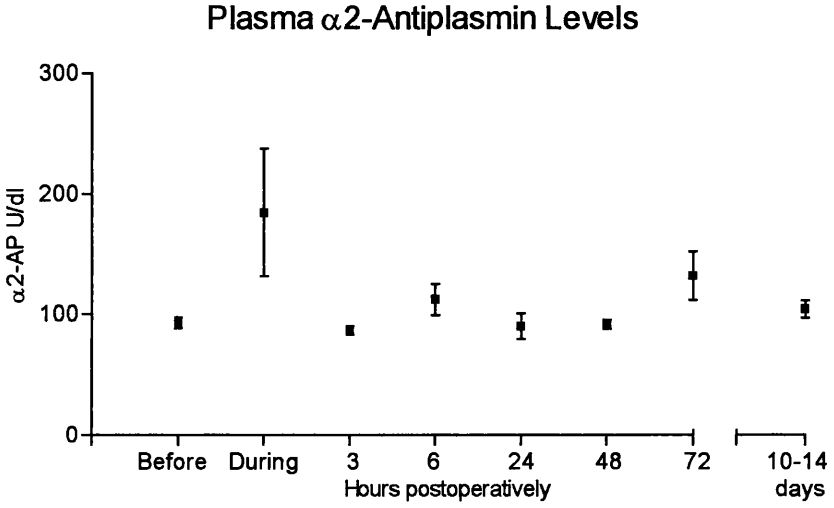
The procoagulant trigger for the observed hypercoagulable state has not been identified. It may be that it is simply a primitive physiological response to stress with a catecholamine-induced activation of the coagulation cascade (Cannon & Gray 1914; Macfarlane 1937; Ingram & Vaughan Jones 1966). The concept of inhibition of fibrinolysis rather than a procoagulant trigger, and particularly the role of PAI-1in this process has been discounted in this study. However other important inhibitors of fibrinolysis are present such as the plasmin - alpha-2-antiplasmin (α_2 -AP) system. We preliminarily looked at the serial changes in α_2 -AP in four patients over the perioperative period using a commercial ELISA (Biopool International Inc., Umeå, Sweden). There was a trend towards increased levels both intraoperatively and postoperatively (figure 10.1a) and although not statitically significant in this small sample (ANOVA p= 0.221) further investigation with larger numbers is clearly warrented.

The concept of inhibition of, or a reduction in, the natural anticoagulants antithrombin III, protein S and protein C and heparin producing a procoagulant state and the effects of surgery and particularly TURP on these systems has not been previously investigated. We preliminarily looked at the serial changes in antithrombin III in 4 patients undergoing TURP using a commercially

available ELISA (Biogenesis, Poole, Dorset, UK). There was a non-significant trend towards reduced levels of antithrombin III postoperatively (figure 10.1b), and this also needs further investigation.

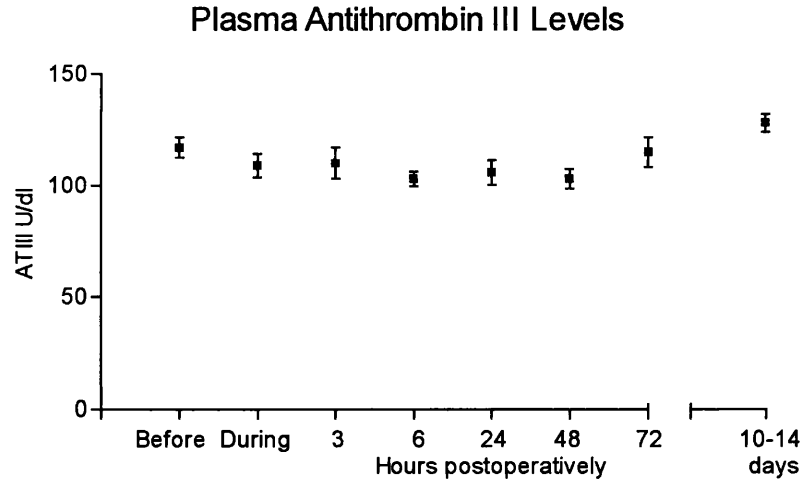
The release of tissue factor (thromboplastin) into the circulation during TURP is inferred by the finding of increased peroperative serum PSA levels. To confirm this, markers of the activation of the coagulation cascade via the 'extrinsic pathway', such as tissue factor pathway inhibitor (TFPI) require evaluation over the perioperative period.

Whether TURP will be replaced by laser prostatectomy, transurethral thermotherapy, transurethral needle ablation or high-intensity focused ultrasound remains to be seen. These innovative techniques all benefit from minimal blood loss. Their effects on coagulation are unknown, and whether they induce an hypercoagulable state justifying anticoagulant prophylaxis requires evaluation.



Mean plasma α2-antiplasmin levels ± SEM bar.
Normal range 70 - 130 U/dl
n = 4 ANOVA p = 0.221

Figure 10.1a



- Mean plasma antithrombin III levels ± SEM bars
  Normal range 70 - 130 U/dl
  n = 4 ANOVA p = 0.172

Figure 10.1b

REFERENCES

Abrams PH, Shah PJR, Bryning K, Gaches CGC, Ashken MH, Green NA. (1982) Blood loss during transurethral resection of the prostate. *Anaesthesia* **37**: 71-73

Adamson AS, Francis JL, Witherow RO'N, Snell ME. (1993) Coagulopathy in the prostate cancer patient: prevelance and clinical relevance. *Ann Royal Coll Surg* **75**: 100-104

Adamson AS, Francis JL, Witherow RO'N, Snell ME. (1994) Procoagulant properties of benign and malignant prostatic tissue. *Br J Urol* **74**: 204-209

Ahsan Z, Cartner R, English PJ. (1993) Coagulation tests in predicting haemorrhage after prostatic resection. *Br J Urol* **72**: 201-206

Alkjaersig N, Fletcher AP, Sherry S. (1959) E-aminocaproic acid: an inhibitor of plasminogen activation. *J Biol Chem* **234**: 832-837

Allen NH, Jenkins JD, Smart CJ. (1978) Surgical haemorrhage in patients given subcutaneous heparin as prophylaxis against thromboembolism. *BMJ* **1**: 1326.

Allen PR, Hughes RG, Goldie DJ, Kennedy RH. (1981) Fluid absorption during transurethral resection. *BMJ* **282**: 740

Allen TD. (1973) Body temperature changes during prostatic resection as related to the temperature of the irrigating solution. *J Urol* **110**: 433-435

Andersen TF, Bronnum-Hansen H, Sejr T, Roepstorff C. (1990) Elevated mortality following transurethral resection of the prostate for benign hypertrophy. But why? *Med Care* **28**: 870 -881

Andersson L. (1964) Antifibrinolytic treatment with epsilon-aminocaproic acid in connection with prostatectomy. *Acta Chir Scand* **127**: 552-564

Andersson L, Nilsson IM, Colleen S, Granstrand B, Melander B. (1968) Role of urokinase and tissue activator in sustaining bleeding and the management thereof with EACA and AMCA. *Ann N Y Acad Sci* **146**: 642-658

Antiplatelet Trialist Collaberation. (1994) Collaberative overview of randomised trials of antiplatelet treatment. 1. Prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ* **308**: 81-106

Aranda A, Pàramo JA, Rocha E. (1988) Fibrinolytic activity in plasma after gynaecological and urological surgery. *Haemostasis* **18**: 129-134

Aro J, Haapiainen R, Rasi V, Rannikko S, Alfthan O. (1990) The effect of parenteral estrogen versus orchiectomy on blood coagulation and fibrinolysis in prostatic cancer patients. *Eur Urol* **17**: 161-165

Astedt B, Lecander I, Brodin T, Lundblad A, Löw K. (1985) Purification of a specific placental plasminogen activator inhibitor by monoclonal antibody and its complex formation with plasminogen activator. *Thromb Haemost* **53**: 122-125.

Astrup T, Mullertz S. (1952) The fibrin plate method for estimating fibrinolytic activity. *Arch Biochem Biophys* **40**: 346-351

Astrup T, Permin PM. (1947) Fibrinolysis in the animal organism. *Nature* **159**: 681-682

Astrup T, Sterndorff I. (1952) Activator of plasminogen in normal urine. *Proc Soc Exper Biol & Med* **81**: 675-678

Baker KA. (1995) In *Williams Hematology* (ed. E Beutler, MA Lichtman, BS Coller, Kipps TJ), chap. 144, pp. 1533. New York: McGraw-Hill Inc.

Bauer KA, Rosenberg RD. (1987) The pathophysiology of the prethrombotic state in humans: insight gained from studies using markers of hemostatic system activation. *Blood* **70**: 343-350

Bauer KA, ten Cate H, Barzegar S, Spriggs DR, Sherman ML, Rosenberg RD.(1989) Tumour necrosis factor infusions have a procoagulant effect on the hemostatic mechanism of humans. *Blood* **74**: 165-172

Beck E, Duckert F, Ernst M. (1961) The influence of fibrin stabilizing factor on the growth of fibroblasts in vitro and wound healing. *Thromb Diath Haemorrh* **6**: 485-491

Bejjani BB, Chen DCP, Nolan NG, Edson M. (1983) Minidose heparin in transurethral prostatectomy. *Urology* **22 (3)**: 251-254

Bellringer JF, Anson KM, Schweitzer FAW. (1996) Life threatening secondary haemorrhage following endoscopic laser ablation of the prostate in an anticoagulated patient. *Br J Urol* **77**: 463-464

Bennett PN. (1967) Postoperative changes in platelet adhesiveness. *J Clin Pathol* **20**: 708-709

Betkurer V, Guinan P, Verma A, Morillo E, Ablin RJ, Sparkuhl A, Cochin A. (1979) Effect of transurethral resection on coagulation in carcinoma of prostate. *Urology* **13**: 142-144

Bevilacqua MP, Pober JS, Majeau GR, Fiers W, Cotran RS, Gimbrone MA Jr. (1986) Recombinant tumour necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc Natl Acad Sci USA* **83**: 4533-4537

Bithell TC (1993) Thrombosis and antithrombotic therapy. In: *Wintrobe's Clinical Hematology* . Lee RG, Bithell TC, Forester J, Athens JW, Lukens JN (eds). Ninth edition. Chapter 58, pp 1515-1551. Lea & Febiger, Philadelphia.

Bithell TC (1993) Acquired coagulation disorders. In: *Wintrobe's Clinical Hematology.* Lee RG, Bithell TC, Forester J, Athens JW, Lukens JN (eds). Ninth edition. Chapter 57, pp 1473-1514. Lea & Febiger, Philadelphia.

Bjerrehuus I. (1952) Fibrinolytic activity of urine. *Scand J Clin & Lab Invest* **4**: 179-182

Blair SD, Menashi S, Samson D, Greenhalgh RM. (1986) Can the hypercoagulability of surgery be measured? *Br J Surg* **73**: 500 (A64)

Blandy JP. (1972) The complications of transurethral resection. In *Transurethral Resection.* Chapter 7, p. 102. London: Pitman Medical.

Blandy JP. (1978) *Operative Urology.* pp. 149-150. London: Blackwell Publishing.

Bolton DM, Costello AJ. (1994) Management of benign prostatic hyperplasia by transurethral laser ablation in patients treated with warfarin anticoagulation. *J Urol* **151**: 79-81

Braasch W F (1918) Median bar excisor. *JAMA* **70**: 758 - 759

Bradford MM. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248-254

Bricker SRW, Savage ME, Hanning CD. (1987) Peri-operative blood loss and non-steroidal anti-inflammatory drugs: an investigation using diclofenac in patients undergoing transurethral resection of the prostate. *Eur J Anaesthesiol* **4**: 429-434

Britton BJ, Hawkey C, Wood WG, Peele M. (1974) Stress - a significant factor in venous thrombosis? *Br J Surg* **61**: 814-820

Bruce AW, Zorab J, Still B. (1960) Blood loss in prostatic surgery. *Br J Urol* **32**: 422-427

Bryan LS, Reynolds KL. (1984a) Community-acquired bacteremic urinary tract infection: epidemiology and outcome. *J Urol* **132**: 490-493

Bryan LS, Reynolds KL. (1984b) Hospital-acquired bacteremic urinary tract infection: epidemiology and outcome. *J Urol* **132**:494-498

Büller HR, Boon TA, Henny CP, Dabhoiwala NF, ten Cate JW. (1982) Estrogen-induced deficiency and decrease in antithrombin III activity in patients with prostate cancer. *J Urol* **128**: 72-74

Bumpus H C Jr (1927) Punch operation for prostatic obstruction. *Surg Clin North Am* **7**: 1473 - 1478

Butler MJ. (1978) Thrombelastography during and after Elective Abdominal Surgery. *Thromb Haemost* **39**: 488-495

Camiolo SM, Markus G, Evers JL, Hobika GH, DePasquale JL, Beckley S, Grimaldi JP. (1981) Plasminogen activator content of neoplastic and benign human prostate tissues; fibrin augmentation of an activator activity. *Int J Cancer* **27**: 191-198

Cannon WB, Gray H. (1914) Factors affecting the coagulation time of blood II. The hastening or retarding of coagulation by adrenalin injections. *Am J Physiol* **24**: 232-242

Caprini JA, Eckenhoff JB, Ramstack JM, Zuckerman L, Mockros LF. (1974) Contact activation of heparinized plasma. *Thromb Res* **5**: 379-400.

Caprini JA, Zuckerman L, Cohen E, Vagher JP, Lipp V. (1976) The identification of accelerated coagulability. *Thromb Res* **9**: 167-180.

Caprini JA, Arcelus JI, Laubach M, Size G, Hoffman KN, Coats RW 2nd, Blattner S. (1995) Postoperative hypercoagulability and deep-vein thrombosis after laparoscopic cholecystectomy. *Surg Endosc* **9**: 304-309

Caprini JA, Arcelus JI, Traverso CI, Vittum D, Adams P. (1991) Hypercoagulability after laparoscopic cholecystectomy. *Thromb Haemost* **65**: 1347 (A2369)

Carr JM, McKinney M, McDonagh J. (1989) Diagnosis of disseminated intravascular coagulation. Role of D-dimer. *Am J Clin Pathol* **91**: 280-287

Chakrabarti R, Hocking ED, Fearnley GR. (1969) Reaction pattern to three stresses - electropexy, surgery and myocardial infarction - of fibrinolysis and plasma fibrinogen. *J Clin Pathol* **22**: 659-662

Chakravarti A, MacDermott S. (1998) Transurethral resection of the prostate in the anticoagulated patient. *Br J Urol* **81**: 520 - 522

Chanarin I. (1989) Laboratory Haematology. An account of laboratory techniques. Pp 274-275. Churchill Livingstone, London.

Chilton CP, Morgan RJ, England HR, Paris AMI, Blandy JP. (1978) A critical evaluation of the results of transurethral resection of the prostate. *Br J Urol* **50**: 542 - 546

Chmielewska J, Rånby M, Wiman B. (1983) Evidence for a rapid inhibitor to tissue plasminogen activator in plasma. *Thromb Res* **31**: 427-436

Christensen LR, MacLeod CM. (1945) Proteolytic enzyme of serum: characterisation, activation, and reaction with inhibitors. *J Gen Physiol* **28**: 559-583

Cohen E, Caprini JP, Zuckerman L, Vagher P, Robinson B. (1977) Evaluation of three methods used to identify accelerated coagulability. *Thromb Res* **10**: 587-604

Cohen J, McConnell JS. (1984) Observations on the measurement and evaluation of endotoxemia by a quantitative Limulus lysate microassay. *J Infect Dis* **150**: 916-924

Collen D. (1976) Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma. *Eur J Biochem* **69**: 209-216

Collen D, de Maeyer L. (1975) Molecular biology of human plasminogen. I. Physiochemical properties and microheterogeneity. *Thromb Diath Haemorrh* **34**: 396-402

Collins JA, Knudson MM. (1991) Massive transfusion. In: *Transfusion Medicine.* Rossi EC, Simon TL, Moss GS (eds). Pp 419-27. Williams & Wilkins. Baltimore MD.

Colman RW, Robboy SJ, Minna JD. (1972) Disseminated intravascular coagulation (DIC): an approach. *Am J Med* **52**: 679-689.

Colucci M, Balconi R, Lorenzet R, Pietra A, Locati D, Donati MB, Semeraro N. (1983) Cultured human endothelial cells generate tissue factor in response to endotoxin. *J Clin Invest* **71**: 1893-1896

Concato J, Horwitz RI, Feinstein AR, Elmore JG, Schiff SF. (1992) Problems of comorbidity in mortality after prostatectomy. *JAMA* **267**: 1077-1082

Conway EM, Rosenberg RD. (1988) Tumor necrosis factor suppresses transcription of the thrombomodulin gene in endothelial cells. *Mol Cell Biol* **8**: 5588-5592

Cosgriff SW, Leifer E. (1952) Factor 5 deficiency in hemorrhagic diathesis (parahemophilia). *JAMA* **148**: 462-463

Creevy CD. (1947) Hemolytic reactions during transurethral prostatic resection. *J Urol* **58**: 125-131

Dale HH, Walpole GS. (1916) Some experiments on factors concerned in the formation of thrombin. *Biochem J* **10**: 331-362

D'Angelo A, Kluft C, Verheijen JH, Rijken DC, Mozzi E, Mannucci PM. (1985) Fibrinolytic shut-down after surgery: impairment of the balance between tissue-type plasminogen activator and its specific inhibitor. *Eur J Clin Invest* **15**: 308-312

Dastre A. (1893) Fibrinolyse dans le sang. *Archives of Physiology and Normal Pathology* **5**: 661-663

Davis JA. (1987) Detection of a prethrombotic state. In: *Haemostasis and Thrombosis*. Bloom AL, Thomas DP (eds). Second edition, pp 721-738. Churchill Livingstone, Edinburgh.

Davis CL, Chandler WL. (1995) Thrombelastography for the prediction of bleeding after transplant renal biopsy. *J Am Soc Nephrol* **6**: 1250-1255

Dawes J, James K, Micklem LR, Pepper DS, Prowse CV (1984). Monoclonal antibodies directed against human alpha-thrombin and the thrombin-antithrombin III complex. *Thromb Res* **36**: 397-409

de Campos Freire G, de Campos Pachelli L, Cordeiro P, Borrelli M, Menezes de Goes G. (1986) Blood loss during and following transurethral resection. *Prostate* **8**: 87-92

De Gaetano G, Vermylen J. (1973) Effect of aspirin on the thrombelastograph of human blood. *Thromb Diath Haemorrh* **30**: 494-498

De Nicola P. (1957) Thrombelastography. Blackwell Scientific, Oxford.

Doebber TW, Wu MS, Robbins JC, Choy BM, Chang MN, Shen TY (1985) Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF-receptor antagonist Kadsurenone. *Biochem Biophys Res Commun* **127:** 799-808

Doll HA, Black NA, McPherson K, Flood AB, Williams GB, Smith JC. (1992) Mortality, morbidity and complications following transurethral resection of the prostate for benign hypertrophy. *J* Urol **147**: 1566-1573

Donat R, McNeill A, Brame KG. (1996) Anti-platelet and non-steroidal anti-inflammatory drugs in transurethral surgery of the prostate and bleeding complications. *Br J Urol* **77** (Suppl 1): 31

Dorman BH, Spinale FG, Bailey MK, Kratz JM, Roy RC. (1993) Identification of patients at risk for excessive blood loss during coronary artery bypass surgery: thrombelastography versus coagulation screen. *Anesth Analg* **76** : 694-700

DuBose DA, Lemaire M, Basamania K, Rowlands J. (1980) Comparison of plasma extraction techniques in preparation of samples for endotoxin testing by the *Limulus* amoebocyte lysate test. *J Clin Microbiol* **11**: 68-72

Duckert F. (1972) Documentation of the plasma factor XIII defiency in man. *Ann N Y Acad Sci* **202**: 190-199

Egeblad K. (1967) Inhibitors of fibrinolysis: a comparative thrombelastographic study.
Scand J Clin & Lab Invest **20** (Suppl. 98): 1-69

Ekengren J, Hahn RG. (1993) Blood loss during transurethral resection of the prostate as measured by the HemoCue photometer. *Scand J Urol Nephrol* **27**: 501-507

Elliot JS, McDonald JK, Fowell AH. (1963) Blood loss and fibrinolysin levels during transurethral prostatic resection.
J Urol **89**: 62-64

Emmons PR, Mitchell JRA. (1965) Postoperative changes in platelet-clumping activity. *Lancet* **1**: 71-75

Eriksson BI, Eriksson E, Risberg B. (1991) Impaired fibrinolysis and postoperative thromboembolism in orthopedic patients.
Thromb Res **62**: 55-64

Esmon C. (1994) Possible involvement of cytokines in diffuse intravascular coagulation and thrombosis. In: Meade T, ed. *Bailliere's clinical haematology*. Vol7: 3. Pp 453-468. Bailliere Tindall, London.

Essell JH, Martin TJ, Salinas J, Thompson JM, Smith VC. (1993) Comparison of thrombelastography to bleeding time and standard coagulation tests in patients after cardiopulmonary bypass. *J Cardiothorac Vasc Anesth* **7**: 410-415

Faber JE, Hansen M, Genster HG. (1987) Use of blood in transurethral prostatectomy- routine or selective cross-matching? *Scand J Urol Nephrol* (Suppl) **104**: 73-75

Fearnley GR, Balmforth G, Fearnley E. (1957) Evidence of a diurnal fibrinolytic rhythm with a simple method of measuring natural fibrinolysis. *Clin Sci* **16**: 645-650

Feruglio G, Sandberg H, Bellet S. (1960) Postoperative changes in blood coagulation in the elderly. *Amer J Cardiol* **5**: 477-482

Fisch IR, Freedman SH, Pellegrin FA. (1973) Effect of oral contraceptives on the thrombelastogram. *Clin Pharmacol Ther* **14**: 238-244

Fleisher MS, Loeb L. (1915) On tissue fibrinolysins. *J Biol Chem* **21**: 477-501

Foley FEB. (1937) A Hemostatic bag catheter: a one piece latex rubber structure for control of bleeding and constant drainage following prostatic resection. *J Urol* **38**: 134-139

Forrester JC. (1988) Wounds and their management. In: Cuschieri A, Giles GR, Moossa AR (eds.) *Essential Surgical Practice*. Second Edition, pp 3-15, Bristol, John Wright.

Frank RM, Lloyd FA. (1959) A clinical study on the effectiveness of adrenosem in transurethral resection. *J Urol* **82**: 243-246

Freedman M, Van Der Molen SW, Makings E. (1985) Blood loss measurement during transurethral resection of the prostate gland. *Br J Urol* **57**: 311-316

Friedman NJ, Hoag S, Robinson AJ, Aggeler PM. (1969) Hemorrhagic syndrome following transurethral prostatic resection for benign adenoma. *Arch Intern Med* **124**: 341-349

Fuglsig S, Aagaard J, Jønler M, Olesen S, Nørgaard JP. (1994) Survival after transurethral resection of the prostate: a 10-year follow up. *J Urol* **151**: 637 - 639

Fujita K. (1988) Factors influencing bleeding during transurethral prostatectomy. *Clin Ther* (special issue) **10**: 16-19

Gaffney PJ. (1987) *Fibrinolysis.* In: *Haemostasis and Thrombosis.* Eds. Bloom AL, Thomas DP, second edition, pp223-244. Churchill Livingstone, Edinburgh.

Gallimore SC, Hoile RW, Ingram GS, Sherry KM. (1997) *Report of the National Confidential Enquiry into Perioperative Deaths.* 1994/1995 National Confidential Enquiry into Perioperative Deaths, London.

Gans H. (1973) Is primary fibrinolysis a real entity? *Surg Gynecel Obstet* **136**: 975-976

Gatenbeck L, Johansson B. (1984) Bleeding during transurethral prostatectomy. *Acta Chir Scand* (suppl) **520**: 97-100

Gibbs NM, Crawford GP, Michalopoulos N. (1994) Thrombelastographic patterns following abdominal aortic surgery. *Anaesth Intensive Care* **22:** 534-538

Gierhake FW, Volkmann W, Becker W, Schwarz H, Schwick HG. (1970) Faktor-XIII-Konzentration und Wundheilung. *Deutsch Med Wschr* **95**: 1472-1475

Gordon SG, Franks JJ, Lewis BJ. (1975) Cancer procoagulant: a factor X-activating procoagulant from malignant tissue. *Thromb Res* **6**: 127-137

Gormsen J. (1961) Coexistence of hypofibrinogenaemia and thrombosis. *Acta Chir Scand* **121**: 359-366

Gormsen J. (1961) Thrombelastographic findings in surgery. *Acta Chir Scand* **122**: 466-470

Grant PJ, Davies JA, Tate GM, Boothby M, Prentice CRM. (1985) Effects of physiological concentrations of vasopressin on haemostatic function in man. *Clin Sci* **69**: 471-476

Grant PJ, Tate GM, Davies JA, Williams NS, Prentice CRM. (1986) Intra-operative activation of coagulation - a stimulus to thrombosis mediated by vasopressin? *Thromb Haemost* **55**: 104-107

Grathwohl KW, Bruns BJ, LeBrun CJ, Ohno AK, Dillard TA, Cushner HM. (1996) Does hemodilution exist? Effects of saline infusion on hematological parameters in euvolemic subjects. *South Med J* **89**: 51-55

Greene LF. (1971) Use of hemostatic bag after transurethral prostatic resection. *J Urol* **106**: 915-919

Griffiths NJ, Woodford M, Irving MH. (1977) Alteration in fibrinolytic capacity after operation. *Lancet* **2**: 635-637

Guthrie G J (1834) Anatomy and diseases of the neck of the bladder and the urethra. Burgess & Hill, London.

Habib NA, Luck RJ. (1983) Results of transurethral resection of the benign prostate. *Br J Surg* **70**: 218-219

Hagstrom RS. (1955) Studies on fluid absorption during transurethral prostatic resection. *J Urol* **73**: 852-859

Hahn RG. (1987) Influence of variations in blood haemoglobin concentration on the calculation of blood loss and volumetric irrigating fluid balance during transurethral resection of the prostate. *Br J Anaesth* **59**: 1223-1229

Hahn RG. (1988) Ethanol monitoring of irrigating fluid absorption in transurethral prostatic surgery. *Anesthesiology* **68**: 867-873

Hahn RG. (1996) Ethanol monitoring of irrigating fluid absorption (review). *Eur J Anaesthesiol* **13**: 102-115

Hahn RG, Ekengren JC. (1993) Patterns of irrigating fluid absorption during transurethral resection of the prostate as indicated by ethanol. *J Urol* **149**: 502-506

Hahn RG, Essén P. (1994) Blood coagulation status after transurethral resection of the prostate. *Scand J Urol Nephrol* **28**: 385-390

Hale DJ, Robinson JA, Loeb HS, Gunner RM. (1986) Pathophysiology of endotoxin shock in man. In *Handbook of Endotoxin* (ed. Procter RA), vol. 4. pp. 1-17. Amsterdam: Elsevier.

Halverstadt DB, Albers DD, Kroovand RL, Rich R. (1977) Anticoagulation in urological surgery. *Urology* **9**: 617-619

Hammadeh MY, Madaan S, Singh M, Philp T. (1998) Two-year follow-up of a prospective randomized trial of electrovaporization versus resection of the prostate. *Eur Urol* **34**: 188-92

Hargreave TB, Botto H, Rikken GHJM, Hindmarsh JR, McDermott TED, Mjolnerod OK, Petays P, Schalkhäuser K, A Stellos. (1993) European collaborative study of antibiotic prophylaxis for transurethral resection of the prostate. *Eur Urol* **23**: 437-443

Hartert H. (1948) Blutgerinnungsstudien mit der Thrombelastographie, einen neuem Untersuchungsverfahren. *Klin Wchnschr* **26**: 577-583

Hartert H. (1952) Klinische Blutgerinnungsstudien mit der thrombelastographie; physiologische und methodische grundlagen der thrombelastographie. *Deutsches Arch Klin Med* **199**: 284-292

Hartert H, Schaeder JA. (1962) The physical and biological constants of thrombelastography. *Biorheology* **1**: 31-39

Harvey MH, Leese T, Lloyd D, Osborn DE. (1986) Antibiotic prophylaxis and secondary haemorrhage following transurethral resection of the prostate: a prospective trial. *Br J Urol* **58**: 450-452

Hasegawa H. (1983) Estimation of coagulation-fibrinolytic factors in DIC. *Haematol* **49**: 173-180

Haslam RJ, Rosson GM. (1972) Effect of vasopressin on human blood platelets. *J Physiol (Lond)* 219: 36P-39P

Hathaway WE, Mahasandana C, Makowski EL. (1975) Cord blood coagulation studies in infants of high-risk pregnant women. *Am J Obstet Gynecol* **121**: 51-57

Hawkey CJ, Stirling Y, Chakrabarti R, Brozovic M, Cox AG, Meade TW. (1983) Haemostatic changes following surgery. *Thromb Res* **32**: 223-227

Hedlund PO. (1969) A double-blind study. Antifibrinolytic therapy with Cyclokapron in connection with prostatectomy. *Scand J Urol Nephrol* **3**: 177-182

Hedlund PO. (1975) Postoperative venous thrombosis in benign prostatic disease. A study of 316 patients using the 125I-fibrinogen uptake test. *Scand J Urol Nephrol* (Suppl 27): 1-100

Hedlund PO, Blombäck M. (1979) The effect of prophylaxis with low dose heparin on blood coagulation parameters. A double blind study in connection with transvesical prostatectomy. *Thromb Haemost* **41**: 337-345.

Heinert G, Kirchheimer JC, Pflüger H, Binder BR. (1988) Urokinase-type plasminogen activator as a marker for the formation of distant metastases in prostatic carcinomas. *J Urol* **140**: 1466-1469

Hickman JA. (1971) A new technique for the quantitative estimation of fibrinolysis using in vitro (125-I) fibrinogen. *Br J Haematol* **20**: 611-21

Hinshaw LB. (1983) Overview of endotoxin shock. In *Pathophysiology of shock, anoxia and ischemia* (ed. Cowley RA, Trump BV), pp219-235. Baltimore: Williams and Wilkins.

Hjertberg H, Jorfeldt L, Schelin S. (1991) Use of ethanol as marker substance to increase patient safety during transurethral prostatic resection. *Urology* **38(5)**: 423-428

Hnatowich DJ, Virizi F, Rusckowski M. (1987) Investigations of avidin and biotin for imaging applications. *J Nucl Med* **28**: 1294-1302

Hoylaerts M, Rijken DC, Lijnen HR, Collen D. (1982) Kinetics of the activation of plasminogen by human tissue plasminogen activator. *J Biol Chem* **257**: 2912-2919

Hong SY, Pyo SJ, Lee HB. (1990) Effect of serum urokinase on urine fibrinolytic activity. *Nephron* **55(3)**: 287-291

Hosenfeld D, Dünnweber E, Kaiser N. (1969) Das Verhalten des Faktors XIII nach Operationen. 2. *Med Welt* **20**: 1170-1173

Howland WS, Castro EB, Fortner JB, Gould P. (1974) Hypercoagulability. Thrombelastographic monitoring during extensive hepatic surgery. *Arch Surg* **108**: 605-608

Howland WS, Schweizer O, Gould P. (1974) A comparison of intraoperative measurement of coagulation. *Anesth Analg* **53**: 657-663

Huggins C, Neal W. (1942) Coagulation and liquefaction of semen; proteolytic enzymes and citrate in prostatic fluid. *J Exper Med* 76: 527-541

Huggins C, Vail VC. (1943) Plasma coagulation and fibrinogenolysis by prostatic fluid and trypsin. *Am J Physiol* **139**: 129-134

Hunter J. (1794) A treatise on the blood, inflammation and gunshot wounds. In: Palmer JF, ed (1835) The works of John Hunter, FRS., Vol.3. London: Longman, Rees, Orme, Brown, Green & Longman.

Hutten R. (1992) New developments in the detection of haemostatic disorders. In *Recent Advances in Haematology 6*. Hoffbrand AV, Brenner MK (eds). Chapter 2 pp 19-44. Churchill Livingstone, Edinburgh.

Ichinose A, Hendrickson LE, Fujikawa K, Davie EW. (1986a) Amino acid sequence of the a subunit of human factor XIII. *Biochemistry* **25**: 6900-6906

Ichinose A, McMullen BA, Fujikawa K, Davie EW. (1986b). Amino acid sequence of the b subunit of human factor XIII, a protein composed of ten repetitive subunits. *Biochemistry* **25**: 4633-4638

Iglesias J J, Sporer A, Gellman A C and Seebode J J (1975) New Iglesias resectoscope with continuous irrigation, simultaneous suction and low intravesical pressure. *J Urol* 114: 929-933

Infectious disease testing for blood transfusions. NIH Consensus Development Panel on Infectious Disease Testing for Blood Transfusion. (1995) *JAMA* **274(17)**: 1374-1379

Ingram GIC, Jones VR. (1966) The rise in clotting factor VIII induced in man by adrenaline: effect of alpha- and beta-blockers. *J Physiol (Lond)* **187**: 447-454

Iwanaga S, Morita T, Harada T Nakamara S, Niwa M, Takada K, Kimura T, Sakakibara S. (1978) Chromogenic substrates for horseshoe crab clotting enzyme. Its application for the assay of bacterial endotoxins. *Haemostasis* **7**: 183-188

Iwan-Zi:etek I, Zi:etek Z, Kotschy M, Rosc D, Tyloch F, Cwiklinska-Jurkowska M. (1992) Tkankowy aktywator plazminogenu i jego inhibitor oraz inne parametry ukl adu fibrinolizy we krwi chorych operowanych z powodu lagodnego przerostu gruczolu krokowego. *Polski Tygodnik Lekarski* **47**: 1096-1097

Jansen H, Berseus O, Johansson JE. (1978) A simple photometric method for determination of blood loss during transurethral surgery. *Scand J Urol Nephrol* **12**: 1-5

Jenkins AD, Mintz PD. (1981) Optimal blood use in Genitourinary Surgery. *J Urol* **126**: 497-499

Jurgens R, Trautwein H. (1930) Ueber fibrinopenie (fibrinogenpenie) beim erwaschsener nebst bermerkungen uber die herkwast des fibrinogens. *Deutsch Arch Klin Med* **169**: 28-43

Kakkar VV, Cohen AT, Edmonson RA, Phillips MJ, Cooper DJ, Das Sk, Maher KT, Sanderson RM, Ward VP, Kakkar S. (1993) Low molecular weight versus standard heparin for prevention of venous thromboembolism after major abdominal surgery. *Lancet* **341**: 259-265

Kalter ES, Daha MR, ten Cate JW, Verhoef J, Bouma BN. (1985) Activation and inhibition of Hageman factor-dependent pathways and the complement system in uncomplicated bacteraemia or bacterial shock. *J Infect Dis* **151**: 1019-1027

Kang Y, Lewis JH, Navalglund A, Russell MW, Bontempo FA, Niren LS, Starzl TE. (1987) Epsilon-aminocaproic acid for treatment of fibrinolysis during liver transplantation. *Anesthesiology* **66**: 766-773

Kang YG, Martin DJ, Marquez JM, Lewis JH, Bontempo FA, Shaw BW Jr, Starzl TE, Winter PW. (1985) Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. *Anesth Analg* **64**: 888-896

Kang Y, Martin LK, Marquez J, Lewis JH, De Wolf A. (1989) Thrombelastographic monitoring of coagulation during cardiac surgery. *Anesthesiology* **71**: A8

Kass EJ, Sonda P, Gershon C, Fischer CP. (1978) The use of prophylactic low dose heparin in transurethral prostatectomy. *J Urol* **120**: 186-187

Kester RC. (1969) Plasminogen activator in the human prostate. *J Clin Path* **22**: 442-446

Kidess E, Miller H, Preisner A, Briel R. (1979) Factor XIII levels in relation to gynaecological operations. *Arch Gynecol* **227**: 163-169

Kirchheimer J, Binder BR. (1983) Localization and Immunological Characterization of Plasminogen Activators in Human Prostate Tissue. *Haemostasis* **13**: 358-362

Kisker CT, Rush R. (1971) Detection of intravascular coagulation. *J Clin Invest* **50**: 2235-2241

Kluft C, Verheijen JH, Jie AFH, Rijken DC, Preston FE, Sue-Ling HM, Jespersen J, Aasen AO. (1985) The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. *Scand J Clin Lab Invest* **45**: 605-610

Knight MTN, Dawson R, Melrose DG. (1977) Fibrinolytic response to surgery. Labile and stable patterns and their relevance to post-operative deep venous thrombosis. *Lancet* **2**:370-373

Kwaan HC, Barlow GH. (1973) Nature and biological activities of degradation products of fibrinogen and fibrin. *Annu Rev Med* **24**: 335-344

Koh SCL, Pua HL, Tay DHB, Chua SE, Yuen WK, Ng BL, Ratnam SS. (1995) The effects of gynaecological surgery on fibrinolysis, fibrinolytic inhibitor and the haemostatic status as assessed by thrombelastography. *Thromb Haemost* **73**: 1138 (A912)

Kolb G, Fischer W, Seitz R, Muller T, Egbring R, Lange H, Havemann K. (1991) Hemodialysis and blood coagulation: the effect of hemodialysis on coagulation factor XIII and the thrombin-antithrombin III complex. *Nephron* **58**: 106-108

Kolmert T, Norlén H. (1989) Transurethral resection of the prostate. A review of 1111 cases.
International Journal of Urology and Nephrology 21: 47-55

Kösters S, Wand H. (1973) über die beeinflussung des blutverlustes nach prostataoperationen durch prä-operative applikation von antifibrinolytika. *Urologe A* **12**: 295-296

Krausz MM, Utsunomiya T, McIrvine AJ, Allen PD, Levine L, Mannick JA, Shepro D, Hechtman HB. (1983) Modulation of cardiovascular function and platelet survival by endogenous prostacyclin released during surgery. *Surgery* **93**: 554-559

Kreger BE, Craven DE, McCabe WR. (1980) Gram-negative bacteraemia. IV. Re-evaluation of clinical features and treatment in 612 patients. *Am J Med* **68**: 344-355

Kruithof EKO, Vassalli JD, Schleuning WD, Mattaliano RJ, Bachmann F. (1986) Purification and characterization of a plasminogen activator inhibitor from the histiocytic lymphoma cell line U-937. *J Biol Chem* **261**: 11207-11213

Kulatilake AE, Roberts PN, Evans DF, Wright J. (1981) The use of cooled irrigating fluid for transurethral prostatic resection.
Br J Urol **53**: 261-262

Lacey SS, Drach GW, Cox CF. (1971) Incidence of infection after prostatectomy and efficacy of cephaloridine prophylaxis.
J Urol **105**: 836-839

Lattan MA, Bradshaw AE. (1995) Investigation of a thrombotic tendency. In *Practical Haematology*. Sir John V Dacie, Lewis SM (eds). Eighth edition. Chapter 18 pp 351-366. Churchill Livingstone, Edinburgh.

Lecander I, Astedt B. (1986) Isolation of a new specific plasminogen activator inhibitor from pregnancy plasma.
Br J Haematol **62**: 221-228

Le Quesne LP, Lewis AAG. (1953) Postoperative water and sodium retention. *Lancet* **1**: 153-158

Levi M, Hack CE, de Boer JP, Brandjes DPM, Büller HR, ten Cate JW. (1991b) Reduction of contact activation related fibrinolytic activity in factor XII deficient patients: further evidence for the role of the contact system in fibrinolysis in vivo. *J Clin Invest* **88**: 1155-1160

Levi M, ten Cate H, Bauer KA, Büller HR, ten Cate JW, Rosenberg RD. (1991a) Dose-dependent endotoxin induced cytokine release and coagulation activation in chimpanzees. *Thromb Haemost* **65**: 793 (A410)

Levi M, ten Cate H, Bauer KA, Büller HR, ten Cate JW, Rosenberg RD. (1992) Endotoxin-induced effects on fibrinolysis in chimpanzees: activation of fibrinolysis is independent of coagulation activation. *Fibrinolysis* **6**: 121

Levin K, Nyrén O, Pompeius R. (1981) Blood loss, tissue weight and operating time in transurethral prostatectomy. *Scand J Urol Nephrol* **15**: 197-200

Levy GA, Schwartz BS, Curtiss LK, Edgington TS. (1985) Regulatory roles of T mu and T gamma cells in the collaborative cellular initiation of the extrinsic coagulation pathway by bacterial lipopolysaccharide. *J Clin Invest* **76**: 548-555

Lewi HJE, Hales DSM, Mahmoud S, Scott R. (1983) The characteristics of post TUR blood loss: a preliminary study. *Urol Res* **11**: 29-31

Logie JRC, Keenan RA, Whiting PH, Steyn JH. (1980) Fluid absorption during transurethral prostatectomy. *Br J Urol* **52**: 526-528

Lombardo LJ, JR. (1958) Studies in the prevention of fibrinolysis occurring in prostatic surgery. *J Urol* **79**: 507-513

Luke M, Kvist E, Andersen F, Hjortrup A. (1986) Reduction of post-operative bleeding after transurethral resection of the prostate by local instillation of fibrin adhesive (Beriplast). *Br J Urol* **58**: 672-675

Loskutoff DJ, van Mourik JA, Erickson LA, Lawrence D. (1983) Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. *Proc Natl Acad Sci USA* **80**: 2956-2960

Lyberg T, Galdal KS, Evensen SA, Prydz H. (1983) Cellular cooperation in endothelial cell thromboplastin synthesis. *Br J Haematol* **53**: 85-95

Lyth DR, Booth CM. (1990) Does ethamsylate reduce haemorrhage in transurethral prostatectomy? *Br J Urol* **66**: 631-634

Macfarlane RG. (1937) Fibrinolysis following operation. *Lancet* **1**: 10-12

Macfarlane RG, Pilling J. (1947) Fibrinolytic activity of normal urine. *Nature* **159**: 779.

Madsen RE, Madsen PO. (1967) Influence of anaesthesia form on blood loss in transurethral prostatectomy. *Anesth Analg* **46**: 330-332

Madsen PO, Strauch AE, Barquin OP, Malek GH. (1968) The lack of hemostatic effect of polyestradiol phosphate and carbazochrome salicylate in transurethral prostatectomy. *J Urol* **99**: 786-787

Madsen PO, Kaveggia L, Atassi SA. (1964) The effect of estrogens (Premarin) and regional hypothermia on blood loss during transurethral prostatectomy.*J Urol* **92**: 314-316

Madsen PO, Strauch AE. (1966) The effect of aminocaproic acid on the bleeding following transurethral prostatectomy. *J Urol* **96**: 255-256

Madsen PO, Knuth OE, Wagenknecht LV, Genster HG. (1970) Induction of diuresis following transurethral resection of the prostate. *J Urol* **104**: 735-738

Maier RV, Hahnel GB. (1984) Microthrombosis during endotoxinemia: potential role of hepatic versus alveolar macrophages. *J Surg Res* **36**: 362-370

Malenka DJ, Roos N, Fisher ES, McLerran D, Whaley FS, Barry MJ, Bruskewitz R, Wennberg JE. (1990) Further study of the increased mortality following transurethral prostatectomy: a chart-based analysis. *J Urol* **144**: 224-228

Mallet SV, Cox DJA. (1992) Thrombelastography. *Br J Anaesth* **92**: 307-313

Mallet S, Rolles K, Cox D, Burroughs A, Hunt B. (1991) Intraoperative use of aprotinin (trasylol) in orthotopic liver transplantation. *Transplant Proc* **23**: 1931-1932

Mann KG. (1984) Membrane-bound enzyme complexes in blood coagulation. In : Spaet Th, ed. *Progress in Hemostasis and Thrombosis* pp 1 - 24. Saunders, Grunej.

Mannucci PM, Rota L. (1980) Plasminogen activator response after DDAVP: a clinico-pharmacological study. *Thromb Res* **20**: 69-76

Mansfield AO. (1972) Alteration in fibrinolysis associated with surgery and venous thrombosis. *Br J Surg* **59**: 754-757

Marchal G, Leroux ME, Samama M. (1961) Atlas de Thrombodynamographie. Pp 1 - 185. Service de Propagande Édition Information. Paris.

Marder M, Weiner M, Shulman P, Shapiro S. (1949) Afibrinogenemia occuring in case of malignancy of prostate with bone metastases. *N Y State J Med* **49**: 1197-1198

Marder VJ, Budzynski AZ. (1975) Data for defining fibrinogen and its plasmic degradation products. *Thromb Diath Haemorrh* **33**: 199-207

Marder VJ, Martin SE, Francis CW, Colman RW. (1987) Consumptive thrombohemorrhagic disorders. In *Hemostasis and Thrombosis*: *Basic Principles and Clinical Practice*, Colman RW, Hirsh J, Marder VJ, Salzman EW eds., 2nd ed., pp. 975-1015. Philadelphia: JB Lippincott.

Marsh NA, Arocha-Pinango CL. (1972) Evaluation of the fibrin plate method for estimating plasminogen activators. *Thromb Diath Haemorrh* **28**: 75-88

Martin P, Horkay F, Rajah SM, Walker DR. (1991) Monitoring of coagulation status using thrombelastography during paediatric open heart surgery. *Int J Clin Monit Comput* **8**: 183-187.

McCarthy J F (1931) A new apparatus for endoscopic plastic surgery of the prostate, diathermy and excision of vesical growths. *J Urol* **26**: 695-696

McDonagh J. Structure and Function of factor XIII. (1987) In: *Hemostasis and thrombosis. Basic principles and clinical* practise, Colman RW, Hirsh J, Marder VJ, Saltzman EW, eds., 2nd ed., pp 289-300. Philadelphia PA. JB Lippincott.

McGowan SW, Smith GFN. (1980) Anaesthesia for transurethral prostatectomy. A comparison of spinal intradural analgesia with two methods of general anaesthesia. *Anaesthesia* **35**: 847-853.

McNicol GP, Fletcher AP, Alkjaersig N, Sherry S. (1961a) Impairment of haemostasis in the urinary tract: the role of urokinase. *J Lab Clin Med* **58**: 34-46

McNicol GP, Fletcher AP, Alkjaersig N, Sherry S. (1961b) The use of epsilon aminocaproic acid, a potent inhibitor of fibrinolytic activity, in the management of postoperative hematuria. *J Urol* **86 (6)**: 829-837

Mebust WK, Holtgrewe HL, Cockett ATK, Peters PC. (1989) Transurethral prostatectomy: immediate and postoperative complications. A co-operative study of 13 participating institutions evaluating 3,885 patients. *J Urol* **141**: 243-247

Mellbring G, Nilsson T, Bergsdorf N, Wallén P. (1984) Tissue plasminogen activator concentrations in major abdominal surgery. Relationship to postoperative deep vein thrombosis. *Thromb Res* **36**: 331-334

Melchior J, Valk WL, Foret JD, Mebust WK. (1974a) Transurethral prostatectomy: computerized analysis of 2,223 consecutive cases. *J Urol* **112**: 634-642

Melchior J, Valk WL, Foret JD, Mebust WK. (1974b) Transurethral prostatectomy in the azotemic patient. *J Urol* **112**: 643-646

Merskey C, Johnson AJ, Kleiner GJ, Wohl H. (1967) The defibrination syndrome: clinical features and laboratory diagnosis. *Br J Haematol* **13:** 528-549

Mertens BF, Greene LF, Bowie EJW, Elveback LR, Owen CA. (1974) Fibrinolytic split products (FSP) and ethanol gelation test in preoperative evaluation of patients with prostatic disease. *Mayo Clin Proc* **49**: 642-646

Miletich JP. (1995) Activated partial thromboplastin time. In *Williams Hematology.* Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds). Fifth edition. Chapter L34, ppL85-86. McGraw-Hill Inc., USA.

Miller RA, May MW, Hendry WF, Whitfield HN, Wickham JEA. (1980) The prevention of secondary haemorrhage after prostatectomy: the value of antifibrinolytic therapy. *Br J Urol* **52**: 26-28

Milstone H. (1941) A factor in normal human blood which participates in streptococcal fibrinolysis. *J Immunol* **42**: 109-116

Mitchell JP. (1981) *Endoscopic Operative Urology.* p 491. Bristol: Wright PSG.

Morris MJ, Golovsky D, Guinness MDG, Maher PO. (1976) The value of prophylactic antibiotics in transurethral prostatic resection: a controlled trial with observations on the origin of postoperative infection. *Br J Urol* **48**: 479-484

Morrison DC, Ryan JC. (1987) Endotoxin and disease mechanisms. *Annu Rev Med* **38**: 417-432

Morrison DC, Ulevitch RJ. (1978) The effects of bacterial endotoxins on host mediation systems. A review. *Am J Pathol* **93:** 526-617

Mosher DF, Schad PE, Vann JM. (1980) Cross-linking of collagen and fibronectin by factor XIIIa. Localization of participating glutaminyl residues to tryptic fragment of fibronectin. *J Biol Chem* **225**: 1181-1188

Mulcahy JJ, Brandenberg RO, Pluth JR, Greene LF. (1975) Transurethral prostatic resection in patients with prosthetic cardiac valves. *J Urol* **113**: 642-643

Murdock PJ, Owens DL, Chitolie A, Hutton RA, Lee CA. (1992) Development and evaluation of ELISAs for factor XIIIA and XIIIB subunits in plasma. *Thromb Res* **67**: 73-79

Murphy WG, Davies MJ, Eduardo A. (1993) The haemostatic response to surgery. *Br J Anaesth* **70**: 205-213.

Nandi SR, Knox J. (1986) Carcinoma of the prostate: the effects of oestrogens on blood viscosity. *Br J Clin Pract* **40**: 383-385

Nawroth PP, Handley DA, Esmon CT, Stern DM. (1986) Interleukin-1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. *Proc Natl Acad Sci USA* **83**: 3460-3464

Nawroth PP, Stern DM. (1986) Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med* **163**: 740-745

Negus D, Pinto DJ, Slack WW. (1971) Effect of small doses of heparin on platelet adhesiveness and lipoprotein-lipase activity before and after surgery. *Lancet* **1**: 1202-1204

Nesbit R M 1939 Modification of Stern-McCarthy resectoscope permitting third dimensional perception during transurethral prostatectomy. *J Urol* **41**: 646-648

Ng KFJ, Lo JWR. (1996) The development of hypercoagulability state, as measured by thrombelastography, associated with intraoperative surgical blood loss. *Anaesth Intensive Care* **24**: 20-25

Nielsen JD, Gram J, Fabrin K, Holm-Nielsen A, Jespersen J. (1997) Lack of correlation between blood fibrinolysis and the immediate or postoperative blood loss in transurethral resection of the prostate. *Br J Urol* **80**: 105-110

Nielsen JD, Gram J, Holm-Nielsen A, Fabrin K, Jespersen J. (1997) Post-operative blood loss after transurethral prostatectomy is dependent on *in situ* fibrinolysis. *Br J Urol* **80**: 889-893

Niemetz J, Muhlfelder T, Chierego ME, Troy B. (1977) Procoagulant activity of leucocytes. *Ann N Y Acad Sci* **283**: 208-217

Norlén H, Allgen L, Vinnars E, Bedrelidou-Classon G. (1986) Glycine solution as an irrigating agent during transurethral prostatic resection. *Scand J Urol Nephrol* **20**: 19-26

O'Brien JR, Etherington M, Jamieson S. (1971) Refractory state of platelet aggregation with major operations. *Lancet* **2**: 741-743

O'Brien JR, Tulevski VG, Etherington M, Madgwick T, Alkjaersig N, Fletcher A. (1974) Platelet function studies before and after operation and the effect of postoperative thrombosis. *J Lab Clin Med* **83**: 342-354

O'Donnell PD. (1983) Serum acid phosphatase elevation associated with transurethral resection syndromes. *Urology* **22**: 388-390

O'Donnell PD. (1984) Acid phosphatase elevation with symptoms following transurethral prostatic resection. *J Urol* **131**: 684-686

O'Donnell PD. (1990) Effects of intravenous infusion of human prostate tissue substances in dogs. *The Prostate* **16**: 49-56

O'Donnell PD, Jacks DW, Brizzolara JP. (1985) Acid phosphatase levels of irrigant solution during transurethral prostatectomy. *Urology* **25**: 35-36

Oester A, Madsen PO. (1969) Determination of absorpion of irrigating fluid during transurethral resection of the prostate by means of radioisotopes. *J Urol* **102**: 714-719

Oesterling JE. (1992) Abdominal catheter holder to maintain controlled urethral catheter tension post transurethral resection of prostate. *Urology* **40**: 206-210

Office of Population and Census Survey. (1985) Hospital in-patient enquiry. HMSO (series MB4 No27)

Ogston D. (1978) Natural activator of plasminogen. In: *Fibrinolysis: Current clinical and fundamental concepts* Gaffney PJ, Balkuv-Ulutin S (eds) pp1-15. Academic Press, London.

Oliver A, Iglesias JM, Zuazu-Juasoro I, Parramon I, Laguna P, Fontcuberta J. (1991) Activation of coagulation and fibrinolysis in prostatic neoplasms. *Thromb Haemost* **65**: 1054 (Abstr. 1291)

Orlikowski CE, Payne AJ, Moodley J, Rocke DA. (1992) Thrombelastography after aspirin ingestion in pregnant and non-pregnant subjects. *Br J Anaesth* **69**: 159-161

Osterud B, Flaegstad T. (1983) Increased tissue thromboplastin activity in monocytes of patients with meningococcal infections related to unfavourable prognosis. *Thromb Haemost* **49:** 5-7

Parr NJ, Loh CS, Desmond AD. (1989) Transurethral resection of the prostate and bladder tumour without withdrawal of warfarin therapy. *Br J Urol* **64**: 623-625

Pearson BS. (1969) The effects of Trasylol and amino-caproic acid in post-prostatectomy haemorrhage. *Br J Urol* **41**: 602

Pengelly AW. (1985) In *Textbook of Genito-urinary Surgery* (ed. Whitfield HN, Hendry WF), chap. 34, pp.416-417. Edinburgh: Churchill Livingstone.

Pepper H, Lindsay S. (1960) Responses of platelets, eosinophils and total leucocytes during and following surgical procedures. *Surg Gynecol Obstet* **110**: 319-326

Perkins JB, Miller HC. (1969) Blood loss during transurethral prostatectomy. *J Urol* **101**: 93-97

Poller L, Thompson JM, Thomas W, Wray C. (1971) Blood clotting and platelet aggregation during oral progestogen contraception: a follow-up study. *BMJ* **1**: 705-707

Potts WJ, Pearl E. (1941) A study of the platelet count and the coagulation time of plasma and whole blood following operation. *Surg Gynecol Obstet* **73**: 492-494

Quick AJ. (1937) The prothrombin in haemophilia and obstructive jaundice. *J Biol Chem* **109**: LXII - XIV

Raby C. (1966) *Biologie des hemorragies et des thromboses.* Masson, Paris.

Raby C. (1969) Principes du traitement des hypercoagulabilités par une thérapeutique anticoagulante rationnelle. *Presse Med* **77**: 17-19

Raby C. (1976) Control of heparin therapy by thrombelastography. pp 211-218. In Kakkar VV and Thomas DP ed. Heparin: chemistry and clinical usage. Academic Press, London.

Rader ES. (1978) Hematologic screening tests in patients with operative prostatic disease. *Urology* **11**: 243-246

Raina S, Spillert CR, Greenstein SM, Lazaro EJ. (1985) Effect of surgery on tumour-induced accelerated coagulation in a rat carcinoma. *J Surg Res* **38**: 138-142.

Rains AJH & Ritchie DH. (1984) *Bailey & Love's Short Practice of Surgery,* 19th ed., chapter 4, pp. 43-44. London: H.K. Lewis & Co. Ltd.

Rånby M (1982) Studies on the kinetics of plasminogen activation by tissue plasminogen activator.
Biochem Biophys Acta **704**: 461-469

Ràkóczi I, Chamone D, Verstraete M, Collen D. (1980) The relevance of clinical and hemostasis parameters for the prediction of postoperative thrombosis of the deep veins of the lower extremity in gynecologic patients. *Surg Gynecol Obstet* **151**: 225-231

Rapaport SI, Chapman CG. (1959) Coexistent hypercoagulability and acute hypofibrinogenaemia in a patient with prostatic carcinoma. *Am J Med* **27**: 144-153

Redl H, Bahramis S, Leichtfried G, Schlag G. (1992) Special collection and storage tubes for blood endotoxin and cytokine measurements. *Clin Chem* **38**: 764-765

Reilly DT, Burden AC, Fossard DP. (1980) Fibrinolysis and the prediction of postoperative deep vein thrombosis.
Br J Surg **67**: 66-68

Remuzzi G, Rossi EC. (1996) Hematologic consequences of renal failure. pp 2170 - 2186. In: Brenner & Rectors *The Kidney*. Eds. Brenner BM. 5th edition. Philadelphia. WB Saunders Company.

Rivers RPA, Hathaway WE, Weston WL (1975) The endotoxin-induced coagulant activity of human monocytes.
Br J Haematol **30**: 311-316

Robertson GSM, Everitt NJ, Burton P, Flynn JT. (1993) Evaluation of current practices in routine preoperative crossmatching for transurethral resection of the prostate. *J* Urol **149**: 311-314

Robinson JA, Klondnycky ML, Loeb HS, Racie MR, Gunnar RM. (1975) Endotoxin, prekallikrein, complement and systemic vascular resistance. Sequential measurements in man. *Am J Med* **59**: 61-67

Robinson MRG, Cross RJ, Shetty MB, Fittal B. (1980) Bacteraemia and baceriogenic shock in district hospital practice.
Br J Urol **52**: 10-14

Robinson MRG, Arudpragasam ST, Sahgal SM, Cross RJ, Akdas A, Fittal B, Sibbald R. (1982) Bacteraemia resulting from prostatic surgery: the source of bacteraemia. *Br J Urol* **54**: 542-546

Robson CJ, Sales JL. (1966) The effect of local hypothermia on blood loss during transurethral resection of the prostate. *J Urol* **95**: 393-395

Rodriguez-Erdmann F. (1974) The syndrome of intravascular coagulation. *Postgrad Med* **55**: 91-98

Rodzynek JJ, Leautaud P, Martin T, Schoenfeld P, Wettendorf P, Delcourt A. (1983) The transfer test: a new screening procedure for thrombotic diseases. *J Surg Res* **35**: 227-233

Rohrer MJ, Natale AM. (1992) Effect of hypothermia on the coagulation cascade. *Critical Care Medicine* **20**: 1402-1405

Roos NP, Ramsey EW. (1987) A population-based study of prostatectomy: outcomes associated with differing surgical approaches. *J Urol* **137**: 1184-1188

Roos NP, Wennberg JE, Malenka DJ, Fisher ES, McPherson K, Anderson TF, Cohen MM, Ramsey E. (1989) Mortality and reoperation after open and transurethral resection of the prostate for benign prostatic hyperplasia. *N Engl J Med* **320**: 1120 -1124

Ruttman TG, James MFM, Viljoen JF. (1996) Haemodilution induces a hypercoagulable state. *Br J Anaesth* **76**: 412-414

Rylatt DB, Blake AS, Cottis LE Massingham DA, Fletcher WA, Masci PP, Whitaker AN, Elms M, Bunce I, Weber AJ. (1983) An immunoassay for human D dimer using monoclonal antibodies. *Thromb Res* **31**: 767-778.

Sack E, Spaet TH, Gentile RL, Hudson PB. (1962) Reduction of postprostatectomy bleeding by epsilon-aminocaproic acid. *N Engl J Med* **266 (11)**: 541-543

Sagar A, Beckett P. (1975) Thrombelastographic studies with low-dose heparin. *J R Coll Surg Edinb* **20**: 391-397

Sahli W. (1885) Über das Vorkommen von Pepsin und Trypsin im normalen menschlichen Harn. *E Pflüger, Archiv f Physiologie* **36**: 209-229

Samaha RJ, Bruns NC, Ross GJ Jr. (1973) Chronic intravascular coagulation in metastatic prostate cancer. *Arch Surg* **106**: 295-298

Scott-Blair GW, Burnett J. (1968) On the rates of coagulation and subsequent softening of bovine and human blood and of thrombin fibrinogen. *Biorheology* **5**: 163-176

Seale RA, Jampolis RW, Bargen JA. (1951) Symposium on surgical aspects of cancer problem: clotting defect in the presence of metastatic carcinoma of the prostate.
S Clin North America **31**: 1111-1119

Serrao A, Mallik MK, Jones PA, Hendry WF, Wickham JEA. (1976) Hypothermic prostatic resection. *Br J Urol* **48**: 685-687

Shainoff JR, Estafanous FG, Yared JP, DiBello PM, Kottke-Marchant K, Loop FD. (1994) Low factor XIIIA levels are associated with increased blood loss after coronary artery bypass grafting. *J Thorac Cardiovasc Surg* **108**: 437-445

Sharifi R, Lee M, Ray P, Millner SN, Dupont PF. (1986) Safety and efficacy of intravesical aminocaproic acid for bleeding after transurethral resection of prostate. *Urology* **27**: 214-219

Sharnoff JG, DeBlasio G. (1970) Prevention of fatal postoperative thromboembolism by heparin prophylaxis. *Lancet* **2**: 1006-1007

Shearer AJ. (1988) The principles of anaesthesia. pp203-224. In: Essential Surgical Practice. Eds. Cuschieri A, Giles GR, Moosa AR. Bristol, U.K. J Wright.

Shiina H, Himeno Y, Ishibe T. (1992) Organisms in the prostate and antibiotics in the treatment of postoperative infections.
Urology International **48**: 187-190

Siegal T, Seligsohn U, Agahi E, Modan M. (1978) Clinical and laboratory aspects of disseminated intravascular coagulation: a study of 118 cases. *Thromb Haemost* **39**: 122-134

Signori EE, Penner JA, Kahn DR. (1969) Coagulation defects and bleeding in open heart surgery. *Ann Thorac Surg* **8**: 521-529

Silver IA. (1980) The physiology of wound healing. In: Hunt TK (ed.) *Wound Healing and Wound Infection.* Pp 11-28. New York, Appleton-Century-Crofts.

Singh M, Tresidder GC, Blandy JP. (1973) The evaluation of transurethral resection of the prostate. *Br J Urol* **45**: 93-102
Slade N, Andrews GL, Tovey GH, Wilson F. (1964) Blood loss in prostatic surgery. *Br J Urol* **36**: 399-405

Sleight MW. (1982) The effect of prophylactic subcutaneous heparin on blood loss during and after transurethral prostatectomy. *Br J Urol* **54**: 164-165

Slichter SJ, Funk DD, Leandoer LE, Harker LA. (1974) Kinetic evaluation of haemostasis during surgery and wound healing. *Br J Haematol* **27**: 115-125

Smart RF. (1984) Endoscopic injection of the vasoconstrictor ornithine-8-vasopressin in transurethral resection. *Br J Urol* **56**: 191-197

Smith RB, Riach P, Kaufman JJ. (1984) Epsilon aminocaproic acid and the control of post-prostatectomy bleeding: a prospective double-blind study. *J Urol* **131**: 1093-1095

Sohn MH, Vogt C, Heinen G, Erkens M, Nordmeyer N, Jakse G. (1993) Fluid absorption and circulating endotoxins during transurethral resections of the prostate. *Br J Urol* **72**: 605-610

Spero JA, Lewis JH, Hasiba U. (1980) Disseminated intravascular coagulation. Findings in 346 patients. *Thromb Haemost* **43**: 28-33

Spiess BD, Tuman KJ, McCarthy RJ, DeLaria GA, Schillo R, Ivankovich AD. (1987) Thrombelastography as an indicator of post-cardiopulmonary bypass coagulopathies. *J Clin Monit* **3**: 25-30

Spiess BD. (1990) Coagulation function in the operating room. In: *Anaesthesiology Clinics of North America: Haemorrhagic Disorders.* pp 481-491. Benumof JI, Spiess BD eds. Philadelphia: Saunders.

Stern M (1926) Resection of obstruction at the vesical orifice. New instruments and a new method. *JAMA* **87**: 1726-1730

Stouthard JM, Levi M, Hack CE, Veenhof CH, Romijn HA, Sauerwein HP, van der Poll T. (1996) Interleukin-6 stimulates coagulation not fibrinolysis, in humans. *Thromb Haemost* **76**: 738-742

Straub PW, Riedler G, Frick PG. (1967) Hypofibrinogenaemia in metastatic carcinoma of the prostate: suppression of systemic fibrinolysis by heparin. *J Clin Pathol* **20**: 152-157

Straub PW. (1971) Chronic intravascular coagulation. Clinical spectrum and diagnostic criteria, with special emphasis on metabolism, distribution and localisation of I131-fibrinogen. *Acta Med Scand* (Suppl) **526**: 1-95

Stump DC, Thienpont M, Collen D. (1986) Purification and characterization of a novel inhibitor of urokinase from human urine. Quantitation and preliminary characterization in plasma. *J Biol Chem* **261**: 12759-12766

Suffredini AF, Harpel PC, Parillo JE. (1989) Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. *N Engl J Med* **320**: 1165-1172

Sullivan NM, Sutter VL, Mims MM, Marsh VH, Finegold SM. (1973) Clinical aspects of bacteremia after manipulation of the genito-urinary tract. *J Infect Dis* **127**: 49-55

Summaria L, Sandesara J, Yang G, Vagher JP, Caprini JA. (1986) *In vitro* comparison of fibrinolytic activity of plasminogen activators using a thrombelastographic method: In vivo evaluation of the B-chain-streptokinase complex in the dog model using pre-titred doses. *Thromb Haemost* **56**: 71-79

Swedenborg J, Olsson P. (1978) Activation of plasma coagulation in vivo by epinephrine and platelet release reaction. An experimental study in the dog. *Thromb Res* **13**: 37-45

Symes JM, Hardy DG, Sutherns K, Blandy JP. (1972) Factors reducing the rate of infection after trans-urethral surgery. *Br J Urol* **44**: 582-586

Symes JM, Offen DN, Lyttle JA,Chaput De Saintonge DM, Blandy JP. (1975) The effect of Dicynene on blood loss during and after transurethral resection of the prostate. *Br J Urol* **47**: 203-207

Szczepañski M, Bardadin K, Zawadzki J, Pypno W. (1988) Procoagulant activity of gastric, colorectal and renal cancer is factor VII-dependent. *J Cancer Res Clin Oncol* **114**: 519-522

Tagnon HJ. (1942) Significance of fibrinolysis in mechanism of coagulation of blood. *J Lab Clin Med* **27**: 1119-1131

Tagnon HJ, Whitmore WF JR, Shulman NR. (1952) Fibrinolysis in metastatic cancer of prostate. *Cancer* **5**: 9-12

Tagnon HJ, Whitmore WF Jr, Schulman P, Kravitz SC. (1953) The significance of fibrinolysis occuring in patients with metastatic cancer of the prostate. *Cancer* **6**: 63-67

Thurston AV, Briant SL. (1993) Aspirin and post-prostatectomy haemorrhage. *Br J Urol* **71**: 574-576

Tillet WS, Garner RL. (1933) The fibrinolytic activity of hemolytic streptococci. *J Exp Med* **58**: 485-502.

Todd AS. (1964) Localization of fibrinolytic activity in tissues. *Br Med Bull* **20**: 210-212

Towler JM, Valerio D. (1978) Dicynene in transurethral resection of the prostate. *Br J Urol* **50**: 547-550

Tscholl R, Straub W, Zingg E. (1980) Electroresection of the prostate in patients treated with heparin. *J Urol* **124**: 221-222

Tuman KJ, Spiess BD, McCarthy RJ, Ivankovich AD. (1987) Effects of progressive blood loss on coagulation as measured by thrombelastography. *Anesth Analg* **66**: 856-863

Tuman KJ, McCarthy RJ, Patel RV, Patel RB, Ivankovich AD. (1991) Comparison of thrombelastography and platelet aggregometry. *Anesthesiology* **75**: A433

van Arsdalen KN, Barnes RW, Clarke G, Smith MJV, Koontz WW. (1983) Deep vein thrombosis and prostatectomy. *Urology* **21**: 461-463

van Deijk WA, van Dam-Mieras MCE, Muller AD. (1983) Activation of factor VII in patients with carcinoma of the prostate. *Haemostasis* **13**: 198-200

van Deventer SJH, Buller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A. (1990) Experimental endotoxaemiain humans: analysis of cytokine release and coagulation, fibrinolytic and complement pathways. *Blood* **76**: 2520-2526

van Oeveren W, Harder MP, Roozendaal KJ, Eijsman L, Wildevuur CR. (1990) Aprotinin protects platelets against the initial effect of cardiopulmonary bypass. *J Thorac Cardiovasc Surg* **99**: 788-797

Varenhorst E, Wallentin L, Risberg B. (1981) The effects of orchiectomy, oestrogens and cyproterone acetate on the antithrombin III concentration in carcinoma of the prostate. *Urol Res* **9**: 25-28.

Vasanthakumar V, Bhan GL, Perera BS, Taft P. (1990) A study to assess the efficacy of chemoprophylaxis in the prevention of endoscopy - related bacteraemia in patients aged 60 and over. *Q J Med* **75** (278): 647-653

Vesey SG, Goble NM, Stower MJ, Hammonds JC, Smith PJB. (1988) The effects of transurethral prostatectomy on serum prostate specific antigen. *Br J Urol* **62**: 347-351

Vinnicombe J, Shuttleworth KED. (1966a) Aminocaproic acid in the control of haemorrhage after prostatectomy. A controlled trial. *Lancet* **1**: 230-232

Vinnicombe J, Shuttleworth KED. (1966b) Aminocaproic acid in the control of haemorrhage after prostatectomy. Safety of aminocaproic acid - a controlled trial. *Lancet* **1**: 232-234

von Hundelshausen B, Stemberger A, Jelen-Esselborn S, Hipp R, Pfab R, Leyh H, Blumel G. (1992) Blutgerinnung und fibrinolyse in der prostatachirurgie. *Fortschr Med* **110**: 126-130

von Kualla KN, Weiner M. (1955) Studies of coagulation and fibrinolysis by new technique of continuous recording. *Blood* **10**: 362-369

Voss R, Matthias FR, Borkowski G, Reitz D. (1990) Activation and inhibition of fibrinolysis in septic patients in an internal intensive care unit. *Br J Haematol* **75**: 99-105

Walton JK, Rawstron RE. (1981) The effect of local hypothermia on blood loss during transurethral resection of the prostate. *Br J Urol* **53**: 258-260

Ward MG, Richards B. (1979) Complications of antifibrinolysis therapy after prostatectomy. *Br J Urol* **51**: 211-212

Wang JS, Lin CY, Hung WT, O'Connor MF, Thisted RA, Lee BK, Karp RB, Yang MW. (1992) Thrombelastogram fails to predict postoperative hemorrhage in cardiac patients. *Ann Thorac Surg* **53(3)**: 435-439

Warren R. (1953) Medical pregress: postoperative thrombophilia. *N Engl J Med* **249**: 99-106

Watson CJE, Deane AM, Doyle PT, Bullock KN. (1990) Identifiable factors in post-prostatectomy haemorrhage: the role of aspirin. *Br J Urol* **66**: 85-87

Weisenthal CL, Meade RC, Owenby J, Irwin RI. (1961) An investigation of Kutapressin as a hemostatic agent in transurethral surgery of the prostate. *J Urol* **86**: 346-349

Whitten CW, Allison PM, Latson TW, Elmore J, Gulden RH, Burkhardt D, Hyndman V. (1991) Thrombelastographic fibrinolysis does not correlate with levels of D-dimer after cardiopulmonary bypass. *Anesthesiology* **75**: A432

Williams JRB. (1951) Fibrinolytic activity of urine. *Br J Exp Path* **32**: 530-537

Wilson J, Grant PJ, Davies JA, Boothby M, Gaffney PJ, Prentice CRM. (1988a) The relationship between plasma vasopressin and changes in coagulation and fibrinolysis during hip surgery. *Thromb Res* **51**: 439-445

Wilson RG, Smith D, Paton G, Gollock JM, Bremner DN. (1988b) Prophylactic subcutaneous heparin does not increase operative blood loss in transurethral resection of the prostate. *Br J Urol* **62**: 246-248

Woodhams BJ, Kernoff PBA. (1983) The application of polyethylene glycol to radioimmunoassays used in haemostasis. *Thromb Res* **29**: 333-341

Wright HP. (1942) Changes in the adhesiveness of blood platelets following parturition and surgical operations. *J Path Bact (Lond)* **54**: 461-468

Yaku H, Yamamoto F, Kawazoe K, Nakajima N, Uchida K, Fujita T. (1988) The change in coagulation factor XIII after open heart surgery. *Kyobu Geka* **36**: 2036-2043.

Yang Q, Cheng J. (1989) Preoperative use of 5-flourouracil to reduce operative bleeding in transurethral resection of prostate. *Urology* **33**: 407-409

Ygge J. (1970) Changes in blood coagulation and fibrinolysis during the postoperative period. *Am J Surg* **119**: 225-232

Young H H (1913) A new procedure (punch operation) for small prostatic bars and contracture of the prostatic orifice. *JAMA* **60**: 253-256

Zeh H, Ott H. (1954) Klinische gesichtspunkte zur entstehung der erworbenen afibrinogenämie und fibrinogenopenie mit hämorrhagischer diathese; beschreibung eines neuen falles. *Acta Haemat* **12**: 106-116

Zuckerman L, Caprini JA, Lipp V, Vagher JP. (1978) Disseminated intravascular multiple systems activation (DIMSA) following thermal injury. *J Trauma* **18**: 432-439

Zuckerman L, Cohen E, Vagher JP, Woodward E, Caprini JA. (1981) Comparison of thrombelastography with common coagulation tests. *Thromb Haemost* **46**: 752-756

Appendix

20 Normal Serum

This is a pooled serum sample taken simultaneously from 20 normal individuals working within the Katherine Dormandy Department of Haemophilia. Nine millilitres of lightly cuffed antecubital venous blood samples are added to 1.0 ml 0.109M tri-sodium citrate, gently mixed and immediately centrifuged at 2000G for 20 minutes at 4^0C . The resulting plasma is then centrifuged as before and the supernatant mixed and subaliquoted into 0.4ml samples and stored immediately in liquid nitrogen until used.

Enzyme-linked Immunosorbant Assays

Principles

A standard solid phase sandwich Enzyme Linked Immunosorbent Assay (ELISA) technique was used for all the assays thus described.

A polyclonal antibody specific for the coagulation factor being assayed (x) is coated onto the wells of a microtitre plate. Samples, including standards of known x content, control samples and unknown samples are pipetted into the wells. During the first incubation the x antigen binds to the immobilised coating (capture) polyclonal antibody on one site. After washing, a biotinylated monoclonal antibody (tag) specific for x is added. During the second

incubation, this antibody binds to the immobilised x captured during the first incubation.

After removal of excess biotinylated antibody, the enzyme Streptavidin-Peroxidase is added. This binds to the biotinylated antibody. After a third incubation and washing to remove the unbound enzyme, a substrate solution is added which is acted upon by the bound enzyme to produce a colour. The intensity of this colored product is directly proportional to the concentration of x present in the original specimen.

Standard operating procedure

Materials

Microtitre plate (Maxisorb Nunc, through Life Technologies, Paisley, UK)

Coat polyclonal antibody

Biotinylated tag monoclonal antibody

Horse-radish peroxidase/streptavidin (HRP-SA, ICN, Thame, UK)

20 normal plasma

0.05M carbonate-bicarbonate coating buffer pH 9.6

Wash buffer - 0.01M phosphate buffer pH 7.4 containing 0.5M sodium chloride and 0.1% Tween 20

Dilution buffer - wash buffer + 3% PEG 8000

Substrate buffer - 0.1M citrate/acetate buffer containing one 10mg tablet of OPD (o-phenylenediamine dihydrochloride, Sigma Chemical Co, Poole, UK).

1.5M sulphuric acid (H_2SO_4)

Equipment

Amersham plate shaker / incubator

Flow Titertek MS-2 plate reader (ICN, Thame, UK)

Denley Wellwash 5 plate washer

Method

3.1 Plate coating - previous day

Coating antibody diluted 1: 1000. 12μl antibody + 12ml coating buffer

3.2 Add 100 μl / well; incubate at 4^0C overnight

3.3 Wash 5 times with wash buffer using plate washer

3.4 Prepare samples from standard curve as shown:

Dilute 50 μl of the 20 normal plasma to 4.0ml in dilution buffer. Prepare a range of standards from this stock.

A	Stock solution	125%
B	0.8ml stock + 0.2ml dilution	100%
C	0.6ml stock + 0.4ml dilution	75%
D	0.4ml stock + 0.6ml dilution	50%
E	0.2ml stock + 0.8ml dilution	25%
F	0.1ml stock + 0.9ml dilution	12.5%
G	0.05ml stock + 0.95ml dilution	6.25%

3.5 Dilute unknown samples to 1:100 and 1:200, in dilutional buffer

3.6 Add 100µl of standards and tests in duplicate to the wells of the coated plate. Use dilution buffer only as blank.

3.7 Cover with plate sealer. Incubate for 1 hour on aplate shaker at room temperature. Then wash with wash buffer 5 times in the Wellwash 5. Tap out excess buffer.

3.8 Dilute biotiylated tag antibody labelled with HRP-SA to 1:1000 as shown: 12µl antidody + 12 ml dilutional buffer

3.9 Add 100µl / well of diluted tag antibody to the plate, cover and incubate for 1 hour at room temperature on the plate shaker.

3.10 Wash the plate 5 times on Wellwash 5

3.11 During washing prepare the substrate solution as shown:

15ml substrate solution + 7 µl 30% hydrogen peroxide

Ensure prepared solution reaches room temperature before use. Keep in dark until required.

3.12 Add 100µl / well of substrate solution to plate. Incubate for 10 minutes on a bench.

3.13 Stop reaction by adding 50µl / well of 1.5 M H_2SO_4. Shake plate on plate shaker to mix.

3.14 Read absorbance at 492nm (filter 4) on Flow Titertek MS-2 plate reader.

Fibrin Plate Method

- **Reagents**

Barbitone buffer - add 11.74g sodium barbitone (BDH AnalaR Laboratory Supplies, Poole, UK) to 430ml 100mmol/l hydrochloric acid.

Barbitone buffered saline (pH 7.4) - 5.67g sodium chloride added to 1L barbitone buffer.

Before use , this is diluted with an equal volume of 154 mmol/L sodium chloride solution (9 g/L NaCL).

Bovine fibrinogen (Chromogenix AB, Mölndal, Sweden)

Bovine thrombin 50NIH u/ml (Diagnostic Reagents Ltd, Thame, Oxon, UK)

Calcium 0.025mol/L ($CaCL_2$)

- **Method**

1. Dilute the fibrinogen in diluted barbitone buffered saline to a concentration of 1.5g/l
2. Pipette 10 ml of the buffered fibrinogen solution into a petri dish and place on a level surface
3. Add 0.5ml of calcium chloride solution
4. Add 0.2ml of thrombin and mix contents immediately by

swirling for 5 seconds. Petri dish is then placed immediately back on a level surface to set - plate sets within 15 - 20 seconds.

5. Leave undisturbed for 20 minutes. Central well can then be cut using a 2mm diameter punch. Plates may be stored at 4^0C for 72 hours.