BLEEDING FROM UPPER GASTROINTESTINAL TRACT AND FIBRINOLYSIS

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DECLARATION

This thesis has been composed and written entirely by myself and has not been submitted previously for any degree. The studies, of which it is a record, were conceived and designed by myself.

Signed

Date

DEDICATION

I must express appreciation to my parents, my wife and our children Amar, Ihssan, Zainub, Yeasr, and Sumia for tolerating the many hours of absence caused by my interest and involvement in this subject and to them this thesis is dedicated.

SUMMARY

UPPER GASTROINTESTINAL BLEEDING AND FIBRINOLYSIS

About 30,000 people are admitted to hospital in the United Kingdom each year with upper gastrointestinal tract (GIT) bleeding and about 3,000 of those will die. Rebleeding after admission to hospital is one of the major factors contributing to this mortality. One factor which may cause rebleeding is lysis of the fibrin haemostatic plug which seals a leaking vessel. Previous studies have shown that fibrinolytic activity in the stomach and duodenum was increased in patients with peptic ulcer, was localised around the ulcer, and could be released by trauma to the stomach. The serum concentration of fibrin degradation products (FDP) was also increased in patients with recent GIT bleeding.

The main aim of this thesis was to measure systemic fibrinolytic activity in a series of patients with acute upper GIT bleeding, and to relate this to clinical outcome, in order to assess its possible role in pathogenesis of bleeding. In this prospective study, 122 patients were studied on the morning after admission to hospital. All had routine endoscopy to establish the likely site of bleeding and the pathological cause of bleeding. Patients were divided into three groups: (1) no transfusion or surgery required; (2) transfusion required; (3) those who required surgery and/or who

died.

Blood was taken from an arm vein for measurement of serum FDP, as well as the fibrinolytic activity of the euglobulin fraction of plasma (fibrin plate lysis area FPLA). The latter test is a global measurement of plasminogen activators and their inhibitors in the euglobulin fraction. Neither test was significantly related to site of bleeding, or to the pathology of the lesion. The FPLA test showed no correlation with outcome. However serum FDP was significantly higher in patients who required transfusion (Group 2) than in patients who did not (Group 1), and patients who died or required surgery (Group 3) had significantly higher levels compared to Group 2 ($p < 0.001$). Thus the serum FDP level was shown, for the first time, to be of prognostic significance in acute upper GIT bleeding. Multivariate analysis confirmed independent prognostic value. A rapid screening test for raised serum FDP (latex agglutination test) was then evaluated in 36 samples from the study. Of the 7 patients with a positive test, 5 required surgery or died. Hence this test may be clinically useful in assessment of severe or recurrent bleeding.

Further investigations were performed to clarify the relationship of serum FDP to outcome of acute GIT bleeding. To evaluate the possibility that blood transfusion may itself raise serum FDP, these were measured before and after blood transfusion in 10

patients: no significant change in levels was observed. Because the serum FDP assay does not distinguish degradation products of fibrinogen from those of cross-linked fibrin, an assay for plasma levels of cross-linked fibrin degradation products (ELISA assay using monoclonal antibodies) was performed in 65 samples from the study. Mean levels were similar in Groups 1 and 2, but significantly elevated in Group 3 ($p < 0.05$). This confirms the association of poor outcome with lysis of cross-linked fibrin, e.g. in haemostatic plugs.

It was concluded that the association of lysis of fibrin with poor outcome after GIT bleeding is consistent with the hypothesis that fibrinolysis may play a role in promoting continued or recurrent bleeding. Further evidence for this comes from encouraging results of clinical trials of fibrinolytic inhibitor drugs, e.g. tranexamic acid.

The second aim of this thesis was to measure systemic fibrinolytic activity in patients with portal hypertension due to liver cirrhosis. In 10 patients with acute bleeding varices elevated serum FDP were again seen in patients who died or required surgery. In 54 patients without acute bleeding varices who attended for elective sclerotherapy, elevated levels of both serum FDP and FPLA were observed, being most marked in Child's Grade C patients, although this was not statistically significant for FPLA. The results of this study suggest that increased fibrinolysis, related to the severity of

cirrhosis, may play a role in bleeding from oesophageal varices, and suggests evaluation of fibrinolytic inhibitor drugs in such patients.

CHAPTER I

INTRODUCTION

Upper gastrointestinal bleeding continues to be a common and serious clinical problem in the author's clinical practice. In attempting to develop some insight into this condition, the literature has been reviewed with the following intentions:

(a) to establish some estimate of the frequency and seriousness of upper gastrointestinal bleeding (UGIT) bleeding in western populations

(b) to determine the factors which have been identified as influencing mortality in these patients

(c) to examine methods of treating this condition and suggest new possibilities for rational management.

1.1 Epidemiology of upper gastrointestinal bleeding

About 30,000 people each year are admitted to hospital in the United Kingdom with upper gastrointestinal bleeding and about 3,000 of these will die (Langman 1985). There is little evidence in the literature to suggest that this condition is becoming less of a problem.

(a) Incidence

Figures released by the health service in Scotland on discharges from hospital are not broken down in sufficient detail to permit assessment of the numbers presenting with UGIT bleeding. However, the Information and Statistics Division of the Common Services Agency of

the Scottish health service does publish details of the number of hospital discharges resulting from "peptic ulcer". These figures show a reduction in the number of admissions from 7,212 to 5,165 males and females between 1979 and 1989. This might be expected in view of the increased use of H_2 receptor antagonists over that decade. There is evidence, however, that this decrease in peptic ulcer admissions has not been accompanied by a decrease in episodes of bleeding. A study of admission rates for peptic ulcer and its complications carried out in Trent Regional Health Authority showed little change in the number of admissions to hospital for treatment of duodenal ulcer between 1972 and 1984 (Bardhan et al 1989). A study of the pattern of admission showed a marked fall in the waiting list for elective treatment - 73% in the period 1979-1984 - and a 40% increase in the number of emergency admissions. Included in this figure was an 8% rise in the number of admissions for haemorrhage.

The rise in the incidence of emergency presentation and decline in elective admission is not mirrored by the reported experience of other western centres. An early study of the effect of cimetidine on the incidence of bleeding, published only in abstract, suggested that the incidence of haemorrhage was decreasing despite an unchanged incidence of perforation (Thompson 1981). In European studies, admission rates for haemorrhage are

reportedly increased in Finland (Tilvis et al 1987) and decreased in Greece (Archimandritis et al 1986). The American experience has been conflicting also. Kurata and colleagues reported a reduction in admissions with haemorrhage (1982) while the Mayo Clinic group reports no change in incidence (Gustavsson et al 1988).

(b) Age

It is clear that the average age of patients presenting with UGIT is increasing. Logan and Finlayson (1976) reviewed the literature and pointed out that less than 10% of patients admitted with UGIT bleeding before 1930 were over 60 years of age whereas in the early 1970's, 50% of patients were over this age. The Trent RHA study (Bardhan et al 1989) found that the number of patients with peptic ulcer over 65 admitted as an emergency increased by 36% while the admission rate with haemorrhage in the over 65's increased by 28%. The biggest increase in this study was found in women over the age of 65 and it must be a possibility that this increase may be related to the more widespread use of non steroidal anti-inflammatory drugs in elderly women with arthritis.

Changing population demography might be expected, therefore, to result in an increasing number of elderly, high risk patients being admitted to hospital with UGIT bleeding. A reducing incidence of peptic ulcer in the community due to improved understanding of the

pathogenesis of this disease and hence improved treatment may eventually work to decrease the number of deaths due to this cause but that effect is not yet apparent.

(c) Mortality

Undoubtedly, great advances have been made in the technology available to investigate and treat UGIT bleeding over the past 25 years. It is commonly held, however, that these advances have not resulted in any great improvement in mortality. Alan and Dykes (1976) summarized the mortality rate of UGIT bleeding in the period prior to 1940. They found that mortality ranged from 1%-22% with a mean of around 10%. Piper and Stiel (1986) summarized the mortality from UGIT bleeding after 1940 and drew a distinction between those treated before endoscopy came into routine use (1940-1973) and the period after 1975. A proper metanalysis was not done but a rough assessment of the figures showed that in the pre-endoscopy era, mortality ranged between 3 and 20%. After endoscopy came into widespread use, reported mortality ranged between 0 and 55%. The approximate mean mortality was about 10% in each case. Clearly, only the broadest of conclusions can be drawn from this paper since heterogeneous subgroups of patients were compared and many different treatment approaches were in use.

Overall, deaths from peptic ulceration numbered 4,259 in England and Wales in 1983 (Taylor et al 1985). This paper confirmed the importance of UGIT haemorrhage as a

threat to the elderly. Ninety five per cent of deaths were in patients over the age of 55 and the mean age of death for men was 69 and for women 74 (Office of Population Censuses and Surveys 1984, Bonnevie, 1978).

Furthermore, Pulvertaft showed in 1968 that both men and women over the age of 55 with a duodenal ulcer have a 25% risk of major haemorrhage during the next decade of life. When they do bleed, they are at greater risk of dying. Kang and Piper (1980) studied 12 published series and confirmed a marked increase in mortality for those over 60 years of age.

While technological advance was not immediately translated into an improved survival, it became clear that endoscopy would have one major impact on the management of the condition: it allowed a distinction to be made between patients according to the lesion which was seen to be bleeding. This was a significant advance. An immediate benefit was the ability to differentiate between bleeding due to peptic ulceration and oesophageal varices. Where varices were present on barium meal and rigid oesophagoscopy confirmed that they were bleeding, mortality was high. A series of studies from Higgin (1947), Atik and Simcone (1954), Nachla and colleagues (1955), Cohn and Blaisdell (1958), Taylor and Jonz (1959), Merrigan and colleagues (1960) and Orloff (1962) showed that mortality in these patients ranged from 34% to 80%. Hunt and colleagues (1983) showed, however, that

the use of early, flexible endoscopy in these patients allowed an aggressive treatment regimen to be implemented and they reported improved mortality in varices patients from 35% to 17% in the periods 1972-1977 and 1977-1982 respectively. During the same two time periods, mortality from bleeding duodenal ulcer improved from 13% to 6% and, in bleeding gastric ulcer, mortality also improved from 34% to 3%. It was apparent from this study that an aggressive management policy could affect outcome of these patients. In addition to this encouraging observation, the information on the presentation and pattern of bleeding that was being obtained from direct observation down the endoscope was providing important insights into the pathogenesis of the disease. A number of factors influencing mortality could be defined.

1.2 Patient-related factors influencing mortality

Several authors have attempted to relate clinical and investigational findings to outcome and it is these which will form the main part of the review in this section.

(a) Clinical findings

There is disagreement as to whether the mode of presentation of gastrointestinal haemorrhage has prognostic significance. Northfield (1971) found that presentation with haematemesis alone was associated with a greater chance of further bleeding than presentation with melaena with or without haematemesis. Johnston et al (1974) found no such difference while MacLeod and

Mills (1982) found that patients presenting with haematemesis had a significantly lower incidence of further haemorrhage than those presenting with melaena alone or with haematemesis.

Several workers have found that a history of recent alcohol ingestion prior to admission is associated with a more favourable outcome (Morgan et al 1977; MacLeod and Mills 1982). This may be due to the high incidence of erosive gastritis in this group of patients (MacLeod and Mills 1982) and may not apply to patients admitted with peptic ulceration. Blood group O patients with gastric ulcer or duodenal ulcer are more likely to bleed and at an earlier age than other groups (Berg 1969).

Other clinical factors of importance are the presence of shock on admission. Balint (1977) pointed out the prognostic significance of an admission systolic blood pressure <100 mm Hg. This fact had been appreciated since Avery Jones' classic studies in the 1950's. Northfield and Smith (1970) at an early stage in the study of this problem stressed the importance of central venous pressure measurement in identifying those patients who were continuing to bleed. Low haemoglobin level on admission, as a sign of prolonged bleeding, has been found also to correlate with a poor outcome (MacLeod et al 1982, Himal et al 1974).

Further ominous clinical features were the association between UGIT bleeding and burns (Curling's

ulcer) and head injuries (Cushing's ulcer) and sepsis. This appears to be an area where prophylactic use of antacids and H_2 receptor antagonists have had a positive effect in reducing the incidence of bleeding.

(b) Age

The increased risk of death with increasing age has already been alluded to. Recurrent bleeding is also a problem with elderly patients.

Mortality significantly increases in patients over 60 years of age. MacLeod and Mills (1982) in Glasgow found that in all cases of upper gastrointestinal haemorrhage regardless of cause, 18% of patients under 60 years of age had further haemorrhage compared to 34% of the patients over that age although this has not been confirmed by others (Northfield 1971; Allan and Dykes 1976; Morgan et al 1977). Similarly, in the Glasgow study (Macleod and Mills 1982), further haemorrhage from duodenal ulcers was more common in those patients over 60 years of age but no significant difference was apparent for gastric ulcer.

(c) Site of bleeding

It is impossible to discuss mortality rate in this condition without relating outcome to the major changes that have taken place in the investigational technology available for UGIT disorders. Some mention of endoscopy has already been made in the context of mortality. It was pointed out that endoscopy has allowed

differentiation between the major sources of bleeding to take place and has underlined the great differences in outcome between peptic ulcer and variceal bleeding.

In general, patients with bleeding duodenal ulcers have a mortality around 4% while patients with bleeding oesophageal varices have a mortality approximating 50%. If the site of bleeding is known prior to surgical or conservative therapy, mortality is about 5%. If the source of haemorrhage is unknown prior to therapy, mortality is approximately 25% (Capper et al 1964; Himal et al 1974).

This is probably explained by the observation that having a potential bleeding source like peptic ulcer or oesophageal varices does not guarantee that it is the source of bleeding. Forrest and Finlayson (1974) found 60% of patients having peptic ulcers were bleeding from that lesion, 21% of patients with no dyspeptic symptoms were bleeding from a peptic ulcer, 10-20% of patients with a history of peptic ulceration were bleeding from other lesions and 30-50% of portal hypertension patients were bleeding from lesions other than oesophageal varices.

Discussion of the investigation of haematemesis and melaena, particularly by endoscopy, becomes clouded by the increasing importance of endoscopy as a prognostic and therapeutic tool and further consideration of the information which can be gained from endoscopy and its

application might best be considered in the next section. Before leaving this part of the discussion, however, it might be useful to summarise.

The number of admissions to western hospitals for treatment of peptic ulcer is decreasing but it appears that the number of admissions for treatment of the complications of this condition is not. In particular, UGIT haemorrhage incidence is probably static. More elderly patients are presenting with this condition and it is known that they have a higher mortality. Some attempt can be made to identify high risk patients on admission from clinical features such as blood pressure and haemoglobin. The presence of risk factors should alert the clinician to the need for action. Possible courses of action are now discussed.

1.3 Management options

The first major decision to be made after the initial steps have been taken to resuscitate the patient concerns early endoscopy. Much debate surrounds the question of timing of endoscopy.

a) Endoscopy: early or not

Protagonists of early endoscopy suggest that the procedure helps in the management of patients with gastrointestinal bleeding. Not only does it identify the site or sites of bleeding but it provides information about continuation of bleeding or signs of recent haemorrhage (Palmer 1969; Katon and Smith 1973; Foster et

al 1978). An alternative view is that because most patients stop bleeding, irrespective of the cause (Schiller et al 1970), endoscopy should be reserved for patients who continue to bleed or who have recurrent bleeding (Winans 1977, Eastwood 1977). Most patients do stop bleeding with conservative measures and the estimated incidence of continued bleeding ranges from 6% to 10% depending on the series. The rebleeding rate can however be as high as 25% and the mortality is greatest in patients who either continue to bleed or rebleed in hospital (Jones 1956). Thus in at least 70% of patients, early endoscopy will not influence the management of the patients as bleeding will stop. This observation may explain the apparent failure of endoscopy to improve mortality. Most studies are too small and therefore are subject to type II error. It is in patients in whom bleeding continues or recurs that early diagnosis and vigorous therapy will be of greatest value. Therefore the timing of endoscopy in the management of acute gastrointestinal haemorrhage has been the subject of discussion (Conn 1981; Eastwood 1981). Controlled studies have failed to demonstrate an improvement in mortality rates in patients submitted to early endoscopy. Until recently the endoscopist was no better than the clinician at predicting whether bleeding would continue or recur.

Obviously, determination of recurrent or continuing bleeding is difficult unless it results in haemodynamic

upset. A logical strategy would be to attempt to visualise the bleeding vessel directly and see if it is bleeding or if the likelihood of recurrent bleeding can be predicted.

b) Stigmata of recent haemorrhage

Signs of recent haemorrhage at endoscopy may indeed correlate with the likelihood of recurrent bleeding. Foster et al (1978) accepted that a lesion had bled only when one or more of the following stigmata were seen at endoscopy: fresh bleeding from the lesion; fresh or altered blood clot or black slough adherent to the lesion; or vessel protruding from the base or margin of an ulcer. These stigmata were found in 69% of patients endoscoped within 12 hours of presentation and in about 40% of patients endoscoped thereafter. Of their 233 patients, 89 had peptic ulcers and stigmata of recent haemorrhage (SRH) were seen in 56% of patients with duodenal ulcers and 80% of those with gastric ulcers. In gastric ulcers with signs of recent haemorrhage the risk of recurrent bleeding was 30% compared to 0% when signs of recent haemorrhage were absent. In duodenal ulcer patients with signs of recent haemorrhage the risk of recurrent bleeding was 63% compared to 5%. Although only one patient without stigmata rebled, stigmata were associated with further haemorrhage in 42% of patients. In this retrospective study the use of clinical factors such as age, history of drug and alcohol ingestion,

concomitant disease and shock proved much less reliable than the endoscopic findings at predicting outcome.

Griffiths et al (1979) found 'visible vessels' in the ulcer crater in 28 of 157 consecutive ulcer patients presenting with gastrointestinal haemorrhage. All 28 patients subsequently required surgery because of uncontrolled bleeding while 79% of the patients without visible vessels in the base of an ulcer settled on conservative treatment. Surgical mortality was only 9% in patients with visible vessels compared to 35% in those patients without visible vessels. The mortality of those patients with visible vessels managed conservatively was 83%. The authors conclude that surgery should be considered when a vessel is identified in the ulcer crater at endoscopy.

An excellent prospective study by Storey et al (1981) examined 292 patients admitted consecutively with acute gastrointestinal haemorrhage. All patients were endoscoped within 24 hours of admission. They defined a visible vessel as an elevated red or blue spot that protruded from the ulcer crater and was resistant to washing and often associated with a red clot. Otherwise, SRH were defined as oozing a fresh or altered blood clot adhering to the ulcer or black spots were seen in an ulcer crater. An independent clinician observed the patient in hospital over the next 7 days for evidence of fresh bleeding. Of the 292 patients, 132 had peptic

ulcer. In 117 patients in whom endoscopic examination was possible, 56 had a visible vessel and 21 other SRH. No such stigmata were present in 40. None of the latter had further bleeding, surgery or died. 56% patients with a visible vessel, had further bleeding, 50% of this group required surgery and operative mortality was 15% (5/34). Of 13 patients with other SRH, 1 had further bleeding and surgery. The paper suggested that a visible vessel carried the greatest prognostic significance. Although the occurrence of further bleeding and mortality was entirely restricted to ulcers with visible vessels, it is important to note that in this study only half these patients had further bleeding.

Harris and Heap (1982) reported a retrospective study on the significance of SRH. Two hundred and eighty three urgent endoscopies were performed for bleeding in 269 patients. One hundred and thirty one had bleeding peptic ulcer (57 gastric, 69 duodenal). Of the duodenal ulcer patients, 52 had SRH of whom 23% rebled and 29% required surgery; 17 had no SRH and 12% rebled and none had surgery. Of the gastric ulcer patients, 44 had SRH of whom 31% rebled and 25% had surgery; 13 had no SRH and none rebled or had surgery. However, not all studies of the prognostic significance of SRH are consistent. A recent report from the World Congress of Gastroenterology (Garrigues-Gil et al 1988) reviewed 207 patients presenting to a single centre with bleeding from an

endoscopically proven duodenal ulcer. The authors were unable to conclude that SRH had any prognostic significance and that only active bleeding at the time of endoscopy was a significant indicator of the need for early surgery. They did, however, point out that their study contained small numbers of patients.

c) Endoscopy or Radiology?

An alternative investigational option is, of course, the barium meal. Prior to the general use of the endoscope, radiology employing barium was the major method in investigation of acute UGIT. A number of studies have compared radiology and endoscopy with the consensus favouring endoscopy. Hoare (1975) examined 158 patients with UGIT and found that because the pre-operative diagnosis was correct in all endoscopy cases there was less delay before surgery. Mortality was improved and he felt that endoscopy was the investigation of choice. Similarly, Stevenson et al (1976) compared double contrast barium meal and fibreoptic endoscopy prospectively in acute upper gastrointestinal bleeding in 53 consecutive patients. Bleeding site was correctly identified by endoscopy in 94% and by radiology in 83%. In 50 patients with a definitive final diagnosis, endoscopy was correct in 100% and radiology in 88%. The trial was abandoned because of better endoscopy figures and endoscopy became the diagnostic method of choice. McGinn et al (1975)

compared endoscopy and radiology in 150 patients with acute UGIT bleeding. One hundred and thirty eight patients had both endoscopy and barium meal. The combination of both methods gave a diagnostic accuracy of 91%. They concluded that radiology seemed adequate for gastric or duodenal lesions whilst endoscopy was preferable for the oesophageal and mucosal lesions. More recently Thoeni and Cello (1980) prospectively examined 100 patients with upper gastrointestinal bleeding with endoscopy and double contrast radiology. Endoscopy detected the primary bleeding site in 93% of patients and led to the correct diagnosis in 91% of all upper gastrointestinal lesions present. Radiography detected primary site in 80% and led to the correct diagnosis in 76% of all upper gastrointestinal lesions. Endoscopy missed lesions in the duodenum and oesophagus most frequently whilst radiography missed oesophageal lesions most frequently. The two investigations combined gave an overall diagnosis accuracy of 99%. The authors suggested that the two methods were complementary in acute bleeding from upper gastrointestinal tract. However it appears that endoscopy has become the prime method of investigation of these patients.

1.4 Treatment of upper gastrointestinal tract bleeding

A number of facts have been deduced from the literature. Although peptic ulcer disease is becoming a less frequent cause of hospital admission, there is no

clear evidence, as yet, that UGIT bleeding is becoming rarer. What is clear is that the average age of patients presenting with this problem is increasing and that mortality is higher in this elderly age group. Patients with clinical evidence of severe or prolonged bleeding, as judged by shock or anaemia, have a poor outlook. Early diagnosis by endoscopy may give useful information in a few cases as to the likelihood that the patient requires surgery but continuous bleeding may be a better indicator than evidence of recent bleeding. Once it has been determined that the patient falls into a poor prognostic group, how then is he to be treated? There are basically two possibilities: the physical methods - surgery, and direct coagulation of the bleeding vessels and pharmacological methods.

1.4.1 Surgical treatment of UGIT bleeding

Conventional surgical treatment with ligation of bleeding vessels is obviously the definitive option where bleeding is uncontrolled by other means. Reference has already been made to the mortality associated with surgery in a number of studies. Mortality is always higher in elderly patients for obvious reasons of intercurrent disease. Most interest in the surgical option in the past few years has centred round the importance of early surgery particularly in the elderly group.

Morris et al (1984) studied 147 patients with proven

duodenal or gastric ulcer who were randomised after stratification for age and site of ulcer to early or delayed surgical management. The criteria for entry to the early group were 4 units blood or plasma to correct acute blood loss in 24 hours, one rebleed, or endoscopic stigmata. The delayed policy group required 8 units blood or plasma expander required to correct acute blood loss in 24 hours, had two rebleeds in hospital or persistent bleeding requiring transfusion of 12 units in 48 hours or 16 units in 72 hours.

One hundred and forty two patients (42 patients aged under 60, 100 patients aged over 60) satisfied the criteria for analysis. The early and delayed groups in each category were well matched for age, sex, pulse, blood pressure and haemoglobin.

In patients aged under 60 there were no deaths in the group receiving early treatment. The groups were closely similar for all prognostic features. One hundred patients aged 60 or over were randomised to early or delayed management. The overall mortality among all 142 patients in the study was 7% (10 deaths) all of the deaths occurred in patients over 60 so that the mortality rate in these patients was 10%. Analysis of mortality in patients over 60 on an intention to treat basis gave a rate of 4% (two deaths) in the early group and a rate of 15% (eight deaths) in the delayed group.

The conclusion of this trial was that for patients

over 60 an aggressive surgical policy is associated with a significant reduction in mortality.

This group has recently published a review of 342 further duodenal or gastric ulcer patients in whom their policy of early surgery has been applied. A mortality of 6% in the over sixties patients was achieved (Wheatley et al 1990).

The importance of early surgery in patients liable to rebleed is relevant to the foregoing discussion on endoscopy and its apparent failure in some studies to effect a reduction in mortality (Dronfield et al 1977, Eastwood et al 1977, Graham 1980). A fall in mortality would not be expected unless early endoscopy was allied to a policy of early, appropriate surgical intervention. This view is supported by the work of Hunt and colleagues (1977). They studied 728 patients in the period 1972-1977 and compared these to 588 patients in the period 1977-1982. They found a reduction in mortality in each diagnostic subgroup in the later period of the study. Only in the gastric ulcer group, however, did this reduction reach statistical significance. They claimed that their achievement was due to the introduction of a special unit for treatment of upper GIT bleeding with a policy of early intervention.

Himal et al (1978) reported a similar result in Montreal when they compared two series of 1963-1971 and 1973-1976. They found that there was a significant

reduction in mortality from 12.5% to 6.7% when peptic ulcer patients were compared. However there was no significant reduction in mortality in varices patients. The authors suggested that the improvement seen in the total series and in ulcer patients specifically was due to more aggressive surgical intervention.

It can be tentatively claimed, therefore, that advances in the diagnosis and early detection of high risk individuals with subsequent aggressive management by special units may lead to reduction of ulcer related mortality.

1.4.2 Physical methods of treatment

a) Cautery

Several physical methods of controlling GI bleeding have been investigated using a variety of modalities. Lyano acrylic tissue glues have failed experimentally (Protell et al 1978) and the spraying of clotting factors on the sites of haemorrhage is unlikely to be effective for brisk haemorrhage (Linscheer et al 1979).

Diathermy, applied endoscopically, although effective in arresting acute haemorrhage, by causing deep and unpredictable tissue injury, and is not suitable for clinical use (Dennis et al 1979).

Heater probes, which apply heat directly to the bleeding vessel, have been used effectively in the control of acute bleeding in gastric ulcers (Storey et al 1983) but have not been widely used in clinical

practice.

Kernohan et al (1984) carried out a controlled study of bipolar electrocoagulation in patients with upper gastrointestinal bleeding. They did not show that bipolar electrocoagulation reduced the incidence of rebleeding in upper gastrointestinal haemorrhage.

Johnstone et al (1982) treated patients with upper gastrointestinal bleeding by monopolar electrocoagulation. They produced full thickness muscle layer damage in 16 of 30 ulcers (53%) and grossly visible serosal change of haemorrhage or whitening overlying the ulcer was found at autopsy in 14 of 30 (47%).

b) Laser Photocoagulation

Blue-green Argon laser light is preferentially absorbed by blood and thus can potentially induce clotting and sealing of blood vessels with less damage to surrounding tissue than other lasers used for haemostasis. However, blood which overlies the target vessel is an effective barrier to transmission of Argon energy. The addition of a coaxial CO_2 gas jet to blow away overlying blood greatly improves the efficacy of Argon treatment (Silverstien et al 1979; Fruhmorgen et al 1976). Animal studies by Johnston et al (1981) have shown that this laser is safe and effective when used endoscopically providing the stomach is not over-distended by the carbon dioxide jet required to clear blood.

Laurence et al (1980) studied the Argon laser in a clinical setting and found it effective in arresting haemorrhage in 48 of 60 patients bleeding from gastric or duodenal ulcers. Laser photocoagulation was seen to stop arterial (spurting) bleeding at endoscopy in 25 of the 36 patients.

Similarly, Swain et al (1981) studied 76 randomized controlled patients with bleeding from peptic ulcer. They showed that Argon laser photocoagulation can significantly reduce mortality from bleeding peptic ulcers accessible to this form of treatment. They also demonstrated a significant reduction in rebleeding rate, and 7 deaths occurred in patients in the control group who had rebled, but there were no deaths in the treated group. Treatment however did not significantly reduce the rebleeding rate in the small group of patients actively bleeding from visible vessels.

Vallon et al (1981) studied 136 patients with bleeding from gastric and duodenal ulcers who were randomly allocated to Argon laser photocoagulation. There were no statistically significant differences between the laser treatment and control groups in terms of rebleeding, the need for surgical intervention, or death.

The Neodymium Yttrium aluminium garnet laser has a wavelength near infra-red and at effective haemostasis energy levels, the depth of penetration of this invisible laser beam is greater than the Argon laser. The Nd YAG laser energy is absorbed by haemoglobin. However, this

absorption is far less efficient than with the Argon laser and its energy is more widely scattered and is mostly absorbed by water and tissue proteins and is converted to heat. The deeper penetration makes it haemostatically effective although animal experiments have demonstrated that it has the potential for full thickness injury (Silverstein et al 1981).

Vantrappen et al (1981) carried out a controlled trial of Nd YAG laser photocoagulation in 227 patients. They were able to coagulate all bleeding lesions and showed a significant reduction in rebleeding rate but not in mortality rate. In another study by Rutgreerts et al (1982), in which 338 patients with bleeding upper gastrointestinal tract were entered, 23 patients who had presented with spurting arterial bleeding were treated by laser. The bleeding ulcer was localized in the stomach in 13 patients and in the duodenum in 10 patients. In 20 patients (87%), the bleeding could be stopped by laser. The haemastasis was permanent in 9 of the 20 patients (45%) whereas 11 of the 20 patients (55%) had recurrent haemorrhage after an interval of 1-36 hours (mean 12 hours). Fourteen of the 23 patients (61%) had to be operated on. Overall mortality in the group of arterial spurters amounted to 7 out of 23 (30%).

MacLeod et al (1983) studied in a prospective single blind controlled trial the efficacy of the Nd YAG laser, significantly reduced the rate of rebleeding and need for emergency surgery in those patients with active bleeding.

Regardless of whether allocated to placebo or laser treatment, none of the 25 patients bleeding from gastric and duodenal ulcers with spots in the ulcer base had further bleeding, required emergency surgery or died. However, only a small proportion of haemaemesis patients were deemed suitable for laser treatment in this study.

Swain et al (1986) examined the efficacy of Nd YAG laser photocoagulation in treatment of bleeding from peptic ulcer. Two hundred and sixty patients with bleeding peptic ulcer of whom 138 patients had stigmata of recent haemorrhage were randomized to be treated by laser or sham treatment.

The results of this trial suggests that the NdYAG laser significantly reduced the rebleeding rate, the need for emergency surgery and mortality. Fifteen of 31 patients rebled in the control group and 4 of 28 patients rebled in the treatment group.

Rutgreerts et al (1987) studied in a randomized trial the efficacy of BICAP electrocoagulation and Nd YAG laser photocoagulation. The study was carried out on 100 patients presenting at endoscopy with peptic ulcers and spurting or oozing vessel or a non-bleeding vessel. All patients received pretreatment with injection of 1:10,000 adrenaline around the ulcer.

In the group with spurting haemorrhage, the BICAP was more effective than laser although the difference between the two groups was not significant. Emergency surgery was required in three patients who presented with arterial

bleeding in the laser group and one in the BICAP group. Nd YAG laser induced arterial spurting from a non-bleeding vessel in 5 patients despite previous injection therapy and laser therapy allowed haemastasis in 3 of these patients. Two patients developed a perforation: 1 patient after laser therapy and another patient after BICAP therapy. The authors concluded that both treatment methods were equally effective.

The importance of these physical methods of treatment can therefore be summarised. Surgery remains the final option in management. When bleeding cannot be controlled by other means, surgery is required. It is associated with a high mortality and is expensive. Knill Jones and colleagues (1990) have recently produced figures which indicate that surgery for UGIT haemorrhage in patients on non steroidal anti inflammatory drugs might cost #2000 per episode. Non operative treatment which might allow patients home earlier than might have been the case if they required surgery is clearly preferable in a time of financial stringency.

The methods dependent on coagulation seem to have a less clearly defined place in management. It appears that laser or electrocoagulation is good at stopping bleeding but not so good at providing a long term solution. The rebleeding rate seems unaffected by the treatment. This type of therapy may find its place as a "first aid procedure" providing temporary control of haemorrhage in unstable patients. The fact that

successfully coagulated vessels have a tendency to rebleed indicates some possibilities for medical therapy. The clot may be dissolved by gastric acid or by natural fibrinolysis and drugs which might prevent this happening have been investigated.

1.5 Medical Treatment for Upper Gastrointestinal Bleeding

(a) Cimetidine/Ranitidine

The development of drugs that specifically block the H_2 receptors for histamine (Black et al 1972) has provided a powerful pharmacological tool for control of gastric acid and hence treatment of peptic ulcer. H_2 receptor antagonists inhibit gastric acid output stimulated by histamine, gastrin, or the vagal pathway and suppress meal-stimulated acid output (Pounder et al 1976).

Cimetidine has been used widely in the treatment of patients with haematemesis and melaena. Many studies of the use of H_2 receptor blockers in upper GIT haemorrhage have been carried out and several of these have shown a benefit of such treatment.

Hoare et al (1979) studied 34 patients bleeding from peptic ulcer and given cimetidine while 32 patients were given placebo. Further bleeding was detected clinically in 24% (8/34) of the treated patients and 47% (15/32) in the placebo group. Cimetidine had no effect on bleeding from duodenal ulcer, but only 2 of 14 patients with gastric ulcer treated with cimetidine rebled, compared

with 10 of 19 patients on placebo. Cimetidine, therefore, may help to prevent haemorrhage from gastric ulcer but not duodenal ulcer. Emergency operations for bleeding ulcers were needed in 4 patients in the cimetidine group and 3 patients in placebo group. One patient on cimetidine and 8 on placebo had emergency surgery for a bleeding gastric ulcer. Only one patient died. He had a duodenal ulcer and was on cimetidine. The number of patients with moderate or severe bleeding who subsequently rebled was not significantly different when treated and untreated groups were compared.

Stiel et al (1984) studied 55 patients with acute bleeding from chronic duodenal ulcers. Twenty nine patients received cimetidine and 26 received a placebo. Rebleeding rate in cimetidine treated patients was 17% (5/29) and 42% (11/26) with placebo treatment. Emergency surgery was required in 10% (3/24) of cimetidine treated patients and 14% (4/26) in placebo treated patients. Cimetidine appeared to influence favourably the course of haemorrhage in patients over the age of 60 years with bleeding from duodenal ulcer with signs of recurrent haemorrhage.

Some studies however have not found a beneficial role for H_2 receptor blockade in upper GIT bleeding.

La Brooy et al (1979) studied 101 patients with upper gastrointestinal tract bleeding. Peptic ulcer was the most common diagnosis. Rebleeding occurred in 21.5%

(11/51) of the patients on cimetidine and 24% (12/50) of patients on placebo. The incidence of rebleeding in patients with peptic ulcers showed that cimetidine was not significantly better than placebo. The only one (2%) death in the series received cimetidine.

Similarly, studies by Siddiqi et al (1979), Carstensen et al (1980), Carr-Locke et al (1984) and Birnie et al (1984) have failed to show any significant improvement in rebleeding rate or mortality with cimetidine. The studies by Carr-Locke and Birnie die however suggest a trend for reduced recourse to surgery in the cimetidine treated group.

Some randomised trials have found that placebo groups have done better than cimetidine treated patients in terms of mortality, rebleeding and emergency surgery.

For example, Macklon et al (1979) found that when 30 patients had completed their trial there had been an overall rebleeding rate of 20%. Of patients on cimetidine, 5 (28%) rebled compared with 1 (8%) out of 12 on placebo.

Other studies by Pickard et al (1979), Meredith et al (1980), and Zuckerman et al (1984) have produced similar results.

Rantidine is approximately four times as potent an inhibitor of gastric acid output on a molar basis as cimetidine. Focon and colleagues (1980) studied the effect of ranitidine on bleeding of the upper

gastrointestinal tract. Six patients were treated by rantidine and 5 patients received placebo. In the treated group one patient rebled and none required surgery or died. In the placebo group, no rebleeding occurred or no surgery was required and none died. Hostein et al (1982) also studied the effect of rantidine in patients with upper gastrointestinal bleeding. Ten patients were in a treated group and 12 patients received placebo. In the treatment group, one patient had persistent bleeding, no surgery was required, and one died. In the placebo group, three patients persisted in bleeding, two patients required surgery and one patient died. Rantidine had a more significant effect on rebleeding, the need for surgery and the mortality rate.

It can be concluded from these studies that small numbers of patients recruited into trials have prevented clear cut answers from being obtained. The effects of H_2 receptor antagonists are probably marginal therefore.

(b) Somatosatin

Somatostatin has been shown to be a potent inhibitor of gastric acid secretion and gastrin release (Bloom et al 1979). It also inhibits the pentagastrin stimulated gastric secretion of acid, pepsin and intrinsic factor in in man (Schrumpf et al 1978). The peptide inhibits both basal and hormone induced secretion, when administered by the intravenous routes and basal gastric secretion when administered intragastrically (Johansson

et al 1978). It also has a stimulative effect on gastric mucous production (Johansson and Aly 1982). Splanchnic blood flow decreases after intravenous infusion of somatostatin (Keller et al 1978; Tyden et al 1979).

Magnusson and colleagues (1985) have studied the effect of somatostatin in treatment of massive upper gastrointestinal bleeding in a randomised double-blind trial in 95 patients. Patients with oesophageal varices were excluded as well as patients with diabetes. Forty six patients, chosen at random, were given a 72 hour infusion of somatostatin, while the remaining 49 patients received an infusion of placebo. On the day after admission, an additional endoscopy was performed at which 8 patients in the somatostatin group and 16 in the placebo group were found to have persistent bleeding. A total of 5 patients in the somatostatin group and 14 in the placebo group underwent surgery. Mortality rate did not differ significantly between the two groups.

Coraggio and colleagues (1984) compared the effects of somatostatin with ranitidine and placebo in a randomised fashion. Patients were allocated to one of 3 groups. The first group received an intravenous injection of 250 mg somatostatin followed by continuous infusion of 250 mg/hour with a peristaltic pump. The second group received ranitidine and the third group placebo. There were 20 patients in each group. The haemorrhage stopped in all the 20 patients treated with somatostatin, 13 of 20 patients treated with ranitidine

and 9 of the 20 patients treated with placebo also had their haemorrhage controlled.

Moreover, no patient treated with somatostatin rebled while rebleeding occurred in 3 of the 13 patients treated with rantidine and in 2 of 9 patients treated with placebo.

Kayasseh et al (1980) also carried out a double-blind trial of somatostatin comparing it with cimetidine in the treatment of severe and persistent gastrointestinal bleeding due to peptic ulcer. Of the 20 patients studied, 10 patients received somatostatin and 10 patients received cimetidine. Bleeding stopped in 8 out of 10 patients treated with somatostatin but in only one patient treated with cimetidine. One out of 5 patients rebled 24 hours after somatostatin treatment had ended and responded to a second course of somatostatin. Emergency surgery was required in one patient in the somatostatin group and in 5 patients in cimetidine group. No patients died in the somatostatin group but 3 patients died after receiving cimetidine

(c) Prostaglandins

Prostaglandins are compounds derived from fatty acids that are found throughout the body. They possess properties that make them uniquely suited for treatment of acute upper gastrointestinal tract haemorrhage. First, they inhibit gastric acid production resulting from a number of stimuli in both animals and humans (Robert et al 1975). In addition, they are cytoprotective in

several animal models and can prevent the development of gastritis and subsequent bleeding caused by stress, shock, aspirin (Konturek et al 1981), indomethacin, absolute alcohol and concentrated hydrochloric acid (Cloud et al 1982; Main et al 1977). In humans they have been shown to be effective in preventing the gastrointestinal blood loss that frequently accompanies indomethacin therapy for rheumatoid arthritis (Johansson et al 1979). Several studies have addressed the question of the efficacy of prostaglandin in controlling acute gastrointestinal tract bleeding caused by duodenitis, gastritis, gastric erosions, gastric ulcers, or duodenal ulcers.

For example, prospective, randomised, double-blind study of the effectiveness of topical prostaglandins E_2 (PGE_2) in altering the outcome in patients with severe upper gastrointestinal tract haemorrhage was carried out by Levine and colleagues (1985).

Of the total of 44 patients entered into the study, 22 patients were randomly allocated to receive either placebo or PGE_2. Nine out of 22 patients were considered treatment failures in the placebo group. Three of these patients had persistent bleeding while 6 had recurrence of haemorrhage during the study. Eleven out of 22 patients in the PGE_2 treated patients also failed the study. Five of these patients exhibited persistent haemorrhage, whereas haemorrhage recurred in 6 during the study period. Emergency surgery was required in 9

patients and these included 5 in the placebo group and 4 in the PGE_2 patients.

It may be concluded, therefore, that the role of acid reducing drugs in the management of UGIT bleeding is far from clear. Somatostatin appears to be effective in stopping bleeding and preventing rebleeding but it is expensive and requires to be given by intravenous infusion. A clear role for H2 receptor antagonists has not really been supported by the published studies and it has not been clearly shown that prostaglandin analogues have a beneficial effect in established bleeding. The role of antifibrinolytic drugs will now be considered.

d) Antifibrinolytic therapy

If clot has developed in a vessel in the base of an ulcer, it may be dissolved by gastric acid or by natural fibrinolytic processes. As mentioned above, the evidence supporting a role for acid reducing drugs in the management of UGIT haemorrhage is far from convincing. The possibility that antifibrinolytic agents might reduce rebleeding, the need for surgery and subsequent mortality in these patients will now be examined.

Three very similar, double blind studies have been carried out on the effectiveness of tranexamic acid in treatment UGIT bleeding (McCormack et al 1973, Biggs et al 1976, Enggrist et al 1979). Each study consisted of about 150 patients randomly allocated to receive active drug or placebo. Trends, which were not significant, were observed in favour of the drug which appeared to

reduce the number of patients requiring surgery. A much larger study, incorporating 775 patients was published by Barer and colleagues (1983). Patients received cimetidine, tranexamic acid or placebo. Again, no significant differences were observed but there was a clear trend towards reduced mortality in patients receiving tranexamic acid.

Another study including 150 patients was reported by Holstein et al (1987). In this case, the tranexamic acid group was observed to require significantly reduced amounts of blood and had significantly reduced operation and rebleeding rates. The dosages of the drug used in this study were identical to those used in the earlier studies and the different result may be a chance finding in a study which had inadequate numbers of patients.

Because of the small sizes of these individual studies, Henry and O'Connell (1989) performed a meta-analysis combining all their data. This showed significant reductions in both rebleeding and mortality with tranexamic acid treatment (table 1.1) supporting the theory that fibrinolysis plays an important role in UGIT bleeding.

1.6 Fibrinolysis

1.6.1 Development of Knowledge

It has been known for many years that human blood possesses fibrinolytic activity. Hunter (1794) records that in 'animals killed by lightning or electricity' or

Table 1.1 Results of individual trials of tranexamic acid for upper gastrointestinal haemorrhage.

Authors	Study group	No. of patients randomised	No. who rebled	No. who had surgery	No. who died	Odds ratios(95% confidence intervals) Rebleeding	Operation	Death
1	(Treated	76	8	NA	3	0.67(0.22		0.97(0.13
	(Control	74	11	NA	3	to 1.98)		to 7.51)
2	(Treated	103	7	7	2	0.30(0.10	0.26(0.09	0.46(0.04
	(Control	97	19	21	4	to 0.80)*	to 0.69)*	to 3.31)
3	(Treated	76	23	10	11	0.66(0.32	0.46(0.18	0.86(0.32
	(Control	73	29	18	12	to 1.37)	to 1.17)	to 2.31)
4	(Treated	25	5	8	5	1.31(0.24	1.21(0.30	0.79(0.16
	(Control	25	4	7	6	to 7.59)	to 4.87)	to 3.73)
5	(Treated	256	58	47	16	1.20(0.77	1.24(0.76	0.43(0.22
	(Control	260	51	40	35	to 1.88)	to 2.02)	to 0.82)*
6	(Treated	94	11	5	4	0.58(0.24	0.32(0.09	0.76(0.15
	(Control	108	20	16	6	to 1.37)	to 0.98)*	to 3.31)
Total		1267	246	179	107			

NA = Date not given * p<0.05 (Henry & O'Connell 1989)

1 Cormack et al (1973) 2 Biggs et al (1976) 3 Engqvist et al (1979)
4 Bergqvist et al (1980) 5 Barer et al (1983) 6 Stael von Holstein et al (1987)

in animals 'who are run very hard, and killed in such a state' the blood does not clot. A partial explanation for this phenomenon was found in 1906 by Morawitz, who noted that the blood from victims of sudden death contained no fibrinogen and could destroy the fibrinogen and fibrin of normal blood. Denis (1838) observed that the blood clots obtained in wet cupping redissolved in less than 24 hours. Green (1887) noted that when fibrin prepared from ox blood had dissolved when incubated in saline it could not be clotted again by thrombin. Dastre (1893), during the course of phlebotomy in dogs, observed a reduction of fibrin yield which he attributed to destruction of fibrin, a process which he named 'fibrinolysis' and Hedin (1903) found spontaneous fibrinolytic activity in the globulin fraction of ox blood. A further addition to knowledge of spontaneous fibrinolytic activity in blood was finally obtained by Macfarlane (1937) who showed that in man fibrinolytic activity in the blood could be provoked by surgical operations.

Fibrinolytic activity in human blood may be derived either from blood cells (especially leucocytes), or from conversion of the circulating plasma protein plasminogen to the active enzyme, plasmin. There is currently much more knowledge of plasmin-mediated fibrinolysis than of cellular fibrinolysis (Bachmann, 1987), hence this review concentrates on the plasminogen-plasmin system which is outlined in Fig. 1.

Plasminogen, plasminogen activators, fibrinolytic inhibitors, and the degradation of fibrinogen and fibrin will now be reviewed in turn.

1.6.2 Plasminogen

(a) Synthesis and Metabolism

The physical properties, turnover, and activation of plasminogen have been summarised in several reviews (Collen and De Maeyer, 1975; Collen and Verstraete, 1975; Robbins, 1978; Bachmann, 1987). Human plasminogen is a single chain glycoprotein with a molecular weight of 88,000 (Wallen, 1978). It is synthesised in the liver. The plasma concentration of plasminogen is around 200 ug/ml (Collen et al 1972). Its half-life in healthy men is 2.2 days (Bachmann, 1987). A rapid rate of synthesis is inferred from the restoration of normal plasma concentrations within 12-24 hours of depletion during thrombolytic therapy with streptokinase.

(b) Structure and Properties

Human plasminogen is a single-chain glycoprotein with a molecular weight of 88,000, containing approximately 2% carbohydrate (Wallen et al 1978; Sjoholm et al, 1973; Wiman and Wallen 1975b). The single-chain molecule has glutamic acid (Wallen and Wiman, 1972; Rickli and Cuendet, 1972) and asparagine (Robbins et al, 1967) as NH_2-terminal and C-terminal amino acids respectively. The plasminogen molecule consists of 790 amino acids, and it

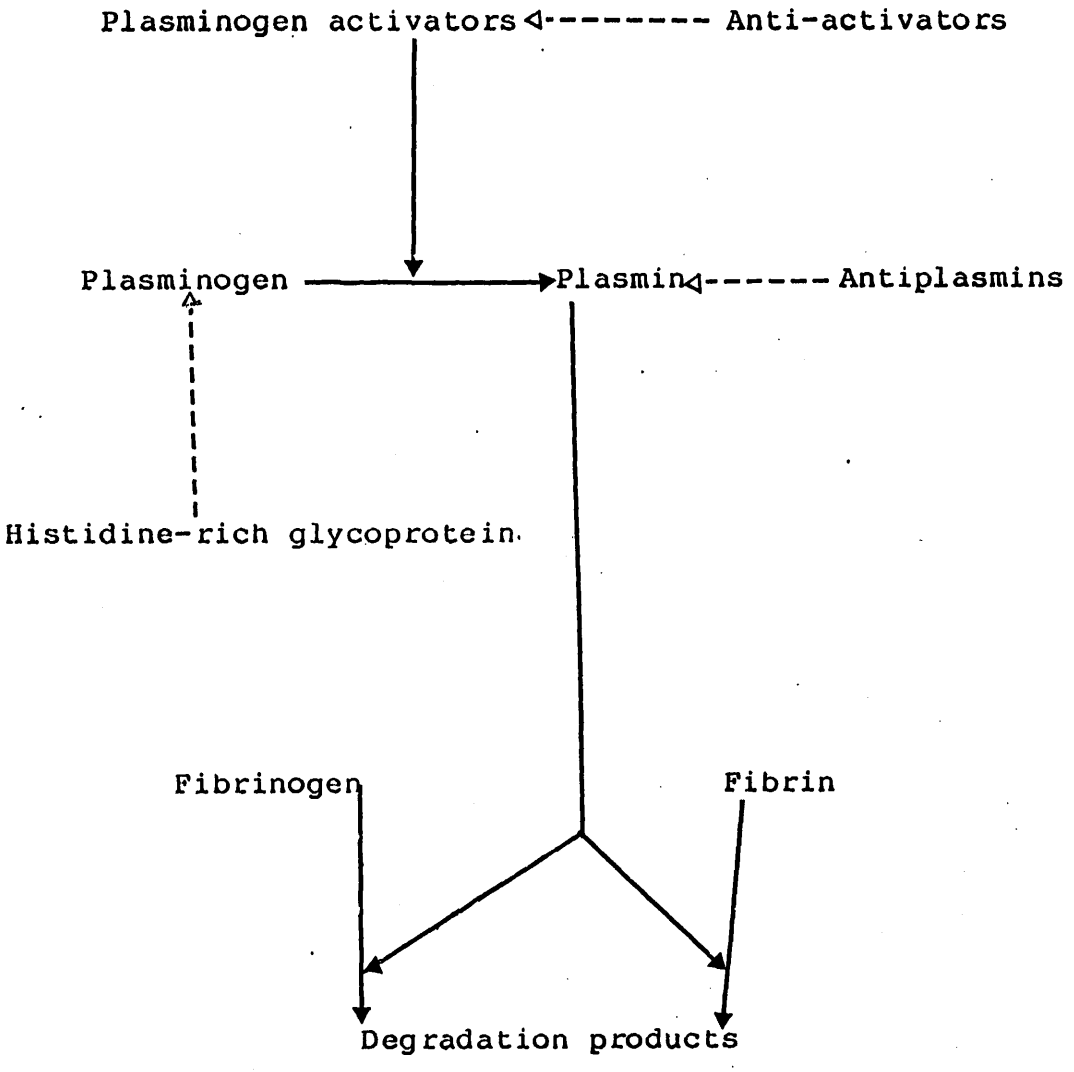

Figure 1.1 The Fibrinolytic System (--- = Inhibitory pathways)

it contains 24 disulfide bridges and 5 homologous triple loop structures known as kringles (Wiman, 1978; Sottrup-Jensen et al, 1978). Native plasminogen has NH_2-terminal glutamic acid ('Glu-plasminogen') but is easily converted by limited plasmic digestion to modified forms with NH_2-terminal lysine, valine or methionine (Wallen and Wiman, 1970; Wallen and Wiman, 1972) which are commonly designated 'lys-plasminogen'.

Affinity chromatography on lysine-sepharose using gradient elution with 6-aminohexanoic acid separates plasminogen into two fractions, type I and type II, in order of their elution from lysine-sepharose (Brockway and Castellino 1972). The first form appears more easily activated than the second and has a larger Stokes radius, as evidenced by gel filtration (Collen and De Maeyer 1975). This heterogeneity is apparently due to differences in the composition of the carbohydrate side chains of plasminogen (Hayes and Castellino, 1979a, 1979b, 1979c). Type I contains both a glucosamine-based oligosaccaride chain on Asp 288 and a galactosamine-based carbohydrate chain on Thr 345, while type II has only the latter. Each of these two fractions can be separated in 6 isoelectric forms with differences in sialic acid content. It has been suggested that these carbohydrate side chains might play a role in the interaction between plasminogen and α2-antiplasmin or fibrinogen (Lijnen et al, 1981; Bachmann, 1987).

(c) Lysine-binding Sites

Structures in plasminogen which specifically bind omega amino-acids such as lysine and 6-aminohexanoic acid are termed the lysine-binding sites. The plasminogen molecule contains 5 of these lysine-binding sites: one binding site with a high affinity for 6-aminohexanoic acid on kringle one and about 4 with low affinity on kringles 2-5 (Markus et al 1978; Bachmann, 1987). These lysine-binding sites are located in the plasmin A-chain (Rickli and Otavsky, 1975).

Plasminogen can specifically bind to fibrin through its lysine-binding sites. It has been found in a purified system (Thorsen, 1975) and in plasma (Rakoczi et al, 1978) that Lys-plasminogen has a higher affinity for fibrin than the intact Glu-plasminogen. The presence of 6-aminohexanoic acid abolished the adsorption of plasminogen to fibrin both in the purified system (Thorsen, 1975) and in plasma (Rakoczi et al, 1978). Thus it is concluded that one of the functions of the lysine-binding sites, and mainly of the high affinity lysine-binding site (Hoylaerts et al, 1981), in plasminogen is to mediate its interaction with fibrin.

As will be discussed below, the lysine-binding sites of plasminogen also mediate its interaction with α_2-antiplasmin, which therefore is a control mechanism in fibrinolysis.

1.6.3 Plasminogen Activators

It has been proposed that endogenous activation of

plasminogen can take place by two pathways, one extrinsic and the other intrinsic. In intrinsic plasminogen activation, all the components involved are present in precursor form in the blood. The extrinsic plasminogen activation system involves the release of tissue plasminogen activator (tPA) into the blood. From the physiological standpoint, the most important source of tPA is the vascular endothelium. The endothelial cells have the capacity to release tPA in response to some stimuli (stress, exercise, venous occlusion, catecholamines, and vasopressin and its analogues) and in accordance with the continuation of the stimulus are able to synthesize further tPA as required (Todd, 1959; Pandofi et al, 1967; Cash, 1975; Bachmann, 1987). Venous occlusion and the vasopressin analogue, desmopressin, have been used as stimulation tests to measure the "fibrinolytic capacity" of subjects and patients (Bachmann, 1987).

a) Tissue-type Plasminogen Activator (tPA), was recognized to exist in many organs, tissues and secretions over 20 years ago (Astrup, 1966). Highly purified preparations were obtained from pig heart (Rickli and Zaugg, 1970; Cole and Bachmann, 1977; Wallen et al, 1978); human uterus (Rijken et al, 1979) and cultured human melanoma cells (Rijken and Collen, 1981). tPA from the latter source was used to develop specific assays, and also allowed cloning and expression of the cDNA for tPA in Escherischia coli (Pennica et al, 1983).

The latter process has been used to produce tPA in sufficient quantities for clinical trials of thrombolytic therapy, e.g. in myocardial infarction (de Bono, 1987). The complete amino-acid sequence of tPA structure has also been defined (Bachmann, 1987).

tPA has a molecular weight of 68,000, and exists at a very low basal concentration in plasma (5 ug/ml; Bachmann, 1987). The molecule has two kringles, one of which has a lysine-binding site which gives it a high affinity for fibrin. Fibrin binds tPA and Glu-plasminogen, allowing a very efficient activation of plasmin on the fibrin surface. Free tPA in plasma is rapidly bound by plasminogen activator inhibitor type I (PAI-1). These mechanisms therefore control extrinsic fibrinolysis and tend to limit its action to fibrin (Bachmann, 1987).

(b) Urinary-type plasminogen activator

The intrinsic system of plasminogen activation involves the activation of the contact system (coagulation factor XII, high molecular weight kininogen, and prekallikrein) to form kallikrein, which then converts circulating single-chain urinary-type plasminogen activator (scuPA, also called pro-urokinase) to two-chain urinary type plasminogen activator (tcuPA, also called urokinase). As with tPA, the amino-acid sequence of uPA and its cDNA nucleotide sequence have been determined, and it has been produced in sufficient quantities for clinical trials of thrombolytic therapy.

uPA has a molecular weight of 54,000, and the basal plasma level is 2-20 ug/ml (Bachmann, 1987). As its name suggests, urokinase was first identified in urine (Sahli, 1885; Macfarlane and Pilling, 1947; Williams 1951; Sobel et al, 1952). It was first isolated from human urine or cultured embryonic kidney cells (Bernik and Kwaan, 1967, 1969; Kucinski et al, 1968).

c) Streptokinase

This exogenous plasminogen activator is a non-enzyme protein. It is produced by the Lancefield group C strains of B-haemolytic streptococci, and activates the fibrinolytic system indirectly. Streptokinase complexes with plasminogen and thereby converts the inactive pre-enzyme into an efficient plasminogen activator. The properties and mechanisms of action of streptokinase have reviewed (Brogen et al, 1973; de Bono, 1987). In recent years thrombolytic therapy with streptokinase has been shown to reduce mortality in acute myocardial infarction, and this has reawakened clinical interest in fibrinolysis (de Bono, 1987).

1.6.4 Inhibitors of Fibrinolysis

The fibrinolytic inhibitors are of two main types: those which inhibit plasmin (plasmin inhibitors, antiplasmins) and those which inhibit plasminogen activation (antiactivators). Many substances have been shown to possess antiplasmin and anti-activator activity, including soy-bean trypsin inhibitor, amino acids such as lysine, 6-aminohexanoic acid, tranexamic acid, and

aprotinin. The last three compounds have been used therapeutically.

a) Endogenous Inhibitors of Plasmin

Platelets and mesothelial cells contain antiplasmins, but these inhibitors are poorly characterised. There are at least 5 well defined proteins which inhibit plasmin in a purified system - namely α_2 macroglobulin, α_1-antitrypsin, inter-α-trypsin inhibitor, antithrombin III-heparin complex, and Cl-esterase inhibitor. The most important physiological inhibitor of plasmin formed in blood, is however a relatively recently described plasma protein called α_2-antiplasmin (Hedner and Abilgaard, 1978). This inhibitor was independently identified by three groups (Collen 1976; Moroi and Aoki, 1976; Mullertz, 1976). Upon activation of plasminogen in plasma, the formed plasmin is preferentially bound to α_2-antiplasmin. Only upon complete activation of plasminogen (concentration in plasma approximately 1.5 umol/l) is the excess plasmin neutralised by α_2-macroglobulin. In the presence of normal concentrations of these two inhibitors, the other plasma protease inhibitors do not play any role in the inactivation of plasmin.

α_2-antiplasmin

α_2-antiplasmin, the main physiological plasmin inhibitor in plasma, is a single chain glycoprotein of molecular weight 70,000 containing approximately 13% carbohydrate (Moroi and Aoki, 1976; Wiman and Collen,

1977). The concentration of α_2-antiplasmin in pooled normal plasma is approximately 1 mM (Moroi and Aoki, 1976; Mullertz and Clemmensen, 1976; Wiman and Collen, 1977). The inhibitor is immunochemically different from the other known plasma protease inhibitors.

α2anti-plasmin forms a very stable 1:1 stoichiometric complex with plasmin which is devoid of protease or esterase activity (Moroi and Aoki, 1976; Mullertz, 1976; Wiman and Collen, 1977). The physiological role of α_2-antiplasmin as an inhibitor of proteases other than plasmin seems negligible (Edy and Collen, 1977; Ohlsson and Collen, 1977).

A structural analysis of the plasmin-α-antiplasmin complex suggested that the stable complex is formed by a plasmin attachment at a specific leucyl-methoionyl peptide bond in the COOH-terminal portion of the inhibitor. A strong properly covalent bond is formed between the Carbonyl group of this specific leucyl residue in the inhibitor (Wiman and Collen, 1979). The turnover of 125I-labelled α_2-antiplasmin was studied in control subjects and in patients during thrombolytic therapy (Wiman and Collen 1979). In the control group α_2-antiplasmin had a plasma half life of 2.64 $\pm$ 0.32 days and fractional catabolic rate of 0.53 $\pm$ 0.09 of the plasma pool per day. During thrombolytic therapy the half life shortened to approximately 0.5 day as a result of formation of plasmin-α_2-antiplasmin complex. The long half-life of the plasmin- α_2-antiplasmin complex was

confirmed by studying the turnover of the purified complex both before and during thrombolytic therapy in patients with thrombotic disease.

The normal concentration of α_2-antiplasmin in pooled normal plasma is approximately 1 umol/l (Moroi and Aoki, 1976; Mullertz, 1976; Wiman and Collen, 1977).

The concentration may decrease to below 30% in severe cases of liver disease or intravascular coagulation (Aoki, 1979) but is normal in patients with cardiovascular, renal or malignant diseases. The inhibitor is temporarily exhausted during thrombolytic therapy with streptokinase (Teger-Nilsson et al, 1977) when measured enzymatically. Residual antigen may however be found immunologically representing complexed or degraded inhibitor or both. α_2-antiplasmin is a weak acute phase reactant (Teger-Nilsson, 1977). Possibly some of the α_2-antiplasmin in plasma is inactive (Mullertz and Clemmensen, 1976).

Histidine-rich Glycoprotein

This glycoprotein of M.Wt. 75,000 is a competitive inhibitor of plasminogen, reversibly binding to its high-affinity lysine-binding site. In plasma, histidine-rich glycoprotein complexes with about 50% of plasminogen, reducing its availability for binding to fibrin. However its inhibition of this interaction is less effective than that of α_2-antiplasmin (Bachmann, 1987).

b) Plasminogen Activator Inhibitors

These have only been characterised in the last 5 years (Bachmann, 1987). Plasminogen activator inhibitor type I (PAI-1) is the major inhibitor of tPA and uPA in plasma, and has a molecular weight of 54,000. It is secreted by endothelial cells, platelets and certain tumour cells. It is a strong acute-phase reactant protein, and high plasma levels are formed after trauma or surgery and in many disease states such as infections, coronary artery disease, and recurrent venous thromboembolism (Bachmann, 1987; Kruithof, 1988). Plasminogen activator inhibitor type 2 (PAI-2) comes from the placenta, leucocytes and monocytes, and high plasma levels are observed during pregnancy. A few other antiactivators have recently been described (Bachmann, 1987).

1.6.5 Physiology of Fibrinolysis

The physiological roles of the fibrinolytic system appear to be (a) maintaining the vascular system free of thrombotic occlusions; (b) maintaining the different exocrine ducts and the urinary tract free from fibrin deposits; and (c) participating in tissue repair.

In the circulation, levels of tPA and plasmin are both very low. Even if the level of tPA rises markedly (e.g. after strenuous exercise, or injection of adrenaline or desmopressin), little free plasmin is formed because it is rapidly inhibited by α_2-antiplasmin. However, if fibrin is present, tPA and Glu-plasminogen

form a complex which facilitates the conversion of clot-bound plasminogen to plasmin. The clot-bound plasmin is partially protected from the action of circulating α_2 antiplasmin, and therefore digests the fibrin. This in turn exposes additional binding sites for the fixation of Glu-plasminogen to fibrin (Bachmann, 1987).

Several factors may modulate this process. PAI-1 is released from platelets and endothelial cells and may stabilize thrombi by inactivating local tPA. Protein C is a plasma protein which inhibits thrombin and hence is an endogenous anticoagulant; it also complexes with PAI-1, thereby reducing the inhibition of fibrinolysis by PAI-1 (Bachmann, 1987).

Degradation of Fibrinogen and Fibrin

When the fibrinolytic system is activated by fibrin formation, plasmin degrades insoluble fibrin to soluble fibrin degradation products (FDP). Plasmin may also degrade circulating fibrinogen to soluble fibrinogen degradation products. The products of fibrinogen and fibrin differ (Niewenhuizen, 1987). When plasmin digests fibrinogen, the first fragments formed are X fragments from the Aα chain of fibrinogen and fragment 1-42 from the Bβ chain. Subsequently, X fragments are cleaved asymmetrically to give one Y fragment and one D fragment; then Y fragments are cleaved to give another D fragment and one E fragment. Fibrin differs from fibrinogen in that it has been cross-linked by factor

XIII, hence its degradation products include cross-linked fragments such as X-oligomers and D-dimer (Niewenhuizen, 1987).

1.6.6 Measurement of the Fibrinolytic System in Plasma (Lowe and Prentice, 1980)

a) - Plasminogen can be measured in plasma either immunologically or by its activity on various substrates (e.g. chromogenic substrates) after activation (e.g. by streptokinase). α_2-antiplasmin can also be measured either immunologically or by its activity against activated plasminogen.

b) - Plasminogen activators can be measured in blood or plasma after removal of circulating inhibitors, which is usually performed by dilution and/or acidification to precipitate the euglobulin fraction, which contains plasminogen and plasminogen activators. Global plasminogen activators are usually measured by the dilute whole blood clot lysis time, the euglobulin clot lysis time, or the lysis area produced by drops of euglobulin fraction applied to fibrin plates (fibrin plate lysis area, FPLA). The latter method has the advantage over clot lysis times in that it is not affected by the patients' plasminogen or fibrinogen levels. Recently, specific assays of tPA activity and antigen have become available, as have assays of PAI-1 activity and antigen. Because PAI-1 is present in the euglobulin fraction, it has recently been appreciated that it is a major determinant of apparent plasminogen activator activity

(Kruithof, 1988).

Fibrin degradation products (FDP) were traditionally measured by estimation of fibrinogen-related antigen in serum, which contains non-clottable FDP (e.g. D and E fragments) but not clottable fibrinogen or FDP (e.g. X and Y fragments). A commonly-used assay is the Wellcome FDP kit which uses the tanned red cell haemagglutination inhibition immunoassay method. A rapid, semi-quantitative latex test is also available (Thrombo-Wellcotest) (Lowe and Prentice, 1980). In recent years, the use of such serum assays has been criticised, because of false high levels due to formation of FDP during clotting of the plasma sample to obtain serum; false low levels due to trapping of FDP in the clot; and insensitivity (Niewenhuizen, 1987). The recent production of monoclonal antibodies against degradation products of both fibrinogen and cross-linked fibrin has allowed sensitive assays to be performed in plasma, as well as the differentiation of fibrinogen degradation from degradation of cross-linked fibrin (Niewenhuizen, 1987).

1.6.7 Pathological Fibrinolysis

In arterial and venous thrombosis, the formation of fibrin results in activation of the fibrinolytic system as shown by raised levels of both FDP and cross-linked fibrin degradation products (Whitaker et al, 1987). Some patients with premature or recurrent thrombosis have a low "fibrinolytic capacity", due either to low release of

tPA from vascular endothelium, or to high levels of PAI-1 (Bachmann, 1987). Marked systemic activation of fibrinolysis occurs in disseminated intravascular coagulation and following streptokinase infusion, as shown by depletion of fibrinogen, plasminogen and 2 antiplasmin, and high levels of both FDP and cross-linked fibrin degradation products (Whitaker et al, 1987). A generalised bleeding tendency occurs in such patients.

Localized bleeding may also be influenced by localized fibrinolysis. This is suggested by the value of fibrinolytic inhibitor drugs (e.g. epsilon aminocaproic acid, tranexamic acid) in several clinical situations. These include menorrhagia, subarachnoid haemorrhage, bleeding after dental extraction in haemophilia, and gastro-intestinal bleeding (Davidson, 1980; Verstraete, 1987, Henry and O'Connell, 1989). The role of fibrinolysis in upper gastrointestinal bleeding is the subject of the present thesis, and is reviewed in the next section.

1.7 The Upper Gastrointestinal Tract and Fibrinolysis

The fibrinolytic system acts as a defence system in several different ways. It helps keep the vascular system free of thrombotic occlusions, and in a similar manner it keeps different exocrine ducts and the urinary tract free from fibrin deposits. In addition, local fibrinolysis probably plays an important role in tissue repair. The presence of fibrinolytic activity in the gastro-intestinal tract (Poller, 1979), is therefore not

surprising.

The possibility that excess fibrinolysis can be an aggravating factor in peptic ulcer bleeding has been suggested. Cox et al (1967) studied gastric fibrinolytic activity by taking blood directly from gastric and peripheral blood vessels in patients with a peptic ulcer and in a parallel control group. The parallel series of patients studied over the same period was a mixed group who had undergone an upper abdominal operation, and from whom blood could therefore be obtained from similar gastric vessels. These patients had a normal stomach and duodenum and had had an operation for gall bladder stones. Free plasmin was found in the gastric vein, plasminogen activator in the mucosa, and fibrinolytic activity in the gastric juice. Eleven out of 13 patients with a peptic ulcer had free plasmin in their gastric vein, but this activity was present in only 5 out of 13 patients from the parallel group. The conclusion was that fibrinolysis in the stomach and duodenum was greater in these patients who had a peptic ulcer than those who had not (Cox et al, 1967). However this was not confirmed by O'Brien et al (1979) who showed that the fibrinolytic activity of blood draining from the stomach of patients with gastroduodenal disease was comparable with the fibrinolytic activity of blood draining from normal stomach. Gastric venous blood from normal and diseased stomachs contains greater amount of plasminogen activator than simultaneously

sampled systemic venous blood. However, gastric venous fibrinolytic activity does not differ between the normal and diseased stomachs.

In a further study, Cox et al (1969) looked for evidence of the release of gastric fibrinolytic activity into peripheral blood after trauma on the stomach. The study was performed on patients who had undergone laparotomy. The first step in the procedure was to pick up the stomach and to exert pressure on the anterior and posterior walls by compression between the fingers and thumb, the finger anterior to the anterior wall and the thumb posterior to the posterior wall from the pylorus to the fundus. This took 30 seconds. Gastric venous specimens were taken immediately after this gastric compression, all the specimens being obtained within 5 minutes. The aim in each case was to take 3 specimens from each patient. In all patients 3 specimens of peripheral venous blood were taken at 10 minute intervals; the first peripheral venous specimen was taken at the same time as the first gastric vein specimen. Fibrinolytic activity in peripheral venous blood and its progressive rise following gastric compression, was observed. It is reasonable to assume that the lytic activity released from the stomach would have reached the arm vein in sufficient concentration within 10 minutes.

Eras and colleagues (1970) studied plasmin activator in the stomach, using samples obtained at surgery from

patients operated on for duodenal ulcer disease. In one patient, additional specimens were obtained from the gastric fundus, antrum, and duodenum. These specimens were taken from an area which appeared grossly normal. Plasminogen activator activity was localized in gastric and duodenal tissue using histological techniques. Plasminogen activator activity was then studied in relation to mucosal and submucosal blood vessels in selected tissues. Proteolytic activity was identified in the surface epithelium of the stomach and duodenum.

Kondo et al (1975) studied the distribution of the fibrinolytic activity in gastric ulcers. In humans gastric ulcer mucosa was surgically removed from patients with gastric or duodenal ulcers and was examined for the distribution of fibrinolytic activity. Fibrinolysis was found in cases with an acute episode of gastric ulcer. The activity was seen to be localized around the ulcer as well as the eroded lesion. In the stomach of those patients developing massive haemorrhage after a long history of gastric ulcer, tissue fibrinolysis was detectable only in those parts close to the ulcer.

A further study reported that high concentrations of systemic serum F.D.P. were also associated with recent gastrointestinal haemorrhage (Poller and Thomson, 1969). Subsequently, Poller (1979) studied patients with haematemesis and melaena, and compared them with patients bleeding in other sites, most of whom were suffering from menorrhagia or having operations. The concentrations of

serum F.D.P. in haematemesis and melaena patients were significantly higher than in other groups, suggesting increased systemic fibrinolysis (Fig 1.2).

Because this increased fibrinolysis may play a role in acute upper gastrointestinal bleeding, several trials of fibrinolytic inhibitor drugs have been performed in this clinical situation as noted in Section 1.3.

Taken together, these studies suggest that tranexamic acid may be beneficial in acute upper gastrointestinal bleeding (Henry and Collins 1988), although it does not yet appear to have become routine therapy for this condition. They also support the hypothesis that excessive fibrinolysis may promote bleeding in this situation.

One way to test this hypothesis is to measure fibrinolytic activity in patients hospitalized with acute upper gastrointestinal bleeding, and to relate such measures to the outcome (i.e. further bleeding requiring blood transfusion or surgery, or resulting in death). Such a natural history study has not been performed. The aim of the first study reported in this thesis was to relate certain tests of systemic fibrinolysis to such outcomes in a large series (over 100) of patients with acute upper gastrointestinal bleeding. The tests chosen were (a) serum FDP, measured quantitatively by the Wellcome FDP kit (Lowe and Prentice, 1980). Serum FDP have been shown to be elevated in acute upper gastrointestinal bleeding although their prognostic

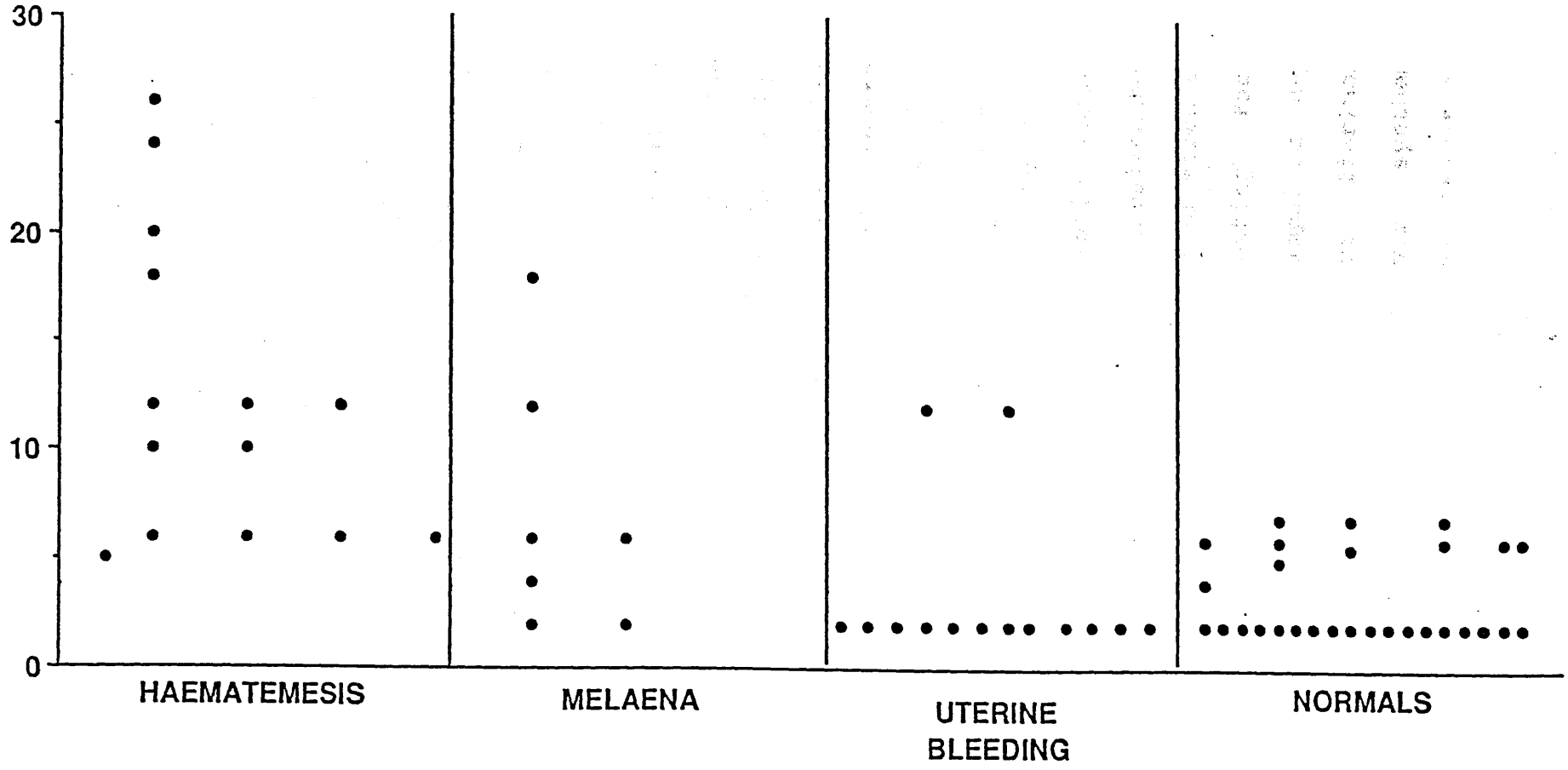

Fig 1.2 F D P measured by TRCHII in patients with haematemisis and other groups (Poller 1979)

significance has not been explored (Poller, 1979) and (b) total plasminogen activators, measured quantitatively by the fibrin plate lysis area test (Lowe and Prentice, 1980), which had been shown to be increased in systemic blood by local compression of the stomach (Cox et al, 1969). Following the demonstration that serum FDP were of prognostic value, retrospective testing on remaining serum samples was performed to determine (c) whether the simple, semiquantitative latex serum FDP test (Thrombo-Wellcotest, Wellcome) was of prognostic value; and (d) whether plasma cross-linked fibrin degradation products (Dimertest ELISA, AGEN) were of prognostic value. These tests were also related to the site of bleeding and the bleeding lesion, as shown by endoscopy. No previous study has investigated these associations.

1.8 Oesophageal Varices and Fibrinolysis

Excessive fibrinolysis may be especially important in the pathogenesis of one particular type of upper gastrointestinal bleeding i.e. bleeding from oesophageal varices in patients with hepatic cirrhosis. Such patients have several defects in haemostasis (Brozovic, 1987). These include thrombocytopenia, impaired hepatic synthesis of clotting factors; synthesis of structurally abnormal fibrinogen and other clotting factors; disseminated intravascular coagulation; impaired hepatic clearance mechanisms; and abnormal fibrinolysis (Brozovic, 1987). The normal liver synthesises plasminogen and α_2- antiplasmin, and is the site of

clearance for plasma plasminogen activators, FDP, and other breakdown products which may activate fibrinolysis (Brozovic, 1987). In cirrhosis, low plasminogen levels might theoretically decrease fibrinolytic potential; however there is usually enhanced fibrinolysis as shown by decreased $\alpha 2$ antiplasmin and increased plasminogen activator levels due to impaired clearance (Brozovic, 1987), and increased serum FDP (Bertaglia et al, 1983).

Bertaglia et al (1983) found higher levels of serum FDP among patients with cirrhosis in those who had bleeding oesphageal varices.

In the second study reported in this thesis, the aim was to study further the role of fibrinolysis in bleeding from oesophageal varices by measuring systemic fibrinolysis (again by fibrin plate lysis area and serum FDP tests) in patients with oesophageal varices, and relate these tests to prognosis, as well as to the Child's grade of severity.

1.9 Conclusions and Aims of Study

(1) Acute upper gastrointestinal bleeding remains a common medical emergency with a significant mortality, requiring further research into its pathogenesis and treatment.

(2) Excessive fibrinolysis may play a role in pathogenesis, however no prospective studies of fibrinolysis have been performed to test this hypothesis.

(3) The aims of the studies reported in this thesis were

as follows:

(a) to measure systemic fibrinolytic activity (total plasma plasminogen activators by the fibrin plate assay, and serum fibrin degradation products) in a large series of patients hospitalised with acute upper gastrointestinal bleeding, and to relate these tests to outcome as well as to site and cause of bleeding;
and (b) to measure these tests in a series of patients with oesophageal varices, and to relate them to presence or absence of bleeding, as well as to the Child's grade of severity.

CHAPTER II

METHODOLOGY

2.1.1 Collection of blood samples

Nine mls of blood was added to plastic tubes which contained 1 ml sodium citrate (3.2%) which had been precooled on melting ice. 2 ml of the sample was added to the FDP tubes at room temperature. Since there is a diurnal variation in plasminogen activator activity, the time was standardised (9-10 a.m.) The drip arm was avoided to prevent dilution of blood. Minimal venous stasis was used to prevent release of tPA. Patients had to be resting and fasting (activity and food release tPA). The citrated samples were immediately refrigerated (4 $^{\circ}$C), centrifuged for 20 minutes (2000 r.p.m.) and the plasma underwent snap-freezing. FDP samples were kept on the bench to allow clot retraction. Therefore the tubes were not shaken. They were centrifuged for 20 minutes at 2000 r.p.m. and then left at -45°C to snap-freeze.

2.1.2 Plasma Plasminogen Activators - Fibrin Plate Lysis Method

Plasminogen activators can be assayed by measuring the mean diameter of the area of lysis around a small volume of plasma euglobulin fraction placed on a plate of fibrin (which is contaminated with plasminogen) after incubation at 37 C. A standard preparation of fibrin avoids the variation in clot lysis time assays due to variable fibrinogen and plasminogen content of the test samples. A further advantage of fibrin plate assay is

that plasma samples may be stored frozen (-20 °C) for assay at a later date, then compared simultaneously, whereas clot lysis assays must be performed immediately. The method described is that of Kluft, Brakman and Veldhuyzen-Stolk (1976), modified by use of Kabi human fibrinogen and Owren's buffer. A device for accurate reading of the diameters of the areas of lysis has been described (Haverkate, 1972). The sensitivity of the plates is checked by testing standard preparations of streptokinase.

2.1.3 Reagents Required

a) Fibrinogen

One gram fibrinogen grade L from Kabi dissolved in 100 ml tris-Cl buffer. Stored frozen in -70 °C freezer in 10 ml aliquots.

b) Thrombin

Fibriquik thrombin reagent from General Diagnostics. Dissolvedin 6 ml Tris-HCl buffer. Stored frozen in -70 °C freezer in 3 ml aliquots.

c) Streptokinase

Streptokinase 100,000 units Topical Varidose from Lederle. Dissolved in 50 ml Tris-HCl buffer, stored frozen in -70 °C freezer in 0.5 ml aliquots.

d) Tris-HC1 buffer

6.06 gm Trizma dissolved in 250 m l distilled water. 400 ml distilled water added to 1.236 ml con. HCl. Add this to the Trizma solutions. Made up to one litre by adding 350 ml distilled water. Adjust pH to 7.4 by

adding more HCl.

e) Owren's Buffer

m HCl	43ml
Na diethylbarbitone	11.756 gm
NaCl	14.7 gm

Heated to 200 ^{o}C and add water to 2 litres.

Adjust pH to 7.4 by adding more HCl.

f) Plastic Dishes

Flat plastic petri dishes (size approximately 100 x 15 mm).

2.1.4 Method for Making Plates

To 90 ml Tris-HCl 10 mls (one aliquot) of fibrinogen was added and mixed in a measuring cylinder. 20 ml of mixture was applied to each plate. The plate was mixed and 0.5 ml thrombin was added. Plates were allowed to sit for at least 15 minutes, before moving. Streptokinase was diluted in 1 ml of tris buffer.

2.1.5 Sample Preparation

9 ml of distilled water was added to a conical test tube. 1 ml of sample was added and the tube put on ice. pH was adjusted to 5.9 using 1% acetic acid and then put in a cold room for 15 minutes to allow the euglobulin fraction to precipitate out. Spinning for five minutes (2000 r.p.m.) in a cold centrifuge to form a button of euglobulin followed. The supernatant was decanted, the euglobulin residue was inverted and left to dry for about 10 minutes.

Reconstitution took place in 1 ml Owren's buffer, 20

ul of sample was added in triplicate and 20 ul of streptokinase to the plate. Incubation at 37 °C for 18 hours followed.

The diameter of the lysis area was read in 2 dimensions on special charts provided (Fig. 2.1). The diameters for the triplicate samples were averaged. The streptokinase control usually read 19 mm. (Fig. 2.2). If it did not then the value of the mean diameter was corrected using the following formula:-

$$\frac{\text{Stand.streptokinase (19mm) x measured mean diameter}}{\text{measured streptokinase diameter}}$$

The fibrin plate lysis area was obtained by the formula,

$$\pi r^2$$

2.2 Serum Fibrin Degradation Products

(Tanned red cell Haemagglutination Inhibition Immunoassay; Merskey et al 1969; Walker et al 1976)

2.2.1 Principle

Red blood cells are tanned then coated with human fibrinogen. These sensitised cells are sensitive indicators of the presence of antibodies to fibrinogen-related antigen (FR-antigen), which cause agglutination. Prior incubation of antifibrinogen serum with a test serum sample containing a sufficient quantity of FR-antigen inhibits this haemagglutination. Serial dilutions of the test sample are incubated with antifibrinogen serum, prior to incubation with the fibrinogen-sensitised red cells. The lowest concentration

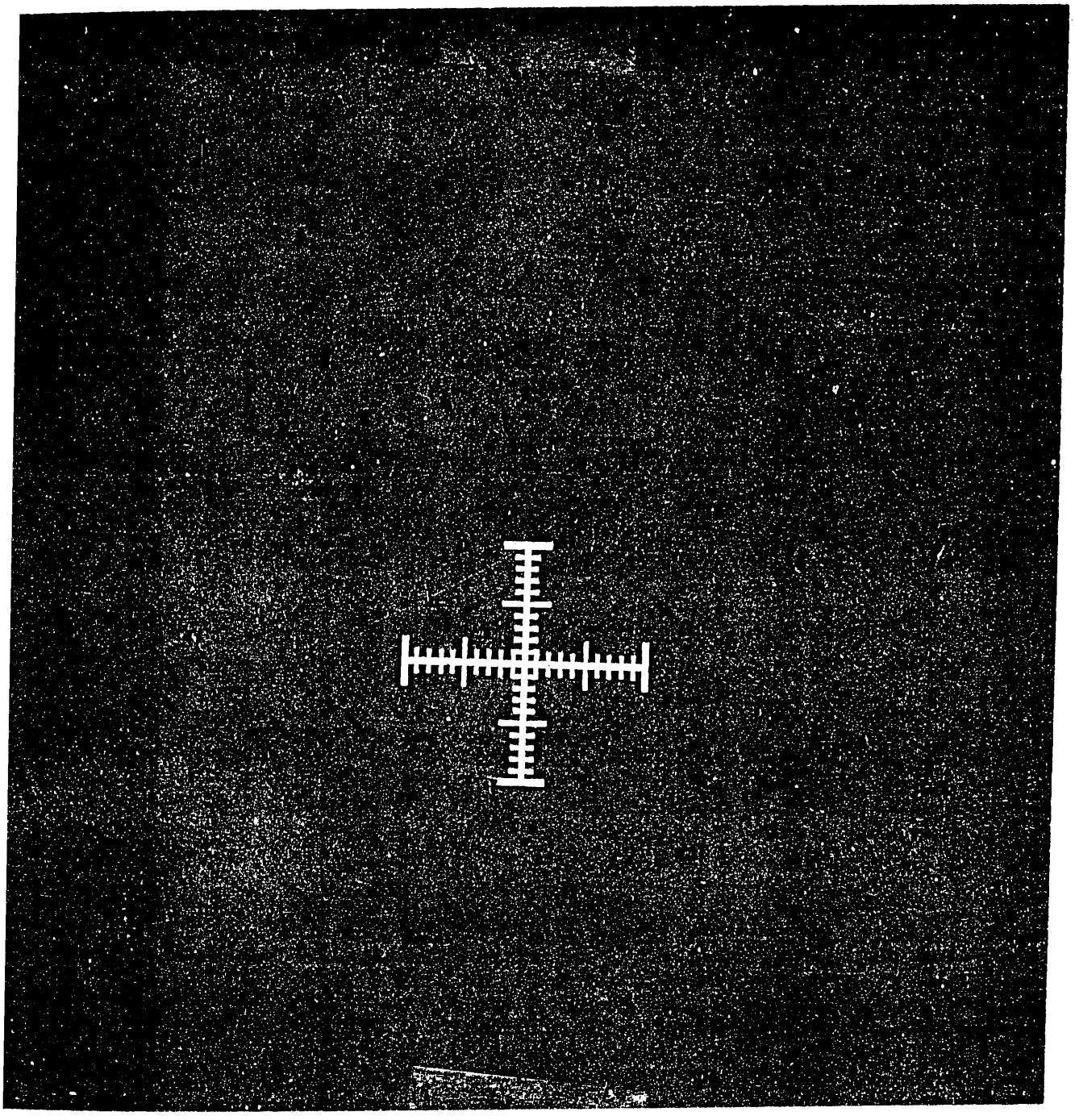

Figure 2.1 Chart illustrating method of measurement of F.P.L.A. in two planes; each point on the axes represents 2 mm

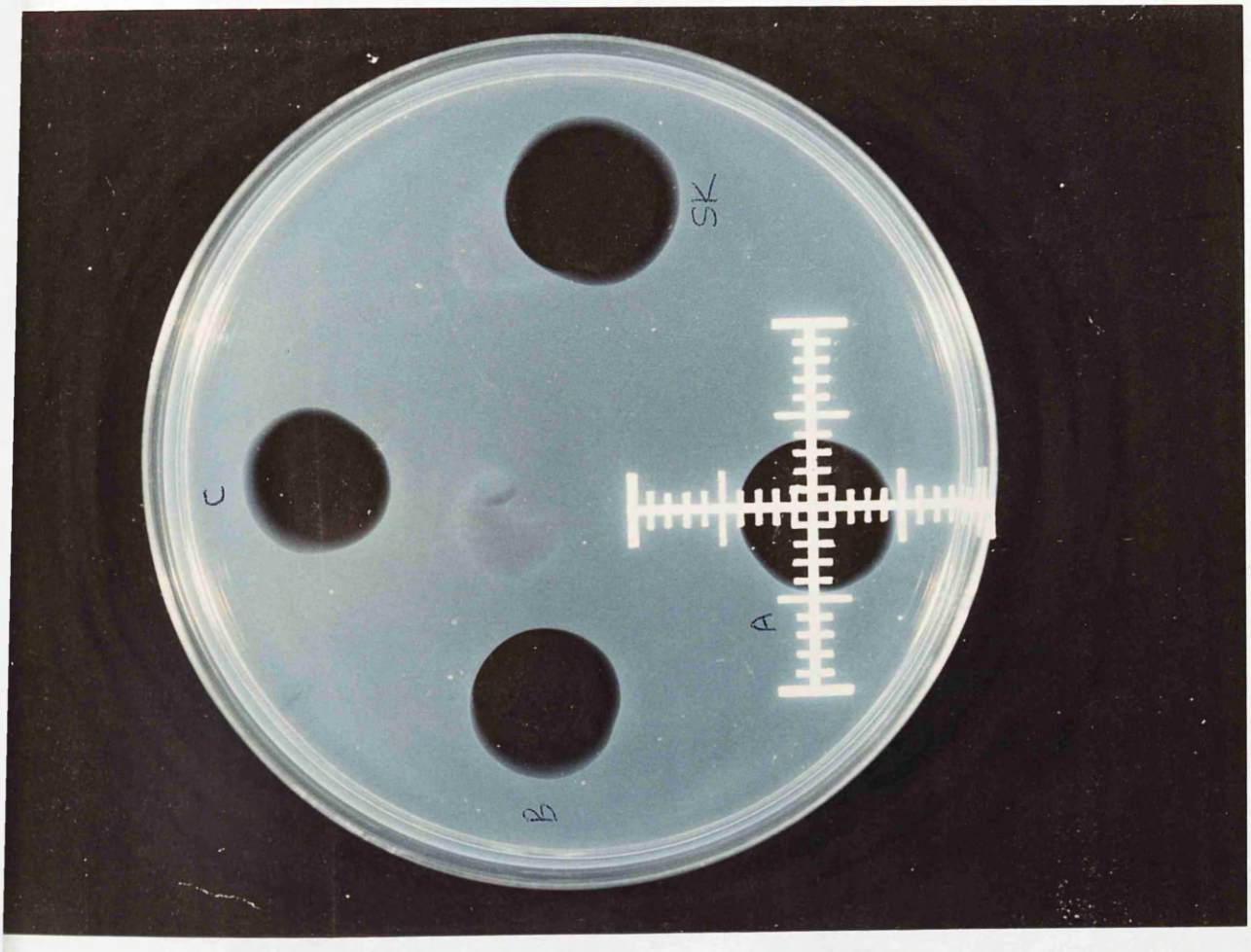

Figure 2.2 A,B,C represents a patient sample in triplicate in 3 areas: The measured mean diameter is corrected using standard streptokinase (usually 19 mm) as the control (sk).

of the test sample to cause complete inhibition of haemagglutination is read and the concentration of the FR-antigen in the test sample is quantified by comparison with a serially-diluted fibrinogen standard.

Reagents These are provided in the Wellcome FDP kit (Wellcome, Beckenham, Kent)

2.2.2 Sample Preparation

Two ml of venous blood was added to a special tube containing thrombin and enzyme inhibitor, incubated at room temperature, then centrifuged to obtain serum (i.e. removal of cross-reacting fibrinogen). Fibrinogen/fibrin related antigen was then measured by the tanned red cell haemagglutination immunoassay.

2.2.3 Reagents required

a) Citrate Buffer

Ten ml of five times concentrated buffer, pH 6.4 containing when diluted 0.1 per cent sodium azide and 0.4 per cent horse serum (pre-absorbed with sheep red cells).

The composition of the working-strength buffer was as follows:

Na_2HPO_4 (anhydrous)	0.186g
KH_2PO_4 (anhydrous)	0.332g
Trisodium citrate	0.735g
Citric acid, 0.5M	sufficient to adjust pH
Sodium azide	0.05g
Horse serum (absorbed)	0.2ml
Distilled water to 50 ml	

b) Fibrinogen Sensitised Cells

The cells were gently centrifuged in a bench centrifuge and the supernatant removed with a pasteur pipette. 2.5 ml of working strength citrate buffer was added to the bottle. The cells were resuspended.

c) Anti-fibrinogen Serum

2.5 ml of working strength citrate buffer was added to a bottle of freeze-dried serum. The dissolved contents were mixed.

d) Fibrinogen Standard

0.5 ml of distilled water was accurately measured and added to the fibrinogen bottle. The contents were mixed to give a solution of 10 mg per ml fibrinogen.

e) Sheep Cells for Absorption

0.5 ml of suspended cells were added to a clean test tube. One tube was prepared for every sample of serum to be tested. Cells were washed in the tubes once with saline and the supernatant discarded leaving only freshly washed packed cells in each tube. Transfer 0.5 ml of clear serum to a tube cntaining the packed cells, mix and allow to stand at room temperature for 30 minutes. Centrifuge the tube and aspirate the clear serum.

f) Reading Plates and Pipettes

Microtiter apparatus (Flow Laboratories Ltd., Irvine, Scotland) was used. One microtiter 'V' plate contains 8 rows of 12 wells, sufficient for assay of 7 samples in parallel with one fibrinogen standard titration. 0.025 ml dropper pipettes are also supplied.

2.2.4 Assay

To each microtiter plate was added (Fig 2.3):

1) Row 1

(Standard and reagent controls). Using 0.025 ml dropper 1 drop of citrate buffer was added to each of wells 2 to 10 and 2 drops in well 11.

2) Row 2 - 8

(7 test serum sample). 1 drop citrate buffer was placed in each of wells 2 to 9 and in well 12. 3) One drop of reconstituted fibrinogen standard was added to wells 1 and 2 of row 1.

4) One drop of undiluted serum (F.D.P. assay) was added to wells 1, 2 and 12 in the row appropriate to the sample under test. The dropper was rinsed in buffer between each sample.

5) A 0.025 ml micro-diluter loop was used to prepare serial dilutions from well 2 to well 9 in each row, discarding a loopful of the final dilution after mixing in well 9.

6) Using a clean dropper, one drop of reconstituted antiserum was added to wells 1 to 10 in row 1 and to wells 1 to 9 of all other rows.

7) The contents of all wells was mixed by gently shaking the plate. The plate was covered and left on the bench for one hour.

8) Using a clean dropper one drop of freshly resuspended fibrinogen-sensitised cells was added to wells 1 to 11 of row 1 and wells 1 to 9 and well 12 in all other rows.

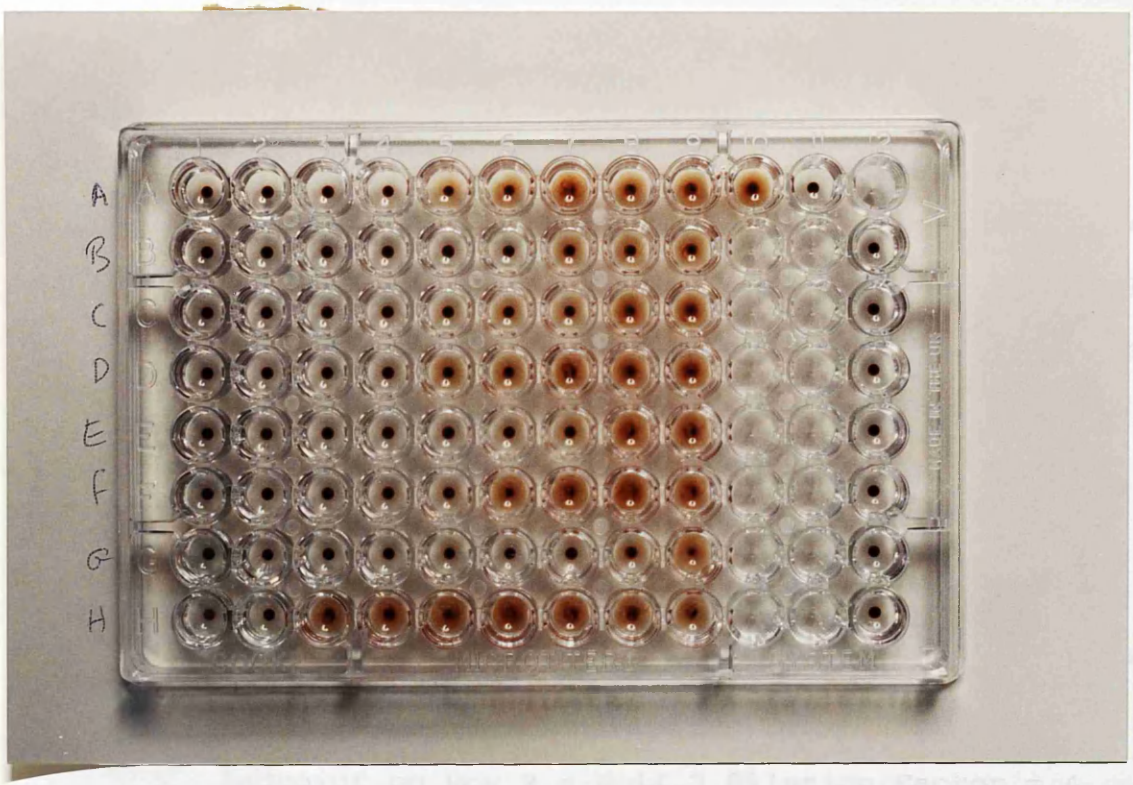

Figure 2.3 F.D.P. Kit: Endpoint in Row 1 = Well 4
Sensitivity of assay = 1.25 ug/ml
Endpoint in Row 6 = Well 3,
Dilution Factor = 4
Thus concentration of F.D.P. in serum tested in Row 6 is 4x1.25 = 5 ug/ml.

9) The contents of the wells were mixed again. The plate was covered and left on a bench (away from direct sunlight or vibration) for a minimum of two hours after which time results were read by inspection of the sedimented patterns in individual wells. The completed plate contained standard and samples as follows:

2.2.5 Calculation of Results

Row 1 To determine sensitivity of assay (fibrinogen concentration at end point).

1	2	3	4	5	6	7	8	9
10	5	2.5	1.25	0.63	0.32	0.16	0.08	0.04

Row 2 to 8 The end point of each sample was read. The dilution factor at endpoint was multiplied by sensitivity of assay to give concentration of serum F.D.P.

1	2	3	4	5	6	7	8	9
1	2	4	8	16	32	64	128	256

e.g. Endpoint in Row 1 = Well 4 sensitivity of assay = 1.25 ug/ml.

Endpoint on Row 2 = Well 3 Dilution Factor = 4

Thus concentration of F.D.P. in serum tested in Row 2 = 4 x 1.25 = 5 ug/ml

2.3 Rapid Latex-Screening Test (Thrombo Wellcotest)

The test is intended for the rapid semi-quantitative testing of human serum for the presence of fibrin/fibrinogen degradation products (F.D.P.).

It is a simple and rapid test which allows the routine investigation of all patients at special risk.

The test is designed as a slide agglutination method in which one drop of sample and one drop of latex suspension are mixed for a period of two minutes by gentle rocking. An agglutinated pattern at the end of the test period indicates the presence of at least 2 ug per ml F.D.P. in the sample under test. The test was performed retrospectively in this study to determine its utility in the prognosis of acute upper gastrointestinal bleeding.

2.3.1 Principle of the Test

Antisera are raised to highly purified preparations of human fibrinogen fragments D and E. After solid-phase absorption to remove antibodies to all other serum proteins, the specific antibody globulins are extracted and used to coat by absorption a suspension of latex particles in glycine saline buffer. The sensitivity of the latex reagent is adjusted so that in the presence of F.D.P. concentrations of 2 ug (fibrinogen equivalent) per ml or greater, the latex particles clump together giving macroscopic agglutination.

2.3.2 Reagents Required (Supplied in Thrombo-Wellcotest, Wellcome, Beckenham, Kent)

a) Latex Suspension

3 ml of a 0.75 per cent suspension of polystyrene latex particles coated with sheep anti-F.D.P. globulin glycine saline buffer containing 0.1 per cent sodium azide and 0.01 per cent thiomersal.

b) Positive Control Serum

1 ml of human serum diluted in glycine saline buffer

containing 0.1 per cent sodium azide to give an F.D.P. concentration of 5-10 ug per ml.

c) Negative Control Serum

1 ml of human serum diluted in glycine saline buffer containing 0.1 per cent sodium azide to give an F.D.P. concentration less than 2 ug per ml.

d) Sample Collection Tubes

20 glass tubes containing soya bean trypsin inhibitor (approximately 3600 NF units per tube) and bovine thrombin (20 NH units per tube) for the collection of two ml whole blood.

e) Glycine Saline Buffer

Two bottles each containing 25 ml of pH 8.2 buffer having the following composition:

Glycine	7.507 gram
NaCl	8.5 gram
NaOH M/5	Sufficient to adjust pH
Sodium azide	1.0 gram
Distilled water to 1.0 litre	

f) Disposable pipettes and mixing rods. Rubber bulb.

g) Test Slide:

One slide with 6 rings to test 3 samples or 2 samples with control.

2.3.3 Handling of Reagents

It is desirable to bring all reagents approximately to room temperature before use. The latex suspension was mixed thoroughly by rapidly inverting the bottle two or three times immediately prior to performing the test.

The control sera were provided diluted ready for use. No further preparation was necessary.

2.3.4 Specimen Collection

This was as for the quantitative FDP method.

2.3.5 Procedures for Assay of F.D.P.

(a) Using the graduated dropper provided with the bottle of buffer, 0.75 ml of glycine saline buffer was placed in a test tube.

(b) Using one of the disposable droppers with the bulb provided, five drops of the serum sample were added to the test tube.

(c) The contents of each test tube which now contain approximately 1.5 dilutions of serum were mixed. One drop from the test tube was transferred to the reaction slide.

(d) The latex suspension was mixed by rapidly inverting the bottle two or three times and then one drop of the suspension was added to each position on the slide.

(e) The slide was gently rocked to and fro for exactly two minutes while looking for macroscopic agglutination. The patterns obtained are clear cut and can easily be recognised under any normal conditions of lighting. The presence or absence of agglutination was determined immediately after rocking the slide for two minutes. If the reaction was allowed to continue for longer, false results might occur due to drying

out of the mixture on the slide.

2.3.6 Reading of Results

The kit is adjusted to a sensitivity of 2 ug per ml. An agglutinated pattern in either position on the slide indicates the presence of F.D.P. at a final concentration of greater than 2 ug per ml in the serum dilution. A positive result in position 1 indicated that F.D.P. were present in the original serum at a concentration in excess of 10 ug. per ml. (Fig. 2.4).

2.4 Measurement of Crosslinked Fibrin Degradation Products with an Immunoassay using Monoclonal Antibodies against D-dimer

The serum F.D.P. assay described above is unable to distinguish between degradation products of fibrinogen and fibrin. It also shows high levels in normal serum due to generation of FDP's during clotting (Nieuwenhuizen, 1987).

The Dimertest (AGEN, Parsippany, New Jersey), provides a simple and precise enzyme immunoassay for crosslinked fibrin degradation products containing the D-dimer (Elms et al 1983).

2.4.1 Reagents

1 - Microtiter plate of 96 wells coated with mouse monoclonal anti D-Dimer antibody and buffer containing 0.1% sodium azide as preservative.

2 - Vial Tag antibody, mouse monoclonal anti-F.D.P. peroxidase conjugate.

3 - D-dimer standard containing purified human D-dimer,

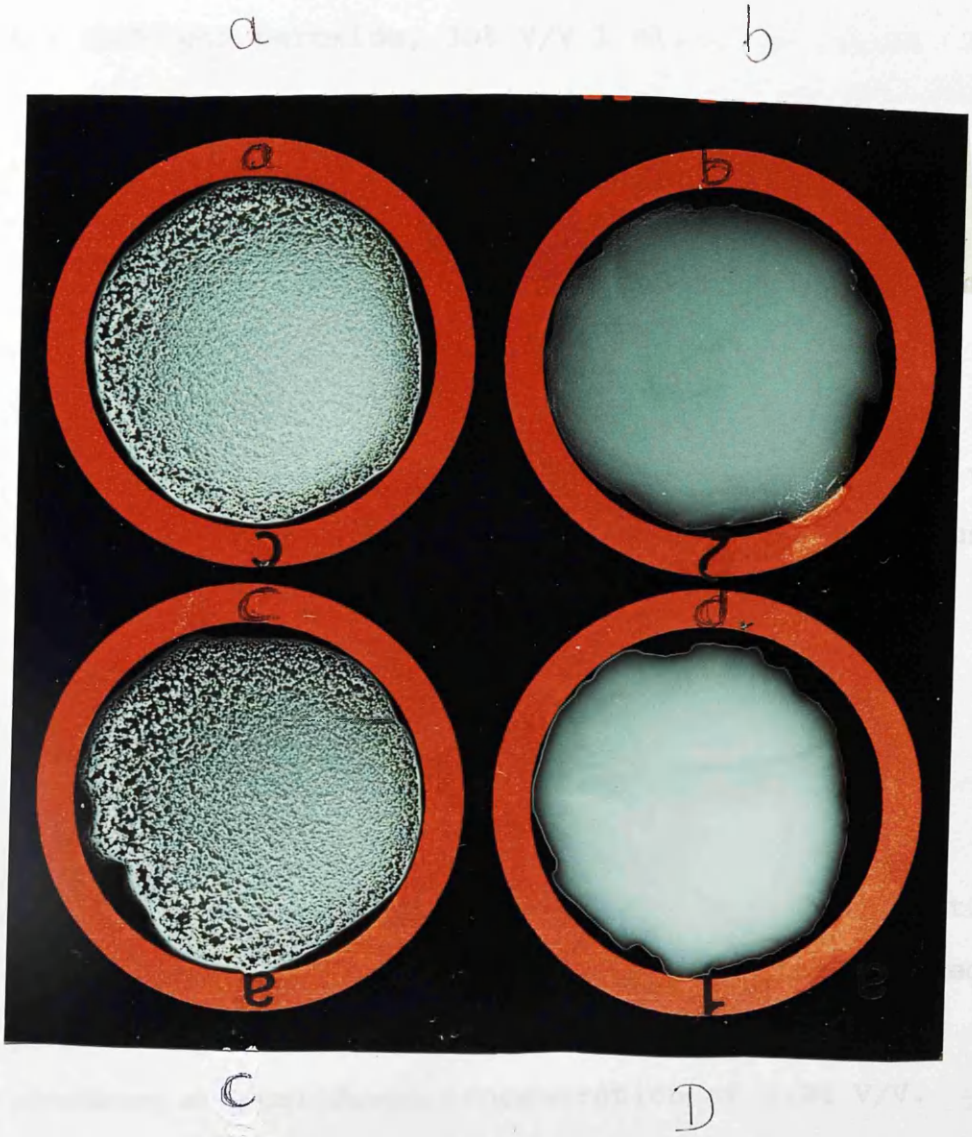

Figure 2.4 Rapid latex screening test (a) control positive (b) control negative (c) patient positive (d) patient negative

with preservative (lyophilized).

4 - Tween 20, 40 V/V 5 ml (green cap)

5 - Vial ABTS substrate, (2,2-Azinobis (3-Ethyl-benzthiazoline solfonic acid) in citrate buffer 10 ml.

6 - Hydrogen peroxide, 30% V/V 1 ml.

7 - Stopping reagent 5 ml (red cap).

8 - Vial diluent 3.5 ml.

9 - Bottle phosphate buffer salts.

Methods for preparation of the above reagents are described by Elms et al (1983).

2.4.2 Specimen Collection

Serum values are generally lower than plasma values (Whitaker 1984). Plasma is preferred, and was used in the present study (prepared from citrated blood).

Frozen plasma samples were thawed at 37 $\overset{\circ}{C}$ and mixed well and centrifuged before assay.

2.4.3 Assay Preparation

- Buffer Reagent

The contents of the phosphate buffer salts bottle and the contents of the Tween 20 vials (5 ml) were mixed in a beaker containing 1 litre distilled water. This produces a final Tween concentration of 0.2% V/V.

- D-dimer standard preparation

(a) The vial of standard was reconstituted with diluent according to the instructions on the vial to give a concentration of 5000 ng/ml.

(b) The standard was left for 10 minutes, then mixed

gently until complete solution was obtained.

(c) A range of serial dilutions of the reconstituted standard was prepared for standard curve construction according to the following protocol:

Reconstituted standard		Dilutent	D-Dimer Concentration ng/ml
A	1.0 ml	-	5000
B	0.2 ml of A	0.2 ml	2500
C	0.2 ml of B	0.2 ml	1250
D	0.2 ml of C	0.2 ml	625
E	0.2 ml of D	0.2 ml	312
F	0.2 ml of E	0.2 ml	156
G	0.2 ml of F	0.2 ml	78
H		0.2 ml	0 (Blank)

A standard curve was included on each occasion the assay was run.

- Washing the coupled plates

(a) Just before use, the microtiter plate was opened.

(b) The plate was inverted and the plate contents shaked out. The plate was washed with buffer reagent, left 2 minutes, then emptied. The plate was blotted by inverting on absorbent material to remove excess liquid.

2.4.4 Assay Procedure

The order of steps was as follows -

(1) Addition of standards and unknown samples.

(a) Addition of 100 ul of buffer reagent to each well.

(b) Addition of 25 ul of standards and unknown plasmas.

Determinations were done in duplicate. The assay range was 20-5000 ng/ml. Samples containing higher levels of D-dimer were diluted in buffer reagent before assay.

(c) Samples were mixed gently and incubate for 1 hour at room temperature.

(2) Tag antibody-enzyme conjugate was added after reconstitution of the Tag antibody by adding 5.5 ml buffer reagent.

(a) Again, the plate was washed 3 times with buffer reagent, as described previously.

(b) 50 ul reconstituted Tag antibody was added to each well and incubated for one hour at room temperature. Again the plate was washed three times as described previously.

(3) Colour Reaction.

(a) 10 ul of hydrogen peroxide was added to the ABTS substrate vial and mixed well.

(b) Pipette 100 ml of this activated substrate was pipetted into each well.

(c) The plate was incubated at room temperature for 20 minutes to allow colour development.

(d) 50 ul stopping reagent was pipetted into each well to stop the reaction.

(e) The absorbance of each well was read at 405-450 mm (optimum 420 mm).

2.4.5 Calculation of Results

Results were plotted using log-linear or log-log data

reduction.

(a) Optical density (O.D.) was plotted against D-dimer concentration. The best curve was drawn through the mean of the duplicate points, rejecting grossly aberrant readings. A typical curve was shown in Fig 2.5.

(b) The mean O.D. for each unknown was calculated and read off the D-dimer concentration from the standard curve.

(c) Samples that read greater than 5000 ng/ml were diluted in buffer reagent for re-estimation.

2.5 Statistical Methods

The statistical analysis used both parametric and non-parametric tests, depending on whether or not it was appropriate to assume that the data were normally distributed. In the former case both paired and two-sample t-tests were used. In the latter case paired analysis used the Wilcoxon signed rank test and unpaired analyses were based on the Wilcoxon Rank sum test. If three or more groups were being compared simultaneously, the analysis used the Kruskal-Wallis test followed up by ranked Wilcoxon rank sum tests. A Fisher's test was used for discrete variables with small numbers in each cell.

A p-value of less than 0.05 was taken as being statistically significant. To determine the independent prediction of outcome by fibrinolytic assays, multivariate analysis was performed using logistic regression analysis on a mainframe computer using the

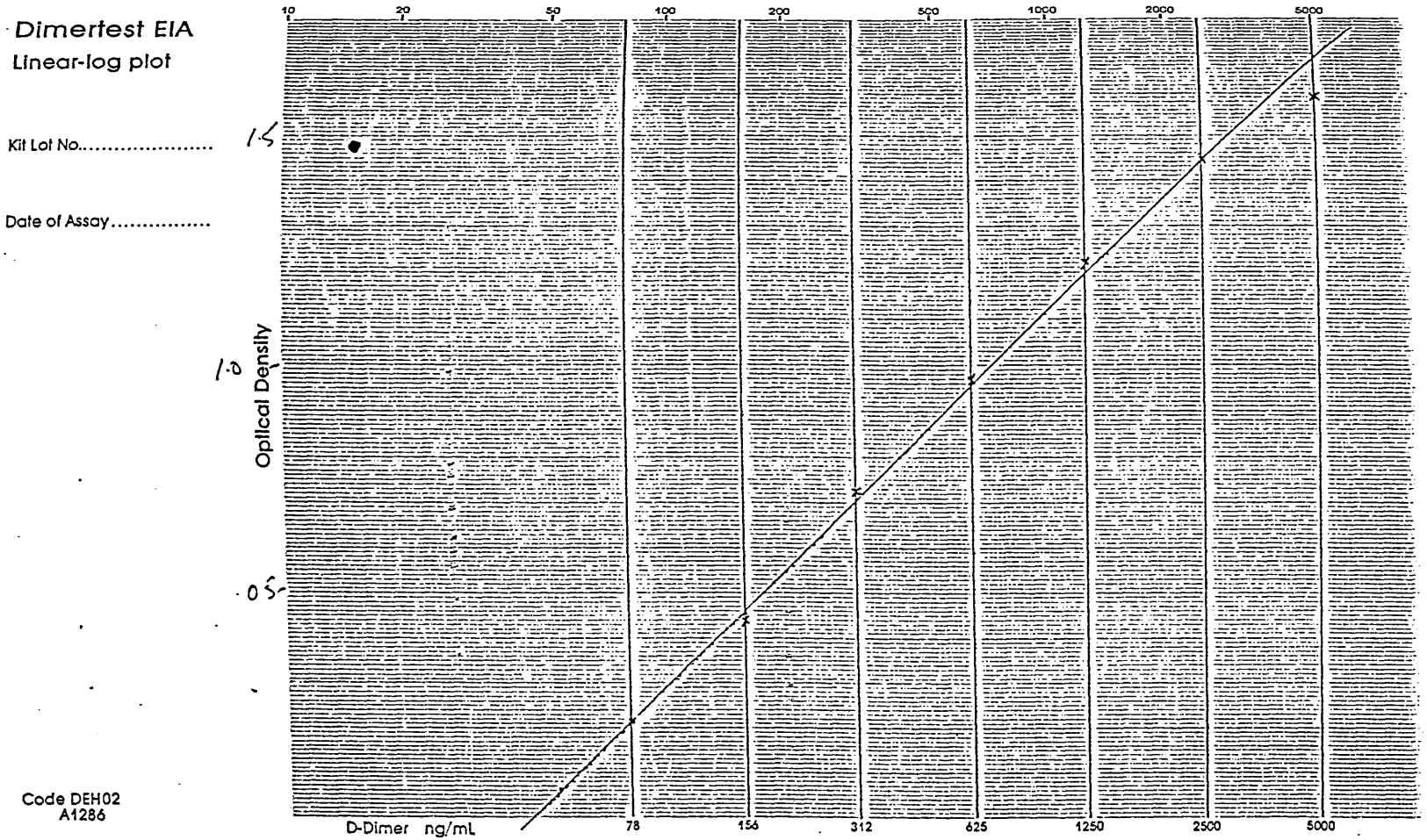

Figure 2.5 The mean optical density for each sample is calculated and the D-dimer concentration is read off from the standard curve.

BMDP statistical package (Dr. G.D. Murray).

CHAPTER III

VARIABILITY OF MEASUREMENTS

3.1 Introduction

Before proceeding to investigate the role of the fibrinolytic system in the pathogenesis of acute upper gastrointestinal bleeding, estimation of the reproducibility of the measurements in the hands of the author was required. In addition, the variability of fibrinolytic activity in relation to sampling time was assessed, to confirm the recognised diurnal variation in plasminogen activator levels (Lowe and Prentice 1980).

3.2 Coefficient of variation of F.D.P. estimation

3.2.1 Method and Subjects

The level of the F.D.P. was measured in 2 volunteers for validation of the accuracy of the technique.

Estimations of the level were made 10 times in each volunteer.

Two healthy male volunteers aged 35 and 45 years who work in the Department of Surgery were studied.

Antecubital venous blood samples were obtained by clean venepuncture with minimal venous occlusion, after proper sterilization of the skin, with plastic disposable syringe. Neither subject was fasted or had normal activity restricted in any way. Samples were taken at 9 a.m.

Twenty mls of blood were taken from each volunteer and evenly distributed between 10 test tubes i.e. 2 mls in each. Each of the 10 test tubes contained soya bean

trypsin inhibitor (approximately 3600 UF units per tube) and bovine thrombin (20 NIH units per tube). The tubes were incubated at room temperature until separation of the serum. As soon as possible, the serum was removed after centrifugation at 2000 r.p.m. for 10 minutes. All serum samples were stored at -45 °C. F.D.P. levels were measured as mentioned previously (Chapter II).

3.2.2 Results

The results of the F.D.P. levels of the two volunteers are shown in Table 3.1

There was no variation of F.D.P. measurement noted in the ten samples in the second volunteer, while analysis of the samples from the first subject had a coefficient of variation of 10.9%. This indicates a satisfactory analytical technique, with the mean level of the coefficient of variation being 5.45%.

3.3 Coefficient of variation of fibrin plate lysis area (FPLA)

For validation of the accuracy of the technique used, the F.P.L.A. levels were measured 10 times in one healthy male volunteer aged 40 years. Antecubital venous blood samples were obtained by clean venepuncture with minimal venous occlusion. Fifty mls of blood were taken. Four and a half mls whole blood was added to each of 10 numbered tubes each of which contained 0.5 ml sodium citrate.

The tubes were put on melting ice until separation of the plasma. Plasma was removed by centrifugation at 2000

Table 3.1 F.D.P. levels for both volunteers.

(Taken on 10 occasions)

No.	Volunteer 1 (µg/ml)	Volunteer 2 (µg/ml)
1	3.7	2.5
2	3.75	2.5
3	3.75	2.5
4	2.5	2.5
5	3.75	2.5
6	3.75	2.5
7	3.75	2.5
8	3.75	2.5
9	3.75	2.5
10	3.75	2.5

r.p.m. for 10 minutes at 4 °C temperature. All plasma samples were stored at -45 °C.

Analysis was by the method discussed in Chapter 2.

The results are shown in Table 3.2.

3.3.1 Results (Table 3.2)

The variation of the lysis area in the 10 samples was within an acceptable range and indicated that the proper technique had been employed. The mean coefficient of variation in this experiment was 6.48%.

The variation in lysis area therefore was within acceptable limits and indicates a satisfactory analytical technique.

3.4 Significance and assessment of diurnal variation in measurement of fibrinolytic activity

Diurnal variation in fibrinolytic activity of blood is well recognised, with a rise in activity during the morning (Fearnley et al 1957; Kanaik et al 1957, 1958; Swinska-Kotschy and Glogowska 1958; Billimoria et al 1959; Buckell and Elliot 1959; Kowarzyk et al 1959; Fearnley 1960; Hajjar et al 1961; Lackner and Sougin-Mibashan 1964; Menon 1966a; Moser and Hajjar 1966). Most of these authors used the F.P.L.A to assess the variations observed in normal subjects' fibrinolytic activity, and in most cases healthy volunteers were used. It has recently been suggested that diurnal variation results from diurnal variations in plasminogen activator inhibitor (Kruithoff, 1988). Variations may also occur as a reaction to stress. They

Table 3.2 The measurement of F.P.L.A. using streptokinase as the standard for lysis area for one volunteer on 10 occasions. The table also illustrates the mean of the 3 samples, on each plate (A.B.C.), the corrected value diameter when compared with the streptokinase standard, and the calculated area of fibrinolysis.

No. of Sample	Streptokinase standard	A	B	C	Mean	Corrected value	Area
1	18	8	9	7	8	8.4	55
2	19	8	8	8	8	8	50
3	19	7	7	8	7.6	7.6	45
4	18	8	6	8	7.3	7.7	46
5	19	7	8	8	7.8	7.8	48
6	19	10	8	7	8.3	8.3	54
7	19	9	8	7	8	8	50
8	19	8	7	9	8	8	50
9	19	9	6	7	7.7	7.7	46
10	19	7	8	9	8	8	50

have been noted to vary both from person to person and in the same individual from day to day (Kowarzyk et al 1960). Fibrinolytic activity is elevated as a result of exercise (Menon 1966b; Menon et al 1967), anxiety (Macfarlane and Biggs 1944; Latner 1947; Truelove 1951) and subcutaneous injection of adrenalin (Truelove 1953).

A diurnal rhythm in the rate of excretion of 17-ketosteroids was first described by Pincus (1943) and in 17 hydroxysteroids by Bliss et al (1953).

The peak concentrations of both groups of steroids arise between 6 a.m. and 8 a.m. after which the level gradually falls to a nadir at 4 p.m. remaining at this level until midnight (Wajchenberg et al 1964). These variations are thought to reflect changes in the rate of corticotrophin secretion by the pituitary glands (Perkoff et al 1959). The variations may be related to but are more consistent than those of fibrinolytic activity (Chakrabarti et al 1964). Significantly higher plasma levels of free 17-OHCS are found in otherwise healthy individuals with anxiety than in tranquil people (Hill et al 1956; Persky 1957), the 8 a.m. level being almost double the normal (Persky et al 1956). Corticosteroids will reduce fibrinolytic activity when this is of a pathological degree e.g. in hepatic cirrhosis (Kwaan et al 1956).

Menon and colleagues (1967) have measured fibrinolytic activity as assessed by euglobulin lysis times and found a loose inverse relationship to plasma

II-hydroxysteroid level. Their subjects had a significant decrease in euglobulin lysis times between 9 a.m. and 4 p.m. They included 5 bedridden ill patients and found that the normal rhythm in fibrinolytic activity was significantly reduced in these patients.

3.5 Diurnal variation in fibrinolytic activity as assessed by Fibrin Plate Lysis Area

3.5.1 Method and Subjects

To check normal levels of F.P.L.A 10 normal male volunteers working in the Royal Infirmary Department of Surgery (age range 25-45 years, mean age 30 years) were examined. None of them were subjected to fasting or had their activity restricted during the study. Venous samples were taken from the antecubital fossa as previously discussed. Three venous samples were taken from each volunteer at 09.00, 12.00 and 17.00 hours. It was assumed that such times would reveal a diurnal rhythm. Four and a half mls of blood was added to test tubes containing 0.5 ml. sodium citrate and this was kept on melting ice until separation of the plasma occurred. The plasma was removed after centrifugation at 2000 r.p.m. for 10 minutes at 4 ^{o}C. All plasma samples were stored at -45 ^{o}C. The analytical methods were as previously discussed.

3.5.2 Results

The results of the F.P.L.A in the blood taken from the 10 volunteers at 09.00, 12.00 and 17.00 are shown in Table 3.3 and Figure 3.1.

Table 3.3 Ten volunteers measurement fibrine plate lysis area at 9.00, 12.00, 17.00 hours for each patients.

No.	9 a.m. (mm^2)	12 noon (mm^2)	5 p.m. (mm^2)
1	85.1	107.6	132
2	45.3	96.1	85.7
3	80.4	90.9	96.2
4	96.2	145	119
5	91	119	102
6	95	151.6	131.8
7	50.24	50.24	55
8	60	100.6	92.7
9	43.3	110.3	96.2
10	50.4	90.13	80.7
Mean	70.6	106.2	99.1
SD	20.9	28.9	23.8
Median	60	100.6	96.2
Sample Range	43.3-96	50-151	55-132

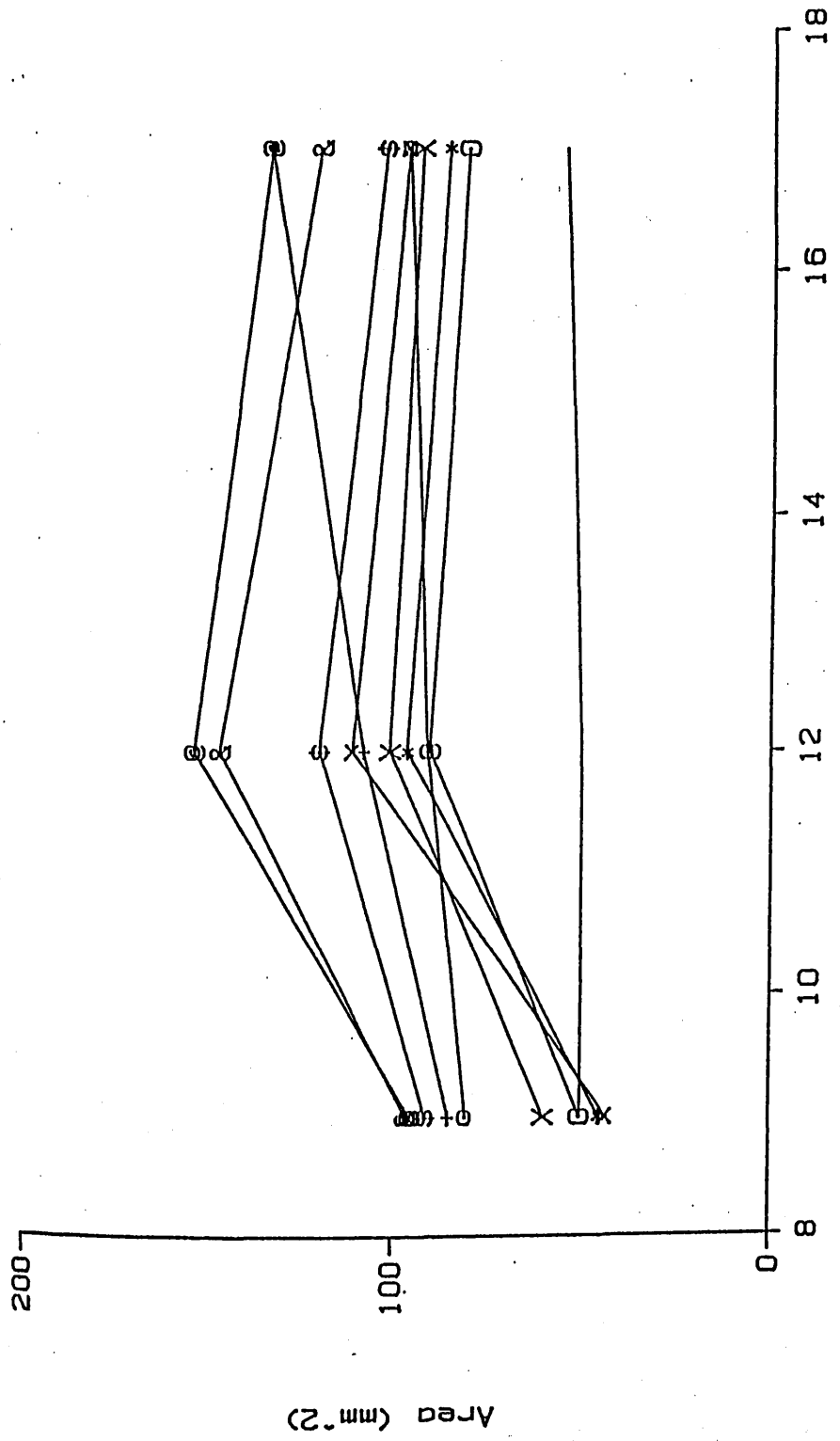

Figure 3.1 Diurnal variation in fibrinolytic activity assessed by fibrin plate lysis area at 9.00, 12.00 and 17.00 hours for each of the 10 volunteers.

The normal range of F.P.L.A at 09.00 ranged from 43.3 mm2 to 95.1 mm2.

The mean was 70 mm2 in this study. Nine volunteers showed an increase in fibrin plate area from 09.00 to 12.00, the other one volunteer did not. Between 12.00 to 17.00 F.P.L.A in most of the volunteers decreased. In only 3 volunteers did the FPLA continue to increase between 12 and 5 p.m.

3.5.3 Statistics

Comparing groups on all data using the Friedman non-parametric test to see if there were group differences gave $P < 0.001$. As there was a difference, the Wilcoxon Signed Ranks test was used to compare pairs.

9 a.m. to 12 p.m.	P = 0.009	Significant
9 a.m. to 5 p.m.	P = 0.006	Significant
12 p.m. to 5 p.m.	P = 0126	

3.5.4 Conclusions

1 - Fibrinolysis increases between 9 a.m. and 12 noon, and starts to decrease after 12 noon.

2 - In the morning there is an increase in blood fibrinolytic activity, followed by a more gradual decrease between 12 noon and 5 p.m. These findings are consistent with the literature reviewed above above.

3 - In some volunteers (2 out of 10) fibrinolysis increased continuously during the day from 09.00 to 17.00.

3.6 Diurnal variation in fibrinolytic activity as assessed by serum F.D.P. level

3.6.1 Methods and Volunteers

To establish the normal level of F.D.P. and to examine diurnal variation 10 volunteers (age range 25-45 years, mean age 30 years) were examined. All of them were men who worked in the Royal Infirmary Department of Surgery, Glasgow. No subject was fasted or had activity restricted during study.

Three venous samples taken from the antecubital fossa from each volunteer at 09.00, 12.00 and 17.00 hours. Samples were assayed by the method discussed in Chapter II. Two mls of blood was added to test tubes as discussed earlier. Each test tube contained soya bean trypsin inhibitor (approximately 3600 UF units per tube) and bovine thrombin (20 NIH units per tube). Each tube was reput at room temperature until separation of the serum occurred. The serum was removed after centrifugation at 2000 r.p.m. for 10 minutes. All serum samples were then stored at -45 ^{o}C.

Analytical method was as previously discussed.

3.6.2 Results

The results of the F.D.P. levels in the 10 volunteers (ages 30-40 years) at 09.00, 12.00 and 17.00 hours are shown in Table 3.4.

The normal variation of F.D.P. ranged from 1.25 ugm - 5 ugm in this study.

However no diurnal variation was observed in the

Table 3.4 F.D.P. level (ug/ml) in serum from 10 volunteers at 09.00, 12.00 and 17.00 hours.

Number	09.00 (µg/ml)	12.00 noon (µg/ml)	17.00 (µg/ml)
1	5	5	5
2	2.5	2.5	2.5
3	3.75	3.75	3.75
4	2.5	2.5	2.5
5	3.75	3.75	3.75
6	1.25	1.25	1.25
7	5	5	5
8	2.5	2.5	2.5
9	5	5	5
10	2.5	2.5	2.5
Mean	3.4	3.4	3.4
Median	2.5	2.5	2.5
SD	1.5	1.5	1.5

level of F.D.P. as shown in these volunteers.

3.6.3 Statistics

There was no statistically significant difference between the 3 groups indicating no diurnal variation in F.D.P. level.

3.7 Conclusions

1 - The coefficient of variation of repeated F.D.P. estimations in 2 volunteers was satisfactory, with the level of the mean coefficient of variation being = 5.45%.

2 - The coefficient of variation of the F.P.L.A technique when measured 10 times in one volunteer was within the acceptable range. The mean coefficient of variation in this experiment was 6.48%.

3 - The normal level of F.P.L.A in this study (no subject was fasted or had their activity restricted) at 9.00 in the morning was 70 mm2 mean (range 43 mm2 - 96 mm2).

4 - A diurnal variation in fibrinolytic activity assessed by FPLA was observed. The level increased in the morning and decreased after 12 p.m. Two out of 10 volunteers had increased fibrinolysis until 5 p.m.

5 - The level of F.D.P. in the serum was 3.4 ug/ml mean (range 1.25 ug/ml - 5 ug/ml).

6 - There was no diurnal variation in fibrinolytic activity as assessed by serum F.D.P. This makes it

potentially more useful as a prognostic test in acute bleeding, compared to the F.P.L.A.

CHAPTER IV

UPPER GASTROINTESTINAL BLEEDING

As the prognostic significance of enhanced fibrinolysis on severity and recurrence of UGIT bleeding has not been clearly identified in human beings, it was decided to study tests of the fibrinolytic activity among a group of patients with haematemesis or melaena admitted to Glasgow Royal Infirmary.

4.1 The Selection Criteria

The selection criteria for inclusion of the patients in the study were as follows

a) Patients admitted with haematemesis or melaena or both regardless of presumptive clinical diagnosis.

b) Patients with blood dyscrasias, or on anti-coagulant, thrombolytic or antifibrinolytic therapy were excluded. Any patient admitted with a history of bleeding more than 24 hours before admission were also excluded.

4.2 Patient Recruitment

In Glasgow Royal Infirmary, patients with UGIT haemorrhage are admitted under the care of the physicians. Patients are admitted to a medical receiving unit where they are stabilised and at the post receiving ward round the next day, the consultant decides if he wishes endoscopy to be carried out. The patient is then referred to the duty endoscopist. Seriously ill patients who require immediate laparotomy obviously

bypass this system and because of the necessity to standardise the time at which blood samples are taken it was not possible to include several patients with active, profuse bleeding admitted and operated upon within a few hours. It was not necessary for the validity of this study to include all patients with UGIT bleeding and no attempt has been made to recruit a consecutive series.

At 09.00 hours ward visits were made for collection of basic data from patients with acute UGIT bleeding for whom endoscopy was requested, plus blood samples. Sampling was left until all patients had been seen, then all samples were taken together (within 10 minutes, hence maximum of 3 at one time). Venous samples of at least 13 mls in a 20 ml syringe were collected as detailed in section 2.1.

4.3 Patient details and information

In this prospective study 122 patients with upper GIT bleeding were included. All the patients were admitted to the Royal Infirmary, Glasgow during the period April 1986 - April 1988. All had been bleeding from the UGIT. They included 73 (60%) men and 49 (40%) women.

For every patient various data were recorded as follows:

4.3.1 History

a) Personal Data

1 - Name, study number, address, telephone number.

2 - General Practitioner's (G.P.) name and address.

3 - Hospital number, date of birth and sex.

4 - Ward number and name of consultant.

5 - Date of onset of bleeding, date of admission and date of the blood sample and follow up.

b) Haemodynamic State

1 - Pulse rate/minute

2 - Blood pressure systolic/diastolic

c) Intravenous Infusion

1 - Units of blood transfused

2 - Units of plasma/colloid

3 - Units of crystalloid

4.3.2 Clinical Data

a) All patients had either

1 - Haematemesis

2 - Haematemesis and melaena

3 - Melaena

b) History of drug-induced bleeding

1 - Non steroid anti-inflammatory drugs (NSAID)

2 - Steroids

c) Alcohol Ingestion

1 - Non drinker

2 - Previous drinker

3 - Current social drinker (less than 10 units/week)

4 - Heavy drinker

d) Smoking: number smoked per day

e) Weight (kg), height (cm) and % ideal body weight

4.3.3 Routine Laboratory Data

a) Haemoglobin

b) Serum bilirubin

c) Serum creatinine

4.3.4 Endoscopic Findings

a) Anatomical diagnosis and site of bleeding

1 - Oesophagus

2 - Stomach

3 - Duodenum

4 - Stoma

b) Pathological Diagnosis

1 - Oesophagus

i Oesophagitis

ii Hiatus hernia

iii Mallary Weiss syndrome

iv Varices

v Carcinoma

vi Peptic ulcer (benign ulcer)

2 - Stomach

i Gastritis

ii Carcinoma

iii Gastric ulcer

iv Gastric erosion

3 - Duodenum

i Duodenitis

ii Duodenal ulcer

c) Diagnosis of Stigmata

1 - Visible vessel

2 - Active bleeding

3 - Slough

4 - Fresh clot

5 - None of above

4.3.5 Laboratory Work

a - Measurement of fibrin plate lysis area (F.P.L.A.) of euglobulin plasma fraction.

b - Measurement of serum fibrin/fibrinogen degradation products (F.D.P.).

c - D-Dimer test for plasma cross-linked F.D.P.

d - Latex test for serum F.D.P.

4.3.6 Treatment

a - Medical treatment (drug therapy)

1 - Antacid

2 - Cimetidine or Ranitidine

3 - Prostaglandine E2

4 - Somatostatin

5 - Tranexamic Acid

b - Laser therapy

c - Surgical treatment

1 - Undersewing of vessel

2 - Undersewing of vessel plus

i Vagatomy and drainage

ii Partial gastrectomy

iii Total gastrectomy

iv Injection of varices

v Transection of oesophagus

vi Laparotomy alone

4.3.7 Outcome

All patients were followed from the evaluation until discharge or death. In hospital survival and date of discharge were recorded, also date of death and post mortem result.

Further bleeding (continuous or re-bleeding) was recorded as well as surgery, laser treatment or blood transfusion.

The definition of recurrent or continuous bleeding was made on clinical grounds. Where the patient had a period of haemodynamic stability and endoscopy showed no active bleeding and the patient subsequently developed further signs of hypotension or tachycardia suggestive of further blood loss or a further haematemesis revealed fresh blood, recurrent bleeding was recorded. If the patient's haemodynamic state continued to require the use of blood or plasma expanders and continued fresh bleeding was found at endoscopy, the patient was judged to have continuous bleeding.

4.4 Results

4.4.1 Clinical Results

The ages of the 122 patients ranged from 19 years to 80 years with a mean of 54 and median of 56 years.

This number comprised 73 (60%) men and 49 (40%) women. The age and sex distribution is shown in fig. 4.1.

Their haematemesis and/or melaena was of variable severity. Severity was judged by requirement of blood

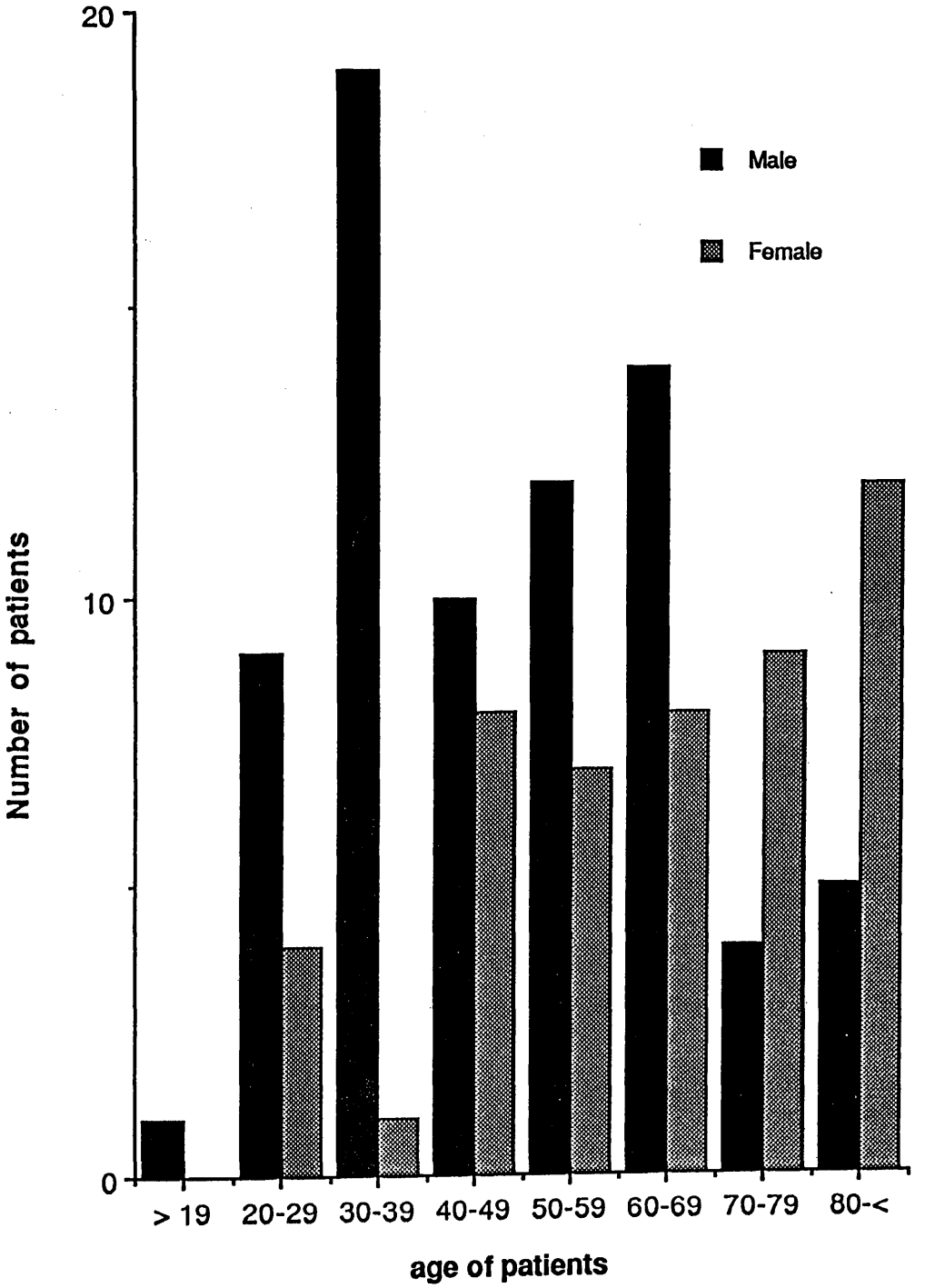

Figure 4.1 Sex and age distribution of UGIT bleeding patients admitted to study.

transfusion. Fifty-six (46%) out of the total 122 patients needed blood transfusion. The total requirements were 302 units of blood, a mean of 5 units per transfused patient. However, the severity of blood loss was very variable among those individuals who required blood transfusion i.e. the minimum requirement was 1 unit and the maximum was 29 units.

Twenty-four (20%) patients had plasma transfusion. In total 172 units plasma were required, within the range 1 to 40 units. The sample median was 6 units.

64 (52%) patients from the total of 122 required in total 1691 units of crystalloid fluid for intravenous infusion, within the range 1 unit to 150 units. The sample mean was 26 units.

Sixty-six (54%) patients from the total had haematemesis only, 52 (43%) had both haematemesis and melaena, and 4 (3%) had melaena only. 45 (37%) had previously had a peptic ulcer, and 54 (44%) had history of previous UGIT bleeding.

Ten (8%) patients did not consume alcohol, 30 (25%) were previous drinkers and had not consumed any within the previous year, 54 (44%) were current social drinkers drinking less than 10 units/week and 27 (22%) were current heavy drinkers. One patient was unsure of his category (Fig. 4.2).

Seventy-three (60%) out of the total 122 patients smoked. The total smoking habit was 1243 cigarettes per day. The mean was 17 cigarettes per day per smoking

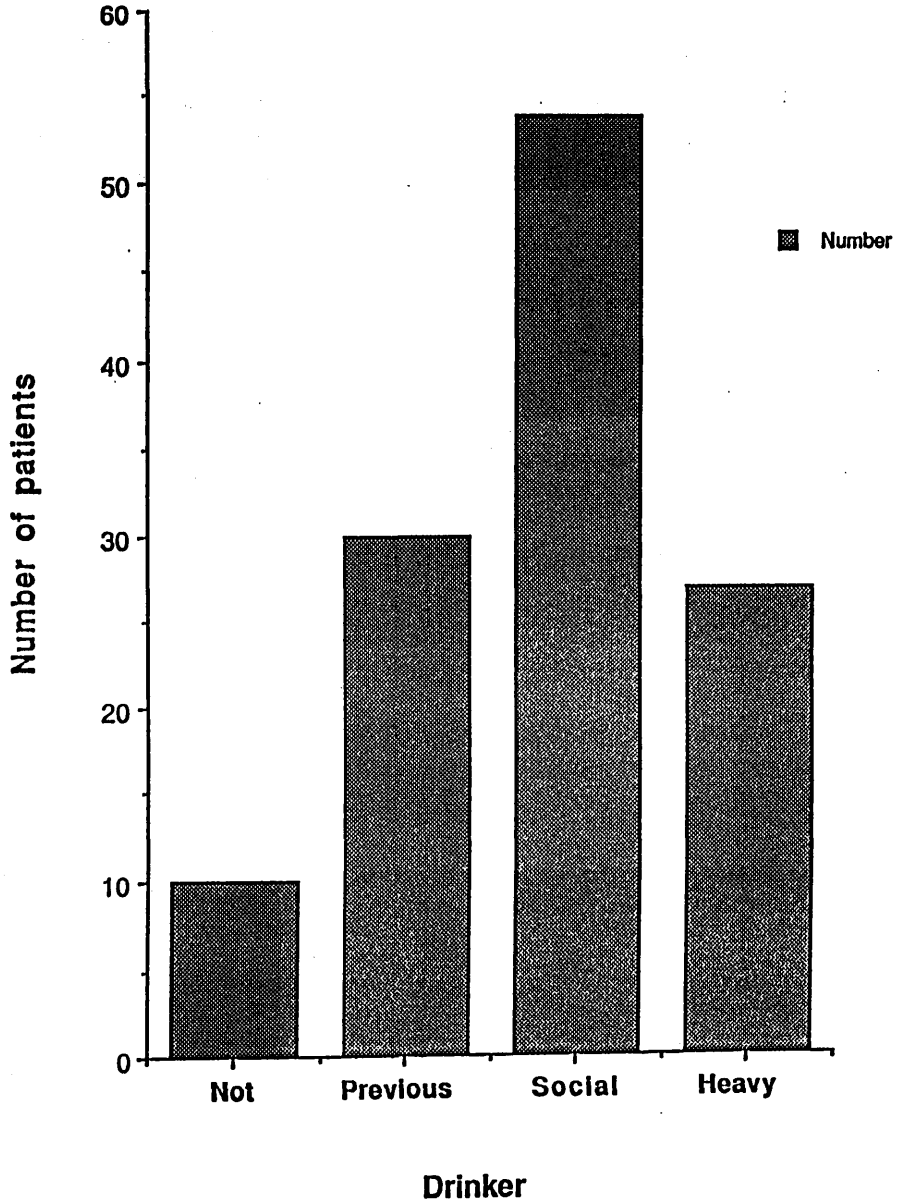

Figure 4.2 Distribution of drinking habits of UGIT bleeding patients according to classification described on p 130.

patient (range 3 to 60 cigarettes per day) and median was 12 cigarettes per day.

The haemoglobin level of patients at admission was important because it is one of the risk factors in upper GIT bleeding (Northfield, 1971; MacLeod et al, 1982). In some patients, especially those with a bleeding peptic ulcer, the lower the haemoglobin level at admittance the greater the mortality rate (Himal et al 1974). Their haemoglobin levels ranged from 5.2 g/dl to 12 g/dl with a mean of 11.96 g/dl and median of 12 g/dl.

The ranges of serum bilirubin in patients varied from 2 umol/l to 150 umol/l with sample mean of 22 umol/l nd median of 10 umol/l.

The serum creatinine level in patients ranged from 20 umol/l to 360 umol/l with a mean of 73 and median of 70 umol/l.

4.4.2 Endoscopic findings

Twenty (16%) patients out of a total of 122 patients were undiagnosed after endoscopy, and 102 (84%) diagnosed.

4.4.3 Anatomical findings (Fig. 4.3)

a) Oesophagus -34 (33%) patients (of these 34 patients there were 24 (71%) men and 10 (29%) women.

b) Stomach - 34 (33%) patients (of these 34 patients 21 (62%) were men and 13 (38%) were women).

c) Duodenum - 32 (31%) patients (of these 32 patients the sex prevalence indicated 18 (56%) men and 14 (44%) women).

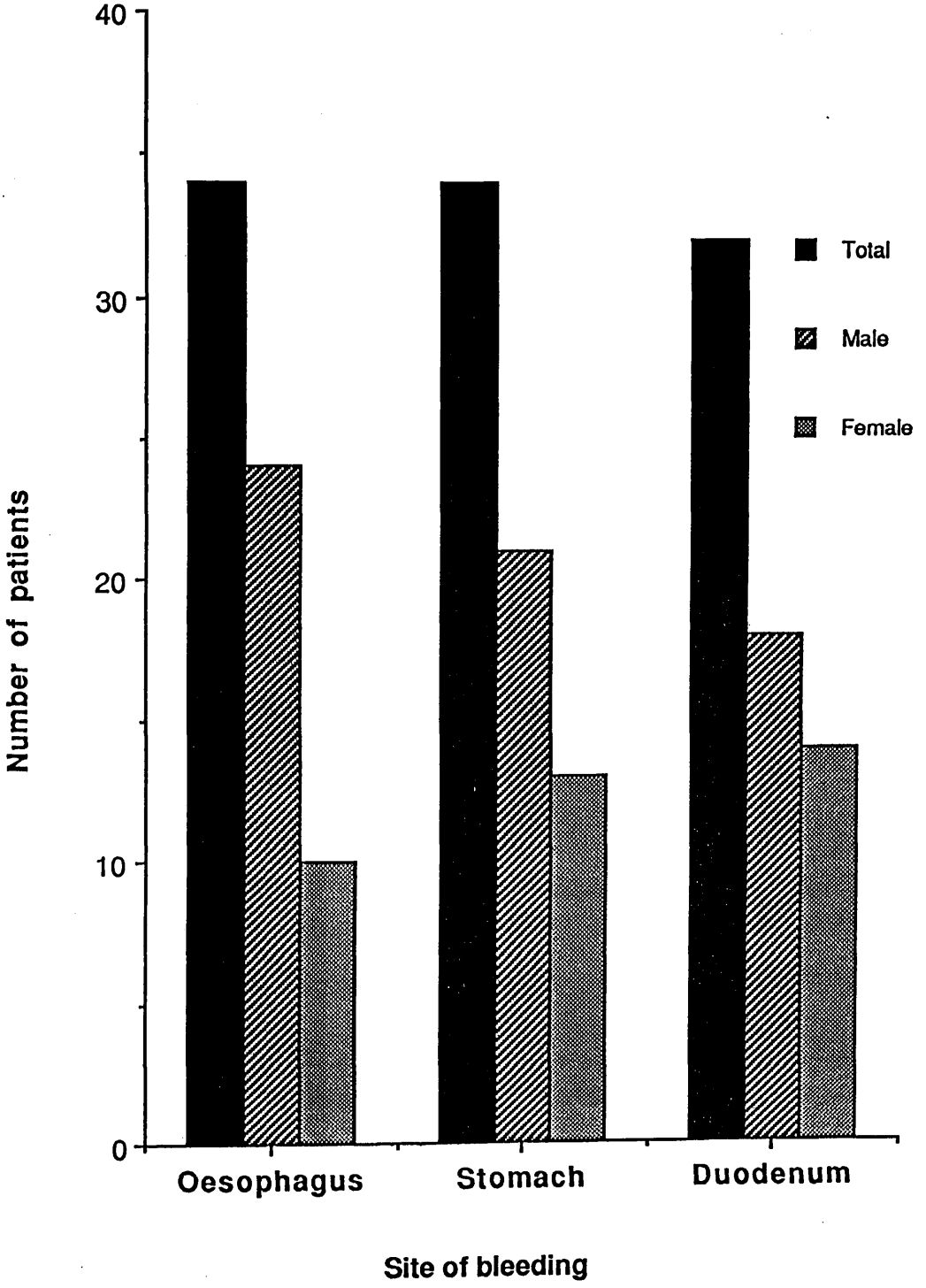

Figure 4.3 Anatomical site of bleeding (oesophagus, stomach, duodenum) and corresponding sex incidence.

d) Stoma - 2 (2%) patients, one man and one woman.

4.4.4 Pathological findings (Figure 4.4)

Pathological investigation indicated a number of causes of bleeding from the oesophagus, stomach and duodenum.

Oesophagus

1 - Oesophagitis This was noted in 3 cases, 2 male (67%) and 1 female (33%). These 3 cases were 2% from the 122 patients who had been bleeding from upper GIT and 3% of 102 diagnosed patients. Of the patients suffering from oesophageal bleeding, oesophagitis was the cause in 9% of the cases.

2 - Mallory Weiss Syndrome

12 patients were diagnosed as bleeding from Mallory Weiss syndrome. Nine (75%) of these were male and 3 (25%) were female. This represented 10% of the total study group.

They also represented 35% of the 34 patients suffering from oesophageal bleeding.

3 - Oesophageal Varices

15 patients were bleeding from oesophageal varices of which 12 (80%) were male and 3 (20%) were female. These patients represented 12% of those with upper GIT bleeding, 15% of those diagnosed, and 44% of oesophageal bleeding.

4 - Four patients (2 male, 2 female) were found to have benign oesophageal ulcer in this survey. Three per cent of UGIT bleeding was caused by this condition and 4% of

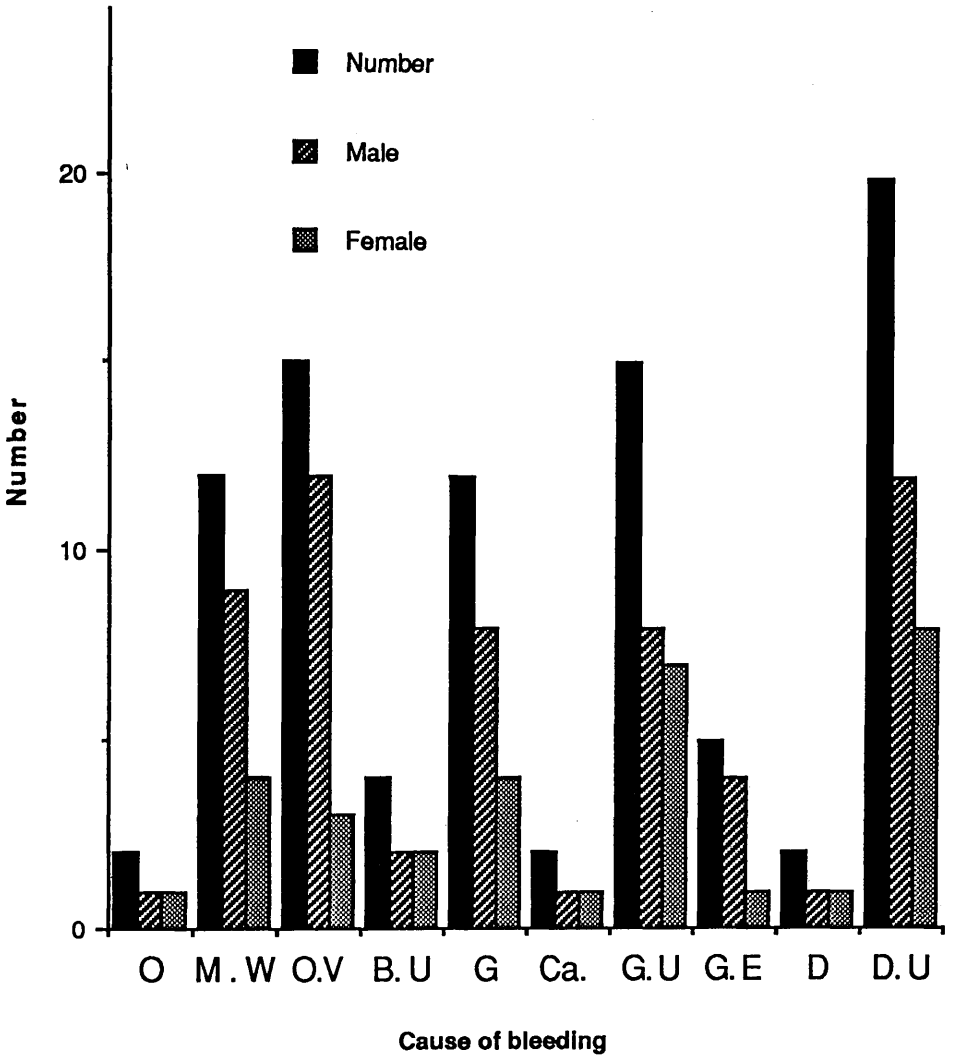

Figure 4.4 Pathological diagnosis of acute upper gastrointestinal bleeding. Total number and corresponding sex incidence.
O: Oesophagitis; M.W.: Mallory Weiss Syndrome; OV: Oesophageal Varices; OU: Benign oesophageal ulcer; GU: Gastric ulcer; G: Gastritis; Ca: Gastric carcinoma; G.E. Gastric erosion; D: Duodenitis; D.U.: Duodenal ulcer.

diagnosed cases. Of these with oesophageal bleeding the 4 patients represented 12%.

Stomach

34 patients from the original 122 monitored were bleeding from the stomach. The sex incidence was 21 (62%) males and 13 (36%) females. The causes were as follows:

1 - Gastritis

This was noted as the sole cause in 12 patients, 8 (67%) male and 4 (33%) female. Of these patients experiencing upper GIT bleeding 10% of the cases were caused by gastritis and of the diagnosed cases this number represented 12%. This condition was the cause of 35% of cases of bleeding from the stomach.

2 - Gastric Erosions

This condition was diagnosed in 5 patients, 4 (80%) male and 1 (20%) female.

This represented 4% of the patients who had been bleeding from UGIT. Of the 34 cases who were bleeding from the stomach these 5 patients represented 15%.

3 - Gastric Ulcer

Gastric ulcer was found in 15 patients of whom 8 (53%) were male and 7 (47%) female. This represented 12% of the total number of cases of UGIT bleeding, and 15% of those diagnosed. From the 34 cases of gastric bleeding this was 44%.

4 - Carcinoma of Stomach

Two patients were found to have a malignant ulcer,

one male and one female.

Duodenum

1 - Duodenal Ulcer

Twenty nine patients had a duodenal ulcer, 15 (52%) male and 14 (48%) female. This appeared to be the cause of 24% of all cases of upper GIT bleeding and 28% of the 102 diagnosed cases of upper GIT bleeding. Of the 32 suffering duodenal bleeding this was the cause of 91% of the cases.

2 - Duodenitis

Three people had the above condition, all patients were male. This accounted for 2.5% of people suffering upper GIT bleeding and 3% of diagnosed bleeding. Duodenitis represented the remaining 9% of cases of duodenal bleeding.

Stomal Ulcer

Two patients were diagnosed as bleeding from a stomal ulcer, one male and one female. Of the 122 cases of upper GIT bleeding this was less than 2%.

4.4.5 Outcome

The 15 patients who had bleeding oesophageal varices were all given sclerotherapy by injection using rigid endoscopy. Five of them died, thus the mortality rate in bleeding oesophageal varices was 33%.

In gastric bleeding, of the 34 patients one was given laser treatment, 28 were given medical treatment, and 5 patients required surgery. Three of these died.

Three patients suffering from a gastric ulcer were

treated by surgery, all survived. One patient with gastric ulcer was given laser treatment but died, and one woman with acute gastritis diedafter medical treatment. Of the 2 patients with a malignant ulcer, one died. Table (4.1) shows that in this study the mortality rate from stomach bleeding was 8.8% and from a gastric ulcer 6.7%.

Twenty nine people suffered from a duodenal ulcer, 8 of these exhibited severe bleeding. Six were treated by surgery, of whom one died; and 2 were treated by laser, of whom one died. One woman given medical treatment died. In total 3 people died from duodenal ulcer, therefore the mortality rate from D.U. bleeding was 10%. No death was recorded from duodenitis. Tables 4.2 and 4.3 summarise the clinical course in those patients who died or required surgery. The case histories are outlined in Appendix 4.

4.5 Discussions

Clearly there are difficulties in drawing too many conclusions about UGIT bleeding from this group. The main difficulty relates to the selection process. Recognition of a diurnal rhythm in fibrinolytic activity meant that only those patients available for sampling at 9.00 a.m. could be included. This was not, therefore, a consecutive series and it is certain that seriously ill patients admitted and proceeding to surgery immediately were not included. It could not be claimed, then, that this series is typical of those admitted to the Royal Infirmary. If anything, it underestimates the severity

Table 4.1 Pathological diagnosis and number of patients who died from upper gastrointestinal bleeding and method of treatment.

Diagnosis	Number of Patients	Injection	Surgery	Laser	Medical Treatment	Died
Oesophageal varices	15	15	-	-	-	5
Gastric Ulcer	15	-	3	1	11	1
Gastric carcinoma	2	-	2	-	-	1
Gastritis	12	-	-	-	12	1
Duodenal ulcer	29	-	5	1	23	3

SERIES NUMBER	DIAGNOSIS	CONTIN. BLEEDING	RE-BLEED	SURGERY	LASER	SCLERO -THERAPY	MEDICAL TREATMENT	DIED
1	O. V.		+			+		+
2	O. V.		+			+		+
3	O. V.		+			+		+
4	O. V.	+				+		+
5	O. V.		+			+		+
6	G. U.		+	+				
7	G. U.	+			+			+
8	G. U.		+	+				
9	G. U.	+		+				
10	GASTRITIS	+					+	+
11	G. Ca.	+		+				+
12	G. Ca.	+		+				
13	D. U.		+	+				
14	D. U.		+	+			+	
15	D. U.		+					+
16	D. U.		+					
17	D. U.		+	+				
18	D. U.	+			+			+
19	D. U.	+		+				
20	D. U.		+	+				+

Fig 4.2 Summary of clinical features of patients dying or requiring surgery.

SERIES NUMBER	DIED	BLOOD TRANSFUSION (UNITS)	F.D.P. ug/ml	F. P. L. A. mm2	CAUSE OF DEATH
1	+	6	160	20	BLEEDING
2	+	6	160	50	ACUTE RENAL FAILURE
3	+	7	20	130	BLEEDING
4	+	10	160	78	BLEEDING
5	+	30	160	60	BLEEDING
6		7	10	95	
7	+	29	80	64	CARDIAC FAILURE
8		2	10	56	
9		3	40	50	
10	+	8	300	20	ACUTE RENAL FAILURE
11	+	8	160	78	ACUTE RENAL FAILURE
12		7	80	60	
13		6	40	75	
14		9	80	45	
15	+	6	40	12	MYOCARDIAL INFARCTION
16		4	20	95	
17		12	80	95	
18	+	6	80	16	BLEEDING
19		6	10	78	
20	+	6	160	60	BLEEDING

Fig. 4.3 Transfusion requirements, F.D.P. and F.P.L.A. results in patients dying or requiring surgery

of the condition as seen at this hospital. A further "skewing factor is that the hospital is a local referral centre for oesophageal varices patients.

However, the current series is not too dissimilar to previously reported series in terms of age, sex and site of bleeding. The incidence of bleeding from UGIT was higher in men than in women (prevalence 60% and 40% respectively). In mean the incidence of bleeding was commonest in young to middle age, but in females the incidence of bleeding increased. Almost certainly, for reasons already mentioned, this series underestimates the mortality of patients with UGIT bleeding admitted to this hospital.

The mortality from oesophageal varices was 33% in this study. In review studies, mortality from oesophageal varices varies considerably, ranging from 13% to 50% were observed: 14% by Alwmark et al (1982), 31% by Kjargaard et al (1982), 21% by Barsoum et al (1982), 32% by Palani et al (1982), 39% by Terblanch et al (1981), 16% by Johnston and Rodgers (1973), 50% by Raschke and Paquet (1973), 13% by Denck (1971).

In those with gastric ulcers, the mortality rate was 7%. In review studies the mortality rates were fairly constant 11% (Barer et al 1982), 13.1% (Hunt et al 1983), 13.9% (Vellacott et al 1982). One person died from severe acute gastritis.

For duodenal bleeding, 3 people died from duodenal ulcer therefore, the mortality rate from duodenal ulcer

bleeding was 10%. In review studies the mortality was 6.2% (Hunt et al 1983) and 9.8% (Vellacott et al 1982). No death was recorded from duodenitis.

Although survival in this series was similar to that reported by other groups it is almost certain that this is an underestimate.

As already mentioned in the introduction, endoscopy can define the source of UGIT bleeding in around 80-90% of cases, and in this study the source of bleeding was defined in 84% of the cases. This is satisfactory, particularly when it is recognised that, in the 122 patients, 11 different endoscopists carried out the examinations. Six of these were surgeons or physicians in training. This accounts for some of the diagnostic categories identified by the endoscopist. For example, "gastric erosions" and "gastritis" are probably different interpretations of the same lesion. It was clearly not the function of the author to influence the endoscopists's assessment of the gastroscopic appearances and patients have been classified exactly as the endoscopist has described them. If "gastric erosion" and "gastritis" groups are combined, it makes little difference to the overall pattern of pathology.

A further difficulty relates to alcohol consumption and its measurement. Any attempt to measure alcohol consumption by asking the patient will inevitably be subject to bias. Patients may underestimate their consumption, they may lie in an attempt to save face and

occasionally, they may exaggerate their intake. The levels of intake chosen in this study (< 10 units/week and > 10 units/week) are somewhat lower than those normally taken to indicate problem drinking. This was done in an attempt to compensate for a systemic tendency to underestimate alcohol consumption. It is accepted that inaccuracy in the estimation of intake of alcohol will occur. Using this method, 60% of patients had been drinking in the week prior to admission.

A proportion of patients (22%) had liver disease. As already mentioned, this will affect some constituents of the clotting cascade. This will be dealt with in due course.

No mention has been made of the drug treatment given to these patients prior to sampling. Three of the oesophageal varices patients received somatostatin, all of the patients who had received blood were given cimetidine or ranitidine prior to sampling and no patient received prostaglandin agonists.

In summary, therefore, the patients recruited into the study were typical of other groups in terms of age, sex and pathology distribution. Mortality was also similar to that seen in other studies but there is a suspicion that the figures obtained underestimate the true mortality seen in Glasgow.

CHAPTER V

FIBRIN DEGRADATION PRODUCTS IN ACUTE UPPER GASTROINTESTINAL BLEEDING

Investigation of the pattern of FDP in the blood of patients with UGIT bleeding occurred in three stages. Initially, serum FDP levels were measured in all 122 patients described in the previous chapter. The standard Wellcome kit assay does not distinguish fibrinogen degradation products from cross-linked FDP and may be affected by clotting *in vitro* during preparation of serum (Nieuwenhuizen, 1987). Therefore an assay for *plasma* levels of cross-linked FDP using specific monoclonal antibodies (Dimertesst ELISA, AGEN) was evaluated retrospectively in stored plasma retained from patients in the prospective study (see section VI). The third part of this chapter stemmed from the realisation that the standard tests are time-consuming and require laboratory assistance. If high serum FDP levels actually predict a poor outcome, the test only has clinical value in so far as it is available to the clinician, on the ward, at all times of the day. The Thrombo-Wellcotest is a simple screening test for high FDP levels and again, its use was evaluated retrospectively in serum stored from the earlier testing.

5.1.1 Serum FDP (Wellcome FDP Kit)

At 09.00 a.m. on the morning after admission and before endoscopy, venous blood was taken from resting, fasting patients with minimal vein occlusion. Samples

were treated as described in section II.

Analysis of FDP results was performed as follows:-

a) Anatomical Site of Bleeding

1) Oesophagus

2) Stomach

3) Duodenum

4) Other (undiagnosed site of haemorrhage)

b) Pathological Causes of Bleeding

1 - Oesophagitis

2 - Mallory Weiss Syndrome

3 - Oesophageal Varices

4 - Oesophageal Peptic Ulcer

5 - Gastritis

6 - Ca. Stomach

7 - Gastric Benign Ulcer

8 - Gastric Erosion

9 - Stomal Ulcer

10 - Duodenitis

11 - Duodenal Ulcer

c) Severity of Bleeding

1 - Patients who had not been transfused

2 - Patients who had a transfusion, without surgery or death

3 - Patients requiring surgery, or patients who died.

5.1.2 Results

a) Site of Bleeding

Table 5.1 shows the FDP results grouped according to

Table 5.1 Measurement of F.D.P. in groups related to anatomical site of bleeding, (others = 20 patients undiagnosed and two patients with stomal ulcer)

Site of Bleeding	Number of patients	Mean Age (year)	Mean FDP (µg/ml)	Median FDP (µg/ml)	SEM FDP (µg/ml)
Oesophagus	34	54	36	10	9.3
Stomach	34	56	39	7.5	2.3
Duodenum	32	51	22	10	6.0
Others	22	58	8	3.5	3.6

clinical severity and indicating site of bleeding: Thirty four patients exhibited bleeding from the oesophagus, 34 patients from the stomach, 32 patients from the duodenum, 2 patients from a stomal ulcer and 20 patients were undiagnosed.

FDP was significantly higher in the groups with bleeding from the oesophagus, stomach or duodenum, compared to the group in which the site of bleeding was undiagnosed (Kruskal-Wallis test, $p < 0.01$). The FDP variations within the first 3 groups were not statistically significant.

b) Pathological Causes of Bleeding (Table 5.2)

Patients with oesophageal varices or gastritis tended to have higher levels than the other pathological groups. No serious conclusions can be drawn because of small numbers in each diagnostic group.

c) Severity of Bleeding (Figure 5.1, Table 5.3)

In the first group (no transfusion), the FDP levels were usually normal. They were higher in group 2 (patients who subsequently required a blood transfusion) and highest in group 3 (those who subsequently required surgery or died). The Kruskal-Wallis test was highly significant ($p < 0.001$). The Wilcoxon Mann-Whitney tests showed significant differences between group 1 and group 2 ($p = 0.05$), group 1 and group 3 ($p = 0.01$) and group 2 and group 3 ($p = 0.01$).

d) FDP level according to medical therapy (Table 5.4)

Three oesophageal varices patients received

Table 5.2 Measurement of FDP in groups related to pathological cause of bleeding in upper gastrointestinal tract.

Pathological Cause	No. of patients	Mean Age (Years)	Mean FDP (µg/ml)	Median FDP (µg/ml)	SEM FDP (µg/ml)
Oesophagitis	3	55	5	5	0
M.W.	12	50	13	5	6.6
Varices	15	58	67	30	17.7
Oesophageal Ulcer	4	64	11	10	3.8
Gastritis	12	43	79	6	64.9
Ca. Stomach	2	64	45	10	35.0
Gastric Ulcer	15	51	15	8	5.1
Gastric Erosion	5	50	11	7	4.1
Stomal Ulcer	2	41	3.75	2.5	1.3
Duodenitis	3	43	5	4	1.2
Duodenal Ulcer	29	52	24	10	6.5

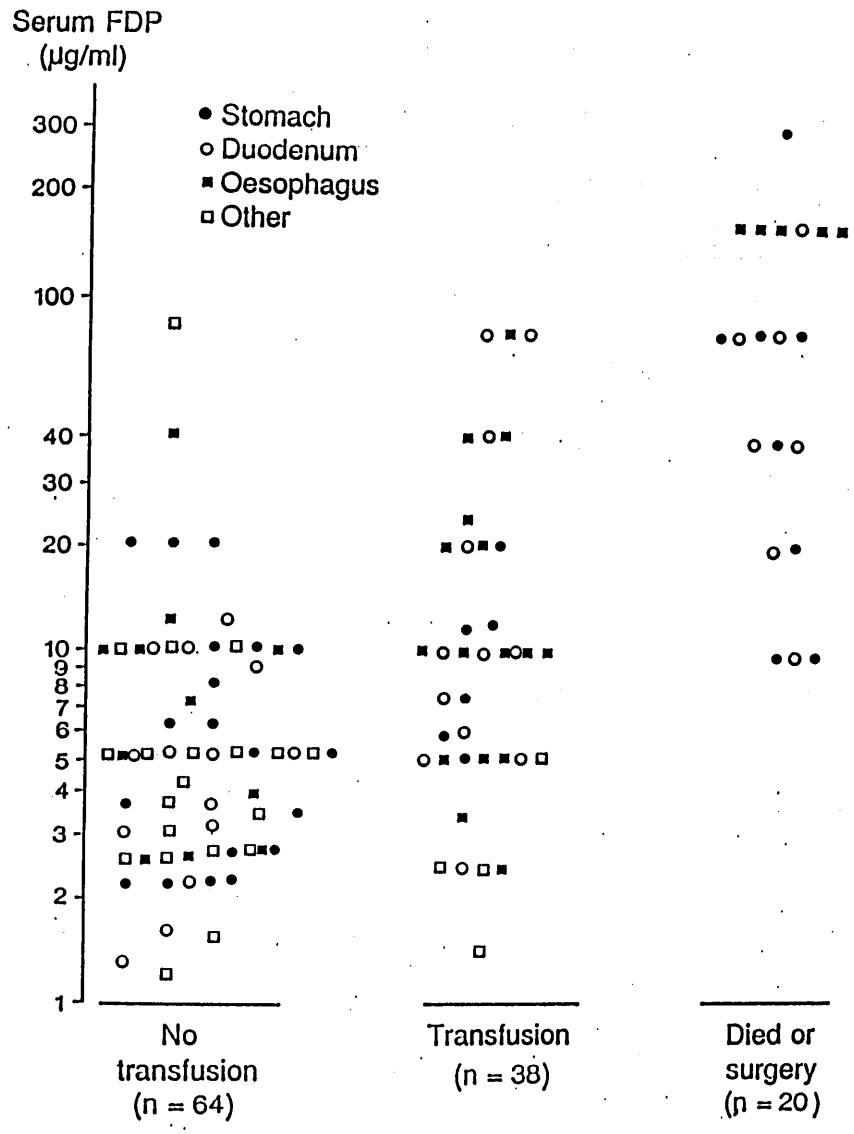

Figure 5.1 Relationship between F.D.P. and severity of bleeding. First group: no blood transfusion; Second group: with blood transfusion; Third group: required surgery or died. Site of bleeding as follows:

Stomach

Duodenum

Oesophagus

Others (undiagnosed, and 2 patients patients with stomal ulcer).

Table 5.3 Relationship between F.D.P. and severity of bleeding. First group: no blood transfusion; second group: with blood transfusion; third group: required surgery or died.

Groups of Patients	No. of patients	Mean Age (years)	Mean F.D.P. (µg/ml)	Median F.D.P. (µg/ml)	S.E.M. F.D.P. (µg/ml)
1 Non-Transfusion	65	56	8.2	5	5.1
2 Transfusion	37	51	15	8.8	3.3
3 Surgery/ Died	20	64	114	80	6.0

Kruskal-Wallis one way analysis of variance group 3 > group 1 or 2 $p < .001$.

Wilcoxon rank sum test 1 vs 2 $p = 0.05$

1 vs 3 $p = 0.01$

2 vs 3 $p = 0.01$

Table 5.4 This contingency table relates the numbers of patients receiving cimetidine to the presence or absence of elevated FDP levels. Cimetidine appears to have no effect on FDP level.

	FDP < 10 µg/ml	FDP > 10 µg/ml	
Cimetidine	45	24	69
No cimetidine	40	13	53
	85	37	122

p > 0.5 X2 = 0.07 D.F. = 1 N.S.

somatostatin before samples. This number is too small to permit a genuine assessment or to assess the effect of this drug on FDP level although it did not appear to have any effect. 69 of the 122 patients had received cimetidine before sampling. The relationship between cimetidine and raised FDP is shown in table 5.2. There appeared to be no association between raised FDP and cimetidine intake. No patient received prostaglandin agonists.

e) Multivariate Analysis

The association between FDP and outcome may reflect mutual associations with other variables of prognostic importance. Therefore, to determine whether FDP were of independent prognostic value, a multivariate logistic regression analysis of good outcome (no transfusion, n = 64) versus poor outcome (transfusion, surgery or death, n = 58) was performed, including not only FDP levels but also other variables shown to be prognostic variables in the literature (age, pulse rate, diastolic blood pressure, haemoglobin level, site of bleeding, and stigmata of active bleeding at endoscopy).

After including these variables, the serum FDP level was still of independent prognostic significance ($p < 0.025$), as were pulse rate ($p < 0.04$) and age ($p < 0.05$).

Although the site of bleeding was not predictive of outcome, patients with oesophageal varices had high FDP levels (Figure 5.1). Therefore the analysis was repeated after excluding the oesophageal group, and the

qualitative results were identical. Serum FDP level was an important independent prognostic factor ($p < 0.001$) as was haemoglobin level ($p < 0.001$). Age ($p = 0.05$) and stigmata of bleeding ($p = 0.08$) were of borderline significance. It was therefore concluded that FDP were of prognostic significance and that this was not only due to the high FDP levels in the oesophageal varices group.

5.2 Study of Plasma D-Dimer in Acute UGIT Bleeding

5.2.1 Methods and Patients

Stored plasma samples were available from 62 patients in the prospective study. The conditions under which they were taken have already been described (section II).

Plasma D-dimer was measured using an ELISA assay (AGEN, Parsippany, New Jersey) as described in Chapter II.

Results were analysed as follows (Table 5.5):

a) Severity

Group 1 - No transfusion

Group 2 - Blood transfusion but no surgery or death

Group 3 - Treated by surgery or died

b) Site of Bleeding

Oesophagus

Stomach

Duodenum

Others (including undiagnosed patients)

Table 5.5 Relationship between D-dimer and severity of bleeding.

Group 1: patients with no blood transfusion

Group 2: patients with blood transfusion but no surgery or died

Group 3: patient requiring surgery or died

Groups	No. of patients	Mean age (Years)	Mean D-dimer (ng/ml)	Median D-dimer (ng/ml)	SEM D-dimer (ng/ml)
Group 1	32	54	130	80	15.9
Group 2	20	56	138	94	32.2
Group 3	10	58	621	460	132.5

Kruskal-Wallis one way analysis of variance Gp 3 > Gp 2 or Gp 1. $p < 0.05$.

5.2.2 Results

Plasma D-dimer levels were measured in 62 patients with upper GIT bleeding. Their age ranged from 30 years to 80 years, with a mean of 52 years and a median of 54 years. This number comprised 36 (58%) men and 26 (42%) women. Twelve patients were bleeding from the oesophagus: 4 patients with Mallory-Weiss tears; 2 patients with oesphageal varices and 1 patient with oesophagitis.

Twenty two patients were bleeding from the stomach: 12 patients with gastric ulcer, 4 patients with gastritis, 2 patients with carcinoma of stomach and 4 patients with gastric erosions.

Eighteen patients were bleeding from the duodenum; 17 patients from duodenal ulcer and 1 patient from duodenitis.

Mean levels of D-dimer were similar in Groups 1 and 2, but significantly elevated in Group 3 (Kruskal-Wallis test, $p. < 0.05$) (Table 5.6, Fig. 5.2).

5.3 Study of Serum F.D.P. Measured by a Rapid Screening Method (Thrombo-Wellcotest) in Acute UGIT Bleeding

5.3.1 Methods and Patients

Stored sera were available from 36 patients with haematemesis (with or without melaena) and melaena. Patients who had been treated by antifibrinolytic drugs, and those with blood dyscrasias were excluded.

The test used is a rapid, latex test for detection of raised fibrinogen degradation products (over 10 ug/ml). The Thrombo-Wellcotest (Wellcome, Beckenham, Kent) is

Table 5.6 F.D.P. (Latex test) was performed on 36 patients with upper gastrointestinal bleeding.

Result Test	Total Patient	Not requiring surgery	Requiring surgery or died
Positive	7	2	5
Negative	29	29	0

Fisher's exact test $p < 0.0001$.

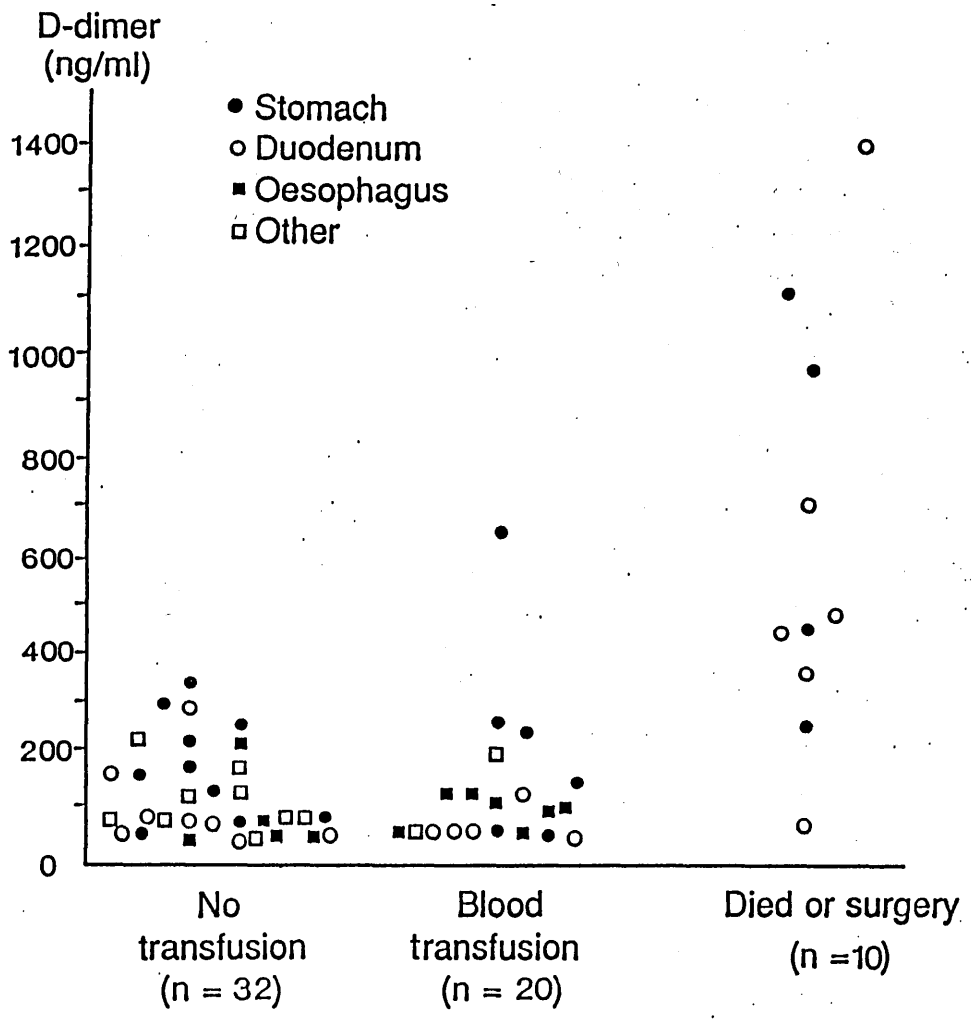

Figure 5.2 Relationship between D-dimer and severity of bleeding. First group: no blood transfusion; Second group: with blood transfusion: Third group: surgery or died. Site of bleeding as follows:

Stomach

Duodenum

Oesophagus

Others (undiagnosed and 2 patients with stomal ulcer).

designed as a slide agglutination method in which one drop of serum sample and one drop of latex suspension are mixed for a period of two minutes by gentle rocking. An agglutinated pattern is seen at the end of the test period (Chapter 2). It is ideally suited for use on the ward.

5.3.2 Sample of blood

As Section 5.1.

5.3.3 Results (Table 5.6)

Thirty-six patients were included in the Thrombo-Wellcotest study. One patient had oesophagitis; 2 patients Mallory-Weiss syndrome; 1 patient had oesophageal varices, and 1 patient had an oesophageal peptic ulcer. From those who had bleeding from the stomach, 3 patients had gastritis, 8 patients gastric ulcer, and 4 patients gastric erosion. Thirteen gastric ulcer, and 4 patients gastric erosion. Thirteen patients had a duodenal ulcer and 1 had a stomal ulcer. In 2 patients the site of bleeding was undiagnosed.

Seven patients showed a positive test for raised fibrinogen degradation products; of whom 5 patients had surgery or died (Fisher's test, $p < 0.0001$). The first patient had a gastric ulcer and died without surgery. The second patient had acute gastritis owing to an overdose of paracetamol and died after medical treatment. The third and fourth patients who had a duodenal ulcer were treated by surgery; and the fifth patient whose duodenal ulcer was treated by laser, died from severe bleeding.

Of the other 2 patients who had positive tests but no surgery, one had severe bleeding and received 6 units of blood. Endoscopic diagnosis showed a posterior large gastric ulcer with necrotic tissue in the ulcer and a visible vessel. The indications for surgery were high, but his general condition was not satisfactory for operation. The patient was put on medical treatment and survived. The second patient had a duodenal ulcer.

5.4 Discussion

Poller (1979) described raised serum FDP levels in a percentage of a small series of patients with acute upper gastro-intestinal bleeding. The main finding of the current, much larger study was that elevated FDP levels were related to the severity of bleeding (Fig. 5.1). The upper limit of the "normal range" for the Wellcome FDP kit varies from 5-10 ug/ml according to laboratory (Lowe and Prentice 1980) and in this laboratory is less than 5 ug/ml. Levels of 10 ug/ml or above were observed in above were observed in 18 of 64 patients in Group 1 (no transfusion), 21 of 38 patients in Group 2 (transfusion but no surgery or death), but in all 20 patients in Group 3 (surgery or death) Fig. 5.1).

Several possible explanations for this prognostic value of FDP levels can be considered.

1) Raised serum FDP levels might result from absorption into the bloodstream of degraded, soluble fragments of blood clot in the lumen of the gastro-intestinal tract. The greater the blood loss,

therefore the higher the serum FDP level may be as a result of the larger amount of blood in the gut.

2) Raised serum FDP levels might arise as a result of blood transfusion, e.g. due to stimulation of intravascular coagulation and hence fibrinolysis by haemolysed red blood cells. This possibility has been addressed in the next chapter.

3) Raised serum FDP levels were higher in Group 3 because of their association with certain sites or pathologies of bleeding (or with other factors such as age) which have a high risk of death or surgery. This may be part of the explanation. Figure 5.1 shows that most patients in whom no site of bleeding was identified at endoscopy had normal FDP levels (less than 10 ug/ml). Conversely, the highest FDP levels were found in patients with oesphageal varices, who have a high risk of death or surgery (Fig. 5.1) This finding is consistent with the study of Bertaglia et al (1983), who found higher levels of serum FDP in 11 patients with bleeding oesphageal varices (mean 30 ug/ml) compared to 13 patients with cirrhosis without bleeding (mean 7 ug/ml). Bertaglia et al (1983) suggested several mechanisms by which intravascular coagulation might be stimulated in cirrhosis, and these are discussed further in Section IX. However, patients with bleeding from the stomach or duodenum who were in Group 3 also had higher levels of FDP than patients bleeding from similar sites in Groups 1 and 2 (Fig. 5.1). Furthermore, multivariate analysis

showed independent predictive value of FDP.

4) Raised serum FDP levels in Groups 2 and 3 reflect increased fibrinolytic activity which promotes continued bleeding by digesting the haemostatic fibrin plugs at the site of bleeding. This possibility is consistent with the metanalysis of the results of controlled trials of the anti-fibrinolytic drug, tranexamic acid, which showed its efficacy in reducing re-bleeding and mortality (Henry and Collins 1988).

The upper limit of the "normal range" for the plasma D-dimer level in this laboratory is 300 ng/ml. Only one patient in group 1 and one patient in group 2 had raised levels, whereas 8 of the 10 patients in group 3 had raised levels (Figure 5.2). These results therefore show a similar prognostic value for plasma D-dimer levels in acute upper gastrointestinal bleeding as for the traditional serum FDP test. Furthermore they indicate that the raised FDP levels reflect lysis of cross-linked fibrin, rather than non-cross-linked fibrinogen. However the test does not distinguish intravascular, haemostatic fibrin from extravascular fibrin (e.g. fibrin blood clot within the lumen of the gastrointestinal tract).

The results of this pilot study suggests that plasma D-dimer levels (which can also be measured rapidly by a latex test) be studied in a larger, prospective study to establish their prognostic value in acute upper gastrointestinal bleeding.

However, the latex test used in the third study is a

simple and rapid test, which takes only two minutes, and which might allow the test to be performed in the investigation of all patients who have upper GIT bleeding.

All patients who were negative avoided surgery and none died. From the 7 patients who were positive, 5 patients required surgery or died. This pilot study is consistent with the results of the quantitative study of FDP levels (Section 5.1), in which FDP levels over 10 ug/ml were associated with a high risk of surgery or death. A further, larger prospective study is suggested to evaluate the prognostic significance of this simple test.

CHAPTER VI

STUDY OF PLASMA PLASMINOGEN ACTIVATOR LEVELS IN ACUTE UGIT BLEEDING

6.1 Introduction

Patients with acute UGIT bleeding have been shown in Chapter V to have increased serum F.D.P. and, presumably elevated fibrinolysis.

It is therefore possible that systemic plasma plasminogen activator levels are raised in such patients.

Plasma plasminogen activator activity was therefore studied by Fibrin Plate Lysis Area (F.P.L.A.) produced by the plasma euglobulin fraction. This test measures the global plasminogen activator level in plasma including the effects of any inhibitors in the euglobulin precipitate (Chapter II).

6.2 Patients and Methods

F.P.L.A. was measured in all 122 patients who were studied prospectively, whose clinical features were described in Chapter IV. Method of blood sampling and assay methods are as described in Chapter II.

6.3 Results

In the prospective study 122 patients with upper GIT haemorrhage were included. Their ages ranged from 19 years to 80 years, with a mean of 54 years and median of 56 years.

This number comprised 73 (60%) men and 49 (40%) women.

The mean level of F.P.L A. in the whole group was 70 + SD 40 mm2. This is very similar to the results for a previous group of patients of similar age studied by the same method in Glasgow Royal Infirmary at the same time of day prior to elective surgery (Blamey et al, 1984). These results suggest that acute UGIT bleeding does not result in any general change in basal plasma plasminogen activator levels.

The F.P.L.A. levels did not differ according to site of bleeding: see Table 6.1.

Furthermore, there were no significant differences between groups when classified as to the type of pathology (see Table 6.2).

Patients were divided by outcome into 3 groups:-

(1) Patients who had no transfusion
(65 patients)

(2) 37 patients who required blood transfusion but did not die

(3) 20 patients who either had surgery or died

Mean F.P.L.A. levels were not significantly different between group 1 and group 2 ($p > 0.3$), or between group 2 and group 3 ($p > 0.2$). However, group 3 was significantly lower than group 1 ($p = 0.02$). (Table 6.3 and Figure 6.1).

6.4 Discussion

The fibrin plate lysis area of the euglobulin fraction of plasma is a global measurement of plasma

Table 6.1 Relationship between F.P.L.A. and site of bleeding (oesophagus, stomach, duodenum and others).

Site of Bleeding	No. of patients	Mean age (years)	Mean FPLA (mm^2)	S.E.M. FPLA (mm^2)	Median FPLA (mm^2)
Oesophagus	34	54	74.9	6.7	78
Stomach	34	56	62.6	6.5	64
Duodenum	32	51	67.9	7.1	70
Others*	22	58	80	9.9	78

* Others = Undiagnosed patients and 2 patients with stomal ulcer.

(Kruskal-Wallis one way analysis of variance). No significant differences between groups.

Table 6.2 F.P.L.A. in relation to pathological underlying cause of bleeding.

Cause of bleeding	No. of patients	Mean Age (years)	Mean FPLA (mm^2)	SEM FPLA (mm^2)	Median FPLA (mm^2)
Oesophagitis	3	55	47	24.8	17
M.W.	12	50	74	13.9	95
Oesophageal varices	15	58	80	9.0	75
Oesophageal ulcer	4	64	64	14.5	54
Gastritis	12	43	77	11.3	78
Ca. stomach	2	64	67	10.6	56
Gastric ulcer	15	51	51	9.3	40
Gastric erosion	5	50	57	20.1	38
Stomal ulcer	2	41	107	38.2	69
Duodenitis	3	43	94	31.2	64
Duodenal ulcer	29	52	65	7.2	68

Table 6.3 Relationship between F.P.L.A. and severity of bleeding (not transfused, transfused and surgery/died).

Group	No. of Patients	Mean FPLA (mm^2)	S.E.M. FPLA (mm^2)	Median FPLA (mm^2)
1) Non-transfusion	65	77.1	9.7	78
2) Transfusion	37	67.3	12.2	74
3) Surgery/Died	20	55.1	13.4	60

(Kruskal-Wallis one way analysis of variance).

Group 1 v Group 2

Group 2 v Group 3 v

Group 1 v Group 3 $p < 0.02$.

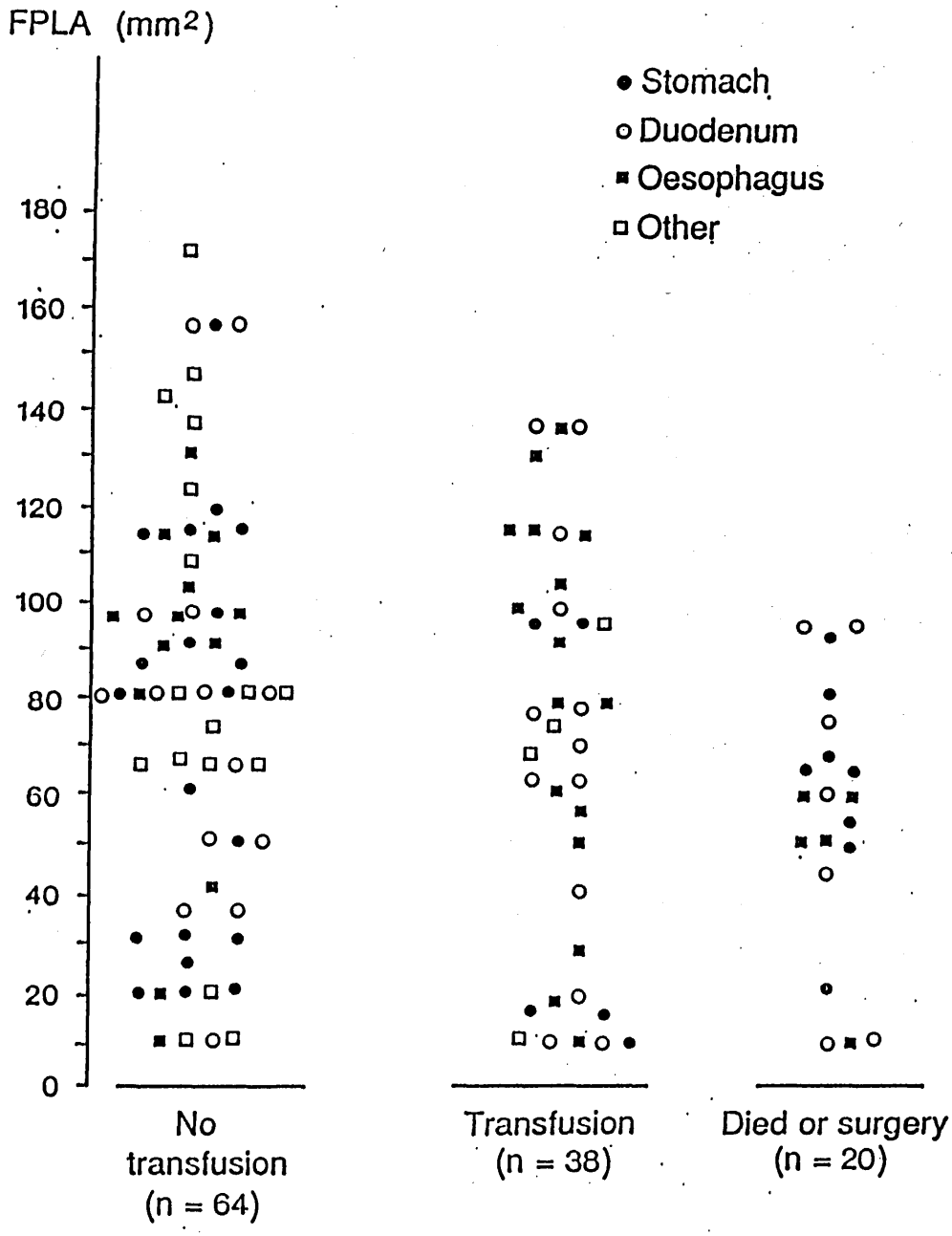

Figure 6.1 F.P.L.A. and severity of bleeding divided into three groups

(1) patients without transfusion

(2) patients with blood transfusion but not requiring surgery or died

(3) patients who had surgery or died.

plasminogen activators, as well as their inhibition by plasminogen activator inhibitors (Brommer, 1988). It may therefore be useful as an estimate of the "fibrinolytic potential" balance between plasminogen activators and their inhibitors (Brommer, 1988). In the present study, the fibrin plate lysis area was determined to test the hypothesis that a systemic increase in plasminogen activator levels might be present in patients with acute upper gastro-intestinal bleeding and might correlate with persistent bleeding. This hypothesis was suggested by previous studies showing fibrinolytic activity in the stomach (Poller, 1979).

Fibrin plate lysis area levels in the whole group of patients with acute gastrointestinal bleeding were similar to levels found by the same method in a group of patients of similar age, studied prior to elective gastrointestinal surgery (Blamey et al, 1984). This finding suggests that, as a group, patients with acute gastrointestinal bleeding do not have a significant increase in plasma fibrinolytic potential. The increased levels of F.D.P. observed (described in Section V) must therefore result from increased <u>local</u> fibrinolytic activity.

Fibrin plate lysis area levels were not significantly associated with <u>site</u> of bleeding (i.e. oesophagus, stomach, duodenum, or the undiagnosed group). This finding suggests that no particular upper gastrointestinal site of bleeding is associated with

increased plasma fibrinolytic potential. Similarly, no significant association with the pathological lesion was observed.

A significant association was observed between severity of bleeding and fibrin plate lysis area. This finding is a determinant of prognosis in acute upper gastrointestinal bleeding. A lower fibrinolytic potential in the poorest outcome group (Group 3) was observed when compared to the best outcome group (Group 1). Two possible explanations are suggested. Firstly, plasminogen activators may have been consumed in adsorption to local fibrin in Group 3 (which had the highest FDP level, see Section V). The second, more probable, explanation is that the ill patients in Group 3 had the highest levels of the acute-phase reactant, plasminogen activator inhibitor (PAI). This would be similar to the finding that, in patients with septic shock, those with high levels of plasma plasminogen activator levels had the poorest outcome (Kruithof, 1988). Measurement of PAI levels would be required to test this hypothesis and, at the time of study, such tests are not available in this laboratory.

In summary, plasma plasminogen activator levels were poorly related to site, pathology, and outcome of acute upper GI bleeding with the exception that the lowest levels were found in the group with poorest outcome.

CHAPTER VII

THE EFFECT OF TRANSFUSION OF STORED BLOOD ON THE FIBRINOLYTIC SYSTEM

7.1 Introduction

As mentioned in the previous section, one explanation for the finding that the more severely ill patients had elevated indices of fibrinolysis is the possibility that stored blood either contains products of fibrinolysis or itself stimulates fibrinolysis. There is no evidence in the literature that this is the case. However, it was necessary to check this possibility and an appropriate study was carried out.

7.2 Aim of Study

The aim of this study was to assess the effect of blood transfusion on the fibrinolytic system as measured by serum F.D.P. levels and plasminogen activator levels (fibrin plate lysis area).

7.3 Patients and Methods

A review of the patients included in the large study reported in chapter III showed that at the time of sampling, no patient in the study had received more than four units of blood. A number of patients who were receiving blood transfusion for elective reasons were recruited. Ten patients (four females and six males) were recruited. Six patients had been admitted electively for assessment of advanced colon cancer and were being considered for chemotherapy. One patient had breast cancer, two patients had oesophageal varices and

had been admitted for elective sclerotherapy. Accordingly, they were stable at the time of admission. On patient was admitted for investigation of anaemia and the subsequent diagnosis was considered to be diverticular disease.

No patient had active bleeding at the time of admission. None had a blood dyscrasia and no patients were on anticoagulants at the time of the study.

All patients were judged by the clinicians in charge of their cases to require blood transfusion and the number of units transfused was recorded.

Blood samples were taken as previously described. All patients wre fasting at the time of sampling which was 09.00 a.m. Transfusion was then started. In nine patients, the time of the post transfusion sample was at least twelve hours later and this sample was taken immediately transfusion was stopped. In one patient only one unit was transfused and sampling took place six hours after the pre-transfusion sample.

Standard methods were used for measurement of F.D.P. in serum and the fibrin plate lysis area was used to measure plasminogen activation (see section II).

7.4 Results.

The results of the F.D.P. levels in serum are shown in table 7.1 and the results of the F.P.L.A. test on plasma are shown in table 7.2. No significant differences are noted in either F.D.P. level or F.P.L.A. after transfusion in stable patients.

Table 7.1 10 patients with blood transfusion: serum F.D.P. before and after blood transfusion.

No. of patient	Blood tranfused (units)	F.D.P. before transfusion (µg/ml)	F.D.P. after transfusion (µg/ml)
1	4	40	40
2	2	80	80
3	3	20	20
4	2	11.2	20
5	2	3.5	5
6	1	2.5	2.5
7	3	20	20
8	2	10	10
9	2	5	5
10	3	10	10
Median		10.6	15.0
S.D.		23.75	23.42

Non significant Wilcoxon signed rank test.

Table 7.2 10 patients with blood transfusion: plasma F.P.L.A. before and after blood transfusion.

No. of Patients	Blood Transfusion (unit)	Before Transfusion mm^2	After Transfusion mm^2
1	4	94.9	86.6
2	2	0	0
3	3	63.6	132.7
4	2	136.8	169.63
5	2	78.5	63.6
6	1	50.2	38.5
7	3	78.5	95
8	2	50.2	54
9	2	106	113
10	3	78.5	83.6
Median		78.5	85.1
S.D.		36.79	48.43

Non significant Wilcoxon signed rank test.

7.5 Discussion

The suitability of these patients to act as subjects for the study of fibrinolytic activity in the post transfused state should be commented upon. Firstly, it should be made clear that these patients were not intended to be "controls" for the UGIT bleeding group. It was necessary to find patients receiving blood for clinical reasons (since there are obvious ethical constraints preventing the use of volunteers willing to be transfused homologous blood). Those patients had to receive several units of blood and obviously post-operative patients who might still have activated fibrinolysis were unacceptable. Patients with profound anaemia seemed to be the most acceptable subjects.

In ten patients who were stable at the time of sampling, no significant increase in FDP level was noted after transfusion of up to four units of blood. The range of level seen in these patients was from 2.5 to 80 ug/ml with a median of 10.6 ug/ml. This is comparable to the levels found in the survey of bleeding patients where patients with non-variceal bleeding had median values of 10 ug/ml when the bleeding was from a duodenal ulcer. The ten patients reported here had similar amounts of blood given to them as in the duodenal ulcer group (although the UGIT bleeding patients went on to have more blood on average) and there was no evidence of alteration of serum FDP level. The one patient who had post-transfusion sampling carried out in the afternoon

when diurnal variation might have been expected to produce a higher value had identical figures for both pre and post-transfusion samples.

Similarly, the patients had FPLA measurements similar to those found in the least ill of the UGIT bleeding patients. The median FPLA was 78.5 mm2 in these ten patients while it was 68 mm2 in the bleeding duodenal ulcer patients. In the bleeding patients who did not require transfusion, median FPLA was 77.1 mm2. In terms then of the levels of the indicators measured, it seems that the present group of ten patients were comparable with the less ill of the study group.

Apart from obvious differences in pathology, the main source of a false negative result in this study would be the possibility that a slower rate of transfusion in the electively transfused patients would allow clearance of FDP and therefore mask the process of fibrin breakdown. Overall, it seems acceptable to conclude that the elevated indicators of fibrinolysis seen in the bleeding group are not due to the transfusion of stored blood.

CHAPTER VIII

FIBRINOLYTIC ACTIVITY IN PATIENTS WITH LIVER DISEASE

8.1 Introduction

It has already been argued in previous sections that increased fibrinolytic activity *in vivo* (raised plasma FDP level) is indicative of severity in patients with UGIT bleeding and that, by itself, the FDP level is a good predictor of outcome in these patients. Inevitably a series of UGIT bleeding patients in Glasgow will include a large percentage of patients with oesophageal varices. This is especially true of the Royal Infirmary because the treatment of this condition is a special interest of the University Surgical Unit in this hospital and it acts as a regional referral centre, accruing patients from all the surrounding Health Board areas.

It might be argued that the oesophageal varices patients in the study might have elevated FDP levels as a result of their liver disease, and because the poor prognosis group contained several such patients, perhaps they had a confounding effect on the results. This possibility is unlikely in view of the fact that the multiple logistic regression analysis reported in section V showed that FDP level was an independent and significant predictor of risk and that this effect was greater than that of site of bleeding. However, the effect of liver disease on fibrinolysis is well documented.

Accordingly, it was decided to carry out a prospective study of fibrinolytic activity in patients with proven cirrhosis, sample in the chronic phase and if possible in the acute phase of further active bleeding.

8.2 Patients and methods

54 patients with biopsy proven cirrhosis of the liver were recruited into this study. All patients were attending the University Department of Surgery on a regular basis for chronic sclerotherapy to oesphageal varices. Clinical examination, including assessment of severity of hepatic failure using Child's classification (Pugh et al 1973), was carried out on each patient. Fasting blood samples were taken at 09.00 a.m. on the morning of admission before endoscopy and before sclerotherapy. No patient had bled in the month before recruitment. Patients in this group were followed prospectively and a proportion of these sclerotherapy patients were admitted subsequently as a result of UGIT bleeding. Ten patients with acute UGIT bleeding due to oesophageal varices were admitted from the chronic sclerotherapy programme and, therefore, they had results of FDP and FPLA test available both pre and post haemorrhage.

Blood samples were taken and assays carried out for serum FDP and plasma FPLA as already described in Chapter II. Results were compared to the 10 healthy controls previously described, as well as to the laboratory normal range.

Table 8.1 Modified Child's classification

Score	1	2	3
Ascites	absent	slight	moderate
Encephalopathy	absent	minimal	severe
Bilirubin (umol/l)	< 34	34-51	> 51
Albumin (g/l)	> 35	28-35	< 28
Prothrombin time (seconds prolonged)	1-4	4-6	> 6

For each characteristic recorded, patients score as indicated

Grade A = < 6

B = 7 - 9

C = > 10

(Pugh et al 1973)

8.3 Results

Of the 54 patients admitted for elective sclerotherapy, there were 37 men and 17 women. The median age was 61 years with range from 31 to 84 years. The results of the laboratory investigations carried out on these patients are shown in table 8.2 Most conventional indicators of liver function were normal or close to normal reflecting patient selection for chronic sclerotherapy.

Serum FDP levels in patients with cirrhotic liver disease showed significant differences between the 3 groups (Fig. 8.1, Table 8.3). The median values of grades a, b and c were 5 ug/ml, 10 ug/ml and 25 ug/ml respectively. Grades a or b did not show a significant increase compared to normal values or to each other, but for groups b and c the difference between the means was highly significant ($p < 0.01$) (Wilcoxon rank sum test).

F.P.L.A. levels were significantly higher in patients with liver cirrhosis compared to expected normal values, but there was no significant difference ($p > 0.1$) between Child's grades (Table 8.4, Figure 8.2). The median F.P.L.A. levels of grades a, b and c were 95 mm2, 95 mm2 and 98 mm2 respectively.

Tables 8.5 and 8.6 show the mean levels of FDP and FPLA according to pathological type of cirrhosis. There were no significant differences between these groups: however, there were few subjects in each of the 3 gruops with non-alcoholic cirrhosis.

Table 8.2 Liver function test in 54 patients with hepatic cirrhosis.

	Range	Mean	Median	Normal range
Serum albumin	20-46 g/l	35.8 g/l	35 g/l	35-45 g/l
Serum bilirubin (total)	10-135 umol/l	42 umol/l	35 umol/l	3-22 umol/l
Plasma thrombin time	13-26 second	19 second	19 second	12-15 second
AST	12-132 u/l	50 u/l	44.5 u/l	12-48 u/l
ALT	12-107 u/l	31 u/l	25 u/l	3-55 u/l
Serum alkaline phosphatase	80-1580 u/l	309 u/l	192 u/l	80-280 u/l

AST = serum aspartate aminotransferase

ALT = serum alanine aminotransferase

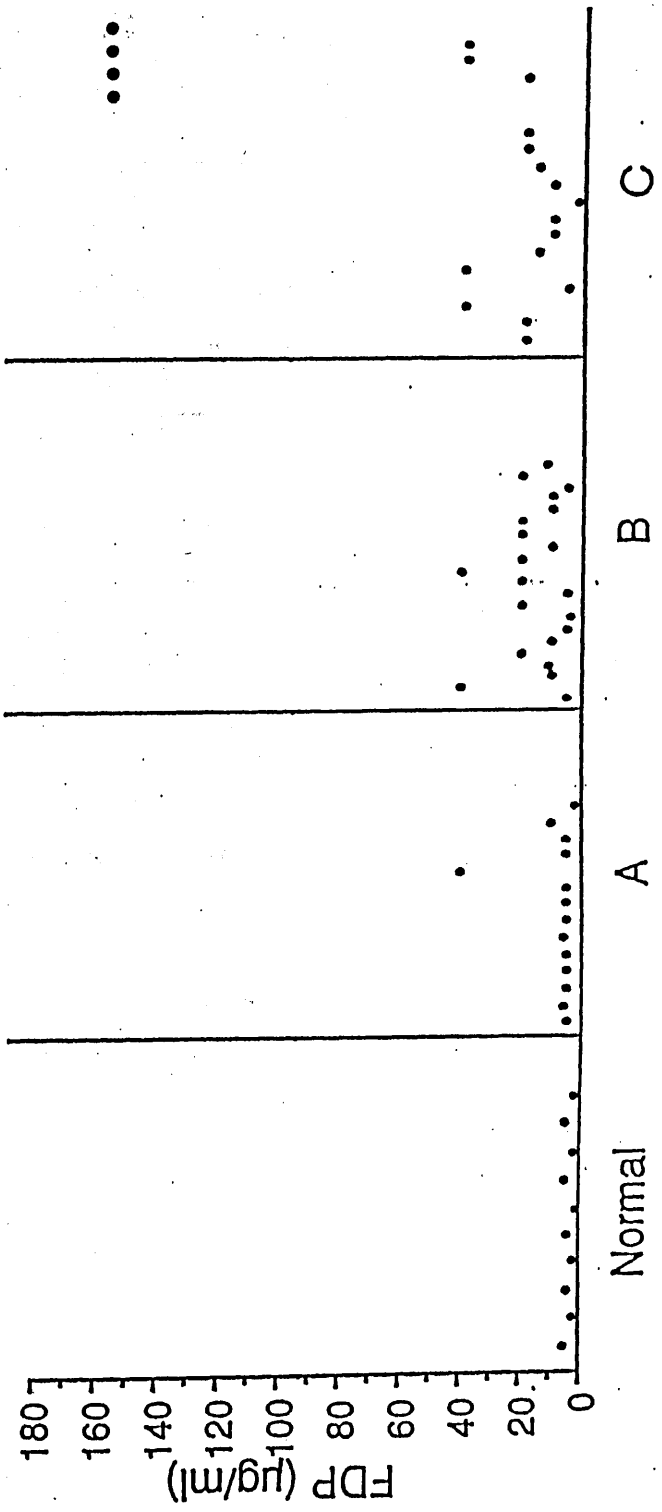

Figure 8.1 Serum F.D.P. in patients with cirrhosis compared to controls. The patients have been split into 3 groups based on the modified Child's classification.

Table 8.3 Child's Classification and measured mean serum FDP in stage A, B, C in patients with liver cirrhosis

Child's Stages	No. of Patients	Mean Age Years	Mean F.D.P. ug/ml	SEM F.D.P. ug/ml	Median F.D.P. ug/ml
A	14	56	8	0.8	5
B	21	55	13	0.4	10
C	19	57	50	2.8	25
Normal	10	30	3.4	0.7	2.5

Laboratory normal range < 10 ug/ml.

Table 8.4 Child's Classification and mean F.P.L.A. in Stage A, B, C in patients with liver cirrhosis.

Child's Stages	No. of Patients	Mean Age Years	Mean F.P.L.A. mm^2	SEM F.P.L.A. mm^2	Median F.P.L.A. mm^2
A	14	56	98	0.8	95
B	21	55	97	0.9	95
C	19	57	107	1.6	98
Normal	10	30	70	3.2	60

Laboratory normal range 47-121 mm2, mean 79 mm2.

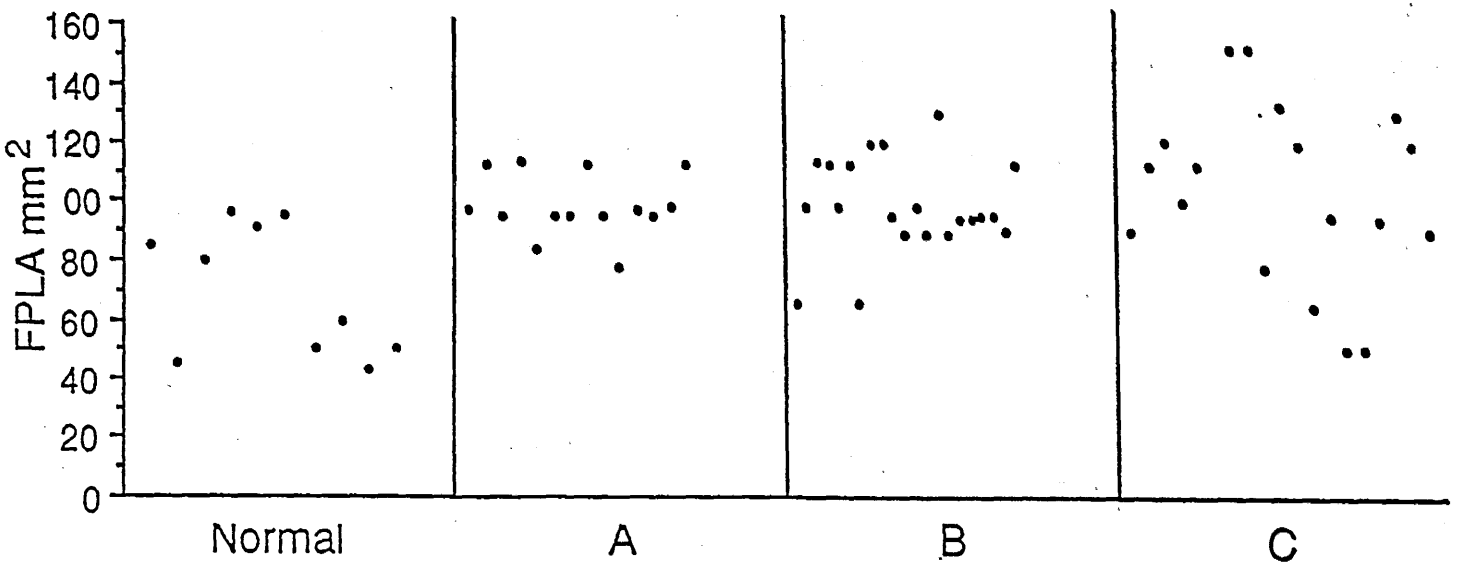

Figure 8.2 F.P.L.A. in patients with portal hypertension compared to controls. The portal hypertension patients have been split into 3 groups based on the modified Child's classification.

Table 8.5 Relationship between serum F.D.P. and pathological diagnosis of liver cirrhosis.

Causes	No. of Patients	Mean Age Years	Mean F.D.P. (µg/ml)	SEM F.D.P. (µg/ml)	Median F.D.P. (µg/ml)
Alcoholic cirrhosis	36	55	13.5	1.9	10
Cryptogenic cirrhosis	8	66	4	0.6	3.5
Primary biliary cirrhosis	6	64	23	6.2	15
Primary sclerosing cholangitis	4	50	10	0	10

Table 8.6 Relationship between F.P.L.A. and pathological diagnosis of liver cirrhosis.

Causes	No. of Patients	Mean Age Years	Mean FPLA (mm^2)	SEM FPLA (mm^2)	Median FPLA (mm^2)
Alcoholic cirrhosis	36	55	104	3.5	98
Cryptogenic cirrhosis	8	66	99	2.4	92
Primary biliary cirrhosis	6	64	97	0.7	96.5
Primary sclerosing cholangitis	4	50	135	1.2	120

Of the patients on the chronic sclerotherapy programme, ten patients were admitted with acute bleeding during the period of this investigation and were available for study. Their ages ranged from 35 to 71 with a median of 61 years. There were 7 men and 3 women.

Three of these patients settled after tamponade and had elective sclerotherapy carried out on the same admission. Seven patients failed to stop bleeding with a Minnesota tube and required emergency sclerotherapy. Two of these patients continued to bleed and died of haemorrhage. The FDP level and FPLA results are shown in table 8.7 and Figure 8.3.

The median FDP in this group was 20 ug/ml and the median FPLA was 95 mm2. FDP level was significantly greater than that seen in the blood samples taken from these patients before haemorrhage ($p < 0.01$ Wilcoxon rank sum test for matched pairs).

8.4 Discussion

This study confirms that patients with hepatic cirrhosis and portal hypertension have elevated levels of plasma plasminogen activator activity (increased FPLA levels), as well as increased levels of serum FDP. Such findings have been previously reported in some other small series (Brozovic 1987). The present study shows that raised FDP levels are related to the severity of cirrhosis, being most marked in Child's grade C. While a trend to higher FPLA levels in Child's grade C was

Table 8.7 Ten patients with acute oesophageal varices bleeding and measurement of serum F.D.P. and plasma F.P.L.A. with mention of diagnosis of liver disease

No.	Sex	Diagnosis	Serum F.D.P. (ug/ml)	Plasma F.P.L.A. (mm2)
1	M	Alcoholic cirrhosis	20	90
2	M	Alcoholic cirrhosis	160	50
3	M	Primary sclerosing cholangitis	10	95
4	M	Alcoholic cirrhosis	10	130
5	F	Alcoholic cirrhosis	20	95
6	F	Primary biliary cirrhosis	40	113
7	M	Alcoholic cirrhosis	5	131
8	F	Primary biliary cirrhosis	40	93
9	M	Alcoholic cirrhosis	20	113
10	M	Alcoholic cirrhosis	160	50
		Mean	48.5	96.0
		SD	59.9	28.4
		Median	20	95

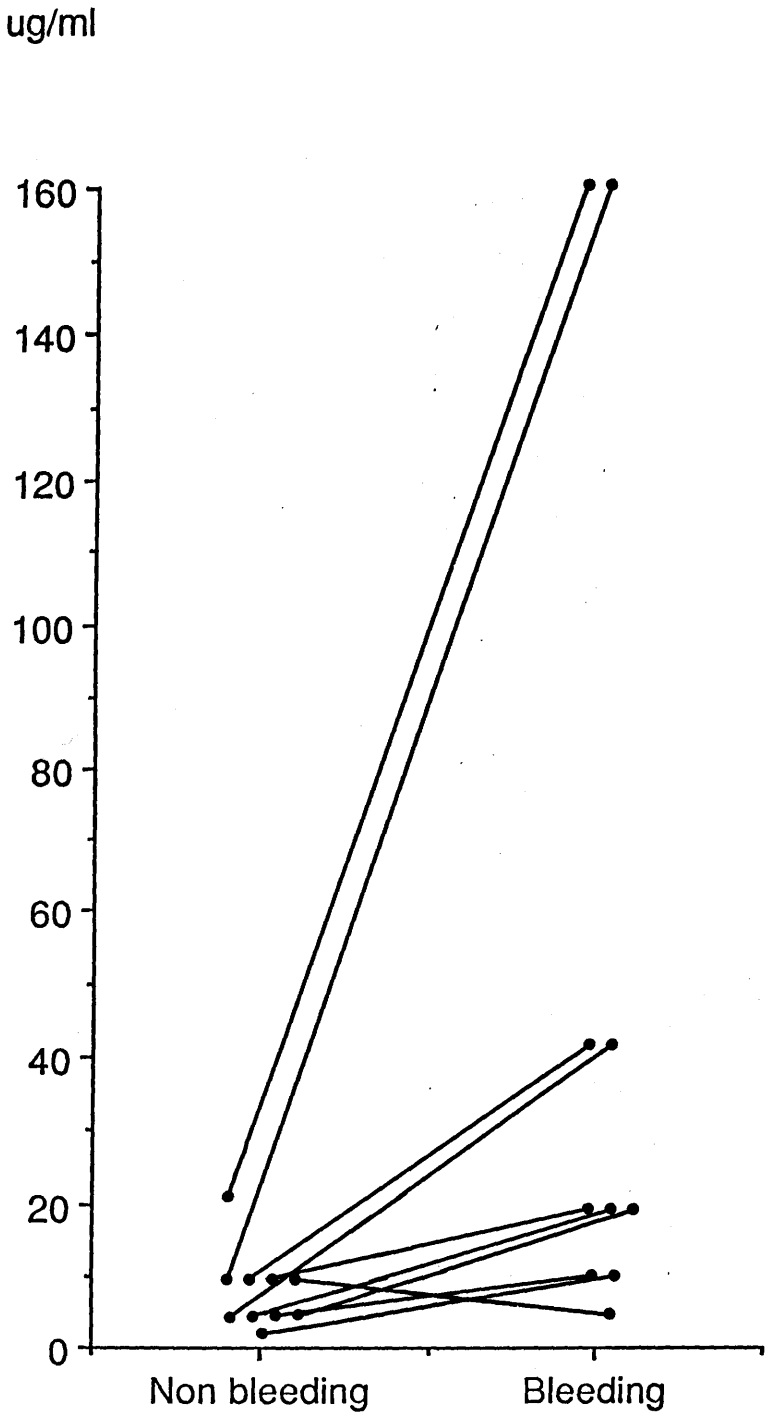

Figure 8.3 Ten patients with liver cirrhosis: serum F.D.P. pre and post bleeding from oesophageal varices.

observed, this was not statistically significant.

The raised FPLA levels in cirrhosis may be due to impaired hepatic clearance of plasminogen activators (Balkuv-Ulutin, 1978), or to enzymes other than plasmin, such as proteases released from blood cells (Latallo et al, 1978). It is also possible that low levels of the recently described plasminogen activator inhibitor type I (PAI-1), which is produced by hepatocytes, endothelial cells and platelets (Kruithoff, 1988) may contribute to the raised FPLA levels.

The association of raised FDP levels with severity of cirrhosis suggests that, as with other haemostatic changes (Brozovic, 1987), increased degradation of fibrin reflects the severity of liver damage. The median FDP level in patients with Child's grade C (25 ug/ml) is similar to that reported in the study of Bertaglia et al (1983). Patients in Child's grade C are also more likely to have acute variceal bleeding but when bleeding supervened in the group of ten patients a further rise in serum FDP was noted.

The relationship between severity of cirrhosis, acute bleeding, and increased FDP levels may be interpreted in several ways. Increased FDP levels may be the result of the disturbances in coagulation and fibrinolysis in severe cirrhosis, or may result from absorption of lysed clot from the gut in patients with acute bleeding. On the other hand, increased fibrinolytic activity may promote breakdown of haemostatic plugs in oesophageal varices,

promoting bleeding. Bertaglia et al (1983) found that 9 of 11 patients with bleeding oesophageal varices due to hepatic cirrhosis had raised serum FDP levels using the same Wellcome method. These authors suggested several other possible mechanisms for activated coagulation and fibrinolysis in such patients, including release of thromboplastins from necrotic liver cells, endotoxaemia; reduced hepatic clearance of activated coagulation factors; reduced antithrombin III levels, and stagnation of blood in collaterals.

Antifibrinolytic drugs have sometimes been used in treatment of bleeding varices (Ratnoff 1977) but no large studies have been performed. A recent textbook of medicine (Weatherall et al, 1987) and a recent review of the management of bleeding due to portal hypertension (Burroughs, 1988) do not even mention this approach to treatment. Further trials of prevention and treatment of bleeding from oesophageal varices are certainly required (Burroughs, 1988). The results of the present study are consistent with a role for fibrinolysis, and suggest evaluation of antifibrinolytic agents in such patients.

CHAPTER IX

DISCUSSION

Although individual studies in this thesis have been discussed as part of the relevant chapter, the nature of the problem addressed requires some general points to be made in a final summing up. The management of UGIT bleeding remains one of the most complex and difficult areas in modern surgery. It is a subject which often causes controversy at scientific meetings and argument in journals. The reason for this, in the author's opinion, is clear. Of all the studies carried out on these patients, most have involved insufficient numbers to allow firm conclusions to be drawn. The use of endoscopy has revealed a new range of prognostic factors - stigmata of recent haemorrhage. Initial enthusiasm for early endoscopy seemed to wane with the observation that a policy of early endoscopy did not apparently reduce mortality. However, as pointed out in the introduction 70% of patients admitted with UGIT haemorrhage will settle without further treatment. If a policy of endoscopy for all is followed, it can only give information of benefit in 30% of cases. Thus, if a treatment given on the basis of endoscopy were to be successful in reducing, for example, the rebleeding rate by a third and we want to be 90% certain of picking up a difference between the treatments at a significant level of 0.05, a study of over 800 patients would be required! (Fleiss 1981).

This comment is not intended as a criticism of all research in this field although papers which describe controlled trials consisting of 10 or 12 patients in each group surely do little to inform the debate. It seems, however, that it is the descriptive, retrospective studies such as the classic work of Avery Jones (1956) which have advanced the understanding of the condition and thereby (possibly) improved prognosis.

An appreciation of the factors which worsen prognosis has allowed earlier surgery in a more appropriate group of patients. The identification of age, shock and anaemia as contributing to a poor prognosis and the resulting tendency of the clinical community to investigate and treat aggressively any patient recognised as falling into a high risk group has probably done more to improve outcome than the efforts of the pharmaceutical industry.

However, the clinical realities of the condition must be recognised. UGIT bleeding is caused by many different pathological processes in different parts of the upper GI tract, is affected by many different patient-associated factors and has many different possibilities for investigation and management. A compromise must be reached between the purist approach which demands hundreds if not thousands of patients in carefully matched study groups and a more intuitive, clinical approach which recognises the limitations of clinically based research. With these comments as background,

several points need to be made about the studies in this thesis.

The introduction identified several studies advocating methods for treatment of patients with UGIT bleeding. None of these seem to be universally applicable and most seem to suffer from the defect mentioned above - that of the statistical type 2 error. H2 receptor antagonists may be useful but their intellectual appeal has not been confirmed in large studies. Most have included small numbers of patients and many are inconclusive. Indeed, the notion of reducing acid secretion in gastritis and gastric ulcer patients many of whom will already have reduced gastric pH is not particularly sensible. The concept that enhanced fibrinolysis in the UGIT is responsible for continued or recurrent bleeding is more appealing and seems to be more widely applicable. The theory was given more support by the paper by Henry and O'Connell (1989) which used metanalysis to deduce that an anti-fibrinolytic drug, tranexamic acid, improved outcome in UGIT bleeding patients. The experimental work reported in this thesis set out to establish how frequently the fibrinolytic system was upset in these patients. Could it be used to predict, for example, which patients would benefit from the use of tranexamic acid?

Accordingly a study of emergency admissions to the medical receiving unit of a general hospital was

arranged. The constraints surrounding collection time for blood sampling (9 a.m.) prevented a strict sequential design for patient accrual and also prevented clear matching of patients into predetermined groups. Despite this, a group of 122 patients with an age and sex composition who were typical of the UGIT bleeding population as a whole was collected. The sample appeared to be a reasonable approximation of the population, and comparable to other reported series.

Clearly, there were several items of information where data could not be collected with precision. For example, patients are unlikely to have been completely honest about alcohol consumption. It seemed reasonable to try to differentiate between heavy drinkers and social drinkers and a deliberately low cut off point of 10 units/week was chosen to make this distinction. No other means of assessing alcohol intake was available to the author, since blood alcohol estimation at the time of sampling only reflects the past few hours' consumption. In the event, it produced a plausible distribution of drinking habits with 44% admitting to social drinking and 22% falling into the heavy drinking category. Drinking category did not contribute any predictive power to the multivariate analysis of prognostic factors.

Another compromise had to be made over laparoscopic accuracy. During the period of the study, the author had no GMC registration and was, therefore, legally prohibited from taking part in the clinical care of the

patients. The diagnoses arrived at for the patients were, therefore, completely the responsibility of the several endoscopists. The author made no attempt to influence the endoscopist in his assessment of the bleeding source. "Gastritis" and "gastric erosions" are probably identical pathologies but it was felt that data collection would be simplified by taking the diagnosis as reported by the endoscopist rather than asking him to select from predefined categories. What this study has shown is that alterations in FDP level in acute UGIT bleeding occur irrespective of pathology, and if this study were to be repeated, it would be reasonable to narrow the diagnostic categories without loss of any information.

Some patients had received medical treatment directed at stopping bleeding by the time blood sampling for FDP measurement was undertaken. In most cases, this was an H2 receptor antagonist. As described, there was no association between receipt of these drugs and raised FDP level. This reflects a tendency amongst junior medical staff to prescribe these drugs in a "blanket" fashion to all patients admitted with a UGIT bleeding problem. No patients received tranexamic acid or prostaglandin agonists.

The biggest area of dissatisfaction in study design for the author lies in the fact that sampling had to take place at a standard time - 09.00 a.m. Although required by the diurnal variation of the FPLA level, it meant that many seriously ill patients could not be included in the

study. Where clinical urgency required that endoscopy and surgery was carried out without delay, the patient could not obviously wait for a standard sampling time. The endoscopy policy in this hospital, however, ensured that a number of severely ill patients did enter the study. Unlike some other hospitals there is no blanket policy to ensure that all UGIT bleeding patients are endoscoped. Endoscopy is considered in the Royal Infirmary to require consultant referral. This philosophy means that many patients wait until the end of the medical receiving round before an endoscopist is contacted with the request. As a result, it was the author's impression that earlier treatment might have been obtained for some patients. These patients had received blood transfusions before sampling. The study reported in chapter VII supported the impression gained from the literature that transfused blood does not produce an increase in FDP or plasminogen activation. Although many patients went on to have more units of blood, no patient had had more than four units at the time of sampling at 09.00 a.m. The selection of the control patients for this study seems appropriate therefore.

If the selection of patients for study were controlled by expediency, the methods used to study them were not. The methods for measurement of FDP and FPLA are standard techniques which have been in use for two decades including the laboratory used by the author (Lowe

and Prentice 1980). The normal ranges for a local population have been well established and a "normal" control population was not justified in view of the widely accepted nature of the tests as carried out in this laboratory. It should be re-emphasised that the patients described in chapter III were studied to confirm diurnal variation in the FPLA as carried out by the author not to validate the methods per se. It is not strictly correct to describe them as controls, therefore. Their function was to demonstrate that the author could perform the laboratory analysis with sufficient accuracy and also to determine the best time for sampling in view of the diurnal variation in fibrinolytic activity. It was not to demonstrate a normal range which is already in the literature for this laboratory.

A final point relating to patient and method selection should be made with regard to the subjects described in chapter VIII. It was appreciated that a possible confounding factor in this study was the inclusion of so many patients with liver disease. Hepatic disorders are known to cause an elevation in serum FDP levels and it might be claimed that the elevation seen in patients with severe UGIT bleeding was due to severe liver disease (and oesophageal varices) rather than the haemorrhage. The multivariate analysis described in chapter V fails to support this point (oesophageal varices did not predict outcome) but it was felt necessary to establish for certain that bleeding caused

an elevation in FDP in addition to that present due to liver disease. The opportunity to study this was afforded by the fact that this hospital has a large chronic sclerotherapy programme for oesophageal varices patients. Several of these patients were tested during admission for routine sclerotherapy in the knowledge that several of them would be admitted as an emergency during the study. This happened in eleven patients. The rise in serum FDP seen, even in patients with already elevated levels, supports the results of the earlier statistical analysis of chapter V, that raised FDP levels are related to bleeding rather than to pathology.

Having discussed the limitations on patient selection in this study, attention should now be paid to the results. It was clear that an elevation in serum FDP was associated with an increased likelihood of death or surgery. Issue could be taken with the decision to assess severity of illness according to the need for transfusion and the need for surgery or death. It is argued that to patients who, on clinical grounds do not need transfusion are likely not to have had a significant bleed. Patients who die of bleeding clearly have a serious lesion. In this study, seven of the eleven deaths occurred during uncontrolled haemorrhage. Of the other deaths, three were due to acute renal failure secondary to hypovolaemic shock. Two cardiac deaths were arguably contributed to by hypovolaemia and one death due to septicaemia occurred as a result of poor lower limb

circulation and resulting gangrene. All the deaths could arguably therefore be due to the direct or indirect consequences of bleeding. Also the inclusion in this group of the surgical patients is justified on the grounds that in each case an experienced surgeon decided that continued or recurrent bleeding was unlikely to stop without surgical intervention. The group of middle severity, those requiring transfusion, is the one most subject to subjective influences. This group will include patients inappropriately transfused one or two units of blood right up to one patient given 29 units of blood but who was not referred for surgery. Clearly this is a very heterogeneous group reflecting the practices of many clinicians. Despite this, there was a significant increase in FDP level when group 1 was compared with the transfused group. The surgery/death group had a highly significant increase.

Plasminogen activation as determined by FPLA was inversely related to outcome, and an explanation for this observation (high PA1 levels) had been advanced in the relevant chapter. This remains to be tested in future studies. Regardless of the explanation for FPLA changes, it is clear that an elevated FDP level in plasma is a highly sensitive index of severity in UGIT bleeding, and in the 36 patients for whom serum was still available for testing. The simple Thrombo-Wellcotest identified correctly all five patients requiring surgery or who died. The results from this small study give the test a

specificity of 94% and a sensitivity of 100%. These figures are impressive and when it is remembered that this test takes 30 seconds to perform, it suggests that the Thrombo-Wellcotest could be an integral part of the assessment of UGIT bleeding patients. This requires validation in a future study.

It is this aspect of the study which is put forward as being the original contribution of this thesis. The various criticisms concerning patient selection in a sense become irrelevant beside this fact. It does not really matter what the patients died of, the test seems able to predict the most seriously ill. It does not really matter whether the patients came to surgery for continuing primary haemorrhage or for rebleeding, the test was able to determine on the morning following admission whether or not the patient fell into a high risk group. The multivariate analysis confirmed that the predictive power of a raised FDP level was greater than any of the conventional indicators.

Some information has been gained as to the cause of the elevated indices of fibrinolysis. The raised FDP level might occur as a result of absorption of soluble clot fragments into the blood stream from the UGIT, and does not appear to result from blood transfusion.

This study suggests other experimental work. If the origin of the FDP is enhanced fibrinolysis in the stomach, rather than systemically, this possibility could be examined by sampling from gastric vein and a

peripheral artery to obtain an A-V difference for FDP across the stomach. This was considered for this study, but ethical committee permission was not envisaged on the grounds that these samples added a small but significant hazard to the surgical procedure. With the benefit of the results of this study now being available, a stronger case for investigation of these levels could perhaps be made.

The important study which now needs to be done is to assess the effects of tranexamic acid in these patients using the Thrombo-Wellcotest to select a high risk group. If this showed a clear cut benefit, particularly in the varices patients, this would benefit management of acute UGIT bleeding. As it is, the case for enhanced local fibrinolysis playing an important role in breaking down clot formation in bleeding vessels in the UGIT seems stronger as a result of the findings reported in the present thesis.

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Appendix 1

Case Record for patients with upper GI bleeding

Study No. ☐☐☐

Name

Address

Telephone No.

G.P.

Hospital No. ☐☐☐☐☐☐

Date of Birth ☐☐☐☐☐☐

Sex 1. Male 2. Female ☐

Ward ☐☐

Consultant ☐☐

Date of Onset of Bleeding ☐☐☐☐☐☐

Date of Admission ☐☐☐☐☐☐

Date seen ☐☐☐☐☐☐

Haemodynamic state (worst data)

Pulse B/min ☐☐☐

B.P. Systolic/Diastolic ☐☐☐/☐☐☐

Units of blood transfused ☐☐☐

Units of plasma/colloid ☐☐☐

Units of crystalloid ☐☐☐

Clinical features

Haematemesis 1. Yes 2. No ☐

Melaena 1. Yes 2. No ☐

Drugs 1. NSAID 4. More than 1 ☐
2. Steroids 5. None
3. Anticoagulants 6. Other

Smoking Cigs/day ☐☐

Liver disease 1. Yes 2. No ☐

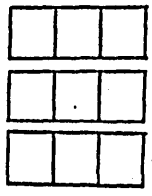
Previous peptic ulcer disease 1. Yes 2. No ☐

Previous GI bleed 1. Yes 2. No ☐

Alcohol

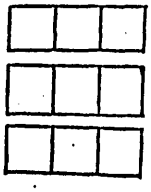
1. None
2. Previous drinker
3. Social drinker
4. Heavy
5. Not known

Height cm.
Weight kg.
% Ideal body wt.



Routine laboratory data

Hb.
Serum Bilirubin
Serum Creatinine



Study No.
Sheet 2.

Name _____

Endoscopy findings

Oesophagus
1. Normal
2. Oesophagitis
3. Hiatus hernia
4. M - W
5. Varices
6. Carcinoma
7. Other
9. Not seen

Stomach
1. Normal
2. Gastritis
3. Carcinoma
4. Gastric ulcer
5. Gastric erosions
6. Other
9. Not seen

Duodenum
1. Normal
2. Duodenitis
3. Duodenal ulcer
4. Other
9. Not seen

Stoma
1. Ulcer
2. Normal
9. Not seen

Source of bleed.
1. Oesophagus
2. Stomach
3. Duodenum
4. Stoma

Stigmata
1. Visible vessel
2. Active bleeding
3. Slough
4. Fresh clot
5. None

Laboratory investigations

Fibrin plate
F.D.P.
Coagulation screen 1. Normal
2. Abnormal
3. Not done

Drug treatment

1. Antacids
2. Cimetidine/Ranitidine
3. Prostaglandins
4. Somatostatin
5. Tranexamic acid
6. More than 1
7. Other
9. None

Further bleeding 1. Yes 2. No

Hb. Day 2
Day 3
Day 4
Day 5
Day 6
Day 7

Total units blood transfused

Surgery 1. Yes 2. No

Operation carried out:

Undersewing of vessel 1. Yes 2. No

Other procedures

1. Vagotomy & drainage
2. Partial gastrectomy
3. Total gastrectomy
4. Injection of varices
5. Transection of oesophagus
6. Laparotomy alone
7. Other
8. More than one

OUTCOME

Hospital survivor	1. Yes 2. No	☐
Date of discharge		☐☐☐☐☐☐
Alive at 1 month	1. Yes 2. No	☐
If Yes ? Further bleed	1. Yes 2. No	☐
Date of death		☐☐☐☐☐☐
Post Mortem	1. Yes 2. No	☐

Appendix 2

Case Record for patients with oesophageal varices

No:

Name:

Hospital No.:

Date of Birth:

Sex:

The sample is taken at:

Date of the first injection:

Dates of the subsequent injections:

Portal hypertension is caused by:

Pugh's modification of Child's classification:

Encephalopathy:

Ascites:

Bilirubin - - - - - - - - - - - - - - - - - mmol/l

Albumin - - - - - - - - - - - - - - - - - - gram/l

Prothrombin - - - - - - - - - - - - - - - - second

Grade:

A - - - - -

B - - - - -

C - - - - -

F.D.P.: - - - -

F.P.L.A.: - - -

Hb

AST

ALT

Appendix 3

Forms used to record F.D.P. and F.P.L.A. values of patients

Name

Address:

Hospital No.

Date of Birth

Sex

Ward

Consultant

Date of Admission

Date of Sample I

Date of Blood Transfusion

Date of Sample II

Diagnosis

F.D.P. before Transfusion

F.D.P. after Transfusion

F.P.L.A. before Transfusion

F.P.L.A. after Transfusion

APPENDIX 4

Abbreviated case histories of patients requiring surgery or who died

Patient No. 80

A 52 year old woman was admitted to the surgical ward with bleeding oesophageal varices. She was treated by sclerotherapy and tamponade with blood transfusion. Her condition stabilised but after 3 days, torrential rebleeding occurred and cardiovascular collapse followed. Despite considerable efforts at resuscitation, she died. Cause of death was haemorrhage.

Patient No. 112

A 35 year old man was admitted to the surgical unit because of oesophageal variceal bleeding. He was treated conservatively but 24 hours later he had a further haematemesis and a sengstaken tube was placed after sclerotherapy. After 3 days, his condition remained poor and he was oliguric. On the 4th day after admission, rigid oesophagoscopy was carried out with injection of 18 mls of ethanolamine into his oesophageal varices. His cardiovascular condition immediately following this procedure was good. He became more alert and orientated but his urine volume remained poor and ultimately he died from acute renal failure.

Patient No. 113

A 58 year old man was admitted to the surgical ward because of haematemesis. He was known to have alcoholic liver cirrhosis. In the previous three years he had been admitted frequently to hospital because of variceal bleeding. He had had repeated courses of elective sclerotherapy for his varices. On admission, he was confused and hypotensive with blood pressure 70/30 and p.r. 120/minute. He was not in hepatic coma. Resuscitation was started and he underwent balloon tamponade with urgent sclerotherapy. He rebled 24 hours after release of the tamponade and he underwent further rigid oesophagoscopy and sclerotherapy but unfortunately died after 24 hours. The cause of death was haemorrhage.

Patient No. 118

A 33 year old man was admitted to the surgical ward because of massive haematemesis. He was known to have alcoholic liver cirrhosis. He underwent resuscitation and his varices were treated by balloon tamponade and sclerotherapy. Cardiovascular collapse caused death despite apparent control of his varices. Post mortem showed that the cause of his death was recurrent massive bleeding.

Patient No. 122

A 35 year old man was admitted to the surgical ward with severe haematemesis. On admission he gave a history of considerable alcohol abuse for some years. At the time of his admission he was transfused 8 units of blood and a sengstaken tube was immediately inserted. This failed to control his bleeding after one day and he also became very unco-operative and pulled his tube out. Endoscopy was undertaken immediately thereafter. This showed oesophageal varices with one varix spurting blood. The patient would not co-operate with the passage of another sengstaken tube and was therefore started on an infusion of intravenous somatostatin. He had been transfused 30 units of blood over two days. Endoscopy was therefore undertaken under general anaesthetic. At this time only old blood could be found in the oesophagus, stomach and duodenum although there was occasional very slight bleeding from mucosal surfaces. Overnight his general condition deteriorated further and he died. The probable cause of death was haemorrhage.

Patient No. 3

A 70 year old lady was admitted as an emergency to the medical receiving unit with a haematemesis. The day after admission she had a further significant bleed and she was transferred for emergency gastric surgery. At operation, the findings were of a benign ulcer on the lesser curve of her stomach. This was dealt with by oversewing and a truncal vagatomy and pyloroplasty was also carried out. She was discharged home on her 12th post-operative day.

Patient No. 15

A 65 year old man was admitted to the surgical ward with evidence of upper GI bleeding. Endoscopy revealed a pre-pyloric gastric ulcer and control of the bleeding was obtained by laser therapy. Thereafter his condition unfortunately deteriorated and he complained of abdominal pain and hypotension. He was transferred to the renal unit because of deterioration in his renal function but unfortunately, despite haemodialysis, he died. He is known to have had hypertension since 1967 and had a myocardial infarction in 1981. The immediate cause of death was congestive cardiac failure. Perforation of his ulcer was not thought likely on clinical and radiological grounds.

Patient No. 40

A 73 year old woman was admitted to the surgical ward because of haematemesis and melaena. She was treated by blood transfusion and a gastric ulcer was confirmed by endoscopy. Two days later she had a further significant bleed and she underwent emergency surgery. At laparotomy a gastric ulcer was found. This was treated with oversewing and trancul vagotomy and pyloroplasty was carried out. She was able to be discharged on the 10th post-operative day.

Patient No. 91

An 85 year old woman was transferred to the care of the surgeons as an emergency with a massive haematemesis following internal fixation of her right hip. She was severely hypotensive on arrival in theatre. The stomach was opened and the source of the bleeding was seen to be a large benign ulcer on the lesser curve of the stomach with a large vessel in its base which was actively bleeding. The vessel was under-run and the ulcer oversewn and vagotomy and pyloroplasty was performed. 10 days post-operatively she was able to go home.

Patient No. 25

A 35 year old was admitted to the surgical ward with a haematemesis. She was hypotensive (B.P. 60/0) on admission. Subsequently her sister admitted that she had taken an unknown quantity of NSAID tablets. Replacement of blood loss was started via a central venous line and she had an upper GIT endoscopy on the same day. Erosive gastritis was confirmed at endoscopy. Massive bleeding was observed. The patient unfortunately died two days later because of acute renal failure secondary to prolonged hypotensive shock.

Patient No. 12

A 53 year old man was admitted to the surgical ward because of haematemesis and melaena. He was known to have gastric cancer for the previous 2 years. On admission he was pale and hypotensive (B.P. 60/40). He received 8 units of blood and his condition stabilized but he had continuous vomiting due to outlet obstruction. Two days later he underwent surgery. A gastro jujenostomy was carried out. Five days later he developed hypotension and chest pain and died after a period of deterioration. Primary cause of death was recorded as acute renal failure.

Patient No. 27

A 30 year old gentleman was admitted to the surgical ward with a 2 day history of haematemesis and melaena. In the past six months he had complained of dyspepsia and occasional vomiting after meals. On admission he was confused and shocked. He was stabilized by vigorous resuscitative measures. Subsequent gastroscopy revealed a cauliflower mass in the lower part of the greater curvature of the stomach extending to the pylorus and on biopsy later proved to be an adenocarcinoma. One week later he underwent gastro jujenostomy and was discharged 7 days later.

Patient No. 28

The patient was admitted to the ward as an emergency a few days after returning home following his coronary artery bypass graft. He had several units of blood in the Lewis Hospital and seemed stable when he came in but within an hour of admission his blood pressure dropped and he became severely unwell.

He was taken immediately to theatre where vagotomy and pyloroplasty and oversewing of an extremely large bleeding vessel in duodenal ulcer was undertaken. Following this he made an excellent recovery and by two weeks was fit enough to return home.

Patient No. 32

A 63 year old man presented with a 2 day history of melaena and a 5 day history of gastric discomfort. In the past, he had undergone a truncal vagotomy and pyloroplasty for duodenal ulcer disease. On examination he was pale but haemodynamically stable. Three days later he was noted to be dizzy when standing and his haemoglobulin on that day was 4.5 gm. per 100 ml. Gastroscopy examination showed visible bleeding from a duodenal ulcer. He was taken immediately to theatre where truncal vagotomy and antrectomy was carried out. He was well at the time of discharge on the 10th post-operative day.

Patient No. 41

An 82 year old woman was admitted to the medical ward because of haematemesis. She has a history of previous myocardial infarction and duodenal ulcer. One day before admission she had had upper abdominal pain with coffee ground vomiting but her condition was stable. However, during her stay in hospital, she deteriorated with signs of shock. She died in the next 24 hours of her admission due to a combination of hypovolaemia and pump failure from myocardial infarction.

Patient No. 46

A 40 year old woman was admitted to the receiving medical ward with a two day history of haematemesis. On the day of admission she had a further significant bleeding for which she was transfused and transferred to the surgical ward. At laparotomy a bleeding duodenal ulcer was discovered which was treated by under-running, truncal vagotomy and pyloroplasty. She was discharged well on 10th post-operative day.

Patient No. 72

A 64 year old man was admitted as an emergency to the medical receiving unit with haematemesis. He had a further significant bleeding shortly after admission. He was transfused and transferred for emergency surgery. At laparotomy a duodenal ulcer was discovered which was freely bleeding. The ulcer was dealt with by oversewing, truncal vagotomy and pyloroplasty. He made an excellent recovery from surgery and was able to go home on the 12th post-operative day.

Patient No. 82

An 80 year old woman was admitted to the surgical ward because of severe haematemesis. On admission she was pale and hypotensive (B.P. 40) and radial pulses were not palpable. A central venous line was established and vigorous resuscitation measures were started. She was taken to the operating theatre where she underwent gastroscopy and an active bleeding duodenal ulcer was detected. Laser photocoagulation was attempted but the patient succumbed due to blood loss.

Patient No. 90

An 84 year old man was admitted to the emergency ward with haematemesis. In the past he had a history of duodenal ulcer which had been confirmed by endoscopy. He continued to have haematemesis and melaena in the next 24 hours for which he received blood transfusion. Surgical intervention was undertaken in view of the continuous bleeding and his age. His duodenal ulcer was treated with oversewing sutures, truncal vagotomy and pyloroplasty. He was discharged well on the 12th post-operative day.

Patient No. 102

A 59 year old man was admitted as an emergency with haematemesis and melaena. Although this was initially of a very minor degree, over the next few hours he had a substantial haematemesis and became shocked. He was therefore taken urgently to theatre for oversewing of a bleeding duodenal ulcer. Post-operatively his legs were noted to have compromised circulation and this was thought to be related to a period of shock during his bleeding. A vascular surgeon felt that embolectomy would not be helpful. He spent some days in I.C.U. following his operation but he did not make a good recovery. The circulation in his lower limbs never became re-established. His condition deteriorated rapidly and finally he died five days post-operatively at which time he was severely hypotensive. Probable cause of death was sepsis related to his ischemic limbs.