GUIDELINES

The rare coagulation disorders – review with guidelines for management from the United Kingdom Haemophilia Centre Doctors’ Organisation


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Summary. The rare coagulation disorders are heritable abnormalities of haemostasis that may present significant difficulties in diagnosis and management. This review summarizes the current literature for disorders of fibrinogen, and deficiencies of prothrombin, factor V, FV + VIII, FVII, FX, the combined vitamin K-dependent factors, FXI and FXIII. Based on both collective clinical experience and the literature, guidelines for management of bleeding complications are suggested with specific advice for surgery, spontaneous bleeding, management of pregnancy and the neonate. We have chosen to include a section on Ehlers-Danlos Syndrome because haematologists may be consulted about bleeding manifestations in such patients.

Keywords: rare coagulation disorders, fibrinogen, prothrombin, FV, FVII, FX, FXI, vitamin K dependent factors, FXIII, Ehlers Danlos syndrome, Factor V+VIII deficiency, molecular genetics

Introduction

The rare coagulation disorders are heritable abnormalities of haemostasis that may present significant difficulties in diagnosis and management to haemophilia centre clinicians. The common feature shared by these disorders is that their overall population frequency is low (with the exception of factor XI deficiency). Consequently, diagnosis and monitoring of affected individuals may require specialist phenotypic and molecular investigations that are not widely available. There may be considerable interindividual variation in bleeding phenotype amongst affected individuals resulting at least in part from the molecular heterogeneity of the rare coagulation disorders. The bleeding risks in affected individuals may therefore be difficult to assess. Since there are few long-term prospective studies of large cohorts of patients, reliable information about clinical management is often scarce. Coagulation factor support may require the prescription of unlicensed treatment products that are not readily available.

Although the rare coagulation disorders are uncommon, most haemophilia centres will have a handful of individuals with one or more disorders. Some centres may have significant number of affected individuals because of the prevalence of these disorders in populations in which consanguineous marriage is common. The objective of this guideline
document is therefore to provide a concise review of each rare coagulation disorder and where possible to provide evidence-based guidelines about diagnosis and management. Particular emphasis is placed on prophylaxis and the management of surgical patients, pregnant mothers and affected neonates. In view of the limitations of the literature describing the rare coagulation disorders, unless specified in the text, the evidence given below is level III (derived from well-designed non-experimental descriptive studies, such as case studies) or IV (based on expert committee reports or opinions and experiences of respected authorities), and the recommendations therefore grade C (an absence of directly applicable clinical studies of good quality). Details of blood products available for each disorder are not listed in these guidelines because this information has recently been published in the updated treatment guidelines from the UKHCD [1].

We have chosen to include Ehlers-Danlos Syndrome (EDS) because it is a rare disorder and affected individuals may present to haematologist with bleeding symptoms.

**General recommendations**

General recommendations based on established management protocols for the more common haemostatic disorders are also applicable to the rare coagulation disorders. Affected individuals should be registered with a haemophilia centre, and all invasive procedures should take place at a centre directly served by the haemophilia centre.

Affected individuals and family members should be offered genetic counselling and screening. It should be recognized that, with the exception of some of the fibrinogen (FII) disorders, the inheritance of the rare coagulation disorders is usually autosomal recessive. Therefore, an affected proband may be part of a kindred with very few affected individuals so that predictive information on phenotype may be scarce. It is, therefore, paramount to determine the molecular defect within affected kindreds to enable comparison with affected individuals from other kindreds reported in case series or in electronic databases.

Best practice is for pregnancy in women with severe rare disorders to be managed in an obstetric unit in a hospital that has a haemophilia centre. If this is not possible, close collaboration between the obstetric unit and haemophilia centre is required. This good communication is also important to ensure appropriate investigation and management of a potentially affected neonate, for example, where the parents are related and already have one affected child or are known to be carriers for one of these disorders. Several of the severe disorders are associated with a significant risk of intracranial haemorrhage in the first week of life.

Paediatricians and neonatologists need to be aware of the increased risk of the rare severe coagulation defects presenting in offspring of consanguineous parents. It is very important that neonates who present with unexpected bleeding be investigated urgently, and then treated vigorously to correct any severe defect. Inadequate or delayed treatment of neonatal intracranial haemorrhage leads to a lethal or poor outcome, with significant long-term disability.

All affected individuals should be vaccinated against hepatitis A and B by the s.c. route if not already immune, and should generally be advised to avoid treatment with medication that interferes with platelet function (salicylates and other non-steroidal anti-inflammatory agents). Paracetamol is a safer analgesic to use.

**Search strategy**

Each section has been written by one or more members of the group who have particular experience (clinical and/or research) of the disorder discussed. In addition to personal archives, the medical literature was searched using appropriate key words to obtain as much published evidence as possible.

**Abbreviations**

- PT, prothrombin time;
- APTT, activated partial thromboplastin time;
- TT, thrombin time;
- LMWH, low-molecular weight heparin;
- Units, we have used IU dL\(^{-1}\), where International standards are available (FII, FVII, FX) and U dL\(^{-1}\) where no International standard was established at the time of preparation (FV, FXI and FXIII). We have used percentage when referring to a publication which reported in this way. In these instances 100% is equal to 100 U dL\(^{-1}\).

**Laboratory investigation of rare bleeding disorders**

**Methodological aspects**

**Introduction** In the investigation of bleeding disorders the results of laboratory tests can be affected by the collection and processing of blood samples and by the selection, design, quality control and interpretation of screening tests and specific assays. These effects have...
important diagnostic and therapeutic implications. The purpose of this section of the guideline is to describe these variables and their potential effects and where possible to make recommendations about methodological aspects of the laboratory investigation of rarer bleeding disorders, although many will be applicable to all disorders.

Sample collection and processing

Sample collection For tests of blood clotting venous blood should be collected gently but rapidly using a syringe or an evacuated collection system when possible. Application of a tourniquet to facilitate collection does not normally affect the results of most tests for bleeding disorders, providing excessive stasis is avoided. By choice the needle should be not more than 21 gauge (for infants a 22 or 23 gauge needle may be necessary). Collection through peripheral venous catheters [2] or non-heparinized central venous catheters [3] can be successful for prothrombin time (PT) and activated partial thromboplastin time (APTT) testing. Local discard procedures should be used where necessary in order to avoid contamination of samples with heparin.

If there is any delay between collection and mixing with anticoagulant or delay in filling of collection system, the blood must be discarded because of possible activation of coagulation. Once blood and anticoagulant are mixed the container should be sealed and mixed by gentle inversion five times, even for evacuated collection systems, and vigorous shaking should be avoided. Any difficulty in venepuncture may affect the results obtained, particularly for tests of platelet function. Prior to analysis the sample should be visually inspected and discarded if there is evidence of clotting or haemolysis.

Anticoagulant, sample filling and container The recommended anticoagulant for collection of blood for investigations of blood clotting is normally trisodium citrate [4,5]. Trisodium citrate (0.105–0.109 m) has been recommended for blood used for coagulation testing in general, including factor assays [5]. One volume of anticoagulant is mixed with nine volumes of blood. Although 0.129 m trisodium citrate has been considered acceptable in the past [6] this is not currently recommended – the results of PT and APTT can be up to 10% longer in the higher strength anticoagulant depending on the reagents used [7]. The samples must be filled correctly, avoiding both under- and overfilling to give accurate results [8]. If the patient has a haematocrit >0.55 L L$^{-1}$ results of PT and APTT can be affected and the volume of anticoagulant should be adjusted to take account of the altered plasma volume [5].

The inner surface of the sample container employed for blood sample collection can influence the results obtained, particularly for screening tests. The tubes should be inert and should not induce contact activation (non-siliconized glass is inappropriate). For factor assays there is evidence that results on samples collected in a number of different sample types are essentially interchangeable [9].

There are specific differences in the haemostatic investigation of neonates and infants when compared with that of older children and adults. The British Committee for Standards in Haematology has recently produced guidelines on the investigation and management of neonatal haemostasis and thrombosis, detailing sampling procedures, laboratory investigation and interpretation of test results [10].

Processing and storage of samples prior to analysis For preparation of platelet-rich plasma to investigate platelet function, samples should be centrifuged at room temperature (18–25 °C) at 150–200 g for 10–15 min, and analysed within 2 h of collection. For most other tests related to bleeding disorders samples should be centrifuged at a speed and time which produces samples with residual platelet counts well below 10 × 10$^9$ L$^{-1}$, for example, using 2000 g for at least 10 min. Centrifugation at a temperature of 18–25 °C is acceptable for most clotting tests. After centrifugation, prolonged storage at 4–8 °C should be avoided as this can cause cold activation, increasing FVII activity [11] and shortening of the PT or APTT

Samples for APTT should be analysed within 4 h of collection [5] and although the PT of some samples may be stable for 24 h or more [12] the stability of test results in subjects with molecular defects is unknown. All investigations should therefore be completed within 4 h of sample collection or plasmas should be deep frozen within this time for future analysis. Clotting factor assay results are generally stable for samples stored at −24 °C or lower for up to 3 months [13] and for samples stored at −74 °C for up to 18 months (results within 10% of baseline defined as stable). Storage in domestic grade −20 °C freezers is normally unacceptable. If frozen samples are shipped on dry ice care must be taken to avoid exposure of the plasma to carbon dioxide, which may affect the pH [14]. Prior to analysis, frozen samples must be thawed rapidly at 37 °C for 3–5 min. Thawing at lower temperatures is not acceptable as cryoprecipitation may occur.
Screening tests The sensitivity of PT and APTT to the presence of clotting factor deficiencies is dependant on the test system employed [15–18]. The degree of prolongation in the presence of a clotting factor deficiency can vary dramatically between reagents. The level of clotting factor deficiency, which should be detected (as an abnormal result) by screening tests has not been defined. The use of different activators in APTT can influence sensitivity [18] and in addition there is marked variation between reagents in respect of phospholipid profile [19]. As for APTT, reagent variation will also influence the sensitivity of PT methods for detecting abnormalities, particularly of FVII or FX. It is useful to repeat borderline results on a fresh sample. It should be noted that the within-subject variation of PT and APTT over time might be 6–12% [20].

For both PT and APTT the degree of prolongation may be small in the presence of mild deficiency and therefore there is a need for adequate quality control procedures and for carefully established accurate normal/reference ranges. In view of the limitations of screening tests it is important that results are interpreted in conjunction with all relevant personal and family history details. Normal screening tests do not always exclude the presence of mild deficiency states.

Reference ranges In order to interpret the result of any clotting test it is important to have data on results of the test in healthy normal subjects. Health is not well-defined and is often a relative term. An age-and sex-matched normal range is the ideal control group. However, such careful selection is not essential for many coagulation tests associated with the investigation of rare bleeding disorders. Samples from normal subjects should be collected, processed and analysed locally using identical techniques to those for patient samples. The literature and reagent manufacturer’s information should only be used as a guide.

In the case of screening tests the reference values will be affected by the test system employed and the possibility that a new batch/lot of reagent from the same manufacturer has a different normal range than previous batches should be considered. For factor assays, results in normal subjects should ideally be similar by different techniques, although this is not always the case. The most suitable assay techniques are those for which the locally established normal range is similar to those reported elsewhere.

The number of normal subjects selected for analysis should not be <25, which is probably adequate for most tests of haemostasis related to investigation of rare bleeding disorders. The results should show a normal distribution. Clear or statistical outliers are probably aberrant results and it is usually acceptable to exclude these. The most frequently used convention is that the reference range should include the central 95% of values, calculated as the mean ± 2 SD as the upper and lower limits respectively. For non-normally distributed values alternative calculations may be used (see Walker [21] for review).

Any reference range should only be used as a guide and aid to clinical interpretation.

Clotting factor assay design The most commonly performed assays for clotting factors for many years have been one-stage clotting assays based on the APTT in the case of FXI and based on the PT in the case of FII, FV, FVII or FX. Guidelines for the assay of clotting factors have been published [22]. A number of general features of the design of one-stage clotting assays, which are necessary to ensure accurate, reliable and valid results are discussed below. In each case the test depends upon the ability of a sample containing the factor under investigation to correct or shorten the delayed clotting of plasma completely deficient in that factor. Such deficient plasmas must contain <1 U dL\(^{-1}\) of the clotting factor under investigation and normal levels of all other clotting factors which could influence the results obtained.

It is important that the amount of factor present in the mixture of deficient and reference/test plasma is rate-limiting in its influence on the clotting time measured by APTT or PT. This requires dilution of a reference or standard plasma of known concentration. Preparation of several different dilutions of the reference plasma allows construction of a calibration curve in which the clotting time response depends on the dose (concentration) of factor present. At lower plasma dilutions/higher factor concentrations the factor under investigation may not be rate limiting and the assay is no longer specific and therefore invalid. It may be necessary to extend the calibration curve by testing additional dilutions when analysing test plasmas with concentrations below 10 U dL\(^{-1}\). At very low concentrations of an individual factor the clotting time of the deficient plasma may not be even partially corrected by addition of diluted test plasmas. Dilutions are selected so that there is a linear relationship between concentration (logarithmic scale) and the response in clotting time (logarithmic or linear scale). The reference curve should be prepared using at least three different dilutions and a calibration curve should be included each time the assay is performed unless there is a clear evidence.
that the responses are so reproducible that a calibration curve can be stored for use on other occasions. The reference plasma should be calibrated by a route traceable back to WHO international standards wherever possible. Test plasmas should be analysed using three dilutions so that it is possible to confirm that the dose–response curve of the test plasma is linear and parallel to the dose–response curve of the reference plasma. It is not acceptable to test a single test dilution as this reduces the accuracy substantially and may lead to important underestimation of the true concentration when inhibitors are present. If a dose–response curve of a test plasma is not parallel to the reference curve, and the presence of an inhibitor such as an antiphospholipid antibody is confirmed or suspected then the estimate of activity obtained from the highest test plasma dilution is likely to be closest to the real concentration, but it should be noted that the criteria for a valid assay cannot be met and results must be interpreted with caution.

Quality assurance

Laboratory tests of blood coagulation require careful application of quality assurance (QA) procedures to ensure reliability of results. QA is used to describe all the measures that are taken to ensure the reliability of laboratory testing and reporting. This includes the choice of test, the collection of a valid sample from the patient, analysis of the specimen and the recording of results in a timely and accurate manner, through to the interpretation of the results, where appropriate, and communication of these results to the referring clinicians.

Internal quality control (IQC) and external quality assessment (EQA) are complementary components of a laboratory QA programme.

Quality assurance is required to check that the results of laboratory investigations are reliable enough to be released to assist clinical decision-making, monitoring of therapy and diagnosis of haemostatic abnormalities.

Internal quality control The IQC is used to establish whether a series of techniques and procedures are performing consistently over a period of time (precision). It is therefore deployed to ensure day-to-day laboratory consistency. It is important to recognize that a precise technique is not necessarily accurate, accuracy being a measure of the closeness of an estimated value to the true value.

Quality control procedures should be applied to ensure immediate and constant control of result generation. Within a laboratory setting the quality of results obtained is influenced by maintenance of an up-to-date manual of standard operational procedures; use of reliable reagents and reference materials; selection of automation and adequate maintenance; adequate records and reporting system for results; an appropriate complement of suitably trained personnel.

For screening tests it is important to include regular and frequent testing of QC material that should include a normal material and at least 1 level of abnormal sample. For factor assays a QC sample should be included with each group of tests. Patient results should only be released if QC results remain within acceptable target limits.

External quality assessment The EQA is used to identify the degree of agreement between one laboratory’s results and those obtained by other centres (accuracy). The primary function of EQA is proficiency testing of individual laboratory testing, but larger EQA schemes can also provide information concerning the relative performance of analytical procedures, including the method principle, reagents and instruments. Continued participation in EQA schemes has been linked to improved laboratory performance [23]. This has been seen not only in the overall performance, evidenced by a reduction of the variability of results between laboratories but also in respect of individual laboratories. Centres undertaking investigations for rare bleeding disorders should participate in an accredited EQA programme where available.

Recommendations

1 Nine parts blood should be mixed with one part 0.105–0.109 M trisodium citrate.
2 Underfilled, haemolysed or partially clotted samples should not be analysed.
3 The collection system used should not induce contact activation.
4 For preparation of platelet poor plasma, samples should be centrifuged at a speed and time that produces plasmas with residual platelet counts below $10 \times 10^9$ L$^{-1}$ (e.g. 2000 g for 10 min).
5 Analysis should be completed within 4 h of sample collection. Alternatively plasmas can be frozen at $-24 \, ^\circ\text{C}$ for up to 3 months or $-74 \, ^\circ\text{C}$ for up to 18 months.
6 The sensitivity of screening tests are influenced by reagents and only techniques that are capable of detecting clinically important abnormalities should be employed.
7 Reference ranges should be established locally using samples from normal subjects, which have
been collected, processed and analysed using identical techniques to those for patient samples. This is essential for screening tests in particular.

The most suitable assay techniques are those for which the locally established reference range is similar to those reported elsewhere.

Quality control samples should be analysed regularly and frequently for screening tests, and with each group of factor assays, and concurrent patient test results only released to clinicians whilst QC results remain within an acceptable target range.

Centres should participate in accredited EQA programmes for all tests in the investigation of bleeding disorders, where available.

One-stage clotting factor assays should be calibrated with reference plasmas traceable back to WHO standards where available.

In factor assays Test samples should be analysed using three dilutions and results are only valid when the dose–response curve of the test is linear and parallel to the reference curve.

Inherited abnormalities of fibrinogen

Background

Fibrinogen is a 340 kDa protein that is synthesized in the liver. It has a plasma concentration of approximately 1.5–3.5 g L\(^{-1}\) and a half-life of around 4 days. The FI molecule is a homodimer, each half consisting of three non-identical polypeptide chains termed \(\alpha\), \(\beta\) and \(\gamma\). The genes for all three chains are located on the long arm of chromosome 4.

Fibrin is produced by proteolytic cleavage of FI by thrombin with the release of fibrinopeptides A and B and generation of insoluble fibrin monomer followed by polymerization. FI is also important in primary haemostasis for normal platelet aggregation.

Definitions

1. Afibrinogenaemia refers to the total absence of FI measured by an antigenic assay.
2. Hypofibrinogenaemia is a decreased level of normal FI.
3. Dysfibrinogenaemia is characterized by structural abnormality of the FI molecule resulting in altered functional properties. Classically the functional assay of FI yields low levels compared with immunological assay but levels may be concordant and the functional level may even be normal.

The definition and classification of abnormalities of FI have changed with increasingly sophisticated methods of analysis. Thus, many cases originally reported as afibrinogenaemia have actually been shown to be cases of dysfibrinogenaemia and many cases of hypofibrinogenaemia are also dysfunctional (hypodysfibrinogenaemia).

There are many acquired causes of dysfibrinogenaemia and hypofibrinogenaemia. These will not be covered in this guideline.

Incidence

Afibrinogenaemia has an estimated prevalence 1:1 000 000 [24]. Data from the UKHCDO from 1999 showed 115 patients registered with 10 having received treatment during that year, three patients were on home treatment.

Geographical variation in prevalence reflects the high incidence in children of consanguineous parents in Muslim countries. Comparative data from registries in Iran, Italy and the UK highlights this geographical variation (patients with levels \(\leq 10\%\) only registered) [24].

The incidence of dysfibrinogenaemia is unknown but is probably under-diagnosed and under-reported.

Clinical phenotypes

Afibrinogenaemia/hypofibrinogenaemia

Bleeding – Afibrinogenaemia is associated with a bleeding tendency of variable severity including life-threatening, spontaneous events, but long periods without problems are also not uncommon. The published literature consists mostly of case reports with two larger case series of afibrinogenaemic patients [25,26]. In the series from Iran [25], bleeding and thrombotic manifestations were reported for 55 patients with afibrinogenaemia. Umbilical bleeding and mucosal bleeding were the most common features. Musculoskeletal bleeding was less common than in haemophilia but still occurred relatively frequently. Three of 55 (5.5%) had central

Prevalence of severe fibrinogen deficiency (level <10% of normal) in different countries.

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<thead>
<tr>
<th>Country</th>
<th>Number</th>
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<tr>
<td>Iran</td>
<td>70</td>
<td>1.5*</td>
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<tr>
<td>Italy</td>
<td>10</td>
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<td>UK</td>
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*Percentage of total number of patients registered with inherited coagulation disorders in each country.
nervous system (CNS) bleeding but the age of onset is not stated. In the other series of 10 patients from two large sibships from Israel, there was again a high incidence of umbilical bleeding with CNS and intra-abdominal bleeding also fairly common. Other authors have also noted the relative infrequency of musculoskeletal bleeding and there are a number of other case reports of CNS bleeding and intra-abdominal bleeding related to splenic rupture [27–29]. In some patients there have been recurrent events.

In hypofibrinogenaemia the bleeding pattern is similar but appears to follow a milder course and bleeding may follow invasive procedures. Pregnancy-related complications – Afibrinogenaemia and hypofibrinogenaemia are associated with recurrent miscarriage, and also with both antepartum and postpartum haemorrhage [30,31]. In one series of 13 pregnancies in six women with afibrinogenaemia, seven ended in spontaneous abortion at 6–7 weeks gestation. This suggests that FI may be important for implantation and this is supported by studies in mice with afibrinogenaemia where failure of embryo implantation has been documented [32].

Thrombosis – Paradoxically for a disorder associated with a significant bleeding tendency there are reports of thrombotic events in patients with afibrinogenaemia. Thrombotic events were recorded in two of 55 (4%) of cases in one series, including one case of sinovenous thrombosis in a 5-year-old boy and ischaemic gangrene in a 15-year-old girl [25]. These events occurred unrelated to FI replacement therapy or other obvious risk factors and the mechanism of thrombosis is obscure. There are also a small number of case reports of thrombotic events in patients with apparent afibrinogenaemia and hypofibrinogenaemia [33,34]. The details of some of these events are not well-defined and others have been reclassified as dysfibrinogenaemias, therefore, the overall incidence of thrombosis in these disorders is unclear.

Other – Impaired wound healing postsurgery has also been reported in afibrinogenaemia.

Dysfibrinogenaemia The literature relating to dysfibrinogenaemia consists predominantly of collections of case reports of patients seen at specialist centres. Most work has been carried out on the molecular analysis of dysfibrinogenaemia with relatively little on clinical aspects. This may have introduced bias, as patients with clinically more severe disease will be over represented.

The clinical phenotype of dysfibrinogenaemia is unpredictable. A compilation of over 250 patients with dysfibrinogenaemia revealed that 53% were asymptomatic, 26% had haemorrhage and 21% had thrombosis, some of whom also had haemorrhage [35]. There is some correlation between molecular defects and clinical phenotype.

Asymptomatic – Patients may present through routine laboratory tests or family studies.

Bleeding – Patients may have bleeding postpartum or with surgery and dental extraction. Bleeding from the umbilical stump and into the CNS and soft tissues are associated with lower levels. Patients may also experience delayed wound healing and dehiscence.

Thrombosis – The ISTH SSC Subcommittee on FI has received reports on 51 cases of thrombosis associated with dysfibrinogenaemia [36]. Only 26 mutations were considered to be unambiguously associated with thrombosis as defined by the following strict criteria: known molecular defect, thrombosis at early age in two or more family members, no other known risk factors, if defect found in another family this family should also exhibit thrombosis.

In the 26 index patients dysfibrinogenaemia was associated with deep vein thrombosis, thrombophlebitis and pulmonary embolus at a young age (mean: 32 years). Severe bleeding in this group was only noted postpartum. In 187 family members, 20 of 99 people with and none of 88 people without dysfibrinogenaemia had had a thrombotic event [36]. The thrombotic risk is increased with acquired risk factors and coinheritance of other thrombophilias [37].

Skin necrosis has been described with some dysfibrinogenaemias (e.g. in those homozygous for FI Marburg). Arterial thrombosis occurs but much less commonly than venous thrombosis [36].

Pregnancy – Of the 15 women in the ISTH database all had had at least one pregnancy and seven had had a postpartum thrombosis. There were 24 spontaneous abortions and six stillbirths with 34 normal deliveries [36]. Pregnant women with dysfibrinogenaemia are at risk of bleeding following vaginal delivery, Caesarean section and with regional analgesia. Bleeding may not correlate well with level of FI or thrombin time but women with undetectable functional FI predictably bleed at delivery and have a high rate of miscarriage.

Diagnosis

Fibrinogen assays should be performed in accordance with BCSH guidelines [38] (http://www.bcshtaguidelines.com/). A FI level derived from the prothrombin time should not be used.
Tests should be interpreted with regard to possible causes of acquired dysfibrinogenaemia. The patient’s age, drug history and liver function tests should be known. Family studies may be helpful.

Afibrinogenaemia There is a marked prolongation of the PT, APTT and thrombin time (TT). The bleeding time is often prolonged. FI levels are undetectable by both functional (Clauss) and antigenic assays.

Hypofibrinogenaemia Coagulation tests are prolonged in proportion to the FI deficiency. TT is the most sensitive test. The total clottable and immunogenic FIs are both reduced to a similar level as the functional FI. It is important to exclude acquired causes of hypofibrinogenaemia and family studies may be helpful.

Dysfibrinogenaemia The TT is usually the most sensitive screening test for dysfibrinogenaemia. TT is usually prolonged but in rare cases may be normal or shortened. It is important to exclude the effect of heparin and interference with fibrin polymerization, for example, by fibrin/fibrinogen degradation products (FDPs) or paraproteins, and thrombin function by a thrombin inhibitor. The reptilase time is usually prolonged but may be shortened or sometimes normal. In some cases the reptilase time is more sensitive than TT. The PT and APTT may be prolonged but are less sensitive than the TT. The sensitivity of coagulation tests to dysfibrinogenaemia are dependent on the specific mutation, reagents and technique [39].

The functional FI by Clauss method will be low. Antigenic measurements may show a decrease in proportion to the Clauss assay or be significantly higher. A definitive diagnosis requires the demonstration of a molecular defect. However, as most dysfibrinogenaemias are dominantly inherited, family studies are useful to confirm inherited rather than acquired dysfibrinogenaemia.

In patients with dysfibrinogenaemia and a history of thrombosis it may be helpful to perform a thrombophilia screen to exclude coexisting prothrombotic defects.

Molecular basis for inherited abnormality of fibrinogen

The three FI subunits (Aβ, Bβ and γ) are encoded by three different genes clustered in a region of 50 kb on chromosome 4 (q28–30), FGA, FGB and FGG. The FGA is 5.4 kb in length and consists of six exons. It encodes two different transcripts by alternative splicing at the 3′-end. The major isoform (z), which represents 99% of mRNA, is coded by exons 1–5. The extended zE variant is produced by the addition of 236 amino acids encoded by exon 6.

The FGB consists of eight exons and is 8.2 kb in length. The synthesis of the Bβ chain is the ratelimiting step in the production of mature FI and there is much research into the effect of polymorphisms within the FGB promoter region on FI levels.

The FGG is 8.4 kb long and has 10 exons. The γ-chain occurs in two isoforms (γ and γ′), which are produced by alternative splicing. The γ isoform constitutes about 15% and contains an additional binding site for FXIII and for thrombin.

Congenital afibrinogenaemia Congenital afibrinogenaemia is an autosomal recessive condition that has been shown to be due to defective FI synthesis.

Although mutations causing afibrinogenaemia have been detected in all three genes, the majority found to date are in FGA. These mutations are mainly deletions, frameshift, nonsense or splicing mutations and several recurrent mutations have been detected, including an 11 kb deletion in the FGA gene and a donor splice mutation in intron 4 + 1 G>T in FGA.

Dysfibrinogenaemia Overall 300 abnormal FIs have been described and over 100 different structural defects identified. These are mainly associated with missense mutations affecting FI structure or function. These mutations are registered on a database [35], which is available on-line. The majority (180) of these mutations are in FGA of which 74 are at (Az 16). Twenty-seven mutations have been reported in the FGB and 75 in the FGG of which 25 affect γ 275. These mutations affect regions associated with all aspects of FI function.

Mutation detection in cases of dysfibrinogenaemia has led to the realization that previously discrete cases share the same molecular abnormality. This has allowed pooling of clinical information concerning any given mutation and thus provides a useful guide for decision-making. For example, FI Bremen (Az Gly17 → Val) is associated with a bleeding disorder and delayed wound healing. Conversely, the majority of individuals with dysfibrinogenaemia [Az Arg16 → His (Bern IV and Milano XI)] were asymptomatic or associated with only a mild bleeding tendency. Individuals (65%) with FGG mutations are asymptomatic with only 5% having bleeding problems and 30%, thrombosis. Certain mutations...
such as Arg$^{554} \rightarrow$ Cys (Chapel Hill III, Paris V and Dusart) are associated with thrombosis but not bleeding [35,40].

While molecular identification of dysfibrinogenaemia is useful step in the assessment of each patient, more accurate and clinically robust information is required for all mutations registered on the database to maximize the utility of this resource.

A mutation database can be viewed at http://www.geht.org/databaseang/fibrinogen/

Treatment principles

The patient’s personal and family history of bleeding and thrombosis are important guides for management. If FI replacement is required a virally inactivated concentrate should be used (grade B recommendation based on level IIb evidence). In general a FI level below 0.5 g L$^{-1}$ is associated with an increased risk of microvascular bleeding [41].

Treatment products

There is no licensed FI concentrate available in the UK. The products that are available in Europe have been listed in the treatment guidelines [1].

A suggested amount of FI required is:

Dose (g) = Desired increment in g L$^{-1} \times$ plasma volume, where the plasma volume is $0.07 \times (1 -$ haematocrit) $\times$ weight (kg)

Therefore, to raise the FI concentration by 1 g L$^{-1}$ a dose of about 30 mg kg$^{-1}$ would be required.

The half-life of infused FI is 3–5 days [42] and, in the absence of consumption, treatment is unlikely to be needed more often than on alternate days. These pharmacokinetic data were established in studies of adult patients and so may not be applicable to children.

Although cryoprecipitate is a good source of FI it should not usually be used, as it is not virally inactivated. Its use may be considered in an emergency situation if no suitable alternative is available.

Fibrin glue may be useful to treat superficial wounds or following dental extraction. There are reports of patients developing anti-FI antibodies after their use [1].

Tranexamic acid can be useful to treat mucosal bleeding or prevent bleeding for procedures such as dental extraction, avoiding the need for blood products. Its use may, however, increase the risk of thrombosis and should be used with caution in people with a personal or family history of thrombosis and avoided when other risk factors such as pregnancy, surgery or immobilization are present.

Treatment

Management of spontaneous bleeding

Afibrinogenaemia – Fibrinogen concentrate is the treatment of choice for significant bleeding. The aim should be to increase the FI level to 1 g L$^{-1}$ and monitor the clinical response. Patients may require repeat infusions dependent on the clinical response and laboratory monitoring. The FI level should be maintained above 1 g L$^{-1}$ until haemostasis is secure and above 0.5 g L$^{-1}$ until wound healing is complete.

Indications for prophylaxis Based on the limited available data it is not possible to make a recommendation on the use of primary prophylaxis. Secondary prophylaxis may be appropriate in cases where there has been potentially life-threatening bleeding because of the risk of recurrence, e.g. intracranial haemorrhage. The frequency and dose of FI concentrate should be adjusted to maintain a trough level above 0.5 g L$^{-1}$. Antifibrinolytic agents may be useful for mucosal bleeding and oestrogen/progesterone preparations have been used in menorrhagia [43]. Antifibrinolytic medication should be used with caution in patients with a personal or family history of thrombosis.

Dysfibrinogenaemia – There are few data on the management of bleeding in patients with dysfibrinogenaemia. The abnormal FI in the patient may be dysfunctional and may interfere with the function of infused FI. Furthermore, abnormal FI may interfere with laboratory assays in patients who have received FI infusions. Raising the measured FI level in the patient to 1 g L$^{-1}$ may, theoretically, not provide adequate haemostasis.

With these considerations in mind, patients who are bleeding are likely to require FI concentrate although in some cases topical fibrin glue or tranexamic acid may be sufficient for superficial or mucosal bleeding avoiding the use of pooled blood products. The functional FI level should be raised to 1 g L$^{-1}$ above baseline level and the clinical response observed. Patients may need repeat infusions dependent on the clinical response and laboratory monitoring. The FI level may need to be maintained above 1 g L$^{-1}$ until wound healing is complete [40].

Management of surgery

Afibrinogenaemia – In the Iranian series [25] postoperative bleeding was reported in 23 of 55 (40%) of untreated cases. It is, therefore, recommended that FI...
levels are increased to 1 \( \text{g L}^{-1} \) and maintained at this level until haemostasis is secure and above 0.5 \( \text{g L}^{-1} \) until wound healing is complete.

**Dysfibrinogenaemia** – Patients with a known bleeding phenotype should be treated with a FI concentrate preoperatively to raise and maintain the FI level to 1 \( \text{g L}^{-1} \) above their baseline level until haemostasis is secure and 0.5 \( \text{g L}^{-1} \) above baseline until wound healing is complete. Venous thrombosis may be precipitated by FI concentrate and so the use of compression stockings is necessary and prophylactic heparin should be considered.

Patients with a thrombotic phenotype should be treated with compression stockings and low-molecular weight heparin (LMWH) dependent on personal and family history in accordance with previous guidelines on inherited thrombophilia [44].

Patients with both a bleeding and thrombotic phenotype are complicated and are likely to require replacement of FI to maintain a level 1 \( \text{g L}^{-1} \) above baseline and use of LMWH.

There are a number of options for asymptomatic people or those who have not had a previous surgical challenge. These are:

1. **Observe and only give FI concentrate if bleeding occurs.**
2. **Treat with FI concentrate as above (if family history is of bleeding).**
3. **Use an antifibrinolytic agent, e.g. tranexamic acid.**

   The decision will depend on the level of functional FI (patients <0.5 \( \text{g L}^{-1} \) are more likely to bleed), past history of haemostatic challenges, family history of bleeding and thrombosis.

   The use of tranexamic acid, for example, to cover dental extraction may avoid the need for pooled blood products but increases risk of venous thrombosis during surgery and should be avoided if there is a personal or family history of venous thrombosis.

**Management of pregnancy** The management depends on the FI level and the personal and family history of bleeding or thrombosis.

**Afibrinogenaemia** – Prophylaxis with FI concentrate is reported to improve pregnancy outcome and prevent postpartum haemorrhage [30,31]. Regular FI infusions are required throughout pregnancy in women with afibrinogenaemia and should be commenced as soon as possible to prevent early fetal loss. Optimal FI levels are not defined but fetal loss and bleeding have been reported in women with FI levels of >0.5 \( \text{g L}^{-1} \), therefore, levels above 1 \( \text{g L}^{-1} \) may be required, particularly toward the end of pregnancy.

**Dysfibrinogenaemia** – During delivery it should be assumed that the neonate has dysfibrinogenaemia and invasive monitoring and procedures should be avoided, particularly if the family phenotype is of bleeding. If tested at birth the interpretation may be complicated by physiological or acquired dysfibrinogenaemia particularly in preterm infants.

Asymptomatic women should be observed closely but no specific treatment is required unless bleeding occurs or the family history suggests bleeding is likely. Standard measures for venous thromboprophylaxis should be followed [45].

Women with a personal or family history of thrombosis should be offered antenatal prophylaxis with LMWH. The delivery should be closely monitored and FI replacement therapy given only if bleeding occurs. The risk of regional anaesthesia is difficult to assess and should be avoided as the woman may be on heparin and haemorrhagic complications cannot be excluded. General anaesthesia, however, increases risk of venous thrombosis and a plan should be made in advance after discussion with the patient and an obstetric anaesthetist.

There are a number of management options for women who have a bleeding phenotype. The decision will depend on the person and family history of bleeding.

Vaginal delivery can often be managed by observation and infusion of a FI concentrate given to raise the FI level to 1 \( \text{g L}^{-1} \) above baseline only if bleeding occurs. If the personal or family history of bleeding is strong a vaginal delivery may need to be covered with FI concentrate prophylactically to raise the FI level 1 \( \text{g L}^{-1} \) above baseline.

If FI concentrate is needed the FI level should be maintained 0.5 \( \text{g L}^{-1} \) above baseline until wound healing has occurred.

Caesarean section is likely to require FI replacement therapy as described for other surgical procedures. Thromboprophylaxis with compression stockings and prophylactic doses of LMWH will be required. The risk of epidural haematoma is increased in patients with a bleeding phenotype both at the time of insertion and removal of an epidural catheter. This type of anaesthesia should therefore be avoided.

**Miscarriage** – There are no studies on the management of women with dysfibrinogenaemia who have recurrent miscarriages. The options are to use prophylactic LMWH and if this fails consider the use of FI replacement. In the absence of any data regarding dosing maintaining a FI level 1 \( \text{g L}^{-1} \) above baseline could be tried.

**Management of thrombosis** – Patients with dysfibrinogenaemia who suffer thrombosis should be managed according to BSCH guidelines [44].
Factor II – prothrombin deficiency

Molecular biology

Prothrombin (FII) is a 72 kDa single chain glycoprotein which is synthesized by hepatocytes. It is one of the vitamin K-dependent coagulation factors and requires post-translational carboxylation to become functionally active. Prothrombin consists of four domains – Gla domain, kringle 1 and kringle 2 domains and a serine protease domain. FXa activates prothrombin on the surface of platelets in the presence of FV and calcium. During cleavage of prothrombin the activation peptide fragment 1 + 2 is released.

Incidence

Prothrombin deficiency is probably the rarest inherited bleeding disorder with an estimated prevalence of 1:2 000 000 in the general population [46]. As with many rare haemostatic disorders the mode of inheritance is autosomal recessive and the condition is therefore seen more frequently where consanguineous marriages are common.

Clinical phenotypes

Two clinical phenotypes are recognized – hypoprothrombinaemia (type I deficiency), in which prothrombin antigen and activity levels are reduced concomitantly, and dysprothrombinaemia (type II deficiency) in which prothrombin activity is reduced but antigen levels are normal. Occasional combined defects (compound heterozygotes) have also been reported.

Complete deficiency of prothrombin is not described suggesting that this is incompatible with life. This is in keeping with mouse knockout models where experimental inactivation of the prothrombin gene results in partial embryonic lethality and neonatal death [47].

Published data on this disorder are very limited and the largest case series reported is from Iran [46]. Prior to the publication of this series only 26 cases of prothrombin deficiency and 22 cases with other prothrombin abnormalities had been described in the world literature [48].

Fourteen patients were included in the Iranian series, 11 of whom had hypoprothrombinaemia with levels of 4–10% and three with dysprothrombinaemia. Haemarthrosis and muscle haematomas were the most frequent severe bleeding manifestations in this group of patients. There was one case of intracranial bleeding and life-threatening umbilical bleeding was reported in two neonates. Postoperative bleeding was also reported in the absence of replacement therapy but there were no cases of postpartum haemorrhage recorded. Mucosal bleeding was also frequently reported but was not severe.

Bleeding manifestations in the 26 cases of hypoprothrombinaemia reviewed by Girolami were similar to the Iranian series and again mucosal bleeding, soft tissue bleeding and haemarthrosis were relatively common [48]. There were also three reported cases of intracranial bleeding. Two other cases of intracranial haemorrhage in hypoprothrombinaemia have been reported more recently in infants aged 4 and 7 months [49].

Bleeding symptoms in patients with dysprothrombinaemias appear to be much more variable and many cases are asymptomatic or have only relatively mild bleeding problems.

Diagnosis

Both the PT and APTT may be prolonged in FII deficiency, depending on the reagent, but it should also be noted that the degree of abnormality may be minimal and results can be within the normal range. A specific FII assay may therefore be required in the presence of clinical suspicion or appropriate family history and normal screening tests. The one-stage clotting assay employing thromboplastin and based on the PT is suitable and is the most widely used technique. There are a number of assays, which employ different snake venoms to convert prothrombin to thrombin. These include Echis Carinatus venom assays that do not require phospholipids and do not differentiate between fully and partially carboxylated forms of FII. Other assays employing Taipan or Textarin venom are phospholipid-dependent.

In hypoprothrombinaemia results of all assays are reduced essentially in parallel [48]. In three subjects considered to be homozygous for hypoprothrombinaemia, FII activity levels were 9–16 U dL\(^{-1}\) by PT-based assay, compared with levels of 43–75 U dL\(^{-1}\) in heterozygous subjects. Normal subjects related to these kindreds and unrelated normal subjects had FII levels in the range 84–130 U dL\(^{-1}\) [48]. This is similar to the normal range of 84–132 IU dL\(^{-1}\) (mean ± 2 SD) in normal subjects tested with human recombinant thromboplastin PT-based assays (S. Kitchen, personal communication).

In dysprothrombinaemias results of PT-based and some venom assays are substantially lower than results of antigen and some other assays. In one recent report
of a family with dysprothrombinaemia FII by Echis Carinatus assay was 2 U dL\(^{-1}\) with results of 26–49 U dL\(^{-1}\) by other FII assay techniques.

Acquired deficiency of prothrombin may rarely be associated with the presence of antibodies and lupus anticoagulant effects. These patients may experience bleeding.

The diagnosis of mild prothrombin deficiency may be especially difficult in premature or young neonates where vitamin K deficiency may complicate assessment. Reassessment after vitamin K replacement may be necessary.

**Molecular defects**

Prothrombin is encoded by a gene on chromosome 11. To date at least 32 different mutations have been identified in patients with prothrombin deficiency and these have recently been reviewed [50]. Seventeen mutations have been described in dysprothrombinaemia, all of which are missense mutations. The mutations in hypoprothrombinaemia are also predominately missense, although nonsense mutations have also been found.

In dysprothrombinaemia the mutations result in amino acid substitutions within the cleavage sites for FXa and the serine protease region of prothrombin, whereas the mutations in hypoprothrombinaemia are often close to the Gla and kringle domains and the A chain.

**Management**

**Treatment options** There are no specific prothrombin concentrates available and prothrombin complex concentrates are therefore treatment of choice. The majority of these products are 3-factor concentrates containing therapeutic quantities of FII, FIX and FX [1]. The 4-factor concentrates, which in addition contain FVII, are also available [1]. These concentrates contain known quantities of these coagulation factors although the potency of the vial is usually expressed in terms of the FIX content. There is usually approximately 1 unit of prothrombin per unit of FIX and this can be used as a basis for dosage calculations. In the absence of an appropriate 3- or 4-factor concentrate, virally inactivated fresh frozen plasma (FFP) is an alternative source of prothrombin.

**Management of bleeding and surgery** It should be noted that there is only very limited published data available on which to base therapeutic decisions.

It is estimated that 1 unit of prothrombin will raise the plasma prothrombin level by 1 IU dL\(^{-1}\). Relatively low levels of prothrombin (20–30 IU dL\(^{-1}\)) are thought to be required for normal haemostasis and doses of 20–30 IU kg\(^{-1}\) have been used previously and seem to be effective. Higher doses may, however, be required in the event of life-threatening bleeding or major surgery, and monitoring of prothrombin levels should be performed. The half-life of prothrombin is around 72 h, which facilitates relatively infrequent dosing, usually every 2–3 days.

**Management of pregnancy** – Other than a small number of reports of postpartum haemorrhage there is no published data on the management or outcome of pregnancy in prothrombin deficiency. It is therefore difficult to make firm recommendations on pregnancy management but it would seem reasonable to increase the prothrombin level to above 25 IU dL\(^{-1}\) prior to delivery.

**Management of neonates and children** – There does not appear to be a high incidence of severe bleeding during the neonatal period although umbilical bleeding has been reported. Prophylactic replacement therapy is therefore not routinely recommended for this age group.

The use of prophylaxis in older children should be based on the frequency and type of bleeding. Where recurrent joint bleeding is a feature prophylaxis should be used to prevent the development of a chronic arthropathy.

**Factor V deficiency**

**Molecular biology**

Clotting FV is a large glycoprotein of MW 249 kDa encoded for by a gene on chromosome 1, which is synthesized by hepatocytes and megakaryocytes. FV and FVIII have approximately 40% amino acid homology in their respective A and C domains and their overall protein structures are identical. Platelets contain approximately 20% of total circulating FV. FV is activated by thrombin and the resulting heterodimer FVa acts as a cofactor for FXa in the conversion of prothrombin to thrombin. At the time of writing 26 separate mutations have been characterized [51]. Twelve of these are located in the exon (exon 13), which encodes the entire B domain. The majority of defects appear to give rise to a quantitative disorder whereas qualitative defects have been reported in approximately a quarter of cases [46,52].

**Clinical phenotype**

Hereditary FV deficiency is a very rare autosomal recessive condition. Owren described the first case, a Norwegian lady, in 1947 [53]. The prevalence of the
homzygous state is approximately 1 per million [54]. Parental consanguinity is often present. It has been reported that in homozygote individuals FV levels range from <1 to 10 U dL\(^{-1}\) with a normal range of 71–125 U dL\(^{-1}\) [55]. Homozygous deficiency is associated with a moderately severe bleeding disorder [55]. It usually presents in childhood with easy bruising and mucous membrane bleeding, in particular epistaxes and oral cavity bleeding. Haemarthroses and muscle haematomas are less of a feature than in FVIII deficiency and are often related to trauma rather than being spontaneous. Gastrointestinal bleeding and haematuria may occur rarely. Postoperative, postdental extraction and postpartum bleeding have been reported. There have been occasional reports of intracranial bleeding especially in the antenatal and neonatal periods [56–59]. Two of these cases were complicated by the development of FV inhibitors following plasma infusions and one of the children died from repeated intracranial haemorrhage.

**Diagnosis**

The FV deficiency is associated with prolongation of both the PT and APTT but a normal TT. Both PT and APTT are corrected by mixing with normal plasma. Deficiency of FV is confirmed by performing a prothrombin time-based FV assay or by immunological assessment of FV levels. Individuals with reduced FV levels should also have a FVIII assay performed to exclude combined FV and FVIII deficiency.

**Management**

There is no FV concentrate available for the treatment of FV deficiency. The only blood product available for FV replacement is FFP. It is recommended that a virally inactivated preparation is used [1] preferably sourced from a country that has no reported case of variant CJD in its donor population or BSE in its cattle. At the time of writing two virally inactivated FFP products are available in the UK. These are methylene blue-treated single unit FFP (MB FFP), prepared by the National Blood Service (England and Wales) and the Scottish National Blood Transfusion Service (SNBTS) and a commercial product [60], a pooled plasma product virally inactivated using a solvent detergent technique (SD-plasma). The mean FV level in units of MB FFP is 80 U dL\(^{-1}\) (NBA, personal communication). As SD-plasma is a pooled product individual units of each batch have the same FV level overcoming the problem of variability of single unit FFP enabling a more consistent dosing strategy. Lot release criteria of this product require that each batch contains at least 70 U dL\(^{-1}\) of clotting FV, FVII, FX, FXI and FXIII.

**Spontaneous bleeding episodes**

The minimum circulating level of FV that has to be achieved for effective haemostasis is likely to vary from individual to individual but it has been reported to be not less than 15 U/dL\(^{-1}\) [46]. In order to achieve this level it is suggested that a dose 15–20 mL kg\(^{-1}\) of FFP is administered to patients presenting with a bleeding episode. Use of agents such as tranexamic acid should also be considered.

It is recommended that the FV level be measured following FFP administration to ensure that the minimum haemostatic level of 15 U dL\(^{-1}\) has been achieved. If not, a further dose of FFP should be given. If bleeding continues despite achieving an adequate FV level a further dose of FFP should be considered. Close monitoring of FV levels is recommended to determine the FV half-life in individual patients and guide further FFP administration when levels fall below 15 U dL\(^{-1}\) if clinically indicated.

In a recent review of the use of SD-plasma in the management of patients with single hereditary factor deficiency a dose of 15 mL kg\(^{-1}\) was used to treat 51 bleeding episodes in 12 patients with single factor deficiencies. This included 34 episodes in six FV-deficient individuals [60]. Bleeds included haemarthroses, haematomas, dental, gastrointestinal and gynaecological haemorrhages. Effective haemostasis was achieved in 49 of 50 episodes assessed (98%) with an average dose of SD-plasma of 4.7 units (940 mL). It has to be appreciated that although a haemostatically effective circulating level of FV may be obtained with a particular dose of FFP this level may not be sufficient to normalize the PT. In one neonatal case an FFP dose of 30 mL kg\(^{-1}\) twice daily was required to achieve correction of the PT [57]. It has been suggested that in cases of severe bleeding not controlled with FFP replacement platelet transfusions may be considered, although proven efficacy of this treatment modality is lacking in the literature [61]. Platelets provide a concentrated supply of FV, which, following \(\gamma\)-granule release upon platelet activation, can presumably bind immediately to surface receptors optimizing prothrombinase complex activity. The use of recombinant activated factor VII (rFVIIa) should also be considered in patients not responding to FFP. This product has recently been used successfully in a FV-deficient patient [62]. However, in patients with FV levels of <1 U dL\(^{-1}\) the FV level may
be a major rate limiting factor in thrombin generation and rFVIIa may not be effective.

Surgery A trial of procedure may be considered in individuals with a partial deficiency of FV undergoing surgical or dental procedures in whom there is no prior history of spontaneous or surgery-related bleeding. FFP should be administered if excessive bleeding occurs. In patients with partial deficiency of FV who have a bleeding history, and in patients with levels of <1 U dL\(^{-1}\), FFP should be administered immediately prior to the procedure. FFP dosages should be those recommended for bleeding episodes. FV levels should be measured following FFP and further units administered prior to surgery if the minimum haemostatic level has not been achieved. The FV level should be observed closely following the procedure and further doses of FV administered to maintain a factor level above 15 U dL\(^{-1}\) until wound healing is established. Close monitoring of cardiovascular function is advised postoperatively because of the possible risk of fluid overload associated with repeated FFP doses, a problem described in some surgical cases [55].

In the study reported by Horowitz and Pehta [60] SD-plasma at an initial dose of 15 mL kg\(^{-1}\) provided effective surgical prophylaxis in seven procedures on five FV-deficient patients. Data available for six of these episodes showed an average increase in FV level of 13 U dL\(^{-1}\) (range: 9–17 U dL\(^{-1}\)). Unfortunately patient baseline FV levels and follow-up FFP dosage schedules postsurgery were not reported.

Pregnancy There are no available data on the management of FV deficiency in pregnancy. In patients with <1 U dL\(^{-1}\) FV we recommend the administration of FFP at the dosage suggested above for surgery as soon as the patient is in established labour, with close monitoring of FV levels. Further doses of FFP should be administered if and when required to maintain minimum haemostatic FV levels until after delivery. If Caesarean section is performed FFP dosing should be continued until wound healing is established. Patients with a partial deficiency and no history of bleeding during invasive procedures could be managed expectantly.

Neonates Severe and moderate FV deficiency can be diagnosed using cord or peripheral blood: FV levels do increase in the first month of life and mild deficiencies may need to be confirmed when the child is older.

Bleeding in the neonatal period appears to be uncommon [55] although it must be emphasized that FV deficiency can be a cause of intracranial haemorrhage in this age group (see above). A small number of single case reports have described this occurrence, with FV levels of the affected neonates varying between 2 and 8 U dL\(^{-1}\) [52,56–58,63]. This diagnosis should therefore be considered for babies presenting with intracranial haemorrhage and abnormal coagulation. Treatment of bleeding in neonates with FV deficiency requires the administration of virucidally treated FFP at a dose of 15–20 mL kg\(^{-1}\) in order to increase the FV level to >15 U dL\(^{-1}\). Fluid overload is a potential problem, particularly where repeated infusions are required and plasma exchange may be necessary in such cases. Platelets provide an alternative source of FV and platelet transfusion may be a valuable therapeutic addition to FFP. As yet, there have been no reports of rFVIIa used in the treatment of neonatal haemorrhage secondary to FV deficiency, although adult experience would suggest this to be worth considering, particularly where fluid balance problems exist [61].

Babies found to have a FV deficiency of <15 U dL\(^{-1}\) should have a cranial ultrasound performed in the first few days of life to exclude intracranial haemorrhage. There is no evidence to suggest that prophylactic infusion of FFP is of value in asymptomatic FV-deficient neonates. Vitamin K can be given i.m. to babies with FV levels >15 U dL U dL\(^{-1}\), oral or i.v. vitamin K can be given to more severely affected individuals.

Factor V inhibitors

Development of alloantibodies to FV in FFP is a potential complication of hereditary FV deficiency. Although very few cases have been reported in the literature this may represent under-reporting. The occurrence of inhibitors, especially transient ones of low level, following FFP replacement therapy may not be uncommon. In the event of a bleeding problem it has been suggested that low-level inhibitors can be neutralized using large amounts of FFP [61]. However, as in the treatment of surgical cases there are concerns over fluid overload in this situation and close cardiovascular monitoring is advised. Intravenous immunoglobulin may be effective in eradicating the FV inhibitor [64].

It has been shown that in acquired FV deficiency due to the development of FV autoantibodies, platelet FV is relatively protected from antibody neutralization [65]. Platelet infusions have been reported to be effective in acquired FV deficiency [66] and may therefore also be an effective treatment alternative in patients with hereditary deficiency.
complicated by inhibitors. Prophylactic use of weekly platelet infusions was effective in one inhibitor patient in stabilizing a subdural haematoma [57]. Use of rFVIIa concentrate should also be considered.

**Combined deficiency of factors V and FVIII**

**Molecular biology**

Combined FV and FVIII deficiency is a rare autosomal recessive disorder which usually arises as a consequence of consanguinity [67]: Oeri first reported it in 1954 [68] and affected individuals have reduced plasma levels of both FV and FVIII. Studies of the inheritance pattern indicated that it was likely to be due to a single gene defect rather than due to coinheritance of separate defects of the FV and FVIII genes. This has been confirmed, the gene being located on the long arm of chromosome 18. The gene encodes a resident protein of the endoplasmic reticulum, the golgi intermediate compartment, termed the ERGIC-53 protein [69–74]. This protein has been identified as playing a major role in intracellular trafficking of certain proteins including FV and FVIII [75–77]. Although it would appear that within hepatocytes FV and FVIII are synthesized normally, defective ERGIC-53 function results in disturbance of the passage of the factors through the cell and impaired release into the circulation. A number of different mutations have been described in this gene leading to combined FV and FVIII deficiency [69,71].

**Clinical phenotype**

Although mild bleeding symptoms such as easy bruising and epistaxis are not uncommon in affected individuals, circulating levels of FV and FVIII are usually sufficient to prevent more severe spontaneous bleeding episodes. However, bleeding is common following surgery, dental extraction and trauma; menorrhagia and postpartum haemorrhage are commonly seen in affected women [78,79]. The bleeding severity observed in affected individuals has been reported as similar to that in individuals with deficiencies of either factor alone at similar levels [79].

**Diagnosis**

The combined deficiency disorder is associated with a prolongation of both the PT and APTT, with the APTT prolongation disproportionate to that of the PT. Both test times are corrected by mixing studies using normal plasma. APTT-based activity assays and antigen assays reveal levels of between 5 and 20 IU dL\(^{-1}\) for both FV and FVIII [67].

**Management**

**Bleeding episodes** The treatment of bleeding episodes is dependant on the nature of the bleed and the affected individual’s FV and FVIII levels. Spontaneous bleeding episodes occurring in patients with combined FV and FVIII deficiency should be treated with both FVIII concentrates and FFP (as a source of FV). For minor bleeding episodes FVIII levels should be raised to at least 30 IU dL\(^{-1}\) and to at least 50 IU dL\(^{-1}\) for more severe bleeds with rFVIII concentrate being the treatment of choice. As for patients with FV deficiency, FFP should be administered in order to increase the FV level to at least 25 U dL\(^{-1}\). The FFP product of choice is virally inactivated FFP.

**Surgical procedures** These should be covered with FVIII concentrates administered 12 hourly to maintain FVIII levels above 50 IU dL\(^{-1}\) and 12 hourly FFP to achieve minimum levels of FV of 25 U dL\(^{-1}\) until wound healing is established.

**Pregnancy** There are no published data on the management of pregnant women with this combined deficiency. FV levels in pregnancy do not consistently increase or decrease whereas FVIII levels will increase throughout pregnancy: any possible bleeding is therefore likely to be dependent on the FV level during labour and postdelivery. In keeping with general recommendations for the management of bleeding disorders in pregnancy, affected women should be managed by an obstetric unit in close liaison with a haemophilia centre. FV and FVIII levels should be confirmed in the third trimester so that the delivery can be planned with regard to haematological intervention. FV levels should be maintained above the haemostatic level of 15 U dL\(^{-1}\) during labour, using virucidally treated plasma as the source of the factor. FVIII levels should remain at >50 IU dL\(^{-1}\) throughout this period. If Caesarean section is carried out, it would be prudent to continue FV replacement until wound healing has healed in women with FV levels of <15 U dL\(^{-1}\). Epidurals can be performed if the woman has FV levels of >15 U dL\(^{-1}\) and FVIII >50 IU dL\(^{-1}\).

**Neonates** Combined FV and FVIII deficiency can be diagnosed in the neonatal period using cord or peripheral blood. Affected babies should receive oral
rather than i.m. vitamin K and go on to receive s.c. childhood vaccinations. They should be managed expectantly and there is no indication for routine prophylaxis with plasma and FVIII. Neonatal intracranial haemorrhage has not been described in this condition.

Factor VII deficiency

Molecular biology

Factor VII is a vitamin K-dependent glycoprotein with a MW of approximately 50 kDa and circulates in plasma in two forms – the majority in a single chain inactive form with a concentration of 10 nmoles L\(^{-1}\) (0.5 \(\mu\)g mL\(^{-1}\)) and a much smaller amount (approximately 10–110 pmoles L\(^{-1}\)) as the active two-chain form. The FVII gene maps to chromosome 13 at 13q34, spans approximately 12 kb of DNA and consists of nine exons encoding a mature protein of 406 amino acids. FVII through its interaction with tissue factor is fundamental to the initiation of coagulation.

Plasma factor VII levels

Factor VII plasma levels are determined by both environmental and genetic factors with the latter accounting for up to one-third of the variation in plasma FVII. Amongst environmental factors, dietary fat intake and the levels of plasma triglycerides are positively correlated with FVII:C levels but age, obesity, diabetes and in women, the use of sex hormones can all influence FVII levels.

Five and possibly six polymorphisms within the human F7 gene have been shown to affect both plasma FVII:C levels and plasma VIIa levels [reviewed in 80]. Of these the most important is the Arg353Gln polymorphism within exon 7, which affects approximately 20% of the UK population. Heterozygosity for this polymorphism is associated with an approximately 25% reduction in FVII:C and VII:Ag levels and homozygosity, an approximately 50% reduction in circulating plasma FVII.

Inherited factor VII deficiency

Factor VII deficiency is the most common of the ‘rare inherited coagulation disorders’. Severe FVII deficiency (FVII:C <2 IU dL\(^{-1}\)) has an estimated prevalence of 1:300 000–1:500 000. FVII deficiency is inherited in an autosomal recessive manner and its frequency is significantly increased in countries where consanguineous marriage is practised. There is a relatively poor correlation between absolute FVII levels and the risk of bleeding with some individuals with very low FVII levels exhibiting very few symptoms whilst others with much higher levels, have a significant bleeding diathesis.

Clinical phenotype

The spectrum of bleeding problems in FVII deficiency is very variable [81]. Epistaxes, gum bleeding, menorrhagia and other mucous membrane-type bleeding are common. Menorrhagia and chronic iron deficiency are frequently seen in women with FVII deficiency. Joint bleeds have been reported in patients with severe FVII deficiency although this is not a consistent finding. In patients with severe FVII deficiency (FVII:C <2 IU dL\(^{-1}\)), bleeding into the central nervous system is common and reported in between 15 and 60% of cases [82]. Such cases often present shortly after birth and this presentation is associated with a high morbidity and mortality.

Diagnosis

The diagnosis of FVII deficiency is suspected following the finding of a prolonged PT, which corrects, unless an inhibitor is present, in a 50:50 mix with normal plasma. The APTT, TT and FI concentration are normal. FVII levels are low at birth and age/gestation-related ranges for FVII are essential if a deficiency is suspected [10]. It is important to exclude vitamin K deficiency or other acquired causes of a clotting disorder before the diagnosis of FVII deficiency is made. A therapeutic trial of vitamin K may be of value. In some cases, family studies may be helpful in establishing the diagnosis of FVII deficiency. The diagnosis of FVII deficiency may be especially difficult in premature or young neonates where vitamin K deficiency may complicate assessment. Reassessment after vitamin K replacement may be necessary.

Functional FVII assays

Functional FVII activity (FVII:C) is measured using a one-stage PT-based assay. In some unusual cases of FVII deficiency, there may be a discrepancy in the levels of FVII:C depending upon the source of thromboplastin [83]. The use of a human thromboplastin is advised on the basis that the results are more likely to reflect in vivo FVII levels. In the functional FVII assay, there is limited conversion of FVII–FVIIa and FVII:C assays, therefore, measure both the inactive zymogen and preformed FVIIa. Blood samples for determination of
FVII:C should not be stored at 4 °C because this can lead to cold activation of FVII and lead to substantial overestimation of the true FVII level [84].

**Immunological FVII assays** FVII antigen (FVII:Ag) is frequently measured using an enzyme-linked immunosorbent assay (ELISA) or Immunoradiometric assay (IRMA) assay and either monoclonal or polyclonal antibodies. Such assays can detect as little as 0.01 IU dL⁻¹ of FVII. Immunological assays should not be used in preference to a functional FVII assay.

**Factor VIIa assays** In samples where there has been no activation of FVII, assays for FVII:C and FVII:Ag should be equivalent. Two direct assays for FVIIa have been reported but for diagnosing FVII deficiency [85,86] there is little if any value in measuring basal FVIIa levels in patient with FVII deficiency.

**FVII deficiency and thrombosis**

Thrombosis in association with FVII deficiency has been reported although the mechanism is unclear [80].

**Inherited factor VII deficiency**

The FVII mutation website (http://www.193.60.222.13/index.htm) lists 120 mutations throughout the FVII gene. In common with many diseases, numerous examples of repeated mutations within the FVII gene have been reported.

**Management**

Current therapeutic options to manage patients with FVII deficiency include fibrinolytic inhibitors, plasma, intermediate purity FIX concentrates (prothrombin complex concentrates), FVII concentrates and rFVIIa. Plasma FVII has a short in vivo half-life of approximately 5 h although this may be shorter during a bleeding episode [87]. For severely affected individuals (FVII:C <2 IU dL⁻¹), rFVIIa is the treatment of choice [1] although it is not licensed, as yet, for this indication.

**Currently available treatments**

**Plasma:** Fresh Frozen Plasma (FFP) has been widely used in the management of FVII deficiency although there is little information available on its efficacy, and it should not be used unless there is no alternative. FFP has been used to manage patients undergoing various surgical operations either by itself or in combination with FVII concentrate [88,89]. In cases in which prolonged administration is required, it is difficult to administer sufficient FFP quickly enough and problems with fluid overload may be encountered. FFP as a source of FVII is not recommended for managing patients with FVII deficiency.

**Factor IX concentrates and prothrombin complex concentrates:** Intermediate purity FIX concentrates contain variable amounts of FVII and have been successfully used to manage patients with FVII deficiency [90–92]. The amount of FVII in these concentrates is very variable but usually indicated by the manufacturer. These concentrates also contain activated forms of FVII, FIX and FX and should be used with caution, as there are reports of both venous and arterial thromboses in association with their use. They should be avoided in patients with liver disease, in patients with major trauma, in patients with antithrombin deficiency and in the neonate whose liver is relatively immature. Prothrombin complex concentrates are not recommended for managing patients with FVII deficiency.

**Factor VII concentrates:** Plasma-derived FVII concentrates (BPL Elstree, UK and Baxter) have been successfully used to manage patients with FVII deficiency with spontaneous bleeds; for patients undergoing a variety of surgical procedures and for prophylaxis against bleeds in children with severe FVII deficiency [93–95]. These products are virally inactivated to reduce the risk of viral transmission.

**Recombinant factor VIIa:** Patients with FVII deficiency can be safely managed using rFVIIa [96–102] and this is the treatment of choice [1] although there is limited published data. The rFVIIa probably has a shorter half-life than that of plasma FVII particularly in children where an increased clearance has been demonstrated and in pregnancy.

**Fibrin glue:** May be effective in facilitating local haemostasis. Currently two products are licensed for although one of these is only licensed for use in liver surgery [1].

**Tranexamic acid:** In practise, 10 mL of a 5% solution of tranexamic acid is used as a mouthwash every 8 hourly. However, this is not commercially available and will require to be formulated by the Pharmacy Department. In women with menorrhagia, tranexamic acid 15 mg kg⁻¹ 8 hourly (in practise 1 g, 6–8 hourly) may be effective when taken for the duration of the menstrual period.

**Acute bleeding episodes** Efficient haemostasis can be achieved with levels of FVII:C in the range of 10–15 IU dL⁻¹. rFVIIa is the recommended choice.
for patients with FVII deficiency requiring replacement therapy.

Factor VII concentrates – Factor VII concentrates have been successfully used to treat a variety of acute bleeding problems:

1. **Mucosal bleeding:** A correction to 40–100% resulted in cessation of bleeding within 15 min in all cases [103].

2. **Intracranial bleeding:** An 8-year-old boy received a dose of 37 IU kg\(^{-1}\) FVII concentrate every 6 h with peak FVII levels of approximately 100% and troughs as low as 4% over the 11-day treatment period. A 37-year-old adult male with intracranial bleeding received alternating doses of 16 and 8 IU kg\(^{-1}\) every 6 h for 10 days with peak FVII levels in the upper 30s (%). The peak FVII level during surgical coverage with FVII concentrate was approximately 100% in all cases, with trough levels ranging from 8 to 65% over treatment periods of 24 h to 16 days using treatment intervals of 6–12 h.

3. **Prophylaxis:** FVII concentrates have been successfully used for prophylaxis against bleeds in children with severe FVII deficiency. Suggested guidelines for the administration of FVII for long-term prophylaxis are in the range of 10–50 IU kg\(^{-1}\) one to three times a week (grade B recommendations based on level III evidence). Although illogical when once considers the short half-life of FVII, in practice it appears successful [103].

rFVIIa – In the study of Mariani et al., rFVIIa was used to treat 17 patients (FVII:C <1% in nine patients and <5% in an additional five patients) with 27 spontaneous bleeding episodes who in addition also underwent seven major and 13 minor surgical procedures [104]. Nine of these patients had FVII levels of <1% and a further five, FVII levels <5%. Fifteen haemarthroses were treated with only a single dose of rFVIIa (14–30 \(\mu\)g kg\(^{-1}\)) and in only one case was rFVIIa ineffective. Seven major surgical procedures were performed in severely affected patients under cover of rFVIIa and no bleeding occurred either during or after surgery. In the case of surgery, rFVIIa was given at 2–3 h intervals for the first 24 h followed by longer intervals (3–8 h) for the remaining postoperative period. All the surgical patients also received tranexamic acid along with the rFVIIa. In one patient rFVIIa became ineffective following the development of an anti-FVII antibody.

Other groups have reported the successful use of rFVIIa with a variety of clinical problems [96,99,101,102]. A dose of 20–25 \(\mu\)g kg\(^{-1}\) administered every 4–6 h appears to be successful in treating the majority of patients with FVII deficiency who are either bleeding or who require treatment prior to and following surgery (grade B recommendations based on level III evidence). This should be combined with tranexamic acid 10 mg kg\(^{-1}\) every 8 h i.v. or 25 mg kg\(^{-1}\) every 8 h orally. For dental surgery, 10 mL of a 5% solution of tranexamic acid can be used as a mouthwash every 8 h for 5–7 days.

In patients receiving 20 \(\mu\)g kg\(^{-1}\) of rFVIIa every 6 h, this leads to peak FVII levels of 430–830 IU dL\(^{-1}\) and trough levels of 30 IU dL\(^{-1}\) (although the results are highly dependent upon the thromboplastin used to assay the FVII). Inhibitors following treatment with rFVIIa have been reported but appear rare [110].

**Pregnancy** There are no published data available on the management of FVII deficiency in pregnancy. Continuous infusion of rFVIIa has been used in one patient with FVII deficiency (FVII:C 3.7 U dL\(^{-1}\)) to provide haemostatic cover for an elective Caesarean section [111]. Pharmacokinetic studies performed prior to the infusion revealed a very high clearance rate (0.208 L h\(^{-1}\) kg\(^{-1}\)) and a short half-life (0.884 h), which the authors attributed to the pregnancy. The patient received an initial bolus or rFVIIa of 13.3 \(\mu\)g kg\(^{-1}\) followed by a continuous infusion initially at a rate of 3.3 \(\mu\)g kg\(^{-1}\) h\(^{-1}\) for 48 h and then at 1.66 \(\mu\)g kg\(^{-1}\) h\(^{-1}\) for a further 48 h. Plasma FVII and FVIIa levels were maintained between 100

\[\text{FVII and FVIIa levels were maintained between 100} \]
and 150 U dL\(^{-1}\) and no bleeding problems were observed.

The neonate  Factor VII deficiency may be difficult to diagnose in the neonatal period because of the low physiological levels in the newborn. For these reasons age- and gestation-related reference ranges must be used [10]. In cases where FVII levels are lower than expected, screening the parents may be of benefit in establishing a diagnosis.

Families in which both parents are known to have FVII deficiency, pregnancy and delivery should be managed in such a way as to minimize the potential risk of bleeding both to the mother and baby. This requires close liaison with the obstetric unit including obstetric anaesthetists and a management plan should be prepared for the delivery and subsequent investigation of the neonate. FVII levels may rise during pregnancy. A FVII assay should be performed prior to delivery. At birth a cord blood sample should be taken for FVII assay.

Cranial ultrasound should be undertaken in severely affected neonates because of the increased risk of intracranial haemorrhage.

Prophylaxis during the neonatal period may be necessary in severely affected neonates although even with appropriate replacement therapy, haemorrhage may occur.

Factor X deficiency

Molecular biology

Factor X occupies a unique position in the coagulation cascade, as the first enzyme in the common pathway of thrombus formation. The gene for FX is 22 kb long and located at 13q34-ter, 2.8 kb downstream of the FVII gene and encodes a protein of MW 59 kDa. FX synthesis occurs in the liver and following secretion into plasma, circulates at a concentration of 10 \(\mu\)g mL\(^{-1}\) as a two-chain molecule. Activation of FX–FXa occurs during the initiation of coagulation by a complex consisting of tissue factor, FVIIa, calcium ions and a suitable phospholipid membrane. However, activation can also take place through FIXa, FVIIIa, calcium ions and acidic phospholipid surfaces. Activation of FX \textit{in vitro} can occur by the direct action of a metalloproteinase found in the venom of \textit{Vipera russelli}. Physiologically, FXa is the most important activator of prothrombin. The presence of the prothrombinase complex (FXa, FVa, Ca\(^{2+}\) and a suitable negatively charged phospholipid membrane) accelerates the conversion of prothrombin to thrombin 280 000-fold.

Inherited factor X deficiency

Severe (homozygous) FX deficiency is inherited as an autosomal recessive disorder with an incidence of 1:1 000 000 in the general population. The prevalence of FX deficiency is greater amongst populations in which consanguineous marriage is common. The prevalence of heterozygous FX deficiency is about 1:500, but individuals are usually clinically asymptomatic [112]. However, some heterozygotes do have a significant bleeding tendency and this may be due to either insufficient enzymatic activity by wild-type FX or inhibition of one of the reactions in the coagulation pathway by a mutant protein.

Clinical phenotype

Although FX deficiency produces a variable bleeding tendency, patients with severe deficiency [FX activity (FX:C) <1 IU dL\(^{-1}\)] tend to be the most seriously affected patients with rare coagulation defects [113].

Patients with FX deficiency may present at any age and severely affected individuals (FX:C <1 IU dL\(^{-1}\)) can present in the neonatal period, with umbilical-stump bleeding. The most frequent symptom in FX deficiency is epistaxis and is seen with all severities of deficiency. Other mucosal-type bleeding is less frequent and occurs mainly in patients with severe deficiencies. Menorrhagia occurs in half the women of reproductive age. Haemarthroses, severe postoperative haemorrhage and central nervous system haemorrhage have been reported. Recurrent haemarthroses may result in severe arthropathy. Moderately affected patients (FX:C 1–5 IU dL\(^{-1}\)) may bleed only after haemostatic challenge, e.g. trauma or surgery. Mild FX deficiency (FX:C 6–10 IU dL\(^{-1}\)) may be identified incidentally during routine screening or family studies. Patients who are only mildly affected may experience easy bruising or menorrhagia.

Diagnosis

The diagnosis of FX deficiency is suspected following the finding of a prolonged PT and APTT which corrects, unless an inhibitor is present, in a 50:50 mix with normal plasma. The diagnosis of FX deficiency is confirmed by measuring plasma FX levels. Five different assays are available for measuring plasma FX levels – the one-stage PT- and APTT-based assays, a chromogenic assay, an assay employing Russell viper venom (RVV) and finally an immunological assay. The one-stage PT- or APTT-based
assay is sufficient for the diagnosis of FX deficiency. In some cases the results obtained using a PT-based FX assay may differ according to the type of thromboplastin used. Chromogenic assays can generate normal values with some dysfunctional FX variants (type II variant: normal immunological FX but reduced functional activity) and should not be used as a screening test for FX deficiency. The full spectrum of tests is only required for detailed protein characterization.

Factor X levels are low at birth and age/gestation-related ranges for FX are essential if a deficiency is suspected [10]. It is important to exclude vitamin K deficiency or other acquired causes of a clotting disorder before the diagnosis of FX deficiency is made. A therapeutic trial of vitamin K may be of value. In some cases, family studies may help in establishing the diagnosis of FX deficiency.

The diagnosis of mild FX deficiency may be especially difficult in premature or young neonates where vitamin K deficiency may complicate assessment. Reassessment after vitamin K replacement may be necessary.

Inherited factor X deficiency

Factor X mutations are thought to be rare because of the central role of FX in the coagulation cascade. The FX knockout mouse has a lethal phenotype, with death occurring either in utero or within a few days of life and this is consistent with the hypothesis that a complete absence of FX is a lethal disorder [114]. A mutational website for FX mutants does not exist but approximately 45 mutations within the FX gene have been reported [115].

Management

Inherited FX is a rare disorder and there are no generally agreed guidelines for the management of this disorder. Current therapeutic options to manage patients with FX deficiency include fibrinolytic inhibitors, plasma and intermediate purity FIX concentrates (prothrombin complex concentrates). rVIIa has been used successfully to treat acquired FX deficiency secondary to amyloidosis [116].

The need for replacement therapy is guided by the particular haemorrhagic episode. The biological half-life of FX is 20–40 h [117], so an adequate level can be achieved with repeated infusions. Factor levels of 10–20 IU dL$^{-1}$ are generally sufficient for haemostasis, even in the immediate postoperative period [118].

Treatment products

Tranexamic acid: Particularly useful for mucosal bleeding. In practise 10 mL of a 5% solution of tranexamic acid is used as a mouthwash every 8 h. However, this is not commercially available and will require to be formulated by the Pharmacy Department.

Fibrin glue: May be effective in facilitating local haemostasis.

FFP: A virally inactivated plasma should be used. 20 mL kg$^{-1}$ followed by 3–6 mL kg$^{-1}$ twice daily, aiming to keep X:C trough levels above 10–20 IU dL$^{-1}$.

Prothrombin complex concentrates (for details see [1]): The calculated required dosage for treatment is based on the empirical finding that 1 IU FX kg$^{-1}$ bodyweight (bw) raises the FX level by 1.5% of normal. Tranexamic acid should not be used concurrently with prothrombin complex concentrates because of the risk of thrombosis. The half-life of FX is 60 h and daily treatment is not usually required. However, in cases where replacement therapy is given, levels should be monitored on a daily basis. Prothrombin complex concentrates should be used with caution, if at all, in patients with concomitant liver disease, large haematomas, major trauma, the neonate and antithrombin deficiency [119].

Concentrates are heat-treated, which significantly reduces the risk of viral transmission.

Mucosal bleeding Tranexamic acid is particularly useful (see above). In women with menorrhagia, tranexamic acid 15 mg kg$^{-1}$ 8 hourly (in practise 1 g, 6–8 hourly) may be effective when taken for the duration of the menstrual period.

Management of the acute bleed No specific concentrates are available and prothrombin complex concentrates are the treatment of choice.

Fibrin glue: May be effective in facilitating local haemostasis.

Plasma: In cases where prothrombin complex concentrates are not available or are contraindicated, FFP can be used (details as above).

Prothrombin complex concentrates: Prothrombin complex concentrates have been used as regular prophylaxis in patients with severe FX deficiency [120]. Kouides et al. reported the case of a patient who received 30 units kg$^{-1}$ of Profilnine administered twice weekly as part of a home treatment programme. If breakthrough bleeding occurred, another dose was administered but no more than two doses in 24 h or on more than 3 consecutive
days. A trough level drawn 48 h postinfusion showed an FX level of 30 U dL$^{-1}$. In a 12-month follow-up there were no bleeding episodes reported. A recent paper [121] reported four cases of FX deficiency in which primary prophylaxis was successfully undertaken using a PCC commencing in the case of one child at the age of 1 month. The four cases reported all had severe FX deficiency and presented within 24–72 h of birth with a severe bleeding diathesis.

Unpublished data at the Royal Free Hospital in a patient with severe FX deficiency (FX:C <1 IU dL$^{-1}$) has shown that 10 U kg$^{-1}$ of HT Defix (SNBTS) given every third day provides effective prophylaxis against bleeds.

$rFVIIa$: rFVIIa has been used to treat amyloid-associated FX deficiency [116] but the data on its use in other patients with FX deficiency is limited. Adequate levels of FX appear to be important for the action of rFVIIa and therefore, in severe FX deficiency rFVIIa may be ineffective [122].

Management of factor X deficiency: X:C >2 IU dL$^{-1}$

Patients with FX levels >10 IU dL$^{-1}$ or a lower level and no significant bleeding history (despite haemostatic challenge) do not require replacement therapy. However, the nature of the surgery and any bleeding history in relation to previous haemostatic challenges must be considered.

Inherited deficiency of the vitamin-K dependent clotting factors

Molecular biology

Coagulation FII, FVII, FIX and FX require $\gamma$-carboxylation of critical glutamic acid residues within their Gla domains to enable binding of calcium and attachment to phospholipid membranes. The $\gamma$-carboxylation reaction is catalysed by the hepatic enzyme $\gamma$-glutamyl carboxylase that requires reduced vitamin K (KH$_2$) as a cofactor. During the $\gamma$-carboxylation reaction, KH$_2$ is converted to vitamin K epoxide (KO), which is recycled to KH$_2$ by the vitamin K epoxide reductase enzyme complex (VKOR) [124]. Heritable dysfunction of $\gamma$-glutamyl carboxylase or of the VKOR complex results in the secretion of poorly carboxylated FII, FVII, FIX and FX that have poor haemostatic function. $\gamma$-Carboxylation is also required for the secretion of functional proteins C, S and Z and the skeletal proteins osteocalcin and matrix Gla protein.

Incidence and inheritance

Hereditary combined deficiency of the vitamin K-dependent clotting factors (VKCFD) has been reported as single case reports in <20 kindreds worldwide. Inheritance is autosomal recessive.
Clinical phenotype

There is wide variation in phenotype in the reported kindreds with VKCFD. Severely affected individuals may present at birth with spontaneous intracranial haemorrhage or umbilical stump bleeding [125–127] or in infancy and childhood with spontaneous haemarthroses and retroperitoneal, soft tissue or gastrointestinal bleeds. Otherwise, presentation may be later in life with easy bruising and mucocutaneous or postsurgical bleeding [128–130]. Severe bleeding is usually associated with activities of the VKCFDs of <5 U dL\(^{-1}\) [125,126]. The phenotype of VKCFD may be more severe if there is also acquired vitamin K deficiency and less severe if patients have received therapeutic vitamin K\(_1\). This may present particular difficulties in establishing a diagnosis of VKCFD in neonates.

Severely affected children may show skeletal abnormalities such as nasal hypoplasia, distal digital hypoplasia, epiphyseal stippling and mild conductive hearing loss [131,132]. These abnormalities are similar to those of warfarin embryopathy and are attributed to dysfunction of the vitamin K-dependent proteins osteocalcin and matrix Gla protein. Although proteins C and S are also abnormal in VKCFD, there are no reports of venous thrombosis.

Diagnosis

Affected individuals show prolongation of the PT and APTT associated with variable reductions in specific activities of FII, FVII, FIX and FX. Care should be taken, particularly in neonates, to exclude causes of acquired vitamin K deficiency or coumarin exposure. The demonstration of normal fasting serum KH2 usually allows exclusion of these diagnoses if there is clinical uncertainty. Serum warfarin assays may sometimes be necessary to exclude factitious coumarin ingestion. VKCFD is associated with comparatively preserved antigenic assays for FII, FVII, FIX and FX and with elevated des-\(\gamma\)-carboxyprothrombin (PIVKA-II) in serum. Elevated serum KO may distinguish VKCFD individuals with a molecular defect in the VKORC complex (designated VKCFD type 2) from those with defective \(\gamma\)-glutamyl carboxylase activity (VKCFD type 1). Reduced activities of proteins C and S have been demonstrated in some kindreds [125,133].

Molecular defects

Two missense mutations in the \(\gamma\)-glutamyl carboxylase gene have been identified in two consanguineous kindreds with severe phenotype VKCFD type 1 [125,127,134]. A locus responsible for VKCFD type 2 has been mapped to 16p12-q21 [135]. Individuals with type 2 deficiency have causative mutations in the recently identified gene encoding a component of the VKOR enzyme complex called VKORC-1 [136].

Management

Vitamin K Most previously reported individuals with VKCFD show partial or complete improvement in clotting factor activity, normalization of the PT and APTT and resolution of bleeding symptoms with oral or parenteral vitamin K\(_1\) [125,126,130–132,134,137]. However, in other kindreds vitamin K was ineffective [129,133,138]. There is no clear relationship from the literature between responsiveness to vitamin K and either severity or molecular basis of VKCFD.

Treatment with oral vitamin K\(_1\) (phytomenadione) is therefore indicated in all patients at diagnosis. In patients who show insufficient response, there is limited experience of weekly parenteral vitamin K\(_1\) 10 mg [125,134].

Management of bleeding or surgery In the rare patients who have responded poorly to vitamin K\(_1\), who require surgery or who have an acute bleed, factor replacement is necessary. There is limited experience of the use of FFP in the treatment of acute bleeds [125,133,137] and a virally inactivated product is therefore the agent of choice. Since FVII has the shortest half-life of the VKCFDs [87], therapy should be monitored using the PT or FVII activity assay.

The 4-factor prothrombin complex concentrates are an alternative to plasma but as these agents have been associated with thrombosis or disseminated intravascular coagulation and should be used with caution in some individuals [119].

Management of pregnancy There is a single report of a pregnancy progressing to term in an individual with severe VKCFD managed with oral vitamin K\(_1\) 15 mg daily throughout pregnancy. Bleeding from an episiotomy wound in this case required FFP [137].

Factor XI deficiency

Molecular biology

Factor XI is a dimeric serine protease whose function in the coagulation pathway was recently clarified. Although usually identified as a ‘contact factor’ it is now realized that the contact pathway is non-physiological. Coagulation is triggered by activation
of the tissue factor–factor VII pathway resulting in thrombin activation. As tissue factor pathway inhibitor modulates the tissue factor pathway, thrombin can activate FXI and thereby recruit the ‘intrinsic’ pathway of coagulation [139–141]. These observations may explain why FXI is less essential to haemostasis than FVIII and FIX, and perhaps why other factors may influence the bleeding tendency in FXI deficiency.

Factor XI deficiency is an autosomally inherited condition, which is particularly common in Ashkenazi Jews in whom the heterozygote frequency is 8% [142]. FXI deficiency has been described in all racial groups, but in general, the incidence of severe deficiency (FXI:C level <10 U dL$^{-1}$, although there is no precise definition) is very low (estimated at 1:1 million) [143].

The FXI gene is 23 kb in length. In the Ashkenazi Jewish population most FXI deficiency is related to two mutations: type II, a stop codon in exon 5, and type III, a missense mutation in exon 9 leading to reduced expression of the FXI molecule. These mutations occur in equal frequency in Jews leading to type II homozygotes who typically have undetectable FXI levels and a more severe bleeding phenotype; type III homozygotes who have a low level of FXI (about 10 U dL$^{-1}$) while compound heterozygotes have levels and clinical expression between these two [144]. Several other mutations have been described, usually in single families; however, common founder effect mutations have been described in French Basques [145] and in 11 UK families with C128X in exon 5 [146].

**Clinical phenotype**

In comparison with haemophilia A and B, bleeding manifestations in FXI deficiency are much less predictable, even in severe deficiency. Spontaneous haemorrhages are very rare. The condition is not recessive, bleeding occurs in people with heterozygous deficiency and is not related to the FXI:C level [147,148]. Bleeding is most commonly provoked by injury or surgery, particularly in areas of the body where there is high fibrinolytic activity (the mouth, nose and genitourinary tracts). Tonsillectomy, dental extractions and sinus surgery are well recognized to result in excessive blood loss. The same individual may bleed after one operation and not after another. There is disagreement about whether the bleeding manifestations breed ‘true’.

Women with FXI deficiency (including heterozygotes) are at risk of menorrhagia [149,150] and bleeding in relation to childbirth.

Bleeding manifestations may depend upon other associated factors such as mild von Willebrand’s disease [151,152]. Associated haemophilia A or B, or platelet disorders have been occasionally reported. However, these observations do not explain all the observed variation in bleeding pattern. This clinical variability makes the management of FXI deficiency potentially difficult.

As with any inherited coagulation disorder it is important to take a full family history and to test any individual at risk (parents, siblings and children). Individuals with very low levels (<10 U dL$^{-1}$) are either homozygous or compound heterozygous. It should be noted that in the Ashkenazi Jewish community as the heterozygote frequency is so high, there is a higher risk of severe deficiency.

**Diagnosis**

The diagnosis depends upon determination of a FXI level below the reference range. The APTT will usually be prolonged, as most reagent-machine combinations are sensitive to mild contact factor deficiencies. It should be noted that the reference range used by many laboratories is incorrect; several authors have noted the lower limit of normal to range from 63 to 80 U dL$^{-1}$ [147–150] so that a cut-off of 50 U dL$^{-1}$ is too low. Bleeding has been reported in individuals with levels between 50 and 70 U dL$^{-1}$. Each laboratory should establish its own reference range. The mean FXI level in a group of normal blood donors was 99 U dL$^{-1}$ with a range of 60–139 U dL$^{-1}$ (S. Kitchen and E. Preston, 1995, personal communication).

In the investigation of possible FXI deficiency it is important to recognize the limitation of some screening tests. The APTT became prolonged at a level below 40 U dL$^{-1}$ in one study [153] but at 50–70 U dL$^{-1}$ in another [18]. In another study, which employed samples from patients with previously identified deficiencies, normal APTT results were obtained in the presence of FXI levels of 22–50 U dL$^{-1}$ with one reagent [17]. Data from published studies and from EQA programmes suggest that most widely used APTT reagents will have prolonged APTT results in samples from patients with FXI below 20–25 U dL$^{-1}$, whereas there is a more mixed pattern of normal and abnormal results when FXI is in the region 25–60 U dL$^{-1}$. The lower limit of normal range for FXI activity is probably between 60 and 70 U dL$^{-1}$ [17,148]. Thus, a normal APTT does not always exclude the presence of a mild FXI deficiency, particularly if marked elevation of FVIII is present.
which may normalize APTT where other factors are reduced [17].

Management

Most individuals with FXI deficiency have few problems. However, it is important that surgery and trauma are managed appropriately. As with mild haemophilia A and B, these individuals tend to forget their potential bleeding risk which results in an increased risk of suboptimal management. In addition, few doctors are familiar with FXI deficiency.

Treatment options in factor XI deficiency

Patients with severe FXI deficiency (FXI:C <10–20 U dL⁻¹) usually bleed in relation to surgery and these patients should therefore receive treatment to increase their FXI levels. Patients with FXI levels between 20 and 70 U dL⁻¹ may bleed and in these patients a bleeding history, especially in relation to any haemostatic challenges and the nature of the proposed surgery, will guide the need for therapy. Some of these individuals may have low von Willebrand factor levels, and it is advisable to measure baseline levels [148].

Fresh frozen plasma: FFP has been used successfully to treat patients with FXI deficiency. However, large volumes of FFP may require to be administered to achieve therapeutic levels, which can lead to fluid overload. FFP given as a dose of 15–20 mL kg⁻¹ appears effective [154,155]. Monitoring of the response is important and a virally inactivated product is recommended. Levels of FXI in patients with severe FXI deficiency (XI:C <15 U dL⁻¹) are unlikely to rise above 30 U dL⁻¹ with FFP.

Factor XI concentrates: FXI concentrates are available to treat FXI deficiency but only on a named-patient basis (for details see [1]). There are concerns about the potential thrombogenicity of FXI concentrates [156,157] and for these reasons, peak FXI levels should not exceed 70 U dL⁻¹. In addition, tranexamic acid should be avoided in patients receiving FXI concentrates.

rFVIIa: rFVIIa has been used successfully to manage adult patients with FXI deficiency undergoing surgery [158], although it is not licensed for this indication.

Tranexamic acid: Tranexamic acid is widely used in FXI deficiency and may be of value in patients with milder forms of the disorder or to supplement the use of plasma and rFVIIa. Tranexamic acid is also of value in the management of women with FXI deficiency and menorrhagia.

Fibrin glue: May be effective in facilitating local haemostasis.

Mucosal bleeding

Tranexamic acid: In practise 10 mL of a 5% solution of tranexamic acid is used as a mouthwash every 8 h. However, this is not commercially available and will require to be formulated by the Pharmacy Department.

In women with menorrhagia, tranexamic acid 15 mg kg⁻¹ 8 hourly (in practise 1 g, 6–8 hourly) may be effective when taken for the duration of the menstrual period.

Spontaneous bleeding

Surgery: Surgery, however minor, should be carried out under the supervision of or in consultation with a haematologist with experience of FXI deficiency. For patients with severe FXI deficiency this will usually be in a haemophilia centre. Several strategies may be used, and the treatment needs to be tailored to the individual circumstances. For patients with severe deficiency, FXI levels of >30 U dL⁻¹ are considered haemostatic for minor surgery and >45 U dL⁻¹ for major surgery [61]. In practise one should aim to obtain FXI levels in the region of 70 U dL⁻¹ prior to major surgery. FXI has a half-life of approximately 52 ± 22 h and daily dosing may not, therefore, be necessary. Daily monitoring of FXI levels is recommended.

Dental extractions may be carried out with oral tranexamic acid alone (1 g q.d.s. starting the day before and continuing for 7 days) even in people with severe deficiency [159].

People with mild FXI deficiency are more difficult to manage because of the variability and unpredictability of the bleeding tendency [148]. Oral tranexamic acid, with or without a virally inactivated FFP should be considered. For major surgery in younger people factor concentrates may be required.

Factor XI concentrates: For patients with severe FXI deficiency (XI:C <10–20 U dL⁻¹) or for those with levels between 20 and 70 U dL⁻¹ but undergoing major surgery, FXI concentrate is advised. In individuals with FXI levels near the upper end of this range the decision will depend upon the bleeding history and the nature of the surgery. The dose should not exceed 30 U kg⁻¹ and peak levels should not exceed 70 U dL⁻¹. Tranexamic acid should probably not be used in patients receiving FXI concentrate because of concerns about the thrombotic risk. Similarly, FXI concentrates should be used...
with caution, if at all, in patients with a history of cardiovascular or cerebrovascular disease. FXI levels should be checked daily.

**Pregnancy**  Women with FXI deficiency are at risk of menorrhagia and may be diagnosed as a consequence of this [160–163]. Their quality of life is often reduced. Bleeding in childbirth and postpartum haemorrhage are potential hazards. Observations of FXI levels in pregnancy are contradictory but changes are generally not clinically significant. Women with FXI levels in the heterozygous range may bleed at delivery.

**Vaginal delivery:** In women with FXI levels between about 15 and 70 U dL\(^{-1}\) and no bleeding history but previous haemostatic challenges, a policy of 'watch and wait' is justified.

For women with FXI levels between about 15 and 70 U dL\(^{-1}\) and a significant bleeding history or no previous haemostatic challenges, tranexamic acid is often used for 3 days with the first dose being administered during labour.

For women with severe FXI deficiency (FXI:C <10–20 U dL\(^{-1}\)) FXI concentrate should be given during labour.

**Caesarean section:** For women with severe FXI deficiency (XI:C <10–20 U dL\(^{-1}\)) FXI concentrate should be given. In patients with FXI levels between about 20 and 70 U dL\(^{-1}\), treatment is dependent upon the bleeding history and the FXI level.

**Epidural anaesthesia:** This is generally discouraged in women with FXI deficiency but may be used in women who have received FXI concentrate and in whom an adequate response (FXI level) has been documented.

**Neonates**  Spontaneous bleeding in the neonatal period has not been reported. No instances of neonatal intracranial haemorrhage resulting from FXI deficiency have been reported.

**Circumcision:** This may result in serious bleeding and may be the first presentation of severe FXI deficiency. For religious circumcision if FXI levels at birth (cord blood) are <10 U dL\(^{-1}\), the procedure should be delayed and the FXI levels checked at 6 months. If the level remains <10 U dL\(^{-1}\), circumcision should be performed in hospital under cover of either FXI concentrate or FFP. However, the input of a Mohel to perform the circumcision is important to fulfil religious requirements and this may require prior coordination with the hospital.

For boys with FXI levels >10 U dL\(^{-1}\) the procedure should be carried out under cover of tranexamic acid (15 mg kg\(^{-1}\), 8 hourly for 3 days). The i.v. preparation is given orally in this situation although this is not a licensed use of the product.

**Management of factor XI inhibitors**

The development of inhibitors to FXI in patients with FXI deficiency following treatment with FXI is rare. rVIIa may be of value in treating these rare patients [158,164].

**Factor XIII deficiency**

**Molecular biology**

Factor XIII (fibrin stabilizing factor) deficiency inherited (autosomal recessive) or acquired is a very rare bleeding disorder with a high prevalence of consanguinity in affected families. In the United Kingdom, the estimated prevalence is one case per 1 million [46]. It is much higher in areas with higher concentration of South Asian immigrants. Similarly, it is much higher in areas of the world with high incidence of consanguinity. The first family, from a consanguineous marriage, with congenital FXIII deficiency was described in 1961 [165].

**Clinical phenotype**

Patients with FXIII <1 U dL\(^{-1}\) are at greatest risk from severe spontaneous bleeding. Those with levels between 1 and 4 U dL\(^{-1}\) are likely to have moderate or severe bleeding. Occasionally patients with levels above 5 U dL\(^{-1}\) may bleed [166]. It should be noted that difficulties in the assay of FXIII complicate the relationship between the FXIII level and clinical features.

Umbilical bleeding, which occurs a few days after birth, is reported in 80% of cases and is very suggestive of the disorder. Thereafter, patients experience a lifelong tendency to severe bruising, muscle haematomas (32%), haemarthroses (24%), intracranial haemorrhage (30%), miscarriages, postnatal bleeding and bleeding after surgery and trauma [167]. FXIII deficiency also leads to a delay in healing of wounds [168]. In the majority of cases the bleeding diathesis is severe, although there are a few cases with mild symptoms reported.

**Diagnosis**

The range of FXIII activity within the normal population is very wide, ranging from 53.2 to 221.3% (mean ± SD: 105 ± 28.56%), of the standard normal plasma value in one report [167] and 51–152 U dL\(^{-1}\) in another study [169].

The bleeding time, PT and APTT are normal. The clot solubility test is a qualitative screening test in
which test plasma is clotted with calcium, thrombin or a combination of both and exposed to a chemical challenge, which lyses the clot unless sufficient FXIII-dependant cross-linking has occurred. The clot may be lysed with 5 mol L\(^{-1}\) urea, 2% acetic acid, 1% monochloroacetic acid or similar. There is no consensus on the relative sensitivity of the different combinations to low levels of FXIII. In a recent study by Jennings et al. [170] clotting with calcium and lysing with urea produced abnormal results when FXIII levels were below 5 U dL\(^{-1}\) (equivalent to 5%). Clotting with 30 U mL\(^{-1}\) thrombin and suspension in 2% acetic acid produced abnormal results when FXIII levels were 10 U dL\(^{-1}\) or less. We recommend use of thrombin/acetic acid as this combination was associated with the highest proportion of abnormal results in the presence of marked FXIII deficiency [170], although the same study identified important effects of the types of reagents used in FXIII screening tests.

Several types of FXIII assay have been reported and at least two are commercially available activity assays. These are based on the cross-linking of glycine-ethylester into a specific peptide [171] or on incorporation of an amine substrate into FI [172], both of which employ thrombin activation of FXIII. ELISA assays have been used to determine antigenic levels of FXIII, but are not widely available. Agreement between results of FXIII assays may be poor and in a series of proficiency testing exercises the results of FXIII assays on the same test sample varied widely between centres, for example, from 3.0 to 130 U dL\(^{-1}\) for one sample [170]. These data indicate that the accurate determination of FXIII is currently problematic. Taken with the variability of screening tests [170] it is clear that the detection and characterization of FXIII deficiency is difficult, and this makes the relationship between clinical severity and the degree of FXIII deficiency unclear.

Specific assays should always be used to confirm abnormal screening test results and can be used for monitoring purposes.

**Prophylactic factor replacement** In view of the high incidence of cerebral haemorrhage it is recommended that all patients with severe FXIII deficiency (<1 U dL\(^{-1}\)) should receive prophylactic replacement therapy with FXIII concentrate from the time of diagnosis. It should also be considered in those with levels <4 U dL\(^{-1}\). Some patients with levels more than 4 U dL\(^{-1}\) may also benefit [166].

The FXIII has a long circulating half-life of 7–10 days and needs to be given at 4–6 weekly intervals [166,173]. It has been suggested that levels of 3–10 U dL\(^{-1}\) are sufficient to prevent spontaneous haemorrhage [166].

It is recommended that 10 units kg\(^{-1}\) bw be given at intervals of 4 weeks. This appears to prevent recurrent clinical problems and bleeding episodes.

**Acute bleeding episodes** Whole blood, FFP, stored plasma and cryoprecipitate have all been used successfully in treatment and are adequate sources of FXIII. Plasma-derived FXIII concentrates are superior to FFP or cryoprecipitate as these provide reliable and high concentrations of FXIII in minimum volume, have fewer contaminating substances, and are virally inactivated [173]. It is recommended in the UK that patients receive a plasma-derived pasteurized FXIII concentrate until a recombinant product is available [1]. Platelets contain FXIII, and in a haemorrhagic emergency platelet transfusions may be helpful [169].

It is recommended that 10–20 units kg\(^{-1}\) bw should be administered, the plasma levels monitored and kept in the normal range until the bleeding has stopped.

It is recommended that ideally FXIII levels should be monitored and in cases of major operation and severe haemorrhage the aim is to obtain normal values.

There are no absolute contraindications. However, the manufacturers advise caution in cases of recent thrombosis and allergic reactions.

**Surgery** Adults should receive 10–20 units kg\(^{-1}\) bw immediately before the operation and ideally, the plasma levels monitored. Further treatment can be given if necessary to keep the level in the normal range for the following 5 days or until the wound has healed completely.

**Pregnancy** The widely held view that women with FXIII deficiency will have inevitable miscarriages, and that affected men are sterile has not been substantiated in the literature [174]. FXIII has an essential role in placent implantation and the continuation of pregnancy [175]. Up to 50% of severely affected pregnant women may miscarry without appropriate treatment. All severely affected girls should be started on monthly infusions of FXIII concentrate from the time of diagnosis and these should be continued throughout pregnancy. FXIII levels in plasma fall throughout pregnancy and should be monitored aiming to keep the trough level >3 U dL\(^{-1}\).

**Neonates** Factor XIII deficiency is a cause of life-threatening haemorrhage in the neonate and babies with factor levels of <3 U dL\(^{-1}\) often present with
bleeding in this period. Persistent bleeding from the umbilical cord is the commonest reported symptom in individuals with severe FXIII deficiency [176]. Intracranial haemorrhage is another well recognized presenting complication of this disorder, as are cephalhaematoma and bleeding after circumcision: the diagnosis should be suspected in any neonate presenting with such symptoms in whom routine coagulation screening tests are normal. FXIII deficiency can be diagnosed from cord or peripheral blood samples using the previously described screening test and confirmed by FXIII assay.

Treatment of an acute bleeding episode requires the administration of FXIII concentrate at a dose of 20 units kg$^{-1}$. Further treatment should be given as required, determined by FXIII levels, if possible, until the bleeding has stopped: if intracranial haemorrhage has occurred then FXIII levels should be maintained in the normal range for a minimum of 2 weeks, which may require a frequency of up to alternate day dosage before reducing treatment to a prophylactic regimen. In the absence of assay data, some clinicians would use daily treatment because of the risk of long-term morbidity if bleeding continues. Further studies are required.

All neonates (and older children) who have been diagnosed as having a FXIII level of <3 U dL$^{-1}$ should commence treatment with prophylactic FXIII concentrate 10 U kg$^{-1}$ given once every 4 weeks. Subsequent dosage and dosage intervals can be varied depending on the pretreatment (>3 U dL$^{-1}$) or 1 h post-treatment FXIII level (>60 U dL$^{-1}$).

Close collaboration and careful investigation is required where there is unexpected severe bleeding in the neonatal period, particularly where the parents are consanguineous.

Inhibitors

The FXIII inhibitors are very rare in congenital FXIII deficiency. Rarely FXIII inhibitors arise in the course of other diseases, and often in relation to chronic therapy with a variety of drugs [167]. Bleeding in these cases may be severe. Several cases have died of cerebral haemorrhage [177].

There is no consensus on management. Treatments attempted include immunosuppression with steroids and cyclophosphamide, administration of large doses of FXIII, and plasma immunoadsorption.

Ehlers-Danlos Syndrome

The EDS is a heterogeneous group of inheritable connective tissue disorders characterized by joint hyperextensibility, skin extensibility and tissue fragility. It is thought that EDS has a prevalence of one in 5000 to one in 10 000. It is known to affect both males and females and all racial and ethnic backgrounds [178].

It is possible that Hippocrates was referring to EDS in his description of the lax joints and multiple scars noted in the Nomads and Scythians. However, the earliest unequivocal account was by Van Meek’ren, a Dutch surgeon, in 1657. It reappears in the medical literature in 1892 when Tscherenogobow, after whom the syndrome is known in Russia, described two cases and proposed that the primary abnormality was in the connective tissue. Ehlers, a Danish dermatologist, published a case report of another patient in 1901. This was rapidly followed by a report by Danlos, a French Physician, and the condition began to be recognized as a distinct entity [179]. A series of further reports were then drawn together by Weber, the ‘doyen of British syndromic diagnosticians’, and the eponym, EDS was coined [180].

The heterogeneity of the condition was soon evident. From the 1960s attempts were made to categorize it and these culminated in the Berlin nosology in 1988. This has now been superseded by the revised nosology agreed in Villefranche in 1997 based on the cause of each type [181].

Classification

There are six major types of EDS (Table 1). Different types of EDS are classified according to the manifestations of signs or symptoms. Rarely a skin biopsy is carried out including more detailed biochemical analysis.

Molecular biology

Patients with EDS exhibit connective tissue abnormalities of cutaneous strength, elasticity, and healing properties. Collagen proteins are multimeric, occurring in trimers. A minimum of 29 genes contributes to the collagen structure, and the genes occur on 15 different chromosomes. There are 19 identifiable different forms of collagen molecules. EDS is caused by variety of abnormalities in the synthesis and metabolism of collagen [182].

Diagnosis

The diagnosis of EDS is based upon clinical findings and on the family history. Since many patients do not fit neatly into one of the specific types of EDS, the
diagnosis is often delayed or overlooked. The commonest presentation to the haematologist will be with bruising. The PT, APTT and TT are normal. The skin bleeding time is usually normal but on rare occasions may be prolonged [183]. Platelet abnormalities have occasionally been reported [184]. Skin hyperextensibility should be tested at a neutral site. It is measured by pulling up the skin until resistance is felt. In young children it is difficult to assess because of abundance of s.c. fat [185,186]. Joint hypermobility should be assessed using the Beighton scale. Joint hypermobility depends on age, gender, family and ethnic background. A score of 5/9 or greater defines hypermobility [186]. The total score is obtained by:

1. Passive dorsiflexion of the little fingers beyond 90°; one point for each hand.
2. Passive apposition of the thumbs to the flexor aspect of the forearm; one point for each hand.
3. Hyperextension of the elbows beyond 10°; one point for each elbow.
4. Hyperextension of the knees beyond 10°; one point for each knee.
5. Forward flexion of the trunk with knees fully extended so that the palms of the hand rest flat on the floor; one point.

Easy bruising manifests as spontaneous ecchymoses, frequently recurring in the same areas, and causing characteristic brownish discoloration. Easy bruising may be the presenting symptom in early childhood. Child abuse should be considered in the differential diagnosis.

Tissue fragility manifests as easy bruising and the presence of dystrophic scars. Scars are found mostly

<table>
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<th>Type</th>
<th>Inheritance</th>
<th>Basic defect</th>
<th>Clinical features</th>
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| Classical (was EDS I gravis + II mitis type) | AD | Abnormality of type V collagen encoded by COL5A1 + COL5A2 genes | Major: skin laxity, typical scars, joint hypermobility  
Minor: velvety skin, molluscoid pseudotumours, muscle hypotonia, easy bruising, hernias |
| Hypermobility (was EDS III hypermobile type) | AD | Unknown | Major: skin laxity, velvet skin, joint hypermobility  
Minor: recurrent joint dislocations, chronic limb and joint pain |
| Vascular (was EDS IV arterial or ecchymotic type) | AD | Structural defects of type III collagen encoded by the COL3A1 gene | Major: arterial/intestinal/uterine fragility or rupture, easy bruising, typical facial appearance  
Minor: hypermobility of small joints, tendon and muscle rupture, club feet, varicose veins |
| Kyphoscoliosis (was EDS VI ocular or scoliosis type) | AR | Deficiency of lysyl hydroxylase, a collagen modifying enzyme | Major: joint laxity, severe muscle hypotonia in infants scoliosis from birth, scleral fragility  
Minor: tissue fragility, easy bruising, arterial rupture, Marfanoid habitus, microcornera, osteopenia |
| Arthrochalasia (was included in EDS VII) | AD | Deficiency of chains in type I collagen due to skipping exon 6 in COL1A1 or COL1A2 gene | Major: severe joint hypermobility with dislocations, congenital bilateral hip dislocations  
Minor: skin laxity, tissue fragility and scarring, easy bruising, muscle hypotonia, kyphoscoliosis |
| Dermatosparaxis (was included in EDS VII) | AR | Deficiency of procollagen I N-terminal peptidase in collagen type I | Major: severe skin fragility, sagging redundant skin  
Minor: soft, dough skin texture, premature rupture of fetal membranes, easy bruising, hernias |

Three rare forms of EDS, with the classical phenotype have been recognized, although their syndromic status is unclear: X-linked EDS, described in a single large UK family (was known as EDS type V); periodontal EDS with prominent gum fragility, AD inheritance (was known as EDS type VIII); EDS with abnormal platelet aggregation, inheritance possibility AR (was known as EDS type X).

Two entities previously thought to be EDS have now been removed from the EDS classification: EDS type IX has been reclassified as occipital horn syndrome, an X-linked disorder of copper metabolism; EDS type XI is now termed familial joint hypermobility.

Table 1. The classification of Ehlers-Danlos Syndromes (EDS) [181] Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

Haemophilia (2004), 10, 593–628 © 2004 Blackwell Publishing Ltd
on pressure points (knee, elbow, forehead, chin) and have a thin, atrophic papyraceous appearance. Frequently the scars become wide and discoloured; wound healing is impaired [185,186].

Mitral valve prolapse and proximal aortic dilatation should be diagnosed by echocardiography, computed tomography (CT) or magnetic resonance imaging (MRI). Mitral valve prolapse is a common manifestation, but aortic dilatation is uncommon. Chronic joint and limb pain is common but skeletal radiographs are normal.

Kyphoscoliosis, arthrochalasia and dermatosparaxis types are considerably less common than the classical, hypermobility, and arterial types [185,186].

Diagnosis

Confirmatory laboratory tests are not generally required in haematological practice. Mutant forms of genes can be detected in cultured skin fibroblasts by biochemical studies. Molecular genetic studies are helpful in Types IV, VI, and for some forms of type VII [187].

Management

There is no specific treatment for bruising or bleeding but measures can be taken to minimize loss of blood and discomfort. Gaping skin wounds, which are common in several types of EDS, should be approached with care. Proper repair of these wounds is necessary to prevent cosmetic disfigurement. The surgeon should be informed and extra sutures may be added and the length of period the sutures are left prolonged by a few days. Tranexamic acid postoperatively has been used to reduce episodes of re-bleeding.

Specialized orthopaedic care is needed for care of the joints and the orthopaedic surgeon may use bracing to stabilize joints. Surgical repair of joints may be necessary at some time. A physiotherapist may prescribe exercises to strengthen muscles and teach the patient how to properly use and preserve their joints. Ascorbic acid up to 4 g daily has been prescribed but there are no controlled studies to show its benefit.

All patients should be offered genetic counselling particularly prior to pregnancy as there is an increased risk of uterine rupture [188].

Cardiac problems may be progressive and should be dealt with by a cardiologist.

Consultation with an ophthalmologist (myopia, retinal tears and keratoconus) and a dentist (periodontitis) may be necessary.

Patients with EDS have reported that local anaesthetic is often ineffective. The diagnosis of EDS should be considered in any patient who complains unexpectedly of pain during their procedure, particularly when an adequate volume of local anaesthetic has been given.

There are reports of correction of the bleeding time or improvement in symptoms in patients treated with desmopressin (DDAVP) [189,190]. There is also a case report of the use of prophylactic DDAVP in labour to prevent haemorrhage in EDS [191]. Although no specific therapies are available to delay or treat the complications of EDS, knowledge of the diagnosis may influence the management of pregnancy, surgery, bleeding and genetic counselling. There are no controlled studies on therapeutic options in relation to bleeding episodes. There is a need for well-conducted clinical studies at every level of management.

Mortality

People with type IV disease may die suddenly because of spontaneous rupture of medium sized arteries.

Useful contacts

Ehlers-Danlos Support Group, 1 Chandler Close, Richmond N, Yorks DL105QQ, UK.

Ehlers-Danlos National Foundation, 6399 Wilshire Blvd, Ste 510, Los Angeles, CA 90048, USA.

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