

Clinical Genetics Services for Haemophilia

This report on Clinical Genetic Services for Haemophilia has been compiled by the Genetics Working Party on behalf of the United Kingdom Haemophilia Centre Doctors' Organisation.

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Preface

During the intervening period since the previous second edition of these guidelines (Ludlam et al, 2005), there have been both significant developments in laboratory genetic techniques and also an increasing awareness of the importance of demonstrating and enhancing the quality of the clinical service. These revised guidelines describe the value of new scientific techniques, such as the use of free fetal DNA in the maternal circulation to determine the sex of the developing embryo, and the potential place for pre-implantation genetic diagnosis. The importance of the quality of genetic counselling, although considered in the previous edition, is given further space here particularly in relation to the training of those who provide the service and the arrangements for helping consultees. An enhanced section on issues around the genetic testing of children offers guidance to those who may be faced with requests from parents requesting genetic testing of girls who are at risk of being carriers.

Perhaps the principal message of this guideline is that there must be not only high quality of care by each member of the healthcare team but that the members of the extended team must work seamlessly together. This collaboration is not only between those providing clinical care and counselling for haemophilia and other clinical services, e.g. obstetric, but also there must be a close and defined working relationship with those with responsibility for the genetic laboratory.

Both the clinical and laboratory service, provided within the UK Haemophilia Genetic Network, must be of demonstrable high quality. The importance of external audit, as part of the UKHCDO audit of Comprehensive Care centres and Clinical Pathology Accreditation, of both these aspects of the service is emphasised and arrangements for these are described.

This edition, as for the previous ones, has been produced by a UKHCDO Working Party whose composition reflects the multidisciplinary professional skill mix which is needed for those providing haemophilia genetic services. I should like to acknowledge the enthusiasm with which all members of the Working Party contributed to the development of these guidelines but also to thank particularly those who primarily work in non-haemophilia specialties. Although this edition of the guidelines was designed to inform service provision within the NHS administrative structure these will inevitably change and in responding to this evolution it is of paramount importance to maintain and if possible enhance the quality of service which can be provided to individuals and families. Whilst many UKHCDO guidelines are compiled for use in the context of the UK healthcare system many are read and used by those providing services in many different countries around the world; in this context it is important to note that some aspects of the guidance is informed by laws pertaining to clinical practise in the UK.

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Chairman

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Executive Summary

The commissioning of Haemophilia Genetic Services in England is subject to guidance in the NHS Standard Contract for Haemophilia (All Ages) – B05/S/a 2013-14. This requires access to genetic services, commissioned and funded as part of the overall Haemophilia Service. Arrangements in Scotland, Wales and Northern Ireland need to be compatible with those in England so that a UK co-ordinated, seamless service is provided to family members who may be dispersed throughout the country.

This report is to inform and offer guidance to commissioners, providers of services, patients, physicians, nurses, genetic counsellors and laboratory scientists on the provision of clinical and laboratory haemophilia genetic services.

The scope of services which should be provided by Haemophilia Centres and the larger Comprehensive Care Haemophilia Centres has been set out historically by the Departments of Health (HSG(93)30, MEL(1994) 29 and DGM(93) 100) and in greater detail in the National Service Specification for Haemophilia and Related Conditions (The Haemophilia Alliance) [2006]. These describe the wide range of clinical and laboratory services that should be offered directly to patients with congenital bleeding disorders and their families. Haemophilia Centres provide the diagnostic laboratory service for bleeding disorders and are responsible for the prevention and treatment of acute bleeds. They also co-ordinate a broad range of other specialist services, e.g. for dental and orthopaedic surgery, HIV and chronic liver disease, necessary for patients. In addition the families of these individuals are likely to include adults and children who may be carriers of haemophilia who themselves may have a haemorrhagic diathesis and require treatment.

This document provides guidance on the range and the standards for clinical and laboratory genetic services which should be offered to patients and their families. In summary these are:

- **Laboratory diagnostic genetic service.** This is provided by a co-ordinated network of laboratories at the larger Comprehensive Care Haemophilia Centres which together form the UK Haemophilia Genetic Laboratory Network. This Network has close links with the broader clinical genetics UK Genetic Testing Network.
- **Genetic counselling for patients and families.** Before any laboratory genetic testing can be undertaken, counselling should be offered by professionals with appropriate training in counselling and specialist experience in heritable bleeding disorders. These counsellors should also work in close association with local clinical genetic services. Counselling and appropriate antenatal investigations and care should be offered to carriers and potential carriers of haemophilia.
- **Genetic data storage, retrieval and disclosure.** Genetic data is potentially very sensitive, personal medical information. It is therefore particularly important that its handling conforms to recent legislation which includes the Data Protection Act and Children's Act. It is likely that in future further national guidance about confidential genetic data will be forthcoming and it will be essential that this can be incorporated into existing arrangements.

The commissioners and providers of the services should ensure that appropriate staff and other resources are available to provide these services

Membership of Working Party

| | |
|--------------------------------------|---|
| Prof John Pasi | Professor of Haematology (Chairman) |
| Dr Keith Gomez | Consultant Haematologist (Secretary) |
| Dr Tony Cumming | Consultant Clinical Scientist in Haematology |
| Dr Alan Fryer | Joint Committee on Genomics in Medicine of the Royal Colleges of Physicians and Pathologists and the British Society for Genetic Medicine |
| Prof Christopher Ludlam | Professor of Haematology and Coagulation Medicine |
| Dr Mike Mitchell | Chairman of Haemophilia Genetics Laboratory Network |
| Ms Dianne Marshall | Advanced Nurse Practitioner in Haemophilia |
| Prof Heather Skirton | Plymouth University and Chair of European Board of Medical Genetics Member of UK Genetic Counsellor Registration Board (2007-2012) |
| Ms Christine Harrington (2009-10) | Nurse Consultant |
| Prof Edward Tuddenham (2009-11) | Professor of Haemophilia |
| Co-opted members | |
| Prof Mike Laffan | British Committee for Standards in Haematology representative Professor of Haematology |
| Ms Rezan Abdul-Kadir | Consultant Obstetrician |
| Acknowledgements | |
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Section 1. Introduction

This guidance document is accented towards haemophilia, however many of the key principles are applicable to other heritable bleeding disorders.

The provision of a clinical and laboratory service for haemophilia and allied disorders has always required a high degree of coordination between those with an expertise in blood coagulation and colleagues in a range of other clinical services. This is particularly true in relation to the genetic aspects of haemophilia and other heritable bleeding disorders. The UKHCDO Genetics Working Party has drawn on the experience of Regional Genetic Centres and the report emphasises the continuing desirability of further developing and maintaining close links with them. The guidelines describe a framework of arrangements for clinical and laboratory haemophilia genetic services which depend upon close collaboration with other specialists to provide a cross-disciplinary, seamless service for patients and their families. This document is accented towards haemophilia, however many of the key principles are applicable to other heritable bleeding disorders.

The arrangements and standards for services have been described in circulars from the Department of Health, UK Haemophilia Alliance Service Specification (The Haemophilia Alliance, 2006) and previously the National Specialist Services definition set no.3 (National Specialised Commissioning Group, 2010) and currently the NHS Standard Contract for Haemophilia (All Ages) – B05/S/a 2013-14. The former was devised by representatives of the Haemophilia Society (representing patients), UKHCDO, specialist haemophilia nurses, Chartered Physiotherapists in haemophilia and social workers, as well as clinical and biomedical scientists working in Haemophilia Centres. The 2010 Service Specification, which has been widely acknowledged as being the standard which patients and families should reasonably expect, forms the basis of the NHS Standard Contract for Haemophilia (All Ages). In this report the Working Party sets out a series of recommendations based on these documents, which aim to promote good clinical practice and provide standards against which services can be audited.

With recent advances in molecular laboratory techniques it is now possible to give the vast majority of individual patients and family members very reliable genetic information. To enable these genetic data to be used to ensure both the optimal treatment of the patient with a bleeding disorder and for reproductive choice in those who may be carriers, there needs to be established a clear and robust framework for systematically acquiring the necessary clinical, personal, family and laboratory information upon which decisions can be made. In this report guidance is offered as to how this information can be collected and recorded as a basis for genetic counselling.

1.1 Genetic counselling

For individuals within a family to make decisions in relation to a heritable disorder skilled non-directive counselling must be available. It is important to distinguish between information giving, education, and counselling; the latter enables each individual to reach their own decisions based on all the appropriate information. The way in which such counselling services may be developed and the necessary training and skills of the counsellors are set out in the guideline. This is merely a starting point for the service and further discussions, in the light of experience gained, will inform its future direction.

1.2 Confidentiality and clinical records

Genetic testing raises many issues of confidentiality and consent. Some of these are generic to all clinical records and are covered by legislation, e.g. Data Protection Act, whereas others are more specific to genetic testing and relate to an individual's understanding of how their genetic information may be used within the family. We have tried to offer guidance for the more common situations based on our understanding of current legislation and good clinical practice. We have recommended the establishment of family genetic files as well as formal genetic family registers in Haemophilia Centres.

1.3 Information and Informed Consent

Even before a blood sample is taken for genetic testing it is essential that the individual understands what investigations are proposed and the potential use to which the result may be put. To help inform patients and family members a Patient Information Leaflet has been developed which sets out some of the background to genetic testing. This can be used as one of the starting points for counselling. A small audit we have undertaken suggests that many patients have found it helpful. It is accompanied by a Consent Form that can act as a record of the individual's agreement as to who should receive the result and where data can be held. These are generic forms and for good Clinical Governance it is appropriate to ensure that they conform to local arrangements, in some instances it may also be necessary for an individual to sign a local hospital consent form.

1.4 Carriers of haemophilia

The arrangements that should be available for haemophilia carriers are described in detail in the guideline. The counselling of potential carriers should take place at an appropriate time and preferably before pregnancy. The management of early pregnancy requires close collaboration between haemophilia physician and obstetrician and in the case of antenatal diagnosis may involve a clinical geneticist. The overall arrangements for antenatal diagnosis require a coordinated input from many members of the haemophilia team including the genetic counsellor. The management of the mother and fetus in later pregnancy in relation to any potential haemorrhagic disorder, e.g. carrier of haemophilia or potentially affected fetus, is not covered in this report.

1.5 Genetic testing of children

Children have particular rights in relation to genetic testing as reviewed in the specific guideline on the value of genetic testing in children (British Society for Human Genetics, 2010). In the present document we seek to inform healthcare staff about some of the important issues related to a child's rights.

1.6 UKHCDO Genetic Laboratory Network

An essential cornerstone of a clinical genetic service is a high quality laboratory service. The Working Party has established the UKHCDO Genetics Laboratory Network (GLN) which is a consortium of laboratories, mostly within Comprehensive Care Haemophilia Centres that work to agreed standards of quality and turn round times. A Network national co-ordinating committee oversees collaboration, adherence to quality standards and the development of the service. The GLN is represented on the Clinical & Scientific Advisory Group of the UK Genetic Testing Network. This ensures appropriate links and close collaboration between haemophilia genetic services and the wider world of clinical genetics. For the laboratory service to be used appropriately and optimally there needs to be effective collaboration

between clinical scientists and clinicians and the guideline offers suggestions on how this can be achieved. A directory of laboratories in the GLN is available on the UKHCDO website. This directory also lists those laboratories with particular expertise in some of the less common heritable bleeding disorders.

1.7 Required resources

Implementation of the recommendations of this report will require additional resources to be invested in haemophilia services. The family files and genetic registers will need to be established to record both factual genetic information and details of clinical consultations with patients and family members. Much of this could be developed with the expertise of genetic counsellors who will bring experience of arrangements from clinical genetics centres. The financing of the laboratory genetic service needs to be from NHS sources and not dependent on research funding for the core staff, equipment and consumables.

1.8 Levels of evidence

Most current guidelines support recommendations with levels of evidence. As there is a paucity of randomised trials for the topics covered in this report, the recommendations are considered to represent, by common consent, good clinical practice. Some aspects, however, of the guidance have statutory authority, e.g. the handling of data.

1.9 Future

Nothing in medicine moves faster than developments in genetics! Although the report offers current guidance on how services should develop for people with haemophilia and their families, arrangements will need to evolve in response to advances in laboratory techniques, new statutes, changes in the way health services are commissioned, and above all by changes within society and its expectations.

Section 2. Provision of services

2.1 *Setting the scene*

The National Service Specification for Haemophilia and Related Conditions states that all individuals with haemophilia (or a related bleeding disorder) and their families should have access to specialised genetic services (The Haemophilia Alliance, 2006). Genetic counselling should be available for all people potentially affected by or at risk of being a carrier of one of these conditions before, during and after the process of genetic analysis. This document sets out proposals for the future direction of genetic counselling provision in haemophilia services.

The provision of genetic counselling varies between haemophilia centres. The involvement of different members of staff in the provision of genetic counselling depends upon their role within the multidisciplinary team, and the skills, knowledge, experience and qualifications held and used by individual members of the team. Centre teams vary in terms of their membership of professionals from social and psychological services, and in the extent to which these practitioners are explicitly involved in genetic counselling. Haemophilia centres also vary in the extent of their engagement with local clinical genetics centres.

All specialist medical and nursing staff within haemophilia centres are expected to have the requisite skills, knowledge and attitudes to enable them to provide information to patients and families on the following:

- Inheritance patterns
- The nature and implications of heritable bleeding conditions
- Treatment and complications
- The options open to family members who may wish to have genetic testing.

The regular audit of Comprehensive Care Centres undertaken by the UKHCDO does incorporate the genetic service but does not formally examine the quality of counselling or the competency of involved personnel. In this document the multidisciplinary UKHCDO Genetics Working Group addresses the governance issues relating to genetic counselling provision within haemophilia centres. This requires the development of a clear, structured and more formalised approach to the audit of genetic counselling conducted within haemophilia centres.

2.2 *Ensuring quality of genetic healthcare services*

Due to advances in molecular genetics, families have options for diagnostic, carrier and prenatal testing, as well as pre-implantation genetic diagnosis. The service provided by Haemophilia Centres should enable families to consider all of the options available to them and to access those services they feel are appropriate. While staff concerned principally with offering treatment and care will have many genetic healthcare skills there may be issues that need to be considered away from the day to day treatment setting. It may therefore be helpful for specialist genetic practitioners to be involved with genetic counselling. This enables the family to consider genetic testing and reproductive issues without disclosing those decisions to professionals offering treatment or care.

Precedents have been set in services for many other types of genetic condition, where specialist health care and specialist genetic counselling are offered in different settings or by different professionals. For example, patients affected by cystic fibrosis are cared for in specialist centres, but genetic counselling is usually offered to such families by separate specialist genetic staff. Similarly, pregnant women seeking genetic information about a potential or actual fetal abnormality are referred to genetic services by the obstetric team responsible for management of the pregnancy. In many centres, joint clinics are held between genetic services and other specialists to serve the needs of families concerned about conditions affecting a particular body system (e.g. joint genetics/ophthalmic clinic, genetic/skeletal dysplasia clinic, genetic/neuromuscular clinic). This enables families to discuss therapeutic options, prognosis for the condition and current reproductive options.

It is essential that those seeking genetic counselling feel free to make decisions that are not constrained by their commitment to existing family members. This professional issue has been identified recently in other areas of healthcare, such as midwifery and is of particular importance when considering the ethical principles of autonomy and justice (Cignacco, 2002). The criteria for offering prenatal genetic testing to individuals include confidentiality and obtaining prior informed consent (Maddox, 1992). These criteria are more effectively fulfilled when a distinction is made between professionals providing clinical management for a condition and those who offer genetic testing. The results of genetic tests should only be disclosed to others with the consent of the consultand. Haemophilia specialists are highly committed to the success of treatment and may, or may not, be conscious of the potential impact of this in the genetic counselling situation. It is also important to consider whether particularly sensitive issues such as paternity can be addressed in a setting where staff and families know each other well. For these reasons families should have the option to access a genetic counsellor who is not directly involved in provision of care for the affected family, and a choice of venue for receiving genetic counselling, either within or outside their haemophilia centre. Reports by the Genetic Alliance UK (Genetic Alliance UK, 2000) have indicated that families should be offered the option of prenatal testing so that they can make decisions relevant to their own situations.

Information on genetic testing and interpretation of results is primarily imparted to patients by haemophilia doctors. However, specialist nurses and psychosocial professionals know patients and families well and therefore have an important role in identifying and reaching individuals who should be offered genetic testing. While these haemophilia specialists would not be expected to have a working knowledge of all genetic conditions, it is appropriate that they have the requisite genetic competences related to bleeding disorders. Guidance on the specific genetics knowledge and skills expected of nurse specialists has been developed for use in European countries and these are directly relevant to haemophilia nurses (Skirton *et al*, 2010). A detailed list of competences and learning outcomes for this group can be found at the website of the European Society of Human Genetics.

While many health professionals use counselling skills in their work, genetic counselling in the United Kingdom is a specialised area of practice with a professional registration system operated by the Genetic Counsellor Registration Board (GCRB). It is anticipated that genetic counsellors will be subject to statutory regulation by The Health and Care Professions Council (HCPC) in the future but a voluntary regulation system is at present still administered by the GCRB. The essential competencies for genetic counsellors have been defined by the Association of Genetic Nurses and Counsellors (AGNC). These are consistent

with the European core competences for genetic counsellors and would apply to genetic counsellors working within a haemophilia centre.

It is not feasible, or necessarily desirable, for all haemophilia specialists to attain the level of practice required for registration as a genetic counsellor, as their work with one group of conditions would not confer generic skills. However, there may be some haemophilia nurses who have a special interest in this area who wish to develop competency to the registration level. This would entail accepting a greater proportion of genetic counselling work and therefore impact on the skill-mix and responsibilities within the haemophilia team. Becoming a registered genetic counsellor also requires knowledge of a wider range of genetic conditions and therefore a period of time working in the regional genetics centre. Strong links with the Regional Genetics Centre would be necessary along with clinical supervision by a registered genetic counsellor.

2.3 Recommendations

- ***Genetic counselling is provided by a multidisciplinary team.***
- ***Maintenance of strong links between haemophilia centres and regional genetics services.***
- ***Education and competency development for haemophilia specialists involved in provision of genetic counselling.***
- ***Identification of a lead professional for genetic counselling within each haemophilia comprehensive care centre to play a key role in service provision, clinical governance, audit, education and liaison with regional genetics services.***
- ***Development of a more structured examination of the genetic counselling service provided by a comprehensive care centre in the UKHCDO triennial audit.***

Genetic counsellors working jointly with haemophilia centres and regional genetic centres may be employed to undertake:

- ***Genetic counselling for members of families with haemophilia and related heritable bleeding conditions.***
- ***Maintenance of a register of families affected by or at risk of heritable bleeding disorders to enable the haemophilia centre to offer a full service to family members***
- ***Clinical supervision for haemophilia centre staff on genetic counselling issues.***
- ***Development of education and training for haemophilia centre staff on genetic counselling issues.***

2.4 Practical issues

Genetic counselling is appropriately provided by many healthcare professionals involved in the care of patients. Named healthcare professionals should be identified to take a lead on the co-ordination of genetic counselling aspects of care. This may be achieved through a variety of approaches.

One option is employment of a specialist genetic counsellor who works within the haemophilia centre on a sessional basis. The specialist genetic counsellor would be mainly based within the Regional Genetic Centre, to enable him or her to access support, supervision and maintain current genetics knowledge. It is essential that a genetic counsellor in this role has the relevant knowledge of heritable bleeding disorders to undertake this co-ordinating

role. Regular education and supervision from haemophilia centre staff is key. An appropriate portion of the salary would be funded by the haemophilia centre.

This approach may not be possible due to practical local or funding issues. A practical solution is to identify a haemophilia nurse specialist with a special interest in genetics who develops their role within the Centre. This person would have strong links with staff of the regional genetics centre, for education, supervision and support, and would be expected to spend some time regularly in the genetics centre. Initially a period of training in the Regional Genetics Centre would be required. Again it is important that clients are offered the choice of seeing the genetic counsellor in the genetics centre rather than in the haemophilia centre.

In both of the above cases, the emphasis is on providing strong links between genetics centre and haemophilia centre, and enabling the genetic counsellor to access education and supervision in both areas. The system would also enable registers to be kept up to date and enable new developments to be available for families rapidly.

It is suggested that a named clinical geneticist be asked to act as the key link person from a clinical genetics perspective in each region.

Section 3. Consent and written information

3.1 Introduction

Seeking informed consent for genetic testing requires careful and considered explanation. The recommendations of the European Society of Human Genetics state that genetic testing should be based on respect for the principle of self-determination of the persons concerned and therefore subject to their express, free and informed consent. No condition should be attached to the acceptance or the undertaking of genetic tests. Informed consent is also required for all types of DNA banking. These recommendations advocate careful consideration of the psychological complexities of testing and a multidisciplinary approach.

The guideline on “Consent and Confidentiality in Genetic Practice” from the Joint Committee on Medical Genetics (JCMG) (Joint Committee on Medical Genetics, 2011) noted the General Medical Council (GMC) guideline that patients may indicate their informed consent either orally or in writing. General Medical Council advice states that written consent is important if there are significant consequences for the patient's employment, social or personal life or where providing clinical care is not the primary purpose of the test, but the judgement of what constitutes significant has to be made on a case by case basis. The JCMG did not wish to prescribe in which situations formal consent forms are used but in the case of haemophilia we recommend that written consent is obtained. The issue of testing children is considered in section 6.

For patients and family members it is recommended that written information is made available and that signed informed consent is obtained for genetic testing.

Within the context of testing for a bleeding disorder a model patient information sheet and consent form is given in Appendix I. This can be adapted to local circumstances and arrangements within each haemophilia centre. A photocopy of the completed and signed form should be given to the patient, a copy of the information sheet and the original of the consent form should be filed in the patient's case notes and a copy of the consent form filed in the family genetic file. Some key elements related to testing should be considered as part of the content of pre-test discussion and follow-up.

3.2 Process

The clinical practitioner should:

- Establish that a bleeding disorder is present in the family and determine its type and severity
- Establish a pedigree/family tree
- Assess understanding, expectations, beliefs and wishes
- Acknowledge the implications of individual and family experiences, values and culture
- Address personal and relationship concerns related to testing
- Provide the opportunity for questions to be asked prior to obtaining consent
- Provide the opportunity for the consultand to present their understanding of the information that has been discussed and its implications for themselves and others.
- Ensure information and its significance is understood and accepted
- Offer a follow-up appointment
- Where the need for ongoing support is identified in the course of the consultation, make appropriate referrals

- Make clear arrangements for imparting the results of testing.

3.3 *Information*

Information provided should include:

- The potential clinical effects of being a carrier or affected person
- Current treatment and implications of the condition
- The mode of inheritance and the individual's genetic risk (for haemophilia A and B leaflets describing X-linked inheritance may be used in addition to the information specific to haemophilia testing)
- The rationale for identifying the genetic defect
- The means by which carrier status is assessed
- What is involved in genetic testing: sample collection; transfer/storage of data; research projects on stored material; insurance issues; risk of error
- Information on the procedures for prenatal and preimplantation testing.

The NHS consent policy and generic forms point to the importance of making written information available to patients to back up the content of face to face discussion. NHS organisations remain responsible for satisfying themselves as to the quality and accuracy of the information they provide to patients (see HSC 2001/023 Good Practice in Consent). A range of patient information leaflets on relevant inheritance patterns and forms of genetic testing are available in many languages from the Genetic Alliance UK website. These have been rigorously assessed by both professionals and patients as appropriate for use and may supplement other individualised written information provided to patients.

Many laboratories seek confirmation that consent has been obtained prior to carrying out genetic analysis and storage of DNA samples. Use of a consent form (such as that in Appendix I) would provide documentary evidence and comply with such a requirement. The responsibility to obtain consent lies with the requesting healthcare professional.

Section 4. Data management

This section provides a detailed background to data collection and storage and consent / right of access to medical records.

Genetic counselling is an essential part of the comprehensive service offered to patients and their families with haemophilia and other heritable coagulation disorders. It is recognised that this should include the offer of counselling and risk assessment to female relatives at an appropriate age and time in those families with X-linked disorders such as haemophilia. Indeed, the Genetic Alliance (UK) (formerly the Genetic Interest Group, GIG), an umbrella organisation of genetic disorder support groups states in its guidelines that, 'systems are needed to facilitate efficient, effective, long-term follow up of service users and their families and contact of at-risk relatives' (Genetic Interest Group, 1998). In terms of specific recommendations, the same GIG document states that 'the service should enable children and young people in a family to be offered the opportunity of referral for genetic information and counselling when appropriate' and that 'services should make direct contact with young adults in affected families when they reach the age of 16, and invite them to use the service'.

In order to enable this process, clinical genetic services have established computerised family recall systems and a system of family genetic records that may be paper based or electronic.

4.1 Family genetic records

In many Haemophilia Centres pedigrees may be compiled but are filed either in the index patient's case notes or manually elsewhere in the Haemophilia Centre. There are advantages in linking members of the same family within the same file and clinical genetics departments keep family-based records for this purpose.

It is recommended that Haemophilia Centres develop family genetic records of patients with haemophilia and other heritable bleeding disorders.

It is recommended that these notes should:

- ***Be organised in a separate 'genetic' file***
- ***Be kept within the Haemophilia Centre***
- ***Contain a family pedigree compiled using standard conventions***
- ***Contain the results of all relevant genetic and phenotypic tests***
- ***Contain informed written consent for genetic studies, sharing of appropriate family information and inclusion on a register***
- ***Contain copies of all pedigree related correspondence***
- ***Be kept confidential and only accessed by authorised staff of the Haemophilia Centre.***

The maintenance of pedigrees will require continued commitment. The pedigree should be updated at least annually, taking advantage of one of the regular clinic visits of the index patient where possible. Note should be taken of any family members who should be offered genetic advice or are reaching an age where it would be appropriate to do so.

At these updates it is important to try and confirm the family relationships that have previously been documented and to add new family members that have been born in the intervening period. Reminders should be put in place to ensure this happens.

4.2 Genetic Family Recall systems

A system needs to be in place to offer follow-up of possibly affected relatives, in particular for the recall and counselling of potential carriers within affected families. As indicated above, this could be achieved by discussing genetic issues at least annually at routine clinic appointments. Clinical genetic departments that do not routinely review families in clinic have generally adopted a recall system, usually computerised, whereby family files are brought up for review at an appropriate time. This acts as a trigger to contact the family and advise that a referral for genetic advice would be appropriate. It would be appropriate for Haemophilia Centres to consider adopting a similar approach.

In conjunction with the development of the family genetic records, it is recommended that a haemophilia genetic recall system is also established in each centre.

This recall system could take the form of a genetic register. In simple terms, a genetic register comprises a list of people affected by, or at risk of genetic disease, linked as families, and linked to a diagnostic index (Dean *et al*, 2000). It is usual for such databases to be computerised. Such a confidential database of families can serve several functions as it allows:

- Regular contact with families
- Planned follow-up in order to offer counselling to at-risk family members at appropriate ages
- Recall of families in the light of genetic research developments.

Such a database could be a genetic add-on to the Haemophilia Centre's general patient management system.

4.3 Contacting relatives to offer genetic counselling

When a pedigree is taken for the first time or when it is updated, the genetic counsellor will seek to identify those other family members to whom the offer of genetic counselling would be appropriate, such as the close female relatives of a male with haemophilia. It is the usual practice in clinical genetic departments to indicate to the patient (or their parents in the case of a child) those relatives to whom this offer would be appropriate. It is usually regarded as the family's responsibility to contact these relatives and alert them to this offer. The Nuffield Council on Bioethics report in 1993 stated that the primary responsibility for communicating genetic information to a family member lies with the individual and not with the doctor (Nuffield Council on Bioethics, 1993). The Medical Ethics Committee of the British Medical Association suggested that in those cases where the individual is unwilling to transmit the information but gives consent for the information to be shared, the genetic centre should approach the relatives through their General Practitioner (GP).

Clinical genetic departments often provide the family with an explanatory letter or information sheet that could be sent to relatives. Certainly it is considered good practice to write to all families following a genetic counselling appointment to provide written confirmation of the risk assessment given during counselling and a summary of the options open to the family, including the possibility of antenatal diagnosis where appropriate.

It is recommended that a post consultation letter is sent to all families indicating the genetic risks, options available and the offer of genetic counselling to other at-risk relatives. The letter should include a recommendation to contact the haemophilia/genetic centre preferably prior to any pregnancy but in the event of a pregnancy, as soon as a pregnancy is confirmed. This should be offered whether or not prenatal diagnosis is a consideration for the family, as it may be necessary to make arrangements for safe delivery of the fetus.

4.4 Storage of data and sharing of that data.

With regard to the storage of data, the Human Genetics Commission (HGC) in its report “Inside Information” (section 4.2, page 69) notes that the storage of information about other persons raises potential data protection issues. The report states, “There is potentially a considerable amount of information about family members on most medical records. However there is potentially far more significant information on records held by clinical genetic centres. This is especially true when family pedigrees are stored in combined files or where genetic registers are held”.

When families are seen in clinic they can be asked to consent for their data, including DNA results, to be stored on a local register and also on the National Register and this is covered in the information sheet and consent form for molecular genetic analysis and the leaflet explaining the National Haemophilia Database.

Consent to information processing is governed by the Data Protection Act. Information processing includes the collection, storage, disclosure, retrieval, destruction and alteration of data. The Joint Committee on Medical Genetics in their recent updated report on “Consent and Confidentiality in Genetic Practice” recommended that family history and clinical information can be shared “with other health professionals (regardless of their geographical location) provided that the sharing of confidential information is necessary for the purposes of health care, and disclosure is between health care professionals who share in their duty of confidence (pursuant to Schedule 3 of the Data Protection Act 1998)”.

4.5 Access to records of a relative

The sharing of stored information discussed above could involve access to medical records or gaining information from colleagues without access to specific records. Whilst referral of a patient implicitly includes consent to review their medical records, there may be occasions when in the genetic counselling of a family, it is important to have access to the records or test results from relatives.

An example would be if a woman was referred for carrier testing because she has a male relative with haemophilia: that male relative’s records and test results may need to be accessed to confirm the diagnosis and familial mutation or alternatively to see whether there was evidence of another bleeding disorder, and, if so, which one. Sometimes the relatives may be deceased.

Under these circumstances, information could be obtained from the patient’s case notes. In terms of seeking information from case notes the legal position for living relatives is broadly that consent can be obtained for access to information from that person.

If the person is alive it is recommended that consent is from them or the person with parental responsibility to access the required information

Access to the health records of the deceased is governed by the Access to Health Records Act 1990. This Act applies only to records compiled on or after 1 November 1991, although the record holder (usually an NHS Trust) does have discretion to permit access to earlier records. Some hospitals exercise this discretion by choosing to allow access to such records only with consent from the spouse, but the information contained in the records may be relevant to the medical management of a blood relative, a possibility not considered in the Act. The Human Tissue Act (HTA) acknowledges this by establishing the concept that those satisfying 'any qualifying relationship' may provide consent for the release of bodily material posthumously for genetic analysis rather than a hierarchy of relatives being applied. Although the HTA rules apply only to cellular material, the recent report on Consent and Confidentiality by the Joint Committee on Medical Genetics (2011) recommends that a similar concept of consent from qualifying relatives be accepted by healthcare facilities to allow access to medical records of deceased patients. Verbal consent from qualifying relatives to staff should be sufficient and this can be documented in the "request for information" letter sent by genetics or haematology departments.

4.6 Disclosure of information about a relative without consent

The above discussion was centred on obtaining information about relatives from medical notes. It is also possible to gain information from colleagues. Although there are many trusted links between departments and laboratories, the established links do not remove the need for consent for both information and sample sharing. However, in exceptional circumstances it should be acceptable under current GMC guidelines to proceed without consent if necessary. An example of such exceptional circumstances would be the case of a pregnant woman presenting at an antenatal clinic and stating that her sister who lives elsewhere is a carrier for haemophilia. If the sister cannot be contacted then, in such circumstances, it should be professionally acceptable for the laboratory that established the diagnosis to share information/samples with those involved in the care of the pregnant woman. The reasons for doing so should be carefully documented.

The recent report from the Joint Committee also highlights the situation where the pregnant lady does not want anyone to know about the pregnancy until test results are available. Seeking consent from a relative may reveal information about the pregnancy and breach the lady's confidentiality. It may be judged that more harm would result by not using the information about the relative than would occur by using their information/sample without confirmation that consent had been obtained.

Disclosure without consent should be carefully considered and documented including the reasons for disclosure and the absence of consent.

Consent for sharing of information with relatives could be achieved prospectively if such information sharing was discussed at the outset of a genetic consultation and consented to. If this is not the case then it is good practice to try to obtain consent retrospectively if this becomes possible e.g. in this example above, if the carrier sister had been abroad.

In order to avoid disclosure of information difficulties the working party recommends the use of an information sheet with written consent for genetic testing. The consent obtained

includes the agreement for sharing the results of genetic tests for the benefit of other family members.

It is good practice to obtain consent for this disclosure whether the other family members are being seen in the same department or another one.

It is good practice to ensure that the proband understands the benefit of keeping the primary Health Care Team informed and also the potential implications of a genetic diagnosis. As mentioned earlier it is recommended that the proband gives consent to information sharing with other Health Professionals.

Another situation is where a relative refuses to consent to the release of important information. The report of the Joint Committee noted that guidance from the Human Genetics Commission, the Nuffield Council on Bioethics and the General Medical Council reaffirms that the rule of confidentiality is not absolute. In special circumstances it may be justifiable to break confidence where the avoidance of harm by the disclosure substantially outweighs the patient's claim to confidentiality

4.7 Genetic testing in persons unable to give consent

The Joint Committee recommend that where genetic testing involves a person who is unable to consent, a consent form should not usually be signed, but it is good practice to document in the medical records why the action was believed to be in the patient's best interests. The report considers the issues in relation to the Mental Capacity Act (2005) and the Adults with Incapacity (Scotland) Act 2000.

Section 5. Pregnancy and prenatal diagnosis

It is good practice to address issues related to the genetics of heritable bleeding disorders before the first pregnancy so that individuals and families are not faced with large amounts of information and potentially difficult decisions in a short period of time during early pregnancy. In addition, laboratories should not be asked to provide results under time pressure if this can be avoided. It is the case, however, that some known or potential carriers of bleeding disorders unavoidably present during pregnancy and in these cases the relevant issues must be addressed urgently.

Good communication between all interested parties is essential to a successful process. This is best co-ordinated by the Haemophilia Centre. Communication should include the pregnant woman, obstetric/fetal medicine unit, laboratories and GP. There may be more than one laboratory involved in providing phenotypic testing, analysis of free fetal DNA (ffDNA) in the maternal circulation for fetal gender determination, molecular diagnosis and karyotype analysis (when prenatal diagnosis for chromosomal abnormalities is also performed).

5.1 Confirmation of diagnosis

The family diagnosis should be confirmed unequivocally and if necessary affected family members should be reinvestigated. This may be particularly relevant if a diagnosis was made some years ago as reinvestigation with modern techniques and assays may yield important information relevant to genetic counselling and management. The coagulation factor level and clinical severity of affected individuals in the family should be reviewed. A definitive confirmation of the family diagnosis and coagulation factor level may not be possible if an affected family member is not available for investigation. The available phenotypic and genetic laboratory data should be critically reviewed. The quality of results should be reviewed with regard to the techniques and controls used. A family tree should be drawn up or the accuracy of an existing family tree confirmed. The status of the pregnant woman can then be confirmed. If the family is affected by a recessive disorder testing of the partner may be helpful.

The diagnosis, coagulation factor level and mutation within the family should be definitively confirmed, if possible, and the family tree updated to ensure that carrier assignment is accurate.

5.2 Counselling

The purpose of genetic counselling is to provide the mother and the family with adequate information to reach a decision regarding prenatal diagnosis and to provide support throughout the process. Genetic counselling should be conducted before pregnancy. For the woman who presents in pregnancy, genetic counselling should be available as early as possible. The environment should offer privacy and comfort. Staff involved should be competent in genetic counselling and knowledgeable about heritable bleeding disorders as described in detail in section 2. In practice, this entails access to more than one professional. If prenatal diagnosis is being considered, appropriate staff from the fetal medicine unit should be involved at an early stage.

Genetic counselling should cover the following topics:

Clinical phenotype: The bleeding disorder in the family should be described along with likely bleeding phenotype and its severity and potential complications including the potential risk of inhibitor development. The expected quality of life of affected children and the impact on the family should be discussed. The efficacy, safety and side effects of current treatment should be covered. It may be necessary to explore the individual's previous experiences of the disorder within the family, particularly in relation to infective complications and severe disability. Partners and individuals who have limited firsthand experience of the disorder will require extensive counselling about the condition and its current treatment and management.

Inheritance: The mode of inheritance of the disorder should be described and the situation of the individual seeking counselling established.

Reproductive Options: The available reproductive options should be discussed.

The options for women with heritable bleeding disorders, in general, include:

- 1.) Not having children
- 2.) Adoption or fostering
- 3.) Conceiving naturally and accepting the outcome of the pregnancy. In this case the majority will not have prenatal diagnosis. However, some may opt for prenatal diagnosis for other reasons such as psychological preparation and planning for place and type of delivery in case of an affected foetus
- 4.) Conceiving naturally and having prenatal diagnosis with the option of termination of affected pregnancy
- 5.) Assisted conception with donor gametes
- 6.) Conceived using IVF and either not having prenatal diagnosis and accepting the outcome or having prenatal diagnosis (including pre-implantation genetic diagnosis) with option of TOP

The advantages and disadvantages of each option should be explored including the psychological effects on other family members and the family as whole.

These discussions will be affected by the individual's and the family's previous experiences of the condition and its complications. Each option should be fully explained including their availability and the procedures involved (how and where they would be performed, accuracy and success rates and potential risks to the fetus and the mother).

Pre-pregnancy counselling should be offered to discuss suitable reproductive options and methods of prenatal diagnosis.

5.3 Prenatal diagnosis

Counselling: All options available for antenatal diagnosis should be discussed with the pregnant woman and, if appropriate, her partner. The risks and benefits of each approach should be discussed and compared. Options may include fetal gender determination by analysis of ffDNA in the maternal circulation with or without ultrasound examination and/or invasive tests for specific diagnosis with chorionic villous biopsy (CVS) or amniocentesis or cord blood sampling (if genetic analysis was not informative). Pre-test counselling is undertaken jointly by appropriate Haemophilia Centre and fetal medicine staff. The mother/couple should be informed about the procedures; how they will be performed, the possibility of not obtaining an adequate sample, non-diagnostic results and potential side effects for both mother and fetus. Processes for any long-term sample storage and quality control should be discussed. It should also be agreed with them what tests will be performed

and in what order. In particular it should be agreed whether tests unrelated to the bleeding disorder will be performed. The latter includes screening for fetal chromosomal and structural abnormalities (e.g. fetal nuchal translucency) during ultrasound examination and of genetic testing for chromosomal abnormalities in the event of invasive testing.

An indication should be given about how long the tests will take to be performed. A crucial part of pre-test counselling is a discussion of what options would be taken by the woman with each possible test outcome and the potential effects of these decisions should be explored.

When planning for invasive testing, the standard precautions for the prevention of rhesus isoimmunisation and haemostatic cover for the procedure, if required, should be discussed along with issues related to maternal and fetal exposure to blood products if relevant.

Communication of results: It should be decided in advance how and where the results of the diagnostic test should be given and who will be responsible for this. Once the results are known the options available to the woman should be discussed. It may be necessary to allow time for the results to be considered before a decision is reached.

Counselling for antenatal diagnosis should be performed by a combination of haemophilia centre and fetal medicine staff.

Procedures and communication between Haemophilia Centre, fetal medicine department, laboratories and GP should be formalised in a written protocol.

5.4 Invasive prenatal diagnostic tests

At present, the definitive (specific) prenatal diagnosis of heritable bleeding disorders can only be achieved through invasive procedures. These include chorionic villus sampling (CVS) or amniocentesis for obtaining fetal materials for genetic analysis or cord blood sampling (cordocentesis) for clotting factor assay. A detailed guideline for amniocentesis and CVS has been published by the Royal College of Obstetricians and Gynaecologists (RCOG) (Royal College of Obstetricians and Gynaecologists, 2010).

Some women may need haemostatic cover, such as desmopressin or coagulation factor concentrates, for these procedures depending on their diagnosis and level of coagulation factor. This should be assessed and organised in advance and administered prior to the procedures appropriately.

5.4.1 Chorionic villus sampling

Chorionic villus sampling (CVS) is currently the method of choice for obtaining fetal materials for the prenatal diagnosis of heritable bleeding disorders. It involves taking a sample of chorionic villi for analysis. The main advantage is that it allows first trimester diagnosis, thus a shorter period of uncertainty and hence avoids late termination of pregnancy if opted for.

Procedure: Written informed consent for the procedure must be taken. The counselling process and the consent should be clearly documented in the patient's notes. Before the procedure, an ultrasound assessment is performed to confirm the viability of the pregnancy, the gestational age, the number of fetuses and the position of the fetus and the placenta. CVS

is usually performed between 11⁺⁰ and 13⁺⁶ weeks of gestation. CVS must be performed under continuous ultrasound guidance. The procedure can be performed using either trans-abdominal or trans-cervical approach. However, most centres use a trans-abdominal approach. This approach takes about 10-15 minutes and is often performed under local anaesthetic. The sample is usually taken either by single needle or double needle aspiration by negative pressure using a syringe or a vacuum aspirator or biopsy forceps. The trans-cervical route involves the use of a speculum and passing a fine forceps or aspiration cannula through the cervix to obtain the sample. Clinicians carrying out this procedure should be trained to the competencies laid down by the RCOG and should use the technique with which they are familiar (Royal College of Obstetricians and Gynaecologists, 2010). Competency should be maintained by carrying out at least 30 ultrasound-guided invasive procedures per annum.

In case of multiple pregnancies, the chorionicity should be established by ultrasound examination early in the first trimester. For dichorionic twins, the role of CVS remains controversial and most authorities suggest that amniocentesis is preferable to CVS to minimise the risk of DNA contamination. For monochorionic twins a single CVS on a definitively monochorionic placenta may be acceptable. CVS or amniocentesis in multiple pregnancy should only be performed by specialist with the expertise to perform these procedures in multiple pregnancies. The principal of sampling in multiple pregnancy involves carefully mapping the pregnancy such that each fetus is clearly sampled separately and that each individual fetus can later be identified if a selective termination of pregnancy is required. The selective termination should be performed by the same specialist who mapped the pregnancy and performed the CVS or amniocentesis.

The material obtained is examined visually to confirm that it is adequate and labelled clearly, especially in a multiple pregnancy. The sample is placed in transport medium. The fetal heart is checked after the procedure and Anti-D given if appropriate. Before leaving, arrangements should be made regarding the method of communication of the result.

Adverse events: The main adverse event related to CVS is miscarriage which is estimated at about 1-2% with an experienced operator (Mujezinovic & Alfirevic, 2007). There are no data comparing miscarriage in pregnancies exposed to CVS to women with no invasive prenatal testing. Meta-analysis of randomised trials comparing CVS (trans-abdominal and trans-cervical) to second trimester amniocentesis showed an excess pregnancy loss after CVS (Alfirevic & von Dadelszen, 2003). However, randomised comparison of trans-abdominal CVS with second trimester amniocentesis in one study showed similar pregnancy loss rates with both procedures (Smidt-Jensen *et al*, 1992).

Fetal limb abnormality has been associated with CVS taken before 10⁺⁰ weeks gestation (Firth *et al*, 1991). Thus, it is recommended that CVS should not be carried out before 10⁺⁰ completed weeks of gestation.

Sampling failure can occur due to technical difficulties. There is a small (<1%) chance of failing to obtain a result from the laboratory test. There is also a very small (less than 1 in 1000) risk of serious infection from inadvertent puncture of the bowel or from contaminants on the skin or the ultrasound probe/gel. Standard procedures for infection control are recommended to avoid this complication. The risk of injury to the fetus is minimal and is decreased by the use of real-time ultrasound guidance.

Each unit should audit the outcomes of invasive procedures performed and advise patients of the respective complication rates.

Laboratory testing: In the case of X-linked disorders the fetal sex should be established initially. If the fetus is female no further tests are done apart from exclusion of maternal contamination. If the fetus is male, tests are performed to establish whether the mutation has been inherited. This may be done by direct mutation analysis, gene tracking techniques or a combination. Results are usually available within 48-72 hours of receipt of samples. Laboratories should be CPA accredited and part of the UK Haemophilia Genetics Laboratory Network.

5.4.2 Amniocentesis

Amniocentesis can also be used for prenatal diagnosis of heritable bleeding disorders. Amniotic fluid contains fetal cells (amniocytes) from which rapid detection of some specific chromosome trisomies can be achieved. Such rapid methods can also identify the chromosomal sex in cases of haemophilia. DNA can be extracted directly and used for PCR-based testing direct mutation detection or linkage analysis. The main disadvantage of amniocentesis compared to CVS is that a termination, if opted for, will occur later in pregnancy and surgical option for termination of pregnancy would not be an option in most NHS hospitals.

Procedure: Written informed consent for the procedure must be taken. This technique is generally performed between 15⁺⁰ to 18⁺⁰ gestational weeks. Before the procedure, an ultrasound assessment is performed to confirm the viability of the pregnancy, the gestational age, the number of fetuses, placental site and the umbilical cord insertion. A fine 20- or 22-gauge needle is inserted through the maternal abdominal wall into the amniotic cavity to obtain a sample (15-20 ml) of the amniotic fluid through needle aspiration. It is recommended that amniocentesis be performed under direct ultrasound control with continuous needle tip visualisation to reduce the chance of obtaining a 'bloody tap' as the presence of blood can interfere with cell culture. This also helps to minimise the risk of fetal trauma, which is rare. Local anaesthetic is usually not required for this procedure. Clinicians carrying out this procedure should be trained to the competencies laid down by the RCOG (Royal College of Obstetricians and Gynaecologists, 2010). The fetal heart is checked after the procedure and Anti-D given if appropriate. Before leaving, arrangements should be made regarding the method of communication of the result.

Amniocentesis should NOT be performed before 15+0 weeks because of the increased risk of miscarriage and fetal talipes (CEMAT Investigators, 1998). Furthermore, early amniocentesis is technically more difficult with higher rates of multiple needle insertions and cytogenetic-culture failure. This may be due to the presence of two separate membranes (amnion and chorion) until the 15⁺⁰ gestational week. For these reasons, early amniocentesis is not recommended and CVS is preferable for achieving early prenatal diagnosis.

Adverse events: The miscarriage risk associated with amniocentesis is around 1% (Tabor *et al*, 1986). Other complications include a small chance (<1%) of not obtaining a definitive diagnosis due to inconclusive results or culture failure and an even smaller risk (<0.1%) of serious infection caused by skin or ultrasound probe/gel contaminants or by inadvertent puncturing of the bowel.

Laboratory testing:

Rapid detection of the sex chromosomes is achieved by fluorescence in situ hybridization (FISH) or increasingly by QF-PCR. This information is usually available within 24-48 hours. DNA is also extracted and used for molecular analysis. However, there is sometimes insufficient DNA present in the sample for analysis. Therefore, the testing may be delayed until cultured cells are available which takes approximately two weeks.

5.4.3 Third trimester amniocentesis

Three to four percent of infants with haemophilia experience a cranial bleed that occurs during labour and delivery (Kulkarni *et al*, 2009;Kulkarni & Lusher, 1999). The best mode of delivery for affected fetuses remains controversial. The traditional recommendation suggests a vaginal delivery, while avoiding a prolonged labour and difficult instrumental deliveries (Lee *et al*, 2006). However, it is not possible to predict which women will have an abnormal labour and subsequently a difficult and/or operative vaginal delivery, all of which increase the risk of cranial bleeding. Therefore, a planned Caesarean section has recently been recommended and increasingly used for delivery of affected or potentially affected fetuses (James & Hoots, 2010;Huq & Kadir, 2011). A planned Caesarean section allows for a controlled delivery and reduces the risk of intracerebral haemorrhage by an estimated 85% compared to vaginal delivery. On the other hand advocates for vaginal delivery argue that section is associated with an increased maternal risk due to haemorrhage and abnormal placentation in subsequent pregnancies (Clark *et al*, 1985).

Recently, prenatal diagnosis by third trimester amniocentesis has been utilised as a possible option that enables appropriate planning of mode and place of delivery for parents who are unwilling to accept the risk of fetal loss associated with earlier prenatal testing (Cutler *et al*, 2013). If the fetus is unaffected, labour and delivery can be managed without any restrictions in the local maternity unit. However, third trimester amniocentesis is an invasive procedure and there is an approximate 1% risk of procedure-related complications such as preterm delivery, premature rupture of membrane and placental abruption (Stark *et al*, 2000;Gordon *et al*, 2002). Complications such as multiple attempts (>5%) and blood-stained fluid (5-10%) are also more common compared with mid-trimester procedures. Furthermore, there is an approximate 1% chance of failing to obtain a sample and a higher culture failure rate in amniotic fluid samples taken in the third compared to the second trimester (10% vs. <1%) (O'Donoghue *et al*, 2007;Hodor *et al*, 2006). There is also the risk of unexpected delivery before the availability of the test result. In addition, a positive diagnosis could become a psychological burden in advance of delivery. It may also raise several ethical and moral dilemmas that are beyond the scope of this guideline. Published data on the use of third trimester amniocentesis in pregnancies affected with bleeding disorders has demonstrated safety and acceptability (Cutler *et al*, 2013)

5.4.4 Cord blood sampling

Fetal cord blood sampling to investigate haemostatic disorders is very rarely used in the UK and should only be considered if all other possible techniques cannot be used or do not give conclusive results. In the vast majority of cases molecular techniques will be available, will give more reliable results with a lower risk of complications and a lower risk of artefact affecting the sample leading to misinterpretation of the results. Fetal cord sampling is a technique that should only be used to investigate severe deficiencies of coagulation factors where an undetectable level would suggest an affected fetus. Fetal cord sampling may be considered if a woman wishes to ensure that she does not have a child affected with severe

haemophilia and a causative mutation cannot be identified. Pre-test counselling should cover the possibility of artefact, incorrect or uninterpretable results.

Procedure: Fetal cord blood sampling should only be considered in tertiary referral fetal medicine units experienced in this technique and capable of performing coagulation assays on fetal blood. Written informed consent should be taken.

Cordocentesis is performed after 18⁺⁰ weeks of gestation. It involves the insertion of a 20-22 gauge needle under continuous ultrasound guidance through the maternal abdomen and uterine walls and into the umbilical cord. A sample of fetal blood is taken usually under continuous ultrasound control from the umbilical vein at the placental insertion of the umbilical cord. The fetal heart is checked after the procedure and Anti-D given if appropriate. Before leaving, arrangements should be made regarding the method of communication of the result.

There is a risk of the sample being contaminated by maternal blood or amniotic fluid which will artefactually affect coagulation factor levels. One practice is to take three 1ml samples and test the first and third for coagulation factor levels to ensure these are consistent. The middle sample is tested for Hb and MCV and compared to the maternal MCV to confirm that the blood is of fetal origin (>120 fl for fetal MCV and ~90 fl for maternal MCV). Other measures can be used to check for maternal blood contamination including the Kleihauer-Betke test showing resistance of fetal haemoglobin to acid elution and/or analysis of molecular markers.

Adverse events: The procedure has a 1-2% risk of miscarriage in experienced departments (Tongsong *et al*, 2000). There is also a risk of cord haematoma or bleeding from the puncture site, which is usually transient but could potentially be significant in the presence of an abnormal coagulation. Fetuses affected with a bleeding disorder are at particular risk of this haemorrhagic complication and this should be taken into consideration when planning for the procedure (Ash *et al*, 1988). Other potential complications include fetal bradycardia (this may require urgent delivery if the procedure is performed after age of viability), infection, premature rupture of membrane and premature birth.

Laboratory testing: Contamination by maternal blood or amniotic fluid will invalidate the assay as coagulation factor levels will be affected. The plasma should be tested for the coagulation factor under investigation and one other control coagulation factor level. Fibrinogen should be tested as a markedly reduced level would suggest activation of the sample and that other measured coagulation factor levels are unreliable. The results should be interpreted with regard to fetal blood normal ranges derived from the appropriate gestation. If two samples have been taken interpretation should only be undertaken if the results are consistent. If the coagulation factor under investigation is undetectable and a control coagulation factor level, and fibrinogen level are within the expected range then a severe deficiency of that coagulation factor is confirmed. The test results would normally be available within 24 hours.

Chorionic villus sampling is the method of choice for specific prenatal diagnosis of haemophilia.

Maternal clotting factor level should be checked prior to any invasive procedures and prophylactic treatment arranged if the level is <50IU/dL.

For RhD negative mother, anti-D is given after the procedure.

Before leaving, arrangements should be made regarding the method of communication of the result.

5.5 *Non-invasive prenatal diagnostic tests*

Prenatal determination of fetal gender is valuable in the management of pregnancies at risk of X-linked genetic disorders such as haemophilia. Knowledge of fetal gender enables carriers of haemophilia to avoid invasive prenatal tests in pregnancies involving a female fetus, thus, avoiding the risks of invasive tests in 50% of cases. Knowledge of fetal sex also allows planning for the management of labour and mode of delivery. Fetal gender determination can be achieved with non-invasive test through ultrasound assessment of fetal external genitalia or by the analysis of ffDNA in the maternal blood.

5.5.1 Ultrasound Assessment

Sonographic fetal gender determination in the late second trimester is based on direct visualization of the external genitalia (Elejalde *et al*, 1985). This can be achieved with close to 100% accuracy if there is good visualisation, and enables detection and exclusion of female gender prior to amniocentesis. A repeat examination may be required if visualisation is not clear due to fetal lie. However, the development of the external genitalia is similar in both sexes until 14⁺⁰ weeks gestation, thus in the late first and early second trimester, ultrasound assessment of fetal sex is based mainly on the direction of the genital tubercle (the "sagittal sign") by measuring, in a mid-sagittal plane, the angle between the genital tubercle and a line drawn through the lumbosacral vertebrae. The fetal sex is considered to be male if the phallus is directed cranially with an angle to the lumbosacral vertebrae $>30^{\circ}$ and female if the phallus is directed caudally with an angle $<30^{\circ}$ (Efrat *et al*, 1999). The accuracy of this technique is limited around at 11⁺⁰ weeks gestation, but it increases with advancing gestation. After 13⁺⁰ weeks, it is accurate in 99% to 100% of cases (Avent & Chitty, 2006) in fetuses without malformed external genitalia. Errors in diagnosis of gender are more likely to occur in the assessment of the female fetus.

5.5.2 Free fetal DNA in the maternal circulation

Analysis of ffDNA in the maternal circulation is an alternative non-invasive means of determining fetal sex. With advances in molecular technology, particularly the development of quantitative real-time polymerase chain reaction (PCR), determination of fetal gender from the analysis of ffDNA in maternal circulation has become highly accurate with 97-100% specificity and sensitivity (Cremonesi *et al*, 2004). Although ffDNA can be detected as early as the 4-5th week of pregnancy, due to its low concentration at this stage the test can be unreliable with a high incidence of false negative results. However, the amount of ffDNA and consequently the sensitivity of the test increase with gestational age. It is, therefore, possible to utilise this technique to accurately identify fetal gender before 11⁺⁰ weeks gestation and avoid CVS in pregnancies involving a female fetus (Chi *et al*, 2006). This test is now incorporated in many units for prenatal diagnosis of haemophilia and pregnancies at risk of other X-linked disorders. It is essential that patients are counselled regarding its accuracy, limitations and the implications of its limitations. Furthermore, it should only be carried out in accredited and experienced laboratories.

Prenatal diagnosis of fetal gender should be offered to all carriers of haemophilia. Ultrasound diagnosis can be used in carriers who will have amniocentesis for specific prenatal diagnosis to exclude female fetuses as well as carriers who decline specific prenatal diagnosis to help management of labour and delivery. An attempt to determine the fetal sex by ultrasound should not be made before 12⁺⁰ weeks gestation because it is relatively inaccurate. FfDNA in the maternal circulation can be used prior to CVS to exclude female fetuses.

It is expected that further advances in fDNA techniques will enable mutation detection in addition to fetal sexing. In a recent study, fDNA analysis was used successfully for non-invasive specific prenatal diagnosis of haemophilia in seven carriers of haemophilia carrying a male fetus by identifying the mutant or wild-type allele inherited by the fetus (Tsui *et al*, 2011). However, this is still a research tool and further work is required before it is considered for clinical practice.

5.5.3 Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is an option for couples who would not consider termination of an affected pregnancy for religious or personal reasons and for those with concurrent infertility. The couple undergo *in-vitro* fertilization (IVF) to identify and transfer unaffected embryos. This reproductive option has been established for severe monogenic diseases over the last 20 years and is now offered in a substantial number of specialized centres. These are listed on the website of the European Society of Human Reproduction and Embryology (ESHRE). Best practice guidelines for PGD have been drawn up by the ESHRE PGD Consortium (Harton *et al*, 2011) and an external quality assurance scheme for molecular Preimplantation Genetic Diagnosis is run through UK NEQAS.

Haemophilia is the third most common X-linked disorder, after Fragile X and Duchenne muscular dystrophy, to be tested by PGD (Harper *et al*, 2010). Initially FISH was used in PGD of X-linked disorders to provide a diagnosis of fetal sex; with re-implantation of female embryos only. This leads to unnecessary disposal of healthy male embryos and reduces the success rate by decreasing the number of embryos suitable for transfer. More recently, disease-specific DNA amplification based tests have become available and these allow the identification of unaffected male embryos which can then be considered for transfer. This also distinguishes female carriers from normal female embryos. While it is not normal practice to exclude female carrier embryos this should be discussed with the parents. Depending on the previous experience of carrier phenotype in the family some couples will wish to exclude these embryos. There is also an extremely small chance of loss or inactivation of the normal X chromosome with the result that the resulting female child could manifest symptoms. Since the purpose of PGD is to avoid an affected child, some couples may not accept that risk however small, and will rank their embryos accordingly. Disease specific tests have been achieved by direct mutation detection in conjunction with testing informative linked markers (Laurie *et al*, 2010; Sanchez-Garcia *et al*, 2006; Michaelides *et al*, 2006; Fiorentino *et al*, 2003) or indirect genetic testing using flanking informative linked markers only (Renwick *et al*, 2010; Gigarel *et al*, 2004). The genetic distance of the linked markers needs to be considered to minimise the chance of an undetected cross-over event; the inclusion of flanking markers within 1Mb of the gene are recommended. Ten years of data collection by the ESHRE PGD consortium has seen the use of >1100 cycles of sex determination for X-linked disorders whilst the number of haemophilia disease specific tests undertaken is growing with just under 100 cycles undertaken (Harper *et al*, 2010).

Preimplantation genetic diagnosis is likely to become a realistic option for more couples at risk of having a child affected by haemophilia or other severe heritable bleeding disorders in the near future. However, PGD remains technically challenging and labour intensive with considerable financial implications, as it entails the use of *in-vitro* fertilisation (IVF). The IVF procedure is associated with significant risks mainly ovarian hyper-stimulation (approximately 1% of cycles started) and multiple pregnancies, with the associated complications of premature births and low birth weights. A 25% multiple pregnancy rate

(MPR) is reported by the ESHRE PGD data collection in 2007. In the UK the HFEA has introduced a policy to reduce the MPR and IVF clinics are working to bring rates below 15% by single embryo transfer and freezing of excess embryos for younger women.

In-vitro fertilization is also associated with high levels of stress and anxiety and the success rate (overall live birth rate) is much lower than spontaneous conception. In PGD cycles, the number of suitable embryos available for transfer is reduced by excluding affected embryos or those with inconclusive results). Latest figures available for PGD in the UK show that in 2009 there were 86 live births resulting in 100 babies. The live birth rate (births per cycles started) in the UK was 30% (data from the Human Fertilisation and Embryology Authority). The mean live birth rate of the ESHRE PGD data collection IX was 22% per cycle started; range 0%-50% (Goossens *et al*, 2009). This overall success rate varies considerably between PGD centres depending on their local IVF and genetic testing protocols. All PGD should be carried out in licensed centres and practice guidelines have been drawn up by the ESHRE PGD Consortium.

Prenatal diagnosis of fetal gender can be achieved accurately by non-invasive methods and should be offered to carriers of haemophilia considering specific diagnosis to exclude female fetuses.

Free fetal DNA in the maternal blood can be used prior to CVS and ultrasound examination prior to amniocentesis.

Knowledge of fetal gender allows appropriate management of labour and delivery.

For carriers who decline prenatal diagnosis, antenatal fetal gender determination should be offered and its importance should be conveyed to the parents.

5.6 Termination of pregnancy

If a woman decides to have a termination of pregnancy the appropriate documents of the Abortion Act must be completed. Dependent upon which clause is being enacted, management would be co-ordinated by either the gynaecology unit or maternity unit. Termination of pregnancy can be performed surgically before 15⁺⁰ weeks of pregnancy and uterine evacuation can be achieved by vacuum extraction with an appropriate-sized curette after cervical preparation with misoprostol or gemeprost. However, most units do not offer surgical option after 13⁺⁰ weeks of gestation. Medical termination is performed by mifepristone followed 36-48 hours later by either misoprostol or gemeprost. It is important that the mother is made aware of the gestation limit for surgical termination for the unit in relation to the likely timing of results from antenatal diagnosis. For a termination after 21⁺⁶ weeks, feticide is employed using an intracardiac injection of KCl to prevent the possibility of a live birth. If selective termination of dichorionic twins is necessary, intracardiac injection of KCl is administered to the affected fetus. This carries a 3% risk of co-twin death (Alvarado *et al*, 2012; Hillman *et al*, 2011). For monozygotic twins, a vaso-occlusive technique such as bipolar cord occlusion or radiofrequency ablation is performed to prevent trans-placental passage of the lethal agent as well as agonal twin-to-twin transfusion at the time of fetal demise and its co-twin sequelae. These procedures should be carried out and co-ordinated by a fetal medicine unit. Counselling and support should be provided by the haemophilia centre as well as the gynaecology unit. Anti-D prophylaxis for Rh negative mothers is administered as appropriate.

Women with bleeding disorders are at a higher risk of bleeding complications during and after termination of pregnancy (Kadir *et al*, 1997; Kadir *et al*, 1998). Coagulation factors should be assessed prior to the procedure and appropriate haemostatic cover should be provided to minimise the risk of bleeding. This is organised and arranged by the haemophilia centre.

When termination of pregnancy is opted for, close collaboration between haemophilia centre, fetal medicine and gynaecology unit is required for the appropriate choice of method of termination and avoidance of bleeding complications

Section 6. Genetic testing in children

Scientific and technological advances have made it possible to establish the causative mutation in most families with haemophilia and in many other heritable bleeding disorders. This has significantly improved the quality of information that can be offered to families by allowing assessment of the risk of inhibitor formation in affected males, precise carrier detection and improved prenatal diagnosis. It is recognised that in adults genetic tests are performed on people who can give informed consent after appropriate counselling but that some people decide that they would prefer not to know their genetic status for a variety of reasons. The recent updated guidance on Consent and Confidentiality in Genetic Practice considers the situation in those persons who cannot give consent for themselves, including children (Joint Committee on Medical Genetics, 2011). A more detailed discussion of the issues of genetic testing in children is provided in the recent guidance from the British Society for Human Genetics (BSHG) which updates the 1994 Clinical Genetics Society report on this topic (British Society for Human Genetics, 2010).

6.1 *Males with haemophilia*

The BSHG guideline notes that where genetic testing in childhood leads to better management of a child's condition (e.g. surveillance or treatment which can be initiated or stopped) the decision to test is unlikely to be contentious. It is thus recommended that all children with haemophilia have their genotype established. This gives useful information regarding the risk of developing an inhibitor and in the future individual genotype will be required if gene therapy becomes a proven treatment option. In addition establishing the genotype allows valuable information to become available to other family members.

6.2 *Females who are potential carriers*

Genetic testing of female children to see whether they are carriers of an X-linked condition, such as haemophilia, is contentious. The BSHG has recently reviewed this difficult area and considered the competing ethical, clinical, parental and individual requirements. The guidance states that where genetic testing is primarily for the purpose of predicting future reproductive risks, a cautious approach should be adopted. In such circumstances testing should normally be delayed until the young person can decide for herself when, or whether, to be tested. The rationale for this recommendation is that testing in childhood removes the opportunity of the future young person to make her own choices about such decisions and that this opportunity should not normally be denied her without good reason. Similar reasoning has been applied to inadvertent carrier testing in prenatal and preimplantation diagnosis for X-linked conditions. Here it is argued that an at-risk fetus or embryo could be sexed and, if female, no further tests need be performed unless the fetus is at risk of being symptomatic (e.g. Turner's syndrome).

However, the situation in haemophilia is more complicated than many other X-linked disorders because carriers may have some symptoms of the condition during childhood. It is important to establish whether carriers have an increased risk of bleeding to ensure appropriate treatment at times of surgery or trauma. There is therefore a medical reason to test baseline coagulation factor levels in carrier girls. This is usually done after one year of age when peripheral venous samples can be easily obtained but the tests should be performed earlier if required for a specific clinical reason. Since such tests need to be performed on more than one occasion to ensure reproducible results, a request for genetic testing to establish whether the girl is a carrier more definitively may well arise. Low coagulation

factors would imply that a girl is a carrier, but normal levels would not exclude it. A genetic test (if negative) might avoid the need for sequential coagulation factor testing and it can therefore be argued that such testing alters the immediate medical management of the child.

Thus, an argument can be made in the case of haemophilia A and B that it may be in the female child's best interests to have definitive carrier testing performed in early childhood in order to prevent repeated venepunctures in a girl who is shown not to carry the mutation (especially as carrier testing can be performed on DNA extracted from a cheek swab/smear). Indeed, in the BSHG guidance (2010) the example of haemophilia is specifically quoted in this context. On the other hand, performing such testing removes the child's future autonomy to make this decision for herself. Other adverse effects to testing during childhood have been proposed, such as actual or perceived stigma or discrimination, as well as poorer understanding of her carrier status as an adult. Some reports suggest that the knowledge of a girl's carrier status has not always been transmitted to her when she is older and performing a test in early childhood passes the onus of informing the girl of her carrier status to her parents/guardians rather than a health professional. It is therefore important to ensure that if testing is performed in early childhood that steps are taken to offer her appropriate information when she reaches adulthood. As the BSHG guidance states, an immediate decision about testing is unlikely to do justice to the complexity of the issues; ample time for discussion and consideration of the timing of test with all relevant parties should be allowed.

Another special consideration is the request to perform neonatal testing on unaffected children born following PGD. The parents may already hold information on the genetic status of the embryo(s) that were transferred so the test will not be supplying the parents with new information but will allow confirmation of the PGD result.

Whatever testing is performed (whether coagulation factor levels and/or genotyping), families should be sent written information of the result, its interpretation and an indication as to whether further tests should be considered or performed in the future. To avoid confusion all people tested should have their own individual case notes and record number and GPs should, where appropriate, be alerted to ensure appropriate follow-up when the child reaches adulthood.

The following are recommended when considering genetic testing in children:

- a) Genetic tests should only be performed after adequate informed consent has been obtained. This should be documented in the medical notes or on a consent form***
- b) Boys with haemophilia should have their genotype established, as this has potential clinical benefit to the boy and his family***
- c) Phenotypic testing of potential carrier girls should be performed around the age of one (unless required earlier for a specific clinical reason) with results confirmed on at least two occasions unless genotyping indicates that the girl is not a carrier***
- d) The complexities around genotypic testing for females who are potential carriers of haemophilia should be carefully discussed and the optimal timing of testing should be discussed with those with parental responsibility***

e) After imparting the results individuals or families should be sent written, confirmatory information regarding the result and interpretation of any tests (genetic or phenotypic). This letter should indicate whether further tests should be considered in the future

f) All individuals tested should have their own set of clinical notes.

Section 7. The Clinical - Laboratory Interface

7.1 *Liaison and communication*

For some Comprehensive Care Haemophilia Centres, the genetics laboratory forms part of the Centre. For other units, however, such services may be geographically separate and formal arrangements need to be in place to ensure appropriate and effective liaison and communication. A close relationship between the coagulation laboratory, the genetics laboratory and the clinical genetic counselling service is fundamental to the provision of a successful genetic diagnostic service. The laboratory-clinical interface is best maintained by regular meetings between the clinical and scientific staff, for example in MDTs, to discuss genetics related issues including individual cases.

Within the comprehensive care centre regular meetings of clinical and laboratory staff from the genetics and coagulation laboratories are essential to review the genetics service, to identify any problems and to ensure the quality of the service.

Such meetings should include audit and review of the results of external quality assurance schemes. Relevant laboratory reports should be reviewed. It is also important that there are good communications with other Haemophilia Centres using the genetic diagnostic services of the Comprehensive Care Centre in order to facilitate the appropriate provision of genetic services to patients and families attending all UK Haemophilia Centres.

A specification for a Haemophilia Genetics Laboratory is set out in Appendix II.

7.2 *Requests for Genetic Testing*

There should be specific laboratory requests (either electronic or paper) for genetic studies in heritable bleeding disorders.

Clinical information: Sufficient information must be made available by the requesting clinician to enable the laboratory to investigate a family appropriately. A referral letter or request form identifying the disorder and its severity (clotting factor levels), the individuals requiring investigation and the investigations required should be provided to the laboratory. The proband should be identified, and other relevant family details provided.

Pedigree: An accurate and appropriately detailed family tree may be an important prerequisite for genetic family studies. A copy of the family tree should be provided to the laboratory, where appropriate, by the clinical team along with the request for investigations. For further details refer to Section 4.

Consent: Genetic testing can only be performed after appropriate informed consent has been obtained. It is preferable for the laboratory to have confirmation that consent has been obtained but receipt by the laboratory of a sample with a request for genetic diagnosis will be taken by the laboratory to indicate that appropriate informed consent has been obtained.

It is the responsibility of the clinician dealing with the particular case, and not the laboratory, to ensure that informed consent is obtained for both testing and storage.

The laboratory should be made aware by the requesting clinician of any restriction on consent, e.g. storage of sample, use of an individual's genetic information in subsequent family studies, storage of results on databases. The completed consent form should be retained in the patient's notes. The laboratory should keep a record of any restrictions, entering them into the database or the genetics file. Refer to Section 3 with regard to confidentiality issues and Section 4 for disclosure of results.

7.2.1 Sample requirements and patient identification:

Details of samples required for laboratory services should be available to all staff members involved in genetic counselling. This information should also be available to outside hospitals / units / centres that may refer patients or samples for investigation. The clinician requesting the investigation should be clearly identified to the laboratory together with the address for reports. The precise sample requirements and the type of anticoagulant may vary from centre-to-centre.

Samples and request forms must be clearly and accurately labelled with:

- 1. The patient's first name and surname***
- 2. The patient's date of birth***
- 3. The patient's NHS or hospital number or other unique identifier.***

This is the minimum patient identification data set required for samples to be accepted for investigation.

Specimens must be clearly and reliably matched with the patient's details on the request. The use of the same hospital number for individuals within the same family is not acceptable. In the case of twins, some unique identifier (i.e. other than date of birth) must be supplied. A unique identifier will also be generated by the laboratory. The date of sample collection should be provided. Inadequately or unlabelled samples or request cards will not be accepted by the laboratory. Samples from each patient or family member should preferably be bagged separately with a separate request for each individual sample.

7.3 *Mutation Data*

Up-to-date mutation data on individual patients and families must be made available by the laboratory to the clinical staff involved in patient management and genetic counselling.

All putative mutations must be assessed for likely pathogenic effect, and validated in accordance with the Association for Clinical Genetic Science (ACGS) practice guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics.

In some cases the family mutation may be known even though the patient may not have been investigated by that centre e.g. as part of studies performed elsewhere. Such data, if known, should be communicated to the laboratory on the request together with a copy of the original report from the previous investigating laboratory. In cases where the family mutation has been identified elsewhere it is recommended practice for the current investigating laboratory to "confirm the mutation", where possible. This is especially important if the mutation was originally identified as part of a research project, rather than as a formal diagnostic report

from an accredited laboratory. If the mutation has not been confirmed in the current investigating laboratory a statement to this effect must be made in the laboratory report.

7.4 Laboratory Database

Accurate and readily accessible records of all stored samples and patient / family studies must be kept for all families with heritable bleeding disorders. Such records should include the results of genetic and phenotypic studies. Mutation information should be maintained on a controlled and confidential database, and appropriately transferred to the patient's notes.

Records must be updated regularly to reflect changes in relevant information that may become available, and effective liaison between clinicians, the genetics laboratory and the coagulation laboratory is a prerequisite. Regular meetings between laboratory and clinical staff to discuss the results of laboratory studies are considered to be essential. Liaison with other centres may be necessary to investigate rare disorders where such expertise does not exist within a specific centre.

In haemophilia A and B it is envisaged that the mutation will be sought in all families. For these reasons, regular updates of sample requirements from family members for outstanding mutation analysis should be made available. This is particularly important for mild cases of haemophilia A or B, who may be seen infrequently.

7.5 Laboratory Reports

Laboratory reports should be timely, accurate and concise. The clinical question being asked should always be restated in the text. Reports should include the following:

- ***Patient identification data***
- ***Disorder and severity, and diagnostic question asked***
- ***Test(s) performed and brief description of techniques used***
- ***Result presented clearly and concisely***
- ***Full and clear interpretation of result***
- ***Authorisation signatures.***

Reports should be referenced so that the original mutational data can be readily accessed if necessary. A key to any nomenclature used should be included. HGVS (Human Genome Variation Society) numbering and nomenclature should be used to describe DNA, RNA and protein sequence variants. Any further tests required or information needed to allow further investigation should be detailed. All reports should be signed and dated by the individual carrying out the laboratory tests, and appropriately authorised, for example by the scientific head of the laboratory.

Reporting of genetic investigations should be in accordance with the best practice guideline on reporting which are currently available on the website of the ACGS.

External Quality Assurance

Member laboratories of the UKHCDO Haemophilia Genetics Laboratory Network are required to participate in the UK NEQAS Blood Coagulation Scheme for the Genetics of Heritable Bleeding Disorders.

Research & Development

A close and effective laboratory-clinical interface is essential to facilitate research and development activities in the genetics of heritable bleeding disorders.

7.6 *Mutation databases on the Internet*

Details of reported mutations in heritable bleeding disorders, together with other important related information, are available at dedicated websites. Examples include (URLs correct at time of publication):

| | |
|--------------------------|---|
| FVIII / haemophilia A: | www.eahad-db.org |
| FIX / haemophilia B: | www.eahad-db.org |
| VWF / VWD: | www.eahad-db.org |
| FXI deficiency: | www.eahad-db.org |
| FVII deficiency: | www.eahad-db.org |
| Rare Bleeding Disorders: | http://www.rbdd.org/ |

Section 8. Genetic Diagnosis and Management of complex cases

This section discusses some of the situations when standard genetic analysis methods might be uninformative and how this might be approached in the clinical setting. An integral part of the process of obtaining consent is discussion of the limitations of the tests being carried out in order that patient expectations are appropriately managed. Some of the alternative strategies described below are labour intensive and time consuming. Results may not be available for several months and realistic details of the timescale and potential outcome should be discussed prior to sampling.

8.1 *No mutation detectable using standard techniques*

The techniques used for genetic analysis in the haemophilias are designed to detect the majority of mutation types that are known to be pathogenic. In this respect haemophilia B is typical of most monogenic disorders in that 75% of mutations affect a single nucleotide (Giannelli *et al*, 1998). The most appropriate method for detecting these abnormalities is amplification of the target sequence by PCR followed by direct sequencing. Gross genetic abnormalities such as inversions or large deletions are less easy to define by this method. Where the deletion affects one or more entire exons then an affected individual can be detected by failure of amplification in the corresponding PCR reactions. However, detection of carriers is more problematic because the presence of the normal allele in the female will support amplification thereby masking the deletion. Large deletions are responsible for 6% of haemophilia B cases. Multiplex ligation-dependent probe amplification (MLPA) is the preferred method for detecting deletions and duplications as it uses specific probes to allow relative quantification of each exon of the gene (Sellner & Taylor, 2004). A deletion is readily detectable in carriers who will have half the amount of the affected exon compared with the rest of the coding sequence. MLPA is unable to detect the exact breakpoints of the deletion but that is not important for clinical management. Complex rearrangements represent only 1% of cases of haemophilia B and remain difficult to detect by the methods described above and require the design of pedigree-specific probes or PCRs. This type of analysis will often take several months and would rarely be available as part of a standard clinical service. Overall standard genetic analysis techniques uncover the cause of haemophilia B in 99% of cases. MLPA kits are also available for analysis of deletions and duplications in Haemophilia A and von Willebrand's disease.

Haemophilia A has a quite different mutation profile because of some unusual features of the *F8* gene. The standard techniques described above were used *en masse* following the characterisation of the gene in 1984. It soon became clear that in nearly half of severe cases a mutation could not be defined by these methods. In 1993 it was demonstrated that 40% of severe haemophilia A was caused by a complex rearrangement involving homologous regions in intron 22 and a region of the X chromosome well away from the gene (Lakich *et al*, 1993). The resulting inversion of the gene cannot be detected by conventional sequencing and requires a specific Southern Blot, or long range PCR, or inverse-shifting PCR technique (Rossetti *et al*, 2008). Whilst still an effective technique Southern Blot has become less popular because of the requirement for radioactivity and the relatively long processing time of 5-7 days. A similar inversion affecting intron 1 causes about 1% of severe cases (Brinke *et al*, 1996). The strategy for genetic analysis in severe haemophilia A therefore includes specific assays for these two inversions followed by the more standard techniques described above. With these advances the causative mutation can be found in >99% of cases of severe haemophilia A. In non-severe disease approximately 5% to 10% of cases remain cryptic

suggesting that there are further genetic mechanisms of haemophilia A awaiting discovery (Klopp *et al*, 2002; Bogdanova *et al*, 2007). *F8* is an extremely large gene at 186 kbp and conventional sequencing strategies that screen the coding regions and intron-exon boundaries cover only about 15 kbp of the gene. This is a limitation of direct sequencing techniques that can only access relatively short stretches of DNA. Advancing techniques in next generation sequencing may allow sequencing of the entire gene (excluding repetitive DNA regions in the introns) and could reveal currently hidden mutations. This technology also has the capacity to analyse other genes of interest simultaneously allowing the possible introduction of a test for a “heritable bleeding disorders” panel of genes.

In a pedigree where no mutation is detectable alternative causes of coagulation factor deficiency should be considered. Acquired defects such as liver disease or vitamin K deficiency are readily detectable as they are associated with multiple laboratory abnormalities although they may occasionally present with an isolated factor deficiency in the early stages. There are two well-described genetic causes of FVIII deficiency other than haemophilia: von Willebrand’s disease (particularly type 2N) and combined FVIII and FV deficiency. Both of these are readily detectable by specific laboratory assays and genetic analysis. Similarly FIX deficiency may be seen as part of a multiple coagulation factor deficiency caused by genetic abnormalities of the γ -carboxylase pathway. This rare condition is mostly due to mutations in the *VKORC1* or *GGCX* genes and is characterised by deficiency to a varying extent of all the vitamin K-dependent factors.

Where other genetic causes or acquired deficiency have been excluded the likelihood is that the cause is haemophilia but without an easily detectable genetic abnormality. Where there is an extended pedigree further studies by linkage analysis may be useful in identifying a genetic disease marker. There are a few well-defined polymorphisms in *F8* and *F9* which may be informative if the relevant obligate carriers in the pedigree are heterozygous. However, it should be remembered that the linkage between the marker and the disease-causing mutation may become less reliable with increasing separation in a pedigree from the index case (Keeney *et al*, 2010; Mitchell *et al*, 2010).

8.2 Using probability to inform potential carriers

In the absence of a defined mutation or an informative genetic marker counselling may still be provided to potential carriers using the rules of Mendelian inheritance and Bayesian methodology to estimate the probability of carriership. More than 50% of cases of haemophilia A are sporadic and studies tracking the origin of the mutated gene back through the pedigree have shown that in 80% of these spontaneous cases the mutation occurred in a germ cell of the maternal grandfather. This results in a pre-test probability of 0.85 that the mother of a sporadic case is a carrier (Becker *et al*, 1996). In daughters of obligate carriers the pre-test probability of 0.5 for carriership can be modified by information from her descendant pedigree. If a potential carrier produces multiple normal male offspring this reduces her probability of being a carrier. The probability of a carrier female having n normal sons is 0.5^n . Thus the overall probability of carriership is arrived at by combining the probabilities from the ancestor and descendant pedigrees (Peake *et al*, 1993).

8.3 Mosaicism

A mosaic is an individual who has genetically different cell lines derived from a single zygote (Kasper & Buzin, 2009). Although the chance of a mutation occurring during any particular cell division is low, the vast number of divisions that take place during a person’s

lifetime means that all individuals should be genetic mosaics to some extent. This is generally not noticeable because it would normally affect a tiny proportion of the total number of cells. However, if a mutation arises during early embryogenesis in selected progenitor cells the consequences can be significant. If these cells are then entirely incorporated into one germ cell layer, or sub-layer, the mutation will manifest in all the tissues derived from that layer but not in tissues derived from other layers. Thus a haemophilia-causing mutation might occur in gonadal tissue, while a normal gene is found in other cell types such as those found in the liver. The haemophilia gene can be transmitted to offspring but there are no clinical effects in the mosaic individual because liver endothelial cells express the normal gene. Similarly peripheral blood, which is the normal source of DNA used in testing, will not show any evidence of the mutation. This is referred to as gonadal or germline mosaicism. A female with gonadal mosaicism would be recognised by having multiple affected or carrier offspring while having a normal genotype in their own blood sample. Rarely such a situation might be difficult to distinguish from chimaerism where an individual has two genotypes arising from the fusion of two zygotes. This would usually result in some phenotypic abnormality such as ambiguous genitalia which would not occur in a mosaic. As mosaicism is difficult to detect by standard tests the incidence is not known for certain. One study found evidence of mosaicism in 13% of haemophilia A carriers (Leuer *et al*, 2001).

Somatic mosaicism occurs when different genotypes exist within the different somatic tissues or organs of a single individual. Thus hepatic endothelial cells might carry a mutation while circulating peripheral blood cells might not. As the liver is the organ for production of clotting factors such an individual would have a clotting factor deficiency. However, the mutation would not be detectable in a peripheral blood sample, nor would it be inheritable as the gametes would be spared. This condition can only be definitively proven by genotyping of specific cell types.

Recommendation:

In a sporadic case of haemophilia absence of the mutation in the peripheral blood of the mother does not exclude carrier status because of the small chance of mosaicism. This possibility should be explained to potential carriers who appear to have no genetic abnormality on blood tests.

8.4 Females with haemophilia

Many female carriers have symptoms consistent with mild disease. Severe or moderate disease in a female generally indicates either inheritance of two abnormal *F8* genes or loss or inactivation of the normal gene (Pavlova *et al*, 2009). Very rarely a low FVIII level in a haemophilia carrier might be further decreased by co-inheritance of an autosomal condition such as type 2N VWD or combined factor V and VIII deficiency. True homozygosity or compound heterozygosity for haemophilia is extremely rare, although it has been reported in regions where consanguinity is more common. The co-existence of an abnormal *F8* gene with an unrelated abnormality of the other X chromosome, such as Turner's syndrome (monosomy X), is a more frequent observation. Such gross chromosomal abnormalities are often associated with mosaicism which goes some way to ameliorating the effect (Kasper & Buzin, 2009).

Inactivation of one of the X chromosomes (lyonisation) in a female cell is a normal process that is necessary to prevent genetic over dosage that would otherwise be caused by over

expression of genes on the X chromosome (Puck & Willard, 1998). As the X chromosome is inactivated in each particular cell is determined randomly in humans the ratio of active paternal and maternal X chromosomes in the body generally follows a normal distribution. If by chance, or because of some chromosomal abnormality, the ratio is skewed towards one chromosome this is referred to as skewed lyonisation. Carriership of haemophilia coupled with extreme skewing resulting in >90% inactivation of the normal chromosome can result in moderate or even severe haemophilia. As silencing of the X chromosomes is achieved through DNA methylation, assays that measure the relative amounts of methylation on each allele are used to indicate the degree of lyonisation. Alternatively measurement of expression of each *XIST* gene which directs methylation on the corresponding X chromosome may be used.

8.5 *Novel mutations*

The primary clinical benefit from databases of mutations lies in providing evidence of pathogenicity from previous reports of a mutation. In this situation a database that accumulates multiple reports of the same mutation is far more useful than those that limit each mutation to a single occurrence. A clear association with a phenotype, particularly the association with inhibitor formation, can be a useful predictor of the phenotype for a newly diagnosed individual. However, if genetic analysis indicates a novel mutation, prediction of the molecular effects is required. Changes which unequivocally result in a null allele, such as most nonsense or frameshift mutations, can be assigned to a severe phenotype without further analysis. Many novel mutations are missense changes and additional techniques detailed in the CMGS best practice guidelines (Bell *et al*, 2008) are then required, such as alignment with homologous sequences to check for evolutionary conservation, or mapping onto known structures. These results should then be corroborated with biochemical data before a conclusion as to the pathogenicity of the putative mutation is reached. Ultimately these conclusions may not be definitive in the absence of gene expression studies which remain largely research tools and rarely available to a clinical service.

8.6 *Testing of carriers in the absence of an affected individual*

Whenever possible the initial genetic analysis in a pedigree should be carried out on an affected individual. This facilitates correlation of the genetic result with biochemical data. In the absence of biochemical data from an affected individual, it may still be possible to predict the clinical severity from the history. As the haemophilias are inherited through a classical Mendelian pattern, an accurate family tree allows calculation of the probability that the consultand carries an abnormal gene. This probability in conjunction with the known or predicted phenotype of an affected individual in the pedigree can then be used to guide genetic analysis. When genetic analysis indicates a previously reported mutation, particularly in association with severe or moderate disease, causality can be assigned with some confidence. However, in pedigrees where the phenotype is mild or the putative mutation is not previously reported it may not be possible to be certain of pathogenicity.

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Royal College of Pathologists
Royal College of Paediatrics and Child Health
Royal College of Obstetricians and Gynaecologists

British Society of Haematology
British Society for Haemostasis and Thrombosis

Some members of UKHCDO, clinical geneticists and other interested individuals

Appendix I

(This space is for heading for hospital and Haemophilia Centre details.) A Word version of this information sheet and consent form for use (and if appropriate modification) is available from UKHCDO Secretariat (lynne.dewhurst@nhs.net)

Information on Genetic Testing and Consent Form for Patients and Families with Bleeding Disorders

Introduction

The purpose of this information sheet is to explain the reasons why you are being offered genetic tests and the consent form you will be asked to sign before these are performed.

Someone from your haemophilia centre has already explained the nature of your disorder to you, and the manner in which it can be passed down through your family. If you require further information, or you are unclear about what you have been told, please ask for clarification or more help.

Genetic testing can tell us which people in your family have the condition and who are ‘carriers’ who might pass the disorder on to their own children. This can be more helpful than simple tests of the defective clotting factor (coagulation factor) where sometimes the level is normal in carriers. With modern genetic techniques it is usually possible to locate the faulty genetic change in each family, although this can sometimes take time. Although many families may have the disorder, it is common for each family to have its own unique genetic change.

- 1. What is the purpose of obtaining a blood sample?** It is very useful to know exactly the genetic change that is causing the disorder in you/your child. Sometimes this helps us to be alerted about how the disorder may respond to treatment in the future. Measurement of the blood coagulation factor level does not always clearly indicate if there is a genetic change present or not; genetic testing is a more accurate way of telling this. For this a special type of blood sample is required from which the genetic material (DNA) can be extracted. A second sample may be taken from you on a separate occasion to confirm the result of the initial test.
- 2. Where will the blood sample be tested?** The tests needed to detect a genetic change are specialised. Some of them are performed locally, but depending upon the nature of you/your child’s disorder, it may be necessary to send the blood sample away to one of a small number of specialised laboratories. In all these, there are strict regulations to ensure complete confidentiality of personal details.
- 3. How long will the test take?** The answers to genetic tests often take some time to obtain. Your doctor will discuss the likely time course with you, as this varies with the disorder. It may take many months if you have one of the less common, or more complicated disorders. You will be informed of progress if it will take a long time to obtain results.
- 4. How long will my blood sample be stored?** Sometimes it may not be possible with existing methods to find the genetic change in your family. In this case, the DNA will be stored until new tests are available. It is usual practice to store DNA samples indefinitely. Other new tests relevant to you/your child’s disorder may arise in the future, which will help us understand more about the mechanisms of you/your child’s disorder.
- 5. What are the risks of genetic testing?** In addition to information on the inheritance of a bleeding disorder, the results from these genetic tests may inadvertently provide other information, such as confirmation of whether a child’s parent is as assumed by the family. Therefore, occasionally

unexpected results about family relationships arise from these tests, which, if known, could cause embarrassment or upset within a family.

The studies performed will be specific for the disorder known to be in your family. They will not exclude all forms of possible bleeding disorders.

6. **What else might be done with my blood sample?** We might want to use your/your child's sample to help develop or refine tests for bleeding disorders. In such cases the blood samples would not be linked back to you/your child. The results would therefore be completely anonymous. It can be very useful to run tests on a series of DNA samples anonymously to compare how common some changes in the DNA are which are not responsible for the condition. If the sample is used for such testing, no one will know whose it is, and there will be no comeback to you and your family.
7. **Who gets to know about the results?** The results will be told to you personally. Your family doctor will be sent the result, unless you withhold consent for this.
8. **Why might it be useful for other members of my family to know about the results?** Information about the genetic change in you/your child is likely to be of benefit to other members of your family. It may, for example, be used to discover if a woman is a carrier and therefore if there is a risk of passing on the disorder to her children. With your permission we would like to be able to make the information about your genetic change available to doctors looking after other people in your family if they ask.
9. **Who should give consent for testing a child?** A child may not provide informed consent until he or she is mature enough to understand the implications of the test being performed. This age varies with the individual child. Generally genetic carrier testing will not normally be carried out before a child is at an age to appreciate the issues and give consent. However, there may be reasons why the results of such a test would be valuable. Information about the genetic change in a child affected by the bleeding disorder may affect treatment and is likely to be of benefit to other family members. In both of these cases the parent or legal guardian of the child will be asked to provide informed consent.
10. **Are my genetic results going to be stored anywhere other than in my hospital and GP case records?** There are local, national and international confidential or anonymised databases, which keep information about genetic disorders of coagulation. We would like to record the information about your gene change. These databases are secure and protected and comply with the recommendations of the Caldicott report (1997) to ensure patient confidentiality and the Data Protection Act.
11. **What will happen if I decide to withhold consent?** You may withhold consent for any or all of the above uses for samples and results. This would not jeopardise your treatment (or that of your child).

Further information on general issues of consent can be found in the Trust's "Consent to Treatment" leaflets for patients and parents. Please ask for a copy if these have not been provided to you.

If you would like to have your blood tested please read the attached consent form.

A) Patient Details

| | |
|---------------------|-----------------------|
| Surname | Consultant |
| Forename | Hospital Number |
| Date of Birth | |

B) Collection and usage of samples

I,.....(print name) give consent for a blood sample to be taken from(myself or name of child) and the genetic material extracted, stored and tested for (specify disorder).

Please initial the boxes below to indicate your consent

- The purposes for obtaining this sample and the potential implications have been explained to me and I have had an opportunity to have my questions answered.
- I have read and understood the above information about genetic testing.
- It is the intention to store the sample indefinitely.
- If no relevant test is currently available, I agree to the sample being stored until such time as an appropriate test is developed and the sample may then be tested.
- I understand that it may be necessary to use part of the sample anonymously for example for quality assurance or development of new tests.

Signed Date.....
(Patient/parent/legal guardian – delete as appropriate)

C) Use and availability of results

- I hereby give consent for clinical and genetic information that may be relevant to other family members to be made available to relevant health care professionals.
- I agree to the results being entered into confidential and/or anonymised databases.

Signed Date.....
(Patient/parent/legal guardian – delete as appropriate)

D) Person obtaining consent

I have explained to the above patient/parent/legal guardian the purpose of obtaining a sample for genetic studies and their implications.

Signed Date.....
Print Name Position.....

A photocopy of the completed form should be given to the patient, the original filed in the patient's case notes and a copy filed in the family genetic record file

Appendix II

The Haemophilia Genetics Laboratory Network and availability of Genetic Diagnostic Services in the UK: Specification for Haemophilia Genetics Laboratory

The Network functions to ensure the provision nationally of a robust and high-quality genetic diagnostic service for the heritable bleeding disorders. A directory of laboratories affiliated to the Network, identifying services available and contact details, is available via the UKHCDO website.

UK laboratories providing a genetic diagnostic service for haemophilia and other heritable bleeding disorders should be affiliated to the UKHCDO Haemophilia Genetics Laboratory Network.

UKHCDO Genetics Laboratory Network-affiliated laboratories are required to comply with the following quality standards:

1. A molecular genetics laboratory, affiliated to a Comprehensive Care Haemophilia Centre, providing an NHS diagnostic service
2. Facilities and expertise to allow the identification of mutations in haemophilia A and B and other heritable bleeding disorders.(including handling risk of infection samples)
3. Ability to assign carriership and make antenatal diagnosis
4. Compliance with locally agreed turnaround times, appropriate to the clinical service. The ability to turnaround urgent samples within two weeks. A three working day turnaround time for prenatal diagnosis cases.
5. CPA accreditation
6. Participation in appropriate genetics external quality control scheme (currently NEQAS)
7. Active collaboration between all haemophilia genetic laboratories to provide a robust service available throughout the year

Assessment of compliance with these standards is an integral part of the external peer-review audit system operated by the UKHCDO for UK haemophilia centres.

Adequate numbers of appropriately qualified HCPC-registered staff are required to provide a high-quality and up-to-date genetic diagnostic service in accordance with the quality standards identified above. Professional direction for the laboratory should be provided by a consultant haematologist, clinical geneticist or a senior HCPC-registered scientist.

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