

Standards of Care

For Fanconi Anaemia affected
Individuals and their Families



Standards of Care for Fanconi Anaemia affected Individuals and their Families

Edited by: Mr Thomas Carroll^d and Prof Ajay Vora^z

Cover design: Mike Finan, Chameleon Design

On behalf of:

UK & Ireland Fanconi Anaemia Clinical Network

Fanconi Hope Charitable Trust (UK Charity Number 1126894)

Paediatric Forum of British Society of Haematology

Address for correspondence:

Prof Ajay Vora

Paediatric Haematology

Sheffield Children's Hospital

Western Bank

Sheffield

South Yorkshire

S10 2TH

UK

e-mail:

Ajay.Vora@sch.nhs.uk

Guideline group:

Dr Sarah Ball^a, Ms Alyson Bradbury^b, Ms Grainne Bourke^c, Mr Thomas Carroll^d, Dr Phil Darbyshire^e, Prof Inderjeet Dokal^f, Dr Sally Feather^g, Dr Josu dela Fuente^h, Dr Mike Gattensⁱ, Alan Gillespie^j, Dr Dorothy Halliday^k, Prof Marcus Hughes^l, Prof Sally Kinsey^m, Mr Jeremy McMahonⁿ, Prof Chris Mathew^o, Dr Stefan Meyer^p, Dr Mary Morgan^q, Dr Leena Patel^r, Prof Susan R Rose^s, Mr Paul Skinner^t, Dr Rod Skinner^u, Dr Colin Steward^v, Dr Holger Tönnies^w, Mr Thomas Westin^x, Dr Paul Veys^y, Prof Ajay Vora^z

Those listed as part of the guideline group are those individuals who directly through their authorship and/or participation at the second UK Fanconi Anaemia Clinical Network meeting (16th/17th October 2008) or indirectly through e-mail correspondence contributed to the final publication of this standards document.

^a Consultant Paediatric Haematologist, St George's University of London

^b Clinical Genetics Specialist Nurse, Sheffield Children's Hospital NHS Foundation Trust

^c Consultant Paediatric Plastic Surgeon, Leeds Teaching Hospitals NHS Trust

^d Consultant Neurosurgeon & FA Parent, Sheffield Teaching Hospitals NHS Foundation Trust

^e Consultant Paediatric Haematologist, Birmingham Children's Hospital NHS Foundation Trust

^f Professor of Paediatric Haematology, Barts & The London School of Medicine & Dentistry

^g Consultant Paediatric Nephrologist, Leeds Teaching Hospitals NHS Trust

^h Consultant Paediatric Haematologist, St Mary's Hospital, London

ⁱ Consultant Paediatric Haematologist, Addenbrooke's NHS Trust, Cambridge

^j Consultant Gynaecologist, Sheffield Teaching Hospitals NHS Foundation Trust

^k Senior Specialist Registrar in Clinical Genetics, Oxford NHS Trust

^l Professor of Molecular Medicine & Genetics, Wayne State University, USA

^m Professor of Paediatric Haematology, Leeds Teaching Hospitals NHS Trust

ⁿ Consultant Maxillofacial Surgeon, Southern General Hospital, Glasgow

^o Professor of Medical Genetics, Guy's Hospital, London

^p Consultant Paediatric Oncologist, Central Manchester & Manchester Children's University Hospitals NHS Trust

^q Consultant Paediatric Haematologist, Southampton University Hospitals NHS Trust

^r Senior Lecturer in Child Health & Honorary Consultant Paediatric Endocrinologist, Central Manchester & Manchester Children's University Hospitals NHS Trust

^s Professor of Paediatrics & Paediatric Endocrinology, Cincinnati Children's Hospital Medical Centre

^t Consultant Colorectal Surgeon, Sheffield Teaching Hospitals NHS Foundation Trust

^u Consultant in Paediatric Oncology & Bone Marrow Transplantation, Newcastle General Hospital

^v Reader in Stem Cell Transplantation, Bristol Royal Hospital for Children

^w Institut für Humangenetik, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Germany

^x Consultant ENT Surgeon, Sheffield Teaching Hospitals NHS Foundation Trust

^y Consultant in Bone Marrow Transplantation, Great Ormond St Hospital for Children

^z Consultant Haematologist, Sheffield Children's NHS Foundation Trust

Fanconi Anaemia affected individuals and families involved in the preparation of this Standards of Care document:

Mr Des Murnane & Mrs Mai Murnane, Dublin, Ireland (FA parents)

Mr Ben Murnane, Dublin, Ireland (FA affected individual)

Mr Bob Dalglish & Mrs Janette Dalglish, Portsmouth (FA parents)

Mr Richard Kawalek & Mrs Jane Kawalek, Manchester (FA parent)

Mr Thomas Carroll & Dr Alison Carroll, Sheffield (FA parents)

Mr David McDowell & Mrs Samantha McDowell, York (FA parents)

The second UK Fanconi Anaemia Clinical Network meeting was also attended by Mr Ralf Dietrich, Germany, of the German FA support group and Mr Christophe Bichet, France (FA affected individual).

Declarations of interest

No author/member of guideline group declared any relevant interest other than being involved or potentially involved in the treatment of families affected by Fanconi Anaemia or being a member of a Fanconi Anaemia affected family.

Acknowledgements

Thanks are expressed to: (1) Dr Edna Maltby, Consultant Clinical Cytogeneticist, Sheffield Children's Hospital, for collating all centres in the UK and Ireland that provide chromosomal breakage testing (see Appendix B); (2) Dr Paul Scriven, Clinical Scientist & Deputy Director of Cytogenetics, Guy's Hospital, London, for comments on the statistical model concerning PGD/HLA-selection/IVF; (3) Prof Helmut Hanenberg, Heinrich Heine University, Germany, and Prof Stella Davies, Paediatric Haematologist, Fanconi Anaemia Comprehensive Care Centre, Cincinnati, USA, for their attendance at and contribution to the second UK Fanconi Anaemia Clinical Network Meeting that led to the production of this standards document.

Disclaimer

While the advice and information in these guidelines is believed to be true and accurate at the time of going to press, the authors, the UK & Ireland Fanconi Anaemia Clinical Network, the British Society for Haematology, the Fanconi Hope

Charitable Trust, and the publishers do not accept any legal responsibility for the content of these guidelines.

Date for guideline review

February 2011

Introduction

The guideline group responsible for this standards document are members of the UK & Ireland Fanconi Anaemia Clinical Network and also included parents of Fanconi Anaemia (FA) affected children. Pubmed was searched systematically for publications in English using the terms Fanconi Anemia/Anaemia. The writing group produced the draft guidelines which were subsequently revised by consensus by all members of the UK & Ireland Fanconi Anaemia Clinical Network. The objective of these guidelines is to provide healthcare professionals with clear guidance on the management of individuals who might have FA, who are established as having FA, and their families. Note that in some cases individual patient circumstances may dictate an alternative approach.

Guideline update

This is the first Standards of Care in Fanconi Anaemia guideline document published in the UK/Ireland. Previous guidelines have been published in the US and in the Netherlands and can be viewed at:

www.fanconianaemia.nhs.uk/clinical-network/standards-of-care/.

Text hyperlinks

Note that cross-referencing hyperlinks within the text are given in **violet** and internet hyperlinks are given in **blue**. Numbered references to publications in the text are hyperlinked to individual entries in the References section.

Summary of key recommendations.

- **Baseline investigations/assessments at diagnosis should be: FBC, blood group and antibody screen, liver and renal function, iron levels, viral screen (including CMV, hepatitis B/C, and varicella), tissue type (including that of siblings and extended family), endocrine assessment, cardiac trans-thoracic ECHO, abdominal ultrasound, audiometry, and referral to a clinical geneticist.**
- **A paediatric haematologist should take overall responsibility for co-ordinating the patient's care in childhood and monitoring for signs of marrow failure or clonal evolution.**
- **There should be arrangements in place for transition of care to an adult setting at around 16 years of age.**
- **A full blood count should be monitored at 3 monthly intervals or more frequently depending on rate of progression of marrow failure. A bone marrow aspirate and trephine should be considered annually for morphological and karyotypic assessment.**
- **Patients and siblings should be HLA typed and, if a matched sibling is unavailable, a preliminary unrelated donor search performed at diagnosis to determine availability of donor so as to reduce the waiting time to transplant when it is indicated. Siblings should be screened for FA prior to using them as donors.**
- **All FA affected individuals should have two to four times a year (depending on blood counts; changing blood counts=4 times) a screen for adverse clonal aberrations (e.g. monosomy 7 and gain of 3q) in peripheral blood cells and once a year in bone marrow cells by interphase FISH.**
- **Patients with severe progressive marrow failure or evidence of transformation to MDS/AML are candidates for intervention, either HSCT or androgen therapy.**
- **HLA matched sibling donor is the treatment of choice in such patients. If a matched sibling is unavailable, patients should be given information about the prevailing risks and outcomes of a 10/10 matched unrelated donor transplant by a transplant specialist to allow them to make an informed choice between transplant and androgen therapy.**

- **Androgens should be used as a bridge to transplant or to avoid transfusion dependency in patients with progressive marrow failure who do not have an appropriately matched donor.**
- **Mismatched unrelated donor or mismatched family donor transplants in patients who do not otherwise have an appropriately matched donor is only indicated in MDS/AML or there has been failure or intolerance of androgen therapy.**
- **Screening for FA within a family requires a DEB/MMC chromosomal breakage test on peripheral blood lymphocytes and/or skin fibroblasts. Confirmation of diagnosis should ideally be duplicative for those tested, i.e., ideally two different testing modalities (chromosomal breakage and genetic analysis) and at two different time points.**
- **Screening for FA should only be arranged through a haematologist or clinical geneticist following appropriate clinical evaluation.**
- **Consideration for screening for FA should be carried out on all individuals with: radial ray/thumb abnormalities, including duplications, irrespective of whether unilateral or bilateral (unless another causative genetic disorder has been identified); failure to thrive/short stature of otherwise unidentifiable cause; combination of café au lait patches and hypopigmented macules; VACTERL association, blood dyscrasias under the age of 45 years; intracranial medulloblastoma under the age of five years; oropharyngeal/anogenital squamous cell carcinomas under 45 years of age; perceived excessive toxicity associated with chemotherapy and/or radiotherapy; and siblings of FA probands.**
- **The possibility of haematologic reverse mosaicism needs to be considered. This should be considered for individuals over the age of five years who have been screened for FA who have a normal blood lymphocyte chromosome breakage test and do not have a sibling proband in whom FA mutations have been identified. In this group, a skin biopsy fibroblast chromosome breakage test should also be performed before a diagnosis of FA is discounted.**
- **Complementation group and mutation testing should be recommended for all FA-affected individuals.**
- **Mutation analysis should be performed on the proband (or at the very least, DNA or fresh tissue from the proband should be stored**

appropriately) if there is a possibility of pre-implantation genetic diagnosis such as: maternal age <45 years with parents who may wish to have a further unaffected child (including PGD/HLA-selection/IVF 'saviour sibling'); and also where there are FA-unaffected siblings/first degree relatives of potential/future child-bearing age.

- Genetic analysis including mutation analysis should be done when specifically requested by the paediatric haematologist for prognostic purposes.
- Screening for hypothyroidism with 8 to 9 am serum TSH and free thyroxine should be done every 6 to 12 months. Overt hypothyroidism should be treated promptly. Treatment with thyroxine should also be considered in children with TSH levels ≥ 3 mU/L, low free thyroxine level, and especially if they are short.
- Screening for glucose intolerance and hyperinsulinemia with single post-prandial glucose and insulin levels should be done at diagnosis and at least annually after age 5 years. Glycosylated haemoglobin (HbA1c) and fructosamine levels may be deceptively normal and are of no use in FA individuals prior to bone marrow transplantation.
- HPV vaccination should be done on all FA patients above the age of one year as soon as the diagnosis of FA has been established.
- Varicella vaccination should be done on all FA patients above the age of one year as soon as the diagnosis of FA has been established and who do not have serological evidence of previous varicella infection, who have yet to progress to moderate/severe bone marrow failure and who are not otherwise immunosuppressed.
- Hepatitis B vaccination should be done on all FA patients from birth as soon as the diagnosis of FA has been established. An FA unaffected sibling who is a tissue match and likely to be a donor at some future time interval to the FA affected proband should also be vaccinated against hepatitis B.
- Flu vaccination with a thioresal/mercury free preparation, in the absence of any specific haematologic contraindication (e.g. peri-haematopoietic stem cell transplant), should be done on all FA patients on an annual basis.

- FA affected individuals should have six-monthly dental check-ups with an NHS dentist or equivalent and should be encouraged to maintain good oral hygiene (tooth-brushing twice daily, dental flossing, use of non-alcohol based mouthwashes).
- Involvement of a paediatric hepatologist is required in the event of concerns over hepatic disease (complications secondary to androgen therapy including hepatocellular tumours, transfusion associated iron overload, post-haematopoietic stem cell transplant graft versus host disease). Interval liver function blood tests and liver ultrasound should be done every three to six months while on androgen therapy.
- Referral to a dietician is required if there are persistent problems with appetite/weight gain or paradoxically, later in the course of FA, with obesity. FA affected individuals/families should also be considered on initial diagnosis for formal dietetic review concerning the importance and content of a 'healthy diet' considering the cancer predisposing nature of the disorder.
- A discussion should be had concerning the use of dietary antioxidants such as alpha lipoic acid and quercetin taking into account the issues presented in [Section 14.5](#).
- All FA affected individuals from the age of 10 years should be offered four times a year screening for oropharyngeal squamous cell carcinoma.
- All FA affected females from the age of 12 years should be offered two times a year screening for anogenital squamous cell carcinomas and precursor lesions with frequency of screening increasing on detection of potential pre-malignant lesions. Screening should be inspection of the external genitalia and vulvoscopy in the first instance progressing to colposcopy of vagina and cervix and anal proctoscopy after the onset of sexual activity. Self-inspection should be encouraged.
- There should be a discussion with all FA adult males from the age of 18 years about the possibility of annual anal cancer screening including proctoscopy, presenting the pros and cons of such screening in the context of the limited information available concerning anal cancer in FA. Frequency of screening would be increased on detection of potential pre-malignant lesions including warts.

- Although penile cancer has yet to be described in FA, self-examination should be encouraged. Any lesions of concern should precipitate a 'two week wait referral' to a urologist.
- In all families, where the FA affected individual is a child, does not have an unaffected HLA matched sibling, and maternal age is less than 40 years, parents should be advised about PGD/HLA-selection/IVF ('Saviour Sibling'). PGD would not be appropriate in women aged 45 years and over.
- FA-affected individuals and their families should be encouraged to contact the available FA-specific support and information resources, i.e., both the UK/Ireland-based Fanconi Hope Charitable Trust (www.fanconihope.org, www.fanconianaemia.nhs.uk) and the US-based Fanconi Anemia Research Fund (www.fanconi.org).
- There should be an attempt to minimise the number of hospital visits, e.g. arranging as many investigations/assessments as possible to be done together during a daycase admission.
- There should also be a clearly understood point of contact, i.e., telephone number(s), including after-hours, for FA patients/families and 24 hour open door access over and above attendance at an accident and emergency department, e.g. to the haemato-oncology ward
- The use of X-ray/CT and radioisotope investigations should be minimised. As much as possible, non-ionising radiation imaging modalities should be used, i.e., ultrasound and MRI.
- If cortisol and ACTH adequacy have not been assessed, stress doses of hydrocortisone should be given before major surgical procedures.

Contents

| | |
|---|-----|
| 1. Introduction | 11 |
| 2. Diagnostic evaluation in Fanconi Anaemia – Overview | 13 |
| 3. Screening for Fanconi Anaemia | 24 |
| 4. Genetic analysis..... | 33 |
| 5. Haematologic follow-up & management of haematologic complications.. | 37 |
| 6. Pre-transplant cytogenetic/molecular cytogenetic follow-up | 48 |
| 7. Endocrinologic follow-up | 50 |
| 8. Renal follow-up | 54 |
| 9. Hearing, developmental and neurologic follow-up | 57 |
| 10. Screening for oropharyngeal/anogenital squamous cell carcinomas..... | 60 |
| 11. Anogenital SCCs in FA | 67 |
| 12. Additional vaccinations | 71 |
| 13. Pre-implantation genetic diagnosis/HLA-selection/IVF | 76 |
| 14. Gastrointestinal, dental, hepatic, and nutritional issues in FA..... | 83 |
| 15. Radial ray and thumb abnormalities | 89 |
| Potential audit projects..... | 93 |
| Appendix A: Abbreviations used in the text..... | 94 |
| Appendix B: Cytogenetics Units in the UK & Ireland providing chromosomal breakage testing..... | 95 |
| Appendix C: Cancer epidemiological data relevant to consideration of FA screening (See section 3.3)..... | 98 |
| Appendix D: Mathematical probability model for success of PGD/HLA- typing/IVF | 102 |
| References | 106 |

1. INTRODUCTION

1.1 What is Fanconi Anaemia?

FA is a rare, (predominantly) autosomal recessive, cancer-predisposing disorder affecting about 150 or more families in the UK (the true extent is unknown) manifested by: (1) a variable presence of congenital anomalies in up to 70% (e.g. thumb and kidney abnormalities); (2) progressive bone marrow failure in childhood usually leading to haematopoietic stem cell transplantation (80% chance); and (3) a predisposition to acute myeloid leukaemia (10% chance), and in particular, oropharyngeal/anogenital squamous cell carcinoma in early adulthood (over 50% in post-haematopoietic stem cell transplant survivors). There are 13 genetic subgroups, also known as complementation groups, reflecting 13 different proteins that can be potentially affected in the Fanconi pathway, an emerging DNA housekeeping mechanism (see [Table 2.4: FA complementation/genetic subtypes](#)). Some of these genetic subgroups have additional issues such as subgroup FA-D1, where affected children are at high risk of brain medulloblastoma and renal Wilm's tumour. Some FA genetic subgroup heterozygotes/'carriers' are also at increased risk of malignancy. *BRCA2*, one of the 'breast cancer genes' is actually part of the Fanconi pathway. A FA-D1 affected child is where both parents are heterozygotes for *BRCA2* with resultant increased breast cancer risk for the mother.

1.2 Fanconi Anaemia, because of its rarity and complexity, is a challenging disease both to diagnose and manage.

Many clinicians outside the paediatric haematology community are unaware of the condition, including paediatricians, hand surgeons, and head & neck cancer surgeons. Accounts provided by families of difficulties experienced reflect this general lack of awareness. In other countries such as Germany, Holland, Spain, and France, FA patients and their families are managed in national FA centres. In the US, Fanconi Anaemia is dealt with in a small number of 'FA comprehensive care centres' with patient numbers in each centre of up to one hundred. There are no such large specialist 'national' or centres in the UK (although the Republic of Ireland has a single treatment centre based in Dublin). Instead the current consensus of best FA care in the UK is in the context of a national FA Clinical Network, sharing

experiences, standards, and treatment protocols^{aa}. These Standards of Care Guidelines will be an important tool in this Clinical Network ensuring that the care of UK FA affected individuals/families is at least as good as in any centre internationally.

1.3 NICE Guidance.

Requirements as per NICE 'Improving Outcomes Guidance for Children and Young People with Cancer' (August 2005) specifically outlines in its key recommendations that all relevant national guidance is followed (see www.nice.org.uk/Guidance/CSGCYP). In subsequent Improving Outcomes Guidances for cancer, the principle of specialist clinician and patient groups coming together to establish national standards of care for rare cancer disorders has been established. A purpose of this document is to meet this key recommendation for Fanconi Anaemia, a genetic cancer predisposition syndrome.

1.4 Guiding principle.

In drawing up the proposal for the UK & Ireland Fanconi Anaemia Registry (UKIFAR), the authors and members of the UK & Ireland Fanconi Anaemia Clinical Network have started from the principle of *'how would I want my child to be managed if they had Fanconi Anaemia'*. This approach is supported by FA-affected individuals/families through the UK & Ireland Fanconi Anaemia 'Family Network' and the Fanconi Hope Charitable Trust.

1.5 UK & Ireland Fanconi Anaemia Registry Project.

As well as setting out standards of care to assist with the provision of care for FA affected individuals and their families, this document provides benchmarks against which care across FA affected individuals and their families can be collected and analysed in the style of a mini-national cancer audit through the UK and Ireland Fanconi Anaemia Registry (UKIFAR) Project.

^{aa} As per inaugural meeting of the UK FA Clinical Network, held 5th March 2008 at Sheffield Children's Hospital.

2. DIAGNOSTIC EVALUATION IN FANCONI ANAEMIA – OVERVIEW

2.1 Introduction.

First described by Guido Fanconi in 1927, Fanconi anaemia (FA) is now one of the most characterized inherited bone marrow failure syndromes¹. It is usually inherited as an autosomal recessive trait but in a small subset of FA cases it can be an X-linked recessive disorder. FA patients display marked clinical heterogeneity. Characteristic features include the progressive development of bone marrow failure (BMF) and an increased predisposition to malignancy. Affected individuals may also have one or more congenital/developmental abnormality including abnormal skin pigmentation (e.g. café au lait spots, hypo-pigmented macules), skeletal (e.g. radial ray anomalies), genitourinary (e.g. dysplastic or horseshoe kidney), and gastrointestinal (e.g. duodenal atresia) abnormalities. A significant subset (~ 30%) of FA patients have no overt somatic abnormalities. The majority of patients present towards the end of the first decade of life. However, increasingly some patients are being first diagnosed in adulthood and many patients diagnosed in childhood are surviving into adulthood making it important for both adult and paediatric physicians to be aware of this disorder.

2.2 History & Examination.

FA is a very variable multi-system disorder. This is highlighted by Table 2.1 below which lists the abnormalities seen in FA patients. In view of the wide range of abnormalities that can be seen in different FA patients it is important in the history and examination to establish which of these might be relevant in each FA patient. A comprehensive history (including the neonatal and developmental history) is important as it may suggest the diagnosis of FA. Equally a comprehensive physical examination is important in order to maximize the chances of picking up the many FA clinical features listed in Table 2.1. FA patients can present to a range of different paediatric and adult specialities. In all disciplines of Medicine it is therefore important to consider the diagnosis of FA if the presentation is with one or more of the features listed in Table 2.1.

| Abnormality | Approximate incidence (%) |
|--|---------------------------|
| Skeletal (e.g. radial ray, congenital hip, vertebral, rib) | 71 |
| Skin pigmentation (café au lait, hyper/hypo-pigmentation) | 64 |
| Short stature | 63 |
| Eyes (e.g. microphthalmia) | 38 |
| Renal & urinary tract | 34 |
| Male genital | 20 |
| Mental retardation | 16 |
| Gastrointestinal (e.g. anorectal, duodenal atresia) | 14 |
| Heart | 13 |
| Hearing | 11 |
| Central nervous system (e.g. hydrocephalus) | 8 |
| No abnormalities | 30 |

Table 2.1: Abnormalities in FA

| Complication | % of patients | Median age (yrs) |
|---|---------------|------------------|
| Aplastic anaemia | 90 | 9 |
| Acute myeloid leukaemia | 10 | 14 |
| Liver disease (including tumours) | 4 | 16 |
| Myelodysplasia | 32 | 17 |
| Cancer (epithelial, usually oropharyngeal/anogenital) | 5 | 17 |

Table 2.2: Complications in FA

In Table 2.2 above are given some of the severe complications of FA. As more data is accumulated from comprehensive registries these figures will vary with time. Note that these figures will also vary depending on how data is presented and how FA populations evolve. For example Cancer (epithelial) is listed as occurring in 5% of

patients overall but will be found with a higher frequency in those FA groups who live into adulthood. Thus as either outcomes improve with respect to management of bone marrow failure or screening for FA in individuals presenting with such epithelial cancers in the general population is considered, the occurrence of such epithelial cancers will inevitably increase (see [Sections 10 and 11](#)). However, the information in Table 2.2 highlights the important point that patients with FA have a greater risk of developing these complications compared with the average population. In some cases the initial presentation of the patient will be with one of these complications. For example, it has become clear that in many FA patients, their first presentation can be with bone marrow failure and most haematologists will now screen all young patients with aplastic anaemia for FA anaemia. However, this is not routine practice; for example, in patients presenting with neck cancer or myelodysplasia, suggesting a continual need for educating professionals in different medical specialities who may encounter FA patients with varying presentations.

2.3 Tests and assessments.

In the diagnostic evaluation for FA a number of tests/investigations are useful. These can be categorised into:

- Peripheral blood tests
- Complementation and genetic subtype analysis
- Skin biopsy (only in selected cases where chromosomal breakage test is negative on peripheral blood)
- Bone marrow examination
- Imaging and related tests
- Special assessments

The results of these tests/evaluations should enable the medical team to build a comprehensive picture of the patient in order to plan optimal management.

2.4 Peripheral blood tests.

2.4.1 Peripheral blood tests required.

In the category of peripheral blood tests the following (Table 2.3) should be undertaken, including a Chromosomal Breakage test.

| | Peripheral blood tests in FA |
|---|---|
| 1 | Blood count (FBC, HbF, MCV, reticulocytes, blood film) |
| 2 | Chromosomal Breakage (based on either MMC and/or DEB). Also test sibs |
| 3 | Blood group & antibody screen |
| 4 | Biochemistry (liver and renal function, iron levels) |
| 5 | Endocrine assessment (including blood glucose) |
| | Viral screen (CMV, hepatitis B/C, varicella status) |
| | Tissue typing (also include siblings/extended family to identify a potential BMT donor) |

Table 2.3: Peripheral blood tests in FA.

2.4.2 Peripheral blood chromosomal breakage test.

In the UK the front line diagnostic test remains the “*DEB-MMC stress*” test. Following exposure to DEB (diepoxybutane) or MMC (mitomycin C) peripheral blood lymphocytes from patients with FA exhibit increased chromosomal breakage compared with normal controls. In some FA patients a reversion of the cellular phenotype (elevated breakage) can occur (up to 10-15%). In these cases, chromosomal breakage testing on skin fibroblasts is indicated. Chromosomal breakage testing should be done in an accredited cytogenetics laboratory. In some parts of Europe a FA screening test using flow cytometry is used based on G2 cell cycle arrest observed in FA cells as compared with normal controls². Guidance concerning screening for FA is outlined in detail in [Section 3](#) below.

2.4.3 HLA typing in family members.

Blood should be taken from siblings for tissue type to identify any potential HLA-matched sibling donors. In the absence of a HLA-matched unaffected sibling, blood should be taken from extended family members starting with grandparents of FA proband if available. (as well as an initial bone marrow registry search through the Anthony Nolan Trust to give an indication of rarity of tissue type).

2.5 FA complementation/genetic analysis.

There are several genetic subtypes/complementation groups in FA. These are detailed below in Table 2.4. This table also gives the location of the FA genes on the different chromosomes, the number of exons in each gene and the total amino acid number in each corresponding protein.

| Complementation Group/gene | Approximate % of FA patients | Chromosome location | Amino acids | Exons |
|----------------------------|------------------------------|---------------------|-------------|-------|
| A (<i>FANCA</i>) | 66 | 16q24.3 | 1455 | 43 |
| B (<i>FANCB</i>) | <1 | Xp22-31 | 859 | 10 |
| C (<i>FANCC</i>) | 12 | 9q22.3 | 558 | 14 |
| D1 (<i>FANCD1/BRCA2</i>) | <1 | 13q12-13 | 3418 | 26 |
| D2 (<i>FANCD2</i>) | <1 | 3p25.3 | 1451 | 44 |
| E (<i>FANCE</i>) | 4 | 6p21.3 | 536 | 10 |
| F (<i>FANCF</i>) | 4 | 11p15 | 374 | 1 |
| G (<i>FANCG</i>) | 12 | 9p13 | 622 | 14 |
| I (<i>FANCI</i>) | <1 | 15q25-26 | 1328 | 35 |
| J (<i>FANCI/BRIP1</i>) | <5 | 17q22 | 1249 | 20 |
| L (<i>FANCL</i>) | <1 | 2p16 | 375 | 14 |
| M (<i>FANCM</i>) | <1 | 14q21.3 | 2048 | 23 |
| N (<i>FANCN/PALB2</i>) | <1 | 16 | 1186 | 13 |

Table 2.4: FA complementation/genetic subtypes

As can be seen from Table 2.4 there are 13 different genes which, when mutated, can result in FA. The mutations in the different genes can be very variable. This makes it very complicated to undertake mutation analysis in FA patients. To date, genetic testing has not been available in the UK and has had to be sourced abroad. Clearly a UK service would be desirable as it would facilitate screening of siblings for FA, earlier prenatal diagnosis, and carrier testing. There are also clinically relevant genotype-phenotype correlations emerging. For example, patients with *FANCD1* and *FANCN* mutations tend to have a high risk of brain tumours and *BRCA2* heterozygotes have high risk of breast cancer. At present, it is possible to get

limited mutation analysis done via groups in either Amsterdam^f or Düsseldorf[#]. Also see genetic analysis providers listed in the Fanconi Anemia Research Fund (FARF) Fanconi Anemia Treatment and Resource Guide 2008

(www.fanconi.org/family/Treatment%20and%20Testing%20Resources%202008.pdf)

. The future costs and provision of these European services are difficult to predict (currently complementation group determination is free 2008/09 in Germany, 650 Euro per gene (allele pair) tested per individual in Amsterdam). In the long run it would be desirable to undertake complementation group and mutation testing for FA in the UK.

2.6 Skin biopsy.

In some FA cases the peripheral blood (PB) chromosomal breakage is either normal or indeterminate. Some of these cases represent somatic mosaics. In such cases a mutation has occurred in an early haematopoietic stem/progenitor cell and this leads to correction of the “FA chromosomal breakage phenotype”. In culture the PB cells appear to be normal yet the patient has underlying FA. In these cases chromosomal breakage analysis of fibroblasts established from a skin biopsy can be very useful as the fibroblasts will still have the increased chromosomal breakage characteristic of FA. Thus in some cases a skin biopsy is needed to make the diagnosis of FA because a somatic reversion event makes the PB lymphocytes behave like normal cells. A skin biopsy is easily performed as a small punch biopsy under local anaesthetic in the older child or adult. It is very unlikely that there would be an indication to perform a skin biopsy for the purposes of FA diagnosis in a child under the age of five years. See also [Sections 3.2 and 3.5](#).

^f **Prof Hans Joenje, PhD**
VU University Medical Center Amsterdam
Department of Clinical Genetics
Van der Boechorststraat 7
NL-1081 BT Amsterdam
The Netherlands
joenje@vumc.nl
Phone: 31-20-444-8270 Fax: 31-20-444-8285
www.english.vumc.nl/afdelingen/404626/1026633

[#] **Prof Helmut Hanenberg, MD**
Heinrich Heine University Medical Center
Children's Hospital
Moorenstrasse 5
40225 Düsseldorf, Germany
Hanenberg@uni-duesseldorf.de
Phone: 49-211-811- 6103 or -6568 Fax: 49-211-811-9436
www.uni-duesseldorf.de/Jahrbuch/2006/medfak/neumed

2.7 Bone marrow examination.

If the blood count is normal there is no urgent need to do an initial bone marrow (BM) examination. When a patient has an abnormal blood count and a diagnosis of FA has been made then it is desirable to do a bone marrow examination. This should include morphology, cytogenetics, and trephine histology. This will enable assessment of the morphology and the cellularity as well as determine if there are any karyotypic abnormalities. It is unclear the frequency with which subsequent marrow examinations should be done although consideration should be given to performing such examinations on an annual basis.

2.8 Imaging and related tests.

2.8.1 Abdominal/renal ultrasound and ECHO.

An abdominal/renal ultrasound and trans-thoracic echocardiogram are always required as initial investigations. The detection of any FA-associated renal or cardiac abnormalities will lead to a renal or cardiac specialist opinion respectively. The absence of any associated cardiac abnormality is required in particular in the context of future anaesthesia.

2.8.2 Brain imaging.

Brain MR imaging is required if there are any significant developmental or neurologic concerns.

2.8.3 Other radiological investigations.

Investigations that involve unnecessary radiation exposure (e.g. CT, bone scanning, skeletal survey X-rays) should be avoided as information required on initial diagnostic evaluation can generally be acquired through either clinical examination or other non-radiation based modalities. An exception to this is the use of isotope assessment of renal function that would be requested only on the opinion of a renal specialist. Individuals affected by FA are considered to be particularly sensitive to sustained radiation exposure over time. The course of their condition is likely to result in significant diagnostic and therapeutic radiation exposure. All radiation interventions should be justified with respect to individual patient benefit and not just be based on routine practice concerning non-FA patients. It is not necessary to rule out or indeed identify every FA-associated abnormality such as a congenital vertebral fusion, only those that are clinically relevant.

2.9 Special assessments.

Special assessments are complementary to the tests described above. They are useful to ensure that all possible abnormalities observed in FA patients are picked up. Some of these assessments may only be necessary once; others may be required on a regular basis. For example, regular dental follow-up is important in FA patients whereas regular ear examinations are not necessary if the first one was normal.

2.9.1 Hearing assessment/audiometry.

A specialist hearing/audiometric assessment is always required on initial diagnosis considering the occurrence of hearing loss in FA (see [Section 9.1](#)).

2.9.2 Dental assessment.

All FA patients should be confirmed to be under a dentist and receive dental checks twice a year considering the apparent higher prevalence of dental problems in the FA population (see [Section 14.2](#)).

2.9.3 Endocrine assessment.

All FA patients should be referred to an endocrinologist on initial diagnosis and undergo a baseline endocrine assessment (see [Section 6](#)).

2.9.4 Oropharyngeal/anogenital cancer assessment.

All FA patients from the age of ten years should be seen four times a year by a head & neck cancer specialist (see [Section 10](#)) independent of any dental assessment.

In addition, all FA female patients from the age of twelve years should have gynaecological cancer specialist follow up twice a year (anal carcinoma screening in males from age of 18 years) (see [Section 11](#)).

2.9.5 Other assessments.

Other assessments should be arranged as appropriate, such as children's hand surgeon if thumb or radial ray abnormality detectable clinically, paediatric developmental neurologist if developmental or neurologic issues, or dietician if problems with dietary intake.

2.10 Genetic counselling.

An FA affected family should be seen by the regional clinical genetics service. The FA screening process should be completed for the affected individual, siblings, and any other relevant family members, as outlined in [Section 3](#). Parents should be specifically advised about family planning issues/reproductive choices. If there is no HLA identical unaffected sibling in the FA affected family, and maternal age is less than 40 years, then the parents should be advised about the option for pre-implantation genetic diagnosis (PGD)/HLA-typing/IVF ('Saviour Sibling') (see [Section 13](#)).

2.11 Patient/family support.

FA-affected individuals and their families should be encouraged to contact the available FA-specific support and information resources, i.e., both the UK/Ireland-based Fanconi Hope Charitable Trust (www.fanconihope.org, www.fanconianaemia.nhs.uk) and the US-based Fanconi Anemia Research Fund (www.fanconi.org). In particular in the context of a cancer diagnosis, information should be provided about contacting Macmillan Cancer Support (www.macmillan.org.uk) and CLIC Sargent (for childhood cancers) (www.clicsargent.org.uk).

2.12 Hospital attendances.

The above list of tests/assessments can be regarded as a check list that should be completed although, by their nature, it will not be possible to undertake all these tests/assessments in a single visit. There should therefore be an attempt to minimise the number of unnecessary hospital visits, e.g. arranging as many investigations/assessments as possible to be done together during a daycase admission. There should also be a clearly understood point of contact, i.e., telephone number(s), including after-hours, for FA patients/families and 24 hour open door access over and above attendance at an accident and emergency department, e.g. to the haemato-oncology ward.

Recommendations

- **A comprehensive history, including details concerning neonatal period, health of siblings, and any family cancer history, should be obtained. Examination should specifically include: blood pressure measurement;**

inspection of skin over whole body for combination of café au lait and hypopigmented macules; inspection of thumbs/thenar eminences and radial forearm; and height/weight measurement/preparation of centile chart.

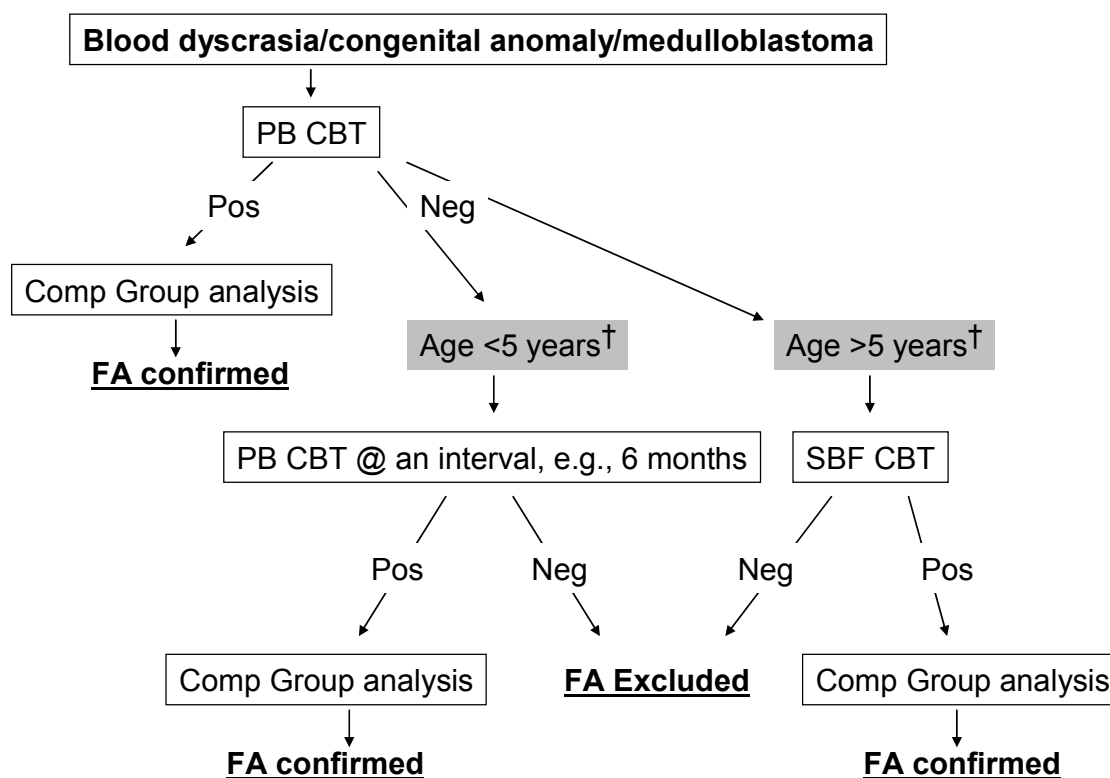
- Screening for FA within a family requires a DEB/MMC chromosomal breakage test on peripheral blood lymphocytes and/or skin fibroblasts. Confirmation of diagnosis should be duplicative for those tested, i.e., ideally two different testing modalities (chromosomal breakage and genetic analysis) and at two different time points (see [Section 3](#) below).
- Baseline investigations/assessments at diagnosis should be: FBC, blood group and antibody screen, liver and renal function, iron levels, viral screen (including CMV, hepatitis B/C, and varicella), tissue type (including of siblings and extended family), endocrine assessment, cardiac trans-thoracic ECHO, abdominal ultrasound, audiometry, and referral to a clinical geneticist.
- All FA affected individuals from the age of 10 years should have screening our times a year for oropharyngeal squamous cell carcinoma (see [Section 10](#)).
- All FA affected individuals should have screening at least twice a year for anogenital squamous cell carcinoma from the age of 12 years for females and from the age of 18 years for males (See [Section 11](#)).
- Other investigations/specialist assessments should be only as indicated, for example: paediatric hand surgeon if radial ray/thumb abnormality, brain MRI/developmental neurologist if delay, ENT surgeon if hearing loss.
- In all families, where the FA affected individual is a child, does not have an unaffected HLA matched sibling, and maternal age is less than 40 years, parents should be advised about PGD/HLA-selection/IVF ('Saviour Sibling'). PGD would not be appropriate in women aged 45 years and over. See [Section 13](#) below.
- FA-affected individuals and their families should be encouraged to contact the available FA-specific support and information resources, i.e., both the UK/Ireland-based Fanconi Hope Charitable Trust (www.fanconihope.org, www.fanconianaemia.nhs.uk) and the US-based Fanconi Anemia Research Fund (www.fanconi.org).

- **There should be a demonstrable attempt to minimise the number of unnecessary hospital visits, e.g. arranging as many investigations/assessments as possible to be done together during a daycase admission.**
- **There should also be a clearly understood point of contact, i.e., telephone number(s), including after-hours, for FA patients/families and 24 hour open door access over and above attendance at an accident and emergency department, e.g. to the haemato-oncology ward.**

3. SCREENING FOR FANCONI ANAEMIA

3.1 Screening for FA.

The screening process for FA is summarised using tentative decision trees as shown in Figure 3.1. As much as possible and depending on circumstances, confirmation or exclusion of FA should be performed in a duplicative manner, both in time, and ideally, testing modality, e.g. positive chromosomal breakage test with subsequent genetic analysis, negative chromosomal breakage on both blood and skin biopsy, etc^{3, 4, †}. It is not unknown for an initial chromosomal breakage test to be false negative⁵. There are also occasional other rare causes of a positive chromosomal breakage test such as Nijmegen Breakage Syndrome that can be confused with FA^{6, 7}. In addition consideration has to be given for reverse mosaicism (see [Section 3.2](#) below).



†Arbitrary cut off on the basis that FA reverse mosaicism is unlikely to occur under the age of 5 years

† 'Although a positive cytogenetic test result is highly indicative for FA, molecular analysis is still required to demonstrate pathogenic mutations in a FA gene. This is essential for adequate clinical management, as this establishes the mode of inheritance, helps to assess prognosis, and allows to exclude diseases with overlapping clinical symptoms.' from *Ameziane et al*⁸.

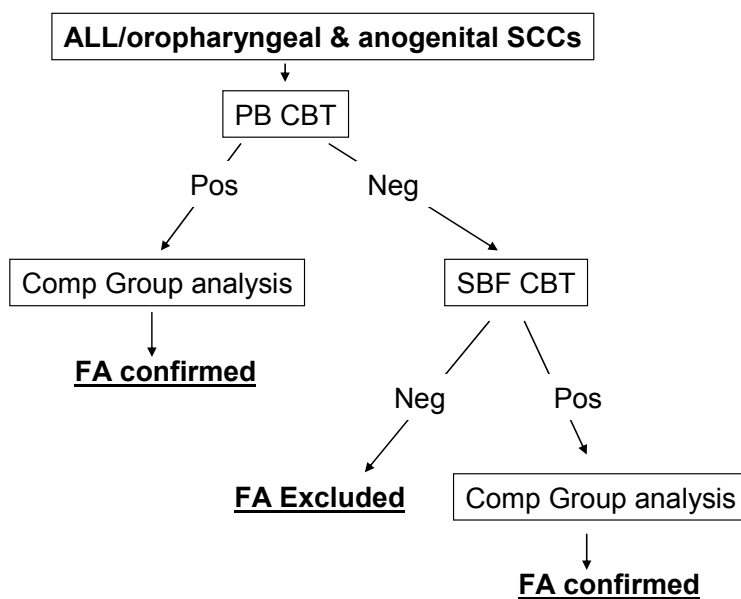
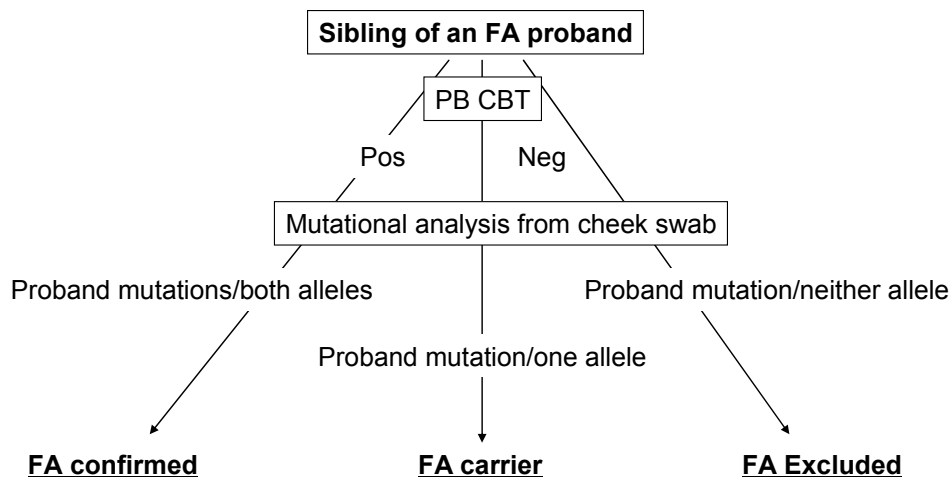


Figure 3.1: Tentative screening trees for FA^f: A (overleaf) Blood dyscrasia (including acute myeloid leukaemia and myelofibrosis)/congenital anomaly/medulloblastoma; B (top) Sibling of an FA proband; C (bottom) ALL/oropharyngeal & anogenital SCCs. Abbreviations are as follows: PB=peripheral blood; CBT=chromosome breakage test; Comp=Complementation; Neg=negative; Pos=positive; SBF=skin biopsy fibroblast; ALL=acute lymphatic leukaemia.

^f Note that ALL, AML, SCCs, does not automatically imply FA screening in all cases indiscriminately. See [Section 3.3](#) for context.

3.2 Reverse mosaicism.

Note that reverse mosaicism is thought to account for the 10-15% of FA affected individuals who subsequently have recovery of haematological parameters and who also may not end up requiring a haematopoietic stem cell transplant^{8, 9, 10}. However reverse mosaicism may only result in partial restoration of the haematological profile and does not exclude the possibility of future acute myeloid leukaemia. Reverse mosaicism is also thought to account for a proportion of FA affected individuals who are only diagnosed in their adult years when presenting with an oropharyngeal/anogenital squamous cell carcinoma. Reverse mosaicism in FA is where a haematopoietic stem cell clone or clones emerge that have spontaneously corrected one copy of the defective Fanc gene. In such circumstances, a chromosomal breakage test performed on a blood sample may not be diagnostically abnormal, but the same test repeated on cultured fibroblasts from a skin biopsy will be positive. In the tentative decision trees presented in Figure 3.1 an arbitrary lower age cut off of five years has been adopted on the basis that haematologic reverse mosaicism is very unlikely to occur in FA affected children under the age of five years.

3.3 When should FA be considered?

The diagnosis of Fanconi Anaemia should be ruled out in the circumstances detailed in Section 3.3.1 to 3.3.9 below. The absence of the typically described features of FA should not exclude screening as such features are absent in 30% of individuals affected by Fanconi Anaemia (see [Table 2.1: Abnormalities in FA](#) above). The spectrum and severity of the FA disease phenotype can vary within families, including identical twins. Screening for FA, once it is considered a possibility or is identified in an individual proband's sibling, should not be delayed but should be done at the earliest opportunity. Screening for FA as early as possible increases the prospects if a family wish to pursue PGD/HLA-selection/IVF (ideally maternal age should be under 35 years, see [Section 13.3](#)). Early diagnosis enables planning for bone marrow transplantation and also putting in place appropriate cancer screening protocols and other interventions such as additional vaccinations and endocrine evaluation. Screening for FA should be done even when a patient presents in a palliative context as the diagnosis of FA has implications for other family members. Considering the heterogeneous nature of FA and the difficulties that can be associated in establishing an FA diagnosis, screening for FA should be only

arranged through a haematologist or clinical geneticist following appropriate clinical evaluation.

3.3.1 Radial ray and thumb anomalies.

The diagnosis of FA should be considered in all individuals with a congenital radial ray or thumb anomaly and who have not been identified as having an alternative causative genetic disorder such as detailed in [Section 15.1](#)^{11, 12}. This is irrespective of whether the anomaly is unilateral or bilateral, mild or severe, and also applies to thumb duplications. Screening for FA or prompt referral to a clinical geneticist for further evaluation of the family should be at the time of identification of the anomaly, prior to any surgical intervention, and should not be delayed. In particular, before any unilateral thumb duplication polydactyly can be labelled 'sporadic', a screen for FA should be negative.

3.3.2 Failure to thrive/short stature.

The diagnosis of FA should be considered in infants failing to thrive and older individuals with short stature, in the absence of another relevant identifiable cause^{11, 12}.

3.3.3 Combination of café au lait and hypopigmented macules.

An infant/child having the combination of café au lait patches and hypopigmented macules should be screened for FA^{11, 12}.

3.3.4 VACTERL association.

The diagnosis of FA should be considered in individuals identified with the VACTERL association. The VACTERL association refers to the combination of vertebral defects (V), anal atresia (A), cardiovascular anomalies (C), tracheoesophageal fistula (TE), renal anomalies (R), and limb defects (L). About 5% of individuals with the VACTERL association have FA¹³.

3.3.5 Blood dyscrasias.

All children/young adults (age under 45 years) presenting with an otherwise unexplained macrocytosis, and/or thrombocytopenia, and/or neutropenia, and/or pancytopenia (including temporary following a systemic illness), and/or myelodysplasia, should be screened for FA. All children/young adults presenting with acute myeloid leukaemia should have the possibility of FA as a diagnosis considered, with screening dependent on individual circumstances such as the presence of FA phenotypic features, including poor chemotherapy tolerance, or the detection of chromosomal aberrations more commonly seen in FA such as of 3q (see [Section 6](#)). There are a number of reports in the literature of FA patients

presenting with acute lymphoblastic leukaemia (including T-cell)¹⁴. Where a diagnosis of FA is being considered in patients with acute lymphoblastic leukaemia, there is a possibility that a peripheral blood lymphocyte chromosomal breakage test may be negative as a result of lymphoid clones having undergone reverse mosaicism in the process of malignant transformation. A skin biopsy fibroblast chromosomal breakage test should in such circumstances be considered.

3.3.6 Intracranial medulloblastoma/renal Wilms tumour.

FA should be considered as a possibility in children five years and under presenting with a posterior fossa medulloblastoma or a renal Wilms tumour, i.e., specifically the FA-D1 complementation group (bi-allelic *FANCD1/BRCA2* mutations)^{15, 16, 17}. FA screening should be performed if one of the following are present: low birth weight; short stature; thumb/radial ray abnormality; skin hyper/hypopigmentation; previous history of malignancy, a sibling who has had a Wilms tumour, brain tumour, or T-cell acute lymphatic leukaemia; or a family history of breast cancer. All cases in the literature reported to date have at least one of the above features although no one particular feature is universal. Note that there is an incongruity between the apparent frequency of *BRCA2* mutations in the population (up to ~1:200) and the FA-D1 complementation group (accounting for only about 1% of FA affected individuals). It is unclear at this point as to what extent women of child-bearing age in *BRCA2* families should be counselled concerning the possibility of a FA-D1 FA affected child. There is also no information in the literature concerning the proportion of children presenting with medulloblastoma or Wilms tumour that might have FA. The number of children to which this screening recommendation applies is likely to be small (see for example age incidence of medulloblastomas in the UK, Appendix B, Figure B.1). In addition, other genetic disorders may be considered for a child with either medulloblastoma or Wilms tumour (see Table 3.2 below).

| Tumour type | Genetic syndrome |
|----------------------------|--|
| Medulloblastoma | Gorlin-Goltz syndrome/Nevoid Basal Cell Carcinoma syndrome (cerebral calcifications, basal cell carcinomas, multiple keratocysts of jaw) Familial Adenomatosis Polyposis (APC gene mutations) |
| Wilms tumour ¹⁸ | WT1 associated syndromes (with genitourinary abnormalities and renal dysfunction) Familial Wilms tumour (autosomal dominant) Childhood overgrowth syndromes (e.g. Beckwith-Weidemann syndrome) |

Table 3.2: Examples of genetic disorders, other than FA, predisposing to either medulloblastoma or Wilms tumour.

3.3.7 Oropharyngeal/anogenital squamous cell carcinomas.

There are reports of FA patients presenting in the first instance with a mucosal squamous cell carcinoma¹⁹. Such individuals are thought to have a milder phenotype or have developed reverse mosaicism. Thus, these individuals may not have the external stigmata or haematological features of FA. Keep in mind that 10% of FA patients have a height greater than the population average and that 30% of FA patients do not have any obvious external features of FA. There is however little data to base any recommendation for FA screening in patients from the general population who present with a mucosal squamous cell carcinoma. Thus, any age cut-off is arbitrary. Certainly, screening for FA should be a consideration in any individual who suffers from successive multi-site mucosal squamous cell carcinomas. We suggest that all individuals aged under 45 years presenting with a squamous cell of mouth/tongue/tonsils/hypopharynx or vulva/penis/anus (in the absence of any other relevant risk factor such as HIV positive or renal transplant recipient) should be screened for FA with particular consideration being given for the possibility of reverse mosaicism. To exclude a diagnosis of Fanconi Anaemia in this patient group, a skin biopsy fibroblast chromosome breakage test would have to be performed. The choice of screening patients presenting with mucosal squamous cell carcinomas under the age of 45 takes into account the smaller numbers of patients in this group as shown in Figures B.2 to B.5 and Table B.1 in Appendix B.

3.3.8 Excessive chemotherapy/radiotherapy toxicity.

Individuals with perceived systemic excessive toxicity in association with chemotherapy and radiotherapy should be screened for FA.

3.3.9 Sibling of an FA proband.

All siblings of an FA proband should be screened for FA. Note that phenotype can vary within a family and that a 'normal' external appearance makes the possibility of FA less likely but does not exclude the diagnosis. See Section 3.4 below.

3.4 Issues surrounding sibling screening.

3.4.1 Screening starts with a peripheral blood chromosomal breakage test.

If the peripheral blood chromosomal breakage test is negative and the proband mutations are known, targeted mutational analysis can be performed in the sibling both to confirm absence of FA and to determine FA carrier status. If the sibling/FA family does not want mutational analysis performed at the time of screening or mutational analysis has been unsuccessful, then a second peripheral blood chromosomal breakage test should be performed if the child is less than five years. If the sibling is over five years, a chromosomal breakage test performed on cultured fibroblasts obtained from skin biopsy should be performed.

3.4.2 Reproductive issues for the sibling of an FA proband.

Siblings are significantly affected over time in a psychological manner and are likely to seek assurance when planning their own families. The importance of being able to identify the FA carrier status of an FA sibling should not be underestimated. The estimated population FA carrier frequency is about 1:300 and is significantly higher in minority populations.

3.4.3 Financial planning issues for the sibling of an FA proband.

Issues surrounding insurance/life assurance/obtaining credit/pensions are sometimes used as reasons not to identify carrier status in genetic disorders. However, in the process of making any such applications, the individual has to declare details of his/her family medical history and is usually specifically asked about the presence of a genetic disorder in first degree relatives. Thus, the sibling of the FA proband will have these issues irrespective. Recent publications have confirmed that the majority (>95%) of FA carriers/heterozygotes do not have as of yet a significant demonstrable cancer risk. Therefore, with respect to siblings, it would be of particular importance to identify the complementation group of the FA

proband, and there may be some advantage in the sibling knowing his/her carrier status.

3.4.4 Timing with respect to determining FA carrier status in siblings.

There is however no immediate benefit to the sibling during childhood years of knowing carrier status or not and ultimately the FA affected family may decide that any unaffected siblings should be allowed to make their own decision with respect to identifying carrier status from the age of 18 years. However, in such circumstances, either the proband mutational analysis must have been performed or DNA from the proband retained and stored for future analysis, otherwise mutation testing would be unavailable in siblings. It would be very challenging and expensive to identify FA carrier status by looking for described mutations for each FA gene in turn in order of frequency in the population without having had a genetic analysis completed on the FA proband.

3.5 Testing centres for chromosomal breakage testing.

Centres in the UK and Ireland that perform peripheral blood chromosomal breakage testing are listed in Appendix 1 (for complementation group/mutational analysis, see [Section 4](#)). Guy's & St Thomas's are the only centre in the UK that performs skin chromosomal breakage testing on skin biopsy obtained cultured fibroblasts (and on amniotic/chorionic tissue for the purposes of antenatal testing) (enquiries to Dr Ian Kesterton, ian.kesterton@gstt.nhs.uk, www.guysandstthomas.nhs.uk/services/managednetworks/genetics/cytogenetics/cytogenetics.aspx).

3.6 Turnaround time for chromosomal breakage testing.

Peripheral blood chromosomal breakage testing usually can take from two to four weeks to perform. Chromosomal breakage on skin biopsy obtained fibroblast culture takes up to six weeks and may be inconclusive. Considering the family anxiety associated with a potential diagnosis of FA, parents or an adult individual being screened for FA should be informed of the result of the chromosomal breakage test and relevant issues surrounding the test within two working days of the test result being available. Results of any sibling testing should be passed on to the affected family in a similar time course.

Recommendations

- **Screening for FA within a family requires a DEB/MMC chromosomal breakage test on peripheral blood lymphocytes and/or skin fibroblasts. Confirmation of diagnosis should ideally be duplicative for those tested, i.e., ideally two different testing modalities (chromosomal breakage and genetic analysis) and at two different time points.**
- **Screening for FA should be only arranged through a haematologist or clinical geneticist following appropriate clinical evaluation.**
- **Screening for FA should be considered in all individuals with: radial ray/thumb abnormalities, including duplications, irrespective whether unilateral or bilateral (unless another causative genetic disorder has been identified); failure to thrive/short stature of otherwise unidentifiable cause; combination of café au lait patches and hypopigmented macules; VACTERL association, blood dyscrasias under the age of 45 years; intracranial medulloblastoma under the age of five years; oropharyngeal/anogenital squamous cell carcinomas under 45 years of age; perceived excessive toxicity associated with chemotherapy and/or radiotherapy; and siblings of FA probands.**
- **Screening for FA, once it is considered a likely diagnosis, or is identified in an individual's sibling, should not be delayed but should be done at the earliest opportunity. Screening for FA should be done even when a patient presents in a palliative context as the diagnosis of FA has implications for other family members.**
- **The possibility of haematologic reverse mosaicism needs to be considered. Thus for individuals over the age of five years who are been screened for FA who have a normal blood lymphocyte chromosome breakage test and do not have a sibling proband in whom FA mutations have been identified, a skin biopsy fibroblast chromosome breakage test should also be performed before a diagnosis of FA is discounted.**
- **The results of a chromosome breakage test should be passed on to individual/family within two working days of the test result becoming available.**

4. GENETIC ANALYSIS

4.1 Genetic analysis – introduction.

Genetic analysis in FA involves first determining which one of the thirteen genes described to date is affected ('complementation group analysis) and then going on to identify the specific mistake in each of the two copies of the affected gene (by gene sequencing)²⁰. Fanconi genes identified to date are listed in [Table 2.4: FA complementation/genetic subtypes](#) above. The details of complementation group analysis and mutation analysis are described in the relevant medical literature with specific approach varying between laboratories. There is no provider of genetic analysis for FA affected families at the time of publication of this standards of care document in the UK or Ireland. Such genetic analysis can be obtained on patients from the UK/Ireland via groups in either Amsterdam^f or Düsseldorf[#]. Also see genetic analysis providers listed in the FARF Fanconi Anaemia Treatment and Resource Guide 2008

(www.fanconi.org/family/Treatment%20and%20Testing%20Resources%202008.pdf)

. In the long run it would be desirable to undertake genetic testing for FA in the UK.

^f **Prof Hans Joenje, PhD**
VU University Medical Center Amsterdam
Department of Clinical Genetics
Van der Boechorststraat 7
NL-1081 BT Amsterdam
The Netherlands
joenje@vumc.nl
Phone: 31-20-444-8270 Fax: 31-20-444-8285
www.english.vumc.nl/afdelingen/404626/1026633

[#] **Prof Helmut Hanenberg, MD**
Heinrich Heine University Medical Center
Children's Hospital
Moorenstrasse 5
40225 Düsseldorf, Germany
hanenberg@uni-duesseldorf.de
Phone: 49-211-811- 6103 or -6568 Fax: 49-211-811-9436
www.uni-duesseldorf.de/Jahrbuch/2006/medfak/neumed

4.2 Consensus is that genetic analysis, i.e., complementation group and mutation testing, should be recommended on all FA-affected individuals.

There is an increasing appreciation of the potential importance of genetic analysis in the management of FA affected individuals and their families^{3, 4, 21}. Knowing the genotype in a particular individual can assist in predicting the severity and spectrum of the disorder including the possibility of reverse mosaicism, contribute to decision-making concerning the timing of haematopoietic stem cell transplantation, be informative about carrier/heterozygote cancer risk, and facilitate antenatal screening²². Genetic analysis is also required if PGD+/-HLA selection/IVF is to be considered. These issues are further outlined below.

4.2.1 Complementation group analysis and FA screening.

Determining complementation group serves to complete the 'duplicative' requirement for confirmation of diagnosis in FA affected individuals (see [Section 3.1](#) above).

4.2.2 Complementation group and carrier cancer risk.

Known carrier (heterozygote) cancer risk is dependent on complementation group. Currently, *FANCD1/BRCA2* heterozygotes are known to have a high risk of breast and ovarian cancer, while mutations in *FANCN/PALB2*, and *FANCI/BRIP1* are associated with a lower risk of breast cancer^{23, 24, 25}. Families from these complementation groups should be referred to a cancer geneticist for consideration of genetic testing and recommendation for screening, i.e., annual breast MRI from the age of 30 to 49 years for female carriers of *BRCA2* mutations, as per NICE Guidance on Familial Breast Cancer (see www.nice.org.uk/CG41). Annual screening for ovarian cancer (i.e., pelvic ultrasound and serum CA125) can also be considered and is available as part of a research trial for carriers of a *BRCA2* mutation (see www.ovacome.org.uk/Ovariancancer/Screeningandprevention/Familialovariancancer), as is screening for prostate cancer in male carriers of *BRCA2*.

4.2.3 Complementation group and prognosis concerning FA affected individuals.

Complementation group can be prognostic with respect to being predictors of early bone marrow failure or leukaemia²⁶. The paediatric transplant haematologist may consider this information helpful with respect to planning a bone marrow transplant, e.g. an earlier transplant might be planned for in the situation of a matched sibling

being available. FA-G patients have a significantly higher risk of acute myeloid leukaemia than FA-A and FA-C. FA-D1 and FA-D2 are severely affected groups with FancD1 patients specifically suffering from, and requiring consideration of screening for development of, intracranial tumours (usually posterior fossa medulloblastomas) and Wilm's tumours.

4.2.4 Determination of complementation group is the current preferred first step in mutational analysis.

Complementation group analysis is generally the first step in most laboratories providing a genetic analysis for FA. If complementation group is identified and fresh tissue under liquid nitrogen/DNA is stored, mutational analysis can always be performed in the future if required. Mutational analysis otherwise is challenging and expensive. Otherwise each pair of potentially affected genes would have to be sequenced in turn. It also can be difficult to determine whether a variation in a gene sequence is a pathologic mutation or a polymorphism of no consequence.

4.2.5 Mutational analysis may be used to complete the screening process in a sibling who has had a normal chromosome breakage test.

On all siblings who have been screened as negative for FA on the basis of a negative chromosomal breakage test and where the two mutations have already been identified in the proband, mutational analysis serves to complete the 'duplicative' requirement in ruling out the diagnosis of FA and also allows FA carrier status to be determined (see [Sections 3.3.9 and 3.4](#) above for a more detailed discussion concerning the issues around sibling FA screening).

4.2.6 Mutation type and prognosis concerning FA affected individuals.

Specific mutations in particular complementation groups can be prognostic with respect to being predictors of early bone marrow failure or leukaemia. The paediatric transplant haematologist may consider this information helpful with respect to planning a bone marrow transplant, e.g. an earlier transplant might be planned for in the situation of a matched sibling being available. FA-A patients with two null alleles have a significant earlier onset of bone marrow failure and higher acute myeloid leukaemic risk than those patients with presence of an altered protein²⁷. FA-C patients with mutations affecting intron 4 or exon 14 have a significant earlier onset of bone marrow failure than mutations affecting exon1^{28, 29}. FA-A patients with the delE12-31 mutation have a significantly higher risk of acute myeloid leukaemia. In addition, concerning the possibility of reverse mosaicism with the implication that a transplant may not be required, absence of compound

heterozygosity on mutational screening would imply that the occurrence of reverse mosaicism is very much an improbability.

4.2.7 Genetic analysis and PGD +/- HLA selection IVF ('saviour sibling').

Mutational analysis should be performed in any family wishing to undergo pre-implantation genetic diagnosis (PGD) (+/- HLA selection/IVF 'saviour sibling'). See [Section 13](#) for further information concerning PGD/HLA-selection/IVF. It should be kept in mind that even a family who has a FA child in a palliative situation, e.g. medulloblastoma, and where the mother is of potential child bearing age (<40 years of age), the parents may subsequently wish to have a further unaffected child by PGD. They will only be able to pursue this option if complementation group analysis with subsequent storage of DNA/tissue under liquid nitrogen or mutational analysis was performed on their initial FA affected child. Mutational analysis should also be done on any FA affected individual/proband in whose family there are first degree relatives of potential or future child-bearing age.

4.2.8 Genetic analysis may be requested otherwise by a clinical geneticist on behalf of an FA affected individual/family.

Providing relevant information and support is part of the responsibility of the clinical genetics service (see [Section 2.10](#)). Irrespective of the other issues concerning genetic analysis outlined above, FA affected families report a strong desire to know as much as possible about their personal situation, including their complementation group and mutations, as part of their coping process. For example, signing off one's complementation group as well as the name of the affected child is seen as the norm on e-mail contributions to the FARF Yahoo notice board used by FA affected families around the world to communicate with each other.

Recommendations

- **Complementation group and mutation testing should be recommended for all FA-affected individuals.**
- **Genetic analysis including mutation analysis should be done when specifically requested by the paediatric haematologist for prognostic purposes.**
- **Genetic analysis including mutation analysis should be done when otherwise requested by the clinical geneticist on behalf of the FA affected individual/family.**

5. HAEMATOLOGIC FOLLOW-UP & MANAGEMENT OF HAEMATOLOGIC COMPLICATIONS

5.1 Introduction.

The ages at onset of bone marrow failure, acute myeloid leukaemia and solid tumours in FA are heterogeneous. Some patients develop progressive marrow failure and require haematopoietic stem cell transplantation in childhood whilst others preserve their blood count into adulthood and are at higher risk of developing solid tumours³⁰

5.1.1 *Cytogenetic abnormalities and haematologic outcome.*

In an IFAR registry study of 388 patients, the actuarial risk of developing hematopoietic abnormalities and MDS/AML was 98% and 52% respectively by 40 years of age³¹. The actuarial risk of developing clonal cytogenetic abnormalities was 67% by 30 years of age. The risk of MDS or AML was higher in persons with a prior clonal cytogenetic abnormality (3% vs 35%) and the actuarial risk of death from hematologic causes was 81% (67% to 90%) by 40 years of age. Clonal cytogenetic abnormalities of sufficient concern to be considered relevant in the context of indications for HSCT are chromosome 3 gains and chromosome 7 losses. See [Section 6](#) for further details concerning these clonal abnormalities. Note that the presence of other isolated clonal cytogenetic abnormalities may be transient and are not in isolation an indication for HSCT.

5.1.2 *Severity of phenotype and haematologic outcome.*

Abnormal radii (but not absent or abnormal thumbs) appear to be the strongest phenotypic predictor of BMF in FA. A described congenital abnormality score (CABS) based on the presence of one or more of (1) developmental delay, (2) cardiopulmonary abnormality, (3) abnormal kidney (i.e., horseshoe, pelvic, ectopic, or absent), (4) abnormal hearing, and (5) abnormal head/microcephaly ('CABS') separates patients into distinct prognostic groups with respect to the risk of bone marrow failure^f. The cumulative incidence of BMF leading to death or transplantation by age ten years ranges from 18% in the lowest risk group (normal radii, CABS=0) to 83% in the highest BMF risk group (abnormal radii, CABS=5)³².

^f The presence of a listed congenital abnormality scores 1. Absence scores 0. There is a net 1.23 fold increase in the hazard of BMF for each unit increase in CABS. Hence, compared with persons classified as CABS=0, persons with CABS =5 were at 1.23⁵, i.e., 2.8-fold higher risk of BMF.

5.1.3 Genotype and haematologic outcome.

In another IFAR registry study, a significantly earlier onset of BMF and poorer survival was found for complementation group C compared with groups A and G and multivariate analysis of overall survival time showed that some *FANCC* mutations defined a poor-risk subgroup (see also [Sections 4.2.3 and 4.2.6](#) for other examples as to how genetic status can have a bearing on haematologic outcome)²⁹.

5.1.4 Haematologic Follow-up.

It is important, therefore, for patients with suspected or proven FA to be referred for follow-up to a paediatric haematologist who should assume responsibility for co-ordinating multi-speciality care and haematological follow-up of the patient. Three monthly full blood counts with consideration of annual marrow aspiration and trephine biopsy should be performed to monitor for signs of marrow failure or development of clonal abnormalities indicating incipient MDS/AML. See [Section 6](#) for details concerning cytogenetic follow-up.

5.2 Management of bone marrow failure.

Figure 5.1 below describes a treatment pathway modified from a recent review³³. Importantly, to meet criteria for bone marrow failure, any cytopaenias must be persistent, and not secondary to another treatable or reversible cause such as infection, medications, or nutritional deficiencies. In particular, infection, including viral, often results in a transient significant reduction in haematologic parameters that generally shows some recovery.

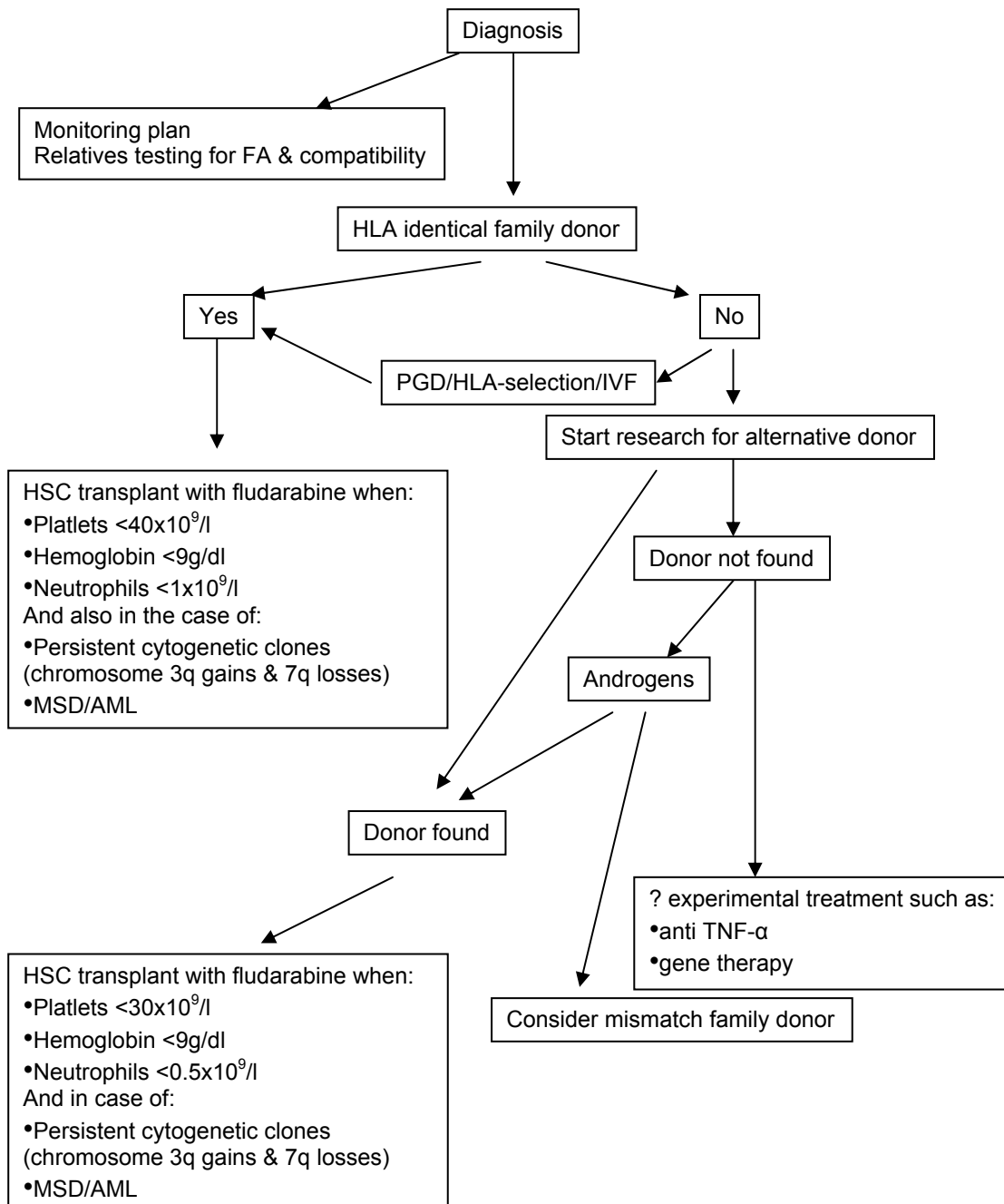


Figure 5.1: An algorithm for treatment decisions concerning haematologic status in FA patients.

5.2.1 Haematopoietic stem cell transplantation as an option.

Treatment should be commenced in patients with progressive marrow failure or signs of progression to MDS/AML. Haematopoietic stem cell transplant (HSCT) is the only curative option and is the treatment of choice in patients with a matched sibling donor. Those for whom a 10/10 matched unrelated donor is available may also proceed to transplant depending on patient and family choice after consultation with a transplant specialist. See [Section 5.5](#) below for detailed discussion.

5.2.2 Androgens as an option.

Androgens may be used as a bridge to transplant or for reducing transfusion dependency in patients who do not have a matched sibling or unrelated donor. See [Sections 5.3 and 5.4](#) below for detailed discussion.

5.2.3 Transfusion as an option.

Transfusions should be given sparingly to avoid sensitisation prior to transplant. The risk of rejection and other transplant related complications is higher in heavily transfused patients with severe aplastic anaemia. Transfused blood components should be matched for an extended red cell phenotype and, if feasible, HLA matched platelets should be used to reduce the risk of sensitisation.

5.3 Androgens and decision to treat.

The decision about whether, when and at what dose to introduce androgens in a Fanconi patient is complex and should only be made by an expert in their use, and then only after very careful consultation with the patient and their parents. Use should be restricted to uncomplicated marrow failure without evidence of clonal haematopoiesis or myelodysplasia. It is suggested that:

- For those with HLA matched sibling donors, stem cell transplantation (SCT) is preferred to the use of androgens when patients become transfusion dependent.
- For those with marrow failure who have 10/10 matched unrelated donors, either androgens or SCT are options. Many doctors prefer SCT but some patients may prefer to try androgens first. There is no evidence of a deleterious effect of androgen therapy on SCT outcome since the introduction of fludarabine-based conditioning therapy in recent studies.
- If androgens are to be used, there may be advantage in starting them before a patient becomes transfusion dependent, e.g. when the platelet count falls to

40-50 x 10⁹/l, but starting at a lower dose, e.g. Oxymetholone 0.5 mg/kg alternate daily. Haemoglobin levels are often the best barometer of response.

- Oxymetholone should be discontinued if there is no response by 6 months (unless there are other causes of cytopaenia such as bacterial or viral infection).

5.4 Considerations in using androgens include:

5.4.1 Response.

Response is relatively slow, often taking 3-6 months, with the fastest response seen in red cells and slowest in platelets. Neutrophils may respond, although most patients with moderate neutropenia seem to get few bacterial infections.

Prophylactic antibiotics may also help, especially during the winter months.

5.4.2 Higher doses.

Higher doses of androgens will often result in higher blood counts but worse side effects. Managing a patient is a trade-off between the two.

5.4.3 Use in girls.

Use in girls is especially controversial, and should be restricted to low dose therapy only (arguably Oxymetholone 0.5-1 mg/kg every second day as a maximum).

5.4.4 Dose reduction.

Many patients in the past have been started on up to 2 mg/kg daily when haemoglobin falls below 8g/dl and platelets below 30 x 10⁹/l consistently until desired response, with cautious dose reduction thereafter. Over 50% of patients will respond but marked side effects are frequent. Many patients can, however, be kept transfusion free (albeit with platelet counts of 30-90 x 10⁹/l) with minimal side effects on as little as Oxymetholone 0.5mg/kg every 3-5 days.

5.4.5 Duration of response.

Most patients who initially respond to androgens will eventually become resistant to therapy, although median duration of response is 7-8 years. Some patients (perhaps especially those who have mosaicism) may be able to stop completely.

5.4.6 Side effects.

Side effects include virilisation (acne, deepening of the voice, facial hair/hair loss), a growth spurt followed by premature closure of the epiphyses, behavioural changes, cholestatic jaundice/transaminitis, hepatic adenoma or hepatoma, peliosis hepatis and hypertension. Some of these side effects may be irreversible.

5.4.7 Monitoring platelet counts.

Platelet counts may fluctuate quite markedly in patients with marrow failure on androgens, perhaps in relation to viral infections. It is wise to try to avoid excessively frequent FBCs or micro-managing androgen doses provided that the patient is consistently transfusion independent.

5.4.8 Hepatic issues (see also [Section 14.3](#)).

Patients on androgen therapy should be monitored regularly with assessment of liver function tests (3-6 monthly) and hepatic ultrasounds (6-12 monthly). If liver transaminases increase to >3 times normal the dose should be reduced until blood tests improve although this is rare on low dose therapy. Androgen associated hepatic adenomas can resolve after androgens are stopped but may persist for years. Adenocarcinomas can also occur.

5.4.9 MDS or AML.

Androgens do not prevent progression to MDS or AML.

5.5 Haematopoietic stem cell transplantation.

Haematopoietic stem cell transplantation (HSCT) for FA remains a challenging undertaking. At present it remains the only curative option for the haematologic complications seen in FA patients. If successful it can prevent progression to Myelodysplasia (MDS) or Acute Myeloid Leukaemia (AML). There is continued debate about the optimal time to offer stem cell transplantation but in general terms this can be summarised as being before regular transfusions have caused iron overload and possibly HLA sensitisation, before the onset of MDS and/or AML, and before the patient's outlook is compromised by persistent infection as a result of neutropenia. There are in addition other disease and donor factors to consider, as well as patient preferences. Table 5.1 summarises present thinking in this area. It is widely accepted that FA patients are highly susceptible to enhanced toxicity caused by DNA cross linking agents as well as radiotherapy (1) and it has been widespread practice in recent years to reduce exposure to such agents within transplant conditioning³⁴. This certainly has contributed to improved survival. There is now a move away from using radiotherapy in conditioning regimes, in part because engraftment in most circumstances occurs satisfactorily without the use of this modality, in part because of concerns about long term toxicity.

| Donor | Transplant Indications |
|---|---|
| HLA identical sibling Or 10/10 or 9/10 volunteer donor Or Matched cord 6/6 or 5/6 | Aplastic anaemia with at least one of the following: <ol style="list-style-type: none"> 1. Need for regular red cell transfusion 2. Neutropenia $<500 \times 10^6/l$ 3. Persistent monosomy 7 or other chromosome 7q losses (see Section 6) 4. Tri- or tetrasomy chromosome 3 in $> 50\%$ of BM cells or other chromosome 3q gains (see Section 6) 5. Presence of leukaemic blasts indicating transformation, i.e., MDS or AML |
| Mismatched family Or <9/10 volunteer donor Or <5/6 matched cord | <ol style="list-style-type: none"> 1. Severe aplastic anaemia unresponsive to androgens or with androgen intolerance 2. Presence of leukaemic blasts indicating transformation, i.e., MDS or AML 3. Note also discussion outlined in Section 6 concerning chromosome 3 gains and chromosome 7 losses |

Table 5.1: Indications for Haematopoietic Stem Cell Transplantation taking into account donor availability and recipient's haematologic condition.

5.5.1 Graft versus Host disease and association with squamous cell carcinoma risk.

It has become clear that there is a high risk of oropharyngeal/anogenital squamous cell carcinomas in long term FA survivors after transplantation and the occurrence of greater than grade 2 skin Graft versus Host disease (GVH) seems to be strongly associated with this late complication (See [Section 10.2.2](#)). The risk of this is now up to 60% in long term survivors in Gluckman's series, hence the need to avoid GVH in post-transplant FA patients³⁵. See [Section 10](#) for further detailed discussion. This has led to the widespread use of some form of T cell depletion in all FA grafts but there is little consensus on T cell depletion methodologies; several have been used with success (see [Section 5.5.7](#) below).

5.5.2 Cord material as a donor source.

The use of cord material as a donor source is still relatively rare in FA. The present view is that a matched cord (6/6 or 5/6) with a good cell dose is likely to produce results comparable to those seen with BM or PBSC but most would still opt for BM or PBSC as donor sources as first choice if they were available.

5.5.3 Mismatched donors.

Mismatched donors (see [Table 5.1](#): Indications for Haematopoietic Stem Cell Transplantation taking into account donor availability and recipient's haematologic condition.), even when T depleted, carry higher risks of morbidity and mortality. Current opinion is to graft at a later stage than patients with matched donors and also to consider a trial of low dose Oxymetholone prior to HSCT. There is evidence that in some patients that the progression of MDS to AML may take some years and if only a very high risk donor is available this may warrant observation to document progression rather than proceeding directly to HSCT. Leave grafting too late will also have a negative impact on survival. Although there seems to be some emerging consensus on how to approach matched sibling grafts and well matched unrelated donor grafts in FA, the precise role of and protocols for mismatched and haploidentical grafts are open to considerable debate although good results have been reported in several small series of patients^{39, 36, 37}. There is little hard evidence to give firm guidance in this difficult area. Probably the best that can be said about patients with FA who do not have good quality family or volunteer donors is that they should be discussed and evaluated individually and recommendations made in the light of current data. Such procedures should only be undertaken by units experienced in grafts for FA and are as much guided by patient choice (having had the opportunity to discuss options) as any other factor. There is no doubt that grafts of this type carry a higher transplant related morbidity and mortality.

5.5.4 Pre-HSCT work-up.

Many of the issues relevant to pre-HSCT will have been covered if prior management of the FA affected individual has been as per laid out in other sections of this Standards of Care document. Note that all patients should have a comprehensive evaluation with particular attention being paid to cardiac, renal (formal GFR), and liver function. There should be some assessment of hepatic venous flow and renal anatomy such as by ultrasound or MRI. All donors should be

screened for FA by chromosomal breakage. Note for potential sibling donors, see also [Section 3.4.1](#). Microbiological prophylaxis, covering pneumocystis and relevant potential viral, fungal, and bacterial pathogens, is as per standard haematologic transplant practice.

5.5.5 Fludarabine.

A significance advance has been the use of Fludarabine in HSCT conditioning regimens in recent years^{38, 39, 40}. In general this highly immunosuppressive agent has been used as a substitute for radiotherapy aiming to promote durable engraftment, again now with good evidence of improved survival (4). The regimens proposed in [Sections 5.5.6 and 5.5.7](#) below all incorporate the use of this agent.

5.5.6 Conditioning Regimen

The suggested conditioning regimen for FA patients undergoing HSCT, other than for those with AML or having a mismatched donor, is given in Table 5.2 below. See [Section 5.5.7](#) for comments on T cell depletion.

Conditioning

Fludarabine 30 mg/kg/day x 5 days: day -10 to -6

And

Cyclophosphamide 10 mg/kg/day x 4 days: day -6 to -2

And

T cell depletion depending on donor type (see [Section 5.5.7](#))

Table 5.2: Suggested conditioning regimen for FA patients other than for those with AML or who have a mismatched donor.

5.5.7 T cell depletion.

The usual T cell depletion methodology in matched grafts would be serotherapy with rabbit ATG 2.5 mg/kg/day from day -3 to day 0. This should be used in all **matched family grafts**. **For volunteer donors**, the three main options for T cell depletion would include Campath 1H 0.2 mg/kg/day from day -9 to -5, rabbit ATG 5 mg/kg/day from day -3 to day 0, or mechanical T cell depletion using a Miltenyi system and PBSC. All these technologies produce profound T cell depletion and also prolonged post graft infection risk due to slower immune reconstitution.

5.5.8 AML.

Current data suggests that HSCT can be successful even in FA patients who develop AML, particularly those in whom the disease process is evolving slowly out of a pre existing MDS^{41, 42}. Again such patients are very rare, perhaps one a year in the UK and they should be discussed on an individual basis with interested clinicians and a treatment plan presented and agreed. It is likely that the addition of low dose total body irradiation (TBI) to conditioning schedules may be of value. Note that patients with cytogenetic abnormality or evidence of MDS only, without AML, should be treated as above without TBI.

5.6 Post graft monitoring.

5.6.1 Use of Cyclosporin for GVH prophylaxis.

It is conventional to continue Cyclosporin only for between 3-6 months post graft and tail off if there is no GVH. Trough levels should be 150-200 ng/ml.

5.6.2 GVH.

Any GVH should be treated aggressively along conventional lines; because, as noted above, this is a major risk factor for the later development of squamous cell carcinoma (see also [Section 10](#)).

5.6.3 Chimerism.

The post graft management of FA patients needs to include regular assessment of donor chimerism. There are instances recorded of the development of MDS and AML in residual host tissue and some cautious recommendations are given for the reversal of mixed chimerism if this is present at a sustained and significant level post graft⁴³. It is recommended that such monitoring of chimerism be undertaken monthly post graft until 6 months, using either FISH or molecular technologies. Any continued mixed chimerism continuing beyond 12 months post graft at >5% host cells in the absence of GVH should lead to consideration of donor lymphocyte infusions (DLIs) in an attempt to achieve full engraftment. This is a rare event and should be discussed on an individual basis. DLIs, if undertaken, should start using very low lymphocyte numbers in view of the risks associated with GVH in FA patients.

5.6.4 Long term follow-up clinics.

In the longer term, as FA patients who have been transplanted may have a higher risk of endocrine failure and secondary cancers, particular attention should be devoted to the monitoring of these problems in long term follow-up clinics.

Recommendations

- A paediatric haematologist should take overall responsibility for co-ordinating the patient's care in childhood and monitoring for signs of marrow failure or clonal evolution.
- Endocrine, renal and clinical genetics involvement should be sought as appropriate. Where available, referral to a multi-professional clinic is recommended to reduce the number of clinic attendances.
- There should be arrangements in place for transition of care to an adult setting at around 16 years of age.
- A full blood count should be monitored at 3 monthly intervals or more frequently depending on rate of progression of marrow failure.
- Patients and siblings should be HLA typed and, if a matched sibling is unavailable, a preliminary unrelated donor search performed at diagnosis to determine availability of donor so as to reduce the waiting time to transplant when it is indicated. Siblings should be screened for FA prior to using them as donors.
- A bone marrow aspirate and trephine should be considered annually for morphological and karyotypic assessment. Ideally, *i*-FISH should be performed on blood and marrow for detection of cryptic chromosome abnormalities.
- Patients with signs of progressive marrow failure or evidence of transformation to MDS/AML are candidates for therapy.
- HLA matched sibling donor is the treatment of choice in such patients. If a matched sibling is unavailable, patients should be given information about the prevailing risks and outcomes of a 10/10 matched unrelated donor transplant by a transplant specialist to allow them to make an informed choice between transplant and androgen therapy.
- Androgens should be used as a bridge to transplant or to avoid transfusion dependency in patients with progressive marrow failure who do not have an appropriately matched donor.
- Mismatched unrelated donor or mismatched family donor transplants in patients who do not otherwise have an appropriately matched donor is only indicated in MDS/AML or there has been failure or intolerance of androgen therapy.

6. PRE-TRANSPLANT CYTOGENETIC/MOLECULAR CYTOGENETIC FOLLOW-UP

6.1 Clonal aberrations and progression to acute myeloid leukaemia (AML) in FA.

Certain clonal chromosomal aberrations, found in bone marrow and blood cells (granulocytes) of FA patients, are closely associated with evolution to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)^{44, 45}.

Abnormal clones with chromosomal aberrations can evolve over time. The original clone gives rise to a second abnormal clone, and this process can continue until malignancy develops. Typically, more than one clone is necessary for a tumor to develop.

6.2 Chromosomal aberrations in AML in FA and non-FA patients.

Such clonal chromosomal aberrations that characterize FA patients are different from those usually seen in non-FA leukaemia patients. Worrisome clonal chromosomal aberrations in FA are usually 'unbalanced translocations' (i.e., net gain of loss of chromosomal material) as compared to the 'balanced translocations' usually seen in non-FA patients. For example, balanced translocations such as t(8;21), t(9;11), t(11;19), and t(15;17) are commonly associated with AML in non-FA patients and have not been described in FA patients.

6.3 Chromosome 3q gains and/or 7q losses are associated with progression to AML in FA.

A gain of material on chromosome 1 can be transient in FA patients. However, abnormal clones with chromosomes 3q gains and/or 7q losses do not tend to go away but rather expand, sometimes rapidly, leading to MDS and leukemia. A gain of chromosome material on 3 is very common in preleukaemic FA patients; a loss of the long arm or of one entire chromosome 7 is also common but less so than abnormalities of 3. An aberration of chromosome 3q can appear quickly with potential disastrous complications. If possible, these patients should go at once to transplant.

6.4 FISH

Certain simple tests, such as I-FISH (interphase fluorescent *in situ* hybridisation), are necessary to detect a dangerous clone with relevant chromosomal aberrations, e.g. a gain of material of chromosome 3q, being often subtle⁴⁶. A simple cytogenetic analysis will often miss such aberrations⁴⁷.

6.5 Peripheral blood (granulocyte) analysis (as well as on any bone marrow cell sample).

Both bone marrow and peripheral blood can be used to detect abnormal clones. Since it is relatively easy to access blood, it is recommended to perform FISH analyses up to four times a year (e.g. three on blood, one on marrow). Peripheral blood I-FISH analysis would be expected to detect all individuals with chromosome 3q gains or chromosome losses although the percentage of positive cells will be less than for bone marrow specimens.

Recommendations

- **All FA affected individuals who have not undergone a haematopoietic stem cell transplant, including those reverted in the bone marrow, should have two to four times a year depending on blood counts an interphase FISH analyses on peripheral blood cells (granulocytes) as a screen for adverse clonal aberrations, e.g. monosomy 7 and gain of 3q. if changing blood counts, rising or falling, peripheral blood granulocyte I-FISH analysis should be done four times per year. A FISH analysis on metaphase and interphase spreads should also be done in addition to conventional cytogenetics on any bone marrow samples.**

7. ENDOCRINOLOGIC FOLLOW-UP

7.1 Endocrine problems

Endocrine problems are reported in over 80% of FA affected individuals^{48, 49}. They include short stature, hypothyroidism, early or delayed puberty, growth hormone (GH) deficiency, abnormal glucose homeostasis with late insulin release, dyslipidemia and osteopenia or osteoporosis. There is no single unifying cause for all these endocrinopathies.

7.2 Height and GH treatment

Almost half of FA affected individuals are shorter than the general population (average adult height -2.1 standard deviation (SD); or about 150 cm for women and 160 cm for men). Approximately 40% of FA children are born small for gestational age (SGA) (average birth weight -1.8 SD). Up to 80% of these remain short, as opposed to 10% children born SGA in the general population. Although licensed in the UK for promoting growth in the latter group, the benefits of GH treatment in FA are questionable and the unknown long term safety in individuals who have an inherent lifelong cancer risk warrants caution⁵⁰. GH replacement can be justified in the small number of FA patients who have unequivocal GH deficiency with growth failure, low GH levels to stimulation tests, low IGF-I and IGFBP-3 levels, and anatomical abnormalities of the pituitary gland on MRI scan (small gland, interrupted stalk).

7.3 Thyroid function

Primary hypothyroidism occurs more commonly than abnormal central TSH release in FA. Thyroid function tests which suggest borderline or mild primary hypothyroidism (borderline low free thyroxine or borderline high TSH) are found in about 60% of FA individuals. Thyroxine treatment in children with these biochemical features and short stature is likely to improve linear growth⁵¹.

7.4 Insulin secretion, glucose homeostasis and lipid profile

Deficient beta cell function (impaired first-phase insulin secretion after eating but high levels by 60 to 120 minutes) leading to impaired glucose tolerance is a common and inherent feature of FA⁵². Diabetes mellitus also occurs more commonly than in

the general population. These comorbidities can worsen with androgen and corticosteroid treatment. They are also associated with abnormal lipid profiles.

7.5 Weight

About 25% of children with FA have low weight for height. However, about 25-30% are relatively overweight for height and this is similar to recent trends observed in the general population. Of those with diabetes, 75% are overweight or obese. Regular physical activity and healthy dietary intake should be encouraged, avoiding concentrated sweets in order to normalize serum glucose and optimize growth.

7.6 Puberty

Delayed pubertal development (no breast development in a girl by 12 to 13 years and no testes development in a boy by 13 to 14 years) is common in adolescents with FA. It can be due to gonadotrophin deficiency or gonadal failure, either from FA itself or chemotherapy and radiation. Relatively early onset of puberty occurs in some children and can attenuate adult height. Treatment to suppress puberty should be considered if a child starts puberty early (before age 11 years) and at a short height to allow additional time to grow. Androgen treatment in children can result in virilisation. Although it can temporarily enhance linear growth, skeletal advancement can outstrip any gain in height and reduce adult height potential.

7.7 Fertility

Developmental anomalies of the reproductive organs are more frequent in subjects with FA than in the general population and include cryptorchidism, hypospadias and unicornuate or hemi-uterus. Males with FA are often infertile. Females have a significantly abbreviated window of fertility and premature menopause. Fertility will in addition likely be significantly affected following a haematopoietic stem cell transplant. An FA-affected teenager or adult should have the issue of sperm or oocyte banking discussed with him/her if circumstances leading to transplant are appropriate.

7.8 Bone mineral density

Impaired bone mineral density (BMD) has been reported in young people with FA and highlights the importance of regular physical activity and ensuring adequate dietary calcium and vitamin D intake.

Recommendations

- **All FA affected individuals should have specialist endocrine evaluation at diagnosis and regular follow-up thereafter with attention to growth, pubertal development and gonadal function, thyroid status, glucose tolerance, lipid abnormality and bone mineral.**
- **Specialists involved should liaise. Whenever possible endocrine tests should be organised at the same time as haematological investigations.**
- **Until adult height is attained, growth (height, weight and head circumference) and pubertal stage should be monitored at 3 to 6 monthly intervals and bone age (from hand and wrist x-ray) assessed every 1-2 years.**
- **Screening for hypothyroidism with 8 to 9 am serum TSH and free thyroxine should be done every 6 to 12 months. Overt hypothyroidism should be treated promptly. Treatment with thyroxine should also be considered in children with TSH levels ≥ 3 mU/L, low free thyroxine level, and especially if they are short.**
- **Patients with exceptional short stature and/or growth failure should be investigated for thyroid hormone, GH and ACTH-cortisol deficiency. In addition to biochemical investigations, a brain MRI should be obtained with emphasis on the pituitary-hypothalamic area.**
- **GH therapy should be used with extreme caution in FA due to the inherent lifelong cancer risk and reserved for the small number in whom GH deficiency is unequivocally documented. GH treatment should be titrated to keep IGF-I levels in the mid-normal range and IGFBP-3 normal or high. GH therapy should be discontinued immediately if routine haematological examination reveals clonal haematopoietic stem cell proliferation.**
- **If cortisol and ACTH adequacy have not been assessed, stress doses of hydrocortisone should be given before major surgical procedures.**

- **Screening for glucose intolerance and hyperinsulinemia with single post-prandial glucose and insulin levels should be done at diagnosis and at least annually after age 5 years. Glycosylated hemoglobin (HbA1c) and fructosamine levels may be deceptively normal and are of no use in FA individuals prior to bone marrow transplantation. HgbA1c may be more useful after bone marrow transplantation.**
- **A 2-hour oral glucose tolerance test (OGTT) (with glucose and insulin levels every 30 minutes) and fasting lipid profile are indicated in those with high post-prandial glucose and insulin levels and obesity or excessive weight gain. FA patients with normal OGTT response should be reassessed every two years and those with mildly abnormal tests annually.**
- **Bone mineral density studies should be performed about one year after HCT and/ or at about age 14 years. Timing of subsequent studies will be influenced by these results and progress with pubertal development.**
- **An FA-affected teenager or adult should have the issue of sperm or oocyte banking discussed with him/her if circumstances leading to haematopoietic stem cell transplant are appropriate.**

8. RENAL FOLLOW-UP

8.1 Congenital renal & urologic abnormalities in FA.

The extent, spectrum, and significance of renal and urologic abnormalities in FA have not been specifically described. Such abnormalities, in particular renal, are thought to occur in the majority of FA affected individuals. The spectrum of renal abnormalities includes: renal dysplasia, renal ectopia, horseshoe kidney, and absence of the kidney. Undescended testis, hypospadias, absence of vas deferens, and hypogonadism are also reported in male FA affected individuals.

8.2 Other renal/urologic issues in FA.

8.2.1 Urinary tract infection/vesico-ureteric reflux.

Vesico-ureteric reflux is relatively common in the paediatric population under the age of three years and will thus be found also in FA patients of similar age. Significant infection episodes of any sort appear to result in reduction in bone marrow haematopoietic stem cell capacity with significant drop in blood counts, usually with some recovery, in association with the development of such infections. It is important therefore to minimize both the number and severity of any such infection episodes, including urinary tract infections. There should be a low threshold for both antibiotic treatment and prophylaxis. The FA affected individual's haematologist should always be made aware of such infection episodes in real-time to ensure a short term closer haematologic follow-up.

8.2.2 Renal associated hypertension.

Secondary hypertension may develop as a result of the described renal issues. A case of an FA patient with congenital renal artery stenosis has also been described. Blood pressure control may be required from a very young age. Blood pressure measurement should be performed on every clinic attendance on an indefinite basis.

8.2.3 Haematopoietic stem cell transplantation related complications.

Haemorrhagic cystitis, renal/urinary tract sepsis, and drug related renal impairment/failure can all occur during the process of haematopoietic stem cell transplantation and are managed within the appropriate specialist context during the in-patient hospital stay.

8.2.4 Renal transplantation.

There are literature case reports of renal transplantation in FA following development of dialysis dependence. This becomes a challenging issue in the context of previous or potential haematopoietic stem cell transplantation.

8.2.5 Malignancy.

Renal Wilm's tumour and adrenal neuroblastoma are associated with the FA-D1 complementation group. Individuals with this complementation group should have quarterly screening renal ultrasound examinations until the age of seven years (as per published guidelines concerning Wilm's tumour screening in pre-disposing disorders). Any concern about the possible development of a tumour should be explored further with MR imaging with a positive finding leading to rapid appropriate oncologic referral.

Recommendations

- **Initial history at diagnosis should include details of any unexplained pyrexial illnesses or documented previous urinary tract infections. Blood pressure measurement should be done on each clinic attendance (e.g. at the quarterly haematologic clinic attendance). In the male, a testicular examination should be performed, both to document size and to determine if either testis is undescended. In a male infant, the ventral aspect of the shaft of the penis and adjacent area of the scrotum should be examined for any severe hypospadias. Any examination of the glans/corona for a mild hypospadias has to wait until a child is older and the foreskin is retractable.**
- **Urinalysis should be performed at diagnosis and at least annually thereafter. Renal function blood tests should also be performed at diagnosis but should be continued annually thereafter if bilateral renal abnormality.**
- **An initial renal (abdominal) ultrasound is required at diagnosis looking for any renal abnormality.**
- **An FA affected individual should be referred to a renal specialist if there is a history of urinary tract infection, hypertension is identified on blood pressure measurement, or if a renal abnormality is demonstrated on imaging.**

- **All FA affected individuals of FA-D1 complementation group require a renal ultrasound four times a year until the age of seven years as a screen for Wilm's tumour and adrenal neuroblastoma.**
- **A referral to a urologic surgeon should be made on discovery of an undescended or 'absent' testis or of hypospadias.**
- **The use of X-ray/CT and radioisotope investigations should be minimal and only on the instruction of a renal specialist who is familiar with FA. As much as possible, non-ionising radiation imaging modalities should be used, i.e., ultrasound and MRI.**

9. HEARING, DEVELOPMENTAL AND NEUROLOGIC FOLLOW-UP

9.1 Hearing.

Hearing loss has been reported in 11.3% and ear malformations in 14.9% of FA patients^{53, 54}. Hearing loss is usually mild and of the conductive rather than sensorineural type. Congenital anomalies include malformed tympanic membranes with islands of ectopic calcification and also abnormal ossicle formation. It is important to identify any hearing loss, including mild, as early as possible to ensure appropriate development of communication skills and progress with schooling. In general, mild hearing loss can be adequately managed using a hearing aid or an assistive listening device. More significant conductive hearing losses should be investigated for the purposes of considering tympanoplasty, ossicular reconstruction, or placement of bone-anchored hearing aid (BAHA). All FA affected individuals, irrespective of age, once the diagnosis of FA is established should have an age-appropriate formal audiological assessment with subsequent referral to an ENT surgeon with otological interest if significant hearing loss is identified. Arrangements should be also made for provision of a hearing aid.

9.2 Congenital anomalies.

Involvement of the brain is suggested to occur in about 8% of FA patients. Developmental delay/intellectual impairment may be a problem in a minority of individuals. Hydrocephalus requiring a CSF diversionary procedure may occasionally be seen. There are a number of reports in the literature concerning anomalies of the intracranial circulation in FA, including internal carotid agenesis leading to moya moya. There may also be incidental congenital anomalies demonstrated on MR imaging of uncertain significance, e.g. partial/complete agenesis of corpus callosum. Failure to meet developmental milestones should lead to paediatric neurologist referral. The indications for brain imaging should be as per the normal paediatric population, i.e., depending on clinical presentation. No routine screening is required. The imaging modality of choice is MR.

9.3 Neurologic complications of haematopoietic stem cell transplantation.

Neurologic complications following bone marrow transplantation are well described, are not particularly different in the FA population as compared to those otherwise undergoing transplantation⁵⁵. Such complications include seizures, brain abscess,

and intra-cerebral haemorrhage. Such complications are managed in the standard manner.

9.4 Medulloblastoma & other brain tumours.

FancD1 and FancN FA complementation groups, accounting for about 1% of FA affected individuals, are associated with increased risk of brain tumours, in particular of posterior fossa medulloblastoma. This increased brain tumour risk is not documented in the other more common complementation groups. If an FA affected individual is identified as being either FA-D1 or FA-N, then he/she should be considered to undergo regular screening MRI of brain, e.g. every six months, at least up to the age of five years. A paediatric neuro-oncologist and neurosurgeon should be involved from an early stage even prior to the development of any intracranial disease. Early small lesions and any post-operative tumour remnants or limited metastatic disease (without drop metastases) could be considered for gamma knife stereotactic radiosurgery^Δ. The treatment of childhood medulloblastoma in the context of FA, in particular considering the associated systemic chemotherapy and radiotherapy sensitivity, is challenging. Treatment regimens are not established. Prognosis is potentially poor.

Recommendations

- **All FA affected individuals, irrespective of age, once the diagnosis of FA is established, should have an age-appropriate formal audiological assessment with subsequent referral to an ENT surgeon with otological interest if significant hearing loss is identified. Arrangements should be also made for provision of a hearing aid.**
- **A small minority of FA patients may have neurologic/developmental issues over and above those complementation groups at high risk for medulloblastoma about or those suffering neurologic complications as a result of bone marrow transplantation. The possibility of a treatable hydrocephalus should be considered. The imaging modality of choice in these patients is MR.**
- **Predicting neurologic outcome to FA parents should be on the basis of clinical progress and not on incidental congenital abnormalities picked**

^Δ Please contact Mr J Rowe, Consultant Neurosurgeon, National Centre for Stereotactic Radiosurgery, Royal Hallamshire Hospital, Sheffield, S10 2JF, Tel 0114 2711900/2713572.

up on brain imaging that may have little bearing on ultimate functional status, e.g. partial agenesis of corpus callosum.

- **FA-D1 and FA-N FA affected individuals should be considered for six monthly surveillance cranial MRIs for the development of intracranial brain tumours, in particular medulloblastomas of the posterior fossa. A paediatric neuro-oncologist and neurosurgeon should be involved at an early stage. Gamma knife stereotactic radiosurgery may be a possibility for early small lesions, post-operative tumour remnants, or limited metastatic disease without drop metastases.**

10. SCREENING FOR OROPHARYNGEAL/ANOGENITAL SQUAMOUS CELL CARCINOMAS

10.1 Head & neck squamous cell carcinomas in FA.

While haematological crises pose the greatest challenge for Fanconi Anaemia (FA) individuals in the first decade, thereafter solid tumours and particularly squamous cell carcinoma of the oropharynx, oesophagus, and anogenital region become increasingly important. Anogenital squamous cell carcinomas are separately covered in [Section 11](#). The head and neck squamous cell carcinomas (HNSCC) in FA occur throughout the mucosal surfaces of the head and neck: larynx, hypopharynx, oropharynx (mostly tongue base and tonsil), and oral cavity. The most common of these sites appears to be the oral cavity (mouth) and the tumours are most often located on the tongue and gum. It is noteworthy that most oesophageal SCCs arise in the proximal two-thirds in FA. Many of these cancers are locally advanced and node positive at the time of presentation.

10.2 Risk of head & neck SCCs in FA.

10.2.1 *The rate of rise of risk of SCC in FA is non-linear.*

The hazard of such tumours increases from 0.7% per year by age of 20, to 5.3%/year at the age of 40, and to about 10%/year at the age of 49^{56, 57, 58}.

10.2.2 *Factors specific to FA patients are known to increase risk of head & neck SCCs.*

Bone marrow transplantation in FA significantly increases the hazard posed by squamous cell carcinomas with both an increased age specific risk by 4.4 fold and tumours occurring at a younger age (median age of onset reduced in the order of seven to sixteen years)⁵⁹. Bone marrow transplantation in the general population also significantly increases the risk of subsequent squamous cell carcinomas (see Table 10.1). In FA, when the competing risk of non-SCC death is removed, 50% of patient who receive transplants are projected to develop SCC by age 29, whereas 50% of patients who do not receive transplants are expected to develop SCC by age 45. As well as bone marrow transplantation itself, radiation, acute graft versus host disease, and chronic graft versus host disease all significantly increase the risk of SCC. For example, 96% of patients who develop severe acute graft versus host disease die or develop an SCC within six years of transplant. On average, 44% of FA patients post-bone marrow transplant are alive and free of SCC for a decade or

longer after transplant. This probability increases to 60% for those patients who have an HLA-identical sibling donor.

| | Cumulative incidence of head & neck SCC without removal of competing risk of non-SCC death (% at year of patient age) | Median age (years) |
|------------|---|--------------------|
| FA/no BMT | 19% at 45 years | 28 years (13-41) |
| FA/BMT | 24% at 25 years 42% at 30 years | 21 years (11-33) |
| Non-FA/BMT | 13% at 39 years | 36 years |

Table 10.1: Cumulative incidence of head &.neck SCC without removal of competing risk of non-SCC death. Abbreviations: BMT=bone marrow transplant

10.3 Relative hazard of mucosal SCC development compared to non-FA population.

It is difficult not to speculate on why FA individuals have such a marked predilection for the development of mucosal SCC as is so clearly illustrated in this work from Rosenberg *et al* published in 2003⁶⁰. Might there be any additional risk factors other than FA itself or haematopoietic stem cell transplantation that might predispose to the development of such mucosal SCCS?

| Cancer Type | Observed/expected | 95% confidence interval |
|-------------|-------------------|-------------------------|
| Head & neck | 706 | 260-1540 |
| Oesophagus | 2362 | 265-8530 |
| Vulva | 4317 | 870-12615 |
| Cervix | 179 | 20-645 |

Table 10.2: Hazard of mucosal SCCs in FA affected individuals compared to the normal population (Rosenberg *et al*⁶⁰)

10.4 HPV & oropharyngeal SCC.

10.4.1 HPV & Oropharyngeal SCC in the general population.

One additional factor for the development of oropharyngeal in FA patients may be Human Papilloma Virus (HPV) oncogenesis. Oncogenic (cancer inducing) subtypes of HPV have an already established role in the aetiology of squamous cell carcinoma of the cervix, vulva, vagina, penis, and anus, in the general population. The International Agency for Research on Cancer conducted a multicentre case-control study of cancer of the oral cavity and oropharynx in nine countries. The authors in reporting this large study concluded that HPV plays a role in many cancers of the oropharynx (approx 25%) and possibly a small group of cancers of the oral cavity[†]. Subsequent published data is consistent with these findings⁶¹.

10.4.2 HPV as a factor in FA SCC oncogenesis.

The limited evidence for HPV oncogenesis in FA is contradictory. Kutler *et al* examined HNSCC arising in FA and a control group of matched tumours occurring in a general population of HNSCC patients⁶². HPV DNA was found in the tumours of 84% of the FA patients compared with 36% of the controls. In a further publication of 16 FA patients who developed cervical, vulvar, and anal SCCs, 54% had a preceding history of vaginal and/or anal HPV-associated condylomas²⁹. However, a subsequent study in FA patients by van Zeeburg *et al* gave conflicting results with 0/16 HNSCC tumours in FA patient being negative for HPV DNA⁶³. Furthermore, the anatomical distribution of HNSCC in FA is different from that seen with HPV related tumours in a general HNSCC population. Oncogenic human papilloma viruses preferentially affects monolayers of epithelium such as exist in the junctional

[†] Human Papillomavirus and Oral Cancer: The International Agency for Research on Cancer Multicenter Study. Herrero R.; *et al*. [Journal of the National Cancer Institute](#), Volume 95, Number 23, 3 December 2003, pp. 1772-1783. **Abstract:** The IARC conducted a multicenter case-control study of cancer of the oral cavity and oropharynx in nine countries. *Methods:* Recruited 1670 case patients (1415 with cancer of the oral cavity and 255 with cancer of the oropharynx) and 1732 control subjects and obtained an interview, oral exfoliated cells, and blood from all participants and fresh biopsy specimens from case patients. HPV DNA was detected by polymerase chain reaction (PCR). Antibodies against HPV16 L1, E6, and E7 proteins in plasma were detected with enzyme-linked immunosorbent assays. Multivariable models were used for case-control and case-case comparisons. *Results:* HPV DNA was detected in biopsy specimens of 3.9% (95% confidence interval [CI] = 2.5% to 5.3%) of 766 cancers of the oral cavity with valid PCR results and 18.3% (95% CI = 12.0% to 24.7%) of 142 cancers of the oropharynx (oropharynx and tonsil combined) with valid PCR results. HPV DNA in cancer biopsy specimens was detected less frequently among tobacco smokers and paan chewers and more frequently among subjects who reported more than one sexual partner or who practiced oral sex. HPV16 DNA was found in 94.7% of HPV DNA-positive case patients. HPV DNA in exfoliated cells was not associated with cancer risk or with HPV DNA detection in biopsy specimens. Antibodies against HPV16 L1 were associated with risk for cancers of the oral cavity (odds ratio [OR] = 1.5, 95% CI = 1.1 to 2.1) and the oropharynx (OR = 3.5, 95% CI = 2.1 to 5.9). Antibodies against HPV16 E6 or E7 were also associated with risk for cancers of the oral cavity (OR = 2.9).

zone of the cervix and crypt epithelium. Squamous cell carcinomas of the head and neck in FA are distributed throughout the mucosal surfaces of the head and neck with the greatest proportion in the multilayer epithelial surfaces of the mouth. Nevertheless, it is possible that susceptibility to HPV infection and HPV oncogenesis is modified in FA. Of interest is that renal transplant recipients have a significantly higher rate of oral cavity HPV DNA detection than the normal population (18% vs 1%), including cutaneous HPV types, and also a higher rate of oral cancer, presumably related to immunosuppression^{64, 65}.

10.4.3 HPV exposure in children.

HPV exposure has been assumed from the age of onset of sexual activity. However, HPV has been isolated in breast milk, accounts for juvenile laryngeal papillomatosis, is detected in 9.4% of tonsillar hyperplasia specimens in children, and has variously been described as detectable in the normal oropharynx of five year olds in 1.2% to 48.1% of individuals^{66, 67}, with the most common subtype being HPV16.

10.4.4 HPV vaccination is recommended in FA.

Whether vaccination against HPV in childhood is of benefit, and if so at what age, is uncertain. A trial is impractical in an FA population. Female FA individuals are likely subsequently to receive vaccination in any event. Offering vaccination to males as well as females seems reasonable. The appropriate age at which any vaccination should be offered in FA is also unknown. The potential benefit to be derived if the risk of SCC can be reduced seems likely to outweigh any risks involved in vaccination. In addition, response to vaccination can be variable post-transplant. For example, MMR has been shown to be suboptimal and short-lived in bone marrow transplant recipients⁶⁸. Therefore HPV vaccination should be discussed with parents about it being given in the first years of life if any benefit to be derived is to be maximised. The consensus recommendation in this document is to vaccinate all FA-affected individuals from the age of one year. Some consideration should be given from five years following HPV vaccination to HPV antibody testing and the possibility of a booster. Details concerning any requirement for a booster injection are likely to become clearer in the medical literature over coming years in the context of general female population vaccination programmes. See also [Section 12.5](#).

10.5 Dental hygiene, alcohol consumption, and smoking/tobacco exposure.

As well as high risk HPV infection, poor dental hygiene, alcohol consumption and smoking/tobacco exposure are also significant risk factors for the development of HNSCC in the general population^{# 69}. Regular dental check-ups reduce the risk of HNSCC[†]. It would be reasonable to assume that FA patients would have an even higher susceptibility with respect to such risk factors. FA affected individuals should be advised against smoking/tobacco products and should avoid alcoholic beverages and alcohol in other preparations such as some oral mouthwashes. The parents of FA affected individuals should be advised not to expose their children to passive smoking, e.g. if one or both parents are cigarette smokers. FA patients should be encouraged to maintain good dental hygiene and to have regular dental check-ups.

10.6 Screening for oropharyngeal SCCs.

Many reports of HNSCC in FA describe patients as presenting with advanced primary tumours with nodal metastases at presentation. Advanced disease is associated with a reduced survival probability even with optimal treatment in a general HNSCC population. Optimal treatment involves the use of adjuvant radical radiotherapy with concurrent chemotherapy. The potent cross-linking agent cisplatin remains the cornerstone of chemotherapy, and is contraindicated in FA.

Radiotherapy is associated with increased toxicity in FA and the potential for radiation induced second cancers remains a concern as survival is prolonged. The high prevalence, limited therapeutic options, and accessibility of SCC in FA make surveillance an obvious strategy to employ in FA. While squamous cell cancers in FA may develop in individuals as young as 10 the risk is highest as maturity is reached. Therefore more intrusive examinations in FA patients who have not yet had a bone marrow transplant may be deferred until these individuals are able to make an informed decision and cooperate with this. If surveillance is to be optimally effective then it should be carried out by clinicians familiar with head and neck carcinoma and its precursors and who are active core members of a Head & Neck cancer MDT. About two-thirds of head and neck SCC lesions in a general HNSCC population arise in association with pre-cancerous change (dysplasia/ intra-epithelial neoplasia). Field carcinogenesis in FA is not well studied but anecdotal reports

[#] The relative hazard for HNSCC development with high risk HPV infection is 63 (95% CI 14-280), with poor dental hygiene is 5.3 (CI 2.5-11.3), with higher than average alcohol consumption is 2.6 (CI 1.3-5.4), and with smoking is 2.4 (1.3-4.1).

[†] The relative hazard for HNSCC development for those having regular dental check-ups is 0.4 (CI 0.2-0.6).

indicate it to be a problem. Where patient cooperation can be obtained endoscopic evaluation of the mucosal surfaces of the head and neck and oesophagus should be performed. The optimal frequency of evaluation is undetermined. The primary considerations are detection of asymptomatic invasive cancers when they are amenable to surgical treatment alone; also the detection of intra-epithelial neoplasia and monitoring for progression. This author's current protocol is to evaluate patients with pre-cancerous lesions at three monthly intervals. This would seem a reasonable starting point for timing follow-up evaluation in FA.

10.7 Detection of intra-epithelial neoplasia.

It is clear that visual inspection alone provides only limited information about the presence and extent of intra-epithelial neoplasia. Where general anaesthesia is employed this may be supplemented with visualisation with Lugol's Iodine. Autofluorescence and photodynamic diagnosis are experimental techniques which show promise as does brush cytology and are able to be employed in an outpatient setting. These latter methods are suitable for further evaluation both in a general HNSCC population as well as FA.

Recommendations

- **All FA affected individuals from the age of 10 years should have four times a year screening for oropharyngeal cancer.**
- **HPV vaccination should be done on all FA patients from the age of one year as soon as the diagnosis of FA has been established. Note that HPV vaccination is not a substitute for an appropriate cancer screening programme in the FA affected individual.**
- **Screening for oropharyngeal cancer in FA should be done by a head & neck cancer specialist who is a core member of a regional head & neck cancer MDT, who has demonstratable experience in head & neck cancer screening, and who has at hand all available techniques concerning cancer screening including endoscopy (pharyngolaryngoscopy).**
- **FA affected individuals should be advised against alcohol consumption (including in some oral mouth washes) and smoking/tobacco products. Parents of FA individuals should be advised not to expose their children to passive smoking.**

- **FA affected individuals should have six monthly dental check-ups with an NHS dentist or equivalent and should be encouraged to maintain good oral hygiene (tooth-brushing twice daily, dental flossing, use of non-alcohol based mouthwashes).**

11. ANOGENITAL SCCS IN FA

11.1 Female genital tract squamous cell carcinoma.

FA affected female individuals have an increased risk of squamous cell carcinomas of cervix, vagina, and vulva (see **Table 10.2: Hazard of mucosal SCCs in FA affected individuals compared to the normal population** (Rosenberg et al). The earliest age for a genital tract squamous cell carcinoma in a FA female is 14 years (vulvar carcinoma). These cancers are commonly considered 'high-risk' HPV-related, are usually preceded by intraepithelial dysplasia, and may occur on a background of epithelial field change. The common high risk HPV types in the general population are 16 and 18 accounting for 70% of invasive squamous cervical cancer. Other HPV types can be associated with significant morbidity, e.g. HPV 6 and 11 are responsible for about 90% of genital warts. Exposure to HPV is common. Approximately 75% of the UK population contract at least one type of HPV in their genital tract. Most infections are transient with no ill effects. A persistent infection is required for the development of HPV-related neoplasia. In the general population, HPV vaccination significantly reduces the incidence of HPV-associated anogenital diseases in young women, i.e., vulvar, vaginal, and perianal lesions, as well as cervical. Such vaccination has already been shown to significantly reduce the risk of development of cervical cancer. HPV vaccination, already licensed for cervical cancer, has recently (September 2008) been licensed for use against vulval and vaginal cancer by the US Food and Drug Administration. Thus HPV vaccination at the earliest opportunity with subsequent screening for genital tract malignancy should be recommended. A pragmatic recommendation is made that screening should commence at the age of 12. Initially screening should include inspection of the external genitalia and vulvoscopy progressing to colposcopy of vagina and cervix after the onset of sexual activity and anal proctoscopy thereafter (see **Section 11.3**). Screening is recommended on a bi-annual basis. There is evidence in the literature of rapid progression of vulval lesions in FA affected individuals, therefore it should be emphasised that immediate medical help seeking between appointments is appropriate if concerns arise. The screener should be a BS CCP accredited colposcopist and ideally a gynaecological oncologist who is a core member of a gynaecological cancer MDT. If this individual is unfamiliar with ano-proctoscopy techniques an appropriate colorectal surgeon should perform this investigation.

11.2 Anal squamous cell carcinoma in the general population.

Anal squamous cell carcinoma is a relatively rare malignancy in the general population having an estimated annual incidence of 14 per 100,000 in the HIV negative population⁷⁰. Its incidence is increased in the HIV positive population with an estimated annual incidence of 137 per 100,000 with a relative hazard of 4.7 (confidence interval 1.3 to 1.7). It is an HPV-associated malignancy (in particular HPV 16). Smoking and above average alcohol consumption are also risk factors. Anal carcinoma is often preceded by a spectrum of anal intraepithelial dysplasia although the presence of anal intraepithelial neoplasia does not automatically imply the development of invasive carcinoma. It is also seen in other immunosuppressed patient groups, other than HIV, such as renal transplant recipients where the relative risk of anal cancer is 10 (and the prevalence of anal HPV infection is 47% and anal intraepithelial neoplasia is 20% versus 12% and <1% respectively for controls)⁷¹. Treatment options include surgery, radiotherapy, and chemotherapy using mitomycin and cisplatin.

11.3 Anal squamous cell carcinoma in FA patients.

It is difficult to determine the extent to which anal carcinoma occurs in the FA population. Anal squamous cell carcinomas are often grouped together with vulvar carcinomas when reported in the literature. There are potentially at least seven reported cases of specifically anal carcinoma in FA patients. There are no cases reported in patients less than the age of twenty years. All cases reported have been in females. In 754 FA affected individuals enrolled in the International Fanconi Anemia Registry from 1982 to 2001, two developed anal carcinoma (and another eight developed vulvar carcinoma)²⁹. Note that in female patients, there are reports implying a field change involving both vulva and peri-anal area. Considering that over 40% of the 725 FA affected individuals involved had died by the age of twenty years from haematological related complications, and that mortality continued in an almost linear manner above the age of twenty, including death from haematologic malignancy and other solid tumours, the two cases of anal carcinoma represent a significantly increased risk for anal carcinoma development as compared to the normal population. An association with HPV is implied. Of 16 FA patients having cervical, vulvar, and anal squamous cell carcinoma, 54% had a preceding history of vaginal and/or anal HPV-associated condylomas²⁹. Therapeutic options are more

limited for FA patients. For example the use of mitomycin and cisplatin would likely be considered contra-indicated. Thus HPV vaccination at the earliest opportunity with subsequent screening for anal carcinoma should be recommended. Screening should be done in females as per outlined in [Section 11.1](#). The issue of screening for anal cancer in FA affected males is much less clear. An arbitrary recommendation for annual anal cancer screening for the FA adult male would seem appropriate with more frequent screening if there was any lesion of concern (including condylomas acuminatum). Any discussion concerning anal cancer screening with an FA affected male should present the pros and cons of screening in the context of the limited information currently available. Note that in renal transplant recipients, anogenital cancers have an age of onset later than all other post-renal transplant malignancies and are twice as frequent in women as men⁷². There is a possibility that as the survival of FA-affected individuals improves, including better management of the oropharyngeal cancer risk, anogenital malignancy in both for FA-affected individuals of both sexes may become a more significant issue.

11.4 Penile squamous cell carcinoma.

Penile squamous cell carcinoma, also an HPV associated malignancy, occurs with an annual incidence of four per million in the population, is seen with an increased frequency in HIV positive men and also men with anal intraepithelial neoplasia, and is often preceded by penile intraepithelial neoplasia^{73, 74}. It has not yet been described in the FA population. Because of its relative rarity in the general population as compared to other squamous cell carcinomas (anus, vulva, vagina, oral cavity, and oropharynx), the possibility of there being an increased risk in the FA population for penile squamous cell carcinoma cannot be excluded. FA adult males should be advised to self-examine. Any changed appearance to the glans, penile or scrotal skin should result in a two week wait referral to a urologic surgeon.

Recommendations

- **All FA affected females from the age of 12 years should have two times a year screening for anogenital squamous cell carcinomas and precursor lesions with frequency of screening increasing on detection of potential pre-malignant lesions. Screening should be inspection of the external genitalia and vulvoscopy in the first instance progressing**

to colposcopy of vagina and cervix and anal proctoscopy after the onset of sexual activity. Self-inspection should be encouraged.

- There should be a discussion with all FA adult males from the age of 18 years about the possibility of annual anal cancer screening including proctoscopy, presenting the pros and cons of such screening in the context of the limited information available concerning anal cancer in FA. Frequency of screening would be increased on detection of potential pre-malignant lesions including warts.
- Although penile cancer has yet to be described in FA, self-examination should be encouraged. Any lesions of concern should precipitate a 'two week wait referral' to a urologist.
- HPV vaccination should be done on all FA patients as soon as the diagnosis of FA has been established, including in children under the age of nine years. Note that HPV vaccination is not a substitute for an appropriate cancer screening programme in the FA affected individual.
- Screening for female anogenital cancers in FA should be done by a gynaecological specialist who is a core member of a regional gynaecological cancer MDT, who has demonstrable experience in gynaecological screening, and who has at hand all available techniques concerning cancer screening including colposcopy and proctoscopy.
- Screening for male anal cancers in FA should be done by a colorectal cancer surgeon who is a core member of a lower gastrointestinal cancer MDT and who has at hand all available techniques concerning cancer screening including proctoscopy.
- FA affected individuals should be advised against alcohol consumption (including in some oral mouth washes) and smoking/tobacco products. Parents of FA individuals should be advised not to expose their children to passive smoking.
- FA affected individuals should be advised on the use of barrier contraception on the basis that FA might make them particularly susceptible to infection and any transforming (dysplasia/malignancy-inducing) effect of HPV.

12. ADDITIONAL VACCINATIONS

12.1 Chicken pox, hepatitis B, and HPV vaccines in FA.

There are four vaccinations, to chicken pox/varicella, hepatitis B, HPV, and to flu/influenza A/B, that should be considered for individuals affected by Fanconi Anaemia (FA) over and above the normal childhood vaccination schedule. The decision concerning the administration and timing of these vaccinations should always be under the supervision of a Fanconi Anaemia specialist, e.g. paediatric haematologist. All such vaccinations have to be repeated, including the normal childhood vaccines, at an appropriate time point following a successful haematopoietic stem cell transplant as decided by the transplanting paediatric haematologist. Note that vaccination against chicken pox or hepatitis B has not been specifically recommended in the FARF Standards of Care Manual as this publication was written in a North American context where all children are routinely vaccinated for chicken pox and hepatitis B as part of their normal childhood vaccination programme, unlike in the UK or Ireland.

12.2 How are these vaccinations given?

All four vaccinations have to be given by injection and are considered safe and effective in normal individuals. All four vaccinations may result in a local injection site area of inflammation with some mild discomfort lasting some days.

12.3 Chicken pox/varicella vaccination.

12.3.1 Vaccine administration.

The chicken pox vaccine is a live vaccine using a weaker strain ('Oka' strain) of the varicella zoster virus that causes chicken pox. It can be given to children from the age of 12 months. For children under the age of thirteen, only one injection is required. For individuals over the age of twelve, two injections are required separated by a six week interval.

12.3.2 Side effects and contraindications.

The chicken pox vaccination, as well as sometimes causing a local injection site area of inflammation, may also result in very mild chicken pox type rash. Infecting a child with an attenuated varicella strain, i.e., the process of varicella vaccination, should not be a concern, as serological evidence of varicella infection in the older child and adult is almost universal. However, because the chicken pox vaccine is a

live vaccine, it should not be given if an individual is potentially immunosuppressed, e.g. on steroid medication. It should also not be given in severe bone marrow failure. There is no requirement for vaccination if serology (not just an apparent past history) confirms previous varicella infection.

12.3.3 Justification for vaccine administration.

The main reason for chicken pox vaccination in FA affected children is that chicken pox infection appears to result in a significant drop in blood counts in at least a third of individuals and in a small minority may result in androgen resistance or the need for haematopoietic stem cell transplantation. Even in the normal population, chicken pox results in a subclinical thrombocytopenia affecting about 30% of individuals infected⁷⁵. In normal children, evidence of prior infection by chicken pox is almost universal by ten years of age⁷⁶. However, about a third of children acquire their exposure to chicken pox between the ages of seven and ten. This is a time at which FA affected children are most likely to have low blood counts and be particularly vulnerable to a chicken pox infection. Chicken pox infection during this period risks significant complication and the possibility of leading to an urgent unplanned bone marrow transplant with a much reduced time window to search for a matched stem cell donor.

12.4 Hepatitis B Vaccination.

12.4.1 Vaccine administration.

The hepatitis B vaccine is not a live vaccine and instead uses a protein from the Hepatitis B virus to stimulate an immune response. Three separate injections are required over a six month period. It can be given from birth onwards.

12.4.2 Justification for vaccine administration.

The main reason for giving the Hepatitis B vaccine is that FA affected individuals will likely require large volumes of blood products, including platelet and red cell transfusions, and will therefore have a small but significant risk of becoming infected with Hepatitis B. Hepatitis B can cause significant liver problems including inflammation of the liver, liver failure, and tumours/cancer of the liver.

12.5 Human Papilloma Virus Vaccination.

12.5.1 Vaccine administration.

The human papilloma virus (HPV) vaccine is not a live vaccine and instead uses a protein from the human papilloma virus to stimulate an immune response. Three separate injections are required over a six month period.

12.5.2 Justification for vaccine administration.

The main reason for giving the HPV vaccine is because HPV is associated with an increased risk of developing a particular cancer type (squamous cell carcinomas) of the mouth/tongue/tonsil and anogenital areas. In the normal population, all squamous cervical cancers in women, and half of mouth/tongue/tonsil, vulval (in women), penile (in men), and anal cancers are caused by/associated with HPV. FA affected individuals are particularly prone to such mouth/tongue/tonsil and anogenital cancers. Although the role of HPV in FA mouth/tongue/tonsil and anogenital cancers is not conclusive, HPV infection is likely to have a contributory role to the development of at least a proportion of these cancers. HPV infection has been traditionally associated with onset of sexual activity. Hence, HPV vaccination was originally only trialled and licensed for girls/women nine years of age and older for the purposes of preventing cervical cancer. HPV vaccination has also shown to significantly reduce vaginal and vulval cancers, including in older women, and has recently been licensed by the US Food & Drug Administration (September 2008) for such use⁷⁷. HPV does appear to occur in younger children independent of any sexual means of transmission (see [Section 10.4.3](#) above). Considering the potential risk/benefit profile, HPV vaccination should be given to all FA affected individuals, as young as possible, in both males and females, ideally shortly after diagnosis, and not just left arbitrarily to when the child reaches nine years of age.

12.5.3 Giving the vaccine off licence to FA children under nine years of age.

When considering the possibility of HPV vaccination to a FA child under nine years of age, discussion with the parents should include that any HPV vaccine administration is off licence and is on the basis of circumstantial evidence only but in a situation where benefits are likely to significantly outweigh any risks.

12.5.4 Recommended preparation.

There are currently two commercial preparations available. Gardasil is a quadrivalent vaccine covering HPV 6, 11, 16 and 18. Cervarix is a bivalent vaccine covering HPV 16 and 18. Although HPV 16 and 18 are the main subtypes seen in cervical cancer, HPV 6 and 11 also have the potential to be cancer causing in the

context of oropharyngeal cancer. In the FA patient group, HPV 6, 11, 16 and 18 have to be all considered as having the potential for contributing to the development of mucosal squamous carcinomas. The quadrivalent preparation Gardasil is therefore recommended in this patient group.

12.5.5 Efficacy, requirements for a booster injection, serological confirmation of antibody response.

The efficacy of the vaccine is considered to be 98% over at least a five year period. There is no current recommendation for context of confirming a post-vaccination antibody response or the requirement/timing of any booster injection.

12.5.6 HPV vaccination and oropharyngeal/anogenital cancer screening.

Note that HPV vaccination is not a substitute for an appropriate cancer screening programme in the FA affected individual. It has not yet been established to what extent HPV contributes to development of oropharyngeal/anogenital squamous cell carcinomas in FA or to what extent HPV vaccination might prevent such cancers. FA affected individuals should have screening examinations every three months from the age of ten years for cancer of the tongue/mouth/tonsil and also anogenital area, by a relevant specialist (e.g. head & neck cancer surgeon for tongue/mouth/tonsil, gynaecologist with oncological interest).

12.6 Flu Vaccination.

The flu vaccine is not a live vaccine and instead uses proteins from the influenza A and B viruses to stimulate an immune response. The vaccine has to be given each year because of the continuous changing nature of the viruses that cause flu. The vaccine is available in the UK from about September onwards. A thioresal (mercury) free preparation is recommended. ***An important reason for giving the flu vaccine is that flu can significantly reduce blood counts***⁷⁸. Further information concerning the flu vaccine can be found at www.immunisation.nhs.uk/files/flu_factsheet.pdf.

Recommendations

- **Varicella vaccination should be done on all FA patients above the age of one year as soon as the diagnosis of FA has been established and who do not have serological evidence of previous varicella infection, who have yet to progress to moderate/severe bone marrow failure and who are not otherwise immunosuppressed.**

- **Hepatitis B (with Hepatitis A) vaccination should be done on all FA patients from birth as soon as the diagnosis of FA has been established.**
- **HPV vaccination should be done on all FA patients as soon as the diagnosis of FA has been established, including in children under the age of nine years. Note that HPV vaccination is not a substitute for an appropriate cancer screening programme in the FA affected individual.**
- **Varicella vaccination should also be done on all FA unaffected siblings under the age of ten years who have not had a history of previous chicken pox infection or whose varicella serology is negative.**
- **Hepatitis B vaccination should also be offered for an FA unaffected sibling who is a tissue match and likely to be a donor to the FA affected proband at some future time interval.**
- **Flu vaccination with a thioresal/mercury free preparation, in the absence of any specific haematologic contra-indication (e.g. peri-haematopoietic stem cell transplant), should be done on all FA patients on an annual basis.**

13. PRE-IMPLANTATION GENETIC DIAGNOSIS/HLA-SELECTION/IVF

13.1 Why might Pre-implantation Genetic Diagnosis (PGD)/HLA-selection/In vitro fertilisation (IVF) be considered for a FA affected family?

Most children affected by Fanconi Anaemia will require a bone marrow transplant at some point, either for bone marrow failure, or following the development of leukaemia. The outcome is best if the (HLA-) matched donor is a brother or sister of the affected child, both in terms of surviving the bone marrow transplant, and also because of the lower risk of graft versus host disease and subsequent chance of head & neck cancer. About 20% of families will be in the position of having a brother or sister who is suitably HLA-matched. The results of bone marrow transplantation in Fanconi Anaemia using an unrelated HLA-matched donor have significantly improved over recent years but overall are not yet as good as from a sibling matched donor. In addition, a proportion of Fanconi Anaemia affected children will neither have a matched sibling donor nor a matched unrelated donor. Bone marrow transplantation in such circumstances is very high risk. Some families may choose to pursue Pre-implantation Genetic Diagnosis/HLA-selection/In vitro fertilisation (PGD/HLA-selection/IVF or 'saviour sibling') as a means of both having a further Fanconi Anaemia unaffected child and a HLA-matched donor for their Fanconi Anaemia affected child^{79, 80}. Successful PGD/HLA-selection/IVF for Fanconi Anaemia was first reported in 2001⁸¹.

13.2 What exactly does PGD/HLA-selection/IVF involve?

The procedure of PGD/HLA-selection/IVF is complex and involves cutting-edge technology. Similar to 'traditional IVF', the father's sperm fertilizes the mother's egg in the 'test-tube' to make an embryo. The embryo is allowed to grow for about three days until it is made up of about eight or more cells. One of these cells is then removed for complex molecular biological analysis for both Fanconi Anaemia and also for HLA-typing. The removal of one of the embryo cells is not thought to be of long-term harm. For each IVF cycle, there may be up to a dozen embryos. Not all of these embryos will survive. For each embryo analysed, there is a three out of sixteen chance it will be both Fanconi Anaemia unaffected and a HLA-match. Further detailed information is provided on providers' websites (see [Section 13.7](#) below).

13.3 PGD/HLA-selection/IVF is a difficult process for the FA affected family.

Some families may struggle with PGD/HLA-selection/IVF as a choice from a religious or philosophical perspective. Those FA affected families embarking on PGD/HLA-selection/IVF may wish to think twice about who they discuss their decision with as some people, even relatives, may have strongly-held negative opinions concerning the process and may as a result be insensitive to the situation the FA affected family find themselves in. Some families will have had the diagnosis of Fanconi Anaemia made too late in terms of fertility of the mother for PGD/HLA-selection/IVF to be an option. Ideally, the mother should be 35 years of age or younger; PGD would not be considered for women 45 years of age and older. Women over the age of 40 years would require assessment by the PGD/IVF provider with respect to ovulatory potential before a commitment could be given to the appropriateness of embarking on PGD/HLA-selection/ IVF. For others, PGD/HLA-selection/IVF may prove to be unaffordable if an application to obtain funding from their local Primary Care Trust has been unsuccessful. PGD/HLA-selection/IVF can be about £7000 per cycle with parents going through at least half a dozen cycles (see Table 14.1 below). The duration of the process is extended. Genetic work-up and HFEA licence approval takes six months before any IVF cycle commences. Typically, parents may go through about two to three cycles per year over a period of two to three years. Duration may be particularly significant if the FA-affected child is already in severe bone marrow failure. The process may be unsuccessful with respect to a 'saviour sibling' although an FA-unaffected child is likely. There is a small risk to the mother as with any type of IVF, e.g. severe ovarian hyperstimulation syndrome (0.5-1% of all IVF cycles), IVF procedural related complications such as internal haemorrhage (1:2500) and pelvic infection (1:500), complications associated with multiple pregnancy, and increased risk of ectopic pregnancy (about twice the normal rate).

The PGD/HLA-selection analysis is also not fool-proof. There is one FA family in the US who went through PGD/HLA-selection/IVF only to have twins both Fanconi Anaemia affected. It is emotionally stressful, but those who do embark on it may feel as if they are doing something positive, both for their Fanconi Anaemia affected child and their family.

13.4 Is PGD/HLA-selection/IVF permitted in the UK?

PGD/HLA-selection/IVF for Fanconi Anaemia is legal in the UK. However, any provider of PGD/HLA-selection/IVF in the UK has to make an application to the Human Embryonic and Fertilisation Authority (www.hfea.gov.uk) for a licence for each family it takes on. This licence application takes up to three months.

13.5 NHS funding for PGD/HLA-selection/IVF?

PGD/HLA-selection/IVF is variably funded within the NHS. A funding application has to be made to the Primary Care Trust (PCT) that covers the area in which the family reside. The funding application should be made by both the haematologist and geneticist together. Some PCTs will fund one to three cycles of PGD/HLA-selection/IVF. Some may refuse funding altogether. There is an Appeals process that parents may choose to engage in if the initial funding application is rejected. An application is currently in process to the National Commissioning Group to have funding for PGD/HLA-typing/IVF in Fanconi Anaemia to be provided centrally.

13.6 Tests required prior to referral for PGD/HLA-typing/IVF

Complementation group analysis must be completed on the FA affected child to identify which FA gene has been affected. Ideally mutation analysis should also be done on the FA affected child and both parents. PGD/HLA-selection/IVF can still be performed if one or both gene mutations are not found as long as the complementation group/specific FA gene has been identified. In addition, if both parents are from a population group in which other genetic disorders are more frequently seen, then both parents should be checked by the clinical geneticist for their carrier status, e.g. cystic fibrosis for Caucasians. Both parents should be tested for Hepatitis B, Hepatitis C, HIV, and syphilis. The mother should be tested for rubella antibodies.

13.7 Accessing PGD/HLA-selection/IVF

13.7.1 UK based providers.

There are currently two healthcare providers in the UK that offer PGD/HLA-selection/IVF for Fanconi Anaemia with both embryo biopsy and the single-cell PGD/HLA-selection analysis done on site. They are CARE Fertility in partnership with Genesis Genetics (see Genesis Genetics Institute below), at Nottingham (see www.carefertilityweb.co.uk/pgd/pgd.shtml) and The Assisted Conception Unit at University College London Hospital (www.conception-acu.com/subpage.cfm?level1Id=3&level2Id=24&level3Id=40). Both are private sector providers but will also accept NHS-funded referrals. The Genesis Genetics laboratory based at Nottingham can also work with other IVF providers around the UK that are local to the FA affected family on request, to minimise disruption to the family.

13.7.2 Providers outside the UK.

The two most experienced providers of PGD/HLA-selection for Fanconi Anaemia in North America are The Genesis Genetics Institute, Detroit, (www.genesisgenetics.org) and The Reproductive Genetics Institute, Chicago (www.reproductivegenetics.com). PGD/HLA-selection/IVF is also available in Paris but requires a referral through the Hôpital St Louis, Paris, France.

13.7.3 Who makes the referral?

The geneticist or haematologist, following complementation group analysis, should make the referral onwards to the appropriate PGD/HLA-selection/IVF provider on the request of the parents.

13.7.4 Who is responsible for making application to the Primary Care Trust for NHS funding?

The geneticist and haematologist should also make a joint application to the family's Primary Care Trust for funding. Such a joint application should specifically include donor type dependent haematopoietic stem cell transplant survival outcomes, relative frequency of graft vs host disease and association with oropharyngeal/anogenital malignancy, as well as the details of the model presented in Section 15.8 concerning chances of success for PGD/HLA-selection/IVF[†].

[†] For example: 'FA is a rare cancer genetic disorder that is characterised by bone marrow failure (median age 10 years), acute myeloid leukaemia (median age 16 years), and increased risk of solid tumours, in particular, of oropharyngeal/anogenital squamous cell carcinomas (SCCs). Haematopoietic stem cell transplantation (HSCT) is required in about 90% of individuals. Peri-HSCT mortality and graft versus host disease (GvsHD) are significantly increased using a donor other than a matched-sibling donor (mortality up to 50%, severe GvsHD up to 70%) as compared to matched

13.8 Statistical Model

Presented below is a summary of a model that details the chances of success for a women in the 30-35 year old age group of having a FA unaffected sibling match. The full model is presented in Appendix B. Two aspects of the probability analysis are presented below that serve to demonstrate the complexity. Firstly, each embryo is an independent event. To illustrate this in a simple way, the chances of an unaffected HLA match are not 3/16 per IVF cycle but 3/16 per successfully biopsied viable embryo, so the probability of an unaffected HLA match per IVF cycle is higher (i.e., each embryo is a separate roll of the dice). Secondly, each IVF cycle for a population of women is 'sampling without replacement'. For example, for a first IVF cycle in 100 women, if 10% of women had a successful pregnancy, then the second IVF cycle would be 10% of 90 women, and the third cycle would be 10% of 80 women. Thus cumulative success for three IVF cycles would be about 29%. These two concepts mean that a small chance per embryo could end up as a reasonable chance over a number of cycles.

| | |
|---|------------------|
| The mean number of embryos biopsied and tested per IVF cycle (1) | 7.6 |
| The percentage of embryos reaching the blastocyst stage (1) | 66% |
| The theoretical chance of an FA unaffected HLA match (unless recombinational events are otherwise significant) | 3/16 (0.1875) |
| The actual estimated chance of an FA unaffected HLA match (2) | 0.15 |
| The probability of a successful clinical pregnancy for a monogenic PGD IVF cycle ending in a blastocyst embryo transfer (1) | 38% |
| The probability of a successful clinical pregnancy for a PGD/HLA-selection IVF cycle ending in a blastocyst embryo transfer (3) | 29% |

Table 13.1: (1) Data provided by CARE Fertility in partnership with Genesis Genetics, based at Nottingham, for women of mean age 30.5+/- 4.2 years undergoing pre-implantation genetic diagnosis. (2) Corrected as per 8th Data Collection of European Society of Human Reproduction & Embryology PGD Consortium (see Appendix B)⁸². (3) As provided by the FARF on behalf of the Reproductive Genetics Institute (Chicago).

sibling donors (mortality <5%, severe GvsHD <5%). Both donor type other than matched sibling and the occurrence of graft versus host disease significantly increase the risk of subsequent oropharyngeal/anogenital cancers. The availability of a matched sibling donor would also lead to earlier transplantation in a situation where the FA affected child is in better general health and before there is a high risk of leukaemia.'

| | Chance of successful pregnancy | Cost of PGD/HLA-typing/IVF | Total cost |
|-------------------------|---------------------------------------|-----------------------------------|-------------------|
| Cost of genetic testing | | £1500 | |
| Cost of PGD work-up | | £1500 | |
| One cycle | 16.2% | £7300 | £10300 |
| Three cycles | 41.1% | £21900 | £24,900 |
| Five cycles | 58.6% | £36500 | £39,500 |
| Eight cycles | 75.6% | £58400 | £61500 |

Table 13.2: Chances of success and costings for PGD/HLA-typing/IVF^A.

13.9 Issues associated with a successful pregnancy

The management of a successful pregnancy should be based on the best health of mother and baby. Umbilical cord blood collection has to be compliant with licensing requirements as laid down by the Human Tissue Authority. The haematologist should make application to the NHS Blood and Transplant (NHSBT) service for a cord blood collection in the event of a successful pregnancy (see www.nhsbt.nhs.uk/). The NHSBT should provide an appropriate staff resource to be available at the time of delivery for the umbilical cord blood collection. Some consideration may have to be given to the circumstances surrounding planning delivery, e.g. maternity hospital access.

Recommendations

- **In all families, where the FA affected individual is a child, does not have an unaffected HLA matched sibling, and maternal age is less than 40 years, parents should be advised about PGD/HLA-selection/IVF ('Saviour Sibling'). Note that mothers aged 40 and above would require assessment by the PGD/IVF providers with respect to ovulatory potential before a commitment could be given to the appropriateness of embarking on PGD/HLA-selection/ IVF. PGD would not be appropriate in women aged 45 years and over.**

^A Note that the costs listed are approximate and were accurate at the time of preparation of this document.

- **If parents opt to explore further the possibility of PGD/HLA-selection/IVF, referral onwards should be made at the earliest opportunity, in particular considering the maternal ‘fertility clock’ and the subsequent duration of PGD molecular work-up and HFEA licence application.**

14. GASTROINTESTINAL, DENTAL, HEPATIC, AND NUTRITIONAL ISSUES IN FA

14.1 Congenital gastrointestinal abnormalities

About 7% of patients with FA have gastrointestinal tract anatomic abnormalities including oesophageal atresia with or without tracheo-oesophageal fistula, duodenal atresia, anal atresia, or ectopic anus. Most of these anomalies are picked up and treated in early infancy often before the diagnosis of FA is made. However, patients with FA may experience subsequent complications of both these anatomic abnormalities and their surgical treatment such as stricture formation, disturbed oesophageal motility, gastro-oesophageal reflux, tracheomalacia, malabsorption, and faecal incontinence. Persistent gastrointestinal symptoms in the FA individual require referral to a paediatric gastroenterologist/paediatric general surgeon.

14.2 Dental health

FA affected individuals may be at increased risk of tooth decay and gum disease (although some of the FA literature reports may more appropriately reflect cultural background)^{83, 84}. FA patients that have had haematopoietic stem cell transplantation are at higher risk than non-transplanted patients⁸⁵. Poor dental hygiene and lack of regular dental follow-up are known independent risk factors for development of oropharyngeal squamous cell cancer in the general population. Periodontal disease may also predispose to the gingival pocket becoming a reservoir for HPV for the oral mucosa. Note that minor congenital dental abnormalities may also be present in FA.

14.3 Hepatic issues

Liver disease in FA is generally a complication of treatment rather than related to the FA directly. Involvement of a paediatric hepatologist is required in the event of concerns over hepatic disease. The main categories of liver disease seen in FA are secondary iron overload associated with repeated red cell transfusions (see Section 5), graft versus host disease post-haematopoietic stem cell transplant (see Sections 6 and 7), and hepatic complications resulting from treatment with androgenic steroids. Hepatic complications resulting from treatment with androgenic steroids are detailed in Section 14.3.1 to 14.3.3 below. Table 14.1, adapted from the FARF

FA Care Guidelines, summarises recommended screening and management of FA patients on androgenic steroids.

14.3.1 Peliosis hepatis.

Peliosis hepatis are cystic dilatations of the hepatic sinusoids that can occur at any time during treatment with androgens⁸⁶. Peliosis hepatis can cause life-threatening haemorrhage. They may regress on androgen withdrawal.

14.3.2 Hepatitic injury.

Androgen therapy may result in cholestatic jaundice or hypertransaminasaemia that usually regresses on withdrawal of such therapy.

14.3.3 Hepatocellular tumours.

FA patients on androgen therapy may develop hepatocellular adenomas and carcinomas^{87, 88}. Such tumours may regress on withdrawal of androgen therapy. Surgical resection or radiofrequency ablation may be necessary, in particular prior to haematopoietic stem cell transplant, considering the haemorrhage risk on a background to thrombocytopenia (liver MRI is specifically indicated in the pre-transplant work-up if there is a history of androgen treatment).

14.4 Nutritional Issues.

Poor appetite and dietary intake is frequent among FA affected individuals. Paradoxically, obesity with hyperlipidaemia on a background of diabetes mellitus may be a challenge later on the course of FA in the later teenage years/early adulthood (see [Section 6](#) for discussion on endocrine issues). Ongoing clinical follow-up of FA patients should include enquiries into diet and gastrointestinal symptoms and should also include weight and height measurement plotted on appropriate growth curves. A dietician assessment is required if there are concerns raised by either FA patient or parent or in the mind of the clinician. It would be good practice, on initial diagnosis, for the FA affected individual/FA family to be seen by a dietician to explore the importance and content of a 'healthy diet' in the context of a cancer predisposing disorder. FA affected individuals should be ensured to have adequate intake of Vitamin D and Calcium. FA patients should specifically avoid consumption of alcohol and caffeine, both of which have been shown to increase chromosomal breakage in FA cell culture models⁸⁹.

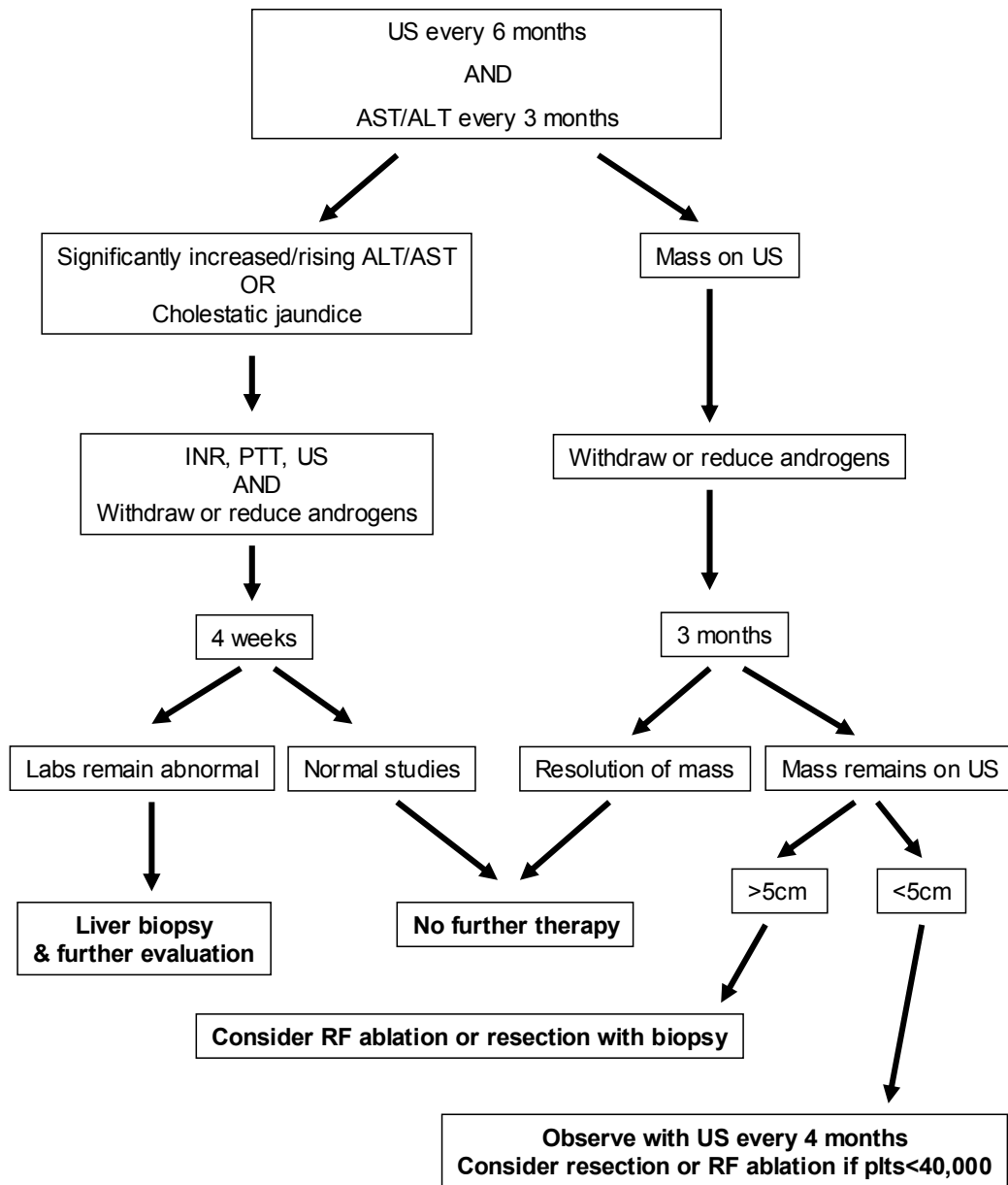


Table 14.1: Management of potential hepatic complications in the FA patient on androgen therapy. Abbreviations are as follows: AST=aspartate transaminase; ALT=alanine transaminase; INR=international normalised ratio; APTT=activated partial thromboplastin time; US=liver ultrasound; RF=radiofrequency; plts=platelets.

14.5 Dietary antioxidants.

14.5.1 FA & oxidative stress

FA is recognised as a disorder in which there is impairment of mechanisms protecting against oxidative injury and evidence of increased oxidative stress^{90, 91}.

Du *et al* describe FA as ‘the only genomic instability syndrome that is uniquely sensitive to oxidative stress’ and provide comprehensive review of oxidative stress in the context of FA haematopoiesis⁹². In addition, it maybe that such increased oxidative stress in FA contributes to activation of cellular stress pathways, in particular the NF-kappaB/TNF alpha cascade contributing to haematopoietic suppression and other systemic features such as poor appetite and exaggerated systemic and haematopoietic responses to viral and bacterial infections and also contributing to leukaemogenesis^{92, 93}. It is this hypothesis that has formed the basis for a recent Etanercept (a TNF alpha antagonist) trial in FA.

14.5.2 Dietary antioxidants.

Dietary antioxidants have been shown in some general situations to improve redox status. Some dietary antioxidants such as rutin/quercetin, alpha lipoic acid, co-enzyme Q10, and resveratrol, are also known to reduce TNF alpha production. Dietary antioxidants are of relatively low cost, do not require a prescription, and are easily obtained, e.g. over the Internet. The risk profile is low and they are well tolerated although they should probably be avoided where an individual is considered at high risk from a bleeding diathesis. There is however only very limited information concerning pharmacokinetics/dosage in the paediatric population. Costs for dietary antioxidants may not be covered by NHS funding and this may discriminate against families on low incomes^Δ.

14.5.3 Is there a role for dietary anti-oxidants in FA?

There has been only limited exploration of the potential role of dietary antioxidants in FA, either in laboratory models or clinical studies. There is some case report-type evidence with also anecdotal support in the FA community (personal communications) to suggest some benefit from the use of rutin (quercetin

^Δ For example, particular rutin and coenzyme Q10 preparations are specifically excluded from NHS funding in England under Statutory Instrument 2004 No 629 NHS (General Medical Services contracts) (Prescription of drugs etc) Regulations 2004. Funding for specific dietary antioxidants may have to go through an individual NHS Trust’s Drugs & Therapeutics Committee with some evidence concerning possible benefit and also that the quality of the agent supplied has been manufactured to pharmacological standards.

rutinoside)⁹⁴. In addition, there have been two reports of parentally administered dismutase providing some benefit in some FA patients^{95, 96}. Alpha lipoic acid (dose 200mg) has been previously administered to children affected by Down's syndrome, a condition in which there is also increased oxidative stress, with significant improvement in redox profile⁹⁷. Furthermore, alpha lipoic acid (dose 200-300mg orally per day) has been used in adult cancer patients in clinical trials with both improvement in redox profile and some symptom response⁹⁸. Considering the otherwise high morbidity/mortality in FA individuals and the circumstantial evidence concerning oxidative stress in FA, the risk benefit scenario would appear to be in favour of encouraging the use of dietary antioxidants among FA affected individuals. Clinicians treating FA individuals should be more than just non-committal about the use of these agents. Taking into account the current extent of medical literature on dietary antioxidants, the use of these agents in the context of FA should not be misrepresented or mistakenly inferred to FA individuals/families as 'complementary medicine'. However, considering the uncertain benefit of dietary antioxidants in FA, any use should ideally be in the context of a clinical study. In the absence of a clinical study, for FA affected individuals/families wishing to pursue dietary antioxidants, it would be appropriate to consider alpha lipoic acid 200mg daily in the first instance. Clinicians are advised to check any relevant new literature reports on dietary antioxidants that may be subsequent to the date of this document.

Recommendations

- **Persistent gastrointestinal symptoms, in particular in the context of previous surgery for a congenital gastrointestinal abnormality, requires referral to a paediatric hepatologist/general surgeon.**
- **FA affected individuals should be advised against alcohol consumption (including in some oral mouth washes) and smoking/tobacco products. Parents of FA individuals should be advised not to expose their children to passive smoking.**
- **FA affected individuals should have six monthly dental check-ups with an NHS dentist or equivalent and should be encouraged to maintain good oral hygiene (tooth-brushing twice daily, dental flossing, use of non-alcohol based mouthwashes).**
- **Involvement of a paediatric hepatologist is required in the event of concerns over hepatic disease (complications secondary to androgen**

therapy including hepatocellular tumours, transfusion associated iron overload, post-haematopoietic stem cell transplant graft versus host disease). Interval liver function blood tests and liver ultrasound should be done every three to six months while on androgen therapy. Liver MRI is specifically indicated in pre-haematopoietic stem cell transplant work up if there has been prior androgen treatment.

- Referral to a dietician is required if there are persistent problems with appetite/weight gain or paradoxically, later in the course of FA, with obesity. FA affected individuals/families should also be considered on initial diagnosis for formal dietetic review concerning the importance and content of a 'healthy diet' considering the cancer predisposing nature of the disorder, including ensuring adequate vitamin D and calcium intake.
- A discussion should be had concerning the use of dietary antioxidants such as alpha lipoic acid and quercetin taking into account the issues presented in Section 16.5.

15. RADIAL RAY AND THUMB ABNORMALITIES

15.1 Fanconi Anaemia and radial ray/thumb abnormalities.

Congenital radial ray and thumb abnormalities are often seen in individuals affected by FA. Such abnormalities may be unilateral or bilateral, mild (e.g. reduced thenar bulk) or severe. There is little information however to what extent FA accounts for such congenital radial ray and thumb abnormalities in the general paediatric population. Other conditions to be also considered before any congenital radial ray and thumb abnormality (unilateral or bilateral, mild or severe) is labeled 'sporadic' includes Duane radial ray syndrome (Okihoru Syndrome), Holt-Oram Syndrome, Trisomy 13/18, and TAR (thrombocytopenia and absent radius) Syndrome⁹⁹.

15.2 Classification of congenital radial ray and thumb abnormalities.

Congenital radial ray and thumb abnormalities associated with FA are classified within the following groups-Radial Ray Deficiency (including radial longitudinal deficiency and thumb hypoplasia) and Thumb Duplication^{100, 101}. These are shown in the three tables below.

| | Radial Dysplasia or Radial Longitudinal Deficiency (Heikel Classification) |
|----------|--|
| Type I | Mild radial shortening of the radius without considerable bowing. Minor radial deviation of the hand is apparent |
| Type II | Miniature radius with distal and proximal physeal abnormalities and moderate deviation of the wrist |
| Type III | Partial absence of the radius (most commonly the distal portion) and severe wrist radial deviation |
| Type IV | Complete absence of the radius with the hand tends having a perpendicular relationship to the forearm |

Table 15.1: Radial Dysplasia or Radial Longitudinal Deficiency (Heikel Classification)

| Hypoplastic thumb (Blauth Classification) | |
|---|--|
| Type I | Small thumb (functions normally) |
| Type II | Small, unstable thumb with adducted contracture of the first web space, lack of thenar muscles and laxity of the ulnar collateral ligament |
| Type III | Skeletal hypoplasia with abnormal carpometacarpal joint, intrinsic muscles absent, rudimentary extrinsic muscles |
| Type IV | 'Pouce flottant' |
| Type V | Total absence |

Table 15.2: Hypoplastic Thumb (Blauth Classification)

| Thumb Duplication (Wassel Classification, based on the radiological level of the duplication) | |
|---|--|
| Type I | Bifid distal phalanx |
| Type II | Duplicated distal phalanx |
| Type III | Bifid proximal phalanx |
| Type IV | Duplication of proximal phalanx resting on broad metacarpal (most common type) |
| Type V | Bifid metacarpal |
| Type VI | Duplicated metacarpal |
| Type VII | Duplication with triphalangeal thumb |

Table 15.3: Thumb Duplication (Wassel Classification, based on the level of the duplication)

15.3 Any congenital radial ray or thumb abnormality identified clinically necessitates a screen for Fanconi Anaemia.

Such a screen for FA should not be delayed and should be done on identification of the congenital radial ray or thumb abnormality (see [Section 3](#) above).

15.4 Referring to a children's hand surgeon.

Where a diagnosis of FA has been established, any child with a congenital radial ray or thumb abnormality requires early referral to a children's hand surgeon. Hand function and aesthetics will be assessed by the specialist children's hand surgeon in conjunction with any necessary radiological tests (radiographic examination is not necessary for referral).

15.5 Consideration of Surgery.

The aim of treatment for radial ray anomalies is to improve upper limb and hand function and appearance. The management strategy varies depending on the severity of the deformity and the associated functional impairment. The timing of surgery is coordinated within the multidisciplinary team. It is dependent on the complexity of the hand deformity, the presence of other anomalies requiring surgery, combined with anaesthetic and technical considerations. The majority of children will have corrective hand surgery between the ages of six months and two years. Significant bone marrow failure does not usually exist at this stage to preclude such surgery. Staged procedures are necessary for those with a combination of radial longitudinal deficiency and thumb hypoplasia/aplasia. Preoperative splinting and manipulation are beneficial. Note that if cortisol and ACTH adequacy have not been assessed, stress doses of hydrocortisone should be given before major surgical procedures preoperatively.

Recommendations

- **Any congenital radial ray or thumb abnormality identified clinically necessitates a screen for FA (unless another underlying predisposing genetic disorder has been confirmed). Such a screen for FA should not be delayed and should be done on identification of the congenital radial ray or thumb abnormality.**
- **In particular, a thumb duplication/polydactyly should not be labeled as 'sporadic' until an FA screen has been negative.**

- **Where a diagnosis of FA has been established, any child with a congenital radial ray or thumb abnormality requires referral to a children's hand surgeon for further evaluation of function and aesthetics (radiographic examination is not required to make the referral).**

POTENTIAL AUDIT PROJECTS

- FA screening has been recommended in this document as a standard for young presentation of squamous cancers of oropharynx and anogenital area. Consideration should be given to establishing a database to include uptake/results so as to subsequently report pick up rates and the detection of *in situ* disease.
- HPV vaccination has been recommended in this document for off licence use in FA affected individuals as young as the age of one year as well as being repeated again at one year post-bone marrow transplant. No specific safety or efficacy data exists for HPV vaccination in FA affected individuals. The UK FA Clinical Network should aim to monitor adverse reactions and also efficacy/longevity of immune response in those FA affected individuals receiving the vaccine.

APPENDIX A: ABBREVIATIONS USED IN THE TEXT

| | |
|---------|--|
| ACTH | Adrenocortical trophic hormone |
| AML | Acute myeloid leukaemia |
| BMD | Bone mineral density |
| BMF | Bone marrow failure |
| BMT | Bone marrow transplant |
| CBT | Chromosomal breakage test |
| CMV | Cytomegalovirus |
| CSF | Cerebrospinal fluid |
| DEB | Diepoxybutane |
| DLIs | Donor lymphocyte infusions |
| ENT | Ear, nose, and throat |
| FA | Fanconi Anaemia |
| FARF | Fanconi Anemia Research Fund |
| FBC | Full blood count |
| FISH | Fluorescent in situ hybridisation |
| GH | Growth hormone |
| GVH | Graft versus host |
| GvsHD | Graft versus host disease |
| HbF | Hemoglobin F (foetal) |
| HLA | Human leucocyte antigen |
| HNSCC | Head & neck squamous cell carcinoma |
| HPV | Human papilloma virus |
| HSCT | Haematopoietic stem cell transplant |
| IGF | Insulin-like growth factor |
| IGFBP | Insulin-like growth factor binding protein |
| IVF | In vitro fertilisation |
| MCV | Mean corpuscular volume |
| MDS | Myelodysplastic syndrome |
| MDT | Multidisciplinary team |
| MMC | Mitomycin C |
| MMR | Mumps measles rubella |
| MR | Magnetic resonance |
| NHS | National Health Service |
| NHSBT | NHS Blood & Transplant Service |
| NICE | National Institute of Clinical Excellence |
| OGTT | Oral glucose tolerance test |
| PB | Peripheral blood |
| PBSC | Peripheral blood stem cells |
| PCT | Primary Care Trust |
| PGD | Pre-implantation genetic diagnosis |
| SBF | Skin biopsy fibroblast |
| SCC | Squamous cell carcinoma |
| SCT | Stem cell transplantation |
| SD | Standard deviation |
| SGA | Small for gestational age |
| TBI | Total body irradiation |
| TNF | Tumour necrosis factor |
| TSH | Thyroid stimulating hormone |
| UKIFAR | UK and Ireland Fanconi Anaemia Registry |
| VACTERL | See Section 3.3.4 |

APPENDIX B: CYTOGENETICS UNITS IN THE UK & IRELAND PROVIDING CHROMOSOMAL BREAKAGE TESTING

Regional Cytogenetics Unit
St Mary's Hospital Manchester
Hathersage Road
Manchester
M13 0JH

Cytogenetics Service
Southmead Hospital
Westbury on Trym
Bristol
BS10 5NB

Nottingham Cytogenetics Service
City Hospital
Hucknall Road
Nottingham
NG5 1PB

Yorkshire Regional Cytogenetics Unit
Ashley Wing
St James's University Hospital
Beckett Street
Leeds
LS9 7TF

Cytogenetics Unit for Wales
Institute of Medical Genetics
University Hospital of Wales
Heath Park
Cardiff
CF14 4XW

North West Thames Regional Cytogenetics Service
NWLH NHS Trust
Level 8V
Kennedy-Galton Centre
Watford Road
Harrow
Middlesex

HA1 3UJ

Institute of Human Genetics
Department of Cytogenetics
International Centre for Life
Central Parkway
Newcastle Upon Tyne
NE1 3BZ

Oxford Regional Genetics Service
Oxford Medical Genetics Laboratories
Oxford Radcliffe Hospitals NHS Trust
The Churchill
Old Road
Headington
Oxford
OX3 7LJ

Guy's and St Thomas' Hospital NHS Trust
Cytogenetics Department
5th Floor
Guy's Tower
Guy's Hospital
London
SE1 9RT

Northern Ireland Regional Cytogenetics Centre
Floor A
Belfast City Hospital Trust
Lisburn Road
Belfast
BT9 7AB

West Midlands Regional Cytogenetics Service
Birmingham Women's Hospital
Edgbaston
Birmingham
B15 2TG

North of Scotland Regional Cytogenetics Service
Department of Medical Genetics
Medical School

Foresterhill
Aberdeen
AB25 2ZD

Sheffield Regional Cytogenetics Service
Sheffield Children's NHS Foundation Trust
Western Bank
Sheffield S10 2TH

APPENDIX C: CANCER EPIDEMIOLOGICAL DATA RELEVANT TO CONSIDERATION OF FA SCREENING (SEE [SECTION 3.3](#))

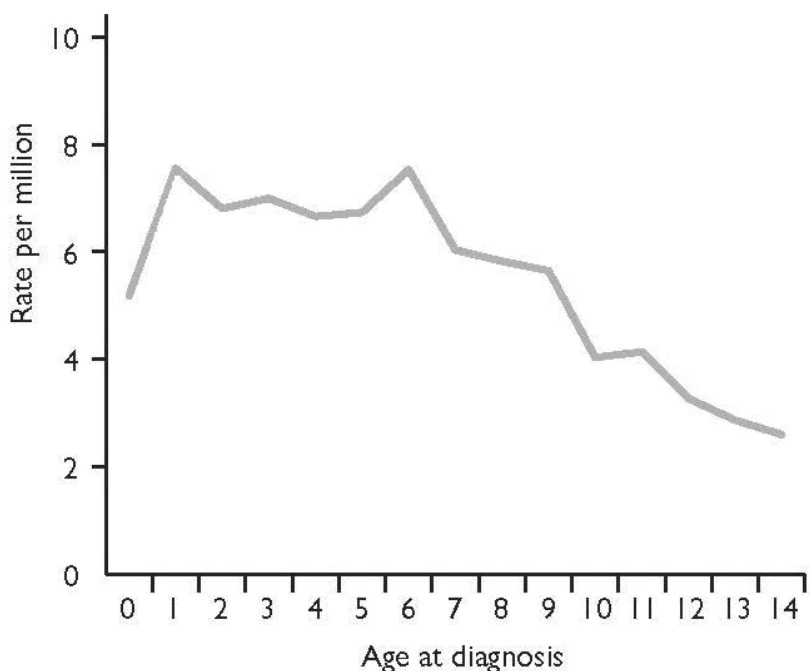


Figure B.1: The age incidence of cerebral PNET/medulloblastoma among children in the UK over the period 1962-1997 (source: Cancer Research UK). Note that the annual birth rate is approximately 600,000.

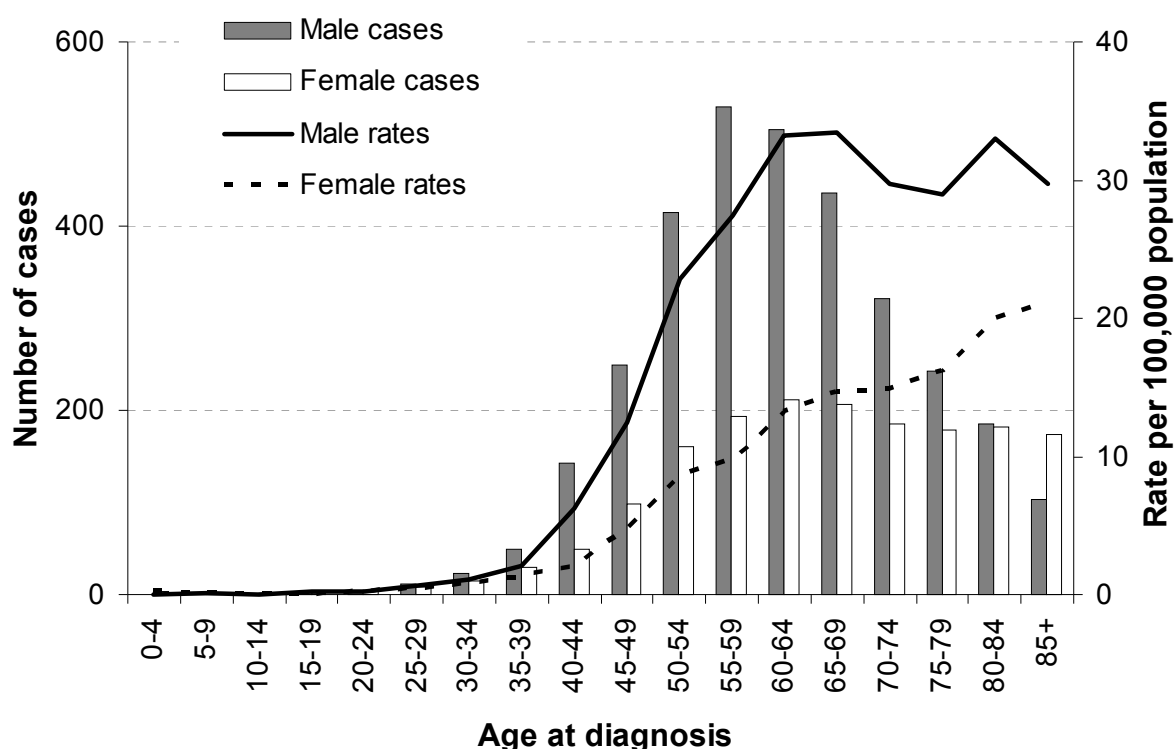


Figure B.2: The number of new cases and age specific incidence rates, by sex, of oral cancer in the UK in 2005 (source: Cancer Research UK).

| Oral Cancer | | |
|-------------|------------|--------------|
| Age | Male cases | Female cases |
| 0-4 | 0 | 3 |
| 5-9 | 1 | 1 |
| 10-14 | 0 | 1 |
| 15-19 | 4 | 1 |
| 20-24 | 4 | 5 |
| 25-29 | 12 | 8 |
| 30-34 | 23 | 15 |
| 35-39 | 49 | 30 |
| 40-44 | 142 | 49 |

Table B.1: The number of new cases of oral cancer in the UK in 2005 by age showing the extent to which such oral cancers occur in age groups under 45 years (source: Cancer Research UK).

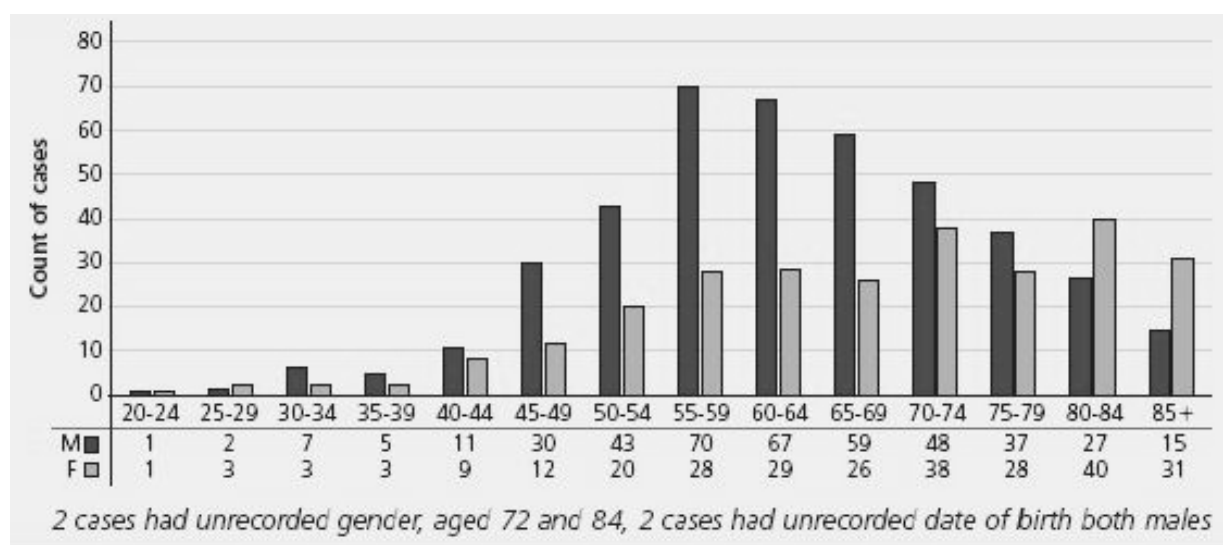


Figure B.3: The number of new cases of oral cancer in England and Wales from October 2005 to November 2006 (source: DAHNO/National Head & Neck Cancer Audit). This data is estimated to cover 48% of oral cancers occurring in England and Wales during the period concerned.

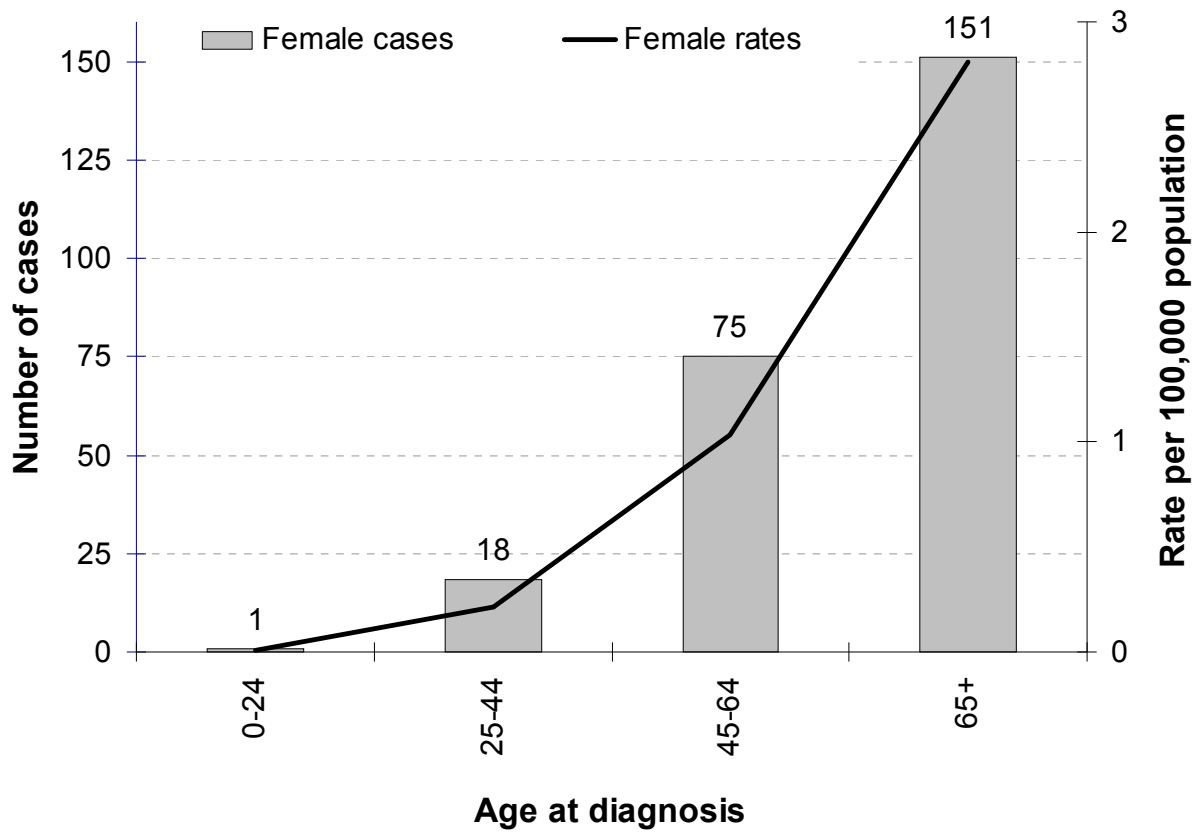


Figure B.4: The number of new cases per year and age specific incidence rates of vaginal cancer in the UK over the period 2001-2005 (source: Cancer Research UK)

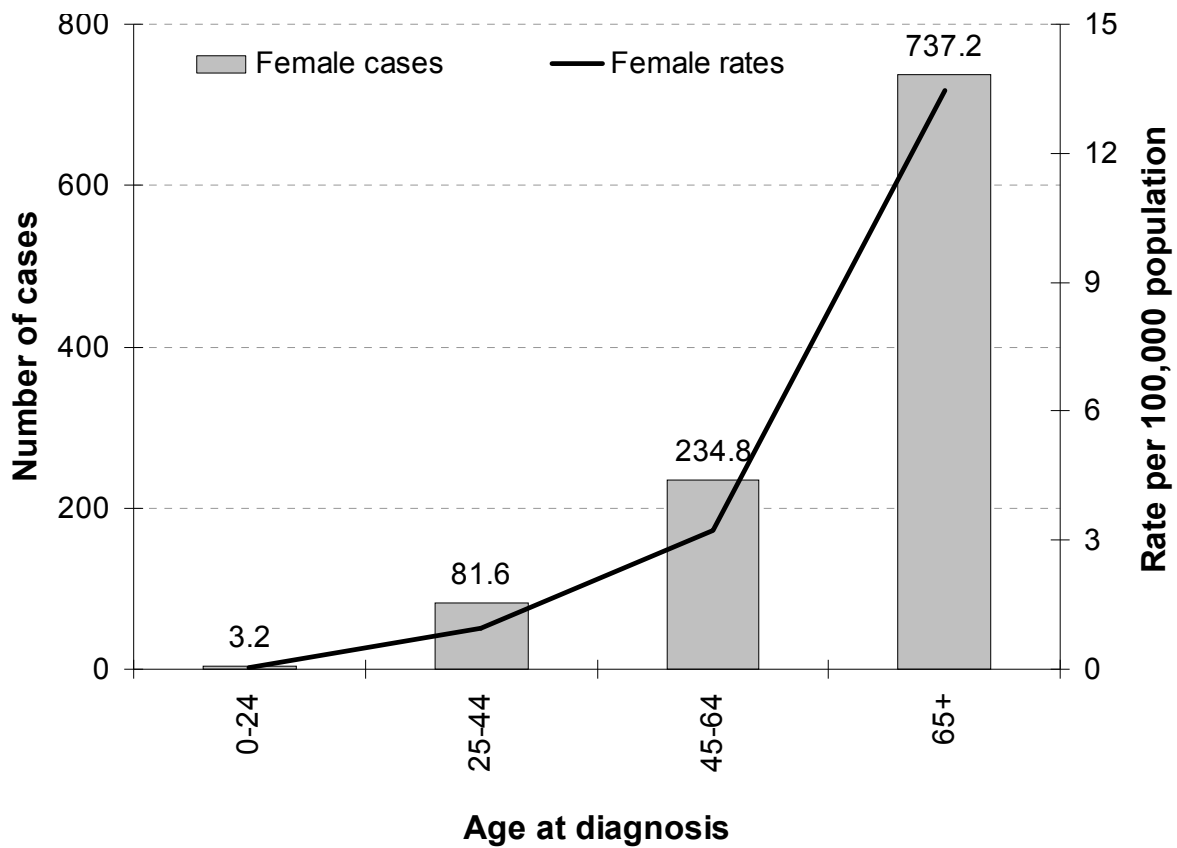


Figure B.5: The number of new cases per year and age specific incidence of vulval cancer in the UK over the period 2001-2005 (source: Cancer Research UK).

APPENDIX D: MATHEMATICAL PROBABILITY MODEL FOR SUCCESS OF PGD/HLA-TYPING/IVF

- The mathematical theory applied here is based on the assumption of independence between events, i.e. that the probability of one embryo being FA unaffected and a tissue match is independent of another embryo being unaffected and a tissue match, also that each cycle is an independent event.
- The probability calculation for subsequent cycles presumes 'sampling without replacement', i.e., a successful pregnancy excludes a couple from participating in subsequent IVF cycles.
- The probability calculation uses the data presented in [Table 13.1](#): (1) Data provided by CARE Fertility in partnership with Genesis Genetics, based at Nottingham, for women of mean age 30.5+/-4.2 years undergoing pre-implantation genetic diagnosis. (2) Corrected as per 8th Data Collection of European Society of Human Reproduction & Embryology PGD Consortium (see Appendix B). (3) As provided by the FARF on behalf of the Reproductive Genetics Institute (Chicago).. Data is provided by CARE Fertility in partnership with Genesis Genetics, based at Nottingham, for women of mean age 30.5+/-4.2 years undergoing pre-implantation genetic diagnosis.
- A correction factor based on the ESHRE PGD Consortium data collection VIII is used to take into account that the actual yield in monogenic PGD is about 80% less than the theoretical yield on the basis of embryos successfully diagnosed⁸². For example, in spinal muscular atrophy, only 547 embryos were transferable out of a total of 867 diagnosed, which falls approximately 20% short of the theoretical 3/4 chance for an autosomal recessive disorder.
- The probability of a successful clinical pregnancy for a PGD/HLA-selection IVF cycle has to be lower than that for a monogenic PGD IVF cycle. The FARF quote results from the Reproductive Genetics Institute (Chicago) on their experience with FA families undergoing PGD/HLA-selection IVF. Twelve families underwent 42 IVF cycles. In total, 24 embryos were transferred. Seven pregnancies have resulted. Six healthy babies have been born (including a set of twins). Two pregnancies resulted in miscarriage. No details were given concerning maternal age. The RGI suggest that the

chances of achieving a successful birth were 16% per IVF cycle and 29% if an embryo was transferred. This is unpublished data presented by the RGI to the FARF FA family meeting at Camp Sunshine in the USA, August 2008. This is the only data that is available concerning PGD/HLA-selection in FA.

Mean number of embryos successfully biopsied and tested = 7.6

Percentage of biopsied embryos reaching blastocyst = 66%

Yielding on average 5.02 embryos surviving to blastocyst stage.

Prob(a given embryo is unaffected by FA and a tissue match) = $3/16 = 0.1875$

With correction, this becomes $0.1875 \times 0.8 = 0.15$

Call this Prob(suitable) = 0.15

Therefore Prob(not suitable) = $1 - 0.15 = 0.85$

Assuming independence between the probabilities that each embryo is suitable:

Prob(at least one suitable in a given cycle) = $1 - \text{Prob}(\text{none suitable in a given cycle})$

$$= 1 - (0.85)^{5.02}$$

$$= 1 - 0.44227$$

$$= 0.55773$$

So, if you assume that each of the 5.02 embryos has an independent probability of 0.15 of being unaffected by FA and a tissue match, then the probability of a cycle resulting in at least one "suitable" embryo is 0.55773 (or around 56%).

If the pregnancy rate is 29% per embryo transfer, then the probability of pregnancy with a "suitable" embryo is simply $(0.55773) \times (0.29) = 0.1617$

The next stage of the calculation can be considered as a geometric distribution, i.e. the probability distribution of the number X of Bernoulli trials needed to get one success.

If the probability of success on each trial is p , then the probability that the k th trial (out of k trials) is the first success is

$$\text{Prob}(X=k) = (1 - p)^{k-1}p \quad \text{for } k = 1, 2, 3 \dots$$

Here, success is a pregnancy and so $p = 0.1617$

And so the probability of a pregnancy on the first cycle is

$$(1 - 0.1617)^0(0.1617) = 0.1617$$

The probability of pregnancy on the second cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^1(0.1617) = 0.1356$$

The probability of pregnancy on the third cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^2(0.1617) = 0.1137$$

The probability of pregnancy on the fourth cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^3(0.1617) = 0.0953$$

The probability of pregnancy on the fifth cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^4(0.1617) = 0.0799$$

The probability of pregnancy on the sixth cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^5(0.1617) = 0.0670$$

The probability of pregnancy on the seventh cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^6(0.1617) = 0.0561$$

The probability of pregnancy on the eighth cycle (having not become pregnant on a previous cycle) is

$$(1 - 0.1617)^7(0.1617) = 0.0470$$

You simply sum these probabilities together to give the cumulative probabilities,

so the cumulative probability of success if one cycle is available is 0.1617

if three cycles are available is 0.4110

if five cycles are available is 0.5861

if eight cycles are available is 0.7562

Applying this to a population of 100 women starting out, on average, one would expect 16 to become pregnant on the first cycle, 41 to have become pregnant by three cycles, 59 women to have become pregnant by five cycles, and 76 women to have become pregnant by eight cycles.

REFEREN

- ¹ Dokal I. Fanconi Anemia is a highly penetrant cancer susceptibility syndrome. *Haematologica* 2008;93:486-488.
- ² Miglierina R, Le Coniat M, Gendron M, Berger R. Diagnosis of Fanconi's anemia by flow cytometry. *Nouv Rev Fr Hematol* 1990;32:391-393.
- ³ Ameziane N, Errami A, Le'veille' F, Fontaine C, de Vries Y, van Spaendonk RML, de Winter JP, Pals G, Joenje H. Genetic subtyping of Fanconi Anaemia by comprehensive mutation screening. *Hum Mut* 2008;29:159-166.
- ⁴ Casado JA, Callén E, Jacome A, Río P, Castilla M, Lobitz S, Ferro T, Muñoz A, Sevilla J, Cantalejo A, E, Cervera J, Sánchez-Calero J, Badell I, Estella J, Dasí A, Olivé T, Ortega JJ, Rodríguez-Villa A, Tapia M, Molinés A, Madero L, Segovia JC, Neveling K, Kalb R, Schindler D, Hanenberg H, Surrallés J, Bueren JA. A comprehensive strategy for the subtyping of patients with Fanconi anaemia: conclusions from the Spanish Fanconi Anemia Research Network. *J Med Genet* 2007;44:241-249.
- ⁵ Dokal I, Chase A, Morgan NV, Coulthard S, Hall G, Mathew CG, Roberts I. Positive diepoxybutane test in only one of two brothers found to be compound heterozygotes for Fanconi's anaemia complementation group C mutations. *Br J Haematol* 1996;93:813-816.
- ⁶ New HV, Cale CM, Tischkowitz M, Jones A, Telfer P, Veys P, D'Andrea A, Mathew CG, Hann I. Nijmegen breakage syndrome diagnosed as Fanconi anaemia. *Pediatr Blood Cancer* 2005;44:494-499.
- ⁷ Rao VB, Kerketta L, Korgaonkar S, Ghosh K. Differentiation of Nijmegen breakage syndrome from Fanconi anemia. *Genet Mol Res* 2007;6:622-626.
- ⁸ Gross M, Hanenberg H, Lobitz S, Friedl R, Herterich S, Dietrich R, Gruhn B, Schindler D, Hoehn H. Reverse mosaicism in Fanconi anemia: natural gene therapy via molecular self-correction. *Cytogenet Genome Res* 2002;98:126-135.
- ⁹ Lo Ten Foe JR, Kwee ML, Rooimans MA, Oostra AB, Veerman AJ, van Weel M, Pauli RM, Shahidi NT, Dokal I, Roberts I, Altay C, Gluckman E, Gibson RA, Mathew CG, Arwert F, Joenje H. Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. *Eur J Hum Genet* 1997;5:137-148.
- ¹⁰ Soulier J, Leblanc T, Larghero J, Dastot H, Shimamura A, Guardiola P, Esperou H, Ferry C, Jubert C, Feugeas JP, Henri A, Toubert A, Socie G, Baruchel A, Sigaux F, D'Andrea AD, Gluckman E. Detection of somatic mosaicism and classification of Fanconi anemia patients by analysis of the FA/BRCA pathway. *Blood* 2005;105:1329-1336.
- ¹¹ Gillio AP, Verlander PC, Batish SD, Giampietro PF, Auerbach AD. Phenotypic consequences of mutations in the Fanconi anemia FAC gene: an International Fanconi Anemia Registry study. *Blood* 1997;90:105-110.
- ¹² Glanz A, Fraser FC. Spectrum of anomalies in Fanconi anaemia. *J Med Genet* 1982;19:412-416.
- ¹³ Faivre L, Portnoi MF, Pals G, Stoppa-Lyonnet D, Le Merrer M, Thauvin-Robinet C, Huet F, Mathew CG, Joenje H, Verloes A, Baumann C. (2005): Should chromosome breakage studies be performed in patients with VACTERL association? *Am J Med Genet* 2005;137:55-58.
- ¹⁴ Yetgin S, Tuncer M, Guler E, Duru F, Ali Kasifolu M. Acute lymphoblastic leukemia in Fanconi's anemia. *Am J Hematol* 1994;45:94.
- ¹⁵ Alter BP, Rosenberg PS, Brody LC. Clinical and molecular features associated with biallelic mutations in FANCD1/BRCA2. *J Med Genet* 2007;44:1-9.
- ¹⁶ Hirsch B, Shimamura A, Moreau L, Baldinger S, Hag-alshiekh M, Bostrom B, Sencer S, D'Andrea A. D. Association of biallelic BRCA2/FANCD1 mutations with spontaneous chromosomal instability and solid tumors of childhood. *Blood* 2004;103:2554-2559.
- ¹⁷ Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, Neveling K, Kelly P, Seal S, Freund M, Wurm M, Batish SD, Lach FP, Yetgin S, Neitzel H, Ariffin H, Tischkowitz M, Mathew CG, Auerbach AD, Rahman N. Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nat Genet* 2007;39:162-164.
- ¹⁸ Scott RH, Stiller CA, Walker L, Rahman N. Syndromes and constitutional chromosomal abnormalities associated with Wilms tumour. *J Med Genet* 2006;43:705-715.
- ¹⁹ Koo WH, Knight LA, Ang PT. Fanconi's anaemia and recurrent squamous cell carcinoma of the oral cavity: a case report. *Ann Acad Med Singapore* 1996;25:289-292.
- ²⁰ Chandra S, Levrán O, Jurickova I, Maas C, Kapur R, Schindler D, Henry R, Milton K, Batish SD, Cancelas JA, Hanenberg H, Auerbach AD, Williams DA. A rapid method for retrovirus-mediated identification of complementation groups in Fanconi anemia patients. *Mol Ther* 2005;12:976-984.
- ²¹ Shimamura A, D'Andrea AD. Subtyping of Fanconi anemia patients: implications for clinical management. *Blood* 2003;102:3459.
- ²² Faivre L, Guardiola P, Lewis C, Dokal I, Ebell W, Zatterale A, Altay C, Poole J, Stones D, Kwee ML, van Weel-Sipman M, Havenga C, Morgan N, de Winter J, Digweed M, Savoia A, Pronk J, de

- Ravel T, Jansen S, Joenje H, Gluckman E, Mathew CG. Association of complementation group and mutation type with clinical outcome in fanconi anemia. European Fanconi Anemia Research Group. *Blood* 2000;96:4064-4070.
- ²³ Berwick M, Satagopan JM, Ben-Porat L, Carlson A, Mah K, Henry R, Diotti R, Milton K., Pujara K, Landers T, Dev Batish S, Morales J, Schindler D, Hanenberg H, Hromas R, Levrano O, Auerbach AD. Genetic heterogeneity among Fanconi anemia heterozygotes and risk of cancer. *Cancer Res* 2007;67:9591-9596.
- ²⁴ Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF, Stratton MR. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* 2007;39:165-167.
- ²⁵ Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, Chagtai T, Jayatilake H, Ahmed M, Spanova K, North B, McGuffog L, Evans DG, Eccles D, Easton DF, Stratton MR, Rahman N. Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat Genet* 2006;38:1239-1241.
- ²⁶ Faivre L, Guardiola P, Lewis C, Dokal I, Ebell W, Zatterale A, Altay C, Poole J, Stones D, Kwee ML, van Weel-Sipman M, Havenga C, Morgan N, de Winter J, Digweed M, Savoia A, Pronk J, de Ravel T, Jansen S, Joenje H, Gluckman E, Mathew CG. Association of complementation group and mutation type with clinical outcome in fanconi anemia. European Fanconi Anemia Research Group. *Blood* 2000;96:4064-4070.
- ²⁷ Adachi D, Oda T, Yagasaki H, Nakasato K, Taniguchi T, D'Andrea AD, Asano S, Yamashita T. Heterogeneous activation of the Fanconi anemia pathway by patient-derived FANCA mutants. *Hum Mol Genet* 2002;11:3125-3134.
- ²⁸ Gillio AP, Verlander PC, Batish SD, Giampietro PF, Auerbach AD. Phenotypic consequences of mutations in the Fanconi anemia FAC gene: an International Fanconi Anemia Registry study. *Blood* 1997;90:105-110
- ²⁹ Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H, Auerbach A. D. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). *Blood* 2003;101:1249-1256.
- ³⁰ Alter BP. Cancer in Fanconi anemia, 1927-2001. *Cancer* 2003;97:425-440
- ³¹ Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. *Blood* 1994;84:1650-1655.
- ³² Rosenberg PS, Huang Y, Alter BP. Individualized risks of first adverse events in patients with Fanconi anemia. *Blood* 2004;104:350-355.
- ³³ Dufour C, Svahn J. Fanconi anaemia: new strategies. *Bone Marrow Transplant* 2008;41 Suppl 2:S90-S95.
- ³⁴ Casado JA, Nunex MI, Segovia JC, Ruiz de Almodovar JM, Bueren JA. Non-homologous end-joining defect in Fanconi anemia hematopoietic cells exposed to ionizing radiation. *Radiat Res* 2005;164:635-641.
- ³⁵ Gluckman E, Rocha V, Ionescu I, Bierings M, Harris RE, Wagner J, Kurtzberg j, Champagne MA, Bonfirm C, Bittencourt M, Darbyshire P, Fernandez MN, Locatelli F, Pasquini R. Results of unrelated cord blood transplant in Fanconi anemia patients: risk factor analysis for engraftment and survival. *Biol Blood Marrow Transplant* 2007;13:1073-1082.
- ³⁶ De Medeiros CR, Bitencourt MA, Zanis-Neto J, Maluf EC, Carvalho DS, Bonfim CS, Funke VM, Setubal DC, Farah N, Pasquini R. Allogeneic hematopoietic stem cell transplantation from an alternative stem cell source in Fanconi anemia patients: analysis of 47 patients from a single institution. *Braz J Med Biol Res.* 2006;39:1297-1304.
- ³⁷ Rossi G, Giorgiani G, Comoli P, Nobili B, Salvaneschi L, de Stefano P, Maccario R, Locatelli F. Successful T-cell-depleted, related haploidentical peripheral blood stem cell transplantation in a patient with Fanconi anaemia using a fludarabine-based preparative regimen without radiation. *Bone Marrow Transplant* 2003;31:437-440.
- ³⁸ Baker JM, Lewis VA, Fernandez CV, Duval M, Crooks BN, Yuille K, Freedman MH, Doyle JJ, Dror Y. Allogeneic hematopoietic stem cell transplantation of patients with FA and high risk features using fludarabine without radiation. *Pediatr Blood Cancer* 2009 (published online 20th January 2009 ahead of print).
- ³⁹ Chaudhury S, Auerbach AD, Kernan NA, Smal TN, Prockop SE, Scaradavou A, Heller G, Wolden S, O'Reilly RJ, Boulad F. Fludarabine-based cytoreductive regimen and T-cell depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anemia. *Br J Haematol* 2008;140:644-655.
- ⁴⁰ Tan PL, Wagner JE, Auerbach AD, Defor RE, Slungaard A, Macmillan ML. Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing

genotypically identical donor hematopoietic cell transplantation. *Pediatr Blood cancer* 2006;46:630-636.

⁴¹ Ayas M, Al Jefri A, Al Seraihi A, Al Mahr M, Rifai S, Al Ahmari A, Khairy A, El Hassan I, El Solh H. Allogeneic stem cell transplantation in Fanconi anemia patients presenting with myelodysplasia and/or clonal abnormality: update on the Saudi experience. *Bone Marrow Transplant* 2008;41:261-265.

⁴² Philpott NJ, Marsh JC, Kumaran TO, Yardumian A, Lawler M, McCann SR, Gordon-Smith EC. Successful bone marrow transplant for Fanconi anaemia in transformation. *Bone Marrow Transplant* 1994;14:151-153.

⁴³ Lawler M, McCann SR, Marsh JC, Ljungman P, Hows J, Vandenberghe E, O'Riordan J, Locasciulli A, Socie G, Kelly A, Schrezenmeier H, Marin P, Tichelli A, Passweg JR, Dickenson A, Ryan J, Bacigalupo A. Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe Aplastic anaemia (SAA): indication for routine assessment of chimerism post SCT for SAA. *Br J Haematol* 2009 (published online 9th January 2009 ahead of print).

⁴⁴ Neitzel H, Kühl JS, Gerlach A, Ebell W, Tönnies H. Clonal chromosomal aberrations in bone marrow cells of Fanconi Anemia patients: results and implications. *Monogr Hum Genet* 2007;15:79-94.

⁴⁵ Tönnies H, Huber S, Kühl JS, Gerlach A, Ebell W, and Neitzel H. Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor. *Blood* 2003;101:3872-3874.

⁴⁶ Tönnies H, Huber S, Volarikova E, Gerlach A, Neitzel H. Interphase FISH assay for the detection of MDS- and AML-associated chromosomal imbalances in native bone marrow and peripheral blood Cells. *Monogr Hum Genet* 2007;15:95-109.

⁴⁷ Meyer S, Fergusson WD, Whetton AD, Moreira-Leite F, Pepper SD, Miller C, EKSaunders, White DJ, Will AM, Eden T, Ikeda H, Ullmann R, Tuerkmen S, Gerlach A, Klopocki E, Tönnies H. Amplification and translocation of 3q26 with overexpression of EVI1 in Fanconi Anemia-derived childhood acute myeloid leukemia with biallelic *FANCD1/BRCA2* Disruption. *Genes, Chromosomes & Cancer* 2007;46:359-372.

⁴⁸ Giri N, Batista DL, Alter BP, Stratakis CA. Endocrine abnormalities in patients with Fanconi anemia. *J Clin Endocrinol Metab* 2007;92:2624-2631.

⁴⁹ Wajrajch MP, Gertner JM, Huma Z, Popovic J, Lin K, Verlander PC, Batish SD, Giampietro PF, Davis JG, New MI, Auerbach AD. Evaluation of growth and hormonal status in patients referred to the International Fanconi Anemia Registry. *Pediatrics* 2001;107:744-754.

⁵⁰ Watanabe S, Mizuno S, Oshima LH, Tsunematsu Y, Fujimoto J, Komiya A. Leukemia and other malignancies among GH users. *J Pediatr Endocrinol* 1993;6:99-108.

⁵¹ Eyal O, Blum S, Mueller R, Smith FO, Rose SR. Improved growth velocity during thyroid hormone therapy in children with Fanconi anemia and borderline thyroid function. *Pediatr Blood Cancer* 2008;51:652-656.

⁵² Elder DA, D'Alessio DA, Eyal O, Mueller R, Smith FO, Kansra AR., Rose SR. Abnormalities in glucose tolerance are common in children with fanconi anemia and associated with impaired insulin secretion. *Pediatr Blood Cancer* 2008;51:256-260.

⁵³ Santos F, Selesnick SH, Glasgold RA. Otolgic manifestations of Fanconi anemia. *Otol Neurotol* 2002;23:873-875.

⁵⁴ Vale MJ, Dinis MJ, Bini-Antunes M, Porto B, Barbot J, Coutinho MB. Audiologic abnormalities of Fanconi anaemia. *Acta Otolaryngol* 2008;128:992-996.

⁵⁵ Uckan D, Cetin M, Yigitkanli I, Tezcan I, Tuncer M, Karasimav D, Oguz KK, Topcu M. (2005): Life-threatening neurological complications after bone marrow transplantation in children. *Bone Marrow Transplant* 2005;35:71-76.

⁵⁶ Alter BP. Fanconi's anemia, transplantation, and cancer. *Pediatr Transplant* 2005;9(Suppl 7):81-6

⁵⁷ Rosenberg PS, Alter, BP, and Ebell W. Cancer risks in Fanconi anemia: findings from the German Fanconi Anemia Registry. *Haematologica* 2008;93:511-517.

⁵⁸ Rosenberg PS, Socie G, Alter BP, Gluckman E. Risk of head and neck squamous cell cancer and death in patients with Fanconi anemia who did and did not receive transplants. *Blood* 2005;105, 67-73.

⁵⁹ Deeg HJ, Socie G, Schoch G, Henry-Amar M, Witherspoon RP, Devergie A, Sullivan KM, Gluckman E, Storb R. Malignancies after marrow transplantation for aplastic anemia and Fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. *Blood* 1996;87:386-92.

⁶⁰ Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood* 2003;101:822-826.

⁶¹ Tran N, Rose BR, O'Brien CJ. Role of human papillomavirus in the etiology of head and neck cancer. *Head Neck* 2007;29:64-70.

- ⁶² Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, Huvos AG, Giampietro P, Levran O, Pujara K, Diotti R, Carlson D, Huryn LA, Auerbach AD, Singh B. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst.* 2003 Nov 19;95(22):1718-21.
- ⁶³ Van Zeeburg HJ, Snijders PJ, Joenje H, Brakenhoff RH. Re: Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst.* 2004;96:968.
- ⁶⁴ Rose B, Wilkins D, Li W, Tran N, Thompson C, Cossart Y, McGeechan K, O'Brien C, Eris J. Human papillomavirus in the oral cavity of patients with and without renal transplantation. *Transplantation* 2006;82:570-3.
- ⁶⁵ Makitie AA, Lundberg M, Salmela K, Kyllonen L, Pukkala E. Head and neck cancer in renal transplant patients in Finland. *Acta Otolaryngol* 2008;128:1255-1258.
- ⁶⁶ Smith EM, Swarnavel S, Ritchie JM, Wang D, Haugen TH, Turek LP. Prevalence of human papillomavirus in the oral cavity/oropharynx in a large population of children and adolescents. *Pediatr Infect Dis J* 2007;26:836-840.
- ⁶⁷ Kojima A, Maeda H, Kurahashi N, Sakagami G, Kubo K, Yoshimoto H, Kameyama Y. Human papillomaviruses in the normal oral cavity of children in Japan. *Oral Oncol.* 2003;39:821-828.
- ⁶⁸ Spoulou V, Giannaki M, Vounatsou M, Bakoula C, Grafakos S. Long-term immunity to measles, mumps and rubella after MMR vaccination among children with bone marrow transplants. *Bone Marrow Transplant* 2004;33:1187-1190.
- ⁶⁹ Rosenquist K. Risk factors in oral and oropharyngeal squamous cell carcinoma; a population-based case-control study in southern Sweden. *Swed Dent J Suppl* 2005;179:1-66.
- ⁷⁰ D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, Jacobson LP. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. *Acquir Immune Defic Syndr* 2008;48:491-499.
- ⁷¹ Patel HS, Silver AR, Northover JM. Anal cancer in renal transplant patients. *Int J Colorectal Dis* 2007;22:1-5.
- ⁷² Penn I. Cancers of the anogenital region in renal transplant recipients. Analysis of 65 cases. *Cancer* 1986;58:611-616.
- ⁷³ Goodman MT, Hernandez BY, Shvetsov YB. Demographic and pathologic differences in the incidence of invasive penile cancer in the United States, 1995-2003. *Cancer Epidemiol Biomarkers Prev* 2007;16:1833-1839.
- ⁷⁴ Kreter A, Brockmeyer NH, Weissenborn SJ, Gambichier T, Stücker M, Altmeyer P, Pfister H, Wieland U. Penile intraepithelial neoplasia is frequent in HIV-positive men with anal dysplasia. *J Invest Dermatol* 2008;128:2316-2324.
- ⁷⁵ Ali N, Anwar M, Majeed I, Tariq WU. Chicken pox associated thrombocytopenia in adults. *J Coll Physicians Surg Pak* 2006;16:270-272.
- ⁷⁶ de Melker H, Berbers G, Hahné S, Rümke H, van den Hof S, de Wit A, Boot H. The epidemiology of varicella and herpes zoster in The Netherlands: implications for varicella zoster virus vaccination. *Vaccine* 2006;24:3946-3952.
- ⁷⁷ Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, Tang GWK, Ferris DG, Steben M, Bryan J, Taddeo FJ, Railkar R, Esser MT, Sings HL, Nelson M, Boslego J, Sattier C, Barr E, Koutsky LA. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *NEJM* 2007;356:1928-1943.
- ⁷⁸ Rice J, Resar LM. Hematologic abnormalities associated with influenza A infection: a report of 3 cases. *Am J Med Sci* 1998;316:401-403.
- ⁷⁹ Bielcorai B, Hughes MR, Auerbach AD, Nagler A, Loewenthal R, Rechavi G, Toren A. Successful umbilical cord blood transplantation for Fanconi anemia using preimplantation genetic diagnosis for HLA-matched donor. *Am J Hematol* 2004;77:397-399.
- ⁸⁰ Grewal SS, Kahn JP, MacMillan ML, Ramsay NK, Wagner JE. (2004): Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood* 2004;103:1147-1151.
- ⁸¹ Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. *Jama* 2001;285:3130-3133.
- ⁸² Goossens V, Harton G, Moutou C, Scriven PN, Traeger-Synodinos J, Sermon K, Harper JC. ESHRE PGD Consortium data collection VIII: cycles from January to December 2005 with pregnancy follow-up to October 2006. *Hum Reprod* 2008;23:2629-2645.
- ⁸³ Acikgoz A, Ozden FO, Fisgin T, Acikgoz G, Duru F, Yarali N, Albayrak D. Oral and dental findings in Fanconi's anemia. *Pediatr Hematol Oncol* 2005;22:531-539.
- ⁸⁴ Tekcicek M, Tavil B, Cakar A, Pinar A, Unal S, Gumruk F. Oral and dental findings in children with Fanconi anemia. *Pediatr Dent* 2007;29:248-52.

-
- ⁸⁵ Yalman N, Sepet E, Aren G., Mete Z, Kulekci G, Anak S. The effect of bone marrow transplantation on systemic and oral health in Fanconi's aplastic anemia. *J Clin Pediatr Dent* 2001;25:329-33.2
- ⁸⁶ Tsigotis P, Sella T, Shapira MY, Bitan M, Bloom A, Kiselgoff D, Levin M, Libster D, Hai AA, Gesundheit B, Or R, Slavin S, Resnick I. Peliosis hepatis following treatment with androgen-steroids in patients with bone marrow failure syndromes. *Haematologica* 2007;92:106-110.
- ⁸⁷ Touraine RL, Bertrand Y, Foray P, Gilly J, Philippe N. Hepatic tumours during androgen therapy in Fanconi anaemia. *Eur J Pediatr* 1993;152:691-693.
- ⁸⁸ Velazquez I, Alter BP. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. *Am J Hematol* 2004;77:257-267.
- ⁸⁹ Pincheira J, Bravo M, Lopez-Saez JF. Fanconi's anemia lymphocytes: effect of caffeine, adenosine and niacinamide during G2 prophase. *Mutat Res* 1988;199:159-65.
- ⁹⁰ Pagano G, Degan P, d'Ischia M, Kelly FJ, Nobili B, Pallardó FV, Youssofian H, Zatterale A. Oxidative stress as a multiple effector in Fanconi anaemia clinical phenotype. *Eur J Haematol*. 2005;75:93-100.
- ⁹¹ Lloret A, Calzone R, Dunster C, Manini P, d'Ischia M, Degan P, Kelly FJ, Pallardó FV, Zatterale A, Pagano G. Different patterns of in vivo pro-oxidant states in a set of cancer- or aging-related genetic diseases. *Free Radic Biol Med* 2008;44:495-503.
- ⁹² Du W, Adam Z, Rani R, Zhang X, Pang Q. Oxidative stress in Fanconi anemia hematopoiesis and disease progression. *Antioxid Redox Signal* 2008;10:1909-1921.
- ⁹³ Li J, Sejas DP, Zhang X, Qiu Y, Nattamai KJ, Rani R., Rathbun KR, Geiger H, Williams DA, Bagby GC, Pang Q. TNF-alpha induces leukemic clonal evolution ex vivo in Fanconi anemia group C murine stem cells. *J Clin Invest* 2007;117:3283-3295.
- ⁹⁴ Pagano G, Korkina LG. Prospects for nutritional interventions in the clinical management of Fanconi anemia. *Cancer Causes Control* 2000;11:881-889.
- ⁹⁵ Izakovic V, Strbakova E, Kaiserova E, Krizan P. Bovine superoxide dismutase in Fanconi anaemia. Therapeutic trial in two patients. *Hum Genet* 1985;70:181-182.
- ⁹⁶ Liu JM, Auerbach AD, Anderson SM, Green SW, Young NS. A trial of recombinant human superoxide dismutase in patients with Fanconi anaemia. *Br J Haematol* 1993;85:406-408.
- ⁹⁷ Gualandri W, Gualandri L, Demartini G, Esposti R, Marthyn R, Volontè S, Stangoni L, Borgonovo M, Fraschini F. Redox balance in patients with Down's syndrome before and after dietary supplementation with α -lipoic acid and L-cysteine. *Int J Clin Pharm Res* 2003;23:23-30.
- ⁹⁸ Mantovani G, Macciò A, Madeddu C, Mura L, Massa E, Gramignano G, Lusso MR, Murgia V, Camboni P, Ferrelli L. Reactive oxygen species, antioxidant mechanisms, and serum cytokine levels in cancer patients: impact of an antioxidant treatment. *J Environ Pathol Toxicol Oncol* 2003;22:17-28.
- ⁹⁹ Cox H, Viljoen D, Versfeld G, Beighton P. Radial ray defects and associated anomalies. *Clin Genet* 1989;35:322-330.
- ¹⁰⁰ Buck-Gramcko D. Congenital malformations of the hand and forearm. Churchill Livingstone 1998.
- ¹⁰¹ Gupta A, Kay SP, Scheker L. The growing hand; diagnosis and management of the upper extremity in children. Mosby 2000.