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Fanconi Anemia

Dutch national guideline: Standards of Clinical Care

Draft: September 4, 2007

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Appendix 1: Diagnostic guidelines for pediatric pancytopenia

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References

The following appendices have been omitted from the English version of this guideline:

 Informed consents: DCOG registration and Fanconi registry

 Task Force Supportive care DCOG

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The DCOG Committee on Fanconi anemia appreciates notification on the use of this guideline by other cooperative groups / centres / countries as well as suggestions to improve this guideline: m.bierings@umcutrecht.nl

Disclaimer

This guideline represents the expert opinions of a group of Dutch doctors involved in the care of Fanconi anemia patients. We do not accept liability for possible errors, every user should check the guideline against national regulations.

1.0 RATIONALE

Fanconi anemia is a rare inherited disease, characterized by the following clinical problems and risks:

1. congenital abnormalities, albeit the absence of anomalies does not exclude Fanconi anemia
2. an increased risk of marrow failure (generally between the ages of 5 and 15)¹
3. an increased risk of hematological malignancies as acute myeloid leukemia (generally between the ages of 5 and 15) and solid tumors (in early adulthood)²⁻⁴
4. other acquired abnormalities, in particular endocrinopathy.⁵

In The Netherlands, currently 30 families with one or more affected members are known.

FA is both clinically and genetically heterogeneous. Until now 13 complementation groups or genetic subtypes have been documented (FA-A, B, C, D1, D2, E, F, G, I, J, L, M, N).⁶⁻¹¹ The genes connected to these subtypes have all been identified.^{7;9;10} For several of these genes mutations have been described that seem to differ in their clinical consequences. All subtypes show autosomal recessive inheritance, with the exception of FA-B which is X-linked.¹² In The Netherlands the 322delG mutatie in the *FANCC* gen is relatively common.¹³ This mutation leads to a relatively mild phenotype.

This is the first edition of the Dutch guideline on diagnosis and treatment for FA patients. There was sofar no national consensus on transplant-indications and conditioning regimens. Long-term follow up guidelines and registration were not available at a national level.

This guideline aims at implementing a systematic approach to diagnostics, treatment and follow-up for FA patients in The Netherlands. The approach builds on the successes obtained in the past decennia for pediatric cancer, using successive clinical trials and careful registration and analysis of results obtained.

The aim is improved care for these patients, resulting in improved survival and quality of life. Registration of follow-up will be implemented at the Dutch Childhood Oncology Group offices in The Hague. This will improve the knowledge of this disease in the Dutch cohort of patients. We hope and anticipate that this approach will serve as a starting point to participate in international studies on this disease.

2.0 FANCONI ANEMIA: INTRODUCTION AND BACKGROUND

Guido Fanconi, a Swiss pediatrician, was the first to report the disease in 1927 as a clinical entity. He described a family with three affected siblings with congenital anomalies and progressive marrow failure at the age of 4-5 years.

2.1 Fanconi anemia: signs and symptoms

Clinical problems in patients with FA can be categorized in 4 groups:

1. Congenitale anomalies. See Table 2.1 for a Summary. Since congenital anomalies can be variable, from almost complete absence to very distinct the diagnosis is often considered after the patient has presented with trombocytopenia.¹⁴ In patients receiving chemotherapy for leukemia or a solid tumor who experience unexpected severe side-effects (mucositis, prolonged neutropenia) FA should be considered, also in the absence of congenital anomalies.

Tabel 2.1. Congenital anomalies in Fanconi anemia

Organ system	Anomalies
Skeleton	Radial hypoplasia, hypoplastic thumb, dislocated hip, scoliosis, vertebral anomalies, small for age, microcephaly
Kidneys	Ectopic position (pelvic position), horseshoe kidney, mono-kidney
Skin	Hyper- and hypopigmentation, café-au-lait spots
Eyes	Microphthalmia
Genitals	Boys: hypogonadism, hypospadias, cryptorchidism Girls: underdeveloped genitals, uterus anomalies
Brain	Mental retardation (usually mild), hydrocephalus, cysts
Intestines	Anorectal or duodenal atresia, tracheo-oesophageal fistula
Heart	VSD, pulmonary artery stenosis, aortic stenosis, coarctatio
Ears	Hear-difficulties, abnormal ears, narrow external auditory canal

2. Bone marrow failure: Develops characteristically between the age of 5 to 10 years, often starting with thrombocytopenia and/or anemia.¹⁵ It presents as a form of aplastic anemia, thus in all pediatric cases of aplastic anemia FA should be tested for, especially in the presence of congenital anomalies.

3. Increased risk for malignancies:

- Hematological malignancies: in particular an increased risk for myelodysplasia and acute myeloid leukemia (estimated risk: 10-15%, depending on genotype, currently not well documented). The risk increases from the age of 10 years (median age to develop AML: 14 years).^{16;17}
Note: In subtypes D1 and N life expectancy is < 5 years, main causes of death being AML, Wilms tumor and medulloblastoma.
- Liver tumors (both adenomas and hepatomas, in particular in patients on treatment with androgens)
- Solid tumors: in particular ENT malignancies, but also esophageal, anal and in women cervical, vaginal and vulvar. Median age: 25 years. ENT tumors in particular in the mouth (65%). Both graft-versus-host disease (GvHD) and radiotherapy as part of the conditioning for stem cell transplantation further increases the risk.^{2;18;19}

4. Endocrinological abnormalities: hypothyroidism, growth hormone deficiency, diabetes mellitus, insufficient puberty, early menopause, infertility and osteoporosis.²⁰

2.2 FA: diagnostics

A **chromosomal breakage analysis** (figure 1) confirms the diagnosis FA. In rare cases this test can be positive in other disorders, like “Nijmegen breakage syndrome”.²¹

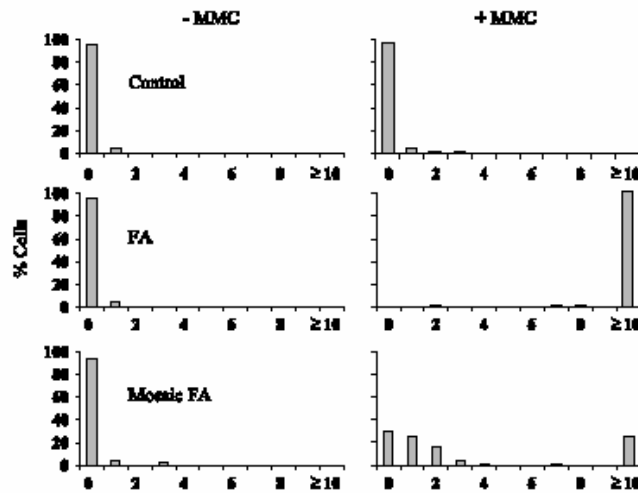
In a chromosome breakage analysis stimulated lymphocytes are exposed to mitomycin C (MMC) or diepoxybutane (DEB). The number of chromosomal aberrations is then quantified and compared to results obtained in a healthy control. If the test proves negative, reverse mosaicism must be considered in case of high clinical suspicion. The test then needs to be repeated using fibroblasts.^{22:23}

In mosaic patients genetic reversion at the disease locus of a hematopoietic stem cell has caused functional correction of the defect often resulting in improved hematopoiesis. If reversion occurs early in life e.g. during fetal development, the “corrected” cells may eventually take over all hematopoiesis, so that a diagnostic test on T lymphocytes may be completely negative. FA can still be diagnosed using skin fibroblasts.

Because the cytogenetic test for FA may be difficult to interpret, it is strongly advised to refer to an experienced diagnostic lab with a track record on FA.

Figure 2.1. Mitomycin C (MMC)-induced chromosomal breakage assay in phytohemagglutinin stimulated T-lymphocyte cultures

The MMC-assay distinguishes between FA patients (middle panel) and a healthy control (upper panel). The lower panel depicts the situation in a mosaic patient: a proportion of the cells (at least 30%) appear normal whereas the remaining cells show increased chromosomal breakage. This situation may require confirmation of the diagnosis in fibroblasts.



Another method to diagnose FA uses **flow cytometry**, to demonstrate spontaneous or MMC-induced G2-arrest, which is characteristic for FA.²⁴

The next step is **DNA diagnosis**. This is of importance since a) the chromosomal breakage assay is not completely specific, b) the mode of inheritance should be established (autosomal recessive versus X-linked), and c) for some subtypes and mutations genotype-phenotype relations have been noted, which may be important for prognosis and follow-up. DNA diagnostics is also important for adequate genetic counseling and prenatal diagnosis. Table 2.2 summarizes the various FA subtypes and genes. Currently well over 95% of all mutations can be detected.

Appendix 3 describes the practical procedure to obtain a diagnosis at the DNA level. The form is also available online: www.dnadiagnostiek.nl [Note: English translation in preparation]

2.3 Genetic heterogeneity in FA.

Genetic subtype	Relative prevalence ^a (%)	Gene symbol	Chromosomal location	Protein References ^b (amino-acids)
A	64	<i>FANCA</i>	16q24.3	1455 1,2
B	2	<i>FANCB</i>	Xp22.31	859 3
C	9	<i>FANCC</i>	9q22.3	558 4
D1	3	<i>FANCD1/BRCA2</i>	13q12.3	3418 5
D2	3	<i>FANCD2</i>	3p25.3	1451 6
E	2	<i>FANCE</i>	6p21.3	536 7
F	2	<i>FANCF</i>	11p15	374 8
G	9	<i>FANCG</i>	9p13	622 9
I	2	<i>FANCI</i>	15q25-26	1328 10-12
J	3	<i>FANCI/BRIP1</i>	17q22-24	1249 13,14
L	<1	<i>FANCL</i>	2p16.1	375 15
M	<1	<i>FANCM</i>	14q21.3	2014 16
N	<1	<i>FANCN/PALB2</i>	16p12.1	1186 17,18

^a Estimated worldwide prevalence, based on 250 FA patients from Europe, Africa, China, India, Australia, U.S.A. and South-America; for all these patients pathogenic mutations were demonstrated in the indicated disease genes. In The Netherlands, so far eight subtypes (A, C t/m I) have been observed, C and A being the most prevalent.

^b References: 1, Lo Ten Foe et al. 1996; 2, FA-breast cancer consortium, 1996; 3, Meetei et al. 2004; 4, Strathdee et al. 1992; 5, Howlett et al. 2002; 6, Timmers et al. 2001; 7, De Winter et al. 2000a; 8, De Winter et al. 2000b; 9, De Winter et al. 1998; 10, Dorsman et al. 2007; 11, Sims et al. 2007; 12, Smogorzewska et al. 2007; 13, Levitus et al. 2005; 14, Levran et al. 2005; 15, Meetei et al. 2003; 16, Meetei et al. 2005; 17, Xia et al. 2007; 18, Reid et al. 2007.

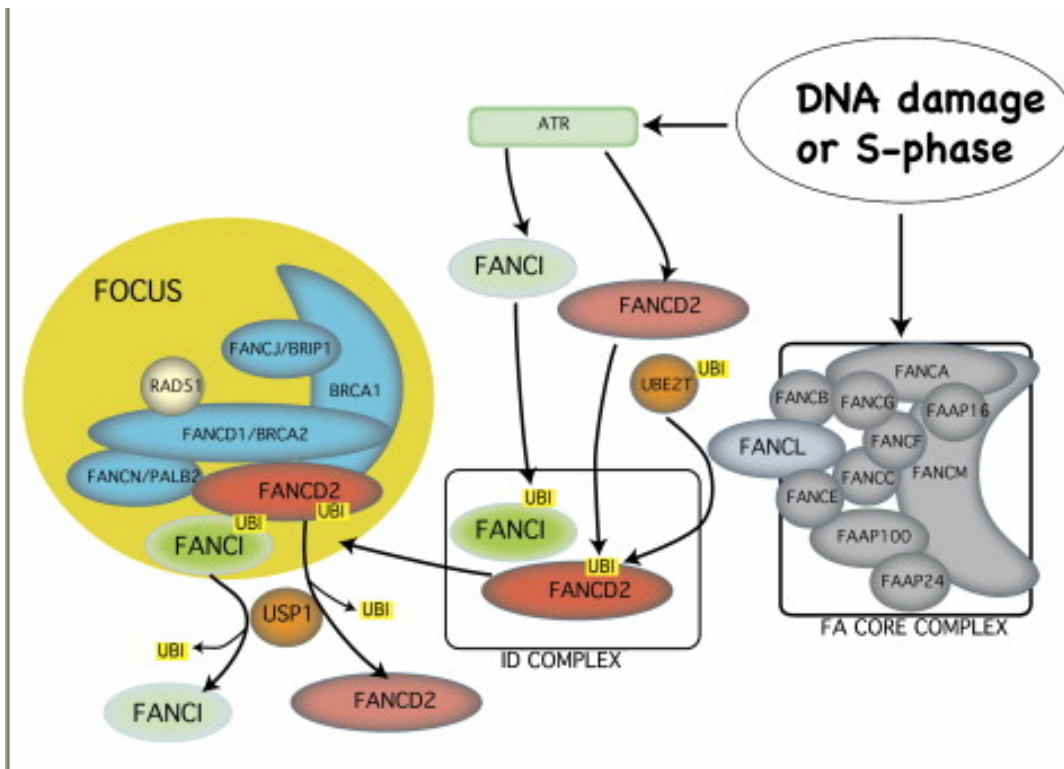
2.4 FA proteins and function

It has become clear that the proteins encoded by the FA genes cooperate in a complex cellular cascade or pathway (figure 2.2). The function of this pathway is to prevent mutations that might result from the specific types of spontaneous or induced DNA damage. FA may therefore be regarded as a DNA repair disease.

The FA pathway may be sub-divided in proteins that are active 'upstream' or 'downstream' of the monoubiquitination event. Defects in the downstream part of the pathway (especially *FANCD1/BRCA2* and *FANCN/PALB2*) seem to have much more severe functional consequences than defects in the upstream part.

Figure 2.2.

The FA proteins collaborate in a pathway that acts to prevent DNA mutations. The central reaction in this pathway is the activation of FANCD2 and FANCI by monoubiquitination (UB). An intact core complex is essential, which is responsible, probably through FANCL, for the transfer of ubiquitin onto FANCD2 and FANCI (Grompe and Van de Vrugt, 2007). FANCD1 (BRCA2) and FANCN (PALB2) play a role 'downstream' of this activation step, as does FANCI/BRIP1. FANCI functions in association with the breast cancer susceptibility gene product BRCA1 (not shown in the figure). Patients with mutations in both alleles of FANCD1 or FANCN have a much more severe prognosis than the other FA subtypes, as these patients most often die before the age of 5 due to AML, medulloblastoma or Wilms tumors. Female carriers of one mutant allele of FANCD1, -N or -J appear to have an increased risk of breast cancer (Walsh and King 2007); for the other FA genes so far no such association has been established (scheme according to Grompe and Van de Vrugt, 2007).

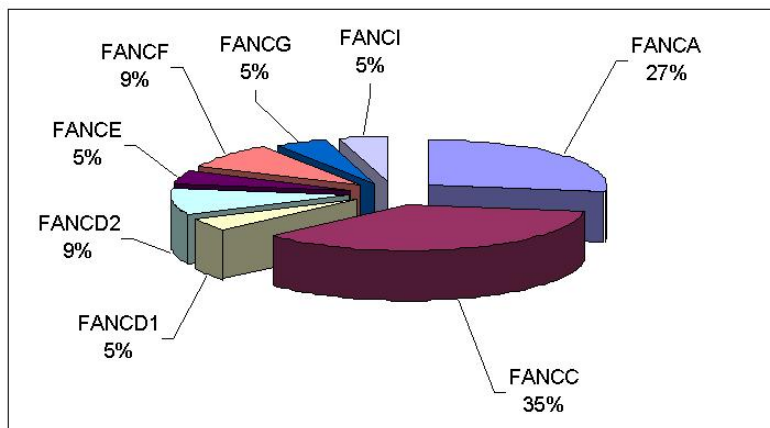


2.4. Genotype-phenotype correlations

There is a clear difference in clinical severity of the disease when comparing the subtypes D1 and N (associated with the downstream part of the FA pathway) with the other subtypes. D1 and N patients develop serious symptoms early in life with a risk for leukemia, solid tumors such as medulloblastoma or Wilms tumor before the age of 5 years.^{29,30}

For the remaining subtypes the nature of the mutations seems to be of more importance than the subtype in its own right. For example, the splice-site mutation IVS4+4A>T mutation in *FANCC*, which is common in people of Ashkenazy Jewish ancestry, causes a very severe clinical phenotype, whereas c.67delG, a frameshift mutation in exon 1 (previously denoted 322delG), the most common mutation in the Netherlands, is associated with a relatively mild phenotype.

Figure 2.3. FA subtypes in the Dutch population (n=22).



The distribution of FA groups in the Netherlands differs from the worldwide distribution. Worldwide *FA-A* has the highest prevalence (\pm 65-70%) whereas in The Netherlands *FA-C* is the most prevalent. The great majority of *FA-C* patients of Dutch ancestry are homozygous for the frameshift mutation c.67delG (previously termed 322delG).

2.5 Stem cell transplantation for marrow failure in FA: background and considerations

Allogeneic stem cell transplantation is the only curative option for aplastic anemia in FA patients and can prevent development of MDS and/or AML. The cumulative incidence for leukaemia is approximately 10% at the age of 25.^{33,34} As mentioned before, in the Netherlands FA type C with a homo- or heterozygous 322delG mutation has a high prevalence. This mutation apparently leads to a mild phenotype, also with regards to hematological problems. However, malignancies have been diagnosed in this group, both AML as well as solid tumors. The need for early allogeneic stem cell transplantation may be limited for this group but so far the registration of follow-up has been rather incomplete.

An allo-HSCT is performed preferably before frequent transfusions are required, reducing toxicity by iron overload and HLA sensitisation. Prolonged aplasia, leading to an immune deficiency will result over time in colonisation with yeasts, fungi and viruses. This partly explains better transplant results in young patients.

There are clearly risks involved in allogeneic stem cell transplantation.^{35,36} FA patients require adapted conditioning regimens to prevent severe toxicity. Graft-versus-host disease (GvHD) is a highly unwanted condition for FA patients. There are indications that GvHD (in a lesser degree radiotherapy as well) can contribute to solid tumors in young adulthood, although such cancers are also seen in patients not having been transplanted.³⁷

Until recently transplant results were highly dependent on the presence of a HLA-identical sibling donor. Using sibling donors survival of 60-70% has been reported. Solid tumors can potentially influence long-term survival.^{38,39} In younger patients 80% survival can be reached.^{40,41} Adapted conditioning regimens generally employ low dose cyclophosphamide.^{42,43} An important question is if radiotherapy can be avoided using fludarabine based regimens. This aims at reducing the risk of developing solid tumors over time.^{44,45} Graft-versus-host disease prevention may prove of even more importance.

Using unrelated donors until recently survival was limited to 20-30%.^{47-49,52} These poor results can be explained by combined toxicities of conditioning, rejection and infection. Fludarabine-based conditioning has improved the short-term prognosis in unrelated stem cell transplantation considerably.^{50,51} Using fludarabine survival was 57% versus 17% in non-fludarabine containing conditioning regimens.⁵² Another recent study found in high-risk FA patients (5 aplastic anemia, 5 MDS, 5 AML patients) 13 of 15 patients surviving, using a fludarabine-based conditioning regimen.⁵¹ In this study three patients suffered a relapse MDS or AML, of whom 1 patient required a second transplant.

There is currently no consensus on the use of T-cell depletion, ATG and radiotherapy. In some centres autologous back-ups are kept in case of unrelated transplantation. This could be of use in case of non-engraftment, although the vitality is causes concerns^{53,56}. Autologous cells may be useful if gene-therapy for FA becomes available.

Cord blood transplants, using sibling donors have been applied, leading to good engraftment. A recent paper on unrelated cord blood transplantation shows results comparable to older results using unrelated transplant, also showing improved survival using fludarabine.⁵⁷

It is technically feasible to select non-affected, HLA-identical embryos for implantation. In vitro fertilisation combined with pre-implantation genetic diagnostics has been done successfully in the US, utilizing sibling cord blood cells to treat a FA patient.⁵⁸

2.6 Progression of marrow failure to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)

Since AML has a poor prognosis in FA patients⁵⁹ there is a need for early detection of imminent leukemia in the absence of clear marrow failure

Various parameters can be potentially useful for this purpose:

1. Morphology. Development of myelodysplastic syndrome (MDS) appears in \pm 7% of Fanconi anemia patients, and is an important predictor of poor prognosis.^{60,61} MDS must be separated from discrete dysplastic features, often found in patients with marrow failure. This phenomenon could explain why not all patients described with “MDS” progressed to overt leukemia.

2. Clonal cytogenetic abnormalities: the most commonly found abnormalities concern chromosome 1q abnormalities, monosomy 7, 5q- and trisomy 8. Especially monosomy 7 is often regarded as ominous for poor prognosis and thus considered a transplant indication. This is not so clear: the presence of clonal abnormalities in the absence of dysplasia is unclear. Not only the appearance but also the disappearance of abnormal clones has been observed. Alter et al. found in 41 patients that those with clonal abnormalities had a worse outcome than those without (5-years survival 40 vs. 94%), but dysplasia proved a better predictor (9 vs 92%) than cytogenetic alterations.^{62,63} Another study found in 20 patients specifically monosomy 7 (n=9) as well as 1q abnormalities (n=4).⁶⁴

3. Additional molecular tests: Recently Tönnies et al. reported their findings on gain of chromosome 3q26q29 in the marrow of Fanconi anemia patients (with a normal peripheral blood karyotype) using FISH.⁶⁵ In 53 patients 25 had a clonal abnormality, of which 18 atri- or tetrasomy of chromosome 3. In 16 patients marrows were tested sequentially, showing an increase of 3q gain over time. In 3 patients a trisomy 3 progressed to tetrasomy of chromosome 3. None of these 3q abnormalities disappeared on follow-up. Eight of these patients also had a monosomy 7 (in 6 patients this developed in the abnormal 3q clone) as a secondary phenomenon. 3q gain appeared significantly associated with the development of AML, MDS and prognosis. This study awaits confirmation in other patient cohorts but seems hopeful to use this screening assay to monitor early signs of development of leukemia.

3.0 AIMS

This guideline aims at implementing uniform standards of care for the diagnosis, treatment and follow-up of FA patients in The Netherlands. This will help in reaching optimal survival as well as quality of life for these patients.

More detailed aims:

1. Retrospective as well as prospective registration of Fanconi anemia patients in The Netherlands, their medical problems as well as their treatment.

This concerns clinical as well as laboratory data such as age, sex, MMC-results, molecular diagnostics, congenital anomalies, hematology results over time, use of androgens, transfusions, stem cell transplantation data, solid tumors, endocrinopathy and causes of death. These data will be recorded in a database at the DCOG office.

2. Uniform diagnostic protocols for FA patients, including DNA diagnostics.

3. Central review of blood and marrow samples at the DCOG reference laboratory. Creating a cell repository for blood and marrow samples of FA patients at the DCOG. This bank aims at future research initiatives in need of samples.

4. Standard of Care guideline for Fanconi anemia in The Netherlands.

5. Long term follow-up of Fanconi anemia patients according to a structured follow-up scheme, to register course of the disease and complications such as malignant disorders.

6. To provide researchers, in The Netherlands and abroad the opportunity to add studies to the clinical guideline. Enabling large international trials to access data on the Dutch cohort of FA patients.

4.0 FANCONI ANEMIA: INITIAL DIAGNOSTICS

A Diagnostics of (pan)cytopenia see Appendix 1.

B Diagnostics for Fanconi anemia

Fanconi anemia must be diagnosed with a chromosomal breakage assay, using stimulated lymphocytes in the presence of mitomycin-C (MMC) or diepoxybutane (DEB). The extent of chromosomal breakage is quantified and compared to that observed in normal lymphocytes. In the case of negative findings and strong clinical suspicion this test should be repeated using (skin biopsy) fibroblasts.

Once the diagnosis has been established molecular subtyping should be done.

Addresses as well as procedures for these diagnostic tests can be found in the appendix.

Tabel 4.1 Tests at diagnosis

Organ system	History and physical findings aiming at:
Growth	Height, weight, head circumference
Skeleton	Dysmorphic features, especially: radius, thumb, vertebral column, hips, ears, external auditory meatus, eyes, external genitals
Skin	café au lait spots, hyper- and/or hypo-pigmentations
ENT	Describe shape of ears, external auditory meatus and check hearing
Heart	Murmurs, signs of heart failure
Intestinal tract	- oesophageal and/or, duodenal atresia, anal atresia - Associated with oesophagus-atresia: GE reflux - Associated with duodenal-atresia: stomach pain, blind-loop syndrome, motility disturbances - Associated with anal atresia: soiling, encopresis
Psycho-motor development	(Mild) Mental retardation in 10% of patients
Renal	21% has congenital anomaly; ± 25% has high blood pressure
CNS / eyes	23% has eye-anomalies; cerebral anomalies
Genitalis	Boys: hypogonadism, puberty Girls: vagina-anomalies, puberty, menstruation
Family history	Girls > 15 yrs: gynaec check-ups: (pre) malignant disorders Consanguinity, other (possibly) affected children ? Stillbirths ? Family history of cancer ?

Radiology

- Skeletal status aiming at inborn anomalies of the skeleton
- Ultrasound of the abdomen (kidneys: form and position, girls: internal genitals)
- Botdensitometry
- X-left hand (skeletal age)
- ECG, heart ultrasound (consultation of the cardiologist)
- If indicated: MRI cerebrum

Comment [S1]: We would argue for not to include I.X ray skeletal status and bone density in the basic work up to reduce radiation exposure.

Laboratory Tests

- Full blood count, reticulocytes, cellular indices, manual-differentiation
- Fetal hemoglobin
- Mitomycine-C or DEB-test
(to be repeated on fibroblasts in case of negative findings and strong clinical suspicion)
- Molecular diagnosis, see Appendix
- Renal function tests (glomerular as well as tubular)
- Liverfunction assays
- Viral status HSV, EBV, VZV, Hep.A-E+, CMV, (in case of transplant or frequent transfusions)
- Endocrinological assays: IGF1, IGF-BP3, fT4 and TSH, LH, FSH, testosterone or oestradiol (dependent on sexe), PTH and vitamin D
- Glucose, if indicated HbA1c (unreliable if transfused)
- Iron status

Comment [S2]: We would argue for
1.Flow cytometry cell cyclus G2 arrest
2.MMC induced chromosomal breakage
Tests can be performed alternatively or sequentially

Comment [S3]: Two options:
1. First: Complementation group,
Second: mutation analysis
2. Mutation analysis
Which option to recommend is dependant on agreements with respective laboratories//costs// insurance coverage

Comment [S4]: Include Parvovirus B 19, HIV

Comment [S5]: We would recommend to leave out HbA1c

Comment [S6]: We would be more precise here: Serum ferritin

Consultations

- Cardiologist: aimed at excluding congenital heart anomalies
- Audiologist: evaluation of hearing
- Ophthalmologist: microphthalmia, strabismus, visus
- Dental care
- Orthopedics/plastic surgery for potential correction of the radius and/or other skeletal deformities
- >15 years: 3-monthly ENT screening for solid tumors
- > 15 years: for girls / women: yearly gynaecologist: inspection of the vulva/vagina,cervixcytology, menstruationpatron, early menopause. Possibly hormonal substitution, obstetric counseling and advice.
- In case of child-wish: perinatology counseling.
- In case of pregnancy: perinatology care.
- Clinical genetics (counseling, antenatal screening, etc.): if wish for children

Comment [S7]: Age dependant recommendation
10-15 years Annual ENT consultation/ screening and in case of any clinical suspicion
>15 years Biannual ENT consultation and in case of clinical suspicion
(needs to be determined in detail)

In case of marrow failure:

- Bone marrow: morphology, karyotyping, FISH for chromosome 3 and monosomy 7, Send material to the DCOG reference lab.Note: FISH chromosome 3 diagnostics eg: dr. A. Nieuwint, cytogenetics laboratory, VU medisch centrum or: mw Dr B Beverloo, cytogenetics Erasmus Medisch Centrum Rotterdam(see Appendix).
- Marrow biopsy: morphology
 - If indicated: HLA-typing, referral to transplant centre

Comment [S8]: FISH 3, 7 not sufficient, would recommend 1. Complete cytogenetic analysis + 2. more specific diagnostics as SNP etc. Needs to be determined with respective research centers. Reference center/ exact bone marrow diagnostic will be announced at a later date.

In case of siblings/family

- Screen siblings for Fanconi anemia (Note: MMC-test negative in carriers, mutation analysis informative)
- Genetic counseling
- HLA-typing
- In case of pregnancy of FA patient-parents and in case of pregnant FA patient consider:
 - prenatal diagnostics
 - preservation of cord blood cells

5.0 TREATMENT

5.1 Aplastic anemia: classification

We refer also to the DCOG guideline on aplastic anemia. Aplastic anemia is characterized by a hypocellular marrow without signs of myelodysplastic syndrome. According to the severity of the disease it is labeled “moderate”(MAA), “severe” (SAA) or “very severe” (VSAA). These categories are based on peripheral blood values.

Table 5.1. Aplastic anemia. Classification of severity.

Aplasia: grade	Marrow cellularity	Neutrophils	Reticulocytes (N : 5-25 promille)	Trombocytes
Moderately severe aplastic anemia (MAA)	hypocellular	500- 1500 x 10 ⁶ /l	< 40 x 10 ⁹ /l	20 – 100 x 10 ⁹ /l
Severe aplastic anemia (SAA)	< 30 %	< 500	< 20 x 10 ⁹ /l < 10 promille	< 20 x 10 ⁹ /l
Very severe aplastic anemia (VSAA)	< 30 %	< 200	< 20 x 10 ⁹ /l < 10 promille	< 20 x 10 ⁹ /l

5.2 Therapeutic measures for marrow failure

It is of great importance to consider stem cell transplantation in case of progressive marrow failure leading to transfusions of increased infection rates. Also in case other risk factors are identified (see Table 5.3) stem cell transplantation should be considered since this provides the only curative option for marrow failure in FA. Other measures, mentioned below can be considered to bridge the period to a transplants. In the currently unlikely situation of not having a stem cell donor these measures can also be used.

Therapeutic options, other than transplant:

- **Blood transfusions:** In case of serious lymphocyte deficiency all cellular blood products should be irradiated. Irradiation kills viable lymphocytes in the transfusion product, thus preventing transfusion-associated graft-versus-host disease, a very serious condition. A lymphocyte count < 500 x 10⁶ /l. is a indication to irradiate blood products. If available single-donor units should be employed to decrease donor-exposition. In Parvo B-19 recipients B-19 negative blood should be used. In case of iron overload chelation therapy should be considered.
- **Androgens.** In ± 60% of all patients a response to androgens can be expected, in particular with regards to anemia and trombocytopenia. The effect on granulocytes is usually less pronounced. ⁶⁶ The drug most often used is oxymethalon, 2-5 mg/kg/day. In case of response the dosage can be gradually reduced after 3 months of therapy (eg. 10-20% every 2-4 months). If however after 2-4 months therapy no response is seen, the chance of response is low. It should then be considered to stop therapy. There are relatively few data on oxandrolon, a less virilising drug than oxymethalon. Danazol is also used, although it is considered to be less virilising comparison studies are lacking. The risk of developing liver tumors is a well-known side effect of these drugs. The incidence is not well known. ⁶⁷ Regular ultrasound check-up of the liver are advised while on androgen therapy. Skeletal age should be done twice yearly. Due to their potential liver toxicity androgens should not be given if stem cell transplantation is planned to be performed soon. Androgen side-effects are summarized in table 5.2. ⁶⁸

Comment [S9]: Stronger advice: „should“, „ aim at“

Comment [S10]: Response to therapy has been observed up to 6 months of therapy. Therefore we recommend to continue androgen therapy –if well tolerated- for six months and to then stop therapy in case of sustained non response.

Comment [S11]: At least biannual

Comment [S12]: We would leave frequent skeletal age out to reduce radiation exposure.

Table 5.2 Androgens: side effects

Masculinisation
Growth-spurt with premature closure of growth plates
Behaviour changes
Cholestatic icterus and/or elevated liver enzymes
Liver adenoma, hepatoma or hepatocellular carcinoma
Peliosis hepatis
Elevated blood pressure

- Hematopoietic growth factors. In case of profound neutropenia (eg $<500 \times 10^6$ granulocytes) G-CSF 5 microgram/kg/day subcutaneously should be considered. Sometimes a 3 times per week schedule can be sufficient (alternate day). The goal is a granulocyte count $> 1000 \times 10^6$ with the lowest possible G-CSF dose. Most hematologists are cautious to use G-CSF in case of clonal cytogenetic abnormalities, since a possible acceleration of these abnormalities using G-CSF can not be excluded.
- Prophylaxis of infections: To prevent infections prophylaxis can be employed. In case of neutropenia there is an increased risk for fungal and yeast infections. Cotrimoxazole should be used in case of lymphopenia to prevent pneumocystis carinii pneumonia.
 - Anti-fungal prophylaxis (eg itraconazole) if granulocytes $<500 \times 10^6/l$
 - Cotrimoxazole as PCP-prophylaxis (18 mg/kg, 3x per week).

5.3 Stem cell transplantation: indications (Not part of European guidelines, no comments)

In case of the occurrence of either of the next symptoms a stem cell transplantation should be considered (summarized in table 5.3):

- progressive aplastic anemia (towards VSAA) with transfusion requirement and/or increased risk of infectious complications or:
- signs of malignant degeneration: development of myelodysplasia and/or persistent / progressive clonal abnormalities: gain of chromosome 3q or persistent/progressive monosomy 7.

It is currently uncertain whether stable, moderately severe aplastic anemia without signs of malignant degeneration and without the need for blood transfusions or an increased susceptibility for infections justifies stem cell transplantation, considering the morbidity/mortality risks of a transplant procedure.

Comment [S13]: If advising PCP prophylaxis, we would choose strict criteria to start prophylaxis as T lymphocyte deficiency is not to expect in FA. Anti fungal maybe better voriconazole// needs to be determined.

Table 5.3. Stem cell transplantation for FA: indications.

Donor	Transplant indication:
HLA-identical (sibling) donor	<p>Aplastic anemia with:</p> <ol style="list-style-type: none"> 1) need for transfusions (risk of HLA sensibilisation and iron overload) 2) neutropenia $< 500 \times 10^6/l$ with increased susceptibility for infections 3) tri- or tetrasomy chromosome 3 in $> 50\%$ of cells or persistent/ progressive monosomy 7 4) myelodysplastic syndrome (not meaning: discrete dysplasia) with or without clonal cytogenetic abnormalities 5) presence of leukemic blasts (transformation towards AML) 6) high-risk mutations like IVS4 mutatie in the <i>FANCC</i> or <i>N</i> gene or mutation in the <i>FANCD1/BRCA2</i> gene <p>NB: Stable MAA or an isolated clonal cytogenetic finding other than indicated is not a clearcut transplant indication. It does require marrow evaluation on a regular basis.</p>
MUD-donor (1-2 mismatches, depending on cell source, see note below table)	<p>Aplastic anemia with:</p> <ol style="list-style-type: none"> 1) marrow failure, need for transfusion or increased risk for infections (granulocytes $< 200 \times 10^6/l$) or increased infection rate. 2) progressive clonal abnormalities: tri- of tetrasomy chromosome 3 in $> 50\%$ of cells, or development of persistent/progressive monosomy 7 3) myelodysplastic syndrome (not meaning: discrete dysplasia) with or without clonal cytogenetic abnormalities 4) presence of leukemic blasts (transformation towards AML) 5) high-risk mutaties zoals een IVS4 mutatie in het <i>FANCC</i> of <i>N</i> gen of een mutatie in het <i>FANCD1/BRCA2</i> gen <p>NB: An isolated clonal cytogenetic finding other than indicated is not a clearcut transplant indication.</p>
Mismatched related/unrelated	<p>Aplastic anemia with:</p> <ol style="list-style-type: none"> 1) myelodysplastic syndrome (not meaning: discrete dysplasia) with or without clonal cytogenetic abnormalities 2) presence of leukemic blasts (transformation towards AML) 3) transfusions or increased infections.

NOTE

The definition employed here for “matched” and “mismatched” is: matched is any donor where a T-cell depletion would not be employed if marrow is the stem cell source or its equivalent (in terms of matching) for cord blood (5/6 or 6/6). Mismatched is any match where T-cell depletion would be employed in case marrow would be the stem cell source (in case of cord blood : 4/6 match). In case of cord blood both matching and cell dose should be considered, according to standard criteria.

5.4 Testing the donor for Fanconi anemia

Family donors should be tested with chromosomal breakage assays. If these assays prove normal this will suffice. Carriers can be donors, since there is no indication that this is an additional risk factor for marrow failure. Carriers can only be identified by DNA-diagnostics.

5.5 Stem cell transplantation: conditioning regimen.

Currently, radiotherapy can and should be avoided in conditioning schedules for Fanconi anemia. There is sufficient evidence that fludarabine based conditionings should be preferred. Graft-versus-host disease should be prevented/avoided since it may play a role in long term acquisition of solid tumors. Table 5.5 describes the proposed conditioning regimen. It has been agreed by the DCOG Task Force Stem cell transplantation.

Table 5.5 Conditioning

Donor type	Conditioning	GVHD-prophylaxis
HLA-identical sibling donor	Fludarabine 30 mg/m ² /d; x 5 (day -10 t/m -6) Cyclophosphamide 10 mg/kg x 3 (day -4, -3, -2) If frequent transfusions add: ATG (Genzyme, rabbit, 2,5 mg/kg/d; 4 days)	Ciclosporin A (aim at trough levels: 0.15-0.20 mg/l) Methotrexate day 1 and 3: 15 and 10 mg/m ² / d.
Matched unrelated donors	Fludarabine 30 mg/m ² /d x 5 (day -10 t/m -6) Cyclophosphamide 10 mg/kg x 3 (day -4, -3, -2) ATG (Genzyme, rabbit, 2,5 mg/kg/d; 4 days)	Ciclosporin A (aim at trough levels: 0.15-0.20 mg/l) Methotrexate day 1 and 3: 15 and 10 mg/m ² / d.

In case of cord blood or haplo-identical transplant additional support measures will be required, to be discussed with the transplant centre, or with the protocol chair.

6.0 LONG-TERM FOLLOW-UP OF FANCONI ANEMIA PATIENTS

6.1 Solid tumors, general introduction

It is essential to check FA patients regularly for solid tumors.^{2,16,69} This concerns squamous cell carcinoma of the head and neck region (65% oral cavity, 10% oropharynx, 10% hypopharynx, 10% larynx, and 5% unknown), but also the esophagus as well as the anogenital region are frequently involved. Vulva carcinoma and cervix carcinoma are increasingly diagnosed (also: 6.3.3) The cumulative risk of head and neck cancer is estimated to be 14% at the age of 40. The risk is increased for all FA patients but increases further after stem cell transplantation, especially if immunosuppressive therapy was used for graft-versus-host disease and after radiotherapy.⁷⁰ Second primary tumors are seen in 63% of FA patients.⁶⁸ It is currently not clear what the role for human papilloma virus in the etiology of these cancers.^{71,72} Liver tumors are mainly related to the use of androgens.

An important argument in favour of screening is the fact that premalignant findings can be treated (eg using surgery) whereas chemotherapy and/or radiotherapy, necessary in more advanced stages generally leads to complications due to the inherent DNA repair problem of FA patients.

Given the high frequency of squamous cancers of the head in the head and neck region, inspection of the oral cavity and oropharynx every three months by a head and neck surgeon are advised. For female patients a yearly gynecological inspection is indicated after the menarche. Suspicious lesions should be biopsied and in general a more aggressive policy of biopsy and early intervention should be followed for these patients.

As preventive measures smoking and alcohol are to be avoided. We advise HPV vaccination. Although the exact role of HPV has not been established, some tumors might be prevented

6.2 Head and neck cancers

The most relevant problem for FA patients in the head and neck region is the risk of developing cancer, in particular squamous cell carcinomas (HNSCC). As mentioned earlier, FA patients are at high risk to develop squamous cell carcinoma, especially in the oral cavity.^{2,3} The cumulative incidence during life is estimated to approach 100%.

Since life expectancy for FA patients has improved due to improved treatment options for marrow failure, the risk for squamous cell carcinoma increases. It should be emphasized that these tumors can appear early in life. Patients as young as 11 years have been described whereas the peak incidence of sporadic patients is at the age of 60-65. Research has shown that human papilloma virus (HPV) might play a role in the pathogenesis of these cancers.⁷¹ However, in the cohort of patients studied at the VU University Medical Centre in Amsterdam only a small proportion of tumors proved HPV-positive. Unlike a US study showing 21/25 squamous cell carcinomas (head, neck, esophagus and anogenital) in FA patients to be HPV-positive, at the VU University Medical Centre this was only found in 2/15 tumors (manuscript in preparation). This latter percentage does not differ from the prevalence of HPV in tumors of sporadic patients. Also in the 4 existing squamous cell carcinoma cell lines derived from FA patients no HPV could be detected.⁷² There are various explanations for this discrepancy in findings: quality of tissues from the paraffin-archives, the exact test employed. Notwithstanding this there seems to be a geographical difference as well between the prevalences found in sporadic patients in the US and the Netherlands, and this same geographical difference might play a role in FA patients. These findings illustrate the need to generate and test more cellines from FA patients. Prof Brakenhoff at the VU University Medical Centre greatly appreciates being informed if FA patients are diagnosed with

squamous cell carcinoma (for details: see appendices). At this moment we advise FA patients to be vaccinated against HPV. This might in any case prevent a subgroup of squamous cancers. However, vaccination does NOT replace frequent inspections, both in the head and neck region in all patients, and in female patients also of the anogenital region.

Treatment

There are several treatment options for head and neck cancer. Tumors diagnosed early are usually treated with surgery *or* radiotherapy. This leads to limited morbidity with relatively good prognosis. More advanced disease can be treated with surgery combined with radiotherapy. Additionally these tumors can be treated with chemoradiotherapy, combining cisplatin with irradiation. However, FA patients are very susceptible to radiation-induced toxicity (mucositis, dermatitis) and some clinicians have used adjusted dosage. FA patients do not tolerate cisplatin (like MMC a DNA crosslinking agent). This illustrates the problems for therapy. It leaves surgery as the only therapy that can be used in FA patients without extra complications.

Screening, early diagnosis and an aggressive policy of biopsies / excisions are thus advocated for these patients. FA patients and/or their families should notify head and neck surgeons that they are dealing with FA patients necessitating special care. FA patients should be controlled and treated preferentially by head and neck surgeons familiar with FA, and with large oncological experience.

Follow-up investigations.

The high incidence of head and neck cancers, even at an early age has led in the US to frequent inspection from the age of 10. In the Netherlands this is advised from the age of 15. It should be done by head and neck surgeons with oncological experience. This includes in the Netherlands only the academic centres as well as the Dutch cancer institute (NKI/AvL). At the VU University medical centre there is an ongoing research project to detect precancerous lesions that are not visible at clinical inspection with a noninvasive genetic assay. This might be of value to identify specific regions at increased risk, even before visible lesions appear.

Patients should be instructed to check their mouths regularly, also between the screening visits. In case of a suspicious change a head and neck surgeon with oncological experience should be consulted, and the patients need to identify themselves as FA patients.

Comment [S14]: We would argue for aged adjusted from 10 years on (see above)

In case a solid tumor is diagnosed in a patient in the Dutch cohort the protocolcommittee appreciates being notified.

6.3 Endocrinopathy

Endocrinopathy can be primary, associated with Fanconi anemia or secondary to chemotherapy or radiotherapy. Androgens can lead to virilisation.

The origin of endocrinopathies can be central (growth hormone) or peripheral (thyroid, diabetes).

6.3.1. Growth - height. Most FA patients are small (mean height -2.4 SD). In patients with either growth-hormone deficiency or hypothyroidism this can be more pronounced. A small proportion (11.5%) of FA patients have a height > 0 SD. Reduced height can be attributed in some 25% of patients to intestinal problems, indicated by a larger weight than height deficiency. It is not known whether skeletal age retardation results in a prolonged period of growth in FA patients. Not all growth problems can be attributed to endocrinopathies, low height can occur in the absence of endocrinological anomalies

6.3.2. Hypothyroidism. This occurs in $\pm 40\%$ of FA patients, indicating the need for regular checks of thyroid function (every 6-12 months).⁷³

6.3.3. Diabetes mellitus. This is more prevalent in FA patients, although the true prevalence is not known. There is a clear increase in hyperinsulism as well as insulin resistance (70-80%). It is felt that thorough follow-up will show even more diabetes.⁷⁴ The HbA1c level is in non-transplanted patients not reliable due to the high turn-over of hemoglobin, leading to false-negative results. The current advice is to measure glucose in a urine portion, and check a fasting level of glucose and insulin in blood. Save oral glucose tolerance test for second line diagnostics. Hyperinsulism as well as insulin-resistance can be treated initially by life style advices.

6.3.4. Puberty and fertility. Both girls and boys with FA arrive rather late in puberty. Girls have an early menopause and – if fertile – their period of fertility is thus shortened. Men often have azoospermia, though not always. Cryopreservation can be considered.

6.3.5. Growthhormone deficiency. A large share of all patients develops growth hormone deficiency. In general, this is due to neuro-secretory dysfunction. Unfortunately it is unknown whether growth hormone substitution contributes to the risk of developing malignancies. Most doctors are therefore reluctant to use growth hormone in FA patients

6.3.6. Other endocrinological problems. Boys often have small testicles, cryptorchism and hypospadias.

6.4 Gynecology

6.4.1 Fertility and reproduction.

In women with FA menarche is usually late, often from the age of 15. Early menopause is frequent. Hormonal substitution can be considered in case of severe clinical symptoms like flushes. Congenital anomalies of the genital tract are often described in FA patients. Hypogonadism often leads to oligomenorrhoe, leading to decreased fertility. Despite reduced fertility pregnancies occur, even after stem cell transplantation.^{51,52} Prenatal counseling in a perinatology centre is advised, also off course for perinatal care.

6.4.2 Gynaecology

Hypermenorrhoe can occur as a consequence of thrombocytopenia. Irregular vaginal bloodloss can be a sign of cervical neoplasia and should lead to careful evaluation. Oral contraceptives given continuously or levonorgestrel containing intrauterine devices (Mirena®) can be prescribed in usually improve bloodloss. Androgens cause amenorrhoe. In the absence of other explanations hysterectomy or endometriectomy can be a solution for persistent vaginal bloodloss. Tranexaminic acid (Cyklokapron 50 mg/kg/dg in 3-4 doses po) can be considered.

6.3.3 Gyneco - oncology

Cervixcarcinoma, vulvacarcinoma as well as vaginacarcinoma are more frequent and can occur at an early age than in the general population^{2,3}. They can be Human Papilloma Virus associated. Cases reported in the literature are under the age of 25. Yearly screening with inspection of the vulva as well as cervixcytology are recommended. In case of complaints of vulvar itching/pain, irregular blood loss, vaginal excretion or loss of blood after intercourse should lead to gynecologic evaluation.

Recently 2 vaccines have been approved for the market that protect against cervical intraepithelial neoplasia and probably as well against vulvar and vaginal intraepithelial neoplasia caused by infection with HPV types 16 and 18. One of these vaccines also protects against genital warts. Although the follow-up is still limited, protecting antibodies persisted over time.^{53,54} These vaccines, given 3 times with 3 months interval did not cause serious side-effects in a healthy population. Although there is no formal proof for vaccination on the age of 11 in FA patients vaccination is recommended.

6.5. Follow-up scheme for Fanconi anemia patients

Based on data described above we formulate the follow up as described in table 6.3.

Comment [S15]: Would recommend to make a special paragraph on HPV vaccination (also including male). We are in process of determining recommendations on that topic and will report on it at a later date.

This table should be individually adapted in case of concomitant diagnosis or symptoms. In transplanted patients side effects, short- as well as longterm of conditioning and complications should be taken in consideration

Tabel 6.3. Follow-up scheme for Fanconi anemia patients

Minimal follow-up is described

Test	monthly	1 x every 3-4 months	Yearly	If indicated
Full blood count + reti's	X	X		X
In case of cytopenia: BM + chrom. + FISH			TWICE YEARLY	X
Growth: length, weight, puberty stage, sitting height, head circumference		X		
Endocrinology (TSH, FT4, IGF1, IGFBP3, LH, FSH, testosterone or estradiol, PTH, VitD, skeletal age) consultation endocrinologist			X	X
Glucose in urine/portion, serum: fasting glucose and insulin, if aberrant: OGTT			X	X
Post-menopausal DEXA-scans (osteoporosis)			X	X
Dentist			X	X
> 10-15 jaar: ENT consultation, early biopsies of apparent pre-malignant lesions (mouth selfscreening !)		X		X
♀ > 15-20 jaar consultation gynaecology (inspection, PAP-smear and HPV diagnostics)			X	X
♀ > 15-20 jaar screening mamma carcinoma (selfscreening !)		X		X
If on androgens : liverfunction tests as well as live ultrasound		X		X
In case of transfusions: iron status and if overloaded: chelation		X		
Bone density			1 X PER 2 YEARS	

APPENDIX 1: DIAGNOSTICS FOR (PAN) CYTOPENIA

DCOG MARROW FAILURE PROTOCOL

Adapted version for this English protocol

Aims of this protocol:

- Differential diagnostic aid in case of marrow failure
- Criteria for DCOG registration

A differentiation is made between single cell cytopenia versus broader marrow failure. Marrow failure becomes more likely in case of persistent cytopenia (> 3 months).

Step wise diagnostics:

Step 1: Diagnoses to be excluded before marrow failure diagnostic procedures.
Do not yet report the patient to the DCOG.

Step 2: Diagnostics aimed at marrow failure
Patient data and materials (blood, marrow, marrowbiopsy) to be submitted to DCOG

Step 3: Confirmation by molecular techniques

I. SINGLE CELL CYTOPENIA: ERYTHROCYTES

Step 1:

Increased degradation	Hemolytic anemia	Hb-pathy Auto-immune Membrane defects	Microangiopathy Enzyme deficiencies
Consumption	Hypersplenism		
Production	Deficiencies	Iron Vit B12	Folic acid
	Viral infections	CMV, EBV, ParvoB19	
	Medication Chronic illnesses Lead-intoxication		
Minimum diagnostics:	Full blood count, reticulocytes, bilirubin, haptoglobin, LDH, Vit B12, folic acid, HbF, TIBC, ferritin, virusserology		

Step 2: Persistent anemia > 3 months

	DBA	MDS/RA	SA	Pearson	CDA
History					
<i>Pre-dysmaturity</i>	+/-	-	-	+	-
<i>Age at presentation</i>	<1 year	Variable	>1 year	Post-natal	>1 year
<i>Impaired growth</i>	+/-	-	-	+	-
<i>Family history</i>	+/-	-	+/-	-	+/-
<i>Steatorrhoe</i>	-	-	-	+	-
Physical examination	Small height Cong. Abn	Splenomegaly, possibly	-	small, thin, abd distension, retardation	Icterus possibly
Lab					
<i>HbF</i>	HbF ↑ ADA can ↑	HbF ↑	HbF ↑	HbF ↑	bili & LDH ↑ hapto ↓ HbF ↑
<i>ADA in erys</i>					
<i>HEMPAS serum lysis test</i>					+ in type II CDA
Marrow					
<i>Cellularity red cells</i>	↓↓↓	Normal to ↓	normal to ↑	normal	normal to ↑
<i>Dysplasia</i>	-	+	+	-	++
<i>Iron stain</i>	Normal	Normal	ring sideroblasts	ring sideroblasts vacuolisation	Normal macrocytosis, bi/multinucl. cells
<i>Specific characteristics</i>					
<i>Cytogenetics</i>	Normal	clonal / monosomy 7	normal	normal	Normal
Marrow biopsy					
<i>Cellularity</i>	Normal	↑ or ↓	↓	normal to ↑	↑
<i>CD34 staining</i>	Normal	↑	normal	normal	normal
<i>Architecture</i>	Normal	affected	Possibly affected	normal	normal

DBA = Blackfan-Diamond anemia SA = Hereditary sideroblastic anemia
MDS = Myelodysplastic syndrome CDA = Congenital dyserythropoietic anemia
RA = Refractory anemia

Note: TEC (transient erythroblastopenia of childhood) has a peak incidence between 1-3 years of age, usually the history, physical examination and laboratory results are all normal, except for anemia and reticulocytopenia. Normally it will normalize within 3 months, this diagnosis is not to be registered at the DCOG.

Step 3:

Disease	Gene	LOCATION	PRODUCT	%	DIAGNOSTICS IN THE NETHERLANDS (!)
Diamond Blackfan anemia (DBA)	<i>DBA1</i>	19q13.3	Ribo prot	25	VUMC (Gerard Pals or Hans Gille) G.Pals@VUMC.nl or jjp.Gille@VUmc.nl Urgent: 1 mth; normal 3 mth
	<i>DBA2</i>	8p23.2-p22	S19	40	
	<i>onbekend</i>			35	
Hereditary sideroblastic anemia (SA)	<i>ALAS2</i>				Unidade de Anemias Congenitas Hospital Pediatrico de Coimbra Janet Pareira (uhm@hpc.chc.min-saude.pt)
	<i>ATP7</i>				Currently not available
Pearson syndrome	<i>Deletions mitochondr DNA</i>			100	Sheffield Children's Hospital, UK Contact: Jo Martindale MSc MRCPPath Joanne.Martindale@sch.nhs.uk
Congenital dyserythropoietic anemia (CDA)	<i>CDAN1</i>	15q15.1-15.3	Codanin-1		Medical genetics Napoli Head of lab: Prof Achille Iolascon iolascon@dbbm.unina.it
	<i>CDAN2</i>	20q11.2			
	<i>CDAN3</i>	15q22			
	<i>CDNA4-7</i>				Clinically described

II. SINGLE CEL CYTOPENIA MEGAKARYOCYTES / TROMBOCYTES

Step 1:

Destruction	Immune-mediated	
Usage	Hypersplenism Activated coagulation	Micro-angiopathy Kasabach-Merritt Chronic DIC
Production	Deficiencies Viral infections Drugs	Vit B12 EBV, CMV, ParvoB19 eg anti-epileptics Folic acid
Thrombocytopathy	Wiscott Aldrich Syndrome (WAS) Giant platelets	
Minimal diagnostics:	Full bloodcount, MPV, thrombopoietine (TPO), glyocalicin (GC), Vit B12, folic acid, virusserology	

Step 2:

	CAMT	TAR	FA	MDS	Dysmega-Karyopoesis
History					
<i>Dysmaturity</i>	-	-	+/-	-	-
<i>Age at presentation</i>	< 1 year	< 1 year	variable	variable	< 1 year
<i>Disturbed growth</i>	-	-	+/-	-	-
<i>Family history</i>	+/-		+/-	-	+/-
<i>Skeletal abnormalities</i>	-	radius	thumb, radius, other	-	-
Physical examination		Radius aplasia horseshoe kidney Cardiac abn	thumb, aplastic radius café-au-lait spots, other anomalies	sometimes splenomegaly	
Lab					
TPO	↑↑↑	↑↑↑	↑	↑	normal or ↑
GC	↓↓	↓↓	N/↓	N/↓↓	N/↓↓
Lab phase II					
<i>HbF</i>	Normal/↑	normal	↑	↑	normal
<i>MMC-test</i>	normal	normal	Increased susceptibility	normal	normal
Marrow					
<i>Megakaryocytes</i>	absent	absent	↓	↓ or ↑	normal
<i>Dysplasie</i>	-	-	-	++	+++
<i>Cytogenetics</i>	normal	normal	Clonal abn	Clonal abn./ monosomy 7	normal
Marrow biopsy					
<i>Cellularity</i>	decreased	No megakar.	↓	↓ or ↑	normal

CAMT : congenital amegakaryocytic thrombocytopenia
 TAR : thrombocytopenia with absent radius
 FA : Fanconi anemia
 MDS : myelodysplastic syndrome

Step 3:

Disease	Gene	LOCATION	PRODUCT	%	DIAGNOSTICS (IN THE NETHERLANDS !!)
Congenital amegakaryocytic thrombocytopenia (CAMT)	<i>C-MPL</i>	1p35		100	Masja de Haas, Sanquin (research based) M.deHaas@sanquin.nl
Thrombocytopenia with absent radius (TAR)	<i>unknown</i>				Not available
Fanconi anemia (FA)	<i>FANCA</i>	16q24.3	1455	27	This represents the distribution in the Netherlands. Note 1° line diagnostics FA: DNA diagnostics: VUMC (Hans Gille, jjp.Gille@VUMC.nl)
	<i>FANCB</i>	Xp22.31	859		
	<i>FANCC</i>	9q22.3	558	35	
	<i>FANCD1</i>	13q12.3	3418	5	
	<i>FANCD2</i>	3p25.3	1451	9	
	<i>FANCE</i>	6p21.3	536	5	
	<i>FANCF</i>	11p15	374	9	
	<i>FANCG</i>	9p13	622	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
Dysmegakaryopoiesis	<i>GATA-1</i>				GATA-1 analysis shortly available in research setting in Rotterdam Monique den Boer, Sophia Kinderziekenhuis m.l.denboer@erasmusmc.nl 010-46388224 of 010-46388340 (lab)
	<i>FLI-1</i>				
	<i>FOG-1</i>				
	<i>NF-E2</i>				
	<i>Gfi-1b</i>				

III. SINGLE CEL CYTOPENIA: MYELOID SERIES

Step 1:

Destruction	Immune-mediated		
Consumption	Hypersplenism		
Production	Viral infections Deficiencies Drugs	CMV, EBV, Parvo B19 Vit B12 eg anti-epileptics	Hepatitis A, B, C Folic acid
Minimal diagnostics:	Full blood count, Vit B12, folic acid, virusserology, antibodies against neutrophils, immunoglobulins		

Step 2:

	SCN, Kostmann syndroom*	Cyclic neutropenia	Reticular dysgenesis	Shwachman	Dyskeratosis Congenita	Cartilage hair hypoplasia	Chediak-Higashi
History							
<i>Pre-dysmaturity</i>	-	-	+/-	-	-	-	-
<i>Age at presentation</i>	< 3 mths	variable	<3 mths	Variable	variable	variable	Variable
<i>Impaired growth</i>	-	-	+/-	+	-	++	-
<i>Family</i>	+/-	+/-	+/-	+/-	+/-	-	-
<i>Other</i>	Late cord release	Periodic infections		Steatorrhoe			
Physical examination							
					naildystrophia; hyperpigmentation; leukoplakia	Thin, non-pigmented hair	ocular blindness; albinism
Lab							
		ANC varies periodically: 20-24 days	Lymphocytes: decreased counts and impaired function			Lymphocytes: decreased counts and impaired function	granules in neutrophils
Phase II lab diagnostics							
				Exocrine pancreas; Metaphyseal dysostosis		Metaphyseal dysostosis	
Marrow							
<i>Myeloid series</i>	Maturation arrest at promyelocyte level	Myeloid series possibly decreased	Absent myelo- and lymphopoiesis	Hypocellular	Hypocellular	Hypocellular	dysplastic, not hypocellular
Marrow biopsy							
Cellularity: see marrow							

SCN = severe congenital neutropenia

Note: gingival hyperplasia in almost all patients.

Some SCN (severe congenital neutropenia) patients are G-CSF resistant.

Step 3:

Disease	Gene	LOCATION	PRODUCT	%	DIAGNOSTICS (IN THE NETHERLANDS !!)
Severe congenital neutropenia (SCN)	<i>ELA2</i>	19q13.3		90	Contact Dr Marry Bruin (M.Bruin@umcutrecht.nl)
	<i>GCSF-rec</i>			10	Research Rotterdam: Prof dr Ivo Touw (i.touw@erasmusmc.nl or 31 10-4087837)
Cyclische neutropenie	<i>ELA2</i>	19q13.3		90	Contact Dr Marry Bruin (M.Bruin@umcutrecht.nl)
Reticulaire dysgenesie					Currently not available
Shwachman-Diamond	<i>SDBS</i>	7q11	250	90	AMC (Mariel Alders, beep 58960) KG_DNA@amc.uva.nl
Dyskeratosis congenita (DC)	<i>DKC1</i> <i>TERC</i>				Imperial College London Hammersmith Hospital Tom Vulliamy (t.vulliamy@imperial.ac.uk)
Cartilage Hair syndroom	<i>RMRP</i>		267		Diagnosis made clinically
Chediak-Higashi	<i>CHS1</i>	1q42.1-42.2	LYST	100	Segregation analysis of polymorphic markers linked to Chediak-Higashi. Hospital Necker-Enfants Malades Paris Dr G. de Saint Basile (sbasile@necker.fr)

• IV. FAILURE OF SEVERAL CELL LINES

Step 1:

Destruction	Immune-mediated	
Consumption	Hypersplenism Hemofagocytic syndrome	(see appropriate protocol)
Production	Deficiencies Drugs Viral infections Leishmaniasis	Vit B12 eg anti-epileptics EBV, CMV, ParvoB19 Folic acid Hepatitis A, B, C
	Marrow affected by:	Leukemia solid tumor metastases
Inborn errors	Osteopetrosis Methylmalonic acid acidaemie Organoaciduria Aminoaciduria	
Minimal diagnostics:	Full blood count, reticulocytes, bili, haptoglobin, LDH, Vit B12, folic acid, ferritin, fibrinogen, triglycerides, virusserology	

Step 2: In this case the criterium > 3 months present does not apply !

	AA	FA	PNH	MDS	Osteopetrosis
History					
<i>Pre-dysmaturity</i>	-	+	-	-	-
<i>Age at presentation</i>	variable	variable	Variable	variable	< 1 year
<i>Disturbed growth</i>	-	+	-	-	Macrocephalic
<i>Family history</i>	-	+/-	-	-	+/-
<i>Other</i>	-		Abdominal pain		Progressive hearing and seeing problems
Physical examination		thumb radius skeletal abn congenital abn cafe-au-lait spots			Cranial nerves affected, hepatosplenomegaly, macrocephalie
Lab					
		MMC-test	PI-anchored proteins		Extramedullary hematopoiesis (erythroblasts in peripheral blood), hemolysis
Marrow					
<i>Cellularity</i>	↓	↓	Variable	↓ of ↑	
<i>Cytogenetics</i>	*	*	*	clonal + monosomy 7	
Marrow biopsy					
<i>Cellularity</i>	↓	↓	Variable	↓ or ↑	↓
<i>Dysplasia</i>	-	-	-	++	+
<i>Reticulin</i>	-	-	-	Reticulin increased	Reticulin increased

AA: aplastic anemia
FA: Fanconi anemia

PNH: paroxysmale nocturnal hemoglobinuria
MDS: myelodysplastic syndrome

Note: In aplastic anemia cytogenetic abnormalities can develop over time.

Step 3:

Disease	Gene	LOCATION	PRODUCT	%	DIAGNOSTICS (IN THE NETHERLANDS !!)
Congenital amegakaryocytic thrombocytopenia (CAMT)	<i>C-MPL</i>	1p35		100	Masja de Haas, Sanquin (research based) M.deHaas@sanquin.nl
Thrombocytopenia with absent radius (TAR)	<i>unknown</i>				Not available
Fanconi anemia (FA)	<i>FANCA</i>	16q24.3	1455	27	This represents the distribution in the Netherlands. Note 1° line diagnostics FAi: DNA diagnostics: VUMC (Hans Gille, jjp.Gille@VUMC.nl)
	<i>FANCB</i>	Xp22.31	859		
	<i>FANCC</i>	9q22.3	558	35	
	<i>FANCD1</i>	13q12.3	3418	5	
	<i>FANCD2</i>	3p25.3	1451	9	
	<i>FANCE</i>	6p21.3	536	5	
	<i>FANCF</i>	11p15	374	9	
	<i>FANCG</i>	9p13	622	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
	<i>FANCI</i>	15q25	1328	5	
Dysmegakaryopoiesis	<i>GATA-1</i>				GATA-1 analysis shortly available in research setting in Rotterdam Monique den Boer, Sophia Kinderziekenhuis m.l.denboer@erasmusmc.nl 010-46388224 of 010-46388340 (lab)
	<i>FLI-1</i>				
	<i>FOG-1</i>				
	<i>NF-E2</i>				
	<i>Gfi-1b</i>				

Links of interest:

Netherlands: <http://www.dnadiagnostiek.nl/> [English translation in progress]

Europe: <http://www.orphanet.org/>

United states: <http://www.genetests.org/>

APPENDIX 2. LABORATORIES

DNA-diagnostics

Dr J.J.P. Gille and/or Dr. R. M. L. Vervenne-Van Spaendonk
Laboratorium voor DNA- en Eiwitdiagnostiek, Afd Klinische Genetica
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Squamous cell carcinomas

Prof. Dr. R. H. Brakenhoff
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APPENDIX 3: MOLECULAR DIAGNOSIS FA

Before a molecular diagnosis for FA can be considered the diagnosis of a chromosomal instability disease should be firmly established on the basis of a chromosomal breakage test (a suitable test protocol is available upon request). Since the breakage test does not always distinguish between FA and other cross-linker sensitive syndromes (such as Nijmegen breakage syndrome), molecular diagnosis by mutation screening is required to determine the defective gene. As of January 2007 there are 13 genuine FA genes known (*FANCA*, *-B*, *-C*, *-D1 (=BRCA2)*, *-D2*, *-E*, *-F*, *-G*, *-I*, *-J*, *-L*, *-M*, *-N*) while at least two putative FA genes remain to be identified.

Protocol for mutation screening

The tests are performed in steps:

1. Complete mutation screening of the *FANCA* gene by direct sequencing and detection of large deletions and duplications by MLPA.
2. Complete mutation screening of the *FANCC*, *-E*, *-F*, and *-G* genes by direct sequencing. Step 1 and 2 together detect mutations in >95% of all Fanconi anemia patients.
3. Should no pathogenic mutations be found in step 1 and 2, *FANCI* and *-D1(BRCA2)* is subsequently screened for mutations (i.e., the gene defective in FA complementation group D1), or, depending on the mode of inheritance, *FANCB*, which is on the X-chromosome.
4. If step 3 is negative too, the *FANCD2*, *-I*, *-L*, *-M* and *-N* genes are screened. The screening of *FANCD2* requires cDNA from growing cells (lymphoblasts, fibroblasts), because the analysis of genomic DNA is hampered by the presence of pseudogenes.
5. If no mutations can be detected in any of these genes, the analysis will be continued on a research basis, at no further charge. This includes screening of the *NBS1* gene and, if negative, a functional complementation analysis to determine the FA complementation group. Finally, gene cloning studies will be carried out to determine the gene that is defective in the patient.

Since this work directly results from the request for mutation analysis there is no requirement for explicit informed consent to cover this extended analysis. Any new FA genes to be discovered will be added to the list of genes to be screened for mutations (step 1-3).

Note. In cases where lymphocyte mosaicism has been observed or suspected, the cause of the reversion can be determined by comparing the mutations in lymphoblasts and skin fibroblasts²³ (please inquire).

Turnaround time

We aim for turnaround times of 2 months for each step. For research studies no turnaround time can be given.

Prenatal DNA testing (*only available after mutations have been found*) is usually completed within one week.

For further information please contact:

Resie Vervenne-van Spaendonk Ph.D., Laboratory of DNA Diagnostics, VU University Medical Center, Van der Boechorststraat 7, NL-1081 BT Amsterdam, Netherlands - phone: +31-20-4446087; fax: +31-20-4448293
Email: r.vervenne@vumc.nl or jjp.gille@vumc.nl

Sample requirements

For tests 1, 2 and 3: genomic DNA from blood or cultured cells is used, for test 4: cDNA from cultured cells. For (5) and all other tests: cell lines (lymphoblasts and fibroblasts) are needed. For transplanted patients and patients with suspected lymphocyte mosaicism a skin biopsy or fibroblast culture is required.

For complete testing we need:

from patients:

- Blood (EDTA or heparin): 20 ml, or DNA (>20 µg)
- Skin biopsy or fibroblast culture.

from parents and sibs:

- Blood (EDTA or heparin): 5 ml, or DNA: at least 1 microgram

for prenatal DNA testing:

- chorionic villi sample (30 mg) or amniotic fluid (10 ml)
- If not tested previously, DNA or blood from both parents is necessary to assess carrier status

Shipping directions

Note that all materials are kept at room temperature at all times and should never be frozen.

- Complete a 'aanvraagformulier voor DNA- en eiwitonderzoek' form (can be downloaded from www.dnadiagnostiek.nl).
- Shipping at room temperature and in accordance with IATA rules.
- Cultured cells (lymphoblasts or fibroblasts) may be shipped in suspension in tissue culture media with 5% fetal calf serum in 2 ml cryostorage tubes. At room temperature cells remain viable for at least one week.
- To avoid possible complications (logistic or otherwise) please contact us by e-mail before shipping any samples (DNAdiagnostiek@vumc.nl).

Shipping address:

**Dr. R. Vervenne/Dr. J.J.P. Gille,
Laboratorium DNA en Eiwitdiagnostiek
Afd. Klinische Genetica
De Boelelaan 1117
NL -1081 HV Amsterdam
The Netherlands**

Phone: +31-20-4448346

Fax: +31-20-4448293

Email DNAdiagnostiek @vumc.nl

www.dnadiagnostiek.nl for request form. [Note: English translation is in preparation]

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