Appendix I

Practical recommendations regarding surveillance and therapy (adapted to the international consensus treatment guidelines as published by the FARF)

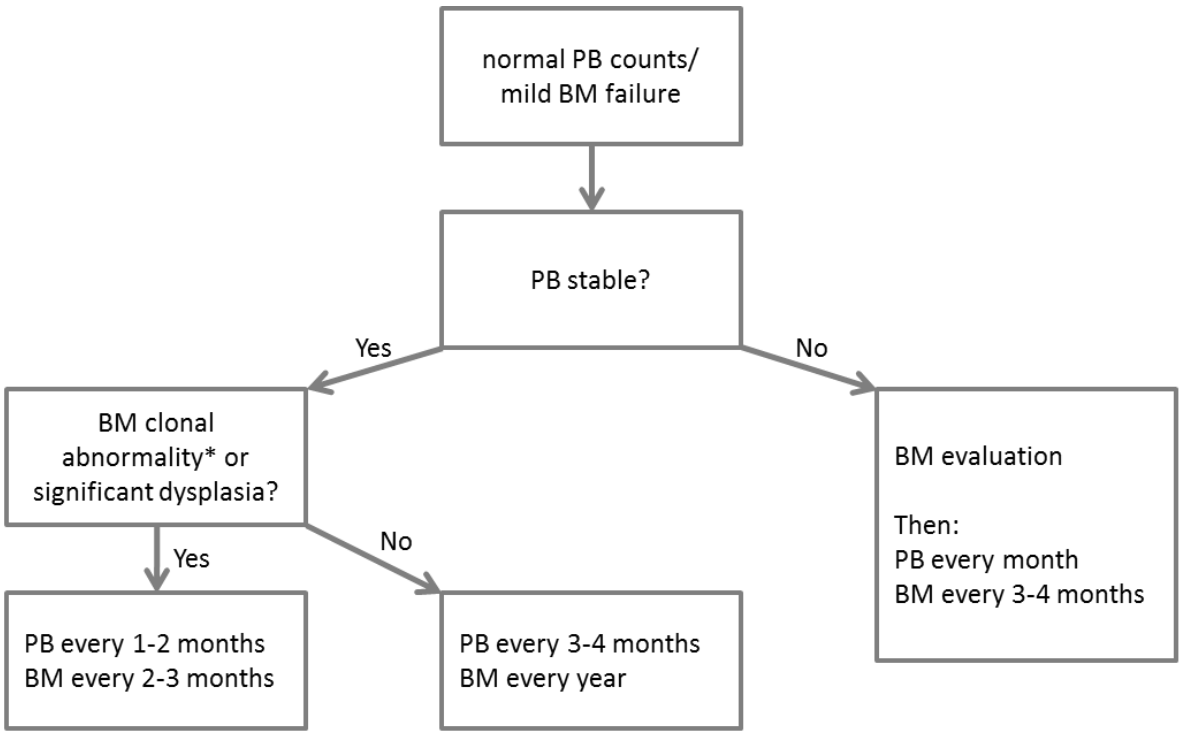
Severity of Bone Marrow Failure

	Mild	Moderate	Severe
ANC	<1,500/mm^3	<1,000/mm^3	<500/mm^3
Platelets	50,000 - 150,000/mm^3	<50,000/mm^3	<30,000/mm^3
Hb	≥8 g/dl*	<8 g/dl	<8 g/dl

*Less than norm for age but >8 g/dl

To meet these criteria for marrow failure, the cytopenias must be persistent and not transient or secondary to another treatable cause, such as infections, medications, PB cell destruction/loss or nutritional deficiencies.

Clinical Monitoring of Bone Marrow Failure



*Specific clonal abnormalities may warrant immediate treatment intervention or closer monitoring.

Figure 1. Suggested monitoring of bone marrow failure

- In stable situations, annual evaluation of the BM is recommended, following the third year of life.
- In case of compliance issues, one can consider skipping annual BM investigations (replacing it by testing for -7/+3q and chromosome 1 anomalies on peripheral blood), but only if all the following criteria are met: (1) stable blood counts; (2) no increase in monocytes; (3) no recent changes in MCV; (4) stable clinical situation; (5) absent known cytogenetic changes;
- BM examination should consist of an aspirate, trephine biopsy, cytogenetics with G-banding and FISH with specific probes for MDS/AML in FA
- Interphase FISH cytogenetic analysis in PB cells with specific probes for MDS/AML in FA (if available) may reduce the frequency of bone marrow examinations to longer than one year; however the approach has not yet been published to be effective.
- A consensus HSCT regimen can be found in Chao et al., Klin Padiatr 2015;227:157-65.

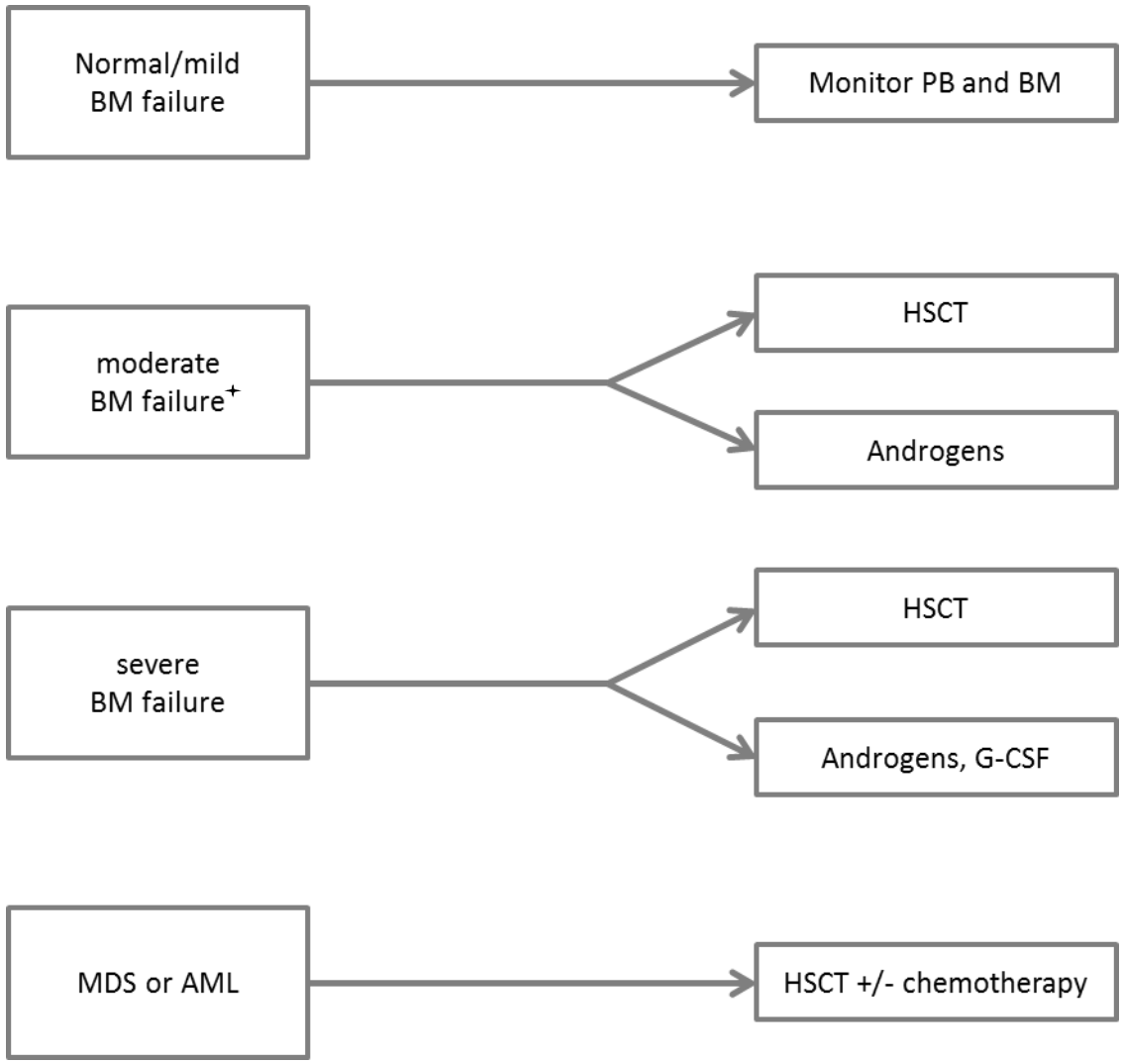
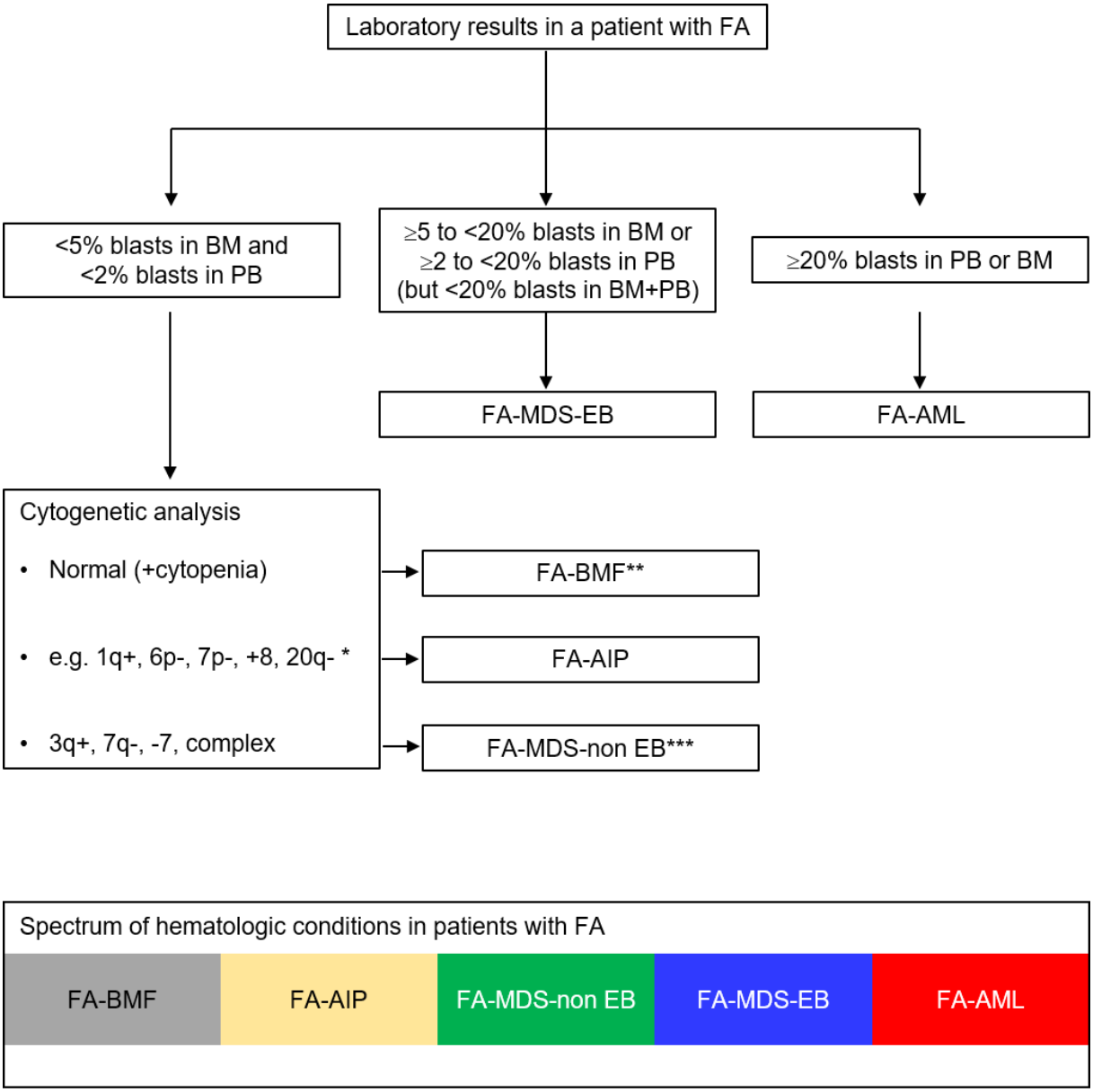


Figure 2. Bone marrow failure algorithm.+ With signs of progression towards severe BMF (for example: declining platelet count, bleeding signs, neutropenia related severe infections).



* all cytogenetic aberrations (exclusion: 3q+, 7q-, -7, complex)
**In cases with rapid rise in BM cellularity FA-MDS needs to be considered
*** In pediatric patients generally Refractory Cytopenia of Childhood (RCC)

Figure 3. Classification of hematologic conditions in Fanconi Anemia (from Behrens et al, *Haematologica* 2021 Nov 1;106(11):3000-3003).

Androgen use in patients with Fanconi Anemia

- The effect of androgen therapy is to increase/stabilize the hemoglobin (it can also improve/stabilize the platelet count)
- Androgen therapy can be considered when the patients develops moderate bone marrow failure or has clinical signs of insufficient counts, however should start when the patient's hemoglobin drops below 8 g/dl or the platelet count falls below

30,000/mm^3. Of course, strategic discussions with the patient and the family about the use of androgens or whether to proceed to transplant need to start earlier. Prior to commencing treatment a bone marrow investigation with cytogenetic evaluation is necessary to rule out MDS/AML or FA-AIP. Discussion with the registry team is recommended.

- In FA-AIP transformation to myeloid neoplasia seems to be infrequent, yet may occur. Therefore androgen therapy in FA-AIP is currently not recommended.
- A few reports in the literature show that both male and female patients can be treated at starting dose of approximately 5 mg/kg/die (in one dose) with an attenuated synthetic androgen, danazol, which produces few virilizing effects. A recent retrospective study did demonstrate the effectiveness of danazol in 7 of 8 FA patients treated (starting dose 3.5-7.7 mg/kg/die).
- Most patients respond within 3-6 months to the initial dose with stabilization or increase in the hemoglobin or platelet levels.
- If a response occurs, the general strategy then is to slowly taper the daily dose in 10-20% decrements every 6 months until a dose is obtained which is still effective and side effects minimal/acceptable.
- The side effect of height and weight gain effectively reduces the individual's dose per kilogram body weight. The "real" dose per kg should be recalculated prior to making dose adjustments.
- Every effort should be made to minimize the side effects by tapering the dose whenever possible.
- Long-term androgen usage at higher doses might lead to testis atrophy in males, due to suppression of the hypothalamic-pituitary-gonadal axis.
- Blood tests of the following hormones are recommended every 3-6 months: LH, FSH, estradiol, total testosterone (if elevated also free testosterone), DHEA-S, progesterone, SHBG (sex hormone binding globulin), AMH (anti-mueller-hormone), 17-OH-progesterone
- For girls before puberty and without menstruation transabdominal ultrasound with measurement of the size of the uterus is recommended every 6-12 months.
- Tanner stages should be regularly determined.
- If no response is seen after 6 months, in the absence of other causes of cytopenias (such as viral or bacterial infection), alternative treatment options need to be considered; Please contact registry team.
- Blood tests for liver function as well as lipid status are recommended every 3-6 months, and a liver ultrasound is recommended every 6-12 months.
- Transaminases do not always correlate with the degree of liver inflammation on liver biopsy. If liver transaminases increase to 3-5 times above normal, the androgen dose needs to be carefully tapered to see if the peripheral blood transaminase levels improve.
- Androgen-associated liver adenomas may develop with long-term androgen treatment, however predominantly with oxymetholone.
- These may resolve after androgens are discontinued, but some may persist even years after androgens are stopped.
- Liver adenomas are not a contraindication for stem cell transplantation.
- If screening tests raise a concern for adenocarcinoma, a liver biopsy using a technique appropriate to the patients bleeding risk should be considered.

- Malignant transformations of liver adenomas only occur after years of androgen treatment.

Side Effects of Androgens

- Virilization (mainly with the use of oxymethalone) (acne, facial hair growth/scalp hair loss, deepening of voice, pubic hair, enlargement of penis or clitoris, painful erection in small boys)
- Growth spurt followed by premature closure of epiphyses and short adult stature
- Hyperactivity and behavioral changes (puberty, aggressiveness)
- Cholestatic jaundice or transaminitis
- Abnormal lipid metabolism
- Hepatic adenoma or hepatoma, hepatocellular carcinoma
- Peliosis hepatis
- Hypertension

Cytokines

- Treatment with G-CSF may be considered if the neutropenia is associated with recurrent or serious infections, particularly if the neutrophil counts persistently fall below 500/mm^3 or fail to rise in response to infection. Treatment should generally be discontinued if the neutrophil count fails to improve after eight weeks of G-CSF therapy.
- A bone marrow aspirate/biopsy with cytogenetics is recommended prior to the initiation of cytokine treatment.
- It is reasonable to monitor the bone marrow morphology and cytogenetics every six months while patients are treated with cytokines.

Transfusion of blood products

- Acute transfusions with red blood cells may be necessary to manage symptoms of anemia or before surgery.
- Platelet transfusions are indicated in case of significant bleeding (thrombocytopenia) or prior to surgery.
- A patient should be transfused to maintain minimal trough hemoglobin levels, usually 7-8 g/dl, to ensure that they remain asymptomatic for the level of activity they choose to maintain.
- A post-transfusion hemoglobin of 10-12 g/dl is generally sufficient to allow for normal activity, growth, and development in children with a 3-4 week interval between transfusions.
- Amicar or related compounds may be used as an adjunct to platelet transfusion in a patient with mucosal bleeding. These drugs are generally contraindicated in patients with hematuria.
- Additional factors that increase bleeding risk should be minimized. Drugs that inhibit platelet function, such as aspirin or non-steroidal anti-inflammatory drugs (e.g., ibuprofen), should be avoided. A soft toothbrush should be used. Stool softeners should be administered if constipation poses a risk of GI mucosal trauma. Activities

carrying a high risk of significant trauma (particularly to the head or trunk) should be avoided. To date there are no data supporting the efficacy of thrombopoietin-mimetic drugs.

Treatment of MDS or AML

- Treatments should be carefully discussed with experts such as study team members of the Fanconi anemia and EWOG-MDS protocols.

HPV vaccination

- HPV vaccination is recommended starting at age 9 because it cannot be excluded that HPV contributes to FA-associated neoplasms. Both genders should be vaccinated.
- Regardless of prior HPV vaccination, patients with FA should be vaccinated after hematopoietic cell transplantation (HCT), when deemed appropriate.
- Ideally, FA patients will be yearly HPV-screened (Cervix/Portio) starting at age 20.

Prevention, Surveillance and Treatment of Head and Neck Squamous Cell Carcinoma

- The high incidence of head and neck SCCs at early age, combined with the limited therapeutic options for FA patients, underscore the importance of regular, lifelong and rigorous surveillance and early surgical interventions in order to achieve cure.
- Early surveillance is regardless of whether the patient has undergone a bone marrow transplant or not.
- As two thirds of head and neck SCCs in FA patients are located in the oral cavity, surveillance should ideally be performed by a specialist and should also include naso-, oro-, and hypopharynx as well as larynx and possibly esophagus, especially in older patients and immediately if there are any signs of reflux or dysphagia.
- Beginning not later than age 10, patients with FA should be examined with a special “Add-on Screening for FA-patients” every six months by an otolaryngologist, oral surgeon, or other doctor who is experienced in head and neck cancer detection and is ideally familiar with FA.
- The “Add-on Screening” applies to the case of a visible lesion: documentation employing mouth photography and a mouth map as well as a brush biopsy (analyzed at the reference cytopathology lab in Düsseldorf, Dr. Martin Schramm) are recommended every three months.
- In case of changes of the visible lesion over time or abnormal results on brush biopsy (ploidy as well as microscopy) a incisional biopsy is necessary.
- Information about the “Add-on Screening for FA-patients” can be found on: www.fanconi.de/scc . Also, teaching videos demonstrating the brush biopsy procedure and all materials and SOPs of the Screening can be found there.
- In case of reasonable suspicion of head and neck cancer, a complete survey (panendoscopy) of the upper aerodigestive tract including representative biopsies or a diagnostic excision of the suspected lesion must be performed.
- Malignant lesions must be treated immediately, as cure can best be achieved via early surgical removal. Further treatment must be discussed with a hematologist/oncologist with experience in FA or via a FA-specialized tumor board (Possibilities:

athttp://www.krebs-praedisposition.de/, https://www.fanconi.org/explore/virtual-tumor-board,https://faeurope.org/).

- The complete tumor extension must be documented and secondary carcinomas must be diagnosed/excluded. Aggressive monitoring by the treating surgeon is required.

Prevention, Surveillance and Treatment of Gynecologic Cancer

- Gynecologists caring for FA patients need to have a good understanding of the unique health issues associated with FA patients at different ages.
- Female FA patients face a variety of gynecological problems such as structural abnormalities, delayed puberty, decreased fertility, early menopause, and a high risk of SCC of the cervix, vagina, vulva, and anus.
- Beginning at age 13, female patients with FA should have annual examinations by a gynecologist for visual inspection of the external genitalia. Once sexually active, or by age 18, female patients with FA should receive comprehensive annual gynecologic exams with cervical cytology testing according to the standard screening program.
- Clinical experts recommend screening for gynecological cancer every 6-12 months because squamous intraepithelial lesions (SIL) can rapidly progress to cancer. Anal pap smears and anoscopy may be considered in female patients with FA who have vulvar disease.
- Suspicious genital tract lesions should be biopsied. Female patients diagnosed with anogenital SCC should be referred immediately to a gynecologic oncologist. Early referral may enable surgical treatment of the cancer, thereby avoiding the risks associated with chemotherapy or radiation for patients with FA .
- FA patients will have a very high susceptibility in all organs for the toxic side effects of platin derivatives and bi- or tri-functional alkylating agents (busulfan, melphalan, mitomycin C). Thus, we strongly recommend avoiding these drugs and encourage discussion of treatment options with FA-specialized tumor board (Possibilities: http://www.krebs-praedisposition.de/, https://www.fanconi.org/explore/virtual-tumor-board, https://faeurope.org/).

Breast Cancer Screening

- Some FA-patients can also develop breast cancer later in life.
- Five of the genes implicated in Fanconi anemia (FA) are breast cancer susceptibility genes: *FANCD1/BRCA2, FANCJ/BRIP1, FANCN/PALB2, FANCO/RAD51C,* and *FANCS/BRCA1*. However, breast cancer risk for individuals with FA who harbor variants in these genes or other FA genes has not been established yet.
- It is unclear whether the current mammography screening recommendations for carriers also apply to individuals with FA, as FA patients have an elevated sensitivity to radiation exposure due to their underlying genetic defects in DNA repair. The long-term risks of radiation exposure must be weighed against the benefits of early detection via mammography.

Fertility and Pregnancy in Female Patients

- Pregnancies have been reported in female patients with FA, in both those who were treated with HCT and those who were not.

- Primary ovarian insufficiency (POI) is common in female patients with FA. It is characterized by a spectrum of low ovarian reserve, declining ovarian function, reduced fertility, and estrogen deficiency.
- It is recommended that female patients with FA be treated either with oral contraceptive pills (if the patient is sexually active and pregnancy is not desired) or postmenopausal hormone therapy, which consists of low to physiologic doses of estrogen and progestins. Either approach is superior to no therapy regarding the effects on bone and other aspects of health.
- In general, the pregnancy rates in untransplanted FA women are in the range of 10%-20%, depending on other manifestations of FA and especially on the hematological parameters and the transplant status.
- Pregnancy in an FA woman is classified as high-risk and menopausal symptoms may present at a young age.

Reproductive Issues in Male Patients

- Male patients with FA may have numerous structural abnormalities of the reproductive system and extremely low sperm count that affect fertility.
- Many male patients with FA may have the following reproductive issues:
    - Delayed puberty
    - Undescended testicles and hypospadias
    - Small testes for their age and pubertal status, most likely reflecting reduced Sertoli cell mass and spermatogenesis
    - Low levels of sex hormone production due to underlying problems with the pituitary gland or hypothalamus
    - Azoospermia

Annual Endocrine Evaluations

- Endocrine abnormalities occur frequently in FA patients. The affected systems often include but are not limited to GH regulation, thyreoid hormons, glucose metabolism, cortisol levels and gonadal hormone productions. Growth should be monitored routinely as well as puberty development.
  Appropriate testing may include: 8:00-10:00 a.m. serum cortisol, TSH and FT4, oral glucose tolerance test (recommended at initial evaluation), HbA1c, 25OH vitamin D.
  If the growth rate is too slow: AM FT4, TSH, IGF-I, IGFBP3, bone age.
  If delayed puberty: LH, FSH, estradiol or testosterone, DXA (bone density scan), bone age
- Use of growth hormone:
  Brain MRI is recommended prior to commencing GH treatment, which is indicated in children who are growth deficient due to proven growth hormone deficiency.

Additional Evaluations

- FA patients need to be evaluated at diagnosis concerning hearing loss or structural ear anomalies.