Chapter 10

Medical Management of Bone Marrow Failure in Telomere Biology Disorders

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Introduction

Bone marrow failure (BMF) is a common complication in telomere biology disorders (TBD), and in particular in classic dyskeratosis congenita (DC). As many as 80% of individuals with DC develop BMF by age 30 years [1, 2]. Many individuals diagnosed with TBD have some degree of abnormality in the complete blood count (CBC) profile which may range from minor findings such as macrocytosis (high mean corpuscular volume [MCV] for age, due to large red blood cells), to mild asymptomatic cytopenias in one or more blood cell lineage, to symptomatic BMF

(also referred to as severe aplastic anemia). Some patients may develop progressive abnormalities (dysplasia) in the bone marrow hematopoietic cells, which may subsequently evolve into myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) [3-5].

The time of onset of BMF is highly variable among individuals. Infants or young children with severe forms of DC may present with progressive BMF early in life even before other signs of DC have appeared, whereas older individuals with TBD (particularly those with variants in *TERC* or *TERT*) may develop blood cell abnormalities much later in life or never. The first cytopenia to appear usually is low platelet count, followed by anemia and/or neutropenia [6, 7].

Definition of Bone Marrow Failure

The diagnosis of BMF is made if the blood counts are persistently below the age-appropriate normal values due to the failure of the bone marrow to produce an adequate amount of normal blood cells. Other causes of low blood counts such as infection, medications, peripheral blood cell destruction or nutritional deficiencies should be ruled out first.

The classification of bone marrow failure in DC and TBD is similar to that for Fanconi anemia and is currently based on the consensus guidelines developed for the management of BMF in Fanconi anemia [8]. Patients who are transfusion dependent for either red blood cells or platelets are considered to have severe disease by default.

Table 1. Classification of bone marrow failure.

Bone Marrow Failure	Mild	Moderate	Severe
Absolute neutrophil count/mm^3	1,000 to less than normal for age	500 - 1000	<500
Platelets/mm^3	50,000 to less than normal for age	30,000 – 50,000	<30,000
Hemoglobin gm/dL	8.0 to less than normal for age		<8.0

The criteria proposed by Camitta and colleagues for diagnosis of immune aplastic anemia can also be applied to denote severe marrow failure in this setting and is defined as: absolute neutrophil count <500/mm^3, platelets <20,000/mm^3, and absolute reticulocyte count <60,000/mm^3.

In an individual with suspected or diagnosed with TBD, a CBC and bone marrow examination should be performed to determine the baseline hematologic status (whether BMF, myelodysplasia, or an abnormal karyotype are present).

Bone marrow examination should consist of both a biopsy and an aspirate. The biopsy is to assess the marrow architecture and cellularity and an aspirate is to determine whether cells within the bone marrow are morphologically normal or abnormal. A sample of the bone marrow aspirate should be sent for cytogenetic evaluation by G-banding and for FISH studies to look for common clonal cytogenetic abnormalities associated with MDS. Next generation sequencing panels for mutations in myeloid cancer genes can be performed, though their significance in the absence of MDS remains unclear. Some degree of cellular morphologic dysplasia is common in individuals with TBD (and in other IBMFS), therefore the presence of minor dysplastic changes in erythroid, myeloid and/or megakaryocyte lineages should not be misconstrued as diagnostic of MDS. A careful evaluation of the baseline bone marrow and subsequent marrows by a hematopathologist with an expertise in marrow failure syndromes is generally warranted to diagnose MDS in TBD.

Monitoring of Bone Marrow Failure

Periodic monitoring of blood counts and bone marrow are important to assess the progression of disease so that timely and appropriate therapeutic intervention can be initiated.

Individuals with non-DC TBD, particularly in the absence of cytopenias or marrow failure, may not require regular bone marrow examination. Guidelines may change over time as new data on the clinical spectrum, heterogeneity of manifestations, progression, treatments, and associated complications become available. There is no consensus on the frequency of bone marrow examination required in individuals with TBD, and it will depend on the clinical background, physician preference, and patient choice. Here we present a potential algorithm, though guidelines may be modified by a treating hematologist and tailored towards individual patient needs as clinically indicated. Individuals with a strong family history of hematologic malignancy, exposure to chemotherapy or radiation, or with a higher risk germline variant may warrant closer follow up.

For patients with:

1. **Normal blood counts and no cytogenetic abnormality**
    - CBC every 6-12 months
    - Bone marrow aspirate/biopsy and cytogenetic testing should be performed once at baseline. If no abnormalities are present on baseline bone marrow and CBC remains normal it is reasonable to repeat bone marrow only if a cytopenia develops.
2. **Stable but mildly low blood counts and no cytogenetic abnormality**
    - CBC every 3-4 months
    - Bone marrow aspirate/biopsy and cytogenetic testing should be performed at baseline. Depending on clinical background, physician preference, and patient choice, bone marrow can be performed at regular

intervals (1-3 years) or can be deferred unless a drop in blood counts occurs.

3. **Blood counts falling or rising:** More frequent monitoring of CBC ± bone marrow evaluation may be indicated.
    - Blood counts may decrease after an episode of infection due to limited bone marrow reserve. Most often the counts will return to the patient's baseline levels within a few weeks after recovery.
    - In patients with progressively declining blood counts without an apparent cause, further investigations with more frequent CBCs and bone marrows for morphology and cytogenetics is indicated. Appropriate plans for intervention should be in place for progressive BMF, or development of MDS or AML.
4. **Clonal cytogenetic abnormality/s:** Presence of a cytogenetic clone by itself (without morphologic evidence of MDS) does not necessarily indicate a diagnosis of MDS. In our experience with TBD, some patients have clonal cytogenetic changes that have persisted for several years (over 10-15 years) without progression to MDS or leukemia. However, MDS associated chromosomal abnormalities should prompt regular monitoring and high risk changes such as monosomy 7 should prompt referral for hematopoietic cell transplantation (HCT).

    General recommendations for clonal cytogenetic abnormalities:
    - Monitor CBC every 1-2 or 2-3 months based on the stability of the blood counts.
    - Repeat bone marrow (with cytogenetics and FISH studies) every 4-6 months for a minimum of 2 -3 times; if the blood counts are stable and the bone marrow clone is unchanged without progression, and the bone marrow morphology is not diagnostic of MDS, it may be safe to revert to annual bone marrow exams.

    Advice for MDS associated cytogenetic abnormalities:

- If MDS associated cytogenetic abnormalities are detected, appropriate plans for HCT should be in place as patients may evolve rapidly to MDS or leukemia. Chromosome 7 changes (in particular monosomy 7) are associated with high risk of leukemic progression and with poor overall survival in BMF and urgent evaluation should be performed for HCT in this situation.

- Other clonal cytogenetic abnormalities such as loss of chromosome 5, trisomy 8, 11q23 translocation, 20q, and 3q abnormalities are known to occur in patients with MDS and in association with transformation to AML. Presence of such clones would need more frequent monitoring of CBC and bone marrow.

Treatment Options for Bone Marrow Failure

Treatment is recommended in patients with persistent severe BMF such as transfusion dependence or blood counts in the moderate-severe range. Immunosuppressive therapy is not recommended for TBD-associated BMF due to lack of demonstrated efficacy. In contrast to acquired aplastic anemia, patients with TBD have a genetic rather than an immune pathophysiology of marrow failure and do not appear to respond to immunosuppressive therapy [9]. Treatment options for TBD-associated BMF include HCT or androgens.

Hematopoietic Cell Transplant (HCT)

HCT is the only curative treatment for BMF or other hematologic complications (MDS, leukemia) in patients with TBD, and is considered the treatment of choice in eligible patients with severe disease. The ideal donor is a matched related donor, proven to not have TBD by physical and laboratory examinations, mutation testing and/or telomere length assay. HCT from an unrelated or alternative source donor such as haploidentical or umbilical cord can be considered for those lacking a fully matched related donor.

However, poor long-term outcomes have been observed in TBD patients post HCT, particularly related to lung toxicity. HCT, while curative of BMF or MDS/AML, does not address the other organ dysfunction seen in TBD. HCT is discussed further in Chapter 13, Hematopoietic Stem Cell Transplantation.

Androgens

Androgens are anabolic steroids that have been in use for a variety of conditions for over 50 years, including for the treatment of acquired BMF and for Fanconi anemia. The published literature of androgen use in TBD has shown varying results but overall suggests that androgen treatment is a reasonable option in patients to improve blood counts, especially hemoglobin. While patients with severe hematologic disease, as fulfilling criteria for severe AA, would typically undergo HCT, some may not be medically eligible (related to concurrent multi-organ disease), lack suitable donors, or choose not to. Additionally, androgens may be a good option for patients with either moderate or severe unilineage cytopenias that could clinically benefit from increased blood counts. Many patients with TBD show a hematopoietic response to androgens with sustained improvement in hemoglobin, platelets and neutrophil counts. Androgens however, have side effects, and patients with TBD seem to be particularly sensitive to the effects of androgens.

The most common side effects reported with androgens are:

- Virilization (or masculinization in females and children) with facial and pubic hair growth, scalp hair loss, penile/clitoris enlargement, deepening of the voice, and acne
- Behavioral changes (e.g. aggression, mood swings)
- Liver toxicity (increase in transaminase and/or bilirubin)
- Alteration in blood lipid profile resulting in abnormally low HDL and high LDL levels
- Growth spurt in children which may result in premature closure of epiphysis (growth plates) and short adult height

- Liver adenomas, peliosis (blood lakes) in spleen and/or liver, and rarely hepatocellular carcinomas

A baseline CBC, hepatic panel, liver and spleen ultrasound, lipid profile, thyroid function, and an x-ray hand for bone age (in a growing child) should be obtained prior to starting therapy. Once the treatment has begun, two to three months at a constant dose is a fair trial, monitoring for hematologic improvement. After the blood counts have stabilized, the androgen dose may be gradually decreased over the next several months (2-4 or 6 months) to the lowest effective dose required to maintain stable blood counts depending on the patient's side effect profile. Close medical supervision and dose adjustments help to achieve the minimum effective dose with least side effects.

Danazol is a synthetic androgen derivative used in the treatment of TBD-related BMF. One prospective clinical trial using 800 mg (16mg/kg in children <50kg) of danazol showed hematologic responses of 79% and 83% after 3 and 6 months of therapy respectively. The majority of patients had *TERT*/*TERC* variants, with only four having other TBD-related variants (3 *DKC1* and 1 heterozygous *RTEL1*) and 6 having no variant identified. Frequent adverse effects were elevation in liver enzymes (41%), muscle cramps (33%), edema (26%) and lipid abnormalities (26%). Liver hemangioma developed in one enrolled patient requiring cessation of therapy [10]. Other retrospective studies and case series have also demonstrated the hematologic efficacy of danazol in patients with Fanconi anemia and TBD/DC with no severe or unacceptable side effects [11-13].

Other androgens such as oxymetholone or nandrolone are also used in TBD-related BMF. Masculinization in females often occurs with oxymetholone and may limit its use. A lower dose is generally recommended in TBD than in patients with Fanconi anemia because patients with TBD may be more sensitive to the effects of androgens. One analysis looking at patients enrolled on a long-term cohort study (n=16) who had received androgens (oxymetholone [n=14], nandrolone [n=1], and fluoxymesterone [n=1]) showed an overall hematologic response rate of 69%; the majority of patients had

variants in *DKC1*, *TINF2*, and *RTEL1* (2 autosomal recessive, 2 autosomal dominant) [14].

Danazol may reduce the rate of telomere attrition in patients with TBD. In a prospective clinical trial, 16/21 (76%) patients assessed at 6 months and 11/12 (92%) patients at 24 months on danazol had a gain in their telomere length, over baseline [10]. Of note, this initial analysis was performed using qPCR to measure telomere length, in lymphocytes and granulocytes combined, whereas flow-FISH using lymphocyte subsets is now considered the clinical standard. Since then, two subsequent retrospective studies looking at androgens in TBD patients have been reported using lymphocytes measured by flow-FISH. One study comparing telomere length over time in 10 androgen-treated and 16 androgen-untreated patients showed no difference in telomere attrition between the two groups [15]. The other study of 7 patients with TBD showed telomere length improvement in lymphocytes by flow-FISH in all patients. Discordant findings may relate to differences in the frequency of specific inherited mutations in each study, as those showing elongation in telomere length tended to have more androgen treated patients with *TERT*/*TERC* variants [13].

Points Regarding Androgen Therapy

1. Androgen treatment does not cure bone marrow failure but can produce a sustained rise in the blood counts for the duration of the treatment. In some patients this may be several years (e.g. 10-15 years or even longer).
2. Blood counts do not generally reach normal values with androgen treatment but may improve to the extent that a previously transfusion-dependent patient may no longer need red blood cell or platelet transfusion support.
3. Androgens are likely to be more effective in patients who have some degree of bone marrow reserve than in those whose marrow reserve is severely depleted. Patients on androgen therapy may become refractory over time if their bone marrow hematopoietic cellular content becomes depleted.

4. Androgens do not prevent or delay the progression to MDS or AML, nor is there evidence they drive progression.

In patients who have not shown a response to the treatment after a trial of up to 6 months, androgens should be discontinued. Occasionally, some patients who initially did not respond to one androgen may subsequently respond to a different androgen.

Monitoring for the Side Effects of Androgens

Patients on androgen treatment should have baseline clinical and laboratory evaluations and at regular follow-up visits while on treatment as outlined in the table below.

Table 2. Recommended clinical and laboratory evaluations for patients on androgen treatment.

Parameter	Before treatment	On treatment
CBC	Baseline CBC	Repeat every 4-6 weeks until counts are stable, then 2-3 months
LFT	Baseline AST, ALT, bilirubin, gamma GT	Every 1-2 weeks for first month then every 6 – 12 weeks
Lipid profile[1]	Baseline cholesterol, LDL, HDL, triglycerides	Every 6 – 12 months
Thyroid function[2]	Thyroid binding globulin (TBG)	May repeat TBG annually
Liver/spleen ultrasound	Baseline	Every 6 months
Bone age (children)	Baseline in a growing child	Every 6 – 12 months until growth plates fuse
Endocrine evaluation	Baseline	Annually
Height and weight	Baseline	Every visit

[1] Persistently low HDL and high LDL levels may be of concern for future cardiovascular risk in patients on long-term (2-5 years or more) androgen therapy. These patients should be regularly followed by an endocrinologist. Lipid levels usually return to baseline values within 3-6 months after stopping androgens.

[2] Thyroid function is not affected by androgen treatment but thyroid binding globulin levels are decreased in patients on androgens.

Patients who have not shown response to androgen treatment after adequate trial or who have become refractory to androgen treatment may consider HCT. There is currently no evidence that androgen treatment increases the risk of future stem cell transplant-related complications.

Other Treatments

Prednisone (5 mg/day or every other day) in combination with androgens was used in the past to delay the early closure of epiphyses (growth plates). This use is no longer recommended because there are no data to support its beneficial effects and prednisone can cause avascular necrosis and early bone loss (osteopenia/osteoporosis).

Cytokines: Hematopoietic growth factors such as G-CSF or GM-CSF can achieve temporary improvement in counts and may be useful in patients with persistent neutropenia (neutrophil count <500/mm^3) in the presence of recurrent or serious infection. However, there is theoretical concern that growth factor use may stimulate the proliferation of a pre-existing clone and malignant transformation. Splenic peliosis and splenic rupture has been observed when G-CSF was used in combination with androgens [16]. Therefore G-CSF or GM-CSF is not recommended in combination with androgens in patients with TBD.

Eltrombopag: Very little data exists on the use of eltrombopag in TBD. In one French study looking at eltrombopag in SAA patients, two were later found to have TBD variants, and neither had responded to eltrombopag [17]. In a NIH clinical trial using eltrombopag in MAA, one TBD patient was included who was deemed a responder [18]. Given the lack of evidence to date, eltrombopag should be considered investigational for patients with TBD and administered in the setting of a clinical trial.

Investigational protocols: Investigational protocols may be considered for patients who are not candidates for HCT and who fail to respond to androgen treatment.

Management Guidelines for Bone Marrow Failure

Clinical management of TBD is complex because several systems may be affected simultaneously to varying degrees, and phenotype may greatly differ between patients.

A treatment approach that works for one patient may not be ideal for another. Therefore, the risks and benefits of available treatments should be discussed with a hematologist with expertise in the care of patients with TBD.

A broad general approach to treatment of BMF is outlined below:

At the time of diagnosis of TBD

- The patient should be evaluated and followed by a hematologist for medical monitoring and management. A detailed assessment of all systems should be undertaken (as per the TBD guidelines) to assess the degree of involvement of other systems. A consult with a hematologist with an expertise in TBD should be sought.
- For patients with any degree of cytopenia, treatment options for bone marrow failure should be discussed in case cytopenias progress to the extent of needing treatment. Early discussion with a HCT team with an expertise in transplanting TBD patients may be considered. HLA-typing and genetic mutation testing of family members for TBD should be considered to assess the availability of a potential HCT donor and exclude any potential affected family member as a donor.
- Families should be referred for appropriate medical counseling as some may wish to have more children and may be interested in pursuing prenatal screening. Preimplantation genetic diagnosis (PGD) and selection of unaffected embryo (who is also an HLA match) for the patient can also be considered.

Normal blood counts or mild bone marrow failure or moderate bone marrow failure

- Monitor CBC and bone marrow as described earlier (see monitoring of BMF) until further treatment is needed.

- Continue discussions regarding treatment options. For patients with declining counts, consider referral to HCT team if not already done; however, HCT need not be undertaken until the development of severe marrow failure or MDS/AML.
- A donor should be identified, most preferably an HLA-identical sibling (proven to be telomere mutation negative), but a matched unrelated donor or alternate donor may also be considered as necessary.
- Consider androgen therapy for patients with clinically significant cytopenias.

Severe bone marrow failure

- Consider HCT for eligible patients
- Begin androgen treatment for patients who are not candidates for HCT due to lack of suitable donor, medical ineligibility, high risk transplant stratus, or unwillingness to undergo HCT.

Severe bone marrow failure unresponsive to androgens and high risk for transplant

- Consider cytokines, supportive care, or investigational protocols

MDS or AML

The diagnosis of MDS in a patient with TBD should be confirmed by a hematopathologist with an expertise in these disorders.

No standard effective therapy other than HCT has been established for MDS or AML associated with TBD.

- Patients should be referred for HCT with or without prior induction chemotherapy
- Phase I/II trials may be considered for patients ineligible for HCT

See Chapter 13, Hematopoietic Stem Cell Transplantation for further information on this topic.

Supportive Care

Some patients with TBD may need red blood cell and/or platelet transfusion support prior to initiation of definitive treatment, before therapy becomes effective, or if other treatments have failed. Timely referral to a transplant center for consideration of HCT should be made for patients who become transfusion dependent.

Anemia: Red blood cell transfusion may be unavoidable and have few immediate adverse consequences. However, chronic red blood cell transfusions may adversely affect transplant outcomes.

Patients receiving many red blood cell transfusions should be monitored for iron overload by at least serum ferritin. T2* MRI of heart and liver or other appropriate studies should be performed if organ damage is suspected. Appropriate treatment with iron chelators such as deferoxamine (Desferal) or deferasirox (Exjade) should be initiated if iron overload is present.

Thrombocytopenia: Platelet transfusions may be indicated in patients with severe thrombocytopenia, or in those undergoing invasive procedures or with mucosal bleeding. Amicar or tranexamic acid may be used as an adjunct to platelet transfusions in patients with mucosal bleeding.

Non-steroidal anti-inflammatory drugs, aspirin and other drugs that inhibit platelet function should be avoided in patients with platelet counts $<50x10^9$/L.

Activities carrying high risk of trauma (e.g. contact sports) should be avoided in patients with platelet counts $<50x10^9$/L.

Neutropenia: G-CSF may be considered in patients with severe neutropenia and concurrent infection. G-CSF should not be used in patients on androgens as the risk of splenic peliosis with rupture may be higher with this combination.

Ongoing Clinical Research

Further studies looking at the use of androgens in patients in TBD are ongoing. Research objectives include: use of lower doses of danazol, assessing the use of danazol for other organ dysfunction in telomere disease such as liver and lung fibrosis, and evaluating the effect of danazol and other androgens on telomere length over time.

Studies looking at ways to reduce post-HCT toxicity using reduced intensity conditioning are also currently underway and may result in more favorable long-term outcomes. Additionally, gene therapy for patients with TBD is currently under investigation.

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