

# Bone Marrow Failure

## I. Aplastic anemia

### A. Definition

1. Reduced bone marrow tissues
2. Fatty replacement of marrow
3. Peripheral blood cytopenia(s)

### B. Etiology

1. 50-75% are idiopathic
2. Approximately 5% are associated with viral hepatitis, although the disease is apparently not directly caused by a known hepatitis virus. It is likely immunologically-mediated. (Brown)
3. 10-15% are associated with drug toxicity
4. Risk ratios for certain exposures defined in a 1993 French study (Baumelou).
5. Patients with aplastic anemia are twice as likely to have HLA DR2 than the general population.

Risk of Exposure for AA Patients		
Exposure	Odds Ratio	95% C.I.
Hepatitis*	6.0	0.7-50
Rheumatoid Arthritis**	5.5	1.4-20.9
Gold salts	4.9	1.9-20
Salicylates	1.8	1.2-2.6
Actaminophen	1.8	1.1-3.0
D-penicillamine	4.9	0.9-27
Colchicine	4.1	1.3-13
Allo/thiopurinol	3.6	1.3-9.7
Chloramphenicol	9.0	0.5-66

\* vs. control group of neighbors

\*\* vs. hospitalized control group

### C. Pathogenesis

1. Autoimmune disease
  - a. T-cell mediated, organ specific destruction of bone marrow hematopoietic cells
    - i. Increased production of interferon- $\gamma$ , tumor necrosis factor, and interleukin-2, all of which are myelosuppressive
    - ii. This profile is characteristic of the TH1 lymphocyte population, similar to what is seen in multiple sclerosis and diabetes
  - b. Immature (CD34+) hematopoietic precursors are destroyed directly and/or experience cell cycle arrest
  - c. The antigens driving this activity are generally not known
2. Is aplastic anemia a clonal disease?
  - a. PNH, which is known to be clonal, occurs in aplastic anemia and vice-versa
    - i. A defect in the glycosylphosphatidylinositol anchor on the cell membrane may develop in 35% - 40% of patients followed long enough. It is usually demonstrable on granulocytes, and rarely on RBCs.
    - b. Some long-term survivors with aplastic anemia develop evidence for clonal hematopoiesis, while some have clonal hematopoiesis at presentation.
    - c. A variety of clonal defects are found, with numeric or structural abnormalities of chromosome 7 the most common, followed by trisomy 8, abnormalities of 13, and deletion of Y.
    - d. The mechanism for development of clonality is unknown, but there is speculation
      - i. Loss of hematopoietic stem cells could restrict the number of available clones
      - ii. Clones could be selected. For example, cells lacking a ligand for cytotoxic lymphocytes would be less susceptible to attack. This might favor the survival of PNH clones, for example. Dunn, et al present evidence favoring this mechanism.
      - iii. Attack by cytotoxic lymphs might induce genetic damage in hematopoietic progenitors.
3. Drugs

- a. Chloramphenicol
    - i. Causes irreversible, fatal marrow suppression in 1/20,000 - 30,000 cases (This is 10-15x the risk of the general population).
      - a). Not related to drug dosage
      - b). Oral route may be more dangerous than parenteral
      - c). Onset may be sometime after cessation of therapy
      - d). The traditional teaching is that the drug causes genetic damage to stem cells. Reported recovery after immunosuppressive therapy lends credence to the notion that regardless of etiology, the final common pathway for marrow damage is immune-mediated.
    - ii. Many patients experience brief, reversible marrow suppression.
      - a). Related to dose and length of treatment
      - b). Marrow depression occurs during therapy.
      - c). Presumably due to disturbed proliferation or maturation of marrow elements
    - iii. Chloramphenicol is a nitrobenzene compound, thus belonging to a class known to be marrow toxic.
    - iv. Spatially, it resembles uridine-5-phosphate.
      - a). Could lead to proposed binding to ribosomes
      - b). Causes reduced mitochondrial protein synthesis in mammalian cells - especially ferrochelatase
    - v. Can induce chromosomal vacuolization
    - vi. Proposed role as a hapten, inducing antibodies against stem cells
  - b. Benzene
    - i. Associated with many hematologic abnormalities in man
      - a). Hemolysis
      - b). AML
      - c). Hypo/hyperplasia
      - d). Myeloid metaplasia
    - ii. Dose related marrow suppression
    - iii. Occurs soon after exposure to benzene
    - iv. In mice, the DNA synthesis of differentiated cells is affected
  - c. Other drugs
    - i. Insecticides - DDT, etc.
    - ii. Toluene
    - iii. Sulfonamides
    - iv. Hydantoins
    - v. Gold
    - vi. Quinacrine
    - vii. Phenylbutazone
4. Radiation
- a. Biologic effectiveness depends on amount and type of radiation, and the radiosensitivity of the irradiated tissue
  - b. Definitions
    - i. 1 rad is the amount of radiation that causes  $10^{12}$  ionizations/gram or releases 100 ergs/gram of tissue
    - ii. 1 grey (Gy) = 100 rad
  - c. About 700 r whole body irradiation is uniformly fatal
  - d. The most radiosensitive tissues are
    - i. Germinal epithelium of the testes
    - ii. Hematopoietic cells

- iii. Gut epithelium
- iv. Basal dermis
- e. Following exposure of the marrow
  - i. Hypoplastic period of 3-6 weeks
  - ii. Recovery may be complete, but typically cytopenias persist, and the marrow appears fibrosed and dysplastic.
  - iii. Erythroid series is the most sensitive, followed by the myeloid, then megakaryocytic series.
  - iv. Lymphocytes are quite sensitive to radiation, some more than others.
  - v. Long-term, low-level exposure has also led to marrow aplasia.
- 5. Viral infection
  - a. Viral hepatitis associated with fulminant aplasia
    - i. Viral induced marrow suppression?
    - ii. More likely, immune mediated
    - iii. Seen with hepatitis B. In cases associated with non-A, non-B hepatitis, hepatitis C does not appear to be the cause.
    - iv. 9/32 patients receiving liver transplants for non-A, non-B hepatitis developed aplastic anemia within 7 weeks, as opposed to none of 1463 patients transplanted for other indications (Tzakis).
  - b. EB viral DNA can be found in the marrow of some affected patients (Baranski)
- 6. Miscellaneous
  - a. PNH
  - b. Pregnancy - case report of remission following normal delivery
  - d. Graft versus host disease
  - e. Lymphoproliferative disease of granular T-lymphocytes (T-LDGL) is an uncommon clonal disorder of cytotoxic T-cells, that may present as aplastic anemia.
- D. Clinical aspects
  - 1. Insidious onset
    - a. Weakness, fatigue
    - b. Fever
    - c. Petechiae, bleeding
  - 2. Physical findings
    - a. Retinal hemorrhage
    - b. Pallor
    - c. Petechiae, purpura, ecchymoses
    - d. No splenomegaly or lymphadenopathy
  - 3. Males and females equally at risk
  - 4. Incidence tends to increase with age
  - 5. Staging system - The International Aplastic Anemia Study Group uses the following criteria to define severe aplastic anemia:
    - a. Granulocytes  $<0.5 \times 10^9/L$
    - b. Platelets  $<20 \times 10^9/L$
    - c. Corrected reticulocyte count  $<1\%$  or absolute count  $<40 \times 10^9/L$
    - d. Severe marrow hypocellularity ( $<25\%$ )
    - e. Moderate marrow hypocellularity ( $<50\%$ ) with  $<30\%$  residual hematopoietic cells
    - f. Severe aplastic anemia defined as (2 of a,b,c) plus either d or e.
    - g. In addition, there must not be "significant" marrow fibrosis.
- E. Laboratory aspects
  - 1. Pancytopenia
  - 2. Red cells
    - a. Normocytic or macrocytic

- b. Retic count very low
- c. Nucleated red cells are not a feature
- 3. Leukocytes
  - a. Granulocytopenia always present, and severity is a prognostic factor
  - b. Monocytopenia usual
  - c. Lymphopenia not usual, but can occur. B and T-cell populations are present
- 4. Thrombocytopenia may persist for years after recovery of the other elements.
- 5. Serum iron and saturation are increased
  - a. Serum Fe clearance is slow with hypoplastic, and rapid with hyperplastic marrows
  - b. Iron incorporation into red cells is subnormal
  - c. Plasma iron turnover is fairly normal, because Fe incorporation into other tissues is somewhat increased.
- 6. Serum and urine erythropoietin is increased over the level found in other anemias with identical hematocrits.
  - a. Older aplastic anemia cells have decreased 2,3-DPG and increased O<sub>2</sub> affinity, which could lead to decreased tissue oxygenation and higher erythropoietin.
  - b. Erythropoietin consumed by active marrow?
  - c. Erythropoietin secretion regulated by some index of red cell production (as opposed to red cell concentration)?
- 7. Occasionally, fetal hemoglobin is increased, and rarely up to 1.5 gm/100ml
- 8. Bone marrow
  - a. Mainly fatty
  - b. Sparse cellularity
  - c. Megaloblastoid changes sometimes
  - d. "Dry tap" not uncommon - need biopsy to make the diagnosis

#### F. Therapy

- 1. Supportive treatment
  - a. Packed red cell transfusion when patient is symptomatic
    - i. Requirement increases with fever, infection, bleeding
    - ii. Hemochromatosis may result from transfusion and increased iron absorption.
  - b. Platelet transfusion
    - i. Eventually, sensitization limits the effectiveness of transfusions
    - ii. Fever and infection shorten platelet survival
    - iii. Epsilon aminocaproic acid, an inhibitor of fibrinolysis, may decrease bleeding in these patients.
  - c. Granulocyte transfusion for bacteremia unresponsive to appropriate antibiotics
- 2. Current treatments of choice
  - a. Bone marrow transplantation
    - i. Cure rate is about 70% for transplantation from an HLA-matched sibling. For unrelated donors or mismatched relatives, the results are not that good.
    - ii. Treatment of choice for patients under age 20 with a matched donor
    - iii. Worth doing in those under age 45 with a matched, related donor
    - iv. Most effective when employed early in the course, before the patient has become sensitized by multiple transfusions.
    - v. Long-term results in those surviving at least 2 years (Deeg)
      - a). Patients without chronic GVHD had 89% survival at 20 years
      - b). Patients with chronic GVHD had 69% survival at 20 years, and experienced more morbidity
      - c). Overall performance status for these patients was very good.
  - b. Immunosuppression

- i. Antithymocyte or antilymphocyte globulin, given with or without high-dose corticosteroids
  - a). Induces hematologic improvement in 40-80% of patients, with comparable increase in one year survival.
  - b). ATG/ALG eliminate T-suppressor cells, which secrete myeloinhibitory substances such as IFN-gamma, TNF- $\alpha$ , lymphotoxin, and transforming growth factor- $\beta$ .
  - c). Elimination of T-suppressor cells by monoclonal antibodies does not produce clinical improvement, implying that ATG/ALG have another mechanism of action. This may be the induction of colony stimulating factor release from T cells.
  - d). Efficacy varies from lot to lot
  - e). Toxicity
    - 1). Fever
    - 2). Urticaria
    - 3). Serum sickness - fever, rash, pruritis, arthralgia, myalgia, headache, nausea, lymphadenopathy. Onset 5 to 29 days into treatment, lasting for several days.
- ii. Cyclosporine
  - a). Fungal metabolite
  - b). Effects
    - 1). Inhibits IL-2 and IFN-gamma production by T cells
    - 2). Blocks induction of IL-2 receptors
    - 3). Does not inhibit elaboration of growth factors
  - c). Comparable effectiveness to ATG. One may be effective where the other has failed.
    - 1). CSA produced survival identical to ATG plus prednisone in a French study (Gluckman)
    - 2). Nonresponders to the initial regimen were crossed over at 3 months
    - 3). ATG/prednisone patients had higher infection rate
    - 4). Overall response rate 36% at 12 months
  - d). Toxicity
    - 1). Hepatotoxicity occurs early and is moderately severe
    - 2). Renal failure
    - 3). Hypertrichosis
    - 4). Gingival hyperplasia
    - 5). Tremor
- iii. Combined treatment
  - a). Frickhofen Regimen
    - 1). ALG 0.75 ml/kg/day via CVP line over 8-12 hours daily x 8
    - 2). Methylprednisolone 5 mg/kg iv or po days 1 to 8 and 1 mg/kg on days 9 to 14, then taper to day 29
    - 3). Cyclosporine 6 mg/kg po bid for 3 months. If no response, discontinue. If response present, continue until a plateau has been maintained for at least 1 month. Then taper over 1 month.
    - 4). Cyclosporine appeared to ameliorate ALG toxicity
    - 5). After 11 years of follow up, it is evident that adding cyclosporine effectively doubles the response rate to treatment, and accelerates the time to response. Overall survival is not

much affected because salvage regimens are good. One-quarter of cyclosporine responders require maintenance therapy.

b). GITMO Regimen

- 1). ALG 15 mg/kg IV d 1-5
- 2). Cyclosporine 5 mg/kg/d x at least 6 months
- 3). Methylprednisolone 2 mg/kg daily x 5, then halve the dose every 5 days until discontinued on day 30
- 4). G-CSF 5 mcg/kg daily x 90 days
- 5). 48/100 patients had complete response, and another 29 had partial response. Overall survival was 87% at 5 years.

iv. High-dose cyclophosphamide given to patients who would have been candidates for allogeneic bone marrow transplantation but lacked a suitable donor produced impressive results (Brodsky). Of 19 patients, 65% had complete remission, 73% no longer required transfusion, and 84% were alive at 2 years.

v. Immunosuppression also works in older patients (age > 60), but there is increased mortality due to the side effects of treatment.

vi. Presence of interferon- $\gamma$  in circulating T-cells of aplastic anemia patients correlates strongly with responsiveness to immunosuppressive therapy (Sloand).

3. Less favored treatments

a. Androgens

- i. Increase both erythropoietin release and sensitivity of marrow stem cells to erythropoietin
- ii. Parenteral therapy preferred as there is a lower incidence of cholestatic jaundice
- iii. Response may be delayed for up to three months
- iv. Switching to another androgen may work when one has failed.
- v. Overall effectiveness is poor, around 10%

b. Splenectomy not rational, although there have been rare remissions following the procedure.

c. cAMP and PgG are inhibitors of colony stimulating activity, and treatment with indomethacin or bethanecol is effective in rare cases.

d. Acyclovir has induced remissions in a limited number of hepatitis-associated cases

e. Cyclophosphamide is effective in occasional cases.

f. High-dose immunoglobulin is reportedly effective in some centers, inducing at least one prolonged remission. Responsive cases are rare, however.

g. GM-CSF produces dose-related increases in neutrophils, monocytes, and eosinophils, especially in less severely affected patients. These effects last only as long as the growth factor is given, however.

- i. Given on days 14 - 28 after ALG treatment, GM-CSF led to quicker recovery of granulocytes and fewer days with fever (Vadhan-Raj).

h. G-CSF is effective in over half of children with aplastic anemia, but counts revert to baseline as soon as treatment is stopped.

i. IL-3 may produce transient increases in all blood cell lines, but has not been adequately studied.

- i. IL-3 for 20 days induced marrow responsiveness to G-CSF in one patient (Geissler)

4. Complications of treatment

- a. The relative risk for developing malignancy is  $\approx 5.5$  following treatment for aplastic anemia

- i. Cases treated by marrow transplantation have increased risk of developing solid tumors
- ii. Cases treated with immunosuppression have the above risk, as well as a high risk of developing myelodysplasia or leukemia.

## II. Fanconi's anemia

A. Syndrome of familial pancytopenia with hypoplastic marrow and various congenital abnormalities, first described by Fanconi in 1927.

### B. Pathogenesis

1. Genetically heterogeneous defects lead to the production of a mutant protein (FAC polypeptide).
2. Defect is inability to maintain/repair damaged DNA.
  - a. Fanconi's anemia cell lines have a high rate of chromosomal breakage when exposed to bifunctional alkylating agents such as mitomycin C, nitrogen mustard, melphalan, cyclophosphamide, cisplatin, diepoxybutane, and 8-methoxypsoralen plus 355nm ultraviolet light. These agents cross-link DNA strands.
    - i. This is the basis for the "DEB test", utilizing diepoxybutane as the cross-linking agent.
    - b. Controversy as to efficiency of DNA cross-link repair in FA
    - c. Oxidative damage to DNA
      - i. Peroxide, superoxide, and hydroxyl radicals likely are toxic to DNA
      - ii. Oxygen-dependent effects can be ameliorated by catalase or superoxide dismutase (SOD).
      - iii. FA cells reported to have 10-40% decrease in SOD activity compared with normals.
  3. Fanconi cells do grow nicely in long-term marrow cultures, implying that the defect may be extrinsic to the cells themselves.
  4. Fanconi cells tend to overproduce TNF $\alpha$  and underproduce IL-6. Treatment of these cells with IL-6 partially corrects the sensitivity to mitomycin C.

### C. Clinical aspects

1. Congenital anomalies
  - a. Low birth weight and growth retardation
  - b. Microcephaly, microphthalmia, microstomia
  - c. Anomalous skin pigmentation in 60-80%
    - i. Hyperpigmented patches (café au lait spots)
    - ii. Hypopigmented patches
  - d. Skeletal abnormalities
    - i. Absent, hypoplastic, or misplaced thumb
    - ii. Polydactyly or syndactyly
    - iii. Absence of radius
    - iv. Absence or misplaced radial artery
  - e. Renal abnormalities - aplasia of one kidney, horseshoe kidney, pelvic kidney
  - f. These anomalies may present in any combination or absent altogether.
2. Hematologic abnormalities
  - a. Slow development of pancytopenia, usually presenting between ages 5 and 10.
    - i. May be delayed until teens.
    - ii. 98% of cases present by age 40.
  - b. Thrombocytopenia usually first
  - c. Bone marrow usually hypocellular
    - i. Hemophagocytosis may be seen
    - ii. Hematopoietic progenitors depleted
  - d. Clonal cytogenetic abnormalities occur in 1/3 of cases.
  - e. Acute nonlymphoblastic leukemia common occurrence

- i. May be preceded by myelodysplastic phase
        - ii. FA may present as AML - be especially wary in children with AML. Can assay peripheral blood lymphocytes for sensitivity to DNA cross linking agents.
        - iii. By 40 years of age, the actuarial risk of developing AML is 50%, and of death from hematologic causes is 80%.
      - f. At least 20% of cases develop cancers. In addition to AML, also see skin, GI, and GYN tumors.
    - 3. Treatment and prognosis
      - a. Androgens may produce reversal of pancytopenia lasting for several years
      - b. In at least one case, hematologic reconstitution was achieved using HLA-identical cord blood from a sibling.
      - c. G-CSF appears to stimulate production of all three cell lines when used chronically.
      - d. Transplantation?
      - e. Usual progression with death after about 5 years
- III. Pure red cell aplasia
  - A. Definition: Anemia caused by isolated depletion of erythroid tissue
  - B. Classification
    - 1. Acute
    - 2. Chronic
      - a. Constitutional
      - b. Acquired
  - C. Acute red cell aplasia
    - 1. Usually reported in patients with hemolytic anemia, probably because it is more noticeable in those circumstances.
    - 2. Etiology
      - a. Frequently preceded by infection
        - i. Parvovirus B19 is most important
          - a). 50% of adult population has antibodies to parvovirus
          - b). Viral structure/function
            - 1). Smallest DNA viruses to infect mammals
            - 2). DNA is single stranded and about 5,000 bases in length
            - 3). Viral replication dependent on infection of actively cycling cells
            - 4). Cytotoxic
            - 5). Heat-stable, implying transmissibility in heat-treated blood products, such as factor concentrates.
        - c). Parvovirus B19 causes a variety of clinical syndromes, depending on the host
          - 1). In normals, especially children, infection causes Fifth Disease (erythema infectiosum), although most de novo infection is clinically silent.
          - 2). In adults, the virus is associated with arthritis and the presence of rheumatoid factor. Up to 90% of RA patients are seropositive for B19 antibody, although no causative role has been established.
          - 3). Intrauterine infection causes fetal death.
      - d). Transient aplastic crisis produced in patients with hemolysis
        - 1). 86% of aplastic crises among Jamaican sicklers associated with recent B19 infection.
        - 2). B19 infection documented as cause of transient aplasia in sickle cell, spherocytosis, thalassemias, enzyme deficiencies,

immune hemolysis, post-hemorrhagic stress, and possibly post-chemotherapy

3). Clinical course

- a)). Severe anemia with abrupt onset
  - b)). Absent reticulocytes
  - c)). Erythroid hypoplasia in marrow, with presence of giant pronormoblasts
  - d)). Recovery usual in 1-2 weeks
  - e)). Occasional marrow necrosis
  - f)). Leukopenia and thrombocytopenia may be present. Patients with spherocytosis have a 35-40% incidence, while sicklers have a rate of 10-15%, possibly due to their hyposplenism.
  - g)). Viral particles present in blood in high titers ( $10^8$  -  $10^{14}$ /mL)
  - h)). "Viral" symptoms - fever, gastroenteritis, headache, myalgia, etc. may or may not be present.
- e). The P blood group antigen is the B19 receptor. Erythrocytes lacking this antigen are resistance to parvovirus infection.
- ii. Other viri may be implicated - mumps, hepatitis, mononucleosis
  - iii. Salmonella, staph, pneumococci, meningococci, mycoplasma
- b. Insect bites
- c. Drug-induced toxic depression
- i. Chloramphenicol, cephalosporins, sulfas, phenytoin, chlorpropamide, etc.
  - ii. May be first manifestation of generalized aplasia

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Allopurinol	Co-trimoxazole	Penicillin
Aminopyrine	Diphenylhydantoin	d-Penicillamine
Arsphenamine	Estrogens	Phenobarbital
Azathioprine	Fenoprofen	Phenylbutazone
Benzene hexachloride	Gold	Procainamide
Bromsulphthalein	Halothane	Salicylazosulfapyridine
Calomel	Isoniazid	Santonin
Carbamazepine	Maloprim (Dapsone and pyrimethamine)	Sodium dipropylacetate
Cephalothin	Mepacrine	Sodium valproate
Chenopodium	Methazolamide	Sulfasalazine
Chloramphenicol	Pentachlorophenol	Sulfathiazol
Chlorpropamide		Thiamphenicol
		Tolbutamide

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Drugs Associated With Pure Red-Cell Aplasia

- d. Questionable role of folate and other vitamin deficiencies, as the disease has occurred with malnutrition
- e. Other associated diseases
  - i. Systemic lupus, juvenile rheumatoid arthritis
  - ii. CLL (B or T), myeloma, lymphoma

## iii. Carcinoma of lung, stomach

Solid Tumors	Hematologic Malignancies
Gastric adenocarcinoma	Chronic lymphocytic leukemia
Breast carcinoma	Chronic myeloid leukemia
Bile duct adenocarcinoma	Idiopathic myelofibrosis
Bronchogenic carcinoma	Hodgkin's disease
Squamous cell carcinoma	Non-Hodgkin's lymphomas
Kaposi's sarcoma	Multiple myeloma
Thyroid carcinoma	Acute lymphoblastic leukemia
Carcinoma of unknown primary	
Thymoma	

## Diseases Associated With Pure Red-Cell Aplasia

## 3. Treatment

- a. Transfuse as needed
- b. Folate not harmful
- c. Await spontaneous remission

## D. Chronic pure red cell aplasia

## 1. Constitutional (Diamond-Blackfan anemia)

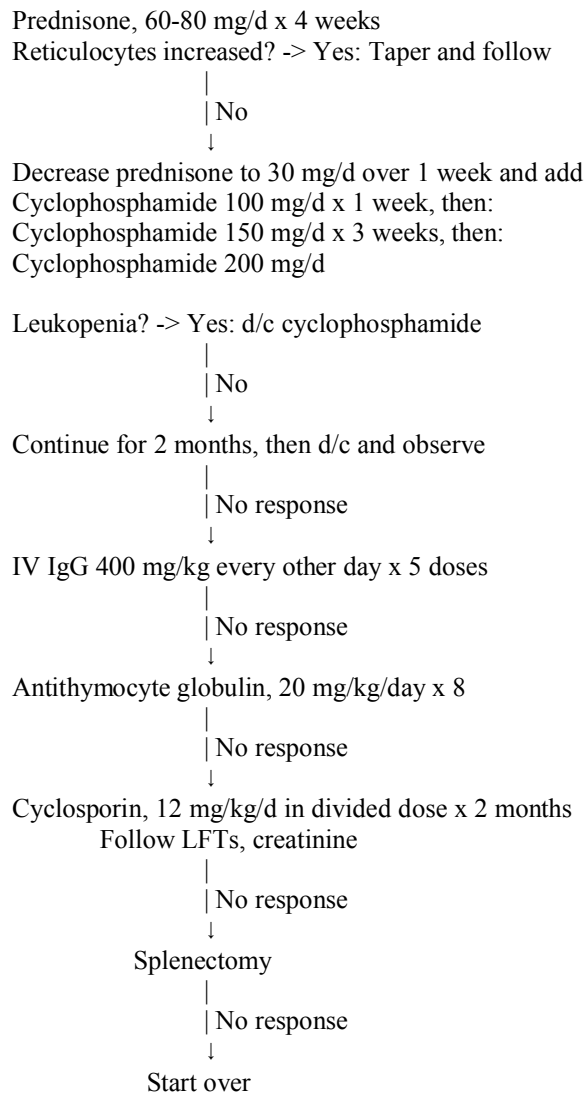
- a. Etiology and pathogenesis
  - i. Occasionally occurs in siblings, suggesting autosomal dominant inheritance
    - a). *RPS19* gene mutated in 25% of cases, although not clear how this inhibits erythropoiesis.
  - ii. Usually sporadic and associated with minor developmental abnormalities
  - iii. Erythroid colonies derived from DBA cell lines show accelerated apoptosis, especially when deprived of erythropoietin (Perdahl).
  - iv. Question of immune mediation has been raised
  - v. Inhibitor of heme synthesis found in serum of some patients
  - vi. Abnormalities of c-kit or its ligand postulated, however 2 patients in literature with normal c-kit (Abkowitz)
- b. Clinical aspects
  - i. Diagnosis in first 2 years of life
  - ii. Anemia usually severe
  - iii. Rib, finger malformations, webbed neck, strabismus
- c. Laboratory aspects
  - i. Normochromic normocytic anemia with reticulocytopenia
  - ii. Occasionally, pancytopenia
  - iii. Bone marrow
    - a). Cellular
    - b). Erythroid hypoplasia, often megaloblastoid
  - iv. Elevated erythropoietin, serum iron
- d. Treatment
  - i. Transfusion, consider deferoxamine
  - ii. Prednisone useful. Sometimes a high dose is required, but others can be maintained on minimal dosage
  - iii. Androgens sometimes help, but to be avoided in children
  - iv. Limited experience with marrow transplantation, but it appears to be effective.

- v. IL-3 reported in 31 cases (Gillio, Olivieri)
  - a). Four responders
  - b). Six had treatment stopped due to adverse effects
- vi. A few cases respond to elevation of prolactin, although why is not clear. Metoclopramide can be used to produce increased prolactin level (Sieff)

## 2. Acquired

- a. Primarily a disease of middle age
- b. Half of cases have thymoma (more common in females)
- c. In up to 20% of cases, PRCA may be a prodrome for myelodysplastic syndrome.
- d. Etiology/pathogenesis
  - i. Association with thymoma suggests immune-mediated rejection of marrow
  - ii. Thymoma associated with other obvious autoimmune disease
    - a). Myasthenia gravis
    - b). Thyroiditis
    - c). Rheumatoid arthritis
    - d). Hypogammaglobulinemia
  - iii. Complement-dependent anti-erythroid or stem cell antibodies demonstrated in a few cases
  - iv. No success in associating this disease with metabolic derangements
  - v. Anti-erythropoietin antibody demonstrated in several cases receiving recombinant EPO
  - vi. Rare association with chronic parvovirus infection in immunodeficient hosts
  - vii. T-cell mediated marrow suppression is the mechanism in CLL and some other cases
    - a). When T<sub>γ</sub> cells (which carry IgG receptors) exceed a certain density in the marrow, hypoplasia or aplasia results.
    - b). In some cases, excess large, granular lymphocytes (LGL) are present. These have NK activity.
- e. Clinical aspects
  - i. Malignant thymoma in about 20% of cases
  - ii. Anergy sometimes present
  - iii. Several cases associated with severe rheumatoid arthritis
- f. Laboratory aspects
  - i. Normochromic, normocytic anemia with low reticulocyte count
  - ii. Normal white count and platelets
  - iii. Bone marrow
    - a). Normoblastic erythroid hypoplasia
    - b). Sometimes see increase in eosinophils and lymphocytes
  - iv. SPEP may show almost anything
  - v. Unusual antibodies may be present
    - a). Cold or warm agglutinins
    - b). Heterophile
    - c). Positive VDRL
    - d). ANA
- g. Therapy
  - i. Transfuse as needed
  - ii. Corticosteroids and androgens
  - iii. Immunosuppression, especially with cyclophosphamide and prednisone
  - iv. Thymectomy indicated when thymic enlargement present
    - a). Up to 60% hematologic response rate
    - b). Irradiation of thymus appears to be unsuccessful

- v. Splenectomy has, in a few cases, induced remission or rendered the patient responsive to other forms of treatment.
- vi. Cyclosporin in high dose can induce a 65% response rate.
- vii. Case report of remission of both thymoma and PRCA with octreotide plus prednisone treatment (Palmieri)
- viii. Protocol, per Sanford Krantz, 10/87:



#### h. Prognosis

- i. Sustained remissions unusual
- ii. Considerable morbidity
- iii. Normal BFU-E formation in marrow culture is a predictor of responsiveness to treatment (Charles).

#### IV. Anemia of chronic renal failure

- A. Etiology/pathogenesis - anemia predictably present when renal function falls below 30% of normal

1. Plasma volume is variable, but overhydration may be contributory
  2. Hemolysis due to altered milieu in uremia
    - a. RBC life span inversely correlated with BUN
    - b. Uremic plasma induces metabolic changes in hexose monophosphate pathway, glutathione, etc.
  3. Ineffective hematopoiesis
    - a. High parathormone levels inhibit red cell production
    - b. Marrow fibrosis sometimes seen with severe hyperparathyroidism
    - c. Suppressive effect of excess aluminum
    - d. Low erythropoietin levels
    - e. Response to exogenous erythropoietin is blunted in uremia
    - f. Subnormal iron utilization
    - g. RBC precursors survive poorly
  4. Nutritional deficiency
    - a. Iron
      - i. GI and GU bleeding
      - ii. Blood loss in hemodialysis coils
    - b. Folate
      - i. Dialyzable
      - ii. Poor diet of uremic patients
  5. Bleeding
    - a. GI and GU mucosal bleeding quite common
    - b. Platelet dysfunction
      - i. Dialyzable factor (guanidinosuccinate?) contributes to poor platelet function
      - ii. Anemia per se decreases platelet function
  6. Deficient erythropoietin production
    - a. Site of renal erythropoietin production still not settled, but probably is tubular cells
    - b. Hypoxia causes a heme-containing protein to enter its deoxy-state, which then induces erythropoietin production.
    - c. Difficult to isolate EPO from renal tissue
    - d. Some EPO found even in anephric individuals. In adults, about 10-15% of production is in liver and possibly other organs.
    - e. As a rule, if renal function is bad enough to produce a BUN >100, renal EPO production is nil.
  7. Anemia associated with dialysis dementia
    - a. Microcytic, hypochromic
    - b. Iron stores normal
    - c. Not all patients are affected
    - d. Presumed cause is aluminum blocking of transferrin receptors on RBC precursors
- B. Laboratory aspects
1. Normochromic, normocytic anemia with retics normal to low
  2. Burr cells and a few spur cells
  3. Hemoglobin affinity for O<sub>2</sub> has a few influences acting on it.
    - a. Acidosis causes decreased affinity
    - b. Decreased glycolysis and dialysis deplete phosphate, causing increased affinity
- C. Treatment
1. Recombinant erythropoietin useful in dialysis and pre-dialysis patients.
    - a. Dose averages 75 U/kg iv or sc tiw
      - i. Relative resistance to EPO correlates with increased PTH level and greater degree of marrow fibrosis (Rao).
    - b. Over 95% of patients achieve hematocrit > 35% within 12 weeks

- c. Functional and cognitive status improves
- d. If you are willing to accept slightly lower hemoglobins (8.5 - 10.5), then 25-30 U/kg tiw is a useful dose
- e. Response to EPO can be predicted by measuring fibrinogen and serum transferrin receptor (Beguin)

	TfR $\geq$ 3500 ng/mL	TfR < 3500 ng/mL
Fibrinogen $\geq$ 400 mg/dL	29% EPO response rate	67% EPO response rate
Fibrinogen < 400 mg/dL	67% EPO response rate	100% EPO response rate

f. Adverse reactions

- i. Increased blood pressure in about 35% of dialysis patients, but rarely in anemic patients with normal renal function
- ii. Iron deficiency (Usually need to supplement iron)
- iii. Myalgias in about 5%
- iv. Seizures in about 5% of dialysis patients

2. Transfuse when necessary

- a. Influence of transfusion on matching for kidney transplant
  - i. Originally thought to sensitize recipient, leading to rejection
  - ii. Subsequently shown that graft survival is enhanced by prior transfusion of 5-10 units RBC
    - a). Pre-sensitization of recipient, leading to better in-vitro organ match?
    - b). Immunosuppressive effect of multiple blood transfusions

3. Iron and folate supplements

4. Androgens

- a. Both increase erythropoietin and induce responsiveness to it
- b. Side effects include hirsutism, fluid retention, cholestasis
- c. Not used much in era of recombinant EPO

V. Anemia of chronic disease

A. Occurs in infection, inflammatory disease, neoplasia. Thus, more appropriately termed "anemia of inflammation."

B. Etiology/pathogenesis

1. Role of inflammatory cytokines

- a. Tumor necrosis factor
  - i. Level increased in cancer, rheumatoid arthritis, infection
  - ii. Administration as an anticancer agent induces anemia
  - iii. Directly inhibits BFU-E, and indirectly inhibits CFU-E through  $\beta$ -IFN
- b. Interleukin-1
  - i. Stimulates T-cell production of  $\gamma$ -IFN, which inhibits CFU-E
  - ii. Stimulates production of G-CSF and GM-CSF, so that granulocytes are spared from suppression by IFN.
- c.  $\gamma$ -Interferon levels elevated in autoimmune and infectious diseases, and the substance is inhibitory to CFU-E

2. Erythropoietin levels are elevated in proportion to the degree of anemia, but less so than in comparably severe anemias of different cause. IL-1 and TNF inhibit EPO secretion in experimental systems.

- a. The expected EPO level as a function of hematocrit is estimated by:

$$[\text{EPO}] = 10^{(4.746 - (0.093 \times \text{HCT}))}$$

3. Altered iron metabolism
    - a. Serum iron and TIBC both decreased
    - b. Absorption of iron is impaired due to defective release from intestinal cells to circulation
    - c. Impaired release of iron from RE sites to transferrin
      - i. Can be demonstrated using radiolabelled hemoglobin
      - ii. Seen in dogs and humans
    - d. These changes take place within a few hours of an inflammatory stimulus, and may last for 10-14 days following the cessation of this stimulus
    - e. Hepcidin mediates this response
      - i. A 25 amino acid peptide made by hepatocytes
      - ii. Downregulates iron absorption in the gut, iron transport across the placenta, and iron release from macrophages
      - iii. Protective against development of iron overload
      - iv. Production is stimulated by inflammation – thus responsible for alterations seen with anemia of inflammation
  4. Relative bone marrow failure exists - subnormal response of marrow to erythropoietin, possibly due to low serum iron (Although  $\gamma$ -IFN induces resistance to the effect of EPO).
  5. RBC survival slightly decreased
    - a. Destruction due to passage through damaged tissue
    - b. Reticuloendothelial hyperplasia
  6. Apoptosis of bone marrow erythroid precursors seen in anemia associated with rheumatoid arthritis, and has been shown to improve with therapy directed against TNF- $\alpha$  (Papadaki).
- C. Laboratory aspects
1. Mild to moderate anemia, sometimes microcytic, hypochromic but usually N/C, N/C
  2. Reticulocyte count in "normal" range
  3. Decreased iron and TIBC, with saturation usually normal
  4. Bone marrow nonspecific
    - a. Reticuloendothelial iron is increased
    - b. Sideroblasts are decreased
  5. Iron kinetics
    - a. Plasma iron turnover normal or increased, as  $T_{1/2}$  is short but plasma level is low
    - b. Incorporation of injected iron into RBCs is fairly efficient
  6. Increased level of zinc protoporphyrin
    - a. Increase occurs when iron metabolism disturbed
      - i. Iron deficiency
      - ii. Myelodysplasia
      - iii. Lead poisoning
      - iv. Chronic inflammation
    - b. Measured fluorometrically
- D. Therapy generally not needed. One must attack the underlying disease process.
1. Nonetheless, erythropoietin treatment has been studied, and is promoted for the treatment of this condition.
    - a. Moderately effective in rheumatoid arthritis
    - b. Responses in about 50% of anemic cancer patients, but large doses may be necessary (150 - 300 U/kg tiw)
    - c. Ludwig, et al developed an algorithm to predict response to EPO in cancer patients
      - i. Treat for 2 weeks, then measure hemoglobin and serum EPO
      - ii. If EPO 100 mU/ml, and the hgb has not risen by at least 0.5 gm/dl, there is a 93% chance that the patient will not respond.

- iii. If the above conditions are not met, there is an 80% chance that treatment will work (defined as an increase in hgb of at least 2 gm/dl within 12 weeks)
- iv. If both the EPO level is < 100 mU/ml and the hgb has increased by > 0.5 gm/dl, there is a greater than 95% chance of a response.

#### VI. Anemia of autonomic dysfunction

- A. Intact autonomic function is required for adequate renal production of erythropoietin.
- B. Patients with autonomic dysfunction tend to mild to moderate anemia
  - 1. Erythropoietin levels do not correlate with hemoglobin concentration
  - 2. The EPO response to anemia is subnormal
  - 3. Low doses of EPO (25 to 50 U/kg tiw) correct the anemia.

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