ORIGINAL PAPER

Acquired aplastic anemia: correlation between etiology, pathophysiology, bone marrow histology and prognosis factors

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Abstract

Aplastic anemia is a clonal disease of stem cell characterized by peripheral blood pancytopenia with hypocellular bone marrow. In most cases acquired aplastic anemia is an autoimmune, T-cell mediated disease (hematopoiesis is mediated by a population of CD8+ T-cells which produce inhibitory cytokines – TNF-α, IFN-γ, IL-6 which suppress hematopoiesis by affecting the mitotic cycle and cell killing by inducing apoptosis). In some cases radiation, medical drugs and chemicals, viruses induce depletion of hematopoietic stem cells by direct toxicity; immune diseases induce complex immune reactions leading to bone marrow failure. Symptoms and signs are represented by fatigue, pallor induces by anemia, infections induce by neutropenia, and bleedings induce by thrombocytopenia. In peripheral blood is present pancytopenia and bone marrow are characterized by hypocellularity, fat cells hyperplasia, residual lymphocytosis, plasmocytosis and mastocytosis. The aim of this study was to establish the correlation between etiology, pathophysiology, bone marrow histology and negative prognosis factors at 16 patients with acquired aplastic anemia (seven with severe aplastic anemia and nine with moderate aplastic anemia) hospitalized in Clinic of Hematology from Craiova between 2003–2008. Eight cases presented idiopathic aplastic anemia and eight cases secondary aplastic anemia (two of them with pure red cell aplasia). Conclusions: The unfavorable evolution, correlated with etiology and pathophysiology, had been seen at the patients with severe idiopathic aplastic anemia and severe secondary aplastic anemia associated with viral infections and insecticides exposure. Pure red cell aplasia was associated in our study with B19 parvovirus infection or malignant thymoma. The negative prognosis factors in acquired aplastic anemia, correlated with laboratory findings and a low survival, were: severe neutropenia, platelets count less than 10 000/μL, corrected reticulocytes less than 1%, hypocellularity of bone marrow <10%, persistence of pancytopenia at 30 days after initiating therapy.

***Keywords*:** acquired aplastic anemia, prognosis factors.

Introduction

Aplastic anemia (AA) is defined as a peripheral blood pancytopenia with a hypocellular bone marrow. Aplastic anemia is considered an autoimmune disease with active destruction of blood-forming cells by lymphocytes, most cases of disease can be pathophysiologically characterized as T-cell-mediated organ specific destruction of bone marrow hematopoietic cells, existing a strong association between AA and antigen HLA–DR2 [1].

Etiology in secondary aplastic anemia is represented by exposure at radiation, drugs and chemicals (cytotoxic agents, Chloramphenicol, non-steroidal antiinflammatory drugs, antiepileptics, gold salts, benzene), viruses (Epstein Barr, hepatitits virus, parvovirus, human immunodeficiency virus), immune diseases (eosinophilic fasciitis, thymoma), paroxysmal nocturnal hemoglobinuria, pregnancy; the rest of cases of acquired aplastic anemia are represented by idiopathic aplastic anemia. The pathophysiologic pathways leading to aplastic anemia are: damage to DNA inducing apoptosis by cytotoxic chemotherapy, irradiation, chemical or physical agents; marrow failure caused by medical drugs (Chloramphenicol, non-steroidal antiinflammatory drugs, antiepileptics, gold salts, antithyroid drugs); complex immune reactions leading to bone marrow failure induced by viruses. The pathogenic mechanism of idiopathic aplastic anemia is still unknown [2].

Beside immune-mediated destruction of marrow hematopoietic progenitor stem cells recent data suggest that disturbed interaction between bone marrow stromal microenvironment and hematopoietic stem cells cause these disorders [3].

Myelosuppressive cytokines, TNF-α, IFN-γ, IL-6, act as final effectors of apoptosis in hematopoietic cells in acquired aplastic anemia [4, 5].

Most patients with aplastic anemia presenting anemia, bleedings or infections, alone or in association, low number of reticulocytes. Peripheral blood usually shows pancytopenia; anemia is common associated with neutropenia and thrombocytopenia. The peripheral blood smear shows paucity of leukocytes and platelets, toxic granulations in neutrophils, erythrocyte macrocytosis, relative lymphocytosis, decreased absolute number of monocytes. Examination of bone marrow (aspiration and biopsy) shows usually hypocellularity,

increased number of fat cells, residual lymphocytosis, plasma cells, stromal elements (fibroblastoid and histiocytic cells). There are more options for the treatment of the patients with aplastic anemia. The younger patients with severe aplastic anemia, which have a HLA-matched sibling donor, are candidates for bone marrow transplantation. For the younger patients with severe aplastic anemia who are not candidates for bone marrow transplantation and the older patients, immunosuppression is the treatment of choice. Patient with severe disease should receive a combination of Anti-Thymocyte Globuline (ATG) 40 mg/kg/day at days 1–4, followed by Cyclosporine A (CsA) for six months at a dose of 12 mg/kg; corticosteroids (1 mg/kg of Prednisone) are added during the first two weeks to ameliorate serum sickness [6–8].

In the patients who receive immunossuppressive therapy, supportive treatment is represented by transfusions, antibiotics, hematopoietic growth factors (G–CSF, GM–CSF). Age and severity of neutropenia are decisive factors to use it. The potential advantages of using G–CSF are faster neutrophil recovery and opportunity to test for white blood cell increments and predict failure [9, 10].

In moderate aplastic anemia, steroids in moderate doses, androgens or CsA may be usefully [11, 12].

The aim of this study was to established the correlation between etiology, bone marrow histology and negative prognosis factors in patients with acquired aplastic anemia.

Material and Methods

We studied 16 patients with acquired aplastic anemia hospitalized in Clinic of Hematology from Craiova, between 2003–2008. The study group included 12 women and four men, aged between 21–63-year-old. The diagnosis of aplastic anemia was established according to the standard criteria (*International Agranulocytosis and Aplastic Anemia Study*, 1987). The severity of aplastic anemia was defined by laboratory findings*:* severe aplastic anemia – bone marrow cellularity less than 30%; two or three peripheral blood criteria – neutrophil count <500/μL, platelets <20 000/μL, reticulocytes <40 000/μL, no other hematologic disease; moderate aplastic anemia – patients with pancytopenia who do not fulfill the criteria of severe aplastic anemia [6].

Differential diagnosis of pancytopenia were made with: congenital marrow failure, paroxysmal nocturnal hemoglobinuria, hypoplastic myelodysplastic syndrome, acute leukemias, Evans syndrome, megaloblastic anemias, hypersplenism. The following parameters were determinate: hemoglobin value, white blood count and leukocyte formula, platelets count, reticulocytes, peripheral blood smear, bone marrow aspirate and biopsy, erythrocyte sedimentation rate, fibrinogen, reactive C-protein, tests of hemostasis, hepatic and renal tests, viral determinations. Patients with severe disease received Cyclosporine A for six months at a dose of 12 mg/kg (with monitoring liver and kidney functions) and supportive treatment represented by transfusions, antibiotics and, in some cases, hematopoietic growth factors (G–CSF). The patients with moderate aplastic anemia received steroids in moderate doses or androgens.

Results

In our study, acquired aplastic anemia was encountered at men as well in woman, with a median age of 42-year-old.

From sixteen patients with aplastic anemia, seven presented severe aplastic anemia and nine moderate aplastic anemia. Eight cases presented idiopathic aplastic anemia and eight cases secondary aplastic anemia. In three cases aplastic anemia followed viral infections (two after hepatitis with C-virus and one after B19 parvovirus infection at a young woman who made a trip in Japan with three months earlier). The patients presented severe pancytopenia and bone marrow histology showed hypocellular marrow with increased fat cells and residual lymphocytosis, plasmocytosis and stromal elements (Figure 1).

Patients with hepatitis-associated aplasia had markers of immune system activation and responding to immunosuppressive therapy. In one case, evolution was infaust, the patient died after 10 months of survival. In one case, severe aplastic anemia appeared after cytotoxic drug (Cyclophosphamide) used for the treatment of a solid cancer, and in a case after insecticides exposure (followed by hepatic failure). A young woman diagnosed with Graves' disease developed a moderate aplastic anemia after six weeks of treatment with Carbimazole 60 mg daily. In a case aplastic anemia was associated to malignant thymoma and in a case with pregnancy. In the case of aplastic anemia associated to malignant thymoma, at a 61-year-old woman with severe anemia (Hb=3.5g/dL), bone marrow biopsy confirmed diagnosis of pure red cell aplasia showed a reduced erythroid series (Figure 2).

Computer tomography exam showed a large fore mediastinum with a good delimited tumor. Surgical intervention was done and macroscopic had been seen an ovoid tumor by 10/6/6 cm, whitish-pink, high consistency, alternance between whitish areas and blackish-grey areas, with more callous zone adequate cholesterolic granulomas on section. Histopathology and immunochemistry established diagnosis of a high-differentiated malignant thymoma. In mediastinal tumor has been seen an epithelial tissue by a thick fibro-connective bag (Figures 3–5).

Tumor cells present pan-cytokeratin (Figure 6) and Ki-67/MIB-1 nuclear factor of cell proliferation in almost 40% from tumor cells (Figure 7). Hemoglobin value was normal after six days from surgical intervention and now, the patient is treated with chemotherapy for malignant thymoma.

In fourteen cases was present global aplastic anemia and in two cases pure red cell aplasia: one case associated with B19 parvovirus infection and one case associated with malignant thymoma.

Neutrophil count were less than 500/μL in seven

cases and more than 500/μL in nine cases; platelets were less than 20 000/μl and more than 20 000/μL in nine cases; reticulocytes were less than 40 000//μL in five cases and more than 40 000/μL in eleven cases. Bone marrow (based on examination of the aspirate smear and biopsy specimen correlated) was hypocellular in all cases, with increased fat cells and lymphocytosis, plasmocytosis and stromal elements (fibroblastoid and histiocytic cells).

Idiopathic aplastic anemia would result from the destruction of hematopoietic cells by an autoimmune attack. In the cases of idiopathic acquired aplastic anemia, possible immune etiology was suggested by disturbance of CD4/CD8 ratio and favorable response after immunosuppressive therapy. In all cases of our study, pancytopenia was severe and bone marrow histology shows hypocellularity, with increased fat cells and lymphocytosis (Figure 8).

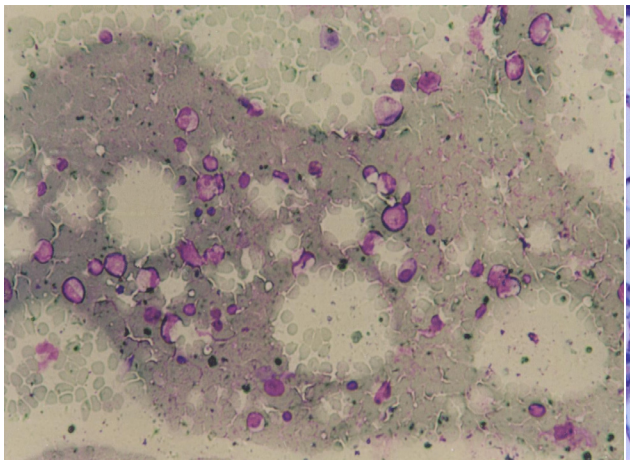

Figure 1 – ***Bone marrow biopsy. Acquired AA associate with C-virus hepatitis (HE stain, ob. ×40).***

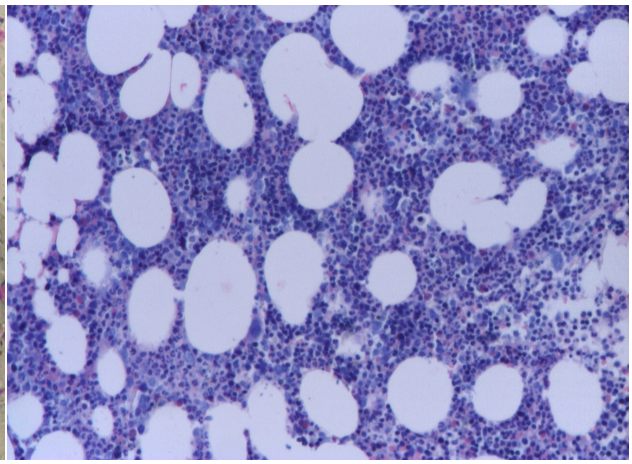

Figure 2 – ***Bone marrow biopsy. Pure red cells aplasia associate with malignant thymoma (Giemsa stain, ob. ×20).***

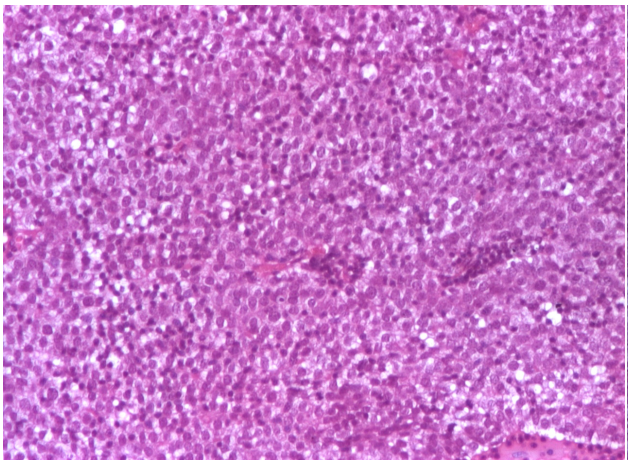

Figure 3 – ***MT (HE stain, ob. ×5).***

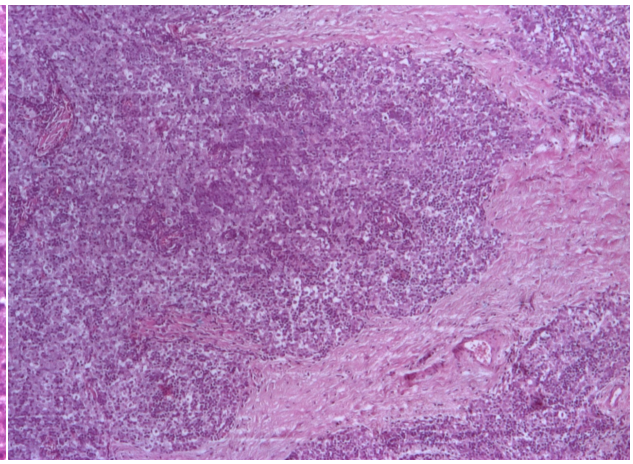

Figure 4 – ***MT (HE stain, ob. ×10).***

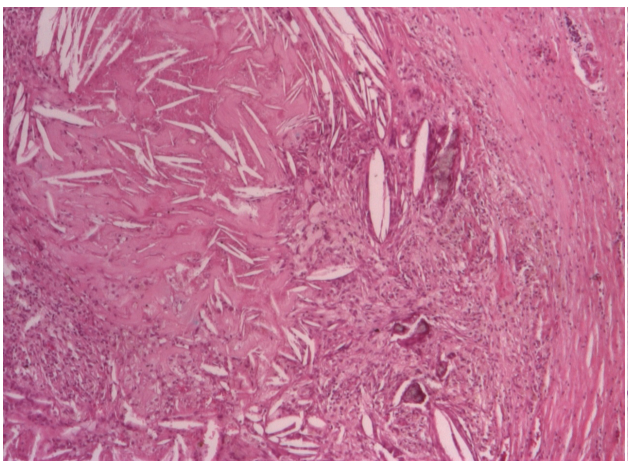

Figure 5 – ***Cholesterol granuloma, ob. ×4.***

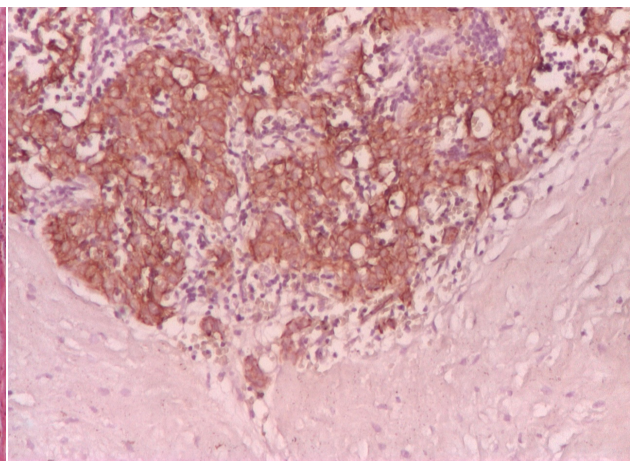

Figure 6 – ***MT (IHC panCK, ob. ×10).***

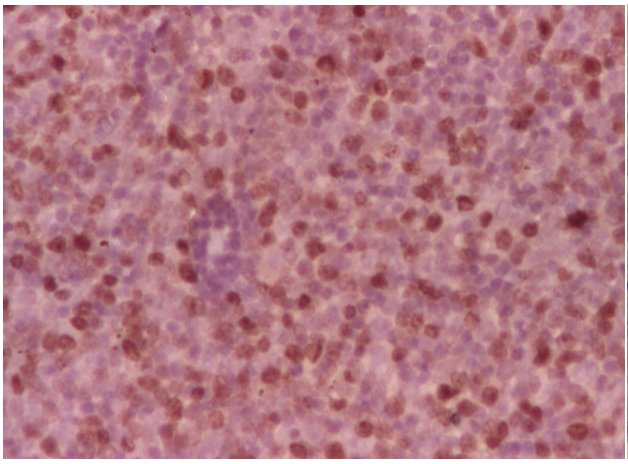

Figure 7 – ***MT (IHC Ki67, ob. ×20).***

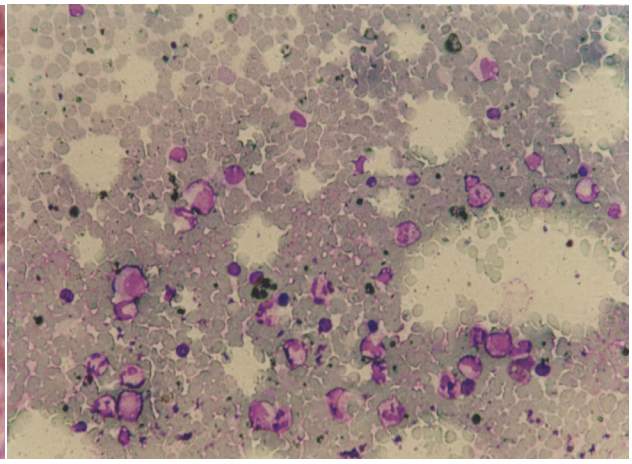

Figure 8 – ***Bone marrow biopsy. Idiopathic acquired aplastic anemia (HE stain, ob. ×40).***

Severity of anemia, etiology, pathogenesis and age were decisive factors for treatment options. Five patients with severe aplastic anemia received Cyclosporine A for six months at a dose of 12 mg/kg (monitoring liver and kidney functions) and supportive treatment and two patients received transfusions, antibiotics and hematopoietic growth factors (G–CSF). Eight patients with moderate aplastic anemia received steroids in moderate doses and one patient received androgens.

The unfavorable evolution had correlated with neutrophil count <500/μL, platelets <10 000/μL, corrected reticulocytes <1%, bone marrow cellularity <10%, persistence of pancytopenia after 30 days after initiating therapy.

Discussion

In the present study, acquired aplastic anemia was more frequent at women than in men (F/M=3:1), with a median age in middle life. In literature, aplastic anemia has been identified as a disease of the young, with two peaks, at age 15–25 years or after age 60 years. It was an equal frequency between idiopatic aplastic anemia and secondary aplastic anemia, and a small predominance for moderate aplastic anemia beside severe aplastic anemia.

Various causes are involved in the pathogenesis of acquired aplastic anemia: viruses, exposure at radiation, drugs, immune diseases, paroxysmal nocturnal hemoglobinuria, pregnancy; the rest of cases of acquired aplastic anemia are represented by idiopathic aplastic anemia [6]. Viruses damage bone marrow directly, by infection and cytolysis of hematopoieic cells, or indirectly, through induction of secondary immune pathways initiation of autoimmune processes leading to depletion of progenitor and stem cells, or destruction of supporting stroma.). Viruses induce severe pancytopenia, leukopenia, atypical lymphocytosis, erythroid macrocytosis, thrombocytopenia [2, 6]. In our study acquired aplastic anemia, after a documented episode of hepatitis, which required an increase in serum aminotransferase level more than three times the upper limit of normal range and had markers of immune system activation, was present in two cases. Both cases presented severe aplastic anemia (one of them very severe aplastic anemia with infaust evolution). Hepatitis associated aplastic anemia is documented in 5–10% of patients with acquired aplastic anemia [13–15]. Immunologic mechanisms are thought to be involved in the pathogenesis of both diseases (cytotoxic T-lymphocytes are thought to cause liver damage in viral hepatitis) [16]. A recent study showed that the Fas/Fas-ligand pathway was involved in this process [17]. The results of immunosuppressive therapy have been reported in small study [14, 18]. Some patients responded to high-dose Cyclophosphamide [19].

Various drugs and xenobiotics (nonsteroidal anti-inflammatory drugs, benzene, cytotoxic agents, Chloramphenicol, antiepileptics, gold salts) induced direct chemical toxicity and immune-mediated destruction The most aplastic anemias associated with medical drugs are idiosyncratic. Their harmful potential depends on the amount of exposure, genetic variations in drug metabolism and the detoxifying capacity of the recipient, the physical properties of the agent, enzymatic pathways that chemically alter the drug. A faulty catabolism of these substances or their metabolites might exert their effects on the stem cell in a more prolonged or intense way thus facilitating (via T-cell activation) damage to the hematopoietic cells. Genetic polymorphisms of some important detoxifying enzymes are associated with low or absent catalytic activity of the protein (cytochromes, glutathione S-transferases, quinoneoxidoreductase) [20–22].

In our study, in one case severe aplastic anemia appeared after cyclophosphamide used for the treatment of a solid cancer. Cytotoxic drugs induced direct chemical toxicity and immunemediated destruction. Busulfan causes depression of bone marrow function and reduces marrow regenerative capacity. Chloramphenicol at ordinary doses determines reversible alterations in erythropoiesis, decreases hematopoietic colony formation, inhibits marrow stromal cell proliferation and production of growth factors; produces also chromosomal abnormalities in white blood cells. Gold salts induce dose-dependent leukopenia, inhibit hematopoietic colony formation *in vitro* [23]. Antithyroid drugs

and Trimethoprim, Sulfamethoxazole are associated with agranulocytosis [24]. In our study, a young woman diagnosed with Graves' disease developed a moderate aplastic anemia after six weeks of treatment with Carbimazole 60 mg daily, with favorable evolution after immunosuppressive therapy. Intermittent exposure to benzene damaged stem cells and marrow stroma. Benzene induces leukopenia, anemia, thrombocytopenia, lymphocytopenia, macrocytosis, acquired Pelger–Huet anomaly, eosinophilia, basophilia. Marrow is usually normocellular, a hypocellular aspect may precede complete aplasia. Chronic exposure is associated with marrow necrosis, fibrosis, edema and hemorrhage [25]. Pesticides and insecticides, especially Chlordane and Lindane, are associated with severe aplastic anemia. Their mechanism of action is represented by direct toxicity of stem cells and immune-mediated destruction. In the present study, in a case acquired aplastic anemia appeared after insecticides exposure, followed by hepatic failure and death.

Acute radiation (γ-rays, α- and β-particles) altered both stem and progenitor cells: altered replicating of progenitor cells, depletion of hematopoietic cells, lymphocytes are directly killed. Histologic aspects include necrosis, nuclear pyknosis and karyorrhexia, nuclear and cytolysis, phagocytosis, congestion, rapidly followed by fatty replacement. Chronic low-level radiation exposure induced lymphocytosis, neutropenia, immature or dimorphic white cells, and giant platelets [6].

Immune diseases are associated with a T-cell mediated tissue specific organ destruction initiated by cytotoxic lymphocyte activation, cytokine production (TNF-α, IFN-γ, IL-6) who suppresses proliferation of stem and progenitor cells. In our case of aplastic anemia associated to malignant thymoma evolution was favorable after surgical intervention, hemoglobin value normalized after six days from surgical intervention and now, the patient is treated with chemotherapy for malignant thymoma.

In the case of moderate aplastic anemia associated with pregnancy, the disease appeared at two months after delivery, with favorable evolution after immunosuppressive therapy. Literature date show that frequency of aplastic anemia in pregnancy is rare, although bone marrow hypoplasia may be relatively common during pregnancy [6].

The evolution of the patients after specific treatment was: three patients obtained complete remission, four partial remission, one transformed in paroxysmal nocturnal hemoglobinuria, one transformed in myelodysplastic syndrome; seven patients with severe aplastic anemia died through infections, bleedings or hepatic failure. Survival to 12 months was 50.6%; in moderate aplastic anemia, the rate of survival was of six years, superior over severe aplastic anemia (10-months survival).

Conclusions

The negative prognosis factors in acquired aplastic anemia, correlated with laboratory findings and a low survival, were: severe neutropenia, platelets count less than 10 000/μL, corrected reticulocytes less than 1%, hypocellularity of bone marrow <10%, persistence of pancytopenia at 30 days after initiating therapy.

The unfavorable evolution, correlated with etiology and pathophysiology, had been seen in the patients with severe idiopathic aplastic anemia and severe secondary aplastic anemia associated with viral infections and insecticides exposure. The prognosis was good in moderate aplastic anemia.

Pure red cell aplasia was associated in our study with B19 parvovirus infection or malignant thymoma, with a good evolution after therapy.

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