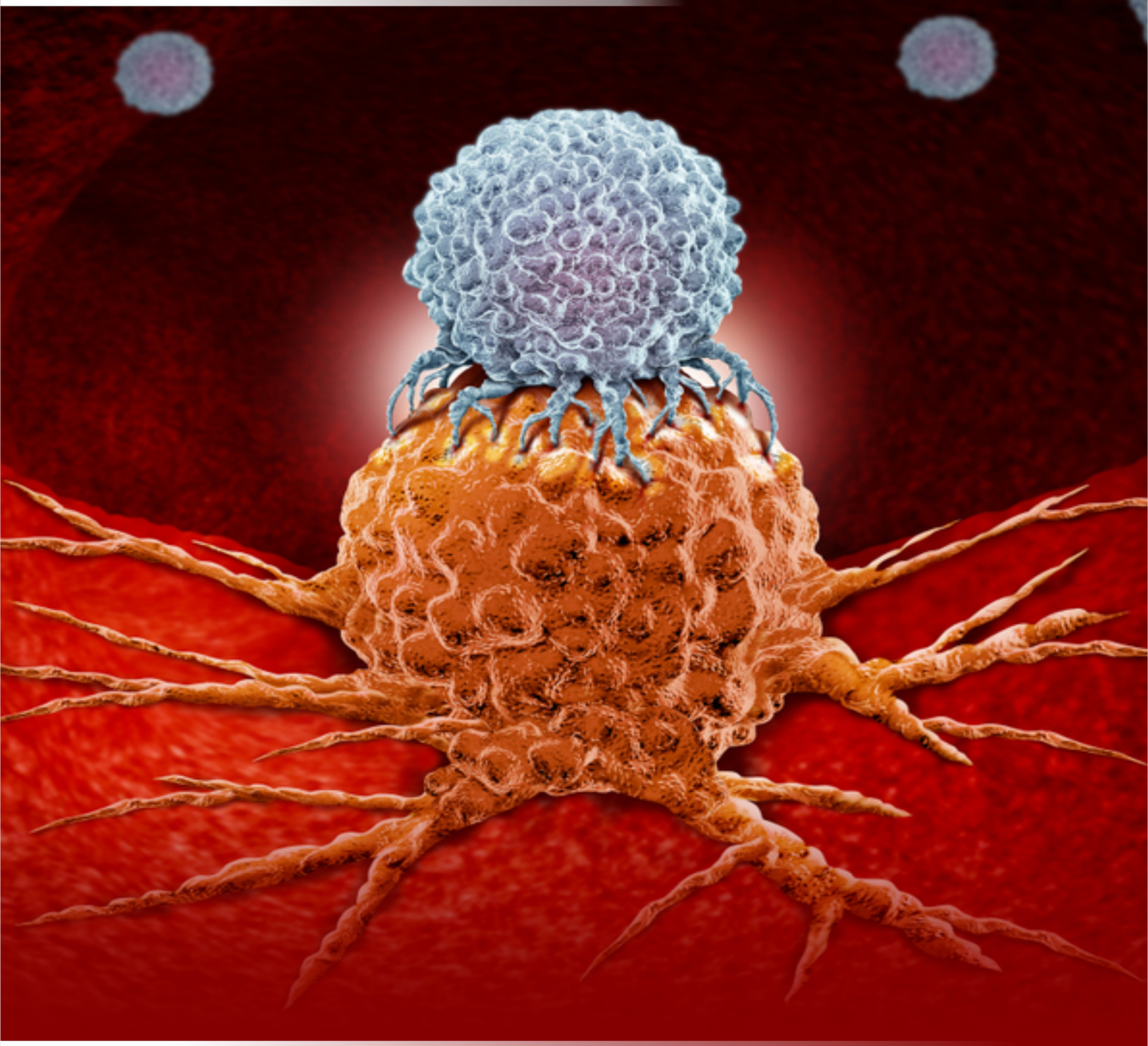
Juniper Publishers
key to the Researchers

ISBN: 978-1-946628-21-3



APLASTIC ANEMIA AS PART OF THE BONE MARROW FAILURE SYNDROME ISSSTE CONSENSUS, 2019

CANCER THERAPY & ONCOLOGY INTERNATIONAL JOURNAL

Aplastic Anemia as part of the Bone Marrow Failure Syndrome ISSSTE Consensus, 2019

Martha Alvarado Ibarra*

Centro Médico Nacional "20 de Noviembre", Ciudad de México

***Corresponding Author**

Martha Alvarado Ibarra. Centro Médico Nacional "20 de Noviembre". ISSSTE. Ciudad de México. 52005003 – 14478, Mexico

Published By

Juniper publishers Inc.

United States

June 21, 2019

Authors names and Affiliations

Martínez Ramírez Mario:	Hospital de Alta Especialidad, Veracruz, Ver
***Alvarado Ibarra Martha:**	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Alcívar Cedeño Luisa María:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Arteaga Ortiz Luis:	Hospital General ISSSTE, La Paz, B.C.
Ávila Castro David:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Chávez Aguilar Lénica Anahí:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
De la Peña Celaya José Antonio:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Estrada Domínguez Patricia:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Fermín Caminero Denisse:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Flores Villegas Luz Victoria:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
González González María de Lourdes:	Hospital Regional de Puebla, Puebla, Pue
González Rubio María del Carmen:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Hernández Ruiz Eleazar:	Hospital Regional "Presidente Juárez de Oaxaca", Oaxaca, Oax
Loera Fragoso Sergio José:	Hospital "Santiago Ramón y Cajal", Durango, Dgo
López Arroyo José Luis:	Hospital Regional, Ciudad Juárez, Chihuahua
Medina Guzmán Liliana:	Hospital General ISSSTE, Querétaro, Qro.
Mena Zepeda Verónica:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Merino Pasaye Laura E:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Miranda Madrazo María Raquel:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Montoya Jiménez Leire:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Morales Adrián Javier de Jesús:	Hospital Regional "Elvia Carrillo Puerto", Mérida, Yucatán
Morales Hernández Alba Edna:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Orellana Garibay Juan José:	Hospital Regional de Alta Especialidad "Centenario de la Revolución Mexicana", Emiliano Zapata, Morelos
Palma Moreno Orlando Gabriel:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Pedraza Colín María Luisa:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Peña Alcántara Paula:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Pérez Zúñiga Juan Manuel:	Hospital Regional "General Ignacio Zaragoza", Ciudad de México
Rojas Castillejos Flavio:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Romero Martínez Eduardo:	Hospital "5 de Diciembre", Mexicali, B.C.
Saavedra González Azucena:	Hospital Regional de Alta Espacialidad "Bicentenario de la Independencia", Tultitlán, Estado de México
Serrano Hernández Cristina:	Hospital Regional, Ciudad Juárez, Chihuahua
Silva Vera Karina:	Hospital General ISSSTE, Tampico, Tamps
Tapia Enríquez Ana Laura:	Hospital General ISSSTE "Presidente Lázaro Cárdenas", Chihuahua, Chih
Tepepa Flores Fredy:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Teomitzi Sánchez Oscar:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Vilchis González Shendel Paulina:	Centro Médico Nacional "20 de Noviembre", Ciudad de México
Villela Peña Atenas:	Centro Médico Nacional "20 de Noviembre", Ciudad de México

Definition

Aplastic anemia (AA) is a bone marrow failure syndrome characterized by cytopenias and hypocellular bone marrow, in which space is replaced by fat tissue in the absence of malignant clonal processes and without an increase in the reticulocyte count [1-4]. The bone marrow failure syndrome refers to a heterogeneous group of diseases that are characterized by a decrease in the number and / or function of the hematopoietic stem cells or the bone marrow microenvironment [1,5,6]. The most representative depiction of a bone marrow failure syndrome is the aplastic anemia [6], an entity closely related to paroxysmal nocturnal hemoglobinuria (PNH) [1,2]. PNH is a clonal disease of the hematopoietic stem cells caused by the acquired mutation of the phosphatidyl-inositol-glycan group A [7]. On the other hand, the term pancytopenia refers to the decrease of the three cell lines in peripheral blood where the reduction of hematopoiesis is not essential; a classic and frequent example is hypersplenism [6].

History

The first cases were described at the end of the 19th century, when the pathologist Paul Erhlich in 1888 described a case of a patient who presented anemia, bleeding, fever and fatty or yellow bone marrow [6]. The term aplastic "aplasique" (from the Greek a: no, and plast: to mold or form) was probably coined by Vázquez and Aubertin when they described a patient's bone marrow in 1904, although the title of the article was pernicious anemia, this was corrected later in the main text of the hematology journal of that time *Le Hemopatié* [4].

Epidemiology

The incidence of aplastic anemia has decreased in recent years. Before 1980 a global incidence of 7 to 25 per million inhabitants per year was reported [8]. With the introduction of diagnostic and severity criteria, the annual incidence decreased significantly [9]. Five-year survival has improved between 70 and 80% [10] after the introduction of immunosuppressive therapy and allogeneic stem cell transplantation. Studies based on specific population groups are difficult to perform due to the lack of registration of aplastic anemia in national health systems [11], but there are isolated statistical studies that estimate an incidence rate that varies geographically. In territories such as Europe, Israel, the United States of America and Brazil, the annual incidence is 2 cases per million inhabitants, while in China, Mexico, Malaysia and Thailand it is higher (between 3.9 to 7.4 cases per million inhabitants) [10]. It affects both sexes equally [5]. There is a variation of bimodal presentation related to age, the first peak between 15 and 25 years of age, and the second after 55 years [4,10].

In Mexico, between 1996 and 2000, an epidemiological study [12] was carried out with the aim of estimating the incidence of aplastic anemia in children registered at the Mexican Institute of Social Security in Mexico City. A total annual incidence of 3.9 cases per million inhabitants was reported. In children under 15 years of age, the average annual incidence was 4.2 per million inhabitants and in those over that age the incidence was 3.8 per million inhabitants. The work of Gutiérrez et al. [11] reported 49% of AA in those over 16 years old, most of those cases were serious with 57%, followed by moderate 23%, and very serious 20%; with a rate of 0.08% of hospital admissions in a period of 10 years (January 1998 to December 2007) in the National Institute of Medical Sciences and Nutrition "Salvador Zubirán", with an average age of 35 years (17 to 38). In 75.5% of the cases the cause of the aplastic anemia was not identified (idiopathic), in the rest of the patients there was a history of exposure to pesticides.

In the international literature, Mexico is among the countries with the highest prevalence of aplastic anemia and, although it is a rare disease, morbidity and mortality are high and result in expensive treatments, mainly due to the risk of hemorrhagic and infectious complications that can lead to death, so the timely and accurate diagnosis will lead to a prompt therapeutic decision that will increase the life expectancy of patients [1,8,10].

Importance of creating a Mexican Consensus for the Management of the Aplastic Anemia

Although there are diverse causes and factors associated with the development of the disease, in most cases it is not possible to identify any etiological cause (around 80%) and it is believed that there is a greater influence of epigenetic factors (environmental aspects, public health, exposure to chemicals and contamination) than genetic [8,13,14]. The causes of a bone marrow failure are diverse and classified according to cell quantification: marrow hypocellularity (such as aplastic anemia, some leukemias and myelodysplastic syndromes) and others with normal or increased cellularity, where normal erythropoiesis is replaced (such as infiltrative tumors of the bone marrow, megaloblastic anemia, etc.) (Table 1) [6]. According to the etiology, AA can be subclassified into hereditary or acquired aplastic anemia (Table 2) [6,2,10,14,].

Table 1: Causes and classification of the bone marrow failure syndrome [3].

Hematopoietic alterations with Hypocellular Bone Marrow	Aplastic anemia
	Some myeloid leukemias and lymphoproliferative syndromes
	Some myelodysplastic syndromes
	Anorexia nervosa

Hematopoietic alterations with Hypercellular or Normocellular Bone Marrow	Ineffective hematopoiesis Myelodysplastic syndrome Deficiency of vitamin B9 and B12 Spinal replacement Metastatic carcinoma Leukemia and lymphoma Multiple myeloma Myelofibrosis Infections Granulomatous diseases Deposit diseases

Table 2: Etiology of Aplastic Anemia [3,4,12,13].

Hereditary	Fanconi's Anemia Diamond-Blackfan Anemia Shwachman-Diamond Syndrome Congenital Dyskeratosis Reticular dysgenesis Associated with Down Syndrome, Dubowitz or Seckel
Acquired	Idiopathic (around 66%) Secondary (around 34%) Related to drugs (chloramphenicol [the most representative], gold salts, penicillamine, carbamazepine, hydantoin, quinidine) Associated with infection by hepatitis virus, Epstein-Barr virus, human immunodeficiency virus (HIV), parvovirus B19 Poisoning with benzene, products associated with metabolism and other aromatic hydrocarbons, insecticides Exposure to radiation (gamma rays) Cytotoxicity by chemotherapy Immune diseases (eosinophilic fasciitis, hypogammaglobulinemia, thymoma, graft versus host disease) Associated with pregnancy

Pathophysiology

Aplastic anemia is associated with the loss of hematopoietic stem cells (HSC) and the resulting decrease of mature blood cells. A decrease of the HSC group below a critical mass, conflicting the demands for self-renewal and differentiation, can lead to pancytopenia [3,15].

Pathophysiological processes that lead to the loss of human hematopoietic stem cells (HSCs) and cause aplastic anemia include [3,15]:

i. Mechanisms by cytotoxic T-cells

ii. Mediator effectors and their role in apoptosis

iii. Telomeres

iv. Clonal evolution

i. Mechanisms by Cytotoxic T cells

The most accepted mechanisms of aplastic anemia are those mediated by autoimmunity directed against hematopoietic stem cells [3,15]. Clinical evidence has been corroborated by multiple studies confirming the clonal expansion of activated T cells directed against marrow progenitors (Figure 1) [15]. Available evidence suggests a large homeostatic dysregulation of the T cells repertory. Among the T lymphocytes subgroups, T CD8+ cells have the best characterized role in aplastic anemia. T CD8+ cells increase in aplastic anemia patients and have selection towards oligo clonal patterns of the Beta chain [15]. Defined auto-antigens against which these T cells react are not clearly known, but the studies of autoantibodies detection in serum suggest multiple potential candidates such as: protein 1 related to the diazepam binding inhibitor (DRS-1), kinectin (KTN1), increased postmeiotic protein 1 (PMS1), heterogeneous nuclear ribonucleoprotein kr (hnRNP K), among others [15].

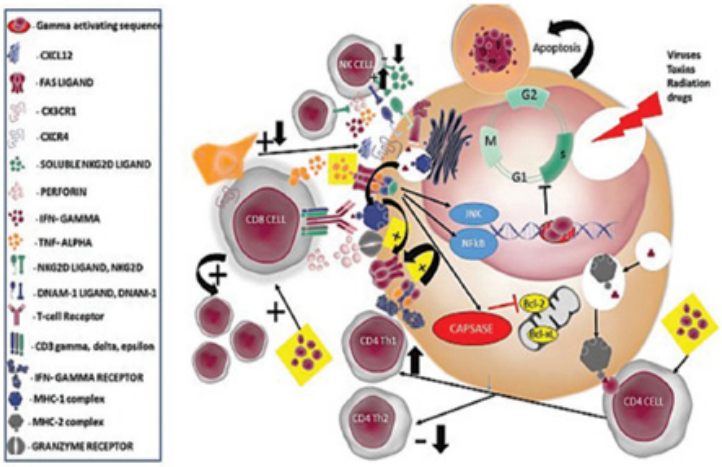


Figure 1: Model of progenitor cell injury mediated by T cell immunity. T cells are actively involved in direct cytotoxicity, especially CD8 cells. IFN GAMMA and TNF ALPHA are the main mediating cytokines to activate cell death / apoptotic machinery, including the FAS / FASL pathway (yellow). The NK cells are involved by means of upregulation of the NKG2D ligands to the progenitor cells, decreasing the soluble ligands [15].

The role of T-helper cells (CD4+) in aplastic anemia is less known compared to their CD8 homologues [15]. All T helper cells subgroups, that is, Th1 IFN-γ producers, Th2 cell IL-4 producers, regulator T-cells (T-reg), and Th17 are involved and play an important role in aplastic anemia [15,16]. Just like T CD8+ cells, T helper cells show clone expansion with restriction in their TCR sequences [15,16]. T-reg cells are reduced and are functionally defective with a suboptimal capacity to suppress the T-effector cells activity. Besides, there is a polarization change towards Th-1 over the Th-2 response, resulting in a higher IFN-γ production, a potent estimulator of T CD8+ cells [15].

Finally, although the contribution of Th17 cells to the pathogenesis of the aplastic anemia is still controversial, they may contribute counteracting the action of T-regulator cells (T-reg) at an early stage [15,17]. Natural killer cells (NK) are key mediator cells in the innate immunity. The NK lymphocytes subgroup is severely reduced in the aplastic anemia, and drastically increased in the response to immunosuppresants [18]. The NKG2D protein-ligand system represents one of the better characterized pathways of the NK cells; an increase of the expression of several NKG2D ligands inducible by stress has been observed, which were capable of preserving the formation of hematopoietic colonies that use antibodies against these ligands [18,19].

ii. Effector Mediators and their role in Apoptosis

The apoptotic fraction of the CD34+ stem cells increases in the aplastic marrow and plays a crucial role in the pathogenesis of the disease. There is higher Fas expression, one of the receptors in the apoptosis pathway of the CD34+ cells; the levels of Fas surface antigen in stem cells are tightly regulated by gamma interferon (IFN-γ) and tumoral necrosis factor alfa (TNF-α), and its expression is potentiated as a result of the exposition of these cells to both of these pathogen cytokines constitutively secreted by activated effector T cells. IFN-γ expression increases in the marrow of aplastic anemia patients. Intracellular levels of IFN-γ in the marrow infiltrative T cells decrease with the response to immunosuppressive therapies (IST), however, they are increased at the beginning of the relapse; these two cytokines transduce an apoptotic signal to activate the caspases cascade, and they positively regulate the transcription factors (such as the interferon regulating factor 1) through the pathways Fas / FasL and TRAIL. The cytokine polymorphisms implicating TNF-α, IFN-γ and other cytokines such as the beta transforming growth factor (TGF-β) are over represented in certain aplastic anemia populations and may have influence over the disease expression independently of the human leukocyte antigen (HLA) [15,19,20].

iii. Telomeres

Aplastic anemia may be seen as an acquired telomeropathy. Telomeric deoxyribonucleic acid (DNA) is unexpendable to maintain the chromosomic stability, and it is lost with every cell replication. When telomere loss occurs, there is cellular senescence. The length of each telomere is maintained by the telomerases, ribonucleic acid (RNA) complexes - proteins adding lost sequences to the DNA at the end of every replication cycle. Telomerases have a high degree of expression in stem cells, and they are overexpressed in periods of high degree of replication [15].

In aplastic anemia patients a telomeric waste in peripheral blood granulocytes and lymphocytes has been observed, and the degree of shortage has been correlated to the disease severity, risk of relapse and decreased survival. The exact mechanism for the telomeric shortage is not well understood yet, however, it seems to be due to damaging hidden effects of the DNA within the context of a reduced expression of telomerase and other undetermined genetic determinants. In this situation, gene mutations in TERC and TERT have been identified, which are in charge of the coding of the telomerase complex components. Telomeric alterations may not be fully attributed to these genes, since these

alterations have been identified in some healthy patients, therefore, an environmental estimulus becomes a necessary assumption to produce the disease [15].

Unlike other congenital telomeropathies where constitutive defects are present, the telomere-telomerase complex is still to be clarified in the aplastic anemia, if a telomeropathy is a damage primary mechanism to the hematopoietic stem cells, or simply a passive epigenetic phenomenon. The importance lays in the possibility to develop new targeted therapies.

iv. Clone Evolution

DNA direct damage and the effects of the telomere waste may promote genomic instability by creating mutations, unbalanced translocations, and aneuploidies. In fact, it has been shown that the accelerated telomerase waste precedes the clone evolution, and therefore, it may work as a predictive biomarker for the future clone evolution. Chromosomal aberrations may, eventually, result in the formation of displastic clones which may evolve in time. These clones develop unique auto-immune escape mechanisms of the T cells, such as the loss of its loci of HLA alleles with over representation in aplastic anemia. Furthermore, these clones may acquire resistance mechanisms for apoptosis and hyper sensibility to the signaling receptors of the growth factor, thus obtaining a survival advantage.

The clone evolution in the aplastic anemia is produced at a rate of 10-15% in a 10-year period. Several karyotype defects with anomalies of chromosome 7, trisomy 8, structural and numeric anomalies of the chromosome 13, Y chromosome deletion and complex cytogenetics have been identified. The type of defect in the karyotype has a prognostic role in the response, and in the clinical results, with anomalies of chromosome 7 predicting worse results, while trisomy 8 points out a favorable prognosis. It is important to highlight that not all cytogenetic anomalies result in a clone evolution; for example, for example, uniparental 6p disomy has not been described in association with myelodysplastic syndromes (MDS) or Acute Myeloid Leukemia (AML) related to aplastic anemia. Besides, some somatic mutations implying several bone marrow defects have been identified, and they are associated to hematologic neoplasias such as ASXL1, DNMT3A, PIGA, BCOR, and their presence has been correlated to a disease with higher risk of transformation to myelodysplasia/AML. Certain mutations in PIGA, BCOR and BCOR1 correlate to a better response to TIS, while mutations in DNMT3A and ASXL1 associated with worst results. Early detection of harmful clones increases the consideration towards a stem cells transplantation in favor of TIS due to the very high risk of disease transformation [15,21,22].

Clinical Presentation and Physical Examination

The onset of the disease can be acute, slowly progressive or insidious, with a gradual reduction of the peripheral cellular count, generating the classic triad: anemic, infectious and purpuric-hemorrhagic syndrome [7]. The clinical presentation is very variable. The severity of symptoms, although sometimes not very representative, will depend directly on the severity of the cytopenias, and on the etiology. Most severe forms usually apperar within the next six to eight weeks from the onset of the disease [6,14]. Most frequent manifestations are 1) hemorrhage, usually mild, that presents with gingivorrhage, petecchiae or metrorrhagia in women, and those related to an anemic syndrome; 2) fatigue, tiredness, tinnitus, paleness and anger, mainly presenting in the elderly [6]. Manifestations are less frequent in adolescents and young adults since the adaptive capacity (cardiac reserve) makes symptoms milder, therefore the diagnosis may be delayed o attributable to other causes, difficulting the etiologic diagnosis [6,14]. Infections as the first clinical manifestation are rare (< 5% of the cases). They mainly present in the advanced forms of the disease and are mainly caused by fungus and bacteria [6].

As part of the physical examination, the most frequent findings are: anemia of diverse grades, with their respective clinical manifestations, which go from moderate paleness to overt manifestations of anemic cor; purpuric-hemorragic syndrome, that presents from escarse manifestations to petechiae and mucous bleeding in any location or gender, and particularly in women in the form of metrorrhagia of different severities, where the most serious situation is a CNS bleeding; we may find variable degrees of jaundice which might be related to jaundice of diverse grades of seriousness, that might be related with different degrees of hemolysis, if there was a relation with PNH, or history of hepatitis virus infections. Regarding infections, they may also vary based on the particularities of the infectious agent, and the bone marrow aplasia severity. Hepatomegaly's, esplenomegalies and lymphadenopathies do not relate to this type of disease, hence, other entities such as, other infections, leukemia or lymphoma, and lympho- or myelo-proliferative syndromes should be investigated instead [2,4,8,11].

Diagnosis

A diagnosis of aplastic anemia is reached by exclusion: No isolated test allows an accurate diagnosis of aplastic anemia, nor is it correlated to any causal agent, so it will be necessary to use several aides to differentiate it from other diseases presenting bone marrow failure signs [22,23].

Clinical Diagnosis

For any age group, a diagnostic approach through the medical history and the physical examination is crucial for the diagnosis [14]. Within the medical history the following background should be emphasized:

i. Family history and/or signs suggestive of any bone marrow failure hereditary syndrome

ii. History of immunizations

iii. Viral infections (cytomegalovirus (CMV), parvovirus, herpes, hepatitis B and C, Einstein Barr virus (EBV), HIV)

iv. Medicines, toxic agents and drugs (Table 3) [24].

Table 3: Drugs known to be associated with the aplastic anemia20.

Drugs groups	Drugs
Antibiotics	Chloramphenicol, sulfonamides, cotriximazole, linezolid
Antiinflammatories	Gold, penicillamine, fenibutazone, indomethacin, diclofan, naproxen, piroxicam, sulfasalazine
Anticonvulsive	Phenytoin, carbamazepine
Antithyroids	Carbimazole, thiouracil
Antidepressants	Phenothiazines
Antidiabetics	Chlorpropamide, tolbutamide
Antimaláricos	Chloroquine
Other	Mebendazole, thiazides, allopurinol

v. Radiation

vi. Other diseases (graft vs host disease (GVHD), collagenopathies, hepatopathies, renal diseases, thymoma

vii. Transfusional

*Consider drugs administrated up to 6 months prior to the diagnosis. In case a suspicious drug is detected, further exposition should be avoided.

Diagnosis by Laboratory and Clinical Tests

In presence of pancytopenia, the following studies should be requested initially (Table 4):

Table 4: Required laboratory and clinical tests for a diagnosis of aplastic anemia [1,26].

Complete blood count with differential
Reticulocyte count
Peripheral blood smear
Blood chemistry
Liver functioning tests, bilirrubins and LDH
Serum levels of vitamin B12, folates and serum ferritin
Immunologic profile
Viral tests (TORCH, HIV, EBV, parvovirfus and Hepatitis B and C, etc.)
FLAER
Coombs
Chest x-ray and/or CAT
Abdominal ultrasound

TORCH: Toxoplasm, herpes, cytomegalovirus, rubella; FLAER, PNH; CAT: Computerized axial tomography.

Among the initial studies, the following criteria should be met to suspect a diagnosis of aplastic anemia (Table 5) [3,11,22,25]. If there is suspicion of aplastic anemia based on lab tests, a bone marrow estimate and a bone biopsy are essential tests to confirm or discard the diagnosis. Findings to confirm a diagnosis in the bone marrow aspirate and the bone biopsy are (Table 6) [3,13,22,25,].

Table 5: Parameters for a diagnosis of aplastic anemia [7,11,21,26].

Complete hemogram	Normochromic, normocytic or macrocytic anemia: Hemoglobin < 10 g/dL, Neutropenia < 1.5 x 10^9/L) Thrombocytopenia < 50 x 10^9/L There must be a decrease of at least two cellular lines

Corrected reticulocytes count	Always decreased <2%[60] The Mexican Norm establishes <1% A manual count is recommended since sometimes the amount is overestimated by the automated count.
Blood smear	No morphologic alterations

Table 6: Findings in the aspirate and bone biopsy for a diagnosis of aplastic anemia [7,11,21,26].

Bone Marrow Aspirate	Marked decrease of the three cellular lines without dysplasia, with a relative increase of mature looking lymphocytes, plasma cells and fatty infiltration.
Bone Biopsy	Cellularity < 25% 95% bone marrow fat Blasts < 2% Reduced or no erythropoiesis (<10 cells showing maturity) Decreased granulopoiesis, with some maturation focci Decreases megakaryopoiesis Increased lymphocytes Slightly increased mastocytes No dysplastic changes in any cellular line No fibrosis There might be physiological cellularity decreased in the elderly

Aplastic Anemia Classification

According to the results obtained in the peripheral blood and the bone marrow tests, the severity of the aplastic anemia is classified using the Camitta [26] modified criteria, established in 1975, which are crucial for the therapeutic decision making and the bases for the prognosis (Table 7) [1,3,26].

Table 7: Camitta modified criteria for Aplastic Anemia [1,7,19].

Severe	• Marrow biopsy • Cellularity < 25% or 25 to 50% with < 30% of hematopoietic residual elements • And at least two of the following: o Peripheral blood ♣ Neutrophils < 0.5 x 10^9/L (< 500/mm^3) ♣ Platelets < 20 x 10^9/L (< 20,000/mm^3) ♣ Reticulocyte count < 20 x 10^9/L (< 1%)
Very severe	• As the former plus: o Neutrophils < 0.2 x 10^9/L(< 200/mm^3)
Not severe	• Cytopenias not meeting the previous criteria

Differential Diagnosis (Table 8)

Paroxysmal Nocturnal Hemoglobinuria (Myelodysplastic Changes)

There is a strong relation between aplastic anemia and paroxysmal nocturnal hemoglobinuria [6,27]. Aplastic anemia patients may develop paroxysmal nocturnal hemoglobinuria (10% risk at five years, even in patients not treated with immunosuppression), and about 20 to 30% of the patients with hemolytic paroxysmal nocturnal hemoglobinuria may develop aplastic anemia [6,25]. Therefore, the aplastic anemia with traces of paroxysmal nocturnal hemoglobinuria should be considered as a unique entity. This disease is less frequent in young patients, but it is not unknown [14] and it represents a diagnostic paradigm as a cause of aplasia. The tests for its detection have been revolutionary in recent years, such as sucrose hemolysis and Ham tests for a cytometric diagnosis. However, the presence of clones with a phenotype similar to that of paroxysmal nocturnal hemoglobinuria may be present in healthy subjects and in patients with aplastic anemia; if present in the latter, it suggests a greater capacity to respond to immunosuppressive treatment [14,22]. To achieve the identification of the clones, in addition, it is recommended to perform studies with biomarkers such as flow cytometry and quantification using anchoring junction of lympho fosfatidil inositol more sensitive than the binding of antibodies to CD59 [22].

Table 8: Differential diagnosis of pancytopenia in young adults [12].

Etiology	Diagnosis	Diagnostic tool
Marrow Hypoplasia	Malignancy (primary hematologic) Metastatic disease (myeloptisis) Hypoplasic myelodisplastic syndrome	Karyotype Flow cytometry Immunohistochemistry PET/CT
Peripheral Destruction / inadequate production	Paroxysmal nocturnal hemoglobinuria Hemophagocytic histiocytosis Drugs Auto-immune diseases	Urianalysis Flow cytometry
Bone Marrow insufficiency Hereditary Syndromes	Congenital dyskeratosis Fanconi's anemia Shwachman-Diamond syndrome Diamond-Blackfan anemia	Chromosome breakage NGS (*Next-generation sequencing*)
Consumption Disorders	Auto-immune mediated pancytopenia Splenic sequestration	Hepato-splenic gammagram
Production Disorders	Nutritional Bariatric surgery, malabsorption, inadequate intake, alcoholism	Levels of copper, zinc
Other	Pregnancy Anorexia nervosa Hypothyroidism Tuberculosis	

PET/CT: Positron Emission Tomography; Urianalysis: NGS: new generation sequencing).

Diagnostic algorithm [28] (Figure 2)

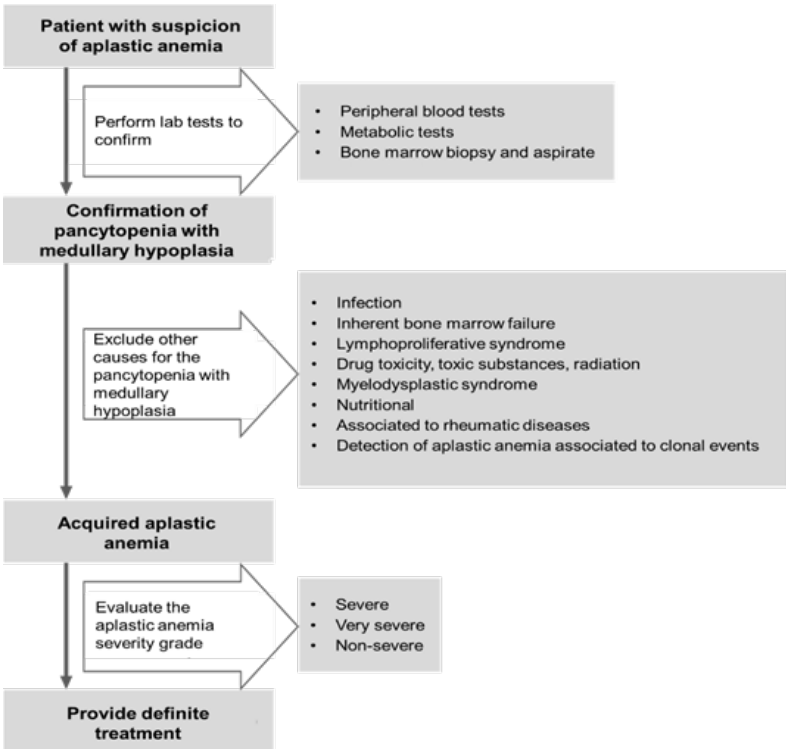


Figure 2: Diagnostic algorithm for aplastic anemia [29].

Treatment

To treat aplastic anemia there are mainly two therapeutical options: hematopoietic stem cells transplantation, and pharmacological immune suppressive treatment [29]. To a large extent, the success of the treatment depends on the accurate and early diagnosis of the disease. Its main objective is to restore hematopoiesis, either based on TCPH or with an immunosuppressive therapy (depending on the choice), besides the preventing and providing early supportive treatment for the complications of a patient with pancytopenia (serious or invasive infections, improvement of cytopenia's and quality of life to reduce morbidity and mortality in this type of cases) [1,29].

The choice of treatment will depend mainly on age (patients <40 years are candidates for HSCT), the severity of the disease and the availability of a compatible HLA donor (related or unrelated) and should be initiated early (shortening the interval between diagnosis and treatment initiation since this is a predictor of survival) [25,29].

Allogeneic or haploidentical transplantation of hematopoietic stem cells

After a diagnosis of aplastic anemia, one of the first actions is the HLA typification of the patient and his family [30], since a hematopoietic stem cell transplantation is the standard treatment in patients under 40 with a compatible donor sibling. Efficacy is estimated between 80 and 90% with a high survival rate (around 90%) [25,22,29,30,31]. Treatment is not recommended during pregnancy [3]. Age is a determining factor for the survival of patients transplanted from HLA compatible siblings. Estimated survival rates at 10 years are 82 to 86%, 72 to 76%, and 53 to 55% for patients from 1 to 20 years, 21 to 40 years, and older than 40 years, respectively [30]. Also, the age of the donor may be an additional prognostic factor. The ideal donor is a male patient, HLA compatible (twins) with loci A, B, C and DRB1, younger than 30 years, with a cytomegalovirus test similar to that of the receptor [30].

Before the transplant a cytogenetic analysis and/or a FISH test are recommended with the aim of determining chromosomic abnormalities. Some have a bad prognosis (as the chromosome 7 suppression), while others (as +Y and +8) are benign and may have influence on the therapeutic response. The identification of a paroxysmal nocturnal hemoglobinuria clone by flow cytometry will help to exclude any hereditary form of bone marrow failure, and to suggest other type of treatment. A negative diepoxybutane test will exclude a case of Fanconi's anemia [30]. Some patients presenting several gene mutations in proteins TERC or TERT may have silent familial antecedents and be asymptomatic or show minimal changes in peripheral blood as macrocytosis. These patients tend to present reduced levels of hematopoiesis and their identification is critical to avoid their selection as familial marrow donors [22]. Another treatment alternative is the transplantation from haploidentical family donors, that may be performed in those patients without an allogenic donor, and who have suspended, at least, a cycle of immunosuppressive therapy or, who rejected an allogenic graft. Although the one-year survival is relatively high, 74%, it should only be performed in the afore-mentioned situations since this type of transplantation is still experimental [30].

Immunosuppressive Therapy

Patients older than 40 years, without criteria of severity or who do not have a compatible HLA donor are the best candidates for immunosuppressive treatment [17,31], and should be started as soon as possible in clinically stable and ideally afebrile patients [9,23,32]. The combination of antithymocyte globulin, cyclosporine A and methylprednisolone is the first-choice scheme due to its effectiveness. The addition of other immunosuppressants such as mycophenolate mofetil (MFM), sirolimus, alemtuzumab and cyclophosphamide (CFA), has not shown improvement in hematological recovery and presenting significant toxicity). Antithymocyte globulin (ATG), obtained from rabbit or horse, produces depletion of T cells in peripheral blood, ganglia and spleen within the first 24 hours and has a prolonged effect [29,33]. It is administered intravenously in 12 to 18 hours (Table 9) [20,29] and possible adverse effects include rash, hypotension, serum sickness (prevented with the use of steroids) and deeper cytopenia's that require transfusional support [24].

Table 9: Summary of the immunesuppresive scheme [24,27].

Drug		Dose	Time
Antithymocyte globulin	Horse	15 mg/kg/day	Infusion 8 h 1-4 days
	Rabbit	3.5 mg/kg/day	Infusion 8 h 1-4 days
Ciclosporin A		5 mg/kg/day every 12 hours	3 to 6 months
Metilprednisolone		25 mg/kg/day IV*	4 days decrease gradually until suspensión in 5 days ATG
Prednisone		1 mg/kg/day PO	Days 6 to 11 de ATG up to 21 days

Randomized prospective studies have compared the effectiveness of the antithymocyte globulin obtained from horse and rabbit and have shown a better hematological response and survival with the one obtained from horse (68 and 96% vs. 37 and 76% respectively) [22]. Approximately two thirds of patients will have hematologic response (in children up to 70-80% but relapses occur in a third of patients). Relapsed or refractory patients can receive a second cycle of immunosuppression, although with a possibility of a response < 20% response [32]. Cyclosporin A, also a T lymphocyte inhibitor, has a greater effect in aplastic anemia when it is associated with anti-thymocyte globulin and

it is recommended to administer it together or after the corticoid suspension for a minimum of 6 months or up to a year at a low dose (until achieving a HR). It is recommended to measure serum levels at 14 days after the treatment initiation and maintaining them between 200 to 400 µg / L, which allows the gradual interruption and prevents adverse events related to treatment [24,29]. Additionally, a Latin American study reported the benefit of the immunosuppressive conditioning prior to a HSCT by improving DFS (79% vs. 61% p = 0.001) and reduces graft failure compared to the use of other schemes (10% vs. 26% p = 0.005) [34].

Other therapies

Monoclonal therapy

Alemtuzumab is a monoclonal antibody that binds to CD52. It was originally used for the treatment of lymphoid neoplasms, but it has been tested in patients with aplastic anemia refractory to treatment who show a four-year overall survival of 67% and a disease-free survival of 37%, with an acceptable safety profile and limited risk of infection [22]. It is also effective as an initial treatment, either alone or in combination with cyclosporin A [35].

Eltrombopag

Eltrombopag (thrombopoietin receptor agonist), initially indicated for the management of idiopathic thrombocytopenic purpura was recently accepted for the control of patients with aplastic anemia [32]. A prospective study analyzed the efficacy of using eltrombopag in patients refractory to conventional immunosuppressive treatment and showed that patients who received it increased the cellularity of the marrow, thus recovering the counting of all the cell lines, suggesting that eltrombopag has a stimulating effect on the hematopoietic stem cell and on early progenitor cells. Subsequently, one trial reported that by adding the drug to the combination of anti-thymocyte globulin and cyclosporin A from the first day of treatment (at a dose of 150mg / day) a complete response was shown with better long-term results, longer hematological recoveries and lower rate of complications, as well as a survival greater than 95% at 18 months of treatment with lower development of cytogenetic abnormalities [30,32].

Androgens

There are no systematized studies showing solid evidence of their benefit, so their use is left to the doctor's judgement. However, androgens have historically been used as an alternative in the palliative management of mild to moderate aplastic anemia in patients <70 years of age. Before the availability of anti-thymocyte globulin and cyclosporine, oxymetholone was used for decades in the treatment of aplastic anemia. In some patients, oxymetalone can stimulate erythropoiesis, but sometimes it can produce a trilineage response [36]. In a randomized trial, women who received this combination regimen had significantly higher response rates, although survival was comparable with the anti-thymocyte globulin-based therapy. Enhanced immunosuppressive therapy with anti-thymocyte globulin and androgens produced a response rate of 77% and a five-year survival of 78% [30], not greater than the conventional immunosuppressive therapy [2], but with a low incidence of clonal events (with oxymetalone) [22].

This drug is available for specific patients (it is not recommended during pregnancy) and it is still useful as an option for those who have suspended for several cycles of anti-thymocite globulin and cyclosporine, or for certain patients in which the standard immunosuppressive treatment is not feasible [9,36]. Oximetalone has liver toxicity and may cause liver dysfunction, jaundice, hepatomas and hepatic peliosis. Hence, it must be used with caution, with regular biochemical and imaging controls. Compared to oximetalone, danazol presents less adverse effects so it is preferred for women and children [22]. Androgens have a special utility in patients with paroxysmal nocturnal hemoglobinuria clones, since there is a possibility of a congenital bone marrow failure if absent [36].

Treatment Algorithm (Figure 3)

Types of response to treatment

About a third of the patients with mild to moderate aplastic anemia present a complete remission of the disease spontaneously and independently of the transfusion. However, the remaining two thirds may be stable for years and progress later to a more severe state [22]. There are three types of responses to treatment classified according to the results obtained after three to four months after this. An important number of patients improve during the first six months (Table 10) [24]. When there is initial treatment failure after 12 months of therapy with the maximum response, it is recommended to hold for three months and to decrease the dose of cyclosporin A [37].

Predictive factors for treatment failure [29] are:

a. Age >18 years

b. Lymphocytes total count <1 x 10^9/L

c. Reticulocytes <25 x10^9/L

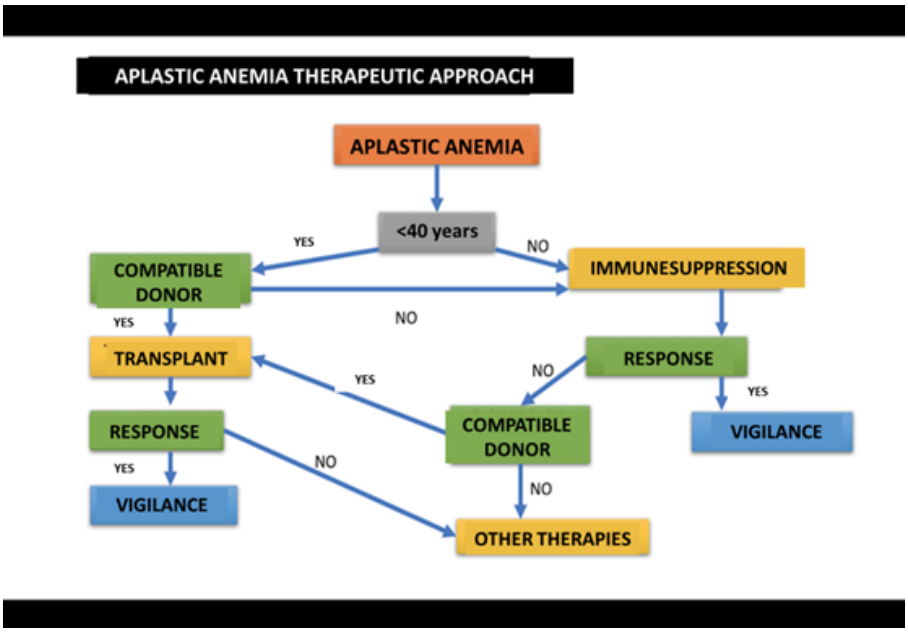


Figure 3: Treatment algorithm proposed by ISSTE physicians [1].

Table 10: Types of response to treatment [20].

Complete Response	Partial Response	Non-Responders
Transfusional Independence	**Transfusional Independence**	**No Transfusional Independence**
• Hemoglobin o 11 g/dL • Platelets o 100 x10^9/L • Neutrophils • > 1.5 x 10^9/L	• Not reaching values for a complete response • Confirmation in two controls (four weeks between each)	• Six months after treatment initiation • Reduction of cyclosporin A

Supportive Treatment

Patients with aplastic anemia are at constant risk of acute and late complications (secondary to cytopenia's or associated with treatment) [5,33], so supportive therapy includes measures that will be carried out from the moment of diagnosis, according to the clinical conditions and the cell count [1], where periodic monitoring and control of the disease will be necessary [24]. Prevention of hemorrhages and prevention / timely treatment of infections are priorities [33].

The support treatment in general comprises 4 important aspects:

i. Transfusion therapy [1,24]

ii. Prophylaxis and management of infections [33]

iii. Iron chelation

iv. Use of stimulating factors

i. Transfusional therapy is essential for the correction of those symptoms related to anemia and thrombocytopenia [29], considering the following measures with respect to the patient conditions [1,18]:

- Maintain hemoglobin levels equal to or greater than 7 g/d that take into account comorbidities and the patient's hemodynamic status; it is advisable to transfuse irradiated and leukoreduced blood products to avoid the development of anti-HLA antibodies and to prevent a graft-versus-host reaction) [1,24].

- Prophylactic transfusion of platelets is recommended when the platelet count is <10 x 10^9/ L or <20 x 10^9/ L in the presence of fever or > 30 x 10^9/ L after the administration of antithymocyte globulin (Never during the administration) [1,24].

ii. Regarding prevention and prophylaxis of infections it is recommended to:

- In case of severe neutropenia, implement personal hygiene measures, isolation, local antiseptics and places with high efficiency air filters (HEPA), if possible.

- Patients with neutrophils <0.5 x 10^9/ L are at high risk of infection and their management is recommended in specialized hospital centers, under strict environmental isolation and the use of prophylactic antimicrobials to impact on survival [33].

Opportunistic infections are typically bacterial causing sepsis, pneumonia, and urinary tract infections. Fungal infestations are the most frequent cause of death, especially in subjects with prolonged and severe neutropenia [35], so prevention and treatment of these complications have a significant impact on the survival, prognosis and quality of life of the patient [33]. Patients with prolonged periods of severe neutropenia (<0.2 x 10^9/L) and with other associated hematopoietic disorders, have a high mortality rate due to *Aspergillus* infection, so antifungal prophylaxis with voriconazole or posaconazole is recommended as first line, leaving fluconazole as a second option that must be maintained at all times as long as neutropenia and/or lymphopenia persist, as well as in the first month after immunosuppressive therapy and/or marrow transplantation [28,33]. Prophylaxis against *Pneumocystis* should be initiated during the period of lymphopenia followed by immunomodulatory therapy [28].

iii. Antibiotic prophylaxis prevents the development of Gram-negative sepsis in patients with severe aplastic anemia, which reduces mortality. The quinolones (ciprofloxacin) and the combination of luminal antibiotics with low absorption level (neomycin and colistin) are effective. On the other hand, beta-lactams or sulfonamides should be avoided due to the potential risk of myelosuppressive effects [33]. Antiviral prophylaxis (acyclovir) is only recommended when patients are going to undergo hematopoietic cell transplantation in order to prevent and treat the reactivation of viremia by cytomegalovirus or Epstein-Barr virus [33].

There are few published data regarding iron chelation in aplastic anemia, the largest study is a 1-year evaluation of patients treated with deferasirox that confirmed that chelation can be administered safely and can reduce serum ferritin. However, a dose adjustment is required, especially in those patients exposed to cyclosporine. In those patients who had a response to immunosuppression or a successful transplant, bleeding is recommended as therapy for iron overload [3].

iv. Regarding the use of growth stimulating factors (GSF), only the GSF-G is indicated during the immunosuppressive therapy or transplantation for the prevention of infections. Erythropoietin has no proven effect during treatment.

Relapse of the Disease

Relapses are more frequent in patients who received immunosuppressive treatment (rate 10 to 40% of patients with good initial therapeutic response), compared with those treated by hematopoietic cell transplantation [5,14,32]. Always keep in mind other causes of pancytopenia and discard them, if this has not been done previously [35]. It is not known with certainty whether modifications of the conventional regimen of immunosuppressive therapy, including danazol, mycophenolate, mofetil, sirolimus or hematopoietic growth factors, positively affect the response to treatment or decrease the relapse rate [32]. Currently, these agents do not have a role in the primary therapy, although some studies suggest that the addition of danazol or growth factors alter the rate of relapse. Alternative immunosuppressive regimens, such as cyclophosphamide or alemtuzumab with or without ciclosporin (for approximately more than a year), are also promising [3,23,35].

Those who received immunosuppressant therapy based on anti-thymocyte globulin with an adequate response and who relapsed, can be given a new cycle with the same scheme, being viable and with good results, generally more favorable than the initial results (response rate from 11 to 65%) [3,23,35]. Other therapies can be considered, such as rituximab, alemtuzumab or cyclophosphamide [3,23], although an excess of relative toxicity has been reported with the latter [3,14].

Transplant in Aplastic Anemia

Introduction and indications of transplantation in Aplastic Anemia

Being a disease mediated by immunity, the response ranges are widely variable depending on factors such as severity, age of the patient and the treatment used. In young patients (under 40 years of age), compatible allogeneic sibling transplantation (HLA) is considered the gold standard, because it is the only curative treatment, with early response rates and a reduction in the risk of clonal evolution [30]; In addition, in those with severe aplastic anemia refractory to treatment or those with recurrent infections, transplantation is the option that offers a better response opportunity. In addition, it is indicated in patients with severe or very severe AA, who have a compatible donor and who are under 40 years of age.

The possibility of having a compatible HLA donor is low, which has led to an increase in the use of haploidentical transplantation worldwide. There are several reports with a limited number of patients, and favorable response rates in those with failure to immunosuppressive therapy or graft failure after a transplantation of an unrelated donor, in which OS rates of up to 67% have been documented at 1 year; However, one of the main limitations of this modality is the high rate of complications such as GVHD and infections [36].

Age plays an important role in the decision to transplant a patient, since the response rate decreases as age increases. This “age effect” is demonstrated in what was reported by EBMT from 2001-2010, finding a 10-year survival of 86%, 76% and 55% in patients aged 1-20 years,

21-40 years and older than 40 years, respectively, being the lower benefit clear in patients older than 40 years. This can be explained due to the high failure rate of the graft and complications such as GVHD that occur in this age group. Furthermore, immunosuppressive therapy in these older patients has comparable response rates with significantly lower complications [36].

Pre-transplant Studies and Interventions

For patients who are candidates for a transplant the following is the minimum recommended:

a. Confirm diagnosis and exclude clonal evolution

b. Evaluation of comorbidities.

c. Selection of the donor, conditioning regimen, as well as the source and dose of hematopoietic stem cells

d. Fertility evaluation

The eligibility criteria commonly used for patients who are candidates for transplantation are mentioned in Table 11.

Table 11: Eligibility criteria.

Eligibility Criteria	Test	Eligible for Transplantation
Patient status	**Medical History and Physical Examination**	**ECOG < 2 and Karnofsky >70%**
Markers for infectious diseases	Serology for hepatitis A, B, C, HIV, CMV, EB virus, Toxoplasmosis	Patients should not have data suggesting active viral infection Confirm vaccination scheme
Cardiac function	Echocardiogram or nuclear medicine study	LVEF >50%
Pulmonary function	Respiratory function test	DLCO > 40%
Renal function	Creatinine and creatinine clearance	Creatinine clearance > 40 ml/min
Liver function	Liver functioning tests	Bilirrubins < 2-3 the upper level
Psychosocial evaluation	Psychologic/psychiatric evaluation	Dependerá de cada centro

ECOG: Eastern Cooperative Oncology Group; LVEF: Left Ventricular Ejection Fraction; DLCO: Diffusing Capacity for Carbon Monoxide; HIV: Human Immune Deficiency Virus; CMV: Cytomegalovirus; EB: Epstein Barr. In addition, an evaluation by social work and nutrition is recommended, this will depend on each site.

Donor Selection

The optimal donor is a related human leukocyte antigen (HLA) donor, that is, one typified as HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 and compatible with the donor 8/8 or 10/10, since these are less associated with acute or chronic graft-versus-host disease [39]. In the event that a related donor is not available, a non-related HLA donor is preferred to a haploidentical donor. The evidence of the response in haploidentical transplantation in aplastic anemia is very limited, however, it can be considered in case of not having any of the aforementioned options [38].

Types of Transplantations

a. Allogeneic hematopoietic stem cell transplantation of related compatible HLA precursors

b. Allogeneic hematopoietic stem cell transplantation of unrelated compatible HLA compatible: it is indicated in ASA after failure to a course of immunosuppressive treatment.

c. Haploidentical hematopoietic stem cell transplantation

Sources of Stem Cells

i. Bone marrow: It is considered the ideal source due to greater probability of graft and lower risk of GVHD in both allogenic and haploidentical.

ii. Peripheral blood: It has been related to a higher risk of GVHD and lower graft, however, it is the most widely used due to the greater ease of collection and fewer complications for the donor.

iii. Umbilical cord and placenta [3].

Conditioning Regimes

The standard conditioning regimen in younger than 40 years of age is based on cyclophosphamide with or without ATG, the European Blood and Marrow Transplantation Registry (EBMT) indicates the use of reduced intensity conditioning in patients older than 60 years, with fludarabine plus cytarabine at low doses and ATG, with good response in overall survival (SG) and a similar incidence of acute and chronic

GVHD to the standard scheme. In regimens that include total body radiotherapy, it has been observed that graft rejection decreases, however it is related to greater pulmonary and cardiac toxicity, concluding that globally, its benefit is controversial [38,39]. The use of rabbit ATG in conditioning therapy was found to be associated with a lower incidence of acute GVHD compared with the use of ATG of equine origin, the latter being more associated with the development of chronic GVHD [37-39].

Mobilization Scheme

The use of colony and granulocyte stimulating factors (CSF-G) for mobilization and subsequent harvesting of hematopoietic stem cells (HSC) has been associated with a higher incidence of GVHD. The incidence of GVHD is reduced when the dose of donor HSC is limited to 2.5 X 108/kg and is more effective when infused after cyclophosphamide 200 mg/kg followed by prophylaxis with cyclosporine and methotrexate, since it reduces the risk to less than 10% [37,38]. Post-transplant complications are shown in the following table (Table 12). The next table shows agents used for GVHD prevention (Table 13). The table of clinical manifestations by organ is presented below (Table 14).

Table 12: Post-transplantation complications [38,39,40].

Phase	Chemotherapy Phase (D-10 to D 0)	Cytopenic Phase (D0 to graft)	Early Recovery (D+5 to D+7)	Early Recovery (D+30 until 6-12 mo PT)*	Late Recovery (>12 mo PT)
Infections	GPC Catheter related	GPC and GNC of GI toxicity VHS Mycotic infections	GPC and GNC resistant mycotic infection CMV reactivation HPV reactivation Other viruses	Viral reactivation Pneumocystis Encapsulated GPC	Viral reactivation (if active GVHD) Encapsulated GPC
Gastrointestinal	Nausea Vomit Diarrhea	Mucositis Diarrhea Nausea Anorexia	If prolonged nausea and/or vomiting, signs of upper GI GVHD		
Hepatic	Transamina-semia	Transamina-semia OVD	Transamina-semia OVD v	Hepatitis reactivation	Hepatitis reactivation
Cardiac	Arrhythmia Water overload	Hypertension due to calcineurin inhibitor	Hypertension due to calcineurin inhibitor	Congestive cardiac failure	Early coronary disease
Pulmonary	Pneumonitis (rare)	Pneumonia Water overload	Idiopathic pneumonia syndrome	Cryptogenic pneumonia	Bronchiolitis obliterans Airway hyperreactivity
Neurologic	Convulsions by busulfan (rare with prophylaxis)				Cognitive dysfunction Short-term memory loss
Endocrine	Hyperglycemia	Hyperglycemia by calcineurin inhibitor	Hyperglycemia by calcineurin inhibitor	Hyperglycemia Hypothyroidism	Metabolic syndrome
Renal	Acute renal lesion Hydroelectrolyte imbalance	Acute renal lesion Hydroelectrolyte imbalance	Acute renal lesion Hydroelectrolyte imbalance	Chronic renal disease	
Acute GVHD			Presentation of fever and skin rash Cytokine storm	Late acute GVHD (it presents with an acute onset of diarrhea or rash)	
Chronic GVHD				It usually presents with the withdrawal of the immune suppression	
Hematologic	Transplant associated TTP (may present as from D+4 to D+60; sometimes up to 2 years post-transplantation) Bleeding (from hemorrhagic cystitis, secondary to cyclophosphamide use or Vk virus infection) Hemosiderosis (increased risk of infection, OVD, liver dysfunction) Lymphomas				
Other					Cataract Secondary Neoplasias (lymphomas)

GPC: Gram-Positive Cocci; GNC: Gram-Negative Cocci; GI: Gastrointestinal; OVD: Occlusive Vascular Disease; CMG: Cytomegalovirus; HPV: Human Papiloma Virus; TTP: Thrombotic Thrombocytopenic Purupura; GVHD: Graft versus Host d#isease.

Table 13: Agents used for GVHD prevention [38-40].

Agent	Dose
Ciclosporin	3mg / Kg IV
Tacrolimus	0.02 mg / Kg IV
Methotrexate	15 mg / m^2 day +1
	10 mg / m^2 day +3, 6 and 11
Methylprednisolone	0.5-1.0 mg / Kg
Mycophenolate mofetil	1.5-3 g / day
Sirolimus	12 mg day -3 after, 4 mg / día
Anti-thymocyte Globulin	2.5 mg / kg / day x 4
Alemtuzumab (anti-CD52)	10 mg / kg / day, for 5 days usually
Cyclophosphamide	50 mg / kg / day, on days +3 and +4

Table 14: Clinical manifestations per transplant organ [38-40].

Organ	Clinical Manifestations
Skin	1. Rash erythematosus-maculopapular, initially involving palms and plants 2. May progress to the whole-body surface 3. May be pruritic and/or painful 4. In severe cases, formation of bullae to desquamation
Liver	5. Cholestasis with or without evident jaundice 6. Higher increase of cholestatic enzymes than transaminases
Gastrointestinal Tract	7. Anorexia, nausea and vomit 8. Diarrhoea, typically liquid and típicamente verdosa y líquida 9. In severe cases, diarrhea may contain fresh blood and mucus, accompanied by abdominal pain and sometimes followed by paralytic ileus

Aplastic Anemia in Pediatric Patients

Definition

It is a rare syndrome characterized by pancytopenia and hypocellularity of the bone marrow, without dysplasia or fibrosis. In the pediatric age, it is particularly important to rule out congenital bone marrow syndromes, which represent 25% to 30% of the cases, as well as hypoplastic myelodysplastic syndrome, which although rare, should be considered in these patients [40].

Epidemiology

Incidence of aplastic anemia in the pediatric population is unknown in our country. At the ISSSTE National Medical Center "20 de Noviembre" there have been sixty eight cases diagnosed with bone marrow failure syndrome, out of which 41% correspond to acquired aplastic anemia patients, 29% bone marrow failure due to congenital disorders, among them (pure red cell aplasia, Fanconi's anemia, congenital neutropenia, congenital amegakaryocytic thrombocytopenia, and Blackfan-Diamond anemia) during the period 2010 to 2017. The rest are attributed to hypoplastic myelodysplastic syndrome and paroxysmal nocturnal hemoglobinuria [41]. It is important to point out along with these findings that Fanconi's anemia is not uncommon in the Mexican population, therefore it is vital to carry out the required studies to rule out a chromosomic instability. Mean age at diagnosis in the treated population in this group is 7.5 years, 53% women and 47% men [1].

There is an important difference between the incidence of aplastic anemia in developed countries and those in development, in such a way that the incidence is to 2 to 3 times higher in the Southeast and East of Asia (3.9-5 cases per million in Thailand, and 7.4 per million in China) than in Europe and the United States (2-2.3 cases per million). This is presumably due to the exposure to chemicals and infections in India, and to the exposition to contaminated water, and other myelotoxic agents in Thailand. It has been described that the age at the onset of the disease is between 11 and 20 years in a hospital in India where 65% were male and 55% presented severe disease [5]. In Mexico City it was estimated that the average incidence in the population registered at IMSS was 4.8 new cases per million in people younger than 15 years, and 4.1 new cases in patients >15 years [40,42].

Etiology

In 70% of the patient's etiology is not determined [40]. It is known that there is an important interrelation between genetic mutations, disease penetration and the interaction of the genetic environment (Figure 4) [40]. Its presentation is attributed to the exposure to a large variety of drugs and chemicals, ionizing radiation and some viruses (Table 15) [40].

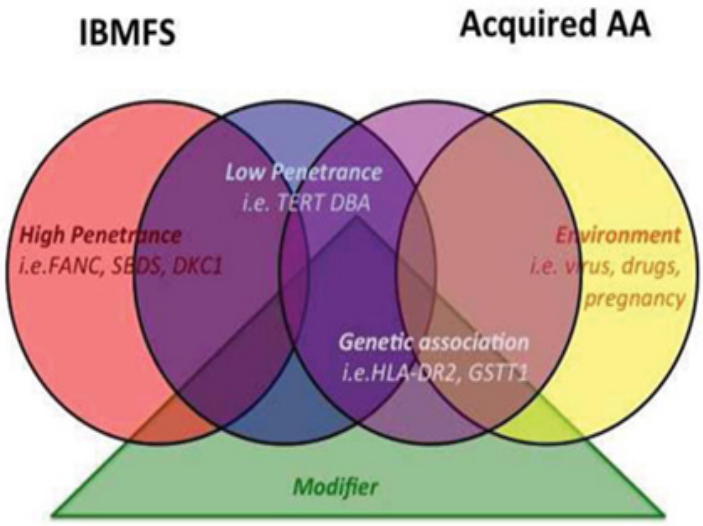


Figure 4: Schematic representation of the relation between genetic mutations, disease penetration, and the genetic environment for the pathogenesis of bone marrow failure syndromes [41]. IBMFS: Inherited bone marrow failure syndromes.

Table 15: Etiologies associated with Acquired Aplastic Anemia [41].

Infections	Hepatitis associated Epstein-Barr virus Cytomegalovirus Parvovirus Mycobacterial infections HIV Herpes virus 6 Virus varicela zoster Measles Adenovirus Others
Nutrition	Copper, folinic acid, and vitamin B12 deficiency
Drugs	Non-steroidal anti-inflammatories Antibiotics Antiseizures Sulfas Gold salts Chloramphenicol Other
Chemicals	Benzene Insecticides Pesticides Solvents
Radiation	
Other associations	Pregnancy Autoimmune diseases (Ej. Systemic erythematous lupus) Graft vs host disease
Idiopathic	Presently called of Immune Origin

Pathophysiology

Recent evidence suggests that acquired aplastic anemia results from an abnormal activation of autoreactive T lymphocyte clones and the suppression of regulator T cells. This IL-2 mediated activation generates the expansion and differentiation of T lymphocytes into effector and memory cells. These proinflammatory T cells produce a variety of cytokines including FAS ligand, interferon gamma, tumor necrosis factor alpha, which induce apoptosis of hematopoietic progenitor cells, alteration in gene regulation and decrease in synthesis of proteins that inhibit cell entry hematopoietic reactions to the cell cycle generating bone marrow failure [41]. Patients with acquired aplastic anemia have a decrease in the number of regulatory T cells (T CD4+, CD25+), which correlates inversely with the severity of the disease. A smaller amount of regulatory T cells is also associated with poor response to treatment [40].

Diagnosis

The clinical suspicion and the diagnostic approach are performed with the same criteria as in the adult population. In the pediatric patient it is important to rule out congenital bone marrow syndromes, especially Fanconi's anemia and congenital dyskeratosis, therefore all patients should be evaluated for chromosomal breaks and telomere lengths. If they are identified, the treatment must be adjusted to these clinical conditions (Table 16) [40]. The severity is measured by the Camitta severity criteria [26] described in 1975, also used in the adult population.

Table 16: Differential diagnosis [41].

Disease to be discarded	Diagnostic Test	Indication
Fanconi's Anemia	Chromosomal breakdown induced by mitomycin	Mandatory Adequate
Congenital dyskeratosis	Measurement of telomere length in leukocytes DKC1, TERC, TERT, TINF2, NOP10, NHP2, TCAB1, RTEL1	If there is clinical suspicion or reduced telomere length
Shwachman-Diamond Syndrome	Pancreatic function	If there is clinical suspicion
Blackfan-Diamond Anemia	Gen SBDS	If there is clinical suspicion
Congenital Amegakaryocytic Thrombocytopenia	c-Mpl	To be considered in younger children
Pearson's Syndrome	Mitochondrial DNA analysis	If there is clinical suspicion

Treatment

In the neonatal bone marrow aplasia, it is important to rule out marrow failure syndromes, immunological alterations such as congenital lupus erythematosus, severe combined immunodeficiency or infections with high mortality, for which there is no consensus of specific treatment and most require finally a hematopoietic stem cell transplantation (HSCT). Early intervention, (immunosuppression <4 weeks, TCPH <12 weeks) is associated with better response [40]. The first-line treatment for pediatric patients with aplastic anemia is hematopoietic stem cell transplantation (HSCT) when a 100% compatible donor is available, with a 5-year general survival with ranges greater than 90%. The unrelated donor transplant is reserved for patients in whom immunosuppressive therapy has failed [40]. Although HSCT is the ideal treatment, the availability of centers for transplantation, the psychosocial and economic conditions of patients often do not make it a viable option in our society, so alternatives to treatment should be sought [41].

Immunosuppressive therapy alters the destruction of hematopoietic stem cells mediated by T lymphocytes by inhibiting their response in multiple processes of their activation, as shown in Figure 5 [40]. For several years, treatment with horse thymoglobulin has been shown to be an alternative treatment. Unfortunately, it is not available in our country, which is why rabbit antilymphocyte globulin has been shown to be a viable option. The dose of 3.5 to 5 mg/kg/day for 5 consecutive days has shown better response rates (56%).44 In recent years, the thrombopoietin analogue, eltrombopag, showed effectiveness in patients with refractory aplastic anemia, which led to a study of its use in the first line of treatment associated with thymoglobulin, with improvement in overall survival, with a 95% survival rate at 18 months after treatment, and a decrease in the development of cytogenetic abnormalities, when it begins on day 1, together with immunosuppressive therapy, up to 6 months or platelet count greater than 50 000 [43,44]. The table below shows pediatric doses for the immunosuppresive treatment for aplastic anemia (Table 17) [45].

Table 17: Pediatric doses for the immunosuppressive treatment of AA [47].

Drug	Dose	Schedule
Thymoglobulin	3.5-5 mg/kg/day	Days 1 to 5
Ciclosporin	1-5mg/kg/day (levels 150-400 ng/ml)	Day 1 to 6 months
Eltrombopag	150 mg/day (>12 years)	Day 1 to 6 months
	75 mg/day (6 – 11 years)	
	Day 1 to 6 months	
Methylprednisolone	1-2mg.kg/day	Day 1 to 2 weeks

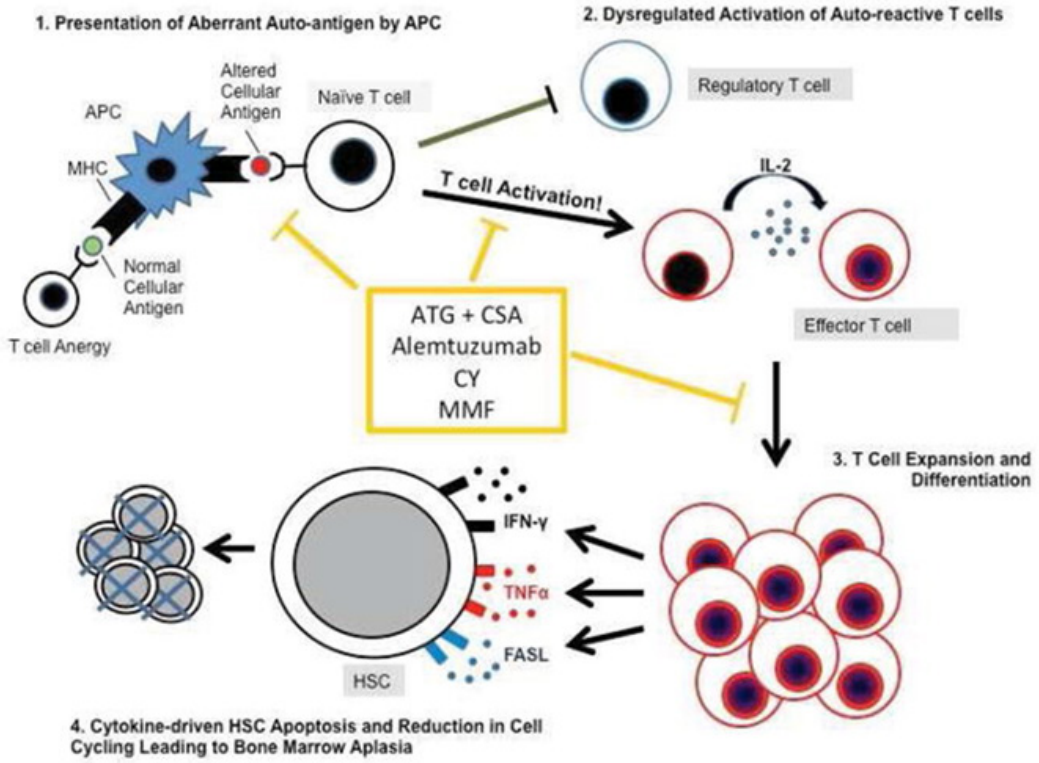


Figure 5: Current evidence suggests that acquired AA results from the aberrant activation of one or more self-reactive T cell clones given the alteration of antigens present in the Major Histocompatibility Complex (MHC) on the surface of antigen-presenting cells [41].

Hematopoietic Stem Cells Transplantation

Bone marrow transplantation from a compatible related donor is the ideal treatment option in patients with severe aplastic anemia, with survival of 75 to 90% [36]. Recommended conditioning regimens include cyclophosphamide (200 mg / kg divided in 4 days) with or without thymoglobulin (7.5 mg / kg); or fludarabine (150 mg / m2SC) associated with low doses of cyclophosphamide. As prophylaxis of GVHD, the use of methotrexate associated with ciclosporin is recommended [45]. If a related donor is not available, it is possible to search for an unrelated donor, where regimens with low-dose irradiation or fludarabine are recommended [36,44]. In case a related donor is not available, a haploidentical transplant can be performed, without reaching yet a consensus for the ideal conditioning in these cases [36]. A marrow transplantation using fludarabine conditioning has shown a global survival of 80% at 2 years, according to the Eurocord report [36].

Other Treatments

Another treatment option for immunosuppressive therapy includes a combination of ciclosporin (5 mg / kg / day), danazol (5-10 mg / kg / day) and levamizol (2.5 mg / kg / day), with a 6-month response rate of 24.3% and 52.9% for very severe and moderate aplastic anemia, respectively. General survival at 5 years of 33.6 and 80.5% for very severe and moderate anemia, respectively [43,46].

Response Evaluation

There are predictors of response, patients whose CD8 cells express IFN-γ have a better response to immunosuppressive therapy with anti-thymocyte globulin plus cyclosporin. An initial absolute reticulocyte count > 25,000/μL have response rates of 90% compared to 65% in those with lower counts [19]. Below is a table showing the response criteria for not very severe and very severe aplastic anemia (Table 18) [36].

Table 18: Response criteria for severe and very severe aplastic anemia [36].

None	Still severe
Partial	Transfusional independence No longer meets the criteria for very severe disease
Complete	Normal hemoglobin for the age Neutrophil count >1.5X10^9 Platelet count >150x10
Response Criteria for Non-Severe Aplastic Anemia	

None	Worsens or not meeting the following criteria
Partial	Transfusional Independence (if previously dependent), or Duplication or normalization of at least one cellular line, or Increase of baseline hemoglobin >30 g/l (if baseline <6,) or Increase of baseline neutrophils >0.5x109 /l (if baseline <0.05), or Increase of baseline platelets >20x109 (if baseline <20)
Complete	Same criteria as for the sever disease

Supportive treatment

Patients with aplastic anemia are at constant risk of acute and late complications; supportive therapy includes measures that will take place as from the moment of diagnosis according to the patient's clinical conditions and cell count.

Transfusions

Red blood cell transfusions will only be reserved for patients with levels below their percentile or < 7 g/dl, and symptoms associated with leucodepleted products to reduce the risk of HLA sensitization; as well as negative CMV products. The European Bone Marrow Transplant Group suggests that irradiated blood products should be received to prevent the graft-versus-host reaction [34]. Consider that repeated transfusions of red blood cells can generate iron overload, which will be suspected when ferritin is > 1000 µg/l persistently or a transfusion volume greater than 200 ml/kg, so iron chelation is important [47,48]. Prophylactic platelet transfusion is recommended when its concentration is < 10 x 10^9 or < 20 x 10^9 with fever. During the administration of anitithymocyte globulin transfuse platelet concentrates to maintain a count equal to or greater than 30 x 10^9

Prevention and treatment of Infections

Typically, infections are bacterial, causing sepsis, pneumonia, and urinary tract infections; Fungal infections are the most frequent cause of death, especially in subjects with prolonged and severe neutropenia [36]. Antifungal prophylaxis with voriconazole is recommended in patients who underwent transplantation from day 1 to day 180, before persistent immunosuppression or total neutrophils < 500 cells/mm3. The posology for prophylaxis is 6 mg/kg every 12 h and maintenance (after 24 h) 4 mg / kg every 12 h, in case of intolerance 3 mg / kg every 12 h. In non-transplanted patients' antifungal prophylaxis with fluconazole (dose 3-12 mg /kg/ day), until reaching a neutrophil count equal to or greater than 1000 cel / mm^3. Pneumocystis jirovechi is still the most frequent causative agent of pneumonia in a lymphopenic patient, therefore prophylaxis with trimethoprim and sulfamethoxazole is recommended 3 times a week during the periods of lymphopenia and deep neutropenia. Antiviral prophylaxis with acyclovir will only be given in case of a transplant with a dosage of 20 mg/kg every 6 hours, with a maximum dose of 800 mg/day [35-50].

Estimulating factors

Its use is recommended when neutrophils are < 500 / µL, but never as single therapy. The use of G-CSF has not shown a significant difference in the overall survival or event-free survival, so its use is recommended only in case of neutropenia associated with infection [42]. Prolonged use of G-CSF (longer than 40 days) may increase the risk of chloral hematopoiesis and malignant transformation, associated with chromosome 7 monosomy [36].

Vaccines

Vaccination is recommended up to one year after the suspension of immunosuppression with inactivated vaccines. Vaccines with live attenuated virus are not recommended in this population because of the risk of relapse of aplastic anemia [40,43]. 15% of patients with severe aplastic anemia develop an PNH clone during the evolution of the disease, which is why it is recommended to perform complementary studies to diagnose them [46].

References

1. Diagnóstico y tratamiento del síndrome de falla Medular en edad pediátrica en tercer nivel de atención médica (2009) Secretaría de Salud, México.
2. Labardini-Méndez JR. Oncoguía: síndrome de falla medular. Cancerología 2011: 125-128.
3. Killick SB, Bown N, Cavenagh J, Dokal I, Foukaneli T, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. British Journal of Haematology 172(2): 187–207.
4. Luzzatto L, Risitano (2018) Advances in understanding the pathogenesis of acquired aplastic anaemia. British Society for Haematology 182(6): 758-776.
5. Núñez J, González-Mesones B, Montes-Gaisán C (2012) Síndromes de fracaso medular: anemia aplásica, eritroblastemias selectivas y anemias diseritropoyéticas. Medicine 11(20): 1229-1237.

6. Martínez-López J, Ayala-Díaz R (2001) Insuficiencia medular. Anemia aplásica. Medicine 8(50): 2625-2631.

7. Salido-Fierrez E, Cabañas-Perianes V, Moraleda-Jiménez JM (2016) Anemia aplásica: hemoglobinuria paroxística nocturna. Medicine 12(20): 1159-1169.

8. Benítez-Aranda H, Vélez-Ruelas MA, Díaz-Cárdenas S, Sánchez-Valle E, Xolotl-Castillo M, et al. (2002) Incidence of aplastic anemia in a defined subpopulation from Mexico City. Hematology 7(4): 229-232.

9. Incidence of aplastic anemia: the relevance of diagnostic criteria. By the International Agranulocytosis and Aplastic Anemia Study (1987) Blood 70(6): 1718-1721.

10. Vaht K, Göransson M, Carison K, Isaksson C, Lenhoff S, et al. (2017) Incidence and outcome of acquired aplastic anemia: real-world data from patients diagnosed in Sweden from 2000–2011. Haematologica 102(10): 1683-1690.

11. Gutiérrez-Serdán R, López-Karpovitch X (2016) Acquired aplastic anemia: a demographic, clinical, and therapeutic survey of a single institution in Mexico City. Rev Hematol Mex 17(2): 81-89.

12. Sánchez Valle E, Vélez Ruelas MA, Benítez-Arana H, Díaz Cárdenas S, et al. (2004) Estudio y tratamiento de la anemia aplásica en México: Una perspectiva sobre el estado actual. Rev Hematol 5 (1): 3-19.

13. Dolberg OJ, Levy Y (2014) Idiopathic aplastic anemia: Diagnosis and classification. Autoimmunity Reviews 13 (4-5): 569-573.

14. DeZern AE, Guinan EC (2014) Aplastic anemia in adolescents and young adults. Acta Hematol 132(3-4): 331-339.

15. Prajwal Chaitanya Boddu, Tapan Mahendra Kadia (2017) Updates on the pathophysiology and treatment of aplastic anemia: a comprehensive review. Expert Rev Hematol 10(5): 433-448.

16. Zoumbos NC, Gascón P, Djeu JY, Trost SR, Young NS (1985) Circulating activated suppressor T lymphocytes in aplastic anemia. N Engl J Med 312(5): 257–265.

17. Hosokawa K, Muranski P, Fenx X, Townsley DM, Liu B, et al. (2016) Memory Stem T Cells in Autoimmune Disease: High Frequency of Circulating CD8+ Memory Stem Cells in Acquired Aplastic Anemia. J Immunol 196(4): 1568–1578.

18. Sloand E, Kim S, Maciejewski JP, Tisdale J, Follmann D, et al. (2002) Intracellular interferon-gamma in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia. Blood 100(4): 1185-1191.

19. Scheinberg P, Nunez O, Weinstein B, Scheinberg P, Biancotto A, et al. (2011) Horse *versus* rabbit antithymocyte globulin in acquired aplastic anemia. N Engl J Med 365(5): 430–438.

20. Zoumbos NC, Gascón P, Djeu JY, Trost SR, Young NS (1985) Circulating activated suppressor T lymphocytes in aplastic anemia. N Engl J Med 312(5): 257–265.

21. Tetsuichi Y (2015) Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med 373: 35-47.

22. Barone A, Lucarelli A, Onofrillo D, Verzegnassi F, Bonanomi S, et al. (2015) Diagnosis and management of acquired aplastic anemia in childhood. Guidelines from the Marrow Failure Study Group of the Pediatric Haemato-Oncology Italian Association (AIEOP). Blood Cells Mol Dis 55(1): 40-47.

23. Guinan EC (2011) Diagnosis and Management of Aplastic Anemia. Hematology 2011: 76-81.

24. García E, Milovic V, Ramos A, et al. (2017) Síndromes de fallo medular. Sociedad Argentina de Hematología, p. 32.

25. Miano M, Dufour C (2015) The diagnosis and treatment of aplastic anemia: a review. Int J Hematol 101: 527 - 535.

26. Hyun Hwa Yoon, Seok Jae Huh, Ji Hyun Lee, Suee Lee, Sung-Hyun Kim, et al. (2012) Should we still use Camitta´s criteria for severe aplastic anemia. Korean J Hematol 2012; 47(2): 126-130.

27. Iwafuchi H (2018) The histopathology of bone marrow failure in children. J Clin Exp Hematop 58(2): 68-86.

28. Peslak SA, Olson T, Babushok D (2017) Diagnosis and treatment of aplastic anemia. Curr Treat Options in Oncol 18(12): 70.

29. Chona Z, Mendoza C, De Baena E, (2015) I Consenso Venezolano de Anemias Aplásicas.

30. Bacigalupo A (2017) How I treat acquired aplastic anemia. Blood 129(11): 1428-1436.

31. Scheinberg P, Young N (2012) How I treat acquired aplastic anemia. Blood 120: 1185-1196.

32. Scheinberg P (2018) Recent advances and long-term results of medical treatment of acquired aplastic anemia: are patients cured? Hematol Oncol Clin N Am 32(4): 609–618.

33. Höchsmann B, Moicean A, Risitano A, Ljungman P, Schrezenmeier H, et al. (2013) Supportive care in severe and very severe aplastic anemia. Bone Marrow Transplantation 48(2): 168–173.

34. Gomez-Almager, Vázquez-Mellado A, Navarro-Cabrera JR, Abello-Polo V, Milovic V, et al. (2017) The Latin American experience of allografting patients with severe aplastic anaemia: real-world data on the impact opf stem cell source and ATG administration in HLA-identical sibling transplants. BMT 52(1): 41-46.

35. Schrier SL, Mentzer WC, Rosmarin AG (2018) Aplastic anemia. Pathogenesis, clinical manifestation and diagnosis uptodate. UpToDate. Actualizado 03 de octubre de 2017.

36. Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, et al. (2009) Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol 149(1): 43-70.

37. Georges G, Storb R (2016) Hematopoietic stem cell transplantation for acquired aplastic anemia. Curr Opin Hematol 23(6): 495-500.

38. Attilla E, Ataca Attilla P, Demirer T (2017) A review of myeloablative *vs*. reduced intensity/non-myeloablative regimens in allogeneic hematopoietic stem cell transplantations. Balkan Med J 34: 1-9.

39. Hematologica (2017) 102: 1291-1298.

40. Hartung HD, Olson TS, Bessler M Acquired Aplastic Anemia in Children. Pediatr Clin North Am 60(6): 1311–1336.

41. Estadísticas del Servicio de Hematología Pediátrica del CMN (2018) "20 de Noviembre" ISSSTE.

42. Diagnóstico y Tratamiento del Síndrome de Falla Medular en la edad pediátrica en tercer nivel de atención médica (2014) Secretaría de Salud, México.

43. Townsley DM, Scheinberg P, Thomas Winkler, Ronan Desmond, Bogdan Dumitriu, et al. (2017) Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia. New England Journal of Medicine. 376: 1540-1550.

44. Su Han Lum, John D Grainger (2016) Eltrombopag for the treatment of asplastic anemia: current perspectives. Drug Design, Development and Therapy 10: 2833-2843.

45. Scheinberg P, Wu CO, Nunez O, Young NS (2008) Long-term outcome of pediatric patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine. J Pediatr 153(6): 814-819.

46. Wang M, Li X, Shi J, Shao Y, Ge M, et al. (2015) Outcome of a novel immunosuppressive strategy of cyclosporine, levamisole and danazol for severe aplastic anemia. Int J Hematol 102(2): 149-156.

47. Peslak SA, Olson T, Babushok DV (2017) Diagnosis and Treatment of Aplastic Anemia. Curr Treat Optiones in Oncol 18(12): 18-70.

48. Samarasinghe S, Webb D (2012) How I manage aplastic anaemia in children. British Journal of Haematology 157(1): 26–40.

49. Brodsky Andrés L, Elena, Graciela Milovic, Vera Ramos, Anahí Rossi, Blanca de los Milagros, et al. (2017) Síndromes de fallo medular. Sociedad Argentina de Hematología, Guías de diagnóstico y tratamiento.

50. Judith CW Marsh, Sarah E Ball, Jamie Cavenagh, Nick Bown, Inderjeet Dokal, et al. (2015) Guidelines for the diagnosis and management of aplastic anemia. Blackwell Publishing Ltd, British Journal of Haematology 147: 43-70.