UNIVERSITATEA DE MEDICINĂ ŞI FARMACIE

„CAROL DAVILA”, BUCUREŞTI

ŞCOALA DOCTORALĂ

DOMENIUL MEDICINĂ

TEZĂ DE DOCTORAT

Conducător de doctorat:

PROF. UNIV. DR. ARION CONSTANTIN-VIRGILIU

student-doctorand:

DR. ZAHARIA (CĂS. JERCAN) CRISTINA GEORGIANA

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ANEMII APLASTICE LA COPIL. ASPECTE ETIOPATOGENICE ȘI TERAPEUTICE ACTUALE

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Cuprins

Introduction

Aplastic anemias constitute re spectrum of severe hematological disorders, characterized by hypoplasia of the bone marrow affecting the three major cell lines. Given the morbidity and mortality associated with these conditions and the complexity of the interventions required in the pediatric population, a thoroughgoing study regarding etiopathogenic mechanisms and optimization of evidence-based therapeutic strategies are required.

The increase in the incidence of congenital bone marrow failure syndromes (IBMFS) - formerly known as constitutional/congenital aplastic anemias, observed in recent decades can be attributed to a combination of factors. First of all, diagnostic techniques have experienced real progress, and the use of advanced sequencing techniques, such as whole exome sequencing (WES) and next-generation sequencing (NGS), have allowed a correct diagnosis of the pathogenic mutations found in this category of disease. WES and NGS techniques enable the detection of rare gene variations and alterations in genome regions that were previously inaccessible using traditional diagnostic methods.

Improved access to specialist medical services and new genetic testing facilities have contributed to the increase in the number of diagnosed cases. The development of the health infrastructure has allowed an earlier and more accurate diagnosis of these conditions, thus reducing the number of underdiagnosed cases. In addition, the increasing availability of outpatient genetic testing has expanded access to these services as well, including for previously underserved populations.

Last but not least, the interaction between genetic predispositions and environmental factors could contribute to the manifestation of the clinical phenotypes of constitutional anemias. Environmental factors, such as exposure to toxins or infectious agents, could act as triggers in the context of a susceptible genetic background, which could explain some of the increased incidence of these conditions.

Viral infections with liver tropism, such as those caused by hepatitis viruses, have been identified as the main etiological factor in the etiopathogenesis of acquired aplastic anemia in numerous cases. These viruses can trigger a disproportionate immune response that leads to the destruction of hematopoietic stem cells (HSCs) in the bone marrow. The pathogenic mechanism often involves the activation of cytotoxic T lymphocytes and the release of cytokines myelosuppressive drugs, which cause hypoplasia of the bone marrow and, consequently, pancytopenia characteristic of aplastic anemia.

The acquired form of aplastic anemia (AA), which represents a diagnostic emergency, is characterized by a significantly increased mortality rate in the absence of immediate treatment, which includes either specific immunosuppressive treatment or hematopoietic stem cell transplantation (HSCT). In the absence of therapeutic intervention, patients present a major risk of severe infections and life-threatening hemorrhages. Immunosuppression, achieved by agents such as antithymocyte globulin (ATG) and cyclosporine (CSA), aims to reduce the cell-mediated immune response that destroys CSH. In refractory or severe cases, allogeneic stem cell transplantation offers a curative chance, reconstituting bone marrow function by repopulating with healthy stem cells from a compatible donor.

I have chosen this topic as a complement to the activity carried out during the first years of residency, which I carried out in the Pediatric Clinic of the Fundeni Clinical Institute. The interest in these pathologies arose as a result of active involvement in the Department of Pediatric Bone Marrow Transplantation.

Starting from 2018, I collaborated, under the guidance of Prof. Univ. Dr. Anca Coliță and Prof. Univ. Dr. Constantin Arion, with the Department of Endocrinology at the Elias Hospital for the monitoring of post-transplant patients, a collaboration that resulted in the publication of 3 articles in specialized journals.

In 2021 I contributed under the guidance of Prof. Univ. Dr. Anca Coliță at the writing of Chapter 53. Acquired and constitutional aplastic anemias, published by EDITURA MEDICHUB MEDIA, under the scientific coordination of Prof. Univ. Dr. Doina Anca Plesca.

In 2021, I published the article on oral mucositis, followed shortly by the article on the experience of the Pediatric Clinic with patients diagnosed with Fanconi Anemia (FA).

In a review of publications about IBMFS with Romanian authors, we found only 2 case reports (from 2022 and 2023) and an extensive analysis by the European Society of Bone Marrow Transplantation (EBMT) regarding hematopoietic stem cell transplantation (HSCT) in patients with SIMC – published in 2024, analysis in which the Fundeni Clinical Institute, Department of Pediatric Bone Marrow Transplant, reported the activity of the last 30 years, in which I actively participated for a period of 10 years.

Analyzing the publications with Romanian authors who reported patients with acquired AA, we identified 1 retrospective study on patients with acquired AA also carried out by EBMT, published in 2020, with data reporting from the Center for Adult Bone

Marrow Transplantation, an article on the experience in HSCT from mismatched donors with adult center experience (2017), the rest of the articles are from 1994 and 2009.

An update is required in the approach to clinical presentation, diagnosing, and treating these pathologies, and I am confident that the results from the two studies conducted over the past eight years meet international standards.

PURPOSE, OBJECTIVES AND WORKING HYPOTHESES

The main objective of the doctoral thesis was the evaluation of pediatric patients with acquired or constitutional forms of aplastic anemia. In this sense, we conducted 2 prospective, observational studies, with specific inclusion and exclusion criteria.

1. Study 1 Analysis of the group of patients with constitutional aplastic anemia / SIMC

The aim of this study was to evaluate SIMC patients in order to identify the clinical-hematological characteristics at the onset and the therapeutic behavior.

The main objectives for this study were:

i. identification of clinical and biological characteristics in relation with the patients' genotype for each SIMC subtype

ii. evaluation of the indication for transplantation for each SIMC subtype and the rate of HSCT according to donor identification

iii. analysis of HSCT results and risk factors associated with patients' evolution for each IBMFS subtype

Secondary Objectives:

- description of the sample analyzed in the study according to hematological parameters at the time of diagnosis
- overall survival of patients who did not undergo HSCT
- survival according to IBMFS subtype.

Working hypotheses for Study 1:

Definition of working assumptions:

1. There is a significant correlation between the presence of clinical abnormalities and younger age at diagnosis
2. Early diagnosis of IBMFS correlates with an increased overall survival rate
3. SIMC patients who undergo HSCT have a higher survival rate

2. Study 2 Cohort analysis of patients with acquired AA

The aim of this study was to evaluate patients with acquired AA in order to identify hematological features and therapeutic response.

The main objectives for this study were:

i. assessment of response to first-line treatment: IST versus MSD
ii. assessment of response to second-line treatment: MUD versus haplo
iii. assessment of risk factors associated with unfavorable evolution

Secondary Objectives:

- analysis of the clinical, biological and etiological characteristics of the patients according to the age group
- distribution of the lot according to degrees of severity
- identifying the genetic peculiarities of AA

Working hypotheses for Study 2:

Definition of working assumptions:

1. There is a significant correlation between the type of ATG used and treatment response
2. TCSH is associated with better response rates compared to IST in the first line of treatment
3. HSCT from a haploidentical donor is associated with similar response rates and toxicity to HSCT from MUD

GENERAL RESEARCH METHODOLOGY

The doctoral thesis includes 2 prospective, observational studies, with specific inclusion and exclusion criteria.

In study 1, we analyzed patients with IBMFS, who were diagnosed and treated in the Pediatric Clinic of IC Fundeni, between 2002 and 2022. The follow-up period was continuous, throughout the duration of the study. The IBMFS diagnosis of patients hospitalized in the Pediatric Clinic of the Fundeni Clinical Institute was carried out by corroborating clinical data, paraclinical results, supplemented in some cases by genetic tests.

Study 2 included patients with acquired AA. In this study, we analyzed patients with AA, who were diagnosed and treated in the Pediatric Clinic of IC Fundeni, between 2002 and 2022. The follow-up period was continuous, throughout the duration of the study.

Both studies were conducted in accordance with the provisions of the Declaration of Helsinki, with approval from the Ethics Committee of the Fundeni Clinical Institute; patient data and photos were anonymized, according to the regulations on the protection of personal

data (General Data Protection Regulation (GDPR). The consent of the relatives was obtained for the publication of the data and photos.

Methods used for statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 29.0.2.0. and illustrated using Microsoft Office Excel/Word 2021. Quantitative variables were expressed as means with standard deviations or medians with interpercentile intervals. Qualitative variables were expressed in absolute or percentage form, and differences between groups were tested using Fisher's Exact Test.

Quantitative variables with normal distribution measured between intervals were tested using the Paired-Samples T-Test.

Chapter 1 of the doctoral thesis covers the characteristics of the most common subtypes of IBMFS in the general population, including Fanconi Anemia, Dyskeratosis Congenita, Shwachman-Diamond Syndrome, Diamond-Blackfan Anemia, and Congenital Neutropenia, which are the most frequent, as well as rarer conditions such as Congenital Amegakaryocytic Thrombocytopenia, GATA2 Deficiency, Congenital Dyserythropoietic Anemia, and SAMD9/SAMD9L mutations.

In both medical practice and specialized literature, the term "Ihherited Bone Marrow Failure Syndromes" is more frequently used than the classic term "Constitutional Aplastic Anemia" because the former encompasses a broader spectrum of hereditary disorders. In contrast, the classic term is more generic and, although it also includes many conditions, does not encompass the diversity and complexity of clinical manifestations encountered in this category of patients. IBMFS are usually diagnosed in specialized centres where multidisciplinary teams are available to manage both bone marrow failure and other clinical implications (e.g., congenital malformations, the risk of malignancies).

IBMFS are usually diagnosed in childhood and are traditionally characterized by pancytopenia, bone marrow hypocellularity, and a predisposition to malignancies/myelodysplastic syndromes (MDS), often accompanied by the presence of congenital somatic malformations. These syndromes result from genetic defects that impair the bone marrow's ability to produce sufficient hematopoietic stem cells, frequently associated with a spectrum of phenotypic abnormalities.

The diagnosis of IBMFS is based on the identification of characteristic physical abnormalities, if present, supplemented by detailed hematological investigations and molecular genetic analyses to detect specific mutations.

Patients with IBMFS have an increased likelihood of developing malignancies throughout their lives. Hematopoietic stem cell transplantation remains the only curative therapeutic intervention capable of fully restoring hematopoiesis, although it does not influence the intrinsic predisposition of patients to the development of malignancies.

From the perspective of the number of affected cell lines, IBMFS can be classified as follows:

- Syndromes with multilineage hematopoiesis impairment: Fanconi Anemia (FA), Dyskeratosis Congenita (DC).

- Syndromes with unilineage hematopoiesis impairment: Diamond-Blackfan Anemia (DBA), Shwachman-Diamond Syndrome (SDS), Congenital Amegakaryocytic Thrombocytopenia (CAMT).

- This category also includes some very rare diseases: Seckel Syndrome, Pearson Syndrome, cartilage-hair hypoplasia (CHH), reticular dysgenesis, thrombocytopenia with absent radius (TAR), Congenital Neutropenia (CN), Congenital Dyserythropoietic Anemia (CDA), SAMD9/SAMD9L germline mutations, and GATA2 deficiency.

In recent years, progress has been made in discovering the genes responsible for the genetic transmission of IBMFS, which has allowed for a better understanding of normal hematopoiesis and how it is disrupted in patients with bone marrow failure.

Chapter 2 details the pathophysiological mechanisms of acquired aplastic anemia (AA) and the etiology of this condition.

Acquired AA is a rare disease with potentially unfavorable outcomes. It is primarily characterized by inefficient hematopoiesis, with bone marrow biopsy typically revealing hypoplasia and fatty vacuolization in the absence of leukemic infiltration or fibrosis.

Etiology of Acquired AA

Establishing a definitive diagnosis and identifying the etiological agent in patients with acquired AA is essential to enable an appropriate and personalized therapeutic approach. This includes a detailed medical history assessment, specific diagnostic tests, and, where possible, identification of causal factors to optimize the treatment strategy and improve the patient’s prognosis.

The etiology of acquired AA can be classified into several main categories based on the involved factors:

1. Idiopathic (in over 70% of cases, the etiological agent cannot be identified)
2. Secondary

a. Medications - usually, the effect is predictable, dose-dependent, and possibly reversible upon discontinuation: 6-mercaptopurine, methotrexate, cyclophosphamide, busulfan; the toxic effect cannot be reliably anticipated and may occur even at doses considered safe for most patients
    i. Antibiotics: chloramphenicol, sulfonamides
    ii. Anticonvulsants: mephenytoin (Mesantoin), hydantoin
    iii. Antirheumatics: phenylbutazone, gold salts
    iv. Antidiabetics: tolbutamide, chlorpropamide
    v. Antimalarials: quinacrine
    vi. Chemical substances: insecticides, dichlorodiphenyltrichloroethane (DDT), pesticides, parathion, chlordane.
b. Toxic exposure: benzene, carbon tetrachloride, glue, toluene.
c. Radiation exposure.
d. Infections: viral hepatitis (non-A, B, C), HIV, Epstein-Barr virus, measles, influenza and parainfluenza viruses, rubella, mumps, CMV, herpes virus, parvovirus B19, adenovirus.
e. Immunological conditions: graft-versus-host disease post-transfusion in an immunocompromised patient, X-linked lymphoproliferative syndrome, eosinophilic fasciitis, hypogammaglobulinemia, systemic lupus erythematosus.
f. Myelodysplastic syndromes (MDS).
g. Thymomas.
h. Paroxysmal nocturnal hemoglobinuria.
i. States of malnutrition: Kwashiorkor, marasmus, anorexia nervosa, pregnancy.

Clinical manifestations in acquired AA include:

- Signs of anemia: extreme fatigue and weakness, pale skin and mucous membranes, shortness of breath, dizziness.
- Signs of thrombocytopenia: cutaneous-mucosal bleeding syndrome (gingival bleeding, nosebleeds, bruising, petechiae, menorrhagia, hematuria).
- Neutropenia: recurrent moderate/severe infections, fever of unknown origin, mouth ulcers.
- Other symptoms: unexplained weight loss, bone/joint pain, abdominal discomfort.

Chapter 3 examines the challenges of establishing a diagnosis in the two conditions and then delves into the specific treatment for each pathology.

It is very important to differentiate between acquired aplastic anemia and IBMFS, as the diagnosis must be made before determining the therapeutic plan for both children and adults. The suspicion of IBMFS diagnosis is based on evaluating the classic triad: medical history, clinical examination, and hematological tests. Confirmation of the diagnosis requires specific tests and genetic analyses. Investigating a case of IBMFS is generally initiated after observing recurrent suggestive changes, typically identified during routine tests conducted periodically for the screening of common conditions.

The diagnosis of acquired aplastic anemia is an emergency due to the acute and potentially fatal nature of the condition. These patients are at an extremely high risk of major hemorrhagic events, including cerebral, gastrointestinal, or pulmonary hemorrhages, due to the severe thrombocytopenia that characterizes this disease. Additionally, the associated pancytopenia exacerbates vulnerability to severe infections and can lead to sepsis, a complication that, without prompt treatment, has a poor prognosis.

Chapters 4-7 present the purpose, objectives, and working hypotheses, the methodology of the scientific research, and the results of the two studies conducted. The obtained results were discussed in comparison with the data available in the literature.

The Conclusions chapter summarizes the results and discussions from each study.

Both in medical practice and in specialized literature, the term "congenital bone marrow failure syndromes" is used more frequently than the classic name of "constitutional aplastic anemia", because the former includes a wider spectrum of hereditary conditions, while the term classic is rather generic and although it also includes many conditions, it does not encompass the diversity and complexity of clinical manifestations encountered in this category of patients. SIMCs are usually diagnosed in specialized centers where multidisciplinary teams are available to manage both the bone marrow failure and the other clinical implications (ex, congenital malformations, risk of malignancy).

IBMFS are usually diagnosed in childhood, and are traditionally characterized by pancytopenia, medullary hypocellularity and predisposition to malignancies/myelodysplastic syndromes (MDS) to which they may associate the presence of congenital somatic malformations. These syndromes result from genetic defects that affect the ability of the bone marrow to produce sufficient hematopoietic stem cells, being frequently associated with a spectrum of phenotypic abnormalities.

The diagnosis of IBMFS relies on identifying characteristic physical abnormalities, when present, along with comprehensive hematological evaluations and molecular genetic analyses to detect specific mutations.

Patients with IBMFS have an increased likelihood of developing malignancies during their lifetime. HSCT remains the only curative therapeutic intervention capable of restoring complete hematopoiesis, without influencing the intrinsic predisposition of patients to malignancies.

From the point of view of the number of cell lines affected, SIMC can be classified as follows:

- syndromes with multilineal damage to hematopoiesis: AF, DC
- syndromes with unilineal damage to hematopoiesis: DBA, SDS, CAMT, this category of conditions also includes some very rare diseases – Seckel syndrome, Pearson syndrome, cartilage and hair hypoplasia (cartilage hair hypoplasia CHH), reticular dysgenesis and thrombocytopenia with absence of the radius (TAR), NC, CDA, SAMD9 germline mutations /SAMD9L, DONE2

In recent years, progress has been made in discovering the genes responsible for genetic transmission in IBMFS, which have allowed a better understanding of normal hematopoiesis and how it is disrupted in patients with bone marrow failure.

IBMFS occur as a result of specific changes or abnormalities/alterations in genes associated with DNA repair, ribosomal structures or their functions, and telomere maintenance. Extensive studies have shown the existence of common cytokine profiles and biological pathways underlying the occurrence of IBMFS .The pro-inflammatory cytokines IL-6 and IL-8, but also TGF-β with an anti-inflammatory role, were identified in some cases of AF and SDS, increased titers of IP-10 and IFN-ɣ were also measured in some patients with AF and DC, and IL-8, IP-10 and IFN-ɣ, appeared with increased serum level in patients with severe form of AA (SAA), thus proving the involvement of IFN-ɣ in the pathogenesis of SAA.

1. STUDY 1 ANALYSIS OF THE PATIENT GROUP WITH CONSTITUTIONAL APLASTIC ANEMIAS / IBMFS

Patients and Methods

The study included 37 patients diagnosed with IBMFS, whose progression was monitored throughout the study. Data collection for the study involved analyzing the clinical records of

the patients, stored in the hospital archives, and a monitoring table of the main clinical and biological parameters, as well as the treatment administered.

Conclusions of Study 1

1. The batch of 37 patients with IBMFS had a median age of 5 years and 8 months, with a sex ratio of 1.4:1, slightly in favor of the male sex, with the predominance of AF 49%, followed by DBA 30%, DC 11%, NC 5%, 5% of patients could not be assigned to SIMC subtypes.
2. Genetic testing performed in 37.8% of patients identified the most frequent gene, FANC-A (in 3 patients), either as a single gene or in association with another FANC gene or with another gene (VPS13B), followed by FANC-F in 2 patients and FANC-N. For DBA patients the most frequently identified gene was RPS19, in 3 patients, followed by RPS26 and RPL5. Genetic testing in the 2 DC patients identified the ACD gene in one of them, and ACD in association with ZCCHC8 in the other. In NC patients the genes identified were: ELANE, in association with CSF3R, SLX4, WRAP53 and in the other case, G6P3.
3. The most frequent malformations encountered were skeletal, in 25 patients (68%), followed by developmental delay in 22 patients (60%), intellectual disability in 15 patients (40%), skin manifestations were present in 11 patients (30%), reno-urinary malformations only in 8 patients (22%), cardiac malformations in 4 patients (11%), as well as gastrointestinal malformations.
4. 19% of patients had no malformations or other suggestive manifestations at the time of onset (7 patients), of which 2/7 with AF, 3/7 with DBA, and 1/7 each with DC and NC.
5. The mean hemoglobin at presentation was 9.84 g/dl, (5.7 – 15.6), with a mean EVM of 90.5 fl (65.7 – 116.3) and a minimum of 5.7 g/dl and a maximum of 15.6 g/dl.
6. In patients with AF, the average age at diagnosis was 7 years, with limits between 1 year and 14 years. They also presented various degrees of anemia (minimum 5.7 g/dl, maximum 15.6 g/dl), the maximum value being in a patient who also associates chronic respiratory failure.
7. Of the 11 patients with DBA, 4 (36%) of them presented severe anemia (7g/dl), 4 presented moderate anemia (7-10 g/dl), and only 3 (28%) presented values over 11 g/dl. The mean hemoglobin value for patients with DBA was 8.2 g/dl (5.7 – 11.8) with limits between 5.7 and 11.8. all patients had reticulocytopenia (100%)
8. The median age of patients who underwent HSCT was 9.5 years. The types of HSCT procedures performed were: MSD (from 10/10 compatible sibling donor), MUD (10/10 compatible unrelated donor), HAPLO (haploidentical donor).

9. Of the 16 procedures performed, 3/16 were MSD, 11/16 were MUD, 2/11 were HAPLO. Regarding donor sex, for all MSD procedures the donors were F (100%), for MUD 09/10 were M, 1/10 were F, and for HAPLO donors were 1/2 of sex F, 1 of sex M.
10. Early complications presented 3/11 patients with MUD and those with HAPLO (2/2) presented early complications. Late complications were presented by 2/11 patients with MUD – thyroid dysfunction, respectively chronic GVHD.
11. Complications at MUD were: 1/11 with PRES, 1/11 with CLS, 1/11 severe intestinal GVHD, 2/11 CMV reactivation. Complications in HAPLO were: 2/2 CMV reactivation, 1/2 acute grade IV GVHD (cutaneous and intestinal).
12. Survival by type of HSCT: Patients with MSD – 3/3 remain in CR, for those with MUD – 3/11 have died (CMV reactivation, intestinal GVHD), 8/11 remain in CR, and for HAPLO, 2/2 patients have died.
13. The survival of patients with HSCT was as follows: 68.75% of patients remain in CR, 31.25% died. TCSH patients at MUD have a 50% survival rate.
14. From the population with FA, 72.22% (13/18 patients) underwent HSCT, with a cumulative survival probability of 94.4% at 6 months and 72.2% at 65 months. The median survival time was approximately 109.2 months (95% CI: 86.9 - 131.5 months).
15. Kaplan-Meier analysis of the population that underwent HSCT showed a cumulative survival probability of 84.6% at 2 months, which decreased to 69.2% at 12 months post-transplant. The median survival time was estimated at 14.08 months (95% CI: 10.65 - 17.51). Due to the small number of events, it was not possible to estimate the median survival time. The 12-month survival probability was 69.2%.
16. Overall survival: At 6 months, the survival probability is 97.3%.

2. STUDY 2 ANALYSIS OF THE PATIENT GROUP WITH ACQUIRED APLASTIC ANEMIAS

Patients and methods

All patients diagnosed with acquired AA, between April 2002 and November 2022, in the Pediatric Clinic of the Fundeni Clinical Institute, were included in the study. The diagnosis of AA was confirmed by PBO (low marrow cellularity), pancytopenia in the peripheral blood in the absence of other malignancies, and after ruling out the diagnosis of SIMC. In 2 patients with a young age at diagnosis, genetic tests were also performed.

Severity criteria:

1. SAA – MO cellularity <25% or 25%–50%, with <30% residual hematopoietic cells and at least two of the following criteria:

- number of neutrophils <0.5×10^3/l
- number of platelets <20×10^3/l or
- number of reticulocytes <20×10^3/l.

2. VSAA – criteria SAA + neutrophil count <0.2×10^3/l.

3. NSAA - does not meet criteria for SAA or VSAA

Inclusion criteria in the group: diagnosis of acquired AA, age between 0-18 years at the time of diagnosis, diagnosis, treatment/monitoring of evolution within the department.

Exclusion criteria: patients with medullary aplasia post-chemotherapy for malignancies, SIMC; data unavailable at the time of diagnosis.

Study variables:

- demographic (age at diagnosis, sex, environment of origin)
- significant hereditary-collateral antecedents
- symptomatology at the onset
- the paraclinical picture at the beginning
- medullary cellularity evaluated by PBO
- etiological factors: hepatitis viruses, CMV, EBV, drug exposure
- degree of severity
- first-line treatment:
    - an immunosuppression: CSA, MMF
    - IST: ATG + CSA
    - TCSH
- response to first-line treatment: complete response, partial response, no response
- the second-line treatment

    - HSCT
- response to second-line treatment: complete response, partial response, no response
- current status

Treatment response criteria

Assessment of response to treatment

- at 3 months:
    - neutrophils still below 0.2 x 10^3/l ◊ the patient has an indication for the TCSH procedure
    - a good/poor partial response and neutrophils above 0.2 x 10^3/l ◊ continue treatment with CSA
- at 4 months:
    - neutrophils still below 0.2 x 10^3/l → the patient has an indication for the TCSH procedure
    - a good/poor partial response and neutrophils above 0.2 x 10^3/l → preparing the patient for TCSH and if at 6 months there is no response, there is an indication for the TCSH procedure
- at 6 months:
    - no response → patients have an indication for the HSCT procedure
    - a good/poor partial response → continue treatment with CSA
    - for patients who are in complete remission ≥ 2 months, CSA should be decreased progressively (10% per month), with weekly blood count monitoring
- any complete remission > 6 months:
    - for patients who are in complete remission ≥ 2 months, CSA should be decreased progressively (10% per month), with weekly blood count monitoring
- at 8 months:
    - a poor partial response → the patient has an indication for the HSCT procedure if he has a 10/10 or 9/10 MUD donor, or another alternative donor
    - a good partial response → continue CSA
- at 1 year:
    - CSA should be decreased progressively (10% per month), regardless of response

Conclusions of Study 2

1. The group of 69 patients with acquired aplastic anemia (AA) had an average age of 9.59 years, with a standard deviation of 4.525, and a sex ratio of M:F = 1.5:1, slightly favoring males, with 57% of patients coming from urban areas.
2. 81% of patients presented with hemorrhagic syndrome as the initial manifestation.
3. 72.5% of patients were diagnosed with idiopathic AA, 16% with AA post-acute hepatitis, 4.3% with AA post-exposure to medications, 5.8% with AA secondary to toxic exposure, and 1.4% with AA secondary to EBV infection. Genetic testing was performed in 2.8% of patients, identifying two genes, LYST and RTEL1 (neither of which is associated with the occurrence of IBMFS).
4. 17.4% of patients were classified with NSAA, 59.4% with severe aplastic anemia (SAA), and 23.2% with VSAA.
5. First-line treatment: 51% received IST, 39% received other combinations of immunosuppressants, and only 10% underwent HSCT.
6. Response to first-line treatment: 34% of patients who received IST achieved complete remission (CR), and 71.4% of patients who underwent HSCT achieved CR.
7. Seventeen patients received second-line treatment, with a median age at transplant of 9 years, and a sex ratio of M:F = 0.71:1.
8. Second-line treatment consisted of HSCT: 37.6% MUD, 43.7% HAPLO, 18.7% MSD, administered on average 113 days after the first-line treatment.
9. Among patients with MUD, 1/6 experienced toxicity from the conditioning regimen, and 1/6 developed severe GVHD; both patients who received HAPLO HSCT experienced graft rejection secondary to BK virus (BKV) infection.
10. For patients with MUD transplants: 2/6 patients died from complications, while 4/6 achieved CR (66%); for HAPLO patients: 2/7 patients died from complications, while 5/7 achieved CR (71.4%).
11. HAPLO transplantation was performed without major toxicities, with a response rate of 71%, slightly higher than that of MUD patients (66%).
12. The median survival time for the entire group of 69 patients, from the time of diagnosis, is approximately 87 months, with a 95% confidence interval between 75 and 99 months.
13. The estimated median survival is 98 months after first-line treatment.
14. The estimated median survival is 92.118 months, with a 95% confidence interval between 68.223 and 116.013 months, after second-line treatment.

Personal Contributions

This study included a large number of patients, both those with congenital conditions and those with acquired disease. It is the only study conducted on these two populations within a pediatric center in Romania. In this regard, two studies were developed, both prospective.

The first study enrolled a significant number of patients over 8 years and is the only study that analyzed patients with Congenital Bone Marrow Failure Syndromes (CBMFS). As a result of these activities, a multidisciplinary team of pediatricians, pediatric onco-hematologists, geneticists, radiologists, cardiologists, pulmonologists, and endocrinologists was formed to monitor these patients from the moment of diagnosis, through the transplant procedure, and afterward.

A national center for the diagnosis and multidisciplinary treatment of CBMFS patients was established at the Fundeni Clinical Institute. The first hematopoietic stem cell transplant (HSCT) procedures for CBMFS patients were performed in our center. The first HSCT procedure from an unrelated donor was conducted on a patient with Fanconi Anemia (FA) in 2013, in collaboration with the National Registry of Voluntary Hematopoietic Stem Cell Donors. The first haploidentic transplant procedures with TCR αβ depletion were performed in our center for FA patients who did not have compatible donors.

The second study concentrated on a large number of patients diagnosed with acquired aplastic anemia (AA). This study analyzed the clinical-biological profile, bone marrow cellularity, disease etiology, severity grading, first- and second-line treatments, treatment response, and overall outcomes. The international collaboration protocol with the study group from MD Anderson led to the first haploidentic transplant procedures being performed on acquired AA patients who did not have compatible donors.

We have successfully consolidated a multidisciplinary team that treats AA patients in Romania, starting from diagnosis and treatment, followed by long-term monitoring.

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