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THE ROLE OF INTESTINAL MICROBIOME AS A PREDICTOR OF INFECTIONS DURING THE TREATMENT OF ACUTE LEUKEMIAS (MYELOID AND LYMPHOBLASTIC)

FINAL DEGREE PROJECT

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Aos meus pais e à minha irmã por serem sempre o meu maior apoio em tudo o que faço.

INDEX

1. ABBREVIATIONS

AML	Acute Myeloid Leukemia
ALL	Acute Lymphoblastic Leukemia
ARSE	European Population Standardized Incidence
WHO	World Health Organization
ARA-C	Cytarabine
BM	Bone marrow
BSI	Bloodstream Infections
PETHEMA	Programa Español de Tratamiento en Hematologia
Ph	Philadelphia Chromosome
SR	Standard Risk
HSCT	Hematopoietic Stem Cell Transplantation
RD	Residual Disease
HLA	Human Leukocyte Antigen
HSC	Hematopoietic Stem Cells
MRD	Minimal Residual Disease
CRM	Complete Morphologic Remission
ICO	Institut Català de Oncologia

2. ABSTRACT

Background: Acute leukemias are a major health problem worldwide, as it represents one of the most frequent types of cancers. For this reason many efforts have been done to improve treatment therapies. Nowadays the chemotherapy agents used are extremely mieloablative making these patients highly susceptible to infection, which remains an important cause of mortality. The delineation of risk factors for infection and the development of screening strategies are of cardinal importance in order to reduce the incidence of infection and overall the mortality in patients with acute leukemias undergoing chemotherapy treatment.

Objectives: The main aim of the present study is to establish an association between changes in the intestinal microbiome diversity composition (before and after treatment) and bacterial infectious complications, in patients undergoing high intensity chemotherapy.

Design: an observational, prospective, longitudinal follow-up without a control group will be performed in Hospital Universitari Dr. Josep Trueta between August 2017 and December 2021

Participants: Patients between 18 and 70 years old, newly diagnosed of acute myeloid leukemia or acute lymphoblastic leukemia

Methods: A non-probabilistic consecutive sampling will be used in this study; we will analyse the diversity of the intestinal microbiome through fecal samples before and after high intensity chemotherapy treatment.

Keywords: acute myeloid leukemia, acute lymphoblastic leukemia, infections, intestinal microbiome diversity

3. INTRODUCTION

3.1. Acute Leukemias

Leukemia is present in the ranking of the 15 most common cancers in man and women worldwide estimated in the 2012 GLOBOCAN report (1).
Acute leukemias are defined as a progressive, malignant disease originated in a hematopoietic precursor, myeloid or lymphoid, characterized by infiltration of the bone marrow, peripheral blood and in some cases other organs. The neoplastic transformation in acute leukemias includes an incontrollable proliferative activity with a loss of differentiation capacity(2).

In order to understand acute leukemias it is important to have a clear idea of the maturation of hematopoietic stem cells. Hematopoietic stem cells continuously replenish all classes of blood cells through a series of lineage restrictions steps that results in the progressive loss of differentiation potential to other cell lineages. Lymphoid lineage cells include lymphocytesT, B and natural killer cells, while myeloid lineage includes megakaryocytes, erythrocytes as well as granulocytes and macrophages (3).

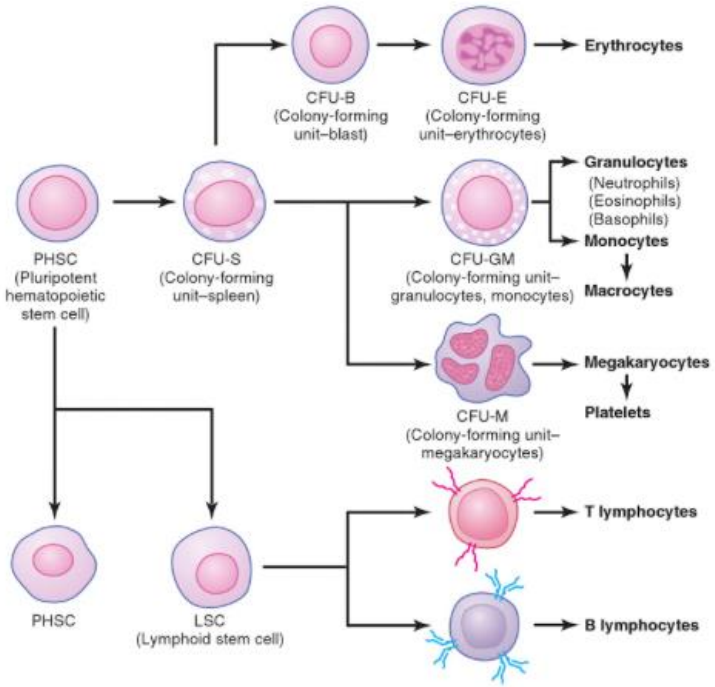


Figure 1: Formation of the multiple different blood cells from HSC in bone marrow(4)

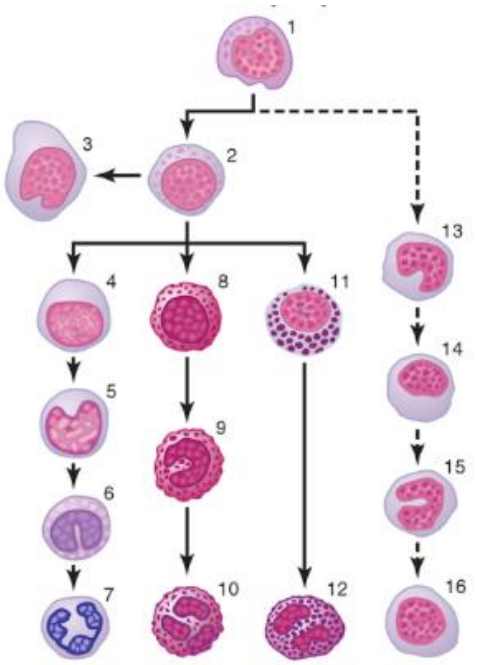
Leukemias can be classified in a numerous ways according to cellular maturity, cellular type or time of presentation.
It is important, due to prognostic reasons, to differentiate *de novo* leukemias, when there is no previously known pathology; and secondary leukemias when there is a previous condition such as a myelodysplastic syndrome o exposure to toxic agents. Therapy-related leukemia defines a specific type leukemia forms that occurs in patients whom had previously received chemotherapy o radiotherapy(2).

Depending on the lineage where the malignant transformation occurred leukemias are classified in **myeloid leukemias** and **lymphoblastic leukemias**. There is a small minority that may express markers from both linages, acute leukemias biphenotypic (2).

Due to the seriousness of the disease it is of primordial importance to correctly diagnose the patient. An extensive differential diagnosis (5) with pathologies that may have similar symptoms and hematological findings, such as leukemoid reactions or infectious processes, it is mandatory.

a. Acute myeloid leukemia

Acute myeloid leukemia is a neoplastic disease characterized by infiltration of the blood, bone marrow, and other tissues by proliferative, **clonal undifferentiated cells of the hematopoietic system** (6) from myeloid lineage (Fig 2).
Last studies published(7) indicate that incidence of AML in Girona between the years of 1994 and 2008 had a crude rate of 3,60 cases per 100,000 inhabitants/year; and an ARSE rate of 3.48 cases per 100,000 inhabitants/year for men and 2.40 cases per 100,000 inhabitants/year for women with a significant male predominance (sex ratio of 1.28).
The median age of diagnosis is 68 years old in Girona(7).



1. Myeloblast
2. Promyelocyte
3. Megakaryocyte
4. Neutrophil myelocyte
5. Young neutrophil metamyelocyte
6. “Band” neutrophil metamyelocyte
7. Polymorphonuclear neutrophil
8. Eosinophil myelocyte
9. Eosinophil metamyelocyte
10. Polymorphonuclear eosinophil
11. Basophil myelocyte
12. Polymorphonuclear basophil

13-16. Stages of monocyte formation

Figure 2 : Maturation of the myelocyte series adapted from(4)

Regarding **etiology** most cases are sporadic, characterized by acquisition of somatic mutations in hematopoietic progenitors that confer a proliferative and/or survival advantage(8); Nevertheless it seems to be an association between heredity, anticancer drugs, high-dose radiation, chemical and other occupational exposures and AML(5,6).

There are more than 100 recurring cytogenetic abnormalities observed in AML and numerous point mutations, making AML challenging from a clinical perspective as well as from a genetic perspective (8).

In order to **classify** AML there are two main systems: the French-American-British (FAB) and the one elaborated by the World Health Organization (WHO).

The FAB’s system, in disuse nowadays, is based on the predominant cellular line of differentiation and the degree of cellular differentiation

Table 1: FABs classification of AML adapted from (5,9)

M0	Undifferentiated acute myeloblastic leukemia
M1	Acute myeloblastic leukemia with minimal maturation
M2	Acute myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia
M4	Acute myelomonocytic leukemia
M4Eos	Acute myelomonocytic leukemia with eosinophilia
M5a	Acute monobastic leukemia
M5b	Acute monocytic leukemia
M6	Acute erythroid leukemia
M7	Acute megakaryocytic leukemia

The current categorization of AML uses the WHO classification, which is based on clinical features and cytogenetic and molecular abnormalities in addition to morphology(5,6).

Table 2: WHO classification of acute myeloid leukemia adapted from(10)

AML with recurrent genetic abnormalities • AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 • AML with inv(16)(p13;1q22) or t(16;16)(p13,1;q22); CBFB-MYH11 • APL with PML-RARA • AML with t(9;11)(p23;q34.1); DEB-NUP214 • AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM • AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); RBM15-MKL1 • Provisional entity: AML with BCR-ABL1 • AML with mutated NPM1 • AML with biallelic mutations of CEBPA • Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS • AML with minimal differentiation • AML without maturation • AML with maturation • Acute myelomonocytic leukemia • Acute monoblastic/monocytic leukemia • Pure erythroid leukemia • Acute megakaryoblastic leukemia • Acute basophilic leukemia • Acute panmyelosis with myelofibrosis

Chromosome alterations at diagnosis are currently the most important independent **prognostic factors**(6); patients are classified as having favourable, intermediate, or poor cytogenetic risk based on the presence of structural and/or numerical aberrations; for patients lacking prognostic cytogenetic abnormalities, outcome prediction uses mutated or aberrantly expressed genes (NPM1, FLT3-IDT, CEBPA)

Age at diagnosis is an important risk factor: advanced age is associated with a poorer prognosis due to the patients' capacity to survive high intensity therapy, pre-existing comorbidities and possibility a more resistant disease (6).

Table 3: Prognostic factors in AML adapted from(2)

FAVORABLE	UNFAVORABLE
AML *de novo*	Secondary AML
Children and young adults	>60 years old
Normal leukocyte count	Leukocytosis
AML promyelocytic	Undifferentiated AML
AML with eosinophilia	Acute erythroid leukemia
t(8;21), t(15;17), inv(16)	Acute megakaryocytic leukemia
Normal karyotype: NPM1 gen mutation without DIT/FLT3	t(9;22), complex karyotype
CEBPA mutation	DIT/FLT3 present

AML is the myeloid malignancy with the poorest outcome, counting with 5-year survival rates of 20.2% in Girona(7).

The **clinical presentation** (5,6) is usually acute, with nonspecific symptoms, a consequence of medullar failure (anemia; leucocytosis leukopenia or leukocyte dysfunction, and thrombocytopenia); half of the patients mention fatigue as the first symptom;
Other frequent findings are:

- Fever;
- Infection (mainly on thorax, lips, perianal and skin);

- Hemorrhage;
- Gingival hypertrophy
- Skin infiltration;
- Hepatomegaly and splenomegaly;
- Lymphadenopathy.

Regarding **hematologic findings** (2,6) anemia is usually present at diagnosis, usually normocytic normochromic; leukocyte count varies but is usually around 15,000/ μL; platelet count is usually low at diagnosis. The morphology of the malignant cell varies depending on the diseases subtype. In AML the cytoplasm often contains primary granules, and the nucleus shows fine, lacy chromatin with one or more nucleoli characteristic of immature cells; abnormal rod-shaped granules named Auer rods are not uniformly present but when they are, myeloid lineage is virtually certain;

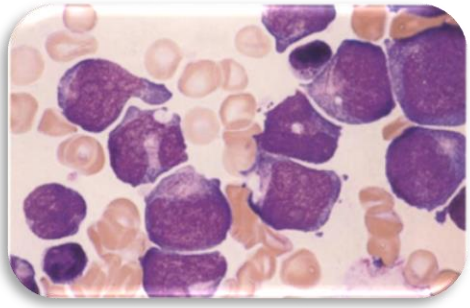


Figure 3: Bone marrow in AML subtype M2, it is possible to see Auer rods in the cytoplasm of two blasts (left of center and upper right) image from (11)

In order to access correctly a patient in this condition is required a complete study of the bone marrow (aspirate and biopsy with cytogenetic, molecular and immunophenotype to correctly classify the disease) as well as a full physical study(5,6). (ANNEX 1)

In many cases **diagnosis** is possible based on examination of the peripheral blood (>20% blasts), however a bone marrow aspirate should always be performed to confirm leukemia subtype and obtain marrow for cytogenetic and immunophenotypic studies (8).

Treatment of AML includes a series of complex chemotherapy regimens that vary according to the protocol used by each hospital.

In hospital Universitari Dr. Josep Trueta in Girona (12) patients are treated following a chemotherapy regimen that consist in an induction cycle (idarubicin and cytarabine), and a consolidation cycle (cytarabine) that varies depending on medullar findings after the induction cycle. (ANNEX 2)

After the consolidation scheme posterior treatment is based on prognostic factors and the evaluation of the patients' current state; another consolidation cycle or a posterior allogeneic transplant may be considered(12).

Chemotherapy regimens may need to be adapted to specific patient characteristics and believes.

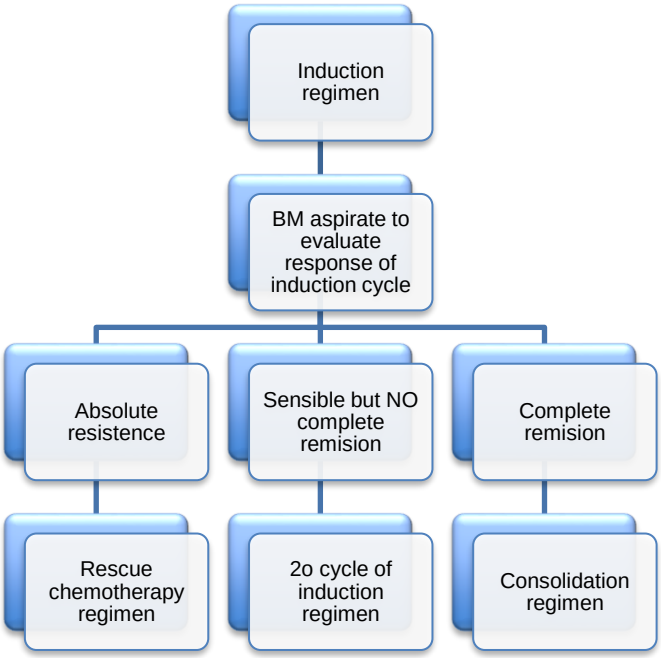


Figure 4: Treatment of AML based on (12)

It is important to know a patient is considered to be in complete remission when all of the following criteria (12) are present:

- Bone marrow normocellular o slightly hypocellular with a proportion of immature cells <5%;
- Absence of Auer rods;
- Absence of extramedullar leukemia;

- Rising levels of neutrophils and platelets.

The presence of minimal residual disease in the cytogenetic and immunophenotype studies does not invalidate the complete remission criteria

b. Acute lymphoblastic leukemia

Acute lymphoblastic leukemia is a neoplastic disease from the lymphoid cells precursors leading to excessive lymphoblasts in bone marrow and other organs(5).

It is the most common malignancy of childhood(8), the majority of cases occur between the ages of 2 and 10; is an infrequent type of leukemia in adults with incidence rates of 0,7-1,8/100.000(5).

In Girona (13) the incidence of precursors B-cell neoplasms between the years of 1994 and 2001 has a crude rate of 1,6 cases per 100,000 inhabitants/year, however this data includes leukemias Pre-B cell (the most frequent subtype) and lymphomas Pre-B cell due to the fact that the WHO classification classifies them in the same section, meaning we don't have exact information of the incidence of acute lymphoblastic leukemia specifically in Girona.

The **etiology** of ALL remains uncertain, regardless exposure to high-dose radiation, some genetic syndromes (Down's, Bloom's and Klinefelter's syndromes) are considered predisposing factors to the development of ALL (5,6).

In order to **classify** this type of leukemias the most commonly used classification is the French-American-British (FAB) classification, which is based on morphologic characteristics of lymphoblasts.

Table 4: FABs classification of ALL based on(5)

L1	Small cells with homogeneous nuclear chromatin, regular nuclear shape, small or no nucleoli; more frequent subtype
L2	Large and heterogeneous cells with variable nuclear chromatin, irregular nuclear shape, 1 or more nucleoli, variable amount of cytoplasm
L3	Large, homogeneous cells with fine chromatin Burkitt-type, abundant cytoplasm with prominent vacuolation

In many cases, leukemic cells appear to represent the clonal expansion of a lymphoid progenitor that has arrested its development at an early stage of B- or T-cell differentiation (8) for this reason an immunological classification divides ALL in subtypes according to if the precursors are lineage-B (the gran majority around 80%) or lineage-T (20%), and how mature these leukemia cells are.

Cytogenetic analysis detects anomalies in 85% of the patients, which offers important prognostic information.

Table 5: Classification of ALL adapted from(6)

Immunologic Subtype	FAB Subtype	Cytogenetic abnormalities
Pre-B ALL	L1, L2	t(9;22), t(4;11), t(1;19)
T-cell ALL	L1, L2	14q11or 7q34
B-cell ALL	L3	t(8;14), t(8;22), t(2;8)

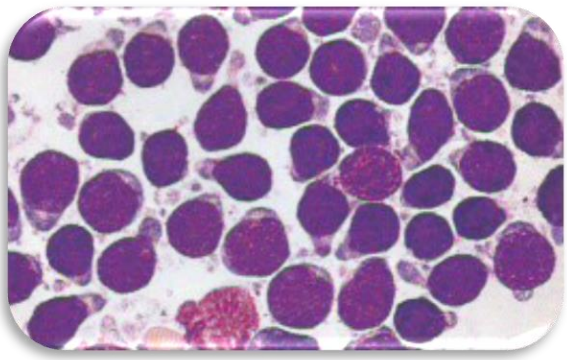


Figure 5: Bone marrow in ALL, shows small blasts that may resemble lymphocytes but are distinguished by their fine chromatin structure and the occasional presence of nucleoli image from (11)

Similar to AML, the **clinical presentation**(2,5) of ALL is usually acute, with nonspecific signs and symptoms, a consequence of medullar failure, that include:

- Asthenia and lethargy;
- Infections;
- Fever;
- Diathesis: purpura, menorrhagia, epistaxis;
- Signs of leukostasis: diffuse pulmonary infiltrates, retinal hemorrhage;
- Bone and joint paint especially in children;

- Generalized lymphadenopathy;
- Splenomegaly;
- Hepatomegaly;
- In a low percentage of cases ALL may debut with central nervous system affection in form of paralysis of cranial nerves y/or intracranial hypertension.

Regarding **hematological findings**(2) at time of diagnosis anemia is almost always present usually normocytic normochromic; leukocytosis in 75% of patients and leukopenia in 15-20%; and thrombocytopenia in most cases.
To correctly diagnose an ALL it is necessary to confirm the presence of more than 20% of lymphoblasts in bone marrow.
Several factors act as prognostic factors in ALL as seen on table 6.

Table 6: Prognostic factors in ALL adapted from(2)

Characteristic	Favorable	Unfavorable
Age	1-9	<1 and >50
Leukocytes (x10^3/L)	<50	>50
Phenotype		Pro-T, early pre-T, Pro-B
Cytogenetic	Hyperdiploid > 50 chromosomes or DNA index >11,5 t(12;21)(TEL-AML1)	Hypodiploid t(4;11)(MLL-AF4) t(9;22)(BCR-ABL) Complex karyotype

The definition of **high risk ALL** (14) include the presence of one or more of the following factors:

- Age between 30 and 50 years old
- Leukocytes> 30x10^9/L in ALL from B-lineage
- Cytogenetic or molecular abnormalities such as reorder of MLL or a complex karyotype
- ALL Pro-B
- ALL Pro-T/ Pre-T o mature T

In order to access correctly a patient in this condition is required a complete study of the bone marrow (aspirate and biopsy with cytogenetic, molecular

and immunophenotype to correctly classify the disease) as well as a full physical study. (ANNEX 1)

Treatment of ALL includes a series of complex chemotherapy regimens that vary according to the protocol used by each hospital; it includes an induction cycle and multiple consolidation cycles.
In hospital Universitari Dr. Josep Trueta in Girona patients are treated following the norms of the PETHEMA group (14), which includes different chemotherapy regimens depending on patients´ age, ALL Ph+ and ALL of mature lineage-B.

Chemotherapy regimens may need to be adapted to specific patient characteristics and believes.
The general chemotherapy (14) regimen for high risk ALL patients consist in an induction cycle (vincristine, daunorubicin, prednisone, L-asparaginase plus intrathecal chemotherapy) and three consolidation cycles with different regimens every three weeks. (ANEXO 3)

- First consolidation cycle: dexamethasone, vincristine, methotrexate and L-asparaginase plus intrathecal chemotherapy
- Second consolidation cycle: dexamethasone, cytarabine and L-asparaginase plus intrathecal chemotherapy
- Third and last consolidation cycle: dexamethasone, vincristine, methotrexate and L-asparaginase plus intrathecal chemotherapy

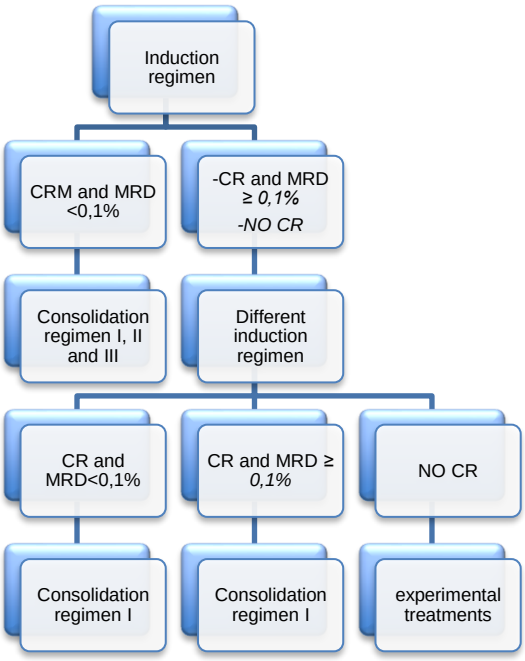


Figure 6: Treatment of ALL based on(14)

While initiating the process it is advised to study the patients brothers and sisters for HLA compatibility due to the fact that an allogeneic-HSCT may be needed at final stages of the treatment.

Regarding the treatment(14) it is important to note the significance of the residual disease and how it affects therapeutic decisions, as well as the fact that patients with a <0,01% of RD after induction o consolidation chemotherapy have higher rates of remission, without the need of performing an allogeneic-HSCT.

Evaluation of RD can be done by means of molecular biology or cytofluorometry.

3.2. Infectious Complications Due To The Disease And Treatment Itself

Infections remains an important cause of morbidity and mortality in patients being treated for acute leukemias(15–17); infection-related mortality rate in this population is 3 to 7,3% during chemotherapy and half of all deaths are attributed to infections with gram-negative bacterias and opportunistic fungi(18).

It is important to differenciate exogenous infections, that occur when a person is exposed to organisms from external sources, and endogenous infections produced by organisms in the person's own microbial flora that spread to normally sterile body sites where disease can ensue (19), because the majority of infections in patients with cancer are of an endogenous source (20).
Infections may progress rapidly and may be difficult to diagnose because of blunted inflammatory response in these patients(18).

Patients with **hematologic maligancies** are at greater risk for neutropenic complications than patients with solid tumors because of the underlying disease process as well as the intensity of the treatment that is required (21).

Patients undergoing these high intensity chemotherapy regimens became neutropenic and consequently severy immunocompromised making them highly susceptible to infection.
We define neutropenia as neutrophil count of <500 cells/mm^3 or a count of <1000 cells/mm^3 with a predicted decrease to <500 cells/mm^3(22).

At least one-half of neutropenic patients who become febrile have an established or occult infection, and at least one-fifth of patients with neutrophil counts < 100 cells/mm^3 have bacteremia; the common sites of infection include the alimentary tract, due to chemoteraphy-induced mucosal damage(22).
The sources of bacterial bloodstream infections in cancer patients can be difficult to ascertain but studies show that may be a result of translocation of intestinal microbes across the gastrointestinal epithelium(23).
It's necessary to understand the demographics of life-threatening and fatal infections by causative organism and how this distribution differs according to different intensity and types of therapy in order to develop preventive measures(15).

Studies have shown that in ALL infections involving the blood, lung, liver and CNS were associated with a high (>60%) proportion of life-threatening of fatal events; which is also related with the causative pathogen(17).
On the other hand factors such as age, initial white blood cell count, gender and ethnicity did no impact on the proportion of infections that were consider life-threatening or fatal.in AML(15).

Knowledge of pharmacokinetic and pharmacodynamics properties of antibiotics helps to optimize antibiotic choice and dosing according to site and severity of infection, host characteristics and improve their efficacy, particularly in those patients with resistant and severe infections(18).

The use of **antimicrobial prophylaxis** remains a discussion topic nowadays, some guidelines defend that the use of antibiotic prophylaxis shouldn't be a routine procedure due to emerging antibiotic resistance and should only be prescribed when neutropenic patients present an on set fever or in afrebile patients who are neutropenic and show signs or symptoms compatible with an infection (22); Others defend that prophylaxis with levofloxacin is effective, well tolerated, and cost-effective, even tough it has no effect on the risk of death(24).

Some evidence suggests that AML patients receiving antimicrobial prophylaxis are at a greater risk of bloodstream infections caused by resistant gram-negative bacterias (17), and also emphasize the emergence of antimicrobial resistance associated with the routine use of prophylactic antibiotics(20,23,25).

A study that aimed to characterize the oral and gut microbiome of cancer patients (26) concluded that a prolonged antibiotic exposure is associated with long-term infectious outcomes among AML patients undergoing chemotherapy, which suggest that the very treatment applied to protect the patient appears to predispose the patient to recurrent infectious-related issues.

It is an important debate to address knowing that infections are a main cause of death in patients being treated for acute leukemias.

In hospital Universitari Dr. Josep Trueta antimicrobial prophylaxis is not a routine procedure in patients in treatment for acute leukemias.

Despite the use or no use of prophylactic antibiotics in patients undergoing chemotherapy we should not forget the importance of the non-pharmacologic interventions that may also mitigate the risk of infection, such as hand-hygiene that is perhaps the most effective measure for limiting the transmission of infection (25).

Regarding **antifungal prophylaxis,** as before mentioned, patients in treatment for acute leukemias are at increased risk for fungal infections because of disease-related and therapy-induced immunosuppression (18,25) due to the fact that these infections are often difficult to diagnose and treat successfully, antifungal prophylaxis may be appropriate(22).

The ideal prophylactic antifungal agent is safe and well tolerated with long-term use, effective against a wide spectrum of fungi, and manufactured as both IV and oral formulations with good bioavailability (25).

In conclusion there is not a one-size-fits-all approach to prophylaxis, every patient undergoing chemotherapy should be evaluated individually and within the context of local microbiologic epidemiology (25).

3.3. Intestinal Microbiome And Cancer

Lately there has been an increasing appreciation for the role the human microbiome plays in many aspects of human physiology, health, and disease. Several studies have showed that although each person has a relatively distinct gastrointestinal microbiome signature, a healthy individual’s microbiome remains relatively stable over time, (26,27) the uniqueness of each individu-

al's microbial may be another feature of the human microbiome specifically associated with health(27), in general a microbiome with **higher diversity** is thought to be an indicator of overall health(23,26).

A fully colonized gut consists of 10^{14} bacteria, that establish a symbiotic relationship with the host and insures normal development and immune homeostasis(28).

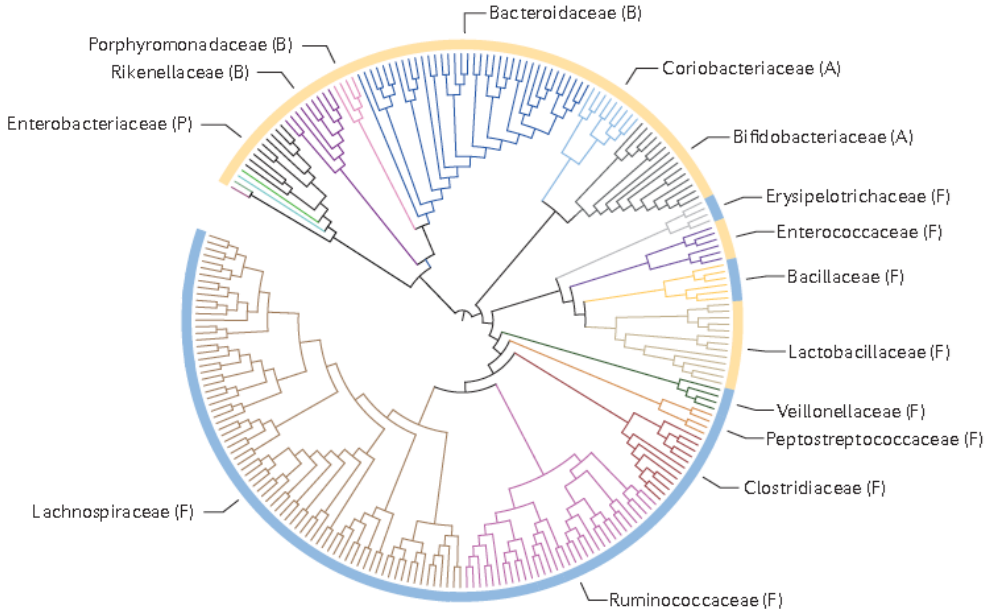


Figure 7: Representative cladogram of the main human intestinal microbiota families and their associated sporulation ability. P(proteobacteria); B(bacteroidetes); A(actinobacteria); F(Firmicutes). Adapted from (29)

A healthy microbiota can also protect against pathogens becoming established at any density in the microbiome, a process referred to as colonization resistance(23). Intestinal dysbiosis, meaning the microbiome composition is grossly perturbed, is thought to be responsible for a number of pathologies(30).

The microbiome present in the distal gut performs myriad functions protecting the host against pathologies due to various reasons it is known that colonization by commensal microorganisms is key to immune development and commensal community keeps in check invading pathogens and prevents them from expressing virulence (23,31).

It is well known that patients in the hospital, due to various reasons, are at risk for colonization and intestinal domination by pathogenic bacteria and that a diverse microbiome provides colonization resistance against many organisms(26).

Regarding the haematological field, the gastrointestinal tract serves as the source for a high percentage of bacteremia in patients with hematologic malignancy(23).

The interaction between the intestinal microbiome and chemotherapy is very complex in one hand the loss of epithelial integrity during chemotherapy markedly increases the risk of bacteremia. Mucositis (mucosal barrier injury) is a major oncological problem caused by chemotherapeutic agents used (16,31), resident bacteria can colonize the ulcer in the ulcerative phase of mucositits leading to the liberation of proinflammatory cytokines. The presence of inflammatory products may exacerbate the mucosal damage and tissue injury that occurs as a result of cytotoxic cancer therapy which could increase the likelihood of bacteria to enter the bloodstream and cause sepsis(23). Oral and small intestinal mucositis translate into a variety of clinical symptoms can be worsened by neutropenia and antibiotics.
It is well established that chemotherapeutic elicit their proapoptotic activity against rapidly proliferating cells populations, meaning not only the tumor cells but also intestinal stem/progenitor cells(31) causing or worsening symptoms.
On the other hand gut microbiome also influences the outcome of cancer therapy by modulating the host inflammatory response(30).

A study that aimed to characterize the oral and gut microbiome temporal variability in hospitalized cancer patients(26) concluded that AML patients undergoing chemotherapy exhibit temporal instability of the stool and oral microbiome diversity, as well as the fact that an high intra-patient temporal variability of oral and stool microbiome among AML patients is associated with increased pathogenic-associated genera abundance.

Another recent study(16) demonstrated that there is a predictive relation between pre-chemotherapy gut microbiome and future risk of BSI in patients with Non Hodgkin Lymphoma receiving allogeneic transplantation, by showing that alpha diversity in fecal samples from patients who developed BSI was significantly lower than alpha diversity from patients who did not develop subsequent BSI.

One of the main aims of microbiome research is to use microbiome as either an indicator for morbidity or to improve human health, an enhanced understating of the kinetics and taxonomic characterization of microbiome stability in acutely ill patients is of paramount importance(26) this way by modulating microbial activities we may boost drug efficacy or alleviate toxicity, two key aspects of chemotherapeutic treatment(30).

4. JUSTIFICATION

The arising knowledge of how the gastrointestinal microbiome interferes in many aspects of health and disease has lead to the questioning of how can we use this information to our advantage and benefit. Nowadays an emerging understanding of the ability of bacterial pathogens, including multidrug-resistant organisms, to colonize and subsequently infect humans is largely dependent on protective bacterial species present in the microbiome(23).

This information becomes especially relevant when we look at data such as the incidence of acute leukemia in our environment and worldwide as well as the list of causes of death in patients in treatment for acute leukemias, as infection is consider one of the leading causes of death(18,23).

The sources of bacterial bloodstream infections in cancer patients can be difficult to ascertain but studies show that may be a result of translocation of intestinal microbes across the gastrointestinal epithelium (23).

Chemotherapy drugs used in the treatment of acute leukemias are extremely myeloablative and it is known that patients with hematological malignancies are at greater risk for neutropenic complications due to the disease process and the intensity of the treatment that it is required(21,32). In conclusion, knowing that infectious count as an important neutropenic complication these patients are extremely prone to infections that may be potentially fatal, for this reason it extremely important to identify risk factors for infection in order to develop screening and prophylactic measures.

A specific recent study (20) concluded that a low baseline α-diversity in the gastrointestinal tract, evaluated in stool samples, is associated with the development of infection during the induction regimen of chemotherapy in AML. However, despite the promising results, these cannot be translated into our study population due to two different reasons: these patients received prophylactic antimicrobials (not a routine procedure in our study population) and the chemotherapy agents used are not the same used by us.

Prophylactic antimicrobials and the type of chemotherapy agents used are two important factors that may alter the intestinal microbiome and for this reason may bias the outcome of infections in patients undergoing chemotherapy treatment for acute leukemias.

For all the above reasons we consider important to evaluate the relation between the gastrointestinal microbiome and infectious complications in our own patients in order to understand if there is a clear association.

Understanding this association could be extremely useful in the future in predict which patients would be more susceptible to infectious complications, in a context of treatment for acute leukemia, allowing us to classify patients as high-risk or low-risk for infections and therefore implement specific measures in order to avoid these situations, such as use of prophylactic antibiotics in the specific group.

In conclusion, the understanding of this relation in a long-term perspective may help to decrease the number of infectious complications in patients with acute leukemia and consequently decrease the mortality rate in these patients.

5. HYPOTHESIS

Different intestinal microbiome diversity composition before and after high intensity chemotherapy treatment in patients newly diagnosed with acute leukemia (myeloid or lymphoblastic) is associated with infectious complications.

6. OBJECTIVES

The main aim of the present study is to establish an association between intestinal microbiome diversity composition (before and after treatment) and bacterial infectious complications, in patients undergoing high intensity chemotherapy.

Secondary:

- To be able to use microbiome as an indicator of morbidity in patients being treated for acute leukemias
- Differentiate patients in 2 groups according to high-risk of infection and low-risk of infection according to microbiome diversity composition
- Implement measures, such as antibiotic prophylaxis in high-risk patients in order to decrease the number of infectious complications and therefor the mortality of these patients.

7. SUBJECTS AND METHODS

7.1. Study Design

This study design will be an observational, prospective, longitudinal follow-up study without a control group. It will be performed in Hospital Universitari Dr. Josep Trueta.

7.2. Subjects Selection

The population of study will be every patient newly diagnosed of acute myeloid leukemia or acute lymphoblastic leukemia: >20% blasts in bone marrow or peripheral blood (WHO) (33) in Hospital Universitari Dr. Josep Trueta.

a. Inclusion and exclusion criteria

Inclusion criteria

- Patients older than 18 years;
- Patients with the diagnosis of Acute myeloid leukemia or Acute lymphoblastic leukemia *de novo*, secondary or therapy-related;
- Patients who agree to participate in the study by understanding and signing the informed consent form (ANEXO 6).

Exclusion criteria

- Patients older than 70 years;
- Patients who do not provide a minimal of one fecal sample before chemotherapy treatment and 1 sample after chemotherapy treatment.
- Patients who do not complete the entire cycle of chemotherapy;
- Patients who are not consider fit to receive high intensity chemotherapy treatment, based on GAH scale (a global evaluation scale developed for patients with haematological malignancies) (ANNEX 4)
- Patients receiving oral or intravenous antibiotics at diagnosis;
- Patients diagnosed with inflammatory bowel disease.

7.3. Sample And Sampling

The income number of patients with the diagnosis of AML in Hospital Universitari Dr. Josep Trueta is approximately 25 patients/year, if we estimate that 28% of patients will be excluded because will not undergo chemotherapy treatment; we narrow the sample to 18 patients/year.
Regarding ALL the income number of patients is approximately 18 patients/year, if we estimate that a 33% of these patients will be excluded because will not undergo chemotherapy treatment; we narrow the sample to 12 patients/year.
Having in consideration that our study includes both patients with AML and ALL that will be treated with high intensity chemotherapy at our center, with a risk of 5% (alpha), the statistical power for bilateral test and a sample size of 30 and the assumption of maximum indeterminacy is 34.93%.

To achieve acceptable power, there are two options:

- Consider a two years study: in this case, with a risk of 5% (alpha), the statistical power for bilateral test and a sample size of 60 (2*30) and the assumption of maximum indeterminacy is 61.10% or even three years, sample size of 90 (3*30) in which case the statistical power will be 78.88%.
- Increase the risk (alpha) up to 15%, in which case the statistical power would be 62.03%. In this second case, however, it would also increase the probability of committing type I error (rejecting the null hypothesis being true).

We decided to increase the duration of the “data collection circuit” to 3 years in order to have an acceptable statistical power.

A non-probabilistic consecutive sampling will be used in this study. The patients will be selected once they have a diagnosis of AML or ALL; since Hospital Universitari Dr. Josep Trueta is the reference centre in the province of Girona we will attend all patients from this geographical area.

7.4. Variables

a. Independent variable

Infectious complications: infectious episodes were classified according to Spanish Society of Infectious Diseases and Clinical Microbiology(32) when a patient presented one of the following criteria:

- Fever: oral temperature >38,3ºC in one occasion, or temperature ≥ 38ªC for at least an hour;
- Microbiologically defined infection: bacteremia or focal infection documented microbiological without positive blood cultures;
- Clinical defined infection: clinical o radiological focality without clear etiology.

Infectious complications will be considered a binary qualitative variable expressed by yes or no.

- Yes: presence of at least one of the previous criteria defining infection;
- No: presence of none of the previous criteria defining infection.

b. Dependent variable

Intestinal microbiome diversity: in order to express this variable we opted to use the Shannon Index, which measures the α-diversity.

α-diversity expresses the mean diversity at a local scale, giving us the number of different types of bacteria present in the samples (34).

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

Where P_i is the proportion of individuals found in species *i*.

Measures of α-diversity in healthy people (20) were realized with a mean Shannon index of 2.

Based on this information we decided to express this variable as a binary qualitative variable:

- Normal or High intestinal microbiome diversity: when Shannon Index ≥ 2
- Low intestinal microbiome diversity: when Shannon Index<2.

c. Confounding variables

- Antifungal prophylaxis: we consider this a binary qualitative variable expressed by yes or no.
  - Yes: the patient initiated antifungal prophylactic treatment before chemotherapy;
  - No.

- Intestinal side effects of chemotherapy: having in consideration the toxic effects of the chemotherapy agents used in this study we consider to be an intestinal side effect of chemotherapy the presence of diarrhea, defined by an increased liquidity or decreased consistency of feces with more than 5 depositions a day without fever.
  We will consider this variable as a binary qualitative variable expressed by yes or no.
  - Yes: presence of diarrhea with over 5 depositions a day without fever;
  - No.

- Age: will be expressed in quartiles.

- Gender: will be expressed as categories woman and man.

7.5. Data Collection

a. Data collection circuit

All the information regarding data collection will be collected in the participant information sheet (ANNEX 5)

Since Hospital Universitari Dr. Josep Trueta is de province reference center all patients with suspected or confirmed diagnosis of acute leukemia (myeloid or lymphoblastic) are derived to our center.
After the certain diagnosis of acute leukemia every patient will be subject to a battery of medical tests (ANNEX 1) in order to correctly classify the disease and access if the patient is fit to endure high-doses chemotherapy treatment by applying the GAH scale, which is a global evaluation scale developed specifically for patients with haematological malignancies.
All this circuit is part of a routine protocol followed by service of hematology in Hospital Dr. Josep Trueta.

In case the patient agrees to participate in the study, after informed consent is signed (ANNEX 7 and 8), we will proceed to the **first fecal sample collection**, before the start of high-dose chemotherapy treatment in order to evaluated the baseline intestinal α-diversity, at day 0 on hospital inpatient admission.

The **second fecal sample collection** will be performed during the neutropenic period (neutrophil count of <500 cells/mm^3 or a count of <1000 cells/mm^3 with a predicted decrease to <500 cells/mm^3).
Based on clinical practice we estimate that neutropenic period starts after the end of chemotherapy treatment and lasts anywhere between 12-20 days.
Having in consideration the interpersonal variability of chemotherapy response we agree that the ideal timing to collect the second fecal samples during the neutropenic period would be 48h after the end of the complete chemotherapy regimen.
The chemotherapy regimens followed are the hospitals' routine protocol.
Due to the fact that the duration of the chemotherapy regimens vary according to the type of acute leukemias and patients characteristics we define the last day of the chemotherapy treatment as "X", meaning the collection will be made in day X+2.

After day 20 post-chemotherapy treatment patients will be classify according

to the presence or not of bacterial infection during this period in 4 groups:

- Patient with the diagnosis of acute myeloid leukemia who **presented signs of infection;**
- Patient with the diagnosis of acute myeloid leukemia who **did not present** signs of infection;
- Patient with the diagnosis of acute lymphoblastic leukemia who **presented signs of infection**;
- Patient with the diagnosis of acute lymphoblastic leukemia who **did not present** signs of infection;

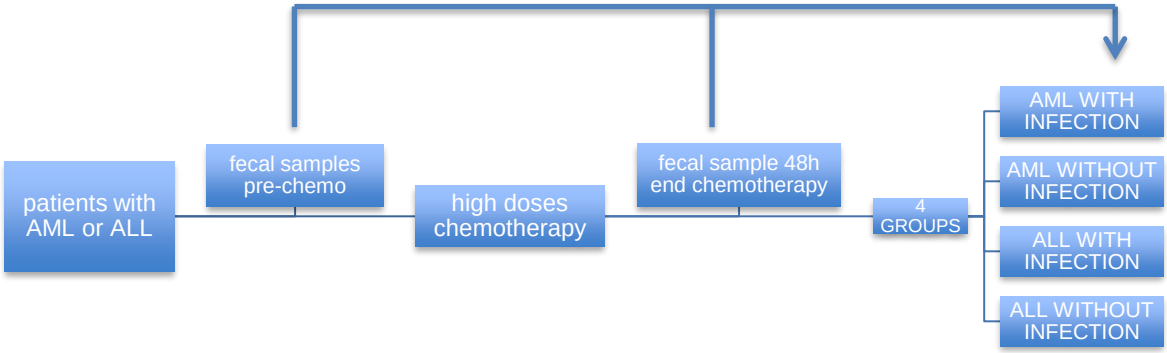


Figure 8: Data collection

During the duration on the chemotherapy and the neutropenic period patients follow a low-bacteria diet to try to minimize the risk of infection.

b. Fecal samples

All the samples will be collected by the patient himself in a sterile container with a screw cap. Rapid transportation to laboratory is important in order to prevent acid production by some bacteria's (bacterial metabolism); in case a delay in transportation is expected the fecal samples should be mixed with a preservative such as phosphate buffer mixed with glycerol or a Cary-Blair transport medium.

In order to access α-diversity, the samples will be process by a microbiologist

in Hospital Universitari Josep Trueta, the following way:

1. Extraction of DNA from the sample with Nucleospin® Soil Kit according to the instructions recommended by the manufacturer;
2. Amplification of the 16S ribosomal RNA (rRNA) V4 region(34) with polymerase chain reaction and sequenced using the Illumina MiSeq platform;
3. Assignation of the 16S ribosomal rRNA into operational taxonomic units using UPARSE pipeline(35);
4. Analysis and visualization of microbiome communities with software R using the Phyloseq package(36);
5. Calculation of the Shannon diversity index.

8. STATISTICAL ANALYSIS

8.1. Descriptive Analysis

In the context of our project, we cannot technically define our variables as independent and dependent due to fact that it is a descriptive follow-up study. However in order to proceed to the statistical analysis we will define the presence of infectious complications as the independent variable and the intestinal microbiome diversity measured by the Shannon Index as the dependent variable, which will be measured in 2 different times.
Our independent variable as well as our dependent and confounding variables are qualitative nominal and are going to be expressed as percentages.

We will elaborate a contingency table for our dependent variable comparing the 2 to different measures in time (before chemotherapy and after chemotherapy).

	Shannon Index	
	Normal/High (≥2)	Low (<2)
Before chemotherapy		
After chemotherapy		

Another contingency table is necessary for our independent variable expressing the cases of AML with and without infection and the cases of ALL with and without infection

		Diagnosis	
		AML	ALL
Infection	Yes		
	No		

We will then proceed to stratify the previous variables according to our con-

founding variables: Antifungal prophylaxis, intestinal side effects of chemotherapy, age and gender.

8.2. Bivariate Analysis

Percentages for categorical variables will be shown in a contingency table, as expressed before and chi-square test ($\mathcal{X}^2$) will be performed to compare the intestinal microbiome diversity before and after chemotherapy treatment in AML and ALL patients with and without infection.

8.3. Multivariate Analysis

Five logistic regression analysis will be estimate to assess the association between the 4 clinical groups and the diversity of intestinal microbiome before and after chemotherapy and the Shannon index categorized in normal/high and low and the diversity of intestinal microbiome.

9. WORK PLAN

9.1. Schedule

Main researchers: 2 Hematology specialists;
Collaborations: nursing staff; a microbiologist; a statistician.

This study is expected to last a total of 53 months (approximately 4,5 years) from August 2017 until September 2021, divided in 5 months of pre-field work, 36 months of field work and data collection plus 3 months for data analysis and 9 months for results interpretation and publication. The activities carried out during this time will be organized in 4 phases, detailed below.

- Phase 0: Preparation and coordination phase (5 months)

During this first phase of the project a detailed protocol will be elaborated with the help of the investigators and collaborators; the hypothesis, objectives, variables and methods will be discussed.
Coordination meetings will be arranged in order to identify and solve any problems or doubts regarding the protocol; all suggestions will be taken in consideration and discussed with the team.
Once the protocol is ready, it will be presented to the Ethical Committee for its evaluation and approval.

- First phase: study conduct (3 years)

Patients' recruitment: patients newly diagnosed with acute myeloid leukemia or acute lymphoblastic leukemia, who meet the inclusion criteria for the study and who will undergo chemotherapy treatment in Hospital Universitari Josep Trueta will be recruited, after signing the informed consent form.

Fecal samples collection: this assignment will consist in the collection of fecal samples for further analysis, the first sample will be collected at admission before the start of chemotherapy treatment; the following sample will be collect-

ed 48h after the last day of chemotherapy, during the neutropenic period (as explained in the data collection chapter)

While the study takes place the data collected from each patient will be register in our database, with the help of the patient data sheet (ANNEX 5).

This collected data will be periodically evaluated and analysed by an external collaborator to control if the protocol is being followed.

During this period a pilot test will be conducted in order to depurate data procedures and detect possible errors.

- Second phase: Data analysis (3 months)

After processing the database, all data will be analysed by our statistician using the statistical tests described in the “Study and Methods” chapter.
The final results will be sent to the main investigators, who will then proceed to their interpretation and discussing, elaborating the conclusions of the study.

- Third phase: Interpretation, publication and dissemination of the results (9 months)

The researchers will write and edit a scientific paper with publishing intention and attend the National Congress of the Spanish Society of Hematology and Haemotherapy in order to disseminate the results.

9.2. Chronogram

	ASSIGNMENT	STAFF	CALENDAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
PHASE 0	◆Study protocol development ◆Presentation to the CEIC ◆Coordination meetings	All research team	2017												
			2018												
			2019												
			2020												
			2021												
PHASE 1	◆Patients recruitment ◆Fecal samples collection ◆Register in database	Haematology department team Microbiologist researcher	2017												
			2018												
			2019												
			2020												
			2021												
PHASE 2	◆Data analysis	Statistician	2017												
			2018												
			2019												
			2020												
			2021												
PHASE 3	◆Interpretation and study writing ◆Publication and dissemination	Main researchers of the haematology department	2017												
			2018												
			2019												
			2020												
			2021												

10.ETHICAL AND LEGAL ASPECTS

This research protocol will be presented to the Clinical Research Ethical Committee (CEIC) of Hospital Universitari Dr. Josep Trueta in Girona for its assessment and approval. Moreover, the recommendations given by the committee will be taken into account to carry out the study.

This study will be conducted in accordance to the Human Rights and to the Ethical Principles established by World Medical Association in the Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects (last actualization, October 2013).

The confidentiality of personal and clinical information of all participants involved in the study will be guaranteed, according to the "Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal". All the information will be only used for the purpose of the research. Patients will always be allowed to modify or destruct any of their collected data study.

According to the "Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica" all participants interested on being part of the study, will be asked to sign voluntarily the informed consent (ANNEX 8). Before being included in it, they will receive all the appropriate information about the study through a personal conversation with the researchers and using the information sheet.

Participants will also be asked to authorize the inclusion of their fecal sample into the "Institut d'investigació biomedical de Girona Dr Josep Trueta" (IdibGi) BioBank. A specific information sheet and informed consent will be given to them (ANNEX 6, 7 and 8).

Furthermore, we will take into consideration the Spanish Organic Law 14/2007, de Investigación Biomédica, which regulates biomedical investigation involving human beings in Spain.

11.LIMITATIONS

Due to our study design and the study target population there are a few undeniable and identifiable limitations such as:

- The sample size of this study could be considered small, but is reasonable for our hospital patients' income. If a larger sample was needed, we could consider engaging other Hospitals from the ICO group into the study and designing a multi-centre study;
- The study design doesn't include a control group meaning that we have no comparison measure with the healthy population;
- Our results can not be generalized to other chemotherapy regimens, due to the impact of these agents in the intestinal microbiome diversity; This takes especial importance when there is not a universally accepted treatment for acute leukemias, meaning each hospital follows his own protocol; This is a limitation hard do access nowadays, we would propose to standardize the acute leukemias treatment as much as possible in Spanish hospitals.
- Our results can not also be applied to other hematological malignancies, since we only focused on patients diagnosed with acute leukemia (myeloid and lymphoblastic);
- The number of fecal samples pre and after chemotherapy may be consider insufficient in number. If positive results are obtained in our study we may consider a larger study analysing a higher number of samples allowing us to describe the evolution of the microbiome intestinal diversity before, during and after chemotherapy regimens.

12.FEASIBILITY

Our research will take place between August 2017 and December 2021 in the Hospital Universitari de Girona Doctor Josep Trueta (HJT), which is the reference hospital of all the territory in the province of Girona.

The personnel whom are going to be part of this study are well trained and has experience on this field.

Before the start of the study, two informative meetings with all the professionals involved will be organized. In these two appointments, the main investigators will explain the objectives of the study, the data that has to be collected and how to do it, remarking the importance of gathering these data into an uniform database.

Regarding the recruitment of the patients, we have estimated that we will collect data over the course of 3 years to reach the desired sample size of the research. We are conscious that we can lose some of the patients mainly due to death during the course of treatment.

For all the reasons exposed, we do think that the research we proposed can be easily brought out without any barriers of knowledge, logistics, experience or budget.

13.IMPACT ON THE NATIONAL HEALTH SYSTEM

If the results of our study are relevant and show that different intestinal microbiome diversity composition before and after chemotherapy treatment in patients newly diagnosed with acute myeloid leukemia or acute lymphoblastic leukemia is associated with infectious complications, we would be able to use the intestinal microbiome composition as a predictor of infection by differentiating high-risk and low-risk patients.

Having in consideration that infection is an important cause of mortality in patients in treatment for haematological malignancies, prophylactic antibiotic treatment (which is not a routine procedure in our hospital) could be prescribed in the high-risk patients groups.

This way we would be doing a thoughtful use of antibiotics, which is an extremely important measure nowadays due to increasing bacterial resistance to antibiotics.

Overall through this measures we would have a decrease in the number of infections in these patients, which would translate in lower rates of mortality among these patients, improving the success rates of treatment.

It is noteworthy that acute leukemias are a common type of cancer, and many of them have low survival rates due to the extremely mieloablative treatment patients have to endure, therefor this subject has an high impact on the national health system.

14. BUDGET

EXPENSES	COSTS (€)
PERSONAL EXPENSES	
- Statistician (x100h, per 30€/h)	3 000 €
EXECUTIVE EXPENSES	
- Material	3 500€
- DNA extraction kit	
- Molecular biology reagents	
- Stool containers	
- Other consumables	
- Ilumina MiSeq (x180, per 70€)	12 600€
PUBLICATION AND DISSEMINATION EXPENSES	
- Publication in open access journal	2 500€
- National congress of the Spanish Society of Hematology and Haemotherapy (2 persons)	1 000€
- Registration	
- Travel	
- Accommodation	
- **TOTAL**	22 600 €

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16. ANNEXES

ANNEX 1: Initial diagnostic evaluation and management od adult patients with acute leukemia

COMPLETE MEDICAL HISTORY

COMPLETE PHYSICAL EXAMINATION

LABORATORY AND RADIOLOGIC STUDIES

- Complete hemogram with blood type
- Peripheral blood smear
- Complete blood count with manual differential cell count
- Chemistry tests (electrolytes, creatinine, BUN, calcium, phosphorus, uric acid, hepatic enzymes, bilirubin, LDH, amylase, lipase, B12, iron)
- Clotting studies (prothrombin partial thromboplastin time, fibrinogen, D-dimer)
- Viral serologies (IgG and IgM for CMV; antibodies for hepatitis A and C; antibodies for HIV; hepatitis B HBsAg)
- HLA study
- Bone marrow aspirate and biopsy (morphology, cytogenetics, flow cytometry, molecular studies)
- Bacteriology (blood cultures; urine tests; fecal samples for fungi studies)
- Cardiology (ECG and echocardiogram)
- PA and lateral chest radiography

INTERVENTIONS FOR SPECIFIC PATIENTS

- Dental evaluation
- Lumbar puncture (for patients with symptoms of CNS involvement)
- Screening spine MRI (for patients with back pain, lower extremity weakness, paresthesias)
- Social work referral and psychosocial support.

ANNEX 2: Chemotherapy drugs used in the treatment of AML

ACUTE MYELOIDE LEUKEMIA		
	IDARRUBICIN	CYTARABINE
Pharmacological group	Analogue of daunorubicin (derived from anthracyclines)	Antimetabolite (pyrimidine antagonist),
Mechanisms of action	Inhibits DNA synthesis by the action of polymerase and topoisomerase II	Inhibition of DNA synthesis by competitive inhibition of DNA polymerase and insertion into DNA
Main toxic effects	• Vomiting; • Mucositis; • Diarrhea; • Elevation of transaminases; • Cardiotoxicity;	• Conjunctivitis; • Photophobia; • Cutaneous maculopapular rash; • Neurotoxicity (dysarthria, ataxia, nystagmus) • Pulmonary edema;

ANNEX 3: Chemotherapy drugs used in the treatment of ALL

ACUTE LYMPHOBLASTIC LEUKEMIA							
	VINCRISTINE	DAUNORUBICIN	PREDNISONE	L-ASPARAGINASE	DEXAME-THASONE	METROTHEXATE	CYTARABINE
Pharmacological group	Plant alkaloid	Derived from anthracyclines	Glucocorticosteroid	Enzyme	Glucocorticosteroid	Antimetabolite (folic acid antagonist);	Antimetabolite (pyrimidine antagonist),
Mechanisms of action	Binds avidly to tubulin, blocks microtubule polymerization and disrupts mitotic spindle formation during mitosis at the M phase of the cell cycle.	Intercalate between the bases in double-stranded DNA, poison topoisomerase II, generate free radicals, and disrupts the functioning of the cell membrane.	Many effects in ALL induce eosinopenia and lymphopenia attributable to a modification in cell production, distribution, or lysis	Hydrolyzes asparagine to aspartic acid and ammonium; Blasts of ALL can not resynthesize asparagine (lack asparagine synthetase) and die.	Many effects in ALL induce eosinopenia and lymphopenia attributable to a modification in cell production, distribution, or lysis	Competitively inhibits the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate (FH_4) this way inhibits the synthesis of new purines.	Inhibition of DNA synthesis by competitive inhibition of DNA polymerase and insertion into DNA.
Main toxic effects	• Neuromuscular alterations (peripheral neuropathy, paresthesias; paresis; neuralgic pain) • Nausea and vomiting; • Diarrhea; • Alopecia; • Hypersensibility; • Stomatitis; • Phlebitis.	• Cardiotoxicity; • Alopecia; • Nausea and vomiting; • Hepatic lesions • Hyperuricemia; • Dermatitis; • Stomatitis; • Phlebitis;	• Increase in appetite; • Hyperglycemia; • Osteoporosis; • Peptic ulcer; • Hirsutism; • Adrenocortical insufficiency; • Cushing's syndrome.	• Fever; • Nausea and vomiting; • Allergic reactions; • Neurotoxicity; • Pancreatitis;	• Gastritis • Peptic ulcer • Hyperglycemia • Hypokalemia • Hypertension • Myopathy • Osteoporosis • Psychic alterations • Increase of intraocular pressure	• Mucosal toxicity; • Nausea and vomiting; • Nephrotoxicity; • Hepatotoxicity; • Neurotoxicity (visual alterations, convulsions, paresthesias, muscular debility, psychosis); • Pulmonary infiltrates and fibrosis;	• Conjunctivitis; • Photophobia; • Cutaneous maculopapular rash; • Neurotoxicity (dysarthria, ataxia, nystagmus) • Pulmonary edema;

ANNEX 4: Global valuation scale (GAH)

ESCALA GAH

Situación funcional

Número de fármacos *(en uso actual; debe tomarse de forma continua al menos 2 semanas; tópicos y oftálmicos sólo si toma más de 3 veces por semana)*	
Velocidad de la marcha Velocidad para recorrer 4 metros a paso normal *(ver instrucciones abajo)*	m/seg

Estado de ánimo ¿En la última semana se sintió deprimido? *(Seleccionar sólo una)*

	Nunca, muy raramente u ocasionalmente (no mas de dos días)		Bastante a menudo, frecuentemente o todo el tiempo (3 a 7 días)

Actividades de la vida diaria (AVD) Necesita ayuda de otros para su vida cotidiana ☐ Sí ☐ No Dispone de cuidador ☐ Sí ☐ No

Tiene alguna dificultad para... *(SIN)*

Comprar objetos personales (p. ej. objetos de aseo o medicinas)		Manejar dinero (p.ej. llevar cuentas o pagar deudas)		Caminar *El uso de bastón o ayudas está permitido*		Realizar trabajo doméstico ligero (p. ej. fregar, levantarse, o limpieza ligera de la casa)		Bañarse o ducharse	
Sí	No	Sí	No	Sí	No	Sí	No	Sí	No

Estado de Salud Subjetivo En general, comparando con otras personas de su edad, diría de su salud que es... *(Seleccionar sólo una)*

	Excelente	Muy buena	Buena	Regular	Mala

Nutrición *(ver instrucciones)*

Peso (kg)		Talla (m), con dos decimales		IMC	

¿Ha perdido algo de peso en los últimos 3 meses?		¿Ha comido menos de lo habitual en los últimos 3 meses debido a pérdida de apetito, problemas digestivos, o dificultad para masticar o tragar?		¿Ha tenido estrés psicológico o alguna enfermedad aguda en los últimos 3 meses?	
Más de 3 kg		Mucho menos		Sí	
No lo sabe					
Entre 1 y 3 kg		Algo menos		No	
No		No			

Estado mental *Realizar las preguntas y recoger la respuesta sin ayuda de calculadora, dietas, documentos personales o cita ajeno a la entrevista.* Correcto/Incorrecto			
	Correcto	Incorrecto	1. ¿Cuál es la fecha de hoy?
	Correcto	Incorrecto	2. ¿Cuál es el día de la semana?
	Correcto	Incorrecto	3. ¿Cómo se llama el sitio donde estamos?
	Correcto	Incorrecto	4. ¿Cuál es su número de teléfono?
	Correcto	Incorrecto	5. ¿Cuál es su edad?
	Correcto	Incorrecto	6. ¿Cuándo nació?
	Correcto	Incorrecto	7. ¿Cómo se llama el presidente del gobierno?
	Correcto	Incorrecto	8. ¿Y cómo se llamaba el anterior presidente del gobierno?
	Correcto	Incorrecto	9. ¿Cuáles son los apellidos de su madre?
	Correcto	Incorrecto	10. Empezando en 20, reste de tres en tres hasta llegar al final.

Comorbilidad y hábitos

Las comorbilidades deberán registrarse como presencia o ausencia de enfermedad. El IMC se categorizará en valores ≥25/<25, y el tabaquismo como Nunca fumador y ex-fumador/Fumador actual.

Diabetes mellitus	Ausencia	Presencia	
Cáncer	Ausencia	Presencia	
Enfermedad pulmonar	Ausencia	Presencia	
Insuficiencia cardiaca	Ausencia	Presencia	
IMC	≥ 25	< 25	
Tabaquismo	Nunca fumador/Ex-fumador	Fumador actual	

ANNEX 5: Patient data sheet

GENERAL DATA

Participant code:

Date of birth (DD/MM/YYYY):

☐ Male

☐ Female

Diagnosis:

TREATMENT DATA

Type of chemotherapy regimen:

First day of the treatment (DD/MM/YYYY):

Last day of the treatment (DD/MM/YYYY):

Other treatments prescribed during the chemotherapy:

CHEMOTHERAPY SIDE EFFECTS

Did the patient present diarrhea with over 5 depositions a day without a fever?

☐ Yes Date (DD/MM/YYYY):

☐ No

INFECTION

Mark in the patient presented any of the following; in case of positive answer add date

☐ Fever (>38ºC)

☐ Microbiological defined infection or bacteremia

☐ Clinical or radiological focality without clear etiology

SAMPLE COLLECTION

Date of the 1st sample collection (DD/MM/YYYY):

Date of the 2nd sample collection (DD/MM/YYYY):

ANNEX 6: Study information for the patient

Hospital Universitari de Girona Doctor Josep Trueta

ICO Institut Català d'Oncologia

HOJA DE INFORMACIÓN AL PACIENTE

EL ROL DE LA MICROBIOTA INTESTINAL COMO PREDICTOR DE INFECCIONES DURANTE EL TRATAMENTO DE LEUCEMIAS AGUAS (MIELOIDE Y LIMPFOBLASTICA).

INTRODUCCIÓN.

Nos dirigimos a usted para informarle sobre un estudio al que se le invita a participar. El estudio ha sido aprobado por el comité ético de investigación clínica del Hospital Universitario de Girona Dr. Josep Trueta.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda evaluar i juzgar si quiere o no participar en este estudio. Para esto lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir después de la explicación. Además, puede consultar con las personas que usted crea convenientes. Nos gustaría proveerle con esta hoja de información en relación a un Proyecto de investigación que se está llevando a cabo en nuestro centro, en el cual está invitado a participar. Por favor, lea con detenimiento la siguiente información antes de tomar una decisión.

PARTICIPACIÓN VOLUNTARIA

Debe saber que su participación en este estudio es voluntaria y que puede decidir no participar y retirar su consentimiento en cualquier momento, sin que esto le suponga una alteración de la relación médico-paciente ni se produzca perjuicio alguno en su tratamiento. Si decide participar en el estudio tendrá que firmar el consentimiento informado después de leer esta hoja de información.

DESCRIPCIÓN DEL ESTUDIO

Una de las principales complicaciones durante el tratamiento de las leucemias agudas (mieloide y limfoblastica) son las infecciones debido a la propia enfermedad y también por los fármacos quimioterapicos agresivos que supone su tratamiento. En estudios recientes se ha evidenciado el rol de la microbiota intestinal en estas infecciones y por esa razón desarrollamos este estudio con el objectivo de establecer una relación entra la microbiota intestinal y las infecciones para que en el futuro podamos disminuir el numero de infecciones, aumento así la tasa de suceso del tratamiento de leucemias agudas.

Este estudio se lleva a cabo en el Hospital Universitario Dr. Josep Trueta y recogeremos datos a lo largo de 3 años.

¿POR QUÉ HA SIDO INVITADO A PARTICIPAR?

Usted ha sido diagnosticado recientemente de leucemia aguda mieloide o de leucemia aguda limfoblastica y va a iniciar tratamiento con quimioterapia a altas dosis, además, cumple los criterios de inclusión de este estudio.

BENEFICIOS Y RIESGOS EN LA PARTICIPACIÓN DEL ESTUDIO

La participación en este estudio es totalmente voluntaria. Es posible que los resultados obtenidos en este estudio tengan poco valor predictivo para usted directamente, pero podrá ayudar a conocer mejor el tratamiento y pronóstico de su enfermedad en los futuros pacientes.

No se prevé ningún riesgo adicional para usted.

Puede negarse a realizar el estudio y puede revocar su consentimiento en cualquier momento, sin tener que dar explicaciones y sin ninguna repercusión en la atención médica que reciba.

TRATAMIENTOS ALTERNATIVOS

La inclusión en este estudio no cambiará la normal estrategia terapéutica.

RESPONSABILIDAD Y SEGURO

Usted estará asegurado ante cualquier daño que pueda sufrir como resultado de su participación en este estudio, de acuerdo con la ley vigente.

CONFIDENCIALIDAD

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se ajustarán por lo dispuesto en la Ley Orgánica 15/1993, del 13 de diciembre de protección de datos de carácter personal. De acuerdo con lo que se establece en la legislación mencionada, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual se tendrá que dirigir a su médico del estudio. Los datos recogidos para el estudio estarán identificados mediante un código y sólo su médico del estudio o colaboradores podrán relacionar estos datos con usted o con su historia clínica. Por tanto, su identidad no será reveada a ninguna persona exceptuando excepciones, en caso de urgencia médica o requerimiento legal.

Sólo se tramitarán a terceros y a otros países los datos recogidos en el estudio, que en ningún caso contendrán información que lo pueda identificar directamente. En caso de que se produzca esta cesión será para la misma finalidad del estudio descrito y garantizando la confidencialidad como mínimo a nivel de protección de la legislación vigente en nuestro país.

El acceso a su información personal quedará restringido al médico del estudio, colaboradores, autoridades sanitarias, al Comité Ético de Investigación Clínica manteniendo la confidencialidad de los mismos de acuerdo con la legislación vigente. El acceso a su historia clínica será solo por lo que hace al estudio clínico.

Al firmar la hoja de consentimiento adjunta, se compromete a cumplir con los procedimientos del estudio que se le han expuesto.

ANNEX 7: Informed consent for participation

Hospital Universitari de Girona Doctor Josep Trueta

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CONSENTIMIENTO INFORMADO.

TITULO DEL ESTUDIO: “THE ROLE OF INTESTINAL MICROBIOME AS A PREDICTOR OF INFECTIONS DURING THE TREATMENT OF ACUTE LEUKEMIAS (MYELOID AND LYMPHOBLASTIC).”

Yo.....
Confirmo que:

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- Han respondido mis preguntas de manera satisfactoria.
- He recibido suficiente información sobre el estudio.

Comprendo que la participación es voluntária y que puedo retirarme del estúdio en cualquier momento.

En consecuencia, doy mi conformidad para entrar en este estudio.

Sí No

Permito al personal del estudio que consulte la mi historia clínica con la finalidad de verificación delos datos.

Sí No

Firma del participante:

Fecha

ANNEX 8: Informed consent for fecal samples

Hospital Universitari de Girona Doctor Josep Trueta

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SERVEI D'HEMATOLOGIA
DOCUMENT DE CONSENTIMENT INFORMAT PER A RECOLECCIÓ DE MOSTRES BIOLÒGIQUES

Justificació
Per un correcte avanç en la recerca de les malalties hematològiques és necessari realitzar estudis per conèixer millor tant les alteracions genètiques com d'una altra índole que presenten els pacients amb sospita de tenir aquest tipus de malaltia i, en conseqüència, aplicar el millor tractament. El nostre centre realitza i participa activament en projectes de recerca, alguns d'ells cooperatius, pels quals cal disposar de mostres biològiques de diferent índole i emmagatzemar-les per estudis posteriors.

Consideracions per a la seva participació
Per disposar de material per projectes de recerca es requereix una donació de sang o d'un altre producte biològic per part del pacient, però de cap manera se li realitzarà cap maniobra afegida a les estrictament necessàries pel diagnòstic i seguiment de la seva malaltia de base. D'aquesta manera, no suposarà cap molèstia afegida. Les mostres s'emmagatzemaran, i exportaran si cal, respectant estrictament les condicions de confidencialitat de les dades i s'assignarà a cada mostra un únic codi que la identificarà a partir d'aquest moment.

Benefici personal
El pacient, d'entrada, no obtindrà cap benefici personal immediat per la seva participació en aquests projectes, ja que el que es pretén és avançar en el coneixement de les malalties hematològiques, els seus aspectes biològics, diagnòstics i pronòstics. Tot això pot tenir en un futur un possible benefici en malalts que pateixin la mateixa malaltia.

Confidencialitat
Tots els resultats de la recerca seran confidencials i assignats a un únic codi del pacient, anònim. Aquests resultats no constaran al seu historial clínic. El seu nom no constarà en cap base de dades derivada dels projectes de recerca. Tota la informació personal que es reculli o es generi dels possibles estudis quedarà protegida d'acord amb la legislació vigent, sent totalment anònima i s'utilitzarà només per fins de recerca científica. En cas de que la informació fos enviada a tercers per ser utilitzada en algun estudi, la informació continuaria sent totalment anònima i no es podria identificar la persona a qui pertany. Els resultats dels projectes de recerca poden ser publicats per a la seva difusió a la comunitat científica. Els resultats genètics del pacient, a més podran ser inclosos en bases de dades consultables, sempre mantenint el seu anonimat.

Participació

La donació del material es totalment voluntària i el no fer-la no tindrà cap repercussió. Per aquesta donació no es percebrà cap compensació econòmica o d'altre tipus.

El donant té dret a sol·licitar en qualsevol moment, i sense necessitat d'especificar-ne el motiu, l'eliminació total de la mostra donada i de la informació relacionada amb la mateixa que estigui emmagatzemada. Els resultats que ja hagin estat utilitzats en la recerca no podran retirar-se. La sol·licitud d'eliminació ha de fer-se per escrit. Per a tot allò no previst en aquest document, s'aplicarà la legislació vigent que fa referència a dades de protecció de dades de caràcter personal (Ley Orgánica 15/1999, de 13 de diciembre) i qualsevol altra que resultés aplicable. Aquesta llei posa de manifest que vostè podrà exercir els dret d'accés, rectificació, cancel·lació i oposició de les dades.