# NATIONAL AUTONOMOUS UNIVERSITY OF MEXICO

MASTER AND DOCTORAL PROGRAM IN MEDICAL, DENTISTRY AND HEALTH SCIENCES

MASTER'S IN CLINICAL DENTISTRY SCIENCES

RESEARCH PROTOCOL:

**IMPACT OF THE USE OF ZINC IN THE PREVENTION OF ORAL MUCOSITIS IN PATIENTS WITH LYMPHOBLASTIC ACUTE LEUKEMIA**

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**Introduction:** Oral mucositis (OM) is a secondary complication of chemo/ radiotherapy, which causes pain, dysphagia and predisposition to infections, being a frequent reason for hospitalization that can affect the prognosis of cancer patients. Various interventions for the prevention of OM have been studied, including the use of zinc, which is a micronutrient that participates in various cellular functions and in wound repair, while showing a reduction in the incidence and severity of OM, so the purpose of the present study is to evaluate the impact of the use of zinc in the prevention of oral mucositis in pediatric patients with acute lymphoblastic leukemia in chemotherapy, comparing it with the use of placebo.

**Objective:** To evaluate the impact of the use of zinc in the prevention, incidence and severity of oral mucositis in patients with acute lymphoblastic leukemia in chemotherapy (induction and consolidation phase) of the National Institute of Pediatrics during the period from 2019 to 2020.

**Methods:** Double-blind randomized clinical trial conducted in pediatric patients with newly diagnosed or relapsed acute lymphoblastic leukemia. The subjects will be randomized to designate them in the control group (Group1 placebo) or intervention group (Group 2 zinc). The zinc/placebo intervention will be administered orally in tablets of 50-100 mg/day, from day 1 of chemotherapy until the end of the consolidation phase (2 to 2.5 months). For the detection of OM will be used the mucositis scale of the World Health Organization and the observation times will correspond to day 1, 4, 7, 14 and 21 of each chemotherapy cycle. Patients in both groups with OM will receive the same treatment (mouthwash with Gelclair®) and will be observed daily until healing to determine the days of recovery.

**Statistical analysis**: Independent t-test and Chi-square test for the difference between the two study groups, ANOVA to compare the groups with respect to the severity of OM.



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## BACKGROUND

The leukemias are a heterogeneous group of neoproliferative processes that are characterized by a clonal expansion of transforming hematopoietic precursors cells, this infiltrate a bone marrow and invade a peripheral blood and other organs.[1] The leukemias has been classified principally by their duration in: acute or chronic and by their cell type are affected in: myeloid, lymphoid and monocytic.[2] The acute lukemias (AL) are the more frequent type of neoplasia in the childhood and are approximated 33% of malignance pediatric illness.[1]

Acute lymphoblastic leukemia (ALL) represents 75% of all cases, being more frequent between 2 and 5 years of age.[1] The worldwide incidence of all types of ranges from 2.0 to 3.5 cases per 100,000 inhabitants per year, while in Mexico 6/100 000 inhabitants are estimated per year;[3] so that Mexico is considered to be one of the countries with a high incidence and mortality rate in LA.[4] The ratio of Lymphoid/myeloid leukemia is 6:1.[5] In a study on the frequency of acute childhood leukemia in Mexico City, it was found that the National Institute of Pediatrics presented 27.6% of all cases, followed by the Pediatrics Hospital of Mexico “Federico Gomez” with 20.2%.[6]

The treatment is based on polychemotherapy, which is the combination of antineoplastic drugs that seek to control cell growth and replication as well as destroy neoplastic cells in different phases of the cell cycle. In general, chemotherapy in patients with LLA consists of three phases: Induction, consolidation and maintenance, during which the prophylaxis of the central nervous system is incorporated, with intrathecal chemotherapy or high doses of chemotherapy.[7]

Induction aims to eradicate more than 99% of leukemic cells, restore normal hematopoiesis and the patient's physical condition. This phase of treatment includes the administration of glucocorticoids, with dexamethasone being the most used because it has better penetration to the central nervous system, associated with vincristine, L-asparaginase and an anthracyclic. With induction, usually more than 90% of patients achieve complete remission. The consolidation phase improves the remission achieved by induction; Commonly the regimens include high doses of methotrexate associated with 6-mercaptopurine. The maintenance phase aims to maintain the remission already achieved. Most regimens include the combination of weekly methotrexate with daily 6-mercaptopurine. The total duration of treatment should be from 2 years to 2 years and six months.[8]

Chemotherapeutic protocols are the result of the collaboration of different study groups, in 2015 Pui et al.,[9] conducted a review of the literature to analyze the impact of collaborative studies on advances in biology and treatment of LAL in children and adolescents, finding a 5-year overall survival rate of 82 to 93.5%. However, the substantial proportion of survivors face long-term complications; Therefore, the development of new protocols is currently aimed at optimizing the use of existing antileukemic agents, by evaluating the pharmacogenetics of normal host cells to avoid insufficient or excessive treatment.[10]

## Oral complications secondary to chemotherapy

The lack of selectivity of antineoplastic drugs produces toxic effects in normal cells, especially in those with high replication rate, such as the cells of the basal layer of the epithelia, in which their renewal capacity is altered resulting in to various complications. That is why in patients with hematologic malignancies such as LLA, oral lesions may occur secondary to direct damage of the lining epithelium by chemotherapy.[6]

The most frequent oral complications that have been reported in pediatric patients under chemotherapy are oropharyngeal mucositis (OM), xerostomia, caries and gingivitis, followed by opportunistic infections, which occur in up to 30% of cases; being more frequent those of fungal origin, although they can also be of bacterial or viral origin (candidiasis, cold sores, aphthous stomatitis and angular cheilitis). In the Mexican population, study by Castellanos et al., On oral lesions in children with acute lymphoblastic leukemia of the National Institute of Pediatrics, found that during the first 8 weeks of onset of chemotherapy, 71% of children developed some oral lesion the most frequent being OM (65%); which in severe grades (III or IV) was associated with neutropenia and malnutrition.[6]

## Oral Mucositis

Oropharyngeal mucositis (OM) is a lesion of the oral and oropharyngeal mucosa that appears as a result of inflammatory changes in epithelial and subepithelial cells caused by the cytotoxic effects of chemo or radiotherapy.[11]

The OM consists of four phases: 1) initial inflammation, 2) vascular and epithelial rupture 3) ulcerative / bacteriological and 4) healing. (7) It begins 3 to 10 days after the start of chemotherapy and may persist for 3 weeks. It reaches its maximum peak around 7 to 14 days and subsequently resolves slowly unless it is complicated by an infection; while, better resolution has been associated with neutrophil recovery.[12-14]

OM occurs at any age; however, it has been observed that the prevalence is higher in pediatric patients than in adults, which can be explained by the higher rate of cellular replication during childhood. It is estimated that 52 to 80% of children undergoing chemotherapy will experience some degree of OM, which varies according to the type of cancer and antineoplastic treatment. In general, children with hematologic malignancies present more frequently with OM than those with solid tumors.[14]

Chemotherapeutic agents are not equally stomatotoxic.[15] The extent of damage depends on the drug used, the duration of treatment, the dose and the route of administration; Therefore, bolus infusion, prolonged or repeated administration, compared with low doses of chemotherapy are associated with an increased risk of developing OM. Drugs that affect DNA synthesis, such as antimetabolites (methotrexate, 5-fluorouracil) and purine analogues (cytarabine), have been shown to cause OM from 40-60%. Etoposide, cyclophosphamide, doxorubicin, daunorubicin, docetaxel and paclitaxel have also been related.[13]

In the ulcerative phase of OM there are complications such as pain, difficulty eating, swallowing and speaking, which may require the use of analgesics and nutritional support.[12] The most severe grades are related to complications such as: deterioration in quality of life, increase in the duration and cost of hospital stay, decrease in the survival rate of patients due to delayed treatment or reduction in the dose of chemotherapy, as well as a higher incidence of secondary infections and may even lead to sepsis and death.[16]

Dental care in pediatric patients with cancer is essential, preventive measures and specific intervention actions are required to improve oral conditions that favor good nutrition and reduce the risk of infections.[16]

Recent research has found that OM is the result of a complex process that begins in the submucosal endothelium and fibroblasts, with the influence of local cytokines and not just direct epithelial cell damage. Thus, through a better understanding of the common intracellular pathway of the endothelium, cell apoptosis and interactions between apoptotic and inflammatory pathways, new treatments have been developed, which although some have been used in the pediatric population, none have been accepted as the treatment of choice.[14]

There are various treatments for OM including medications such as amifostine, palifermin, benzydamine, HCl and pentoxifylline, low intensity laser therapy and various organic products such as zinc; however, the effects of these interventions have not been fully confirmed.[12]

## Zinc

Zinc is a natural mineral and a vital micronutrient in humans that is required for the function of more than 200 metalloenzymes, including alkaline phosphatase and carbonic anhydrase. It also plays a critical role in the regulation of DNA and the synthesis of RNA (through interaction with DNA binding proteins), in the interactions of the hormone-receptor complex and in the second messenger system of cellular signal transduction.[17]

## Therapeutic indications of zinc

Many studies have shown the benefits of zinc supplementation on infections in human populations, such as the reduction in the incidence and duration of acute and chronic diarrhea, the decrease of lower respiratory tract infections in infants and young children, as well as the urinary infections.[20]

The use of zinc in children has been studied mainly for the treatment of diarrhea, since it has been found that the consumption of 10 to 20 mg of zinc per day, during and immediately after an episode of acute diarrhea decreases its duration and severity, as well as the incidence of diarrhea in the next two or three months.[21]

Daily zinc requirements as well as some therapeutic indications are shown in Table 1.

**Table 1. Recommended doses and administration intervals of elemental zinc [18, 19]**

**Dietary zinc requirements by age**

| Age | mg / day |
|---|---|
| <4 | 5- 10 mg |
| 4 - 6 | 10 mg |
| 7 – 10 | 10 mg |
| Teenage men and adults | 15 mg |
| Teenage and adult women | 12 mg |
| Pregnant women | 15 mg |
| Lactating women | 16 - 19 mg |

**Zinc dose indicated for the treatment of Wilson's disease**

| Age | Dose / interval |
|---|---|
| 1 – 6 años | 25 mg twice daily |
| 6 – 16 años | 25 mg 3 times a day |
| >16 años / peso > 57 kg | 50 mg 3 times a day |
| Pregnant women | 25 mg 3 times a day |
| Adults | 3 times a day (maximum dose of 50 mg 5 times a day). |

**Zinc dose indicated for the treatment of acute diarrhea**

| Age | Dose / interval |
|---|---|
| < 6 months | 10 mg daily for 10 - 14 days |
| > 6 months | 20 mg daily for 10 - 14 days |

**Zinc dose indicated for the treatment of respiratory tract infection**

| Age | Dose / interval |
|---|---|
| < 6 months | 10 mg daily for 10 - 14 days |
| > 6 months | 20 mg daily for 10 - 14 days |

**Zinc dose indicated for the treatment of sickle cell anemia**

10 - 15 mg per day

**Zinc dose indicated for the treatment of enterohepatic acrodermatitis**

1 - 2 mg * kg weight / day

## Secondary and adverse reactions

Adverse reactions are rare.[18] Nausea, vomiting and diarrhea may occur.[22] If gastric irritation occurs, zinc can be consumed with food, but foods high in calcium, phytates and phosphorus should be avoided. . Other symptoms that have been reported are: Hypotension, jaundice, pulmonary edema, edema of the mucous membranes of the mouth and stomach, unusual tiredness or weakness and gastric ulceration followed by perforation.[18]

## Drug and other gender interactions

The concomitant use of large amounts of fiber, phosphorus or phytates with zinc supplementation may reduce the absorption thereof by formation of non-absorbable complexes; Foods that contain fiber, phosphorus or phytates should be consumed at least 2 hours after the zinc supplement. Large amounts of iron can inhibit intestinal absorption of zinc, so it should be consumed at least 2 hours after ingesting iron supplements.

Zinc may decrease the absorption of penicillin, tetracycline and quinolone.[18]

## Allergic reaction

Signs of an allergic reaction may include rash, hives, erythema, edema or peeling; fever, wheezing, dyspnea, dysphagia, stertor, labial, lingual or airway edema.[22]

## Manifestations and management of accidental overdosing or intake

In general, zinc is well tolerated and acute poisoning is rare. Excess zinc in the body interacts with free thiol groups in macromolecules, thereby blocking the active sites of enzymes, coenzymes and membrane receptors.[23]

Acute poisoning of zinc sulfate has only been reported by deliberate ingestion,[24, 25] while chronic poisoning is the result of the excess oral zinc supplement that causes secondary copper deficiency.[26]

In case studies, the most common effects associated with prolonged consumption of zinc (ranging from 150 mg to 1-2 g / day) include sideroblastic anemia, hypochromatic microcytic anemia, leukopenia, lymphadenopathy, neutropenia, hypocupraemia and hypoferraemia. Patients often recover to normal blood patterns after zinc suspension with or without copper supplements.

Gastrointestinal toxicity: Gastrointestinal irritation seems to be dose related. Symptoms include nausea (20%), xerostomia (12%), gastrointestinal disorders (10%), abdominal pain, vomiting and diarrhea.[22] Zinc sulfate in amounts of 2 g / day or more can cause gastrointestinal irritation and vomiting.[27]Acute gastrointestinal bleeding was reported in a patient, who required a blood transfusion of 8 units after taking 440 mg of zinc sulfate daily for a week. [28]

Neurotoxicity: Zinc concentrations in the human brain remain in a range of less than 600-800 ng / L. The optimal concentration of free zinc in its ionic form in the cells is around 10 ng / L and when it drops to levels less than 0.06ng / L, apoptosis can be activated; while when levels exceed 60ng / L toxicity can be reached.[29]The symptom that has been reported most frequently is headache.[18]

Hematoxicity: Excessive ingestion of zinc sulfate chronically can induce anemia and leukopenia.[19]

Endocrines: Decreased HDL has been reported in men, while alterations in triglycerides have not been reported.[22]

Immune: In a study with 11 volunteers it was observed that excess zinc (300 mg per day for 6 weeks) was associated with immunodeficiency and inflammatory response.[23]

## Toxicity management

Gastric lavage should be avoided, the administration of chelators such as milk, sodium and calcium at a dose of 50 to 75mg / kg / day is recommended.[22]

The researchers' response to adverse reactions, allergic reactions and accidental intake are described in the investigator's manual.

## Zinc and oral mucositis

The effectiveness of zinc for the prevention of OM was initially studied in patients with head and neck cancer receiving radiotherapy. Ertekin et al. [27] established the dose of 150 mg of zinc sulfate daily in adult patients, finding a reduction in the severity of OM. Lin et al.[30] administered zinc chelate at a dose of 75 to 100 mg/day in adults with oral and nasopharyngeal cancer, finding a reduction in the development of OM in patients with oral cancer.

Other studies have used zinc sulfate mouthwashes in adults with head and neck cancer in radiotherapy and in patients with acute myeloid leukemia in the hematopoietic progenitor cell transplant protocol, finding that there were no differences between the zinc group and the control group.[31, 32] Other studies used L-carnosine zinc mouthwashes in adults with head and neck cancer receiving chemo / radiotherapy and in patients with leukemia in the transplant protocol, obtaining a reduction in the severity of the OM. [33, 34]

To date, only 3 studies have been conducted in patients receiving chemotherapy, (11) one of them was conducted in patients with head and neck cancer; another in patients with myeloid leukemia and the last in patients with myeloid and lymphoid leukemia; being all in adults, who received 150 mg of zinc sulfate daily, resulting in a reduction in the severity of the OM.[12, 37, 38] (See Table 3).

Table 2. Studies that have evaluated the effectiveness of zinc for the prevention of oropharyngeal mucositis in cancer patients receiving radio / chemotherapy.

| Reference | Treatment | Intervention | Type of cancer | Design | Results |
|---|---|---|---|---|---|
| Ertekin et al.[27] (2004) | Radiotherapy | Zinc Sulfate 50 mg / 3 times daily<br>150 mg / day<br>VS. Placebo | Head and neck cancer<br>18 - 71 years old | Randomized clinical trial<br>n = 30<br>6 weeks<br>Criteria of the Radiation Therapy Oncology Group. | OM severity reduction<br>p <0.05 |
| Lin et al.[30] (2006) | Radiotherapy | Zinc chelate 25 mg 2- 4 times / day<br>75 - 100 mg / day<br>VS. Placebo | Nasopharyngeal carcinoma / oral cancer<br>> 18 years | Randomized clinical trial<br>n = 100<br>3 months follow-up<br>Criteria of the Radiation Therapy Oncology Group. | Reduction of the development of severe degrees of OM in patients with oral cancer<br>Grade II p = 0.017<br>Grade III p = 0.0003 |
| Sangthawan et al.[31] (2013) | Radiotherapy | Zinc Sulfate Oral Rinse 50 mg<br>VS. Placebo | Head and neck cancer | Randomized clinical trial<br>n = 104 | Not significant |
| Watanabe et al.[33] (2010) | Chemotherapy Radiotherapy | Zinc solution L-carnosine VS. Rinses with tile | Head and neck cancer<br>35 - 86 years | Randomized clinical trial<br>n = 31<br>No observation period indicated<br>Common terminology criteria for adverse events version 3.0 | OM severity reduction<br>≥grade 2<br>p <0.05 |
| Hayashi et al. [35] (2016) | ADQ HPCT | Zinc solution L-carnosine 75mg / 20 mL 4 times / day VS. 18.75 mg tablets 4 times / day VS. No premedication<br>75 mg / day | Leukemia TCPH<br>22 - 73 years | Comparative study<br>n = 66<br>1 month post transplant<br>Common terminology criteria for adverse events version 3.0 | Reduction of severity of OM ≥ grade 2<br>p <0.01<br>and decreased pain<br>p <0.01 |
| Mansouri et al. [36] (2012) | HDC TCPH | Capsules of 220 mg equivalent to 50 mg of zinc sulfate 2 times / day<br>100 mg / day<br>VS. Placebo | Hematological cancers<br>> 15 years | Randomized double blind clinical trial<br>n = 60<br>3 weeks<br>WHO oral mucositis scale | Not significant |
| Mehdipour et al. [32] (2011) | HDC | 0.2% zinc sulfate VS. Chlorhexidine Gluconate Rinse 0.2% | Myeloid and lymphoid leukemia<br>> 15 years | Randomized double blind clinical trial<br>n = 30<br>2 weeks<br>Spijkervet mucositis scale | Not significant |
| Arbabi-Kalatl et al.[37] (2012) | Chemotherapy | 220 mg capsules equivalent to 50 mg of zinc sulfate 3 times / day<br>150 mg / day<br>VS. Placebo | Head and neck cancer<br>18-79 years | Randomized double blind clinical trial<br>n = 50<br>20 weeks<br>WHO oral mucositis scale | OM severity reduction<br>In week 8, 12, 16 and 20<br>p <0.05 |
| Gholizadeh et al. [38] (2017) | Chemotherapy | 220 mg capsules equivalent to 50 mg of zinc sulfate 3 times / day<br>150 mg / day<br>VS. Placebo | Myeloid and lymphoid leukemia<br>18 - 71 years old | Randomized clinical trial<br>n = 140<br>4 weeks follow-up (week 2 and 4) | Reduction of severity of OM grade 3-4<br>p = 0.004 |
| Rambod et al. [12] (2018) | Chemotherapy | 220 mg capsules equivalent to 50 mg of zinc sulfate 3 times / day<br>150 mg / day<br>VS. Placebo | Myeloid and lymphoid leukemia<br>> 18 years | Randomized triple blind clinical trial<br>n = 86<br>14 days<br>WHO oral mucositis scale<br>Oral Mucositis Index | OM prevention<br>p = 0.01<br>OM severity reduction<br>p = 0.01 |

OM; Oral Mucositis, HDC: High doses of chemotherapy, HPCT: hematopoietic progenitor cell transplantation.

There are currently no studies evaluating the effectiveness of zinc for the prevention of OM in pediatric patients receiving chemotherapy, so the purpose of this study is to conduct a clinical trial that evaluated the impact of the use of zinc for the prevention of OM in children with LAL in chemotherapy.

## PROBLEM STATEMENT

The development of OM in patients with acute leukemia varies between 81.3% and 90% .8 The available literature recognizes the importance of prevention and treatment of OM, since it has an impact on the quality of life and even on the prognosis of disease in patients under chemotherapy. The better understanding of the pathophysiology of this entity has allowed the development of new therapies, among which the use of zinc, which has shown beneficial effects in the prevention of OM in adult patients with leukemia, and because they are not counted with studies in pediatric patients with ALL; The following research question arises:

What is the impact of the use of zinc for the prevention, incidence and reduction of the severity of oropharyngeal mucositis in patients with acute lymphoblastic leukemia in chemotherapy during the period from 2019 to 2020?

## JUSTIFICATION

In the National Institute of Pediatrics, about 100 new cases of ALL enter each year, which will require a multidisciplinary approach throughout the course of cancer treatment, which includes the participation of the stomatologist, who must continually establish preventive and therapeutic measures of complications buccal, among which the OM stands out as a reason for frequent consultation.

Cancer patients will experience pain, limitation and psychological involvement due to oral OM; this debilitating condition also significantly affects nutritional intake, oral care and quality of life, representing up to 21.9% of the reasons for hospitalization.8 OM, if not properly treated or associated with prolonged neutropenia, may end in septic shock and even the death of the patient.

The use of zinc has been studied as a low-cost alternative for OM, so the realization of this study will determine the effectiveness of the use of zinc for the prevention and reduction of complications due to OM in pediatric patients with ALL of the Institute National Pediatrics.

## OBJECTIVE

To assess the impact of the use of zinc in the prevention, incidence and severity of oropharyngeal mucositis in patients with acute lymphoblastic leukemia in chemotherapy (induction and consolidation phase) of the National Institute of Pediatrics and the Children's Hospital of Mexico during the period from 2019 to 2020.

## SPECIFIC OBJECTIVES

1. Determine the incidence of OM in patients with ALL assigned control group (Group 1: oral care + placebo) versus the intervention group (Group 2: oral care + zinc).

2. Identify if there are differences in OM severity between Group 1 and Group 2.

3. Identify if there are differences in the recovery time of the OM between Group 1 and Group 2.

4. Identify if there is an association between the severity of OM and severe neutropenia in both groups.

## HYPOTHESIS

The use of zinc reduces the incidence and severity of OM in pediatric patients with ALL in chemotherapy compared to the control group.

## MATERIAL AND METHODS

Type of study: Randomized, double blind clinical trial.

Duration of the intervention: Induction and consolidation phase of chemotherapy (2 to 2.5 months).

Inclusion Period: from August 2019 to May 2020.

### Study population

Target population: patients of both sexes from 3 to 18 years of age with newly diagnosed or relapsed ALL during the period from 2019 to 2020.

Study population: patients of both sexes from 3 to 18 years of age with recently diagnosed or relapsed ALL treated by the Pediatric Oncology service of the National Institute of Pediatrics and the Children's Hospital of Mexico during the period from 2019 to 2020.

## Selection criteria

Inclusion criteria: Patients aged 3 to 18 years with a recent diagnosis of ALL or relapse who accept by informed consent to participate in the study.

Exclusion criteria. Patients with another disease or systemic condition in addition to ALL. Presence of oral infections such as candidiasis, herpetic gingivostomatitis, reactive oral lesions. Children with Down syndrome, patients allergic to zinc or mannitol.

Elimination criteria: Patients who present with hypersensitivity to zinc or placebo (mannitol), those who develop a systemic condition that modifies their treatment or prevents oral care, as well as patients requesting to leave the study.

## Definition of the variables

| INTERVENTION VARIABLE | | | | |
|---|---|---|---|---|
| | **CONCEPTUAL DEFINITION** | **OPERATIONAL DEFINITION** | **TYPE** | **MEASUREMENT** |
| ZINC | Trace element found in most of the body's cells, having catalytic, structural and cellular regulation functions. | Food supplement based on zinc sulfate in doses of 50-100 mg / day for prevention and treatment of oropharyngeal mucositis . | Qualitative<br>Nominal | 1. Apply<br>2. No apply |

| DEPENDENT VARIABLES | | | | |
|---|---|---|---|---|
| ORAL HYGIENE | Combination of physical and chemical measures to control the formation of bacterial plaque, which is the most important risk factor in the development and evolution of caries and periodontal disease. | Oral hygiene registered with the O'Leary index (bacterial plaque on dental surfaces identified by a developer. It is obtained from the sum of stained dental surfaces / total surfaces, multiplied by 100. | Qualitative<br>Ordinal | 1. Good 0% to 15%:<br>2. Regulate 16% to 49%:<br>1. 3. Poor: 3.50% to 100%: |
| OM RECOVERY DAYS | Days in which some degree of OM is clinically observed until healing. | Number of days from the clinical diagnosis of OM (Grade ≥ 1) to recovery of the oral epithelium. | Continuous | 1,2 ,3 4, 5… |

| INDEPENDENT VARIABLES | | | | |
|---|---|---|---|---|
| ORAL MUCOSITIS | Inflammatory reaction that affects the entire gastrointestinal tract from the mouth to the anus caused by chemo and / or radiotherapy. | Inflammatory reaction of the oropharyngeal mucosa to chemotherapeutic drugs, according to the WHO scale. | Qualitative<br>Ordinal | Grade 0. Healthy<br>Grade 1. Erythema<br>Grade 2. Ulcers<br>Grade 3. Extensive ulcers<br>Grade 4. Ulcers and necrosis. |
| PAIN | Localized and subjective sensory perception that is the result of an excitation or stimulation of specialized sensory nerve endings. | Visual scale of facial expressions | Qualitative<br>Ordinal | 0-2 No pain<br>3-4 Mild<br>5-6 Moderate<br>7-8 Intense<br>9-10 Maximum |

| SOCIODEMOGRAPHICAL VARIABLES | | | | |
|---|---|---|---|---|
| | **CONCEPTUAL DEFINITION** | **OPERATIONAL DEFINITION** | **TYPE** | **MEASUREMENT** |
| AGE | Life time elapsed from birth to the current date. | Life time in years recorded in the clinical record. | Quantitative<br>Discreet | Years<br>0 a 18 |
| SEX | Physiological and sexual characteristics with which men and women are born. | Biological characteristics recorded in the clinical file as female and male. | Qualitative<br>Nominal<br>Dichotomous | 1. Female<br>2. Male |



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| CONFUSORY VARIABLES | | | | |
|---|---|---|---|---|
| ACUTE LEUKEMIA LYMPHOBLASTICS (LAL) | Neoplastic disease that results from a clonal proliferation of lymphoid precursors (lymphoblasts), which infiltrates bone marrow, produces a variable degree of pancytopenia and can compromise different organs and / or systems. | Type of leukemia recorded in the clinical file according to the immunophenotype. | Qualitative Nominal | 1. Pro B leukemia<br>2. Common leukemia<br>3. Pre-B leukemia<br>4. Mature leukemia B<br>5. T leukemia (precursors)<br>6. T leukemia (mature) |
| NEUTROPENIA | Reduction of peripheral blood neutrophil count . | Absolute neutrophil count obtained by laboratory test (blood count). | Qualitative Ordinal | 0. Absence of neutropenia<br>1. Mild: 1,000-1,500 / ml<br>2. Moderate: 500-1,000 / ml<br>3. Severe: <500 / ml |
| OPPORTUNIST INFECTIONS | Infections in immunosuppressed patients, whether of bacterial, fungal or viral origin. | Record of suggestive lesions by C. albicans and determination by means of KOH, suggestive lesions of herpes and determination by PCR, suggestive bacterial lesions and detection through cultures. | Qualitative Nominal | 0. Absent<br>1. Fungal<br>2. Bacterial<br>3. Viral |

## STUDY DESIGN

Double blind clinical trial

**Sampling**: Sequential non-probabilistic until the required number of participants is completed.

**Randomization**: sealed envelopes for group assignment.

**Sample size calculation**

Formula for the difference of proportions (incidence of OM).

$$\frac{\left[Z_{\alpha}\sqrt{2\pi_1(1-\pi_1)} \; - Z_{\beta}\sqrt{2\pi_1(1-\pi_1) + 2\pi_2(1-\pi_2)}\;\right]^2}{(\pi_1 - \pi_2)^2}$$

Assumptions:

α = 0.05
Power = 0.90
Rambod study findings: The effect of zinc sulfate on prevention, incidence, and severity of OM in leukemia patients undergoing chemotherapy.

$Z_\alpha = 1.96$

$Z_\beta = -0.84$

$n = 86$

$\boldsymbol{\pi_1 = 75\%}$

$\boldsymbol{\pi_2 = 47\%}$

$\boldsymbol{\pi_1 - \pi_2 = 28\%}$

$$\frac{\left[1.96\sqrt{2(0.75)(0.25)} - (-0.84)\sqrt{(0.75)(0.25)+2(0.47)(0.53)}\right]^2}{(0.75-0.47)^2} = 46$$

41/5000

15% losses = 53 patients per group

## Statistical Analysis Plan

Descriptive statistics will be used to characterize the population using measures of central tendency and dispersion for continuous variables and distributions of relative and absolute frequencies for categorical variables. Independent t-test and Chi-square test for the difference between the two study groups. Analysis of variance (ANOVA) to compare the two groups with respect to the OM in the five time periods.

# METHODOLOGY

## Randomization

The randomization will be taught by Dr. José de Jesús Figueroa Carbajal, assigned to the Oncology service, who is unaware of the objective of the study.

The randomization technique will be carried out through sealed envelopes, which will indicate the bottle that will correspond to the patient, identified as treatment “A” and “B”. Likewise, to ensure that the groups are balanced, blocks of 6 sealed envelopes containing 3 patients will be made for each group.

## Blinding

The patient and the investigator will be blinded; for this, the 50 mg zinc and placebo tablets will be similar in size, color, shape, weight and taste; the bottles will be classified into codes A and B, which in turn will have the same shape, size and color. All of the above will be done through the Pharmaceuticals GREMAR S.A. de C.V., which is a Mexican company that has a line of food supplements located in Mexico City.

An independent investigator (who will be blinded for the purpose of the study) will be requested to receive the medications (zinc / placebo) prepared by the Pharmaceuticals GREMAR S.A. of C.V., so that it provides to the principal investigator only the bottles coded as "A" and "B". At the end of the intervention, the independent investigator will be asked to reveal the coding of the medications to determine which group (Group 1 placebo or Group 2 zinc) corresponds to treatment A and B respectively.

## Instruments

## WHO OM scale

The oral mucositis scale proposed by the World Health Organization (WHO) in 1979 will be used. This instrument was designed based on objective signs (erythema or ulcer) and subjective signs (mucosal sensitivity, ability to swallow).

The classification includes 5 degrees as follows: grade 0, no changes in the oral cavity are detected; grade I, pain and erythema in mucosa, gums, tongue or palate; grade II, erythema and ulcers, still with a solid diet tolerance; grade III, oral ulcers, tolerance to pasty foods and liquid diets; and grade IV, ulcers, erythema, pain, inability to swallow fluids, impossible oral feeding, and narcotic requirement to relieve pain. The total score of this scale ranges from 0 to 4.

Visual scale of facial expressions

This scale was developed by Bieri in 1990 for use in children from 3 years. It is made up of faces with different expressions that represent a person who is happy because they do not feel pain, and it changes to a sad expression as the pain increases. Each face is assigned a score, to answer it, the patient is asked to select the face that best describes how he feels.

## Standardization

It will be carried out through a series of clinical cases with clinical photographs of different degrees of OM, evaluated using the WHO's OM scale and contrasting the answers with an expert researcher in OM (Dr. Rosaura Gutiérrez Vargas).

The master's student and a social service student will be standardized, the evaluation will be in pairs and independently.

The weighted Kappa coefficient (kp) for the degrees of OM will be calculated, until a minimum of 0.61 to 0.80 is obtained.

## Stages of the study and data collection

The recruitment of patients will be carried out in the Oncology service of the National Institute of Pediatrics and the Children's Hospital of Mexico. Every day you will wonder if a new patient arrived with suspected leukemia or relapse. We will be aware of the result of the bone marrow aspirate confirming the diagnosis of ALL or relapse.

The master's student will present indicating that the reason for their vision is to inform them about the importance of oral health with respect to their current medical treatment and will give them information about the oral care that they should perform for the prevention of oral complications secondary to their disease and / or the drugs they will receive during their treatment. Visual material will be displayed by means of a computer / tablet device that includes information on oral care indicating brushing technique and making mouthwashes with bicarbonate solution. Likewise, it will explain what consists of

oropharyngeal mucositis, how it relates to chemotherapy and the preventive measures they can perform. Finally, the complications that may occur during the OM will be explained, such as: difficulty in eating, speaking or swallowing, as well as the measures that must be taken to allow adequate recovery.

Parents will be invited to participate in the protocol explaining the objectives of the study, as well as its risks and benefits. In case of accepting to participate, informed consent and assent (in children over 12 years old) will be presented in accordance with the characteristics that are written in it.

The intervention will be assigned under the balanced block scheme. Children will be randomly assigned to each of the two treatment groups using sealed envelopes.

Oral examination, staining of dentobacterial plaque will be performed (the characteristics of the plaque revealing solution are described in the researcher's manual) for the determination of the oral hygiene index and explanation of brushing technique on day 0 of chemotherapy.

Patients who require dental caries treatment will be rehabilitated by the stomatology service according to their stomatological diagnosis and their general con A closed bottle containing 100 tablets (treatment A or B) will be provided according to the random assignment. It will be indicated to administer 1 or 2 tablets daily (according to the age of the patient) from day 1 of chemotherapy.

A written reminder will be provided to parents in which they must check each day if the tablet was taken.

Calls will be made every 3 days to verify that oral hygiene and tablet administration are being carried out; In addition, the reminder will be reviewed twice a week and the remaining tablets will be counted in the bottles.

During the course of chemotherapy (induction and consolidation phase) reviews will be performed on days 1, 4, 7, 14 and 21 of each cycle.

## Maneuver

Group 1 (oral care + placebo): gentle tooth brushing with a soft bristle brush 3 times a day and mouthwash with baking soda after each meal (1/4 tablespoon of baking soda dissolved in water to make a mouthwash for 3 minutes ) plus the consumption of 1 or 2 oral placebo tablets (mannitol) from day 1 of chemotherapy.

Group 2 (oral care + zinc): gentle tooth brushing with soft-bristle brush 3 times a day and mouthwash with baking soda after each meal (1/4 of tablespoon of baking soda dissolved in water to make a mouthwash for 3 minutes ) plus the consumption of 1 or 2 oral zinc tablets (50-100 mg / day of zinc sulfate), from day 1 of chemotherapy. (See Flowchart 1).

## Zinc dose determination

The studies described in Table 2, have used zinc doses of 50 to 150 mg divided into 2 to 4 daily doses in adult patients, which was determined from the study conducted by Ertekin et al. Because our study would be the first to evaluate the effectiveness of zinc for the prevention of OM in children, there is no reference dose in previous studies.

Due to the above, an exhaustive review of the literature on zinc requirements, therapeutic doses and toxic doses was made (Table 1), which are summarized below:

- ✓ Diarios Daily zinc requirements in children: 5 - 10 mg / day in children under 4 years, 10 mg / day in children 5 to 10 years and 15 mg / day from 11 years.
- ✓ Dose used to treat diarrhea: 10 to 40 mg / day in children older than 6 months.
- ✓ Treatment for Wilson's disease or zinc deficiency: 50 mg / day in children under 6 years, 75 mg / day from 6 to 16 years and in adults up to 150 mg / day.
- ✓ Toxic dose: 1 to 2 g / day

Considering the above information, as well as the reported doses of the use of zinc for the treatment of OM, it was decided to establish for this study the doses of zinc by age as follows:

- Patients under 10 will receive 50 mg / day (1 tablet daily).
- Patients aged 11 to 18 will receive 100 mg / day (2 tablets daily).

The characteristics of the tablets are described in Annex VIII. Zinc / Placebo features.

## OM treatment

In the case that the patients attend with some degree of OM, the intervention will continue as follows:

For the follow-up of the OM, observations will be made from the beginning of the table until the remission (2 to 3 weeks).

The treatment of the OM will be the same, regardless of the group to which they are assigned, and will consist of:

Mouthwashes with Gelcliar® ® administered 30 minutes prior to food intake until box remission. In addition, oral care measures and zinc / placebo administration will continue.

## Pilot test

The pilot test will be carried out once the protocol is approved by the ethics and research committee of the National Institute of Pediatrics.

Six randomized patients with a recent diagnosis of childhood cancer who start chemotherapy will be selected, data from the clinical record will be obtained to determine if it meets the inclusion criteria. The patient identified as eligible will be visited at his place of hospitalization or in the outpatient office of the Oncology Service.

The intervention will be assigned under the balanced block scheme. Children will be randomly assigned to each of the two treatment groups using sealed envelopes.

The sequence of procedures and the complete description of the instruments can be found in the Annex "Pilot test applicator manual".

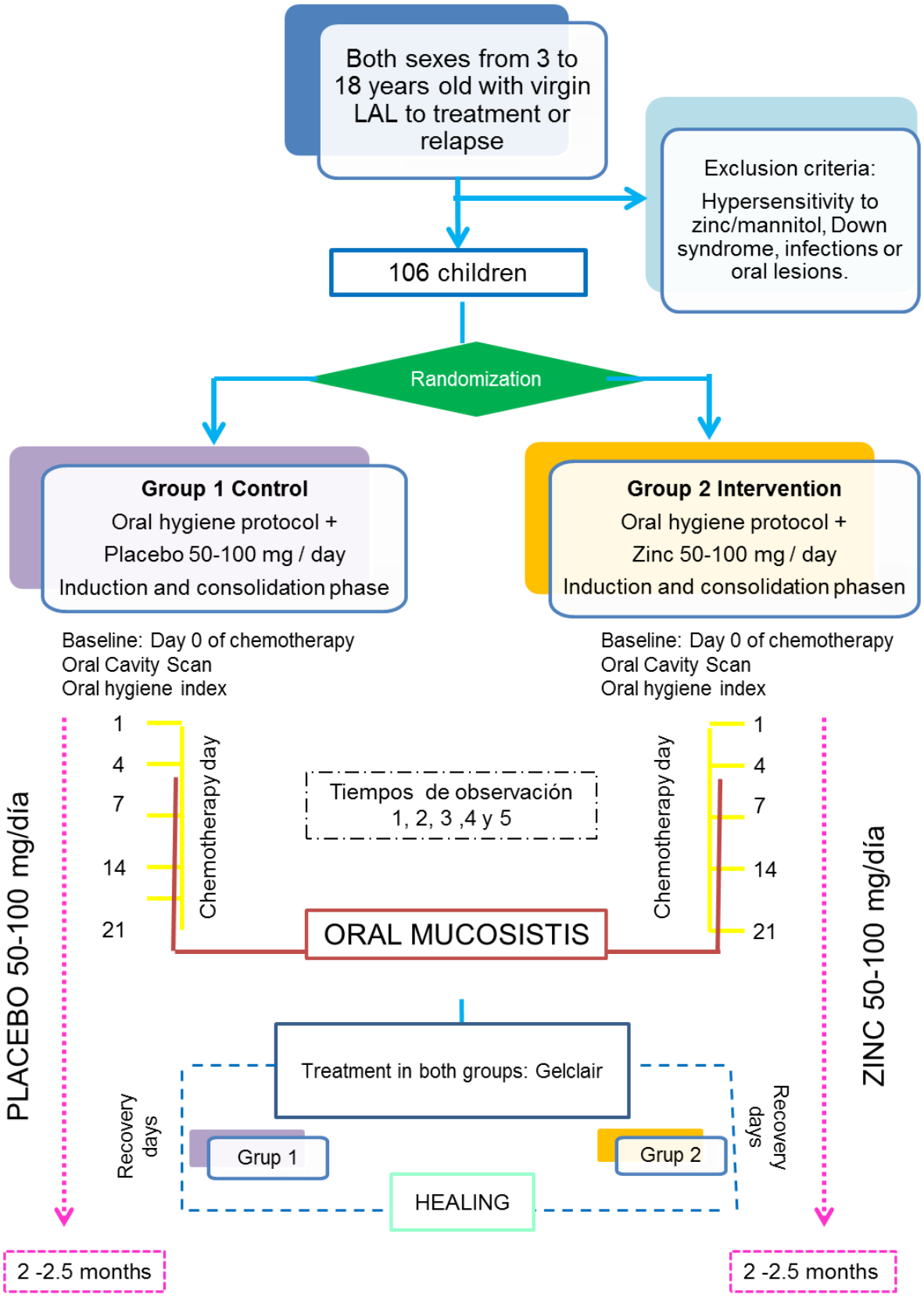
## Information collection method

A data record sheet will be filled out that includes the patient's identification form (name, age, sex), type of ALL, chemotherapy phase and blood count; which will be obtained from the clinical file. It will also include the table for the determination of the WHO OM scale, the oral hygiene index and the opportunistic infection registry.

## Registration and processing method.

The data will be recorded in an Epidata database and analyzed using the STATA 14 statistical program.

**FLOWCHART 1**

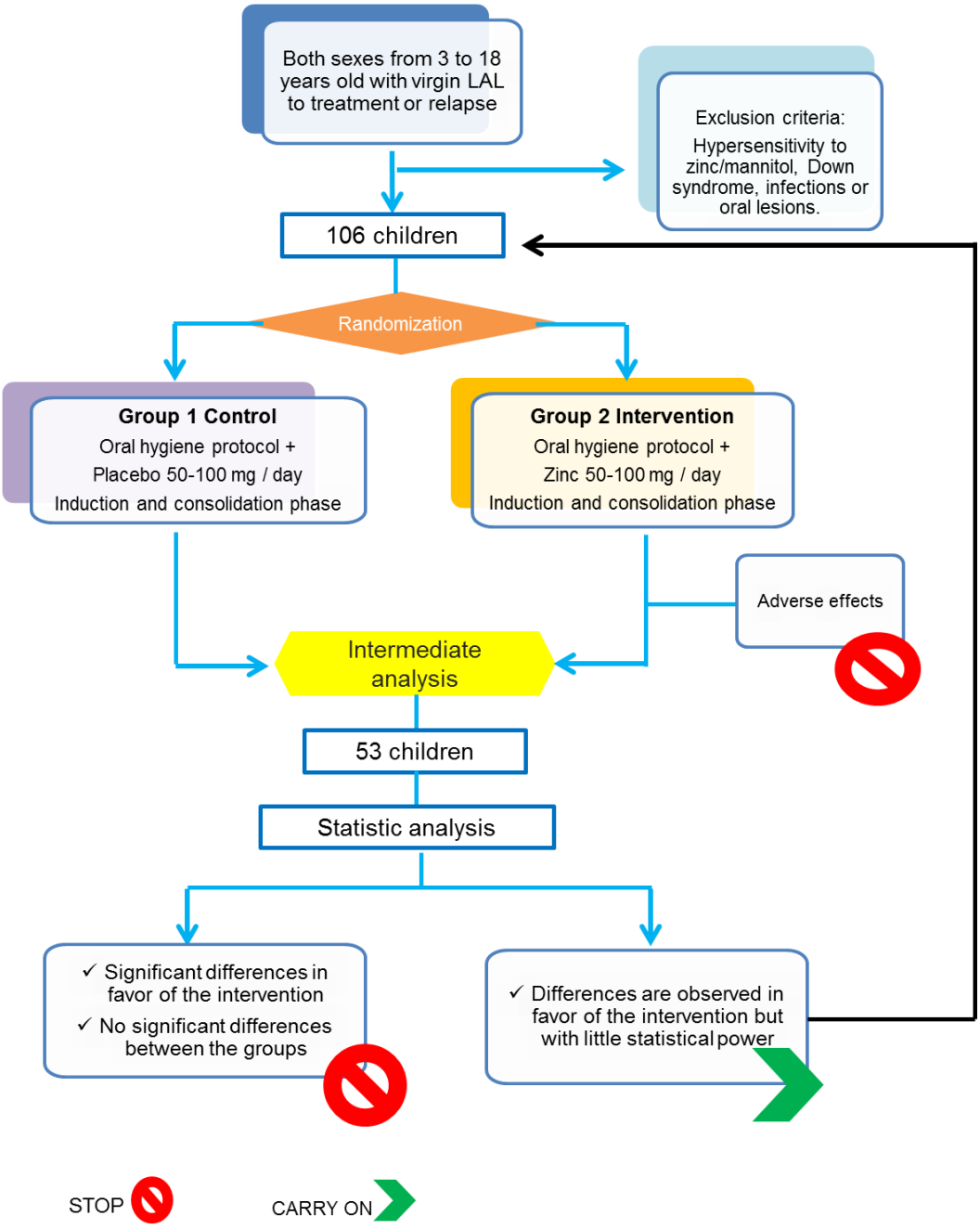





Date October 30th 2019

In the previous study by Rambod et al. (12), a difference in the incidence of OM of 28% was obtained and from this, for the present study the estimated sample size was 106 subjects.

Complying with one of the principles that govern clinical trials, which is to reduce the risk of the operated subjects to the minimum possible, in case of observing adverse reactions the suspension of the study will be indicated; while if there are no complications, recruitment will continue until a sample size of 53 subjects is met and at this point the intermediate analysis will be performed as shown in Flowchart 2.



## ETHICAL CONSIDERATIONS

In accordance with the principles and guidelines established by good clinical practices (BPC), in accordance with the principles set forth in the Helsinki Declaration of 1964 and with the support provided in the General Health Law, in the Regulations of the General Health Law in the area of provision of Health Care Services, which establishes that the individual welfare of the subjects must sometimes prevail in a study, over the interests of science and the community.

This study will be carried out with the strict observation of the recognized scientific principles and respect, handling the affected data anonymously and confidentially.

### Consent

To parents or to those who exercise parental rights of patients, we provide the information related to the study, both verbally and in writing. The master's student made an initial invitation to the parents or guardians of the minor for their child to participate in the study and read the "Informed Consent Letter" to state the purpose of the investigation and make a description of the same In the case of patients older than 12 years, also read the "Informed Assent Letter". The signature of the parents and the minor is requested freely and voluntarily with the "Declaration of Consent / Informed Consent" after the purpose of the investigation has been understood and doubts have been clarified.

### Confidentiality

To verify the confidentiality and privacy of the participants, the data will be stored in databases to which only the researchers of the study have access.

### Risk

The study is classified with greater than minimum risk. The administration of zinc in doses of 50-100 mg / day is considered safe, since no adverse effects have been described at that dose.

### Benefits

Patients participating in the study will have greater clinical control for the prevention of oropharyngeal mucositis, since they will be collected before the start of chemotherapy and notifications will be received to receive care from the Stomatology service in the case of treatment.

Although no difference is found in favor of the intervention and the necessary sea, the suspension of the study, measures will be taken to ensure that participants of both groups receive benefits by receiving educational oral care talks, identification of signs of OM, as well as Oral hygiene attachments and medications for the treatment of OM.

## BIOSECURITY CONSIDERATIONS

In accordance with current regulations, the following is held:

• NOM-052-SEMARNAT-2005

It establishes the characteristics of hazardous waste, also mentions that expired and leftover drugs are considered hazardous waste and therefore, must finally be disposed of responsibly.[41]

• General law for the prevention and integral management of waste.

Article 31. The following hazardous wastes and products used, expired, withdrawn from trade or disposed of and which are classified as such in the corresponding official Mexican standard shall be subject to a management plan:

VIII. In accordance with the above, the remaining or expired doses of the drug (zinc sulfate) should be handled as hazardous waste and its disposal will be as follows:

Leftover zinc tablets will be kept in closed plastic containers and considering that the amount will not exceed 20 bottles (100 tablets per bottle) may be deposited in the SINGREM safe containers (National System for the Management of Packaging and Drug Residues AC.), which are responsible for the final disposition of expired medicines and their leftovers from homes and are located in pharmacies registered in different parts of the city.

## LIMITATIONS

Because the population considered for the present study comes from two national reference centers, it is frequent that they find the cases with major complications (greater risk in terms of the presentation characteristics of LAL or late diagnosis) so they would not necessarily represent to all children of LAL in Mexico.

## FINANCING

**Materials:** Registration formats, intraoral mirror for review, computer equipment, printer.

**Economic**: Laboratory tests (blood count, KOH, PCR, bacterial cultures) will be performed at the National Institute of Pediatrics and the Children's Hospital of Mexico.

The zinc sulfate drug and placebo as well as oral hygiene products and medications for the treatment of mucositis will be obtained through the Field Work Funds of the Master's and Doctorate Program in Medical, Dental and Health Sciences, will not be sponsored by pharmaceutical companies.

**Humans:** Master's student, master's tutor and social service assistant of Oncology.

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**NATIONAL INSTITUTE OF PEDIATRICS**

**LETTER OF INFORMED ASSENT FOR PEDIATRIC PATIENTS**

**Informed Assent Document For Children Over 12 Years**

Name of Principal Investigator: Marta Zapata Tarrés.

The following writing is an invitation for you to participate in the project:

**Impact of the use of Zinc in the prevention of oropharyngeal mucositis in patients with acute lymphoblastic leukemia of the National Institute of Pediatrics.**

Name of the child who takes the Assent._____

Dr. Marta Zapata Tarrés, Pediatric Oncologist of Pediatric Oncology Service and Dr. Bianca Anahí Cristino Sicairos, Pediatric Stomatologist and Master's student, we invite you to participate in this research protocol, we will clarify your doubts, if you do not understand some words or concepts about the procedures, risks and benefits of the investigation, you can ask us and you can take the time you want to reflect if you want to participate or not.

It is necessary that you decide if you want to participate, for this we ask you to read this format and ask any doctor of the study if you have doubts.

**Project description:** Oropharyngeal mucositis is a complication of chemotherapy that occurs in many of the patients with leukemia like you, we can see it as a redness or ulceration (lesion) inside the mouth either in the inner part of the lips, cheeks, tongue, gum, palate and throat. This causes pain, discomfort when chewing and can even prevent eating by mouth.

Zinc is a nutrient that is obtained from food which has helped cancer patients who receive chemotherapy do not have oropharyngeal mucositis, which is less bothersome or heals faster.

This protocol is a study in which the children who participate are divided into two groups, one that will receive zinc tablets and another that will receive placebo tablets (tablet with no real action). Each child when accepting to participate in the study will belong to one of the random groups (as well as when throwing a coin to obtain eagle or sun, in this case treatment A or B); Neither doctors nor parents or children will know what treatment they will be receiving until the end of the study. Therefore, you can receive zinc or placebo and this will only be given by luck, no one will decide which group you will belong to.

The main **purpose** of the investigation is:

- Teach you oral care as a patient with acute lymphoblastic leukemia (ALL) that will start chemotherapy.
- Study whether zinc prevents the appearance of oropharyngeal mucositis in children with ALL.
- Identify from the beginning (early) the picture of oropharyngeal mucositis and treat it.

With this study, useful research data will be obtained and problems that affect your overall health can be detected, such as the difficulty of eating due to having oropharyngeal mucositis.



Date October 30th 2019

You are invited to participate as you meet the criteria such as: Be a patient with a recent diagnosis of ALL between 3 to 18 years of age who will start chemotherapy who accept by consent and/or informed consent participate in the study.

Among the criteria that make participation impossible are: Patients who have another disease in addition to ALL, children with Down syndrome, allergy to any of the medications (zinc and/or lactose). You do not have any of these criteria, so you are a candidate for this protocol.

**Do you have any risk for participating in the study?**

Research has a higher risk than the minimum, this means that although zinc at the dose we will give you (50-100 mg / day) is considered safe; You can also have nausea, vomiting, diarrhea, as well as an allergic reaction (redness, swelling or dry skin; fever, swelling of the tongue, lips and even difficulty breathing). In the event that any of the above symptoms may be caused by the consumption of zinc / placebo, it should be discontinued and immediately tell your parents and them to some of the researchers indicated in this document with name and contact telephone number.

We believe that the expected benefit will be much greater for your health because the objective is to prevent complications of chemotherapy such as mucositis and knowing the results of the study will improve the comprehensive care of other children with ALL.

**What are your benefits?**

Children participating in the study will have greater clinical control (reviews) for the prevention of oropharyngeal mucositis since they will be seen before the start of chemotherapy and in case of requiring attention from the dentists of the hospital they will receive faster attention. We will report directly to the Stomatology service.

Medications (zinc / placebo, bicarbonate, melox, benadryl) and what is necessary for your oral hygiene (brush, paste) will be provided at the beginning of the study.

**Procedures:** The research involves the continuous review of your mouth and the administration of 50 mg zinc / placebo tablets twice a day from day 1 of chemotherapy.

First visit: the data obtained through the clinical file (name, age, sex, diagnosis and name of the parents) will be confirmed. You and your parents (who are present) will be given a talk about the importance of oral hygiene and care for the prevention of oropharyngeal mucositis through an information leaflet and a digital presentation through a computer.

They will be provided with a sealed envelope in which the medicine code A or B will be indicated (remember that we will not know if they will be zinc or placebo), as well as written instructions on how to take the tablets.

The oral examination will be done by the Stomatologist (Dr. Bianca) she will see your mouth inside, from the inside of the cheeks, lips, tongue, palate, gum to the throat. You will also see the teeth and record if they have cavities, in case of detecting that any one needs treatment, your revision will be requested from the stomatology service Finally, to see how your oral hygiene is, the dental biofilm (bacteria that adhere to the teeth) will be painted by placing 2 or 3 drops of pink dye and then you will be given a brush and paste to be able to remove it and teach you how to brush your teeth.

Oral care to prevent oropharyngeal mucositis involves tooth brushing and the use of sodium bicarbonate rinses (1/4 of a tablespoon of bicarbonate dissolved in water to rinse for 3 minutes) after each meal, from day 1 of chemotherapy. The procedures will not cause you harm or pain.

Later visits: Visits will be made on day 1, 4, 7 and 21 of each chemotherapy cycle, to identify data from oropharyngeal mucositis. During the visits you will be asked if you have had any discomfort or difficulty eating, the oral examination will be done as indicated above, the dental biofilm dye (bacteria attached to the teeth) will be applied and the tooth brushing technique will be reinforced.

**What is your participation?**

Your participation (parents or guardians) and yours is very important and will consist of:

1. Go to the hospital for chemotherapy with brush, toothpaste, baking soda and tablets (zinc / placebo) which will be provided at the beginning of the study.
2. Perform gentle tooth brushing 3 times a day and bicarbonate rinses after each meal.
3. Promptly administer 50mg zinc / placebo tablets 2 times a day.
4. Report any concerns such as pain, any complications from the consumption of tablets (zinc / placebo) or inability to perform oral care.

In case of oropharyngeal mucositis: a series of care will be indicated, consisting of:

1. Gentle tooth brushing.
2. Continue consumption of 50 mg zinc / placebo tablets 2 times a day.
3. Mouthwashes with Melox® / Benadryl® solution (3.7g aluminum hydroxide, 4g magnesium hydroxide / diphenhydramine hydrochloride 250 mg) prepared at the moment, mixing 5 ml of Melox plus 5ml of Benadryl, cold, administered 15 minutes prior to food intake for 14 days.

In this case you will be supervised for the next 3 weeks until the picture of the oropharyngeal mucositis is completely cured.

**Who will pay the expenses of the study?**

You will not have to pay anything, all we require is your valuable participation.

The expenses of the study are given by the Master's and Doctorate Program in Dental Sciences of the National Autonomous University of Mexico.

**How will my data be used?**

The personal data will only be known by the researchers or the health personnel who are in charge of you, such as the resident doctor and the nursing staff, the data will be kept confidential (secret) and the publication that is generated will not include your name or that of your parents and family.

Participation in this research is voluntary. You can refuse to participate from the beginning or retire at any time you want, while maintaining the rights you currently have as a patient of the Institute.

**Who can you call if you have questions?**

The procedures will be performed by: Dr. Bianca Anahí Cristino Sicairos (Pediatric stomatologist and master's student), cell 5551061233 and Dr. Marta Zapata Tarres, Pediatric Oncologist, attached to the Pediatric Oncology service, telephone 10840900 ext. 1339, cell: 5554184099, which will answer your questions will clarify your doubts about the procedures, in the same way there is the participation of the President of the ethics committee of this Institute for any clarification, Dr. Alberto Olaya Vargas Chairman of the Committee of Ethics Tel: 10840900 ext. 1581. This proposal has been reviewed and approved by the Research and Ethics Committees of the INP. If you wish to find out more about this committee, contact Dr. Silvestre García De la Puente. Telephone 10840900 ext 1581.

**DECLARATION OF INFORMED ASSENT FOR PEDIATRIC PATIENTS**

By signing below you agree that:

1. You have read this assent format.
2. You have had the opportunity to ask questions and these have been answered.
3. Your participation in the study is voluntary.
4. You will be part of the study with the indicated procedures.
5. You know you can refuse to participate and withdraw whenever you want.
6. If you do not follow your doctor's instructions, you may be withdrawn from the study, without affecting the medical care you receive.

Name of the participating child_____
Mother's Name: _____
Date: _____
Firm: _____

Father's Name: _____ Date: _____
Firm: _____

I have witnessed the exact reading of the consent document for the potential participant and the individual has had the opportunity to ask questions. I confirm that the individual has freely given consent.

**Name of witness 1:** _____
Signature of witness: _____
Address: _____
Relationship with the participant: _____
Date: _____

**Name of witness 2:**_____
Witness Signature: _____
Address: _____
Relationship with the participant: _____
Date: _____

Name of the researcher or who collects the informed consent: _____ Signature: _____
Date: _____

**I received a copy of this consent**

Name and signature: _____
Date: _____

## LETTER OF INFORMED CONSENT FOR PEDIATRIC PATIENTS

Name of Principal Investigator: Marta Zapata Tarrés.

We hereby allow you to invite them to participate in the protocol:

**Impact of the use of Zinc in the prevention of oropharyngeal mucositis in patients with acute lymphoblastic leukemia of the National Institute of Pediatrics.**

Dr. Marta Zapata Tarrés, a pediatric oncologist, attached to the Pediatric Oncology service and Dr. Bianca Anahí Cristino Sicairos (pediatric stomatologist and master's student), invite your child to participate in this research protocol, we will clarify your doubts in case you don't understand some words about the investigation procedures, being free to take the time you want to reflect if you want to participate or not.

**Project description:** Oropharyngeal mucositis is a complication of chemotherapy that occurs with high frequency in patients with leukemia, its identification is through direct observation as a redness or ulceration of the internal mucous membranes of the cheeks, tongue, gum , palate and oropharyngeal (all tissues of the mouth to the throat) which causes pain, difficulty feeding until the inability to feed by mouth (mouth) when it is very severe.

Zinc is a nutrient that is obtained from food which has been seen that if it is provided to patients with various types of cancer who receive chemotherapy it helps to prevent oropharyngeal mucositis or if it is less severe and have a faster recovery.

This protocol is a double-blind randomized clinical trial, which means that study participants will be divided into two groups, one that will receive zinc tablets (Intervention Group) and another (Control Group) that will receive placebo (medication tablets inactive which in this case are lactose monohydrate). Each participant when accepting to be included in the study will be randomly assigned to one of the groups having a 50% chance of being in one or another group (such as throwing a coin to get eagle or sun); Both the study researchers and the participants will not know until the end of the study to which group each patient belongs. Therefore, your child may be included without distinction in the group receiving zinc or in the group receiving placebo.

The main **purpose** of the investigation is:

- Instruct on oral care in patients with acute lymphoblastic leukemia (ALL) who will start chemotherapy.
- Prevent the development of oropharyngeal mucositis with the administration of zinc supplement.
- Early detection of oropharyngeal mucositis and its treatment.

This will allow us to obtain useful data and thus intercept problems that affect the integral health of your child.

Your child is invited to participate as it meets the inclusion criteria: Be a patient with a recent diagnosis of leukemia (ALL) from 3 to 18 years of age who will start chemotherapy and accept by consent and / or informed consent participate in the study.

Among the criteria that preclude their participation are: Patients who attend with another disease besides LAL, children with Down syndrome, known allergy to zinc sulfate and / or lactose. And your child does not have any of these criteria, so he is a candidate for this protocol.



Date October 30th 2019

**Is there any risk?**

The investigation is classified with greater than minimum risk. The administration of zinc (50-100 mg/day) is considered safe; However, your child may have nausea, vomiting, diarrhea, as well as an allergic reaction (redness, swelling or dry skin; fever, swelling of the tongue, lips and even difficulty breathing). In case you consider that any of the above symptoms may be caused by zinc/placebo consumption, stop immediately and inform some of the researchers listed in this document with name and contact telephone number.

We believe that for the health of your child, the expected benefit will be much greater because the objective is to prevent complications of chemotherapy such as mucositis and knowing the results of the study will improve the comprehensive care of children with ALL.

**Benefits for your child:**

Patients participating in the study will have greater clinical control (reviews) for the prevention of oropharyngeal mucositis since they will be seen before the start of chemotherapy and will be given priority to receive care from the Stomatology service in the event that they require treatment.

Medications (zinc / placebo, bicarbonate, melox, benadryl) and what is necessary for oral hygiene (brush, paste) will be provided to your child at the start of the study.

**Procedures:**

The research consists of the continuous revision of the oral cavity and the administration of 50 mg zinc / placebo tablets 1 or twice a day from day 1 of chemotherapy.

First visit: the data obtained through the clinical file (name, age, sex, diagnosis and name of the parents) will be confirmed. They will be given a talk about the importance of oral hygiene and care for the prevention of oropharyngeal mucositis through an information leaflet and a digital presentation through a computing device.

You will be provided with a sealed envelope in which the medication code A or B (zinc / placebo) will be indicated, as well as written instructions on how to provide it to your child.

The oral examination will consist of the observation of the internal part of the cheeks, lips, tongue, palate, gum and oropharynx (throat). The teeth will also be observed and recorded if they have cavities, in case of detecting that any require treatment, an interconsultation will be sent to the stomatology service to request their attention. Finally, to evaluate oral hygiene, staining of the dental biofilm (bacteria that adhere to the teeth) will be performed by placing 2 or 3 drops of pink dye and then brush and paste will be provided to remove it and show the brushing technique.

Oral care to prevent oropharyngeal mucositis involves tooth brushing and the use of sodium bicarbonate rinses (1/4 of a tablespoon of bicarbonate dissolved in water to rinse for 3 minutes) after each meal, from day 1 of chemotherapy. The procedures will not cause damage to the oral mucosa, or symptoms of pain after examination.

Later visits: Visits will be made on day 1, 4, 7 and 21 of each chemotherapy cycle, to identify data from oropharyngeal mucositis. During the visits you will be asked if your child has had any discomfort or difficulty feeding, the oral revision will be done as indicated above, the dental biofilm dye (bacteria attached to the teeth) will be applied and the technique will be reinforced of tooth brushing.

**What is your participation?**

Your participation (parents or guardians) and the child is important and consists of:

1. Go to hospital for chemotherapy with brush, toothpaste, baking soda and zinc / placebo tablets which will be provided at the beginning of the study to perform oral care for your child.
2. Perform gentle tooth brushing 3 times a day and bicarbonate rinses after each meal.
3. Promptly administer 50mg zinc / placebo tablets once a day for children under 10 years and 2 times a day for children from 11 years of age, from the start of chemotherapy to the next 2 and a half months after Your suspension is indicated.
4. Report any concerns such as pain, any complications from the consumption of zinc / placebo tablets or the inability to perform oral care.

In case your child has oropharyngeal mucositis: a series of care will be indicated, consisting of:

1. Gentle tooth brushing.
2. Continue with the consumption of 50 mg zinc / placebo tablets 1 or 2 times a day.
3. Mouthwashes with Melox® / Benadryl® solution (3.7g aluminum hydroxide, 4g magnesium hydroxide / diphenhydramine hydrochloride 250 mg) prepared at the time, mixing 5 ml of Melox plus 5ml of Benadryl, cold, administered 15 minutes prior to food intake for 14 days.

Your child will be supervised for the next 3 weeks until the complete remission of the oropharyngeal mucositis picture.

**Who will pay the expenses of the study?**

You will not have to pay anything, all we require is your valuable participation.

The expenses of the study are foreseen by the Master's and Doctorate Program in Dental Sciences of the National Autonomous University of Mexico.

**Informed consent**

This document is provided to the parents or guardians of minor patients who do not have legal capacity; however, patients from 7 years of age may refuse to participate in the study and in children 12 years of age they will also be provided with an “Informed Assent Letter” in which the objective is explained in a simple way of the study and their permission to participate is requested and in which case they do not accept they will be discharged from the protocol (to respect their decision) even if you as parents have agreed to participate.

**Confidentiality**

The personal data will only be known by the researchers or the health personnel who are in charge of the patient, such as the resident doctor and the nursing staff in charge to provide the best care of your child, the data will be kept confidential and The publication generated will not include the name of the participant or their parents and family members.

Participation in this research is voluntary. You can refuse to participate from the beginning or withdraw at any time, while maintaining the rights your child currently has as a patient of the Institute.

**Who can you call if you have questions?**

The procedures will be performed by: Dr. Bianca Anahí Cristino Sicairos (Pediatric stomatologist and master's student), cell 5551061233 and Dr. Marta Zapata Tarres, Pediatric Oncologist, attached to the Pediatric Oncology service, telephone 10840900 ext. 1339, cell: 5554184099, which will answer your questions will clarify your doubts about the procedures, likewise there is the participation of the President of the ethics committee of this Institute for any clarification, Dr. Alberto Olaya Vargas Chairman of the Committee of Ethics Tel: 10840900 ext. 1581

This proposal has been reviewed and approved by the Research and Ethics Committees of the INP. If you wish to find out more about this committee, contact Dr. Silvestre García De la Puente. Telephone 10840900 ext 1581.

**DECLARATION OF INFORMED CONSENT FOR PEDIATRIC PATIENTS**

I have read the information provided or it has been read to me. I have had the opportunity to ask about it and the questions I have asked have been satisfactorily answered. I voluntarily agree to participate in this research, I understand that I have the right to withdraw from the research at any time without affecting my medical care in any way.

**Rights**

1. You may freely not agree to participate in the protocol.

2. You have the right to receive an answer to any question, clarification or doubt regarding the zinc review and administration procedure.

3. The review, as well as the necessary elements for oral care are completely free.

4. In the event that you voluntarily accept, you can withdraw your consent when you wish or cannot continue with the follow-up.

5. If you decide to withdraw your consent, at any time, it will be understood that you no longer wish to participate in this study and the attention on the services provided in the INP will NOT be affected.

In order to accept to participate in the project, you must answer the following questions.

| | Yes | No |
|---|---|---|
| I have read this consent form. | | |
| Have you understood the informative letter? | | |
| Have you had the opportunity to ask and discuss what your participation and your child's participation is? | | |
| Do you agree to comply with the instructions and report any lack thereof in a way you will see? | | |
| Do you agree that the information obtained be used for research purposes and be disclosed confidentially? | | |
| I accept that my child participate in the study. | | |
| I can choose to have my child participate in the study or to abandon it at any time, communicating it to the researchers. | | |

If you answer NO to any of the questions, this implies that you reject the invitation to participate in the protocol.

**Consent**

**Yes** ☐

**No** ☐

If you decide to participate, sign the letter. You consciously agree to participate in the company of your child in the protocol.

Child's Name: _____
Mother's Name: _____ Date: _____
Signing of consent: _____

Father's Name: _____ Date: _____
Signing of consent: _____
Address:_____
Phone: _____

I have witnessed the exact reading of the consent document for the potential participant and the individual has had the opportunity to ask questions. I confirm that the individual has freely given consent.

Name of witness 1: _____
Signature of witness: _____
Address: _____
Relationship with the participant: _____
Date: _____

Witness Name 2: _____
Witness Signature: _____
Address: _____
Relationship with the participant: _____
Date: _____

Name of the researcher or who collects the informed consent: _____
Signature: _____
Date:_____

**I received a copy of this consent**

Name and signature: _____

Date: _____