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DEALING WITH INFORMATIVE CENSORING IN SURVIVAL ANALYSIS

Jessica Giselle Blanco López

Tutor: Prof.ssa Maria Grazia Valsecchi

Co-tutor: Dott.ssa Emanuela Rossi

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List of abbreviations

AHOPCA	Asociación de Hemato-Oncología Pediátrica de Centro América
ALL	Acute Lymphoblastic Leukemia
BFM	Berlin Frankfurt Münster
BM	Bone Marrow
CD	Cluster Differentiation
CI	Cumulative incidence
CNS	Central Nervous System
CR	Complete Remission
CSF	Cerebral Spinal Fluid
EFS	Event free survival
GHS-2000	Guatemala-Honduras-El Salvador 2000 (Protocol)
HIC	High Income Countries
IOP	International Outreach Program
IPCW	Inverse Probability Censoring Weighting
LIC/LMIC	Low / Middle Income Countries
MISPHO	Monza International School of Pediatric Hematology and Oncology
NCI	National Cancer Institute
NCI-SR	National Cancer Institute -Standard Risk
NCI-HR	National Cancer Institute -High Risk
OS	Overall survival
PB	Peripheral Blood
PODC	Pediatric Oncology in Developing County
POND	Pediatric Oncology Networked Database
SE	Standard Error
SIOP	Société Internationale d'Oncologie Pédiatrique
SOPHO-LIC	Statistical Office Pediatric Hemato-Oncology for Low Income Countries
St Jude	St Jude Children's Research Hospital
USD	United States Dollar
WBC	White Blood Cells count
WHO	World Human Organization

1. INTRODUCTION

Childhood cancer is a worldwide public health concern being a leading cause of death in children. While high-income countries have improved the probability of surviving after cancer diagnosed in pediatric age (it now reaches 80%), children and adolescents with cancer living in low and middle-income countries (LIC/LMIC) have a dismal outcome. This is due to many reasons, including the lack of resources, scarce living conditions and the high priority for public health on communicable diseases. The higher proportion of children in the populations of these countries magnifies the problem. Thanks to the enthusiasm of the pediatric oncology community, many initiatives have been implemented with the aim to improve survival after cancer in children in LIC/LMIC.

One important initiative is the AHOPCA (Asociación de Hemato-Oncología Pediátrica de Centro América) network, which is a group of hospital units specialized in childhood cancer treatment from Central America and the Caribe. These countries faces common difficulties such as widespread poverty (25 to 60% of their populations live below the poverty line), malnutrition, illiteracy, poor infrastructure, difficult access to health services, inconsistent drug availability, lack of supportive care and low priority of cancer treatment if compared to the priority of other health issues (mostly infectious diseases).[1–6]

This network developed from an initial partnership (1986) between Manuel de Jesus "La Mascota" Hospital (Managua, Nicaragua), and three institutions in Europe, the Pediatric Clinic of the University of Milano-Bicocca (Monza, Italy). Followed by the San Giovanni Hospital in Bellinzona (Switzerland) and the Istituto Nazionale di Tumori in Milan (Italy) that lead to the establishment in the late 80ies of the Monza International School of Pediatric Hemato-Oncology (MISPHO). In the same period (1994) St. Jude Children's Research Hospital (Memphis, USA) established a twinning program with the Hospital Benjamin Bloom (San Salvador, El Salvador), in the framework of the International Outreach Program. Almost a decade later, after the participation in MISPHO, and thanks to their geographical and linguistic proximity, the countries of Central America joined formally into the AHOPCA collaborative group.[1,7]

Nowadays, the AHOPCA network promotes the development of shared clinical protocols (mainly focused on cancer chemotherapy), educational programs for physicians and nurses, a more integrated role for psychologist and social workers in the approach to patients and collaborative research. A data management infrastructure has also been developed, the Pediatric Oncology

Networked Database (best known as POND), located at the St Jude and complemented by the statistical support from a small team at the University of Milano-Bicocca named SOPHO-LIC (Statistical Office Pediatric Hemato-Oncology in Low-Income Countries). This facilitates the application of various treatment protocols, the collection of data on diagnosis, treatment and outcome and the evaluation of the results achieved for children affected by cancer in these countries. [2,4,8–14]

In this context, survival analysis is the methodology typically used to describe the outcome in cancer clinical trials and is also used as an indicator of their efficacy in disease management and care. Survival analysis deals with the study of the time elapsed between some initial event defined as a starting point (such as date of diagnosis or start of treatment) and the time of occurrence of some event (failure) of interest (such as disease relapse or death).

A typical complexity of observed survival data is the presence of right censoring on the survival time, which occurs generally when the survival time is shorter than the failure time. Censoring is due to a limitation on the observability of the failure/survival time itself (for this reason it is called administrative censoring) and has to be accounted for in the analysis. Statistical methods in survival analysis were developed mostly to address for the presence of censoring and for the non-symmetric shape of the distribution of survival time. In the classical survival analysis theory, the censoring distribution is reasonably assumed to be independent of the survival time distribution, i.e. censoring is non-informative on the "true" survival time. This assumption implies that the velocity of occurrence of failure can be estimated by considering the survival experience of the non-censored times.

In more complex situations, like treatment abandonment, censoring cannot be directly assumed as independent from the survival experience (informative censoring). In this case, the issue is to account for the information carried from the censoring time on the true survival time. The censoring time could "hide" a survival time which would be observed right after the censoring time if, for example, the patient decided deliberately to leave the treatment/study given his/her very bad conditions and with a dismal prognosis. On the other hand, the censoring time could "hide" a very long survival time if, for example, the patient decided deliberately to leave the treatment/study when his/her conditions were very good and the disease apparently cured.

Treatment abandonment is a relevant problem in LIC/LMIC and, according to the experience of these countries, some of the children who abandon treatment are seen later alive and in complete remission, others return to the clinic with relapse or progressive disease or die, most of them do not return, are not traced again and their status is unknown. Given these considerations, it is clear that abandonment is not the standard administrative censoring and is not independent of the survival experience.

Considering abandonment of therapy as an event (failure) likely leads to underestimate the protocol effect but considering it as administrative censoring can lead to overestimate the effect. In SIOP (Société Internationale d'Oncologie Pédiatrique) a PODC working groups (Pediatric Oncology in Developing Country) recommended to carefully document every abandonment of treatment and, with SOPHO-LIC, suggested to perform the estimation of EFS (event-free survival) in two ways: by treating abandonment as a failure and by censoring.[15]

Several studies on the causes of abandonment of therapy in LIC demonstrated that is highly related to patient's socioeconomic condition, time travel for patients to the specialized clinic, parent's illiteracy, low monthly household income (less than 100 USD) and increased number of household family members.[16–19]

Other studies conducted in LIC/LMIC revealed some other possible causes such as nutritional status, hospital policies (financial burden of treatment) and other cultural aspects that are difficult to document (such as beliefs, feelings, fears). Arora et al. summarized many probable causes and possible solutions such as twinning programs, an increase of financial support, development of adapted protocols to be delivered in specialized clinics, which actually are the measures adopted from AHOPCA network.[20–24]

Thanks to these efforts the prevalence of abandonment has been reduced significantly in El Salvador and Guatemala, where the rate in 2002 was less than 2%, while it remains higher than 10% in other countries (Honduras, Nicaragua).[1,4,25] However, the problem is still present and, given its nature, difficult to be completely solved.

This project aims at estimating the survival outcome of childhood cancer in LIC/LMIC countries where treatment abandonment is a relevant issue with approaches that can deal with the

informative nature of the related censored information. The project will develop the following two points:

1. Handling informative censoring on survival time due to the abandonment of treatment, using the non-standard statistical method of Marginal Structural Model.

2. Comparing the classic with the non-standard statistical methods in evaluating the effects of treatment protocols in children with of acute lymphoblastic leukemia treated in LIC/LMIC.

2. BACKGROUND

2.1. CHILDHOOD CANCER IN LOW AND MIDDLE-INCOME COUNTRIES

Childhood cancer is a worldwide public health concern. The incidence and mortality rates differ from country to country, also depending on the how accuracy of reported data. World Human Organization (WHO) estimates for childhood cancer (<15 years old) a worldwide incidence rate of 8.8 per 100,000 per year and a mortality rate of 4.3 per 100,000 per year. [26]

Möricke et al reported results of consecutive trials; children from HIC (Germany, Austria, and Switzerland) were diagnosed with acute lymphoblastic leukemia, and treated according to BFM-protocols; the 10-year event-free survival (EFS) was improved from 65% (SE = 0.02) for the ALL-BFM 81 study to a 10-year EFS of 78% (SE = 0.01) for the ALL-BFM 95 study.[27] While Navarrete et al reported the results of the AHOPCA-ALL 2008 the estimated 3-year EFS was 59.4% (SE = 1.7)[3], and Magrath et al reported a 4-year EFS of 45% (1986) that improved to 61% in (1997) for one treatment center in India.[28] This gap in survival is due in part to treatment abandonment; as well as the shortage of chemotherapy agents, poor living conditions, late diagnosis and difficult access to a prompt treatment, evidenced by higher rates of mortality and relapse

2.2. BRIDGE THE GAP (MISPHO, IOP, AND AHOPCA)

One of the most encouraging successes in childhood cancer treatment is the improved survival in developed countries among children with acute lymphoblastic leukemia (ALL) that sees nowadays more than 80% survival at 5 years from diagnosis while the corresponding figure was less than 50% in the 60ies. In order to bring these benefits to children that live in LIC/LMIC cooperative efforts have been carried on and main strategies comprehend:

-***Twinning programs***, professionals from institutions in HIC have collaborated with their colleagues in LIC/LMIC. This has promoted the progress of pediatric oncology care, though educational programs (for health workers such as physicians, nurses, and others), development of tailored treatment protocols, implementation of cancer registration and promotion of clinical research.

-***Local sustainability***, through the development of oncology units with the support of the local governments and non-government foundations that facilitate the treatment of pediatric cancer.[4,5,25]

These actions were implemented also in the AHOPCA network which was created in the late 90ies after the experience with MISPHO and with the Outreach program of the St. Jude Children's Research Hospital.[1,5,7]

The countries that constitute the AHOPCA network are Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Dominican Republic and Haiti (that joined recently). The network promotes the development of shared clinical protocols (mainly focused on cancer chemotherapy), educational programs for physicians and nurses, and a more integrated role for psychologist and social workers in clinical units and collaborative research.

A data management infrastructure has also been developed, after a MISPHO pilot program, in collaboration with St Jude. POND, which became the main database. This online network database has incorporated a software that allows real-time monitoring of patients outcomes, shared protocols, and generation of chemotherapy roadmaps. It allows web-based data reporting on diagnosis, treatment, and outcome and may be extended to include data on supportive care, the health-related quality life of children affected by cancer in these countries. [2,4,8–14]

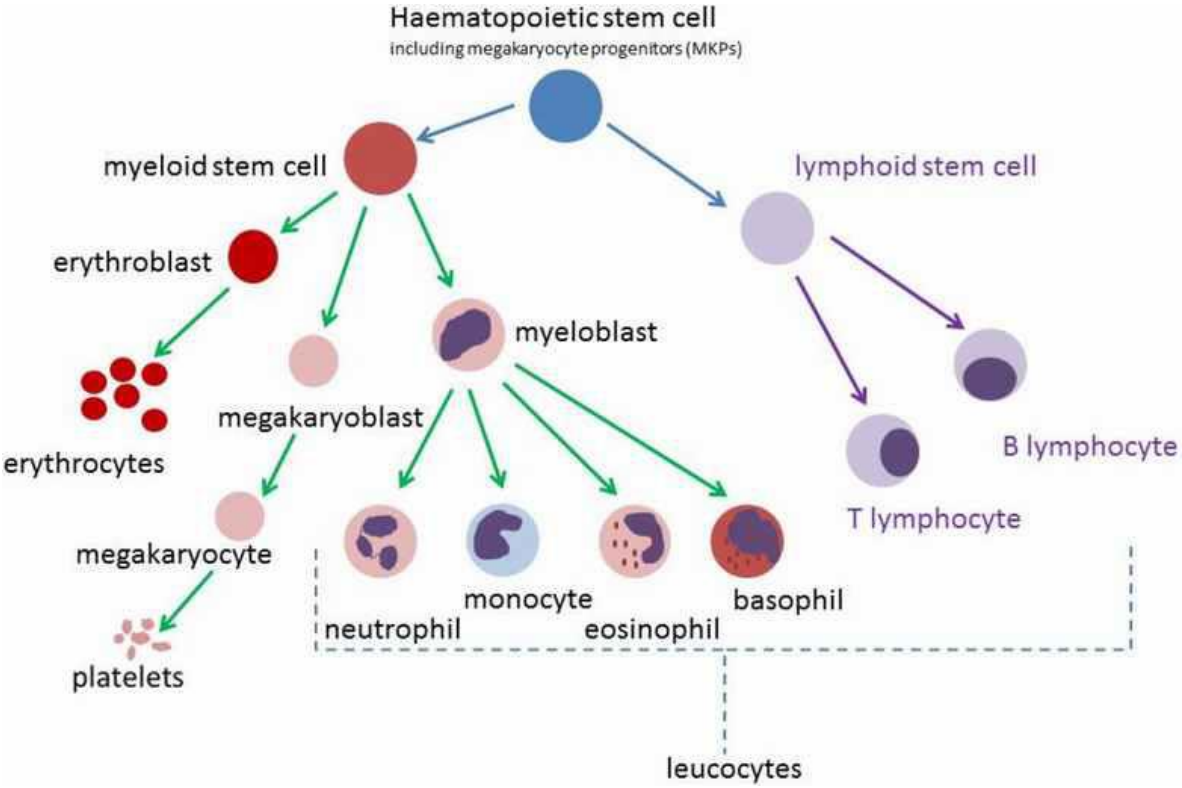
Along these years, there has been teamwork carried out between oncologists, their partners of the twinning institutions (especially Monza and St. Jude), the data managers of the AHOPCA network, the POND developers and the statisticians in SOPHO-LIC. This teamwork has allowed: to develop tailored treatment protocols for various cancers; to collect the data and to report data and discuss results on treatment efficacy to the regular annual meetings of AHOPCA.[4,13]

2.3. CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

The WHO estimates for childhood acute leukemia (<15 years old) a worldwide incidence rate of 2.7 per 100,000 per year and a mortality rate of 1.5 per 100,000 per year. Moreover, for the countries of the AHOPCA network, it estimates an incidence rate of 2.9 per 100,000 per year and a mortality rate of 1.9 per 100,000 per year. [26] Leukemia, overall these countries, constitutes approximately 30% of incident childhood cancers.

Acute lymphoblastic leukemia (ALL) is a malignant neoplasm of the lymphocyte B or T precursor cells (lymphoblasts). The excessive growth of lymphoblasts leads to a decrease of normal hematopoiesis (Figure 1) with a subsequent deficiency of erythrocytes, platelets and normal leukocytes (especially neutrophils).[29]

Figure 1. Normal hematopoiesis.



In the last decades, through the development of molecular biology techniques such as microarray and sequencing analysis of ALL cohorts, the complexity, and heterogeneity of the disease has been revealed. There are many ALL subtypes, characterized by structural and sequence alterations that alter key cellular pathways (cell cycle regulations), cytokine receptors and chromatin modifications (chromosomal translocations, aneuploidy deletions,

and amplifications). [30,31] Although etiology remains unclear, some possible etiologic risk factors are genetic, infectious diseases, radiation, and/or chemical exposures.[29]

2.3.1. DIAGNOSIS

Initial clinical presentation depends on the infiltration of ALL cells into tissues. Virtually any organ system may be involved, but peripheral blood, lymph nodes, spleen, liver, central nervous system (CNS) and skin are the most common sites clinically detected. Patients often present a short history of fatigue, lethargy, weight loss, bone pain, fever and/or spontaneous bleeding. When CNS is involved, clinical presentation can include nausea, vomiting, headache, neuropathies and papilledema. Testicular involvement is usually a painless unilateral mass noted at diagnosis. The physical exam often reveals pallor, lymphadenopathy, splenomegaly, hepatomegaly and signs associated with thrombocytopenia (gingival bleeding, epistaxis, petechiae/ecchymosis).[29]

The initial diagnosis of ALL includes peripheral blood cell count (WBC) with differential hemogram, cytomorphological examination of bone marrow (BM) and cerebrospinal fluid (CSF). With the presence of lymphoblasts in peripheral blood (PB) and/or their presence (≥ 25%) in the bone marrow. ALL can be diagnosed with an appropriate stained of PB or BM, preferably, with May-Grünwald-Giemsa. The French-American-British (FAB) scoring system considers (1) the nuclear/cytoplasmic ratio; (2) the presence, prominence and frequency of nucleoli; (3) the nuclear shape and (4) the cell size to classify ALL in two basic subtypes L1 and L2, these subtypes are more descriptive than specific, but still used when immunophenotyping is not available.[32,33]

Nowadays classification of ALL is based on immunophenotyping by flow cytometry and genotype. Phenotypic evaluations comprehend cytochemical studies such as myeloperoxidase (MPO) or Sudan black B reaction and specific esterase reactions to exclude most cases of acute myeloid leukemia. Additional detection of surface and cytoplasmic markers by flow cytometry identifies the leukemic cell population through monoclonal antibodies identified as Clusters of Differentiation (CD). The commonly used markers for immunophenotyping in acute leukemia are: (1) *General*, CD34, Human leukocyte Antigen-D Related (HLA-DR), terminal deoxynucleotidyl transferase (TdT), CD45; (2) *B-cell* markers, CD10, CD19, cCD22, CD20, cCD79A, CD24, cμ and sIg and (3) *T-cell* markers, CD1a, CD2, cCD3, CD4, CD5, CD7, and CD8. [29,34]

Once the ALL diagnosis is established, other complementary genetic studies such as karyotyping and detection of chromosomal rearrangements are helpful to define features that have prognostic value. Karyotyping detects gross chromosomal alterations in B-cell precursor ALL (B-ALL), hyperdiploidy (>50 chromosomes, occurs in 25-30% of childhood B-ALL) is associated with favorable outcome and hypodiploidy (<44 chromosomes, occurs in 2-3% of childhood B-ALL) is associated with poor outcome. The chromosomal rearrangements create chimeric fusion genes that commonly involve hematopoietic transcription factors, epigenetic modifiers, cytokine receptors and tyrosine kinases. Common B-ALL rearrangements are the t(12;21)(p13;q22) encoding *ETV6-RUNX1* (*TEL-AML*), t(1;19)(q23;13) encoding *TCF3-PBX1* (*E2A-PBX1*), t(9;22)(q34;q11.2) encoding *BCR-ABL1* ("Philadelphia" chromosome) and t(4;11)(q21;q23) encoding *MLL-AF4* fusion; another key genetic alterations are *PAX5*, *IKZF1*, *JAK1/2* and *CRLF2*. T-cell precursor ALL (T-ALL) is characterized by mutations of *NOTCH1* and rearrangements of transcription factors *TLX1* (*HOX11*), *TLX3* (*HOX11L2*), *LYL1*, *TAL1*, and *MLL*.[31]

Initial assessment of CSF at diagnosis is essential for diagnosis and staging, it comprehends CSF-chemistry (protein and glucose), the cell count of nucleated cells and erythrocytes (in a counting chamber) and the cell morphology assessment on a high-quality cytospin preparation. For patients with neurological symptoms, a careful evaluation accomplished with cranial imaging by axial tomography (CT) o magnetic resonance (MRI) is mandatory.[29]

2.3.2. RISK-STRATIFICATION CRITERIA FOR TREATMENT ASSIGNMENT

The intensity of treatment is tailored to the prognostic profile of the patients as defined by clinical and biological features. Patients who are likely to have good prognosis receive a less intensive therapy, patients with high-risk features will receive more aggressive and potentially more toxic treatment due to their lower probability of long-term survival.

The National Cancer Institute (NCI) risk stratification criteria is commonly use, to classify B-cell ALL into Standard risk (WBC count <50,000/μl **and** age 1 to younger than 10 years) and High risk (WBC count ≥50,000/μl **and/or** age 10 years or older).[35] However, stratification criteria should take into account all available characteristics to assign treatment risk. For children with ALL the factors that have demonstrated prognostic value are summarized below:

Patient and clinical disease characteristics:

- *Age at diagnosis*, patients aged at least 1 but younger than 10 years have usually reported a better long-term survival than older children (≥10 years old), adolescents and, than infants (<1-year-old).

- *White blood cell count at diagnosis*, 50,000/μl is used as a cut point between better and poorer prognosis, although the relationship between WBC count and the prognosis is more complex and survival tend to be poorer at increasing WBC count.

- *CNS involvement*, patients who have a non-traumatic diagnostic lumbar puncture may be placed into CNS 1 (CSF with cytospin negative for blasts regardless of WBC count), CNS 2 (CSF with <5 WBC/μL and cytospin positive for blasts) or CNS 3 (CSF with ≥5 WBC/μL and cytospin positive for blasts). Patients with CNS 3 and patients with a traumatic puncture (≥10 erythrocytes/μL) that include blasts have a higher risk of CNS relapse and overall poorer outcome.[36]

- *Testicular involvement*, in early ALL trials, was considered an adverse prognostic factor. With more intensive induction therapy, it does not appear to have prognostic significance.[37]

- *Down syndrome (trisomy 21)*, some studies report lower survival probability for these patients, due in part to an increased treatment-related mortality and higher rates of induction failure.[38]

- *Gender*, some studies report better prognosis for girls than for boys with ALL, one reason is the occurrence of testicular relapses.[39]

- *Race and ethnicity*, survival rates in black and Hispanic children with ALL are lower than in white and Asian children with ALL. Associated factors are ALL subtypes (black children have a higher rate of T-cell), ancestry related genomic variations (e.g. polymorphisms of the *ARID5B* more frequent in Hispanics) and lower adherence to treatment (mostly in Hispanic children).[40,41]

Leukemic characteristics:

- *Immunophenotype*, precursor B-lymphoblastic leukemia (distinguished from mature B-cell ALL –Burkitt) can be subdivided into Common precursor B (CD10 positive and no surface of cytoplasmic Ig), Pro-B (CD10 negative) and Pre-B (presence of cytoplasmic Ig). Patients with common precursor B-cell ALL usually are associated with favorable cytogenetic. Instead, the absence of CD10 is associated with *MLL* gene rearrangements and the presence of cytoplasmic Ig is associated with *TXF3-PBX1* fusion, both with a poorer prognosis. T-cell ALL with appropriately intensive therapy has an outcome similar that of B-cell ALL. Some translocations have been identified in T-cell ALL, high expression of *TLX1/HOX11* is associated with more favorable outcome and *TLX3/HOX11L2* appears to be associated with increased risk of treatment failure. Myeloid antigen expression can be found and it could be associated with *MLL* rearrangements and *ETV6-RUNX1* gene rearrangement, but no independent adverse prognostic significance has been found.[42]

- *Cytogenetic and genomic alterations*, chromosomal abnormalities have been shown to have prognostic significance, especially in precursor B-cell ALL. High hyperdiploidy (51-65 chromosomes) and *ETV6-RUNX1* fusion are associated with favorable outcomes. Others have been associated with poorer prognosis, including Philadelphia chromosome (t(9;22)(q34;q11.2)), *MLL* rearrangements, hypodiploidy and intrachromosomal amplification of the *AML1* gene (iAMP21). Also, a number of polymorphisms of genes, such as *NUDT15*, involved in the metabolism of chemotherapeutic agents have been reported as prognostic factors in childhood ALL.[42]

Response to initial treatment:

- *Cytomorphological evaluation in BM and PB*, evaluate the clearance of the tumor burden in the initial phase of treatment has been shown to be an important prognostic factor. Specifically, the absolute blast count in PB on day 8 (Prednisone response)[27] and the percentage of blasts in the BM identifies the good or poor response to treatment, and are currently used in ALL protocols to define prognosis.[43]

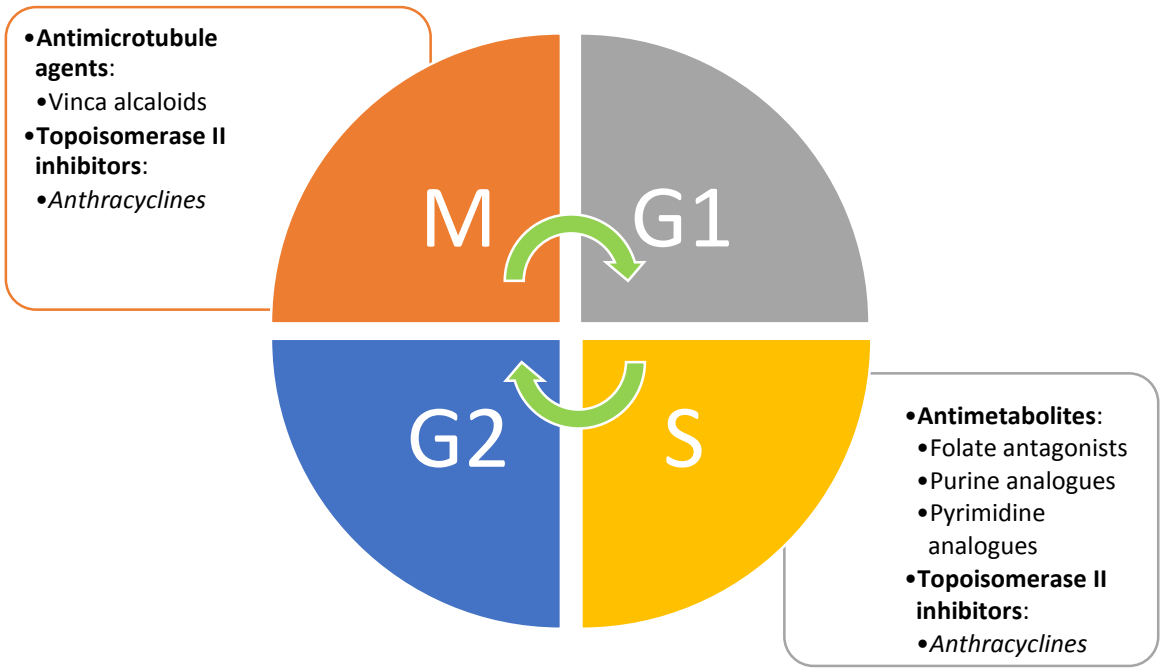
- *Minimal residual disease determination* (MRD), is the accurate and sensitive detection of low frequencies of ALL cells (≤1 ALL cell in 10000 normal cells) in

blood and BM by flow cytometry or polymerase chain reaction (PCR)- based molecular techniques.[44]

2.3.3. TREATMENT

In ALL, frontline therapy relies mainly on combinations of corticosteroids, amino acid or substrate depletion (Asparaginase, methotrexate), alkylating agents and antimetabolites, in addition to metaphase blockers and anthracyclines. In some cases their mechanism of action are cell cycle independent (e.g. corticosteroids, Asparaginase, alkylating agents), some affects multiple phases of the cell cycle (e.g. antitumor antibiotics) and some affects specific phases (e.g. methotrexate, mercaptopurine, cytarabine –S Phase, vinca alkaloids –M Phase) (Figure 2).

Figure 2. Cell cycle and chemotherapy agents' action.



Frontline ALL treatment usually divides into phases:

- Cytoreductive pre-phase
- Induction of remission phase
- Consolidation phase
- Re-intensification
- Maintenance

- Along with CNS directed treatment (intrathecal therapy) and radiotherapy when needed

The cytoreductive pre-phase (typically BFM-oriented protocols) consists of one week of corticosteroids (prednisone or dexamethasone) and one dose intrathecal methotrexate. Patients usually present with hyperleukocytosis (WBC count ≥50,000/μl) and/or tumor lysis syndrome which implies a comprehensive initial treatment (such as this pre-phase). The evaluation of the clearance of peripheral blasts at the end of this phase has been identified as an important prognostic factor.

Through decades with the introduction of diverse chemotherapeutic agents, induction to remission phase has been developed. With the combination of corticosteroids, vinca alkaloids, Asparaginase, intrathecal methotrexate, and anthracyclines most of the patients achieve remission (the disappearance of all signs of cancer). Next phases (consolidation, re-induction, and maintenance –the less intensive phase) vary according to risk stratification and between protocols and are designed and administered to maintain continuous complete remission and prevent relapse. ALL treatment protocols are designed to last 2 years (or up to 3 years in certain protocols/subgroups).

The clinical research allowed the development of other therapies, especially for patients with poor prognosis, such as allogeneic hematopoietic stem cell transplantation (HSTC), specific inhibitors for selective pathways and novel immunotherapeutic approaches that intend to be adaptive and improve the expected survival in the presence of specific biological features.

2.3.4. EXPECTED AND POTENTIAL OUTCOMES

The first goal of any regimen is to attain ***complete remission*** (CR) followed by a ***long-term survival.*** For ALL CR is defined as the disappearance of all the signs of the disease, in the bone marrow (<5% blast cells –M1– with sufficient cellularity and signs of regeneration of normal myelopoiesis), in the CSF and any other sites that were infiltrated at the diagnosis. With an appropriate adherence to current regimens approximately 98% of children with ALL achieve CR [45] .

The events that may occur as treatment failures are first of all the lack of CR due to death during induction or the resistance to treatment. In patients who experience CR, the events

that may occur are a relapse, death or the diagnosis of a second malignant neoplasm either during treatment or after the end of therapy during clinical follow-up. (See section 4 for the detailed definition).

2.3.5. TREATMENT ABANDONMENT

The occurrence of treatment abandonment, as often observed in LIC/LMIC, is of major concern because it prevents the correct administration of the full treatment regimen to the child with cancer and affects the effectiveness of the treatment and prevents observing the patient's next/final state. It is defined as the termination of the relationship between the patient and the treatment center during active therapy. The ***current more specific definition of abandonment of treatment*** for AHOPCA is missing four or more consecutive scheduled weekly visits during active treatment. [3,4,13,14]

Several studies on the causes of treatment abandonment in LIC demonstrated that is highly related to patient's socioeconomic condition. Metzger et al. assessed the outcome of ALL pediatric patients in Honduras; they found that the main cause of failure was treatment abandonment (23%). The Gray's proportional hazard model showed that it was associated with prolonged travel time to the treatment center at Tegucigalpa (> 2 hours) and to a patient's younger age (< 4.5 years). To address the problem of travel time, in 2002 a satellite clinic in the second largest city in the country (San Pedro Sula) was set up and results of this policy are now being investigated.[16]

Bonilla et al. assessed the prevalence and predictors of treatment abandon in cancer patients (diagnosed with leukemia, lymphoma and solid tumors) from El Salvador. They reported that the prevalence of treatment abandonment was of 13%, occurring at a median time of 2 months (from the beginning of therapy) and that it was associated with parent's illiteracy, low monthly household income (less than 100 USD) and increased number of household family members.[17]

Sweet-Cordero et al. assessed predictors of treatment abandon in children with cancer in Guatemala; they evaluated economic, family and community factors through a questionnaire. They found that less than 3 years of elementary school paternal education were strongly associated to treatment abandon. Meanwhile, access to mainstream and strong family support

were associated with adherence to treatment.[18] An instrument was developed to assess socio-economic status of the cancer patient's family in order to support them accordingly.[19]

Other studies conducted in diverse LIC/LMIC revealed some other possible causes such as nutritional status, hospital policies (financial burden of treatment) and other cultural aspects that are difficult to document.[20–24]

3. THE PURPOSE OF THIS THESIS

The project will develop the following two points:

1) Handling informative censoring on survival time due to the abandonment of treatment, using the non-standard statistical method of Marginal Structural Model, in order to obtain an unbiased estimate of the outcome.

2) Comparing the classic with the non-standard statistical methods in evaluating the outcome of treatment protocols of acute lymphoblastic leukemia in low-income countries.

4. MATERIALS AND METHODS

4.1. MOTIVATING EXAMPLE

The protocols to treat ALL shared in the AHOPCA network are based on the St Jude and AIEOP-BFM experiences and tailored according to local resources (usually chemotherapy intensity has to be reduced).

For the purpose of this investigation we focused on the results from Guatemala, El Salvador, and Honduras, because the ALL treatment protocols have been shared since February 2000:

- In the first shared experience, ALL patients were treated according to the protocol denominated GHS-2000 based on the protocol Total XI and Total XIIIB developed by St Jude Children's Research Hospital.

- The second experience consists of the protocols AHOPCA ALL-2008 (El Salvador and Honduras), and LLAG-0707 (Guatemala), both based on the ALL-IBC-BFM 2002 of the International Berlin-Frankfurt-Münster Study Group (BFM).

PATIENTS:

The protocols were designed to treat children and adolescents between 1-17 years old (some infants were included), newly diagnosed with ALL, and with the informed consent of parents or legal guardians. Patients previously treated were excluded. The diagnosis of ALL was based on a morphological assessment of May-Grünwald or modified Giemsa-stained smears of bone marrow and immunophenotyping was performed by flow cytometry with a basic panel. Clinical examination, WBC count, CNS assessment and complementary studies were also performed.

RESPONSE AND EVENTS DEFINITIONS:

Prednisone response: for BFM based protocol is performed on day 8 after receiving 7 days of prednisone and one intrathecal dose of methotrexate (MTX). The presence in the peripheral blood of less than 1,000 blasts/mm^3 defines a good response (PGR) and having at least 1,000 blasts/mm^3 constitutes a poor response (PPR).

Bone marrow assessment: both protocols contemplate the morphological evaluation of the bone marrow (BM) on day 15 and at the end of induction phase (day 36 or 33 according to

each protocol definitions). For BFM based protocols, patients who did not experience complete remission by day 33 were evaluated after phase IB of the study or after the subsequent high-risk (HR) blocks (for LLAG-0707 only). Bone marrow status was categorized as M1 (<5% blasts), M2 (5-24% blasts) or M3 (≥25% blasts).

Complete remission (CR): was defined as M1 BM status with normal peripheral counts and no extramedullary disease at the end of the induction/consolidative phase (according to each protocol definitions).

Resistant disease: was defined as not experiencing CR after completing the induction/consolidative phase of treatment (according to each protocol definitions).

Relapse: was defined as the reappearance of at least 25% blast in BM and/or extramedullary disease after experiencing CR.

Death: event reported as death in induction (if it occurs during induction to remission phase, before evaluating the remission status) and death in continuous CR due to treatment toxicity (if occurs after achieving CR and the patient is in continuous in CR). Death can also occur after another event such as resistant disease or relapse due to disease progression or related to treatment.

Treatment abandonment: defined as missing 4 or more consecutive weekly scheduled visits during therapy, reported as abandonment in induction (if occurs during induction to remission phase –the patient left before achieving CR) and abandonment in CR (if occurs after achieving CR).

Secondary Neoplasm Malignant (SNM): defined as the diagnosis of another malignant neoplasm confirmed by pathology. It may occur during active treatment or, more commonly, at long-term during follow-up.

RISK STRATIFICATION CRITERIA:

Considering the available information for all the patients for the following analysis, the baseline risk was calculated according to the NCI risk stratification criteria. According to this the Standard risk (or NCI-SR) comprehends patients between 1 to 10 years, B-linage and with an initial white blood cell count (WBC) <50,000/mm^3, while the High risk (or NCI-HR) comprehend the rest of the patients.

CNS classification: CNS 1 (cerebrospinal fluid [CSF] without blasts and nontraumatic); CNS 2 (CSF with no more than 5 cells/mm^3 with blasts or the spinal tap was traumatic [>10 red blood cells/mm^3] or performed more than 72 h after the beginning of therapy); CNS 3 (CSF > 5 cells/mm^3 with blasts or cerebral nerve palsy or a cerebral mass).

For GHS-2000 protocol, patients aged at least 1 year but younger than 10 years, a WBC count of less than 50,000/mm^3, B-lineage, hyperdiploidy (DNA index ≥ 1.6 and <1.6), no CNS 3 or testicular involvement, BM M1 on day 15 and 36 were considered Standard risk (SR), and the rest of patients were considered High risk (HR).

For BFM-based protocols, patients aged at least 1 year but younger than 6 years, a WBC count of less than 20,000/mm^3, B-lineage, no CNS 3 or testicular involvement, BM M1 or M2 on day 15, and no HR criteria were considered SR. High risk criteria were: patients aged less than 1 year, PPR, BM M3 on day 15, no CR at day 33, the presence of t(9;22) or t(4;11) positive, hypodiploidy (DNA index < 0·81) and, for AHOPCA ALL-2008, T-lineage. Patients aged at least 6 years or having WBC count of at least 20,000/mm^3 and no HR criteria were considered Intermediate risk (IR). For ALLG-0707 T-lineage and SR patients with BM M3 on day 15, with no HR criteria were also considered IR.

TREATMENT:

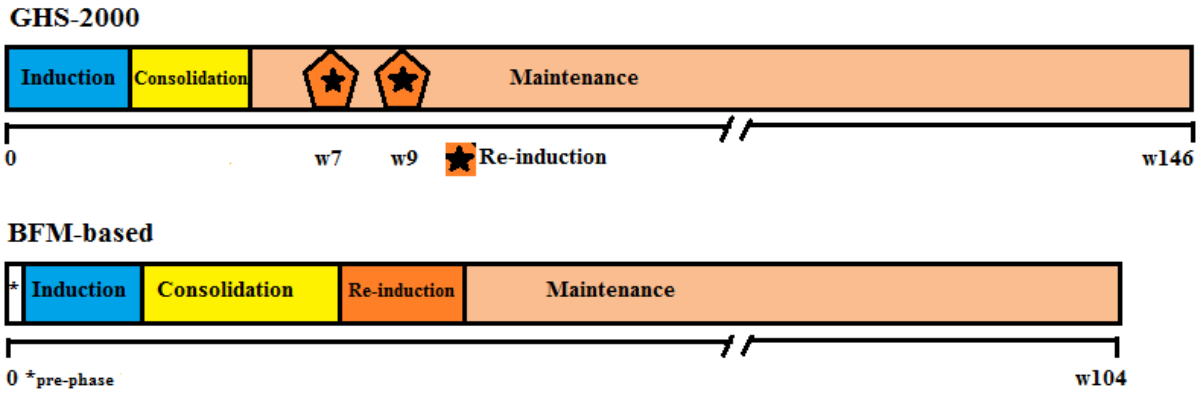
The general design of both protocols was similar, see Figure 3 and Table 1 for details.

Table 1. Treatment protocols GHS-2000 and BFM based protocols.

PHASES	Element/drug & method	GHS - 2000 Dose	GHS - 2000 days	BFM based Dose	BFM based days
Pre-phase	Prednisone PO			60 mg/m^2	1-7
Induction to remission				***Phase IA***	
	Prednisone PO	40 mg/m^2	1-28	40 mg/m^2	8-28
	Vincristine IV	1.5 mg/m^2	1,8,15,22	1.5 mg/m^2	8,15,22,29
	Daunorrubicin IV	25 mg/m^2	1,8,15[a]	30 mg/m^2	8[c],15[c],22[c],29[c]
	L-Asparaginase IM	10,000 UI/m^2	2,3,5,8,10,12, 15[a],17[a],19[a]	10,000 UI/m^2	12,15,18,21,24,27,30,33
				Phase IB	
	Cyclophosphamide IV	1,000 mg/m^2	22	1,000 mg/m^2	36,64
	Cytarabine IV	75 mg/m^2	23-26,30-36	75 mg/m^2	38-41,45-48,52-55,59-62
	6 mercaptopurine PO	60 mg/m^2	22-36	60 mg/m^2	36-64
	Met/Cyt/Hidro[b] IT	By age	1,8[a],15,22[a],29		
	Methotrexate IT			By age	1,8[d],15,22[d],33,45,64[d]
Consolidation	Methotrexate IV	2 [a] g/m^2 (3h)	1,8	2 [e] g/m^2 (24h)	1,15,29,43
	6 mercaptopurine PO	75 mg/m^2	1-14	50 mg/m^2	1-56
	Leucovorin IV	15 mg/m^2	2,16,30,44 (every 6 h x3)	15 mg/m^2	2,16,30,44 (every 6 h x3)
	Met/Cyt/Hidro[b] IT	By age	1,8		
	Methotrexate IT			By age	1,15,29,43
Re-induction			[f]	***Protocol III*** [g]	
	Dexamethasone PO	8 mg/m^2	1-21	6 mg/m^2	1-21
	Vincristine IV	1.5 mg/m^2	1,8,15	1.5 mg/m^2	1,8
	Daunorrubicin IV	25 mg/m^2	1	30 mg/m^2	1,8
	L-Asparaginase IM	10,000 UI/m^2	8,10,12,15	10,000 UI/m^2	1,5,8,12
	Methotrexate IV	2 g/m^2	22	-	-
	Cyclophosphamide IV	-	-	500 mg/m^2	15
	Cytarabine IV	-	-	75 mg/m^2	22-25,29-32
	6 mercaptopurine PO	75 mg/m^2	22-28	50 mg/m^2	22-35
	Met/Cyt/Hidro[b] IT	By age	22		
	Methotrexate IT			By age	22,29
Maintenance			[h]		
	6 mercaptopurine PO	75 mg/m^2	1-7	75 mg/m^2	Once daily
	Methotrexate IV/IM	40 mg/m^2	1	PO 20 mg/m^2	Once weekly
	Dexamethasone PO	8 mg/m^2	1-5	6 mg/m^2	1-7 every 8 weeks
	Vincristine IV	1.5 mg/m^2	1	1.5 mg/m^2	1 every 8 weeks
	Methotrexate IV/IM	2 [a] g/m^2 (3h)	1		
	Cyclophosphamide IV	500 [a] mg/m^2	1		
	Cytarabine IV	300 [a] mg/m^2	1		
	Met/Cyt/Hidro[b] IT	By age			
	Methotrexate IT				
Radiotherapy [i]	≥ 2 years old	18Gys		-	
	≥2-3 years old	-		12 Gys	
	≥3 years old	-		18 Gys	

[a] Doses for high risk patients only. Methotrexate 3 g/m^2 for HR patients
[b] Met: methotrexate; Cyt: cytarabine; Hidro: hydrocortisone
[c] According to protocol and treat arm (risk)
[d] Additional doses for CNS status 2 and 3.
[e] Methotrexate 5 g/m^2 for AHOPCA ALL-2008 HR patients and LLAG-0707 linage T IR patients. For LLAG-0707 HR patients, consolidation phase consisted in polychemotherapy HR-blocks.
[f] During maintenance at weeks 7-9, and for HR patients also at 17-19.
[g] Once for SR-patients, twice for IR-patients and three times for HR-patients. IR and HR-patients received interim phase between each protocol III (that consisted in methotrexate and 6-mercaptopurine PO)
[h] by specific cycles (according to risk) until 120 weeks for girls and 146 weeks for boys.
[i] If indicated according to protocol (CNS status 3, B-linage with WBC count ≥100,000/mm^3 at diagnosis, T-linage with WBC count ≥50,000/mm^3 at diagnosis, hypodiploidy and other HR characteristics).

Figure 3. Treatment basic scheme for GHS-2000 and BFM based protocols.



Main differences are that BFM based protocols have pre-phase (first seven days of therapy with prednisone and one dose of methotrexate intrathecal), response evaluation after pre-phase (count of blasts in peripheral blood), different risk stratification (an intermediate risk was introduced) and the shorter duration of therapy (104 weeks vs 146 weeks for GHS-2000).

DATA COLLECTION AND STATISTICAL ANALYSIS:

The data of the individual patients treated in the two protocols were registered in POND and were periodically reviewed for consistency and completeness by SOPHO-LIC in collaboration with the data managers of the clinical centers.

The statistical analysis considered the following end-points:

- Overall survival (OS) was defined as the time from the beginning of treatment to the date when death (for any cause) or abandonment occurred, or to the date of the last follow-up (censored data).

- Event-free survival (EFS) was defined as the time from the beginning of treatment until the date when the first event occurred among induction failure (defined as either death, abandonment, resistant disease), relapse, death (in CR), abandonment of treatment or occurrence of a SNM, or date of last follow-up if no event occurred (censored data). Of note, abandonment is here considered as treatment failure.

An alternative approach consists in not considering abandonment as a failure in all outcome indicators. This implies to assume that abandonment causes a censored observation which is equivalent to the common right censoring (where we can assume independence from survival).

The probabilities of OS and EFS were estimated using the Kaplan-Meier method with Greenwood standard error (SE).

Competing causes of failure were defined considering resistant disease, relapse, death and treatment abandonment (occurring as first event), as competing risks. Crude cumulative incidences of each cause of failure were estimated using the Aalen-Johansen estimator.

4.2. STANDARD METHODS FOR SURVIVAL ANALYSIS

4.2.1. BASIC NOTATION AND QUANTITIES

In general terms, survival analysis collects statistical procedures for which the ***Outcome*** variable of interest is ***Survival time***. This time variable gives the elapsed time between the starting point (e.g. beginning of the relevant observation due to diagnosis, or treatment start, or achievement of CR, etc.) until the occurrence of the event of interest. This ***Time*** could be measured in years, months, weeks or days. The ***Event*** (also known as ***failure*** if it is negative), could be death, relapse from remission, recovery or any designated experience of interest that may happen to an individual. Sometimes more than one event is considered defining a ***Composite Event***. When analyzing the single events defining the composite events, the correct approach is to adjust for ***Competing risks***.

Censoring is a key analytical problem present in survival data and it is handled by any statistical technique of the entire survival analysis theory. In practice, censoring occurs when we do not know the exact time to the event, but we only know that this time is greater than the censoring time. Censoring acts as a limitation on the observability of the event and thus of the true survival time.

The most common reasons of right censoring are:

- The study ends before a person experiences the event.
- A person is lost to follow-up during the study period.
- A person ***deliberately withdraws*** the treatment (***drop out or treatment abandon***).
- A person is obliged to ***withdraw*** the treatment (e.g. due to an adverse reaction).

Some basic notations:

- The random variable ***T*** denotes the non-negative survival time.
- The scalar ***t*** denotes a generic nonnegative time instant; if we are interested in evaluating a specific period of survival from some point *t* onwards the notation ***T>t*** is used.
- The Greek letter **δ** denotes the status indicator. It is set equal to 1 is the event was observed or 0 if the time was censored.
- The random variable **ε** denotes the event type indicator when in the presence of competing risks. For instance, if the survival time may occur due to two different causes of failure, **ε = 1,2** represents the types of failure.

- Z and **Z** denote a single or a vector of (fixed) covariates.

The survival function:

$$\boldsymbol{S(t)} = Pr\{T > t\}$$

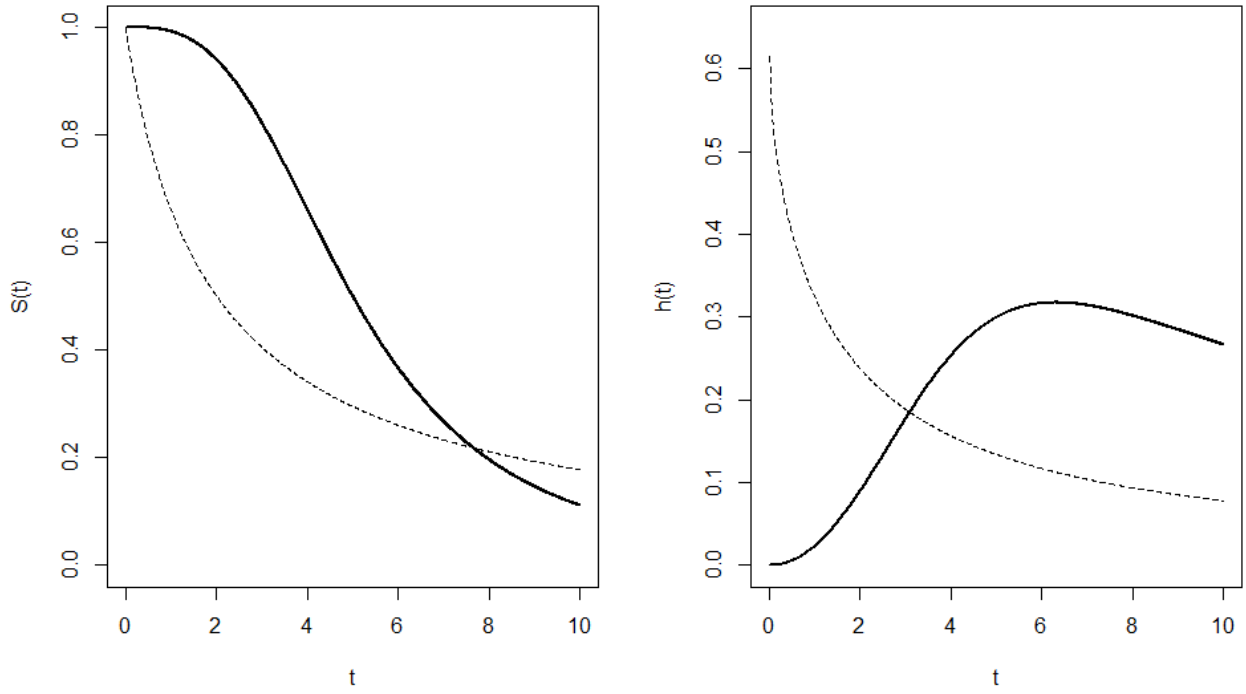
Gives the probability that a person *survives* in time *(not failing)* longer than any specified time **t**. It is a fundamental quantity showing in time the fraction of subjects free from failure. Theoretically ***S(0) = 1***, because at the beginning of the study all subjects are free from failure yet, and ***S(∞) = 0***, because eventually, nobody would survive.

Another meaningful quantity is the instantaneous probability of *failing* at ***t*** conditional on being failure free just before ***t*** represented by the hazard function:

$$\boldsymbol{h(t)} = \lim_{\Delta t \to 0} \frac{P(t < T < t + \Delta t | T > t)}{\Delta t}$$

That is the velocity for the event to occur per unit of time, given that the individual has survived up to time ***t*** (conditional instantaneous failure rate). Unlike the survival function, the hazard function is not a monotone decreasing function starting from 1, but can start at any (nonnegative) time, and increase or decrease over time. Two examples of ***S(t)*** and ***h(t)*** are represented in figure 4, where one hazard function is monotone decreasing function while the other is not monotone.[46,47]

Figure 4. Theoretical survival ***S(t)*** and hazard functions ***h(t)***.



While ***S(t)*** describes the pragmatic survival experience in time, ***h(t)*** provides insight about how the instantaneous rate of the event may change with age or with time elapsed from the origin. The function ***h(t)*** is the vehicle by which mathematical modeling of survival data is carried out. Survival models are, in fact, usually written in terms of the hazard function. Clearly, there is a one-to-one relationship between ***S(t)*** and ***h(t):***

$$\boldsymbol{S}(\boldsymbol{t}) = exp^{(-\mathrm{H}(t))}$$

Where $\boldsymbol{H}(t) = \int_0^t h(u)du$ is the cumulative hazard function.

Standard survival analysis considers composite end-points with events for which competing risks are present. In this case, several types of event may originate the failure time ***T*** and are thought as competing causes. In this context, the cumulative incidence of any event (i.e. ***1-S(t)***) is not the only quantity of interest, in fact, the incidence of each specific type of event and its contribution to the overall incidence is also important. The crude cumulative incidence function of a specific event is the probability in time of observing such event as first, given that also other events are acting. In the presence of two competing risks, we have two different (crude) incidences.

$$F_1(t) = P(T \leq t; \varepsilon = 1)$$
$$F_2(t) = P(T \leq t; \varepsilon = 2)$$

The sum of the incidences of each event gives the incidence of any event whichever occurs first. Similarly the sum of the velocity of development of the two competing risks (cause-specific hazards).

$$h_1(t) = \lim_{\Delta t \to 0} \frac{P(t < T < t + \Delta t; \varepsilon = 1 | T > t)}{\Delta t}$$
$$h_2(t) = \lim_{\Delta t \to 0} \frac{P(t < T < t + \Delta t; \varepsilon = 2 | T > t)}{\Delta t}$$

Non-parametric estimators can address the analysis of the survival time **T** by means of survival function or hazard based function and the impact of covariates can be evaluated through regression models. The commonly utilized non-parametric methods, such as the Kaplan-Meier estimator for the survival function, the Aalen-Nelson estimator for the cumulative hazard function require the assumption of independent censoring. The well-

known Cox semi-parametric model requires independent censoring conditional on covariates.[46,47]

4.2.2. THE KAPLAN-MEIER ESTIMATOR

Kaplan-Meier estimator, also known as the **product limit estimator**, is a non-parametric estimator. It can be used to estimate the survival function from survival data in the presence of censored data assuming independent censoring.

It is often used to measure the fraction of patients living for a certain amount of time after treatment. A plot of the Kaplan-Meier estimate of the survival function is a step function, which, when a large enough sample is taken, approaches the true survival function for that population. The value of the survival function changes at every time when at least one failure is observed and is assumed constant between successive distinct observed failure times.

An important advantage of the Kaplan-Meier curve is that the method can take into account some types of censored data, particularly *right censoring*, which occurs if the final outcome is not observed in a patient within the time window of the study. On the plot, small vertical tick marks can be added to indicate censoring where a patient's survival time has been right censored. When no truncation or censoring occurs, the Kaplan-Meier curve is the complement to one of the empirical distribution function.

Let $t_{(1)}, t_{(2)}, \ldots, t_{(j)}, \ldots t_{(J)}$, be the observed distinct ordered (event or censoring) times. For each t_j we compute the n_j number of subjects "at risk" prior time t_j, and the d_j number of deaths/failures at t_j. Censored individuals before time t_j are not anymore in the risk set n_j. The Kaplan-Meier estimator is the non-parametric maximum likelihood estimate of $S(t)$. It is the product of the following quantities:

$$\widehat{S}(t) = \prod_{j|tj<t} \frac{n_j - d_j}{n_j}$$

In large samples, $\widehat{S}(t)$ is approximately normally distributed with mean $S(t)$ and a variance which may be estimated by Greenwood's formula.[46–48]

$$Var\left(\hat{S}(t)\right) = \hat{S}(t)^2 \sum_{j|tj<t} \frac{d_j}{n_j(n_j - d_j)}$$

4.2.3. COX REGRESSION MODEL

The ***proportional hazards regression model***, most commonly known as the ***Cox model***, is a semi-parametric method used to analyze survival or failure time data. It models the hazard function ***h(t)*** as a function of time and covariates:

$$\boldsymbol{h_i(t)} = h(t; Z_i) = h_0(t)exp(\beta' Z_i)$$

Where $\boldsymbol{h_0(t)}$ is an arbitrary and unspecified baseline hazard function, $\boldsymbol{Z_i}$ is the vector of explanatory variables for the $\boldsymbol{i^{th}}$ individual, and **β** is the vector of unknown regression parameters that is associated with the explanatory variables. The vector **β** is assumed to be the same for all individuals.

The hazard model makes two important assumptions:

i. Proportional hazards: the ratio of the hazards of any two individuals who differ by covariates is constant in time.
ii. The effect of covariate on the hazard is multiplicative.

And in the case of continuous covariate ***x***, it is typically assumed that its effect is *log linear*. Each unit increase in ***x*** results in the proportional scaling of the hazard.

The survival function can be expressed as:

$$S(t; Z_i) = [S_0(t)]^{\exp(\beta' Z_i)}$$

Where $S_0(t) = exp\left(-\int_0^t h_0\,(u)du\right)$ is the baseline survival function. To estimate **β**, Cox introduced the partial likelihood function, which does not depend on the unknown baseline hazard $\boldsymbol{h_0(t)}$ and allows to estimate the parameters **β**.

The partial likelihood of Cox also allows time-dependent explanatory variables when the value for any given individual can change and be updated over time. Time-dependent variables have many useful applications in survival analysis.[46,47,49]

4.2.4. THE NELSON-AALEN ESTIMATOR OF THE CUMULATIVE HAZARD

The **Nelson-Aalen estimator** is a non-parametric estimator of the cumulative hazard rate function. It can be used to estimate the cumulative hazard from survival data in presence of censored data assuming independent censoring. It is expressed by the following formula:

$$\widehat{H}(t) = \sum_{j|tj \le t} \frac{d_j}{n_j}$$

Where d_j is the number of events at t_j and n_j the total number of individuals at risk at t_j.

The cumulative hazard function and its non-parametric estimator own a meaningful interpretation only in the case of survival analysis with repeated events representing the cumulative number of expected events in time.[50,51]

4.2.5. THE AALEN-JOHANSEN ESTIMATOR

The **Aalen-Johansen estimator** is a non-parametric estimator of the crude incidence of a competing risk. It can be used to estimate the crude incidence function from survival data in the presence of competing risks with censored data, assuming independent censoring from the survival time **T**.

This estimator is the sum of unconditional probabilities of failure due to the event of interest in time, obtained by multiplying the probability of having survived any event by the cause-specific hazard of the event of interest.

$$\hat{F}_1(t) = \sum_{j|tj \le t} \hat{h}_1(t_j) \cdot \hat{S}(t_j)$$

Where

$$\hat{h}_1(u) = \frac{d_{1j}}{n_j}$$

Where d_{1j} is the number of events of type 1 at t_j and n_j the total number of individuals at risk at t_j. Of note, this estimator is not equivalent to the Kaplan-Meier estimator after censoring the observations at the times of all competing events which is known to overestimate the crude incidence.

The occurrence of a competing event prevents that the event of interest occurs as first and thus cannot be handled with artificial right censoring as, by definition, censoring is a state that instead does not prevent the specific event from occurring later. For instance, death from

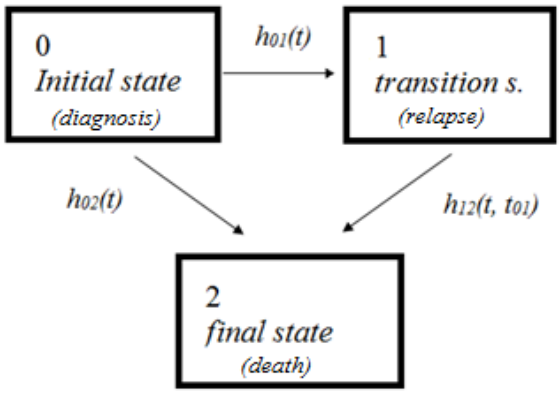
cause 1 prevents death from cause 2. In other words, any subject who experienced death from cause 1 will never fail for cause 2.[52,53]

4.2.6. MULTISTATE MODELS

Studies in cancer may have complex end-points; with different types of events which may also occur in sequence. In leukemia, for example, we might be interested in the occurrence of relapse and of death (with or without relapse), as causes of treatment failure.

Instead of analyzing the time to a single event, subsequent events may be analyzed in a multistate framework, which includes competing risks as a special case. In this framework each event is a state that can be transitory (e.g. relapse) or absorbing (e.g. death). [54]

The basic multistate model is a three state model with transitions possible from state 0 (initial) to state 1 (transitory) or 2 (absorbing), and from state 1 to state 2.



This model may be defined via two proper random variables:

1) The sojourn time, T_0, spent in the initial state 0 $\quad T_0 = \inf_{t>0}(X(t) \neq 0)$
2) And the time, T, to the absorbing state $\quad T = \inf_{t>0}(X(t) = 2)$

Where the random variable *X(t)* defines the state occupied at *t*. Thus, T_0=T corresponds to a $0 \rightarrow 2$ transition at T, and T_0<T corresponds to a $0 \rightarrow 1$ transition at T_0 and $1 \rightarrow 2$ transition at T.

The velocity of the transition between states are defined by the following the transition hazards. The $0 \rightarrow 1$ intensity is described by:

$$h_{01} = \lim_{\Delta t \to 0} P(X(t + \Delta t) = 1 | X(t) = 0)/\Delta t$$

The $0 \rightarrow 2$ rate is:

$$h_{02} = \lim_{\Delta t \to 0} P(X(t + \Delta t) = 2 | X(t) = 0)/\Delta t$$

In a markovian process[55] the 1→2 rate is:

$$h_{12}(t) = \lim_{\Delta t \to 0} P(X(t+\Delta t) = 2 | X(t) = 1)/\Delta t$$

Besides the transitions intensities the most important quantities of interest are the state and transition probabilities, which are functions of the three transitions rates. Specifically the probability of staying in state 0 from time s up to t is:

$$P_{00}(s,t) = P(X(t) = 0 | X(s) = 0) = exp\left(-\int_s^t h_{01}(u)du\right) s < t$$

Under Markov, the probability of remaining in state 1 from s up to t is:

$$P_{11}(s,t) = P(X(t) = 1 | X(s) = 1) \ = exp\left(-\int_s^t h_{12}(u)du\right) \ s < t$$

The probability of being in state 1 at t, conditional on being in state 0 at s is:

$$P_{01}(s,t) = P(X(t) = 1 | X(s) = 0) = \int_s^t P_{00}(s,u) h_{01}(u) P_{11}(u,t|u)du$$

The probability of being in state 2 at t, conditional on being in state 1 at time s is:

$$P_{12}(s,t) = P(X(t) = 2 | X(s) = 1) = 1 - P_{11}(s,t)$$

Finally, the probability of being in state 2 at time t, conditional on being in state 0 at time s is:

$$P_{02}(s,t) = \ P(X(t) = 2 | X(s) = 0) =$$

$$\int_s^t h_{01}(u) \exp(-\int_s^u (h_{01}(v) + h_{02}(v))dv) \, P_{12}(s,t)du + \frac{\int_s^t h_{02}(u) \exp(-\int_s^u (h_{01}(v)+h_{02}(v))dv)du}{\exp(-\int_s^u (h_{01}(v)+h_{02}(v))dv)}$$

Generally, the estimation of the transition hazards is carried out by the Nelson-Aalen estimator or the Cox model, while the estimates of the prediction probabilities by simply replacing integrals with sums and replacing hazards by their estimates (Aalen-Johansen estimator).[55]

4.3. NON-STANDARD METHOD OF SURVIVAL ANALYSIS

4.3.1. THE INVERSE PROBABILITY OF CENSORING WEIGHTING METHOD

The inverse probability of censoring weighting (IPCW) method is here shown in relation to the problem of non-independent censoring.

With reference to our context, the method is based on the idea to recreate the potential population one would observe in the absence of abandonment. One may observe that, if the probability of abandon in time depends on some known and measured covariates, and if, conditional on these covariates, abandon is not associated with the outcome, the observation of a patient who abandons can be represented, at each time *t*, by the one belonging to a fully observed patient with a similar characteristic profile. Thus IPCW can be applied in situations where both independent (e.g. administrative) and non-independent censoring (e.g. abandonment) are present.

In practice, this is achieved by adding a weight to the observation to these patients (fully observed) in order to represent also those one would observe if *no abandonment* occurred.

The estimates obtained by using an IPCW estimator are unbiased with respect to the potential quantity one would estimate on data with *non-abandonment* if the following assumptions are met:

i. The model for the probability of abandon in time is estimated consistently (e.g. no unmeasured confounders are present);

ii. At each time *t*, the probability of abandon is independent of the potential outcome conditionally on some observed characteristics. This is known as "coarsening at random" (CAR) assumption;

iii. At each time *t*, the probability of not abandoning treatment is non-zero. This is known as "positivity" assumption.

The application of the method to the data can be summarized in the following three steps:

1. Modeling of the conditional probability of abandon. In principle, the IPCW approach can be applied in any context where the outcome is not observed for every subject.

In the context of survival analysis, however, one additional complication is present: the weights have to be computed for each distinct failure time. To this purpose, standard approaches for survival analysis can be adopted. Once the predictors of abandonment have been identified (call *X* this vector of covariates), a Cox model considering abandon (call it *C*) as the event of interest can be fitted to the data. Alternative regression models for time-to-event data may also be considered (e.g. Aalen additive model). The model estimates are then used to compute, for each patient and time *t*, the probability of not abandoning up to *t* given the covariates:

$$\Pr(C > t|X).$$

2. Compute the weights for each patient at each time t. The weights are calculated as:

$$W(t) = 1/\Pr(C > t|X).$$

This is called "unstabilized weights". To avoid numerical problems, sometimes is convenient to adopt a modified version of the weights (called "stabilized weights"):

$$W(t) = \Pr(C > t)\ /\Pr(C > t|X).$$

3. Estimate potential quantities using IPCW weighted model for the outcome. This can be, for example, a Kaplan-Meier estimator. It is the same model one would fit to the complete data, except that each observation at each time *t* is weighted by:

$$W(t) = 1/\Pr(C > t|X).$$

For example, the IPC weighted Kaplan-Meier estimator can be defined as:

$$\hat{S}_{IPCW}(t) = \prod_{j:t_j<t} 1 - \frac{\Sigma_i \delta_i(t_j) w_i(t_j)}{\Sigma_i r_i(t_j) w_i(t_j)}$$

Where $\delta_i(t_j)$ and $r_i(t_j)$ are, respectively, the status indicator and the at-risk indicator for the patient *i* at time t_j.[56,57]

5. RESULTS

5.1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

The GHS-2000 protocol was applied in Guatemala, Honduras and El Salvador from February 2000 to June 2008 (Guatemala shifted to the BFM based protocol in July 2007); 1,496 patients were enrolled in this protocol and were considered eligible and evaluable. For the BFM based protocols, enrollment period was considered until June 2015; 2,148 patients were enrolled into these protocols in the same countries and were considered eligible and evaluable. The main demographic and clinical patient's characteristics are summarized, by protocol and by country in tables 2 and 3.

Table 2. Demographic and Clinical characteristics by Protocol

	GHS-2000		**BFM-based**		**TOTAL**	
	N	***%***	**N**	***%***	**N**	***%***
TOTAL	**1496**		**2148**		**3644**	
Gender						
Female	**646**	*43.2*	**963**	*44.8*	**1609**	*44.2*
Male	**850**	*56.8*	**1185**	*55.2*	**2035**	*55.8*
Age (years)						
[0-1)	**4**	*0.3*	**17**	*0.8*	**21**	*0.6*
[1-6)	**694**	*46.4*	**994**	*46.3*	**1688**	*46.3*
[6-10)	**424**	*28.3*	**488**	*22.7*	**912**	*25.0*
[10-15)	**311**	*20.8*	**478**	*22.3*	**789**	*21.7*
[15-18)	**63**	*4.2*	**171**	*7.9*	**234**	*6.4*
WBC count						
0-20000	**940**	*62.8*	**1217**	*56.7*	**2157**	*59.2*
20000-50000	**225**	*15.0*	**338**	*15.7*	**563**	*15.4*
50000-100000	**140**	*9.4*	**218**	*10.2*	**358**	*9.8*
≥100000	**186**	*12.4*	**290**	*13.5*	**476**	*13.1*
No data	**5**	*0.3*	**85**	*4.0*	**90**	*2.5*
Immunophenotype						
B-lineage ALL	**1356**	*90.6*	**2001**	*93.1*	**3357**	*92.1*
T-Lineage ALL	**117**	*7.8*	**143**	*6.7*	**260**	*7.1*
No data	**23**	*1.5*	**4**	*0.2*	**27**	*0.8*
CNS positive						
Yes (3)	**72**	*4.8*	**180**	*8.4*	**252**	*6.9*
No (1,2)	**1416**	*94.7*	**1959**	*91.2*	**3375**	*92.6*
Not assessed	**8**	*0.5*	**9**	*0.4*	**17**	*0.5*
BM status d15						
M1	**1149**	*76.8*	**1239**	*57.7*	**2388**	*65.5*
M2	**119**	*8.0*	**528**	*24.6*	**647**	*17.8*
M3	**26**	*1.7*	**241**	*11.2*	**267**	*7.3*
Not assessed	**202**	*13.5*	**140**	*6.5*	**342**	*9.4*
NCI Risk						
SR	**850**	*56.8*	**1116**	*52.0*	**1966**	*54.0*
HR	**646**	*43.2*	**1032**	*48.0*	**1678**	*46.0*

Demographic and clinical characteristics at diagnosis were similar for both protocols, except for CNS involvement and the proportion of M3 on day 15, that are higher for BFM based protocols. Also, the proportion of some characteristics (age, CNS involvement and BM15 response) were slightly different between countries, which reflects their particularities.

Table 3. Demographic and Clinical characteristics by Country

	Guatemala		**Honduras**		**El Salvador**		**TOTAL**	
	N	***%***	**N**	***%***	**N**	***%***	**N**	***%***
TOTAL	**1418**		**1231**		**995**		**3644**	
Gender								
Female	**614**	*43.3*	**525**	*42.7*	**470**	*47.2*	**1609**	*44.2*
Male	**804**	*56.7*	**706**	*57.3*	**525**	*52.8*	**2035**	*55.8*
Age (years)								
[0-1)	**1**	*0.2*	**7**	*0.6*	**13**	*1.3*	**21**	*0.6*
[1-6)	**571**	*40.3*	**571**	*46.4*	**546**	*54.9*	**1688**	*46.3*
[6-10)	**404**	*28.5*	**242**	*19.7*	**266**	*26.7*	**912**	*25.0*
[10-15)	**341**	*24.0*	**280**	*22.8*	**168**	*16.9*	**789**	*21.7*
[15-18)	**101**	*7.1*	**131**	*10.6*	**2**	*0.2*	**234**	*6.4*
WBC count								
0-20000	**854**	*60.2*	**682**	*55.4*	**621**	*62.4*	**2157**	*59.2*
20000-50000	**209**	*14.7*	**190**	*15.4*	**164**	*16.5*	**563**	*15.4*
50000-100000	**143**	*10.1*	**122**	*9.9*	**93**	*9.3*	**358**	*9.8*
≥100000	**212**	*14.9*	**148**	*12.0*	**116**	*11.7*	**476**	*13.1*
No data			**89**	*7.2*	**1**	*0.1*	**90**	*2.5*
Immunophenotype								
B-lineage ALL	**1315**	*92.7*	**1123**	*91.2*	**919**	*92.4*	**3357**	*92.1*
T-Lineage ALL	**96**	*6.8*	**96**	*7.8*	**68**	*6.8*	**260**	*7.1*
No data	**7**	*0.5*	**12**	*1.0*	**8**	*0.8*	**27**	*0.8*
CNS positive								
Yes (3)	**158**	*11.1*	**53**	*4.3*	**41**	*4.2*	**252**	*6.9*
No (1,2)	**1255**	*88.5*	**1173**	*95.3*	**947**	*95.2*	**3375**	*92.6*
Not assessed	**5**	*0.3*	**5**	*0.4*	**7**	*0.7*	**17**	*0.5*
BM status d15								
M1	**816**	*57.6*	**940**	*76.4*	**632**	*63.5*	**2388**	*65.5*
M2	**341**	*24.1*	**119**	*9.7*	**187**	*18.8*	**647**	*17.8*
M3	**131**	*9.2*	**95**	*7.7*	**41**	*4.1*	**267**	*7.3*
Not assessed	**130**	*9.1*	**77**	*6.2*	**135**	*13.6*	**342**	*9.4*
NCI Risk								
SR	**762**	*53.7*	**573**	*46.6*	**631**	*63.4*	**1966**	*54.0*
HR	**656**	*46.3*	**658**	*53.4*	**364**	*36.6*	**1678**	*46.0*

Guatemala, Honduras, and El Salvador are considered low-middle income countries. This means that the GNI (Gross National Income) per capita (calculated using the World Bank Atlas methods) is between 1,026 to 4,035 USD. The reported HDI (Human Development Index) for Guatemala, Honduras, and El Salvador are 0.627, 0.606 and 0.666 respectively. Nutritional status and other socio-economic conditions, that were shown to be related to

protocol outcome[16–19], are summarized by country in table 4. The amount of missing data on these variable is high especially in Honduras (availability of phone at home, nutritional status, type of family, parents' literacy, family income) and limits the possibility of comparison. In the subsequent analyses, these variable will be used to adjust for country-specific characteristics, whenever feasible.

Table 4. Socio-economical characteristics by Country

	Guatemala		**Honduras**		**El Salvador**		**TOTAL**	
	N	***%***	**N**	***%***	**N**	***%***	**N**	***%***
TOTAL	**1418**		**1231**		**995**		**3644**	
Living conditions								
Urban	**335**	*23.6*	**253**	*20.6*	**158**	*15.9*	**746**	*20.5*
Suburbs	**386**	*27.2*	**310**	*25.2*	**431**	*43.3*	**1127**	*30.9*
Rural	**697**	*49.2*	**484**	*39.3*	**403**	*40.5*	**1584**	*43.5*
No data			**184**	*14.9*	**3**	*0.3*	**187**	*5.1*
Time to reach the center (h)								
[0-2)	**428**	*30.2*	**340**	*27.6*	**322**	*32.4*	**1090**	*29.9*
[2-4)	**363**	*25.6*	**239**	*19.4*	**500**	*50.2*	**1102**	*30.2*
[4-more)	**627**	*44.2*	**471**	*38.3*	**172**	*17.3*	**1270**	*34.9*
No data			**181**	*14.7*	**1**	*0.1*	**182**	*5.0*
Phone at home								
Yes	**704**	*49.7*	**409**	*33.2*	**316**	*31.8*	**1429**	*39.2*
No	**714**	*50.3*	**340**	*27.6*	**676**	*67.9*	**1730**	*47.5*
No data			**482**	*39.2*	**3**	*0.3*	**485**	*13.3*
Nutritional status								
Adequate	**646**	*45.5*	**179**	*14.5*	**180**	*18.1*	**1005**	*27.6*
Undernourished	**663**	*46.8*	**71**	*5.8*	**215**	*21.6*	**949**	*26.0*
No data	**109**	*7.7*	**981**	*76.7*	**600**	*60.3*	**1690**	*46.4*
Family type								
Integrated	**1056**	*74.5*	**307**	*24.9*	**475**	*47.7*	**1838**	*50.4*
Disintegrated	**359**	*25.3*	**665**	*54.0*	**518**	*52.1*	**1542**	*42.3*
No data	**3**	*0.2*	**259**	*21.0*	**2**	*0.2*	**264**	*7.2*
Parent literacy								
High*	**383**	*27.0*	**81**	*6.6*	**291**	*29.2*	**755**	*20.7*
Low*	**658**	*46.4*	**154**	*12.5*	**423**	*42.5*	**1235**	*33.9*
Unknown	**377**	*26.6*	**996**	*80.9*	**281**	*28.2*	**1654**	*45.4*
Family income monthly (USD)								
≥325	**208**	*14.7*	**12**	*1.0*	**84**	*8.4*	**304**	*8.3*
<325	**735**	*51.8*	**77**	*6.3*	**372**	*37.4*	**1184**	*32.5*
Unknown	**475**	*33.5*	**1142**	*92.7*	**539**	*54.2*	**2156**	*59.2*

**Educational systems are similar in the different countries. High: parents who received secondary or advanced education; Low: parents who did not receive any education or only received elementary school.*

5.2. OUTCOME

The outcome results are summarized by protocol in table 5. Overall 91.5% of patients experienced CR and death in induction was about 5%. After complete remission, relapse occurred in 24% of patients, mostly during therapy and in the bon morrow. Death in CCR was about 4%

Table 5. Overall Patients Outcome by Protocol

EVENTS	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients Enrolled	**1496**		**2148**		**3644**	*100*
Events during remission induction						
Death in induction	**82**	*5.5*	**108**	*5.0*	**190**	*5.2*
Abandonment	**61**	*4.1*	**34**	*1.6*	**95**	*2.6*
Resistant Leukemia	**15**	*1.0*	**11**	*0.5*	**26**	*0.7*
Patients who achieved remission	**1338**	*89.4*	**1995**	*92.9*	**3333**	*91.5*
Events in complete remission						
Death in CCR	**74**	*5.0*	**77**	*3.6*	**151**	*4.1*
Abandonment	**195**	*13.0*	**105**	*4.9*	**300**	*8.2*
Second Malignant Neoplasm	**4**	*0.3*	**1**	*0.1*	**5**	*0.1*
Relapse	**366**	*24.4*	**511**	*23.8*	**877**	*24.0*
Phase of relapse						
During therapy	*232*	*15.5*	*335*	*15.6*	*567*	*15.5*
After completion of therapy	*134*	*9.0*	*176*	*8.2*	*310*	*8.5*
Site of relapse						
Bone Marrow	*231*	*63.3*	*323*	*63.2*	*554*	*63.2*
Extra – medullary	*106*	*28.9*	*140*	*27.4*	*246*	*28.1*
Bone marrow plus extra-medullar	*20*	*5.5*	*41*	*8.0*	*61*	*7.0*
Missing	*9*	*2.5*	*7*	*1.4*	*16*	*1.8*
Alive in complete remission	**556**	*37.2*	**1244**	*57.9*	**1800**	*49.4*
Lost to follow-up after completion of therapy/Transferred	**143**	*9.6*	**57**	*2.7*	**200**	*5.5*

Details on timing of abandonment are summarized by country in table 6; overall it occurred at a median time of 109 days. Of interest, treatment abandonment was reduced from 17.1% in the GHS-2000 to 6.5% in the BFM-based protocols

Table 6. Treatment abandonment (first event) by Protocol and country.

		Guatemala	Honduras	El Salvador	TOTAL
GHS-2000	*N. Patients*	**482**	**514**	**500**	**1496**
	Median follow-up (years)	*9.8*	*8.2*	*9.0*	*9.1*
	Treatment abandonment	**17.6%**	**21.2%**	**12.4%**	**17.1%**
	Median time to abandon (days)	***151***	***124***	***90***	***126***
BFM-based	*N. Patients*	**936**	**717**	**495**	**2148**
	Median follow-up (years)	*3.6*	*3.4*	*3.6*	*3.5*
	Treatment abandonment	**2.7%**	**13.4%**	**3.6%**	**6.5%**
	Median time to abandon (days)	***86***	***73***	***63***	***76***

Outcome results by country are shown in tables 7-9. In Guatemala mortality in induction and in CCR did not vary between protocols while it decreased consistently in El Salvador. The proportion of patients who relapsed was similar in the two protocols and by country. Of importance, the rate of abandonment markedly decreased in all countries with the BFM-based protocol, especially in CCR.

Table 7. Guatemalan Patients' Outcome by Protocol

EVENTS	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients Enrolled	**482**		**936**		**1418**	
Events during remission induction						
Death in induction	**32**	*6.6*	**57**	*6.1*	**89**	*6.3*
Abandonment	**18**	*3.7*	**1**	*0.1*	**19**	*1.3*
Resistant Leukemia	**5**	*1.0*	**4**	*0.4*	**9**	*0.6*
Patients who achieved remission	**427**	*88.7*	**874**	*93.4*	**1301**	*91.8*
Events in complete remission						
Death in CCR	**12**	*2.5*	**43**	*4.6*	**55**	*3.9*
Abandonment	**67**	*13.9*	**24**	*2.6*	**91**	*6.4*
Second Malignant Neoplasm	**1**	*0.2*	**1**	*0.1*	**2**	*0.1*
Relapse	**109**	*22.6*	**208**	*22.2*	**317**	*22.4*
Alive in complete remission	**156**	*32.4*	**567**	*60.6*	**723**	*51.0*
Lost to follow up /Transferred	**82**	*17.0*	**31**	*3.3*	**113**	*8.0*

Table 8. Honduran Patients' Outcome by Protocol

EVENTS	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients Enrolled	**514**		**717**		**1231**	
Events during remission induction						
Death in induction	**28**	*5.4*	**37**	*5.2*	**65**	*5.3*
Abandonment	**23**	*4.5*	**27**	*3.8*	**50**	*4.1*
Resistant Leukemia	**8**	*1.6*	**5**	*0.7*	**13**	*1.0*
Patients who achieved remission	**455**	*88.5*	**648**	*90.3*	**1103**	*89.6*
Events in complete remission						
Death in CCR	**35**	*6.8*	**21**	*2.9*	**56**	*4.6*
Abandonment	**86**	*16.7*	**69**	*9.6*	**155**	*12.6*
Second Malignant Neoplasm	**0**	*0.0*	**0**	*0.0*	**0**	*0.0*
Relapse	**127**	*24.7*	**174**	*24.3*	**301**	*24.4*
Alive in complete remission	**161**	*31.4*	**362**	*50.5*	**523**	*42.5*
Lost to follow up /Transferred	**46**	*8.9*	**22**	*3.0*	**68**	*5.5*

Table 9. Salvadoran Patients' Outcome by Protocol

EVENTS	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients Enrolled	**500**		**495**		**995**	
Events during remission induction						
Death in induction	**22**	*4.4*	**14**	*2.8*	**36**	*3.6*
Abandonment	**20**	*4.0*	**6**	*1.2*	**26**	*2.6*
Resistant Leukemia	**2**	*0.4*	**2**	*0.4*	**4**	*0.4*
Patients who achieved remission	**456**	*91.2*	**473**	*95.6*	**929**	*93.4*
Events in complete remission						
Death in CCR	**27**	*5.4*	**13**	*2.6*	**40**	*4.0*
Abandonment	**42**	*8.4*	**12**	*2.4*	**54**	*5.4*
Second Malignant Neoplasm	**3**	*0.6*	**0**	*0.0*	**3**	*0.3*
Relapse	**130**	*26.0*	**129**	*26.1*	**259**	*26.0*
Alive in complete remission	**239**	*47.8*	**315**	*63.6*	**554**	*55.7*
Lost to follow up /Transferred	**15**	*3.0*	**4**	*0.8*	**19**	*1.9*

5.3. SURVIVAL ANALYSIS WITH THE STANDARD APPROACH

5.3.1. ABANDONMENT AS EVENT

The following analysis considers abandonment as failure of the approach to cure and as such abandonment is counted as an event. By contrast curves are estimated also considering abandonment as non informative censored observation.

For GHS-2000 protocol, with a median observation time of 9.1 years, *considering abandonment as an event*, the 5-year event-free and overall survival estimates obtained by the Kaplan-Meier method were 49.7% (SE = 1.3%) and 55.8% (SE = 1.3%), respectively, while c*ensoring abandonment*, the 5-year EFS and overall survival were 61.6% (SE = 1.3%) and 64.7% (SE = 1.3%), respectively. The 5-year cumulative incidence (CI) rates for death, relapse and treatment abandonment, estimated by Aalen-Johansen method, were 10.3% (SE = 0.6%), 22.8% (SE = 1.1%) and 17.1% (SE = 0.9%) respectively. (Figure 5A and 6A)

In the BFM-based protocols, with a median observation time of 3.8 years, when *abandonment was considered an event*, the 5-year event-free and overall survival estimates were 52.7% (SE = 1.3%) and 62.3% (SE = 1.3%), respectively, while *censoring abandonment*, the 5-year EFS and overall survival were 56.8% (SE = 1.4%) and 65.7% (SE = 1.3%), respectively. The 5-year CI rates for death, relapse and treatment abandonment were 9.1% (SE = 0.4%), 31.6% (SE = 1.5%) and 6.6% (SE=0.9%) respectively. (Figure 5B and 6B)

Figure 5. Overall Event-free survival of (A) GHS-2000 and (B) BFM-based protocols, with treatment abandonment as event and censored.

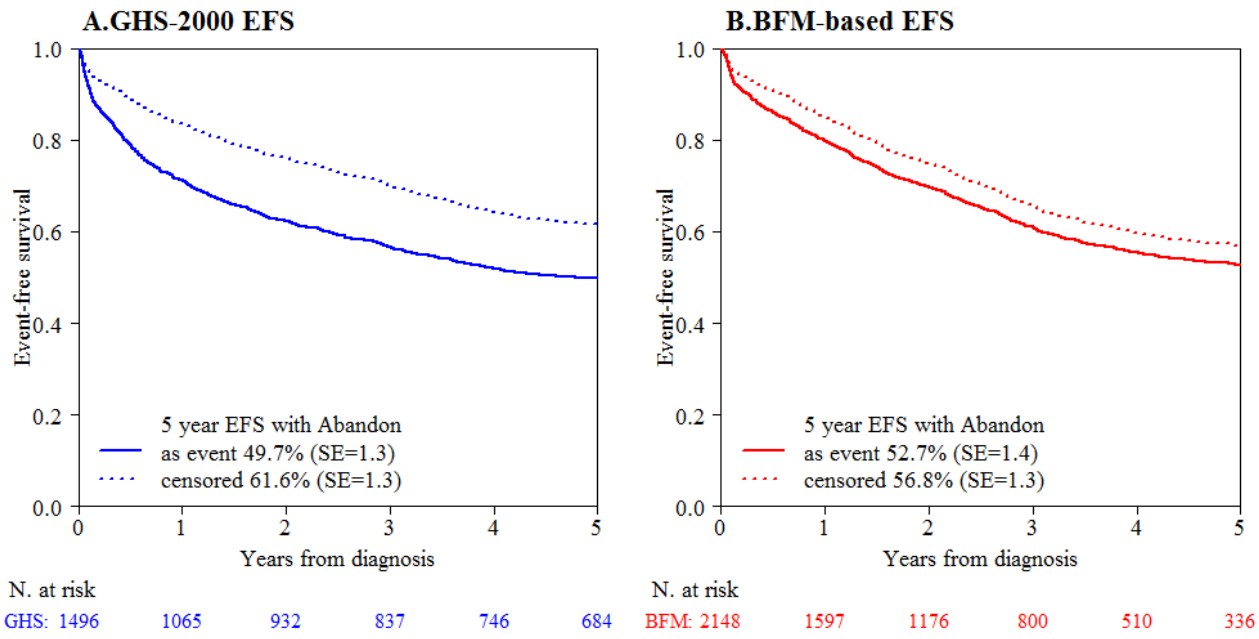
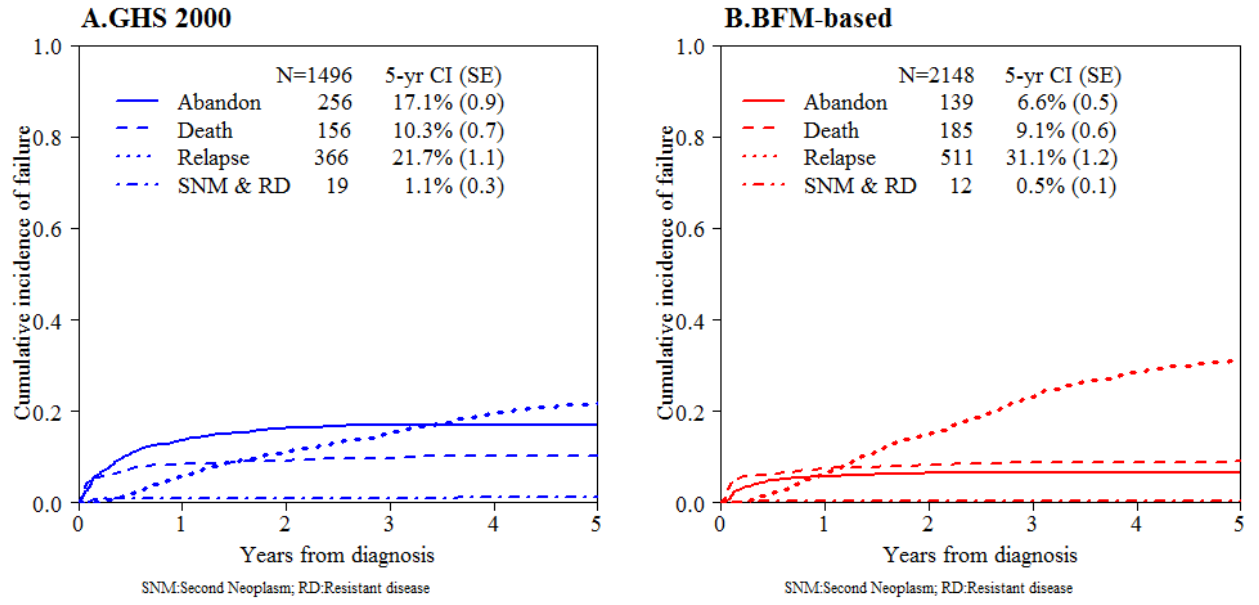


Figure 6. Cumulative incidences of treatment abandonment, death and relapses according to (A) GHS-2000 and (B) BFM-based protocols.

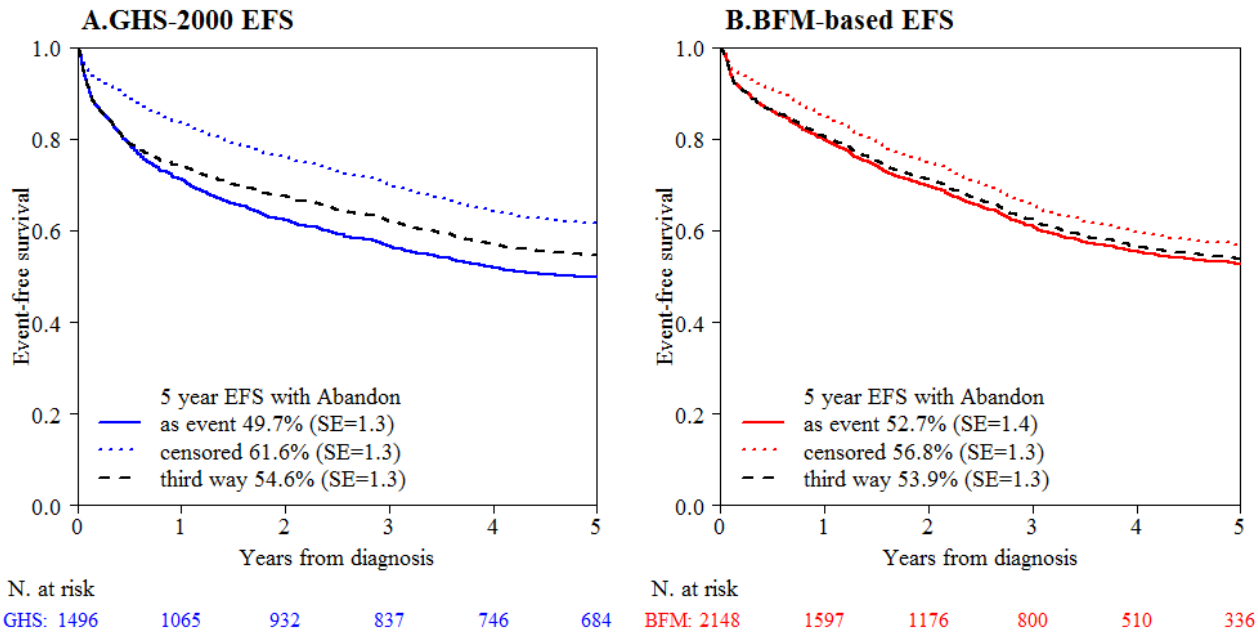


5.3.2. CONSIDERING PHASE OF ABANDOMENT

Patients who abandon treatment before maintenance (during the most intensive treatment) are very likely to die of the disease, or relapse and die while those who abandon after finishing the most intensive chemotherapy are more likely to survive. Thus an alternative approach where it is reasonable to assume that abandonment is an even or is censored according to the phase in which it occurred.

Given the lack of tested cut off, taking into account the design of both protocols and the median time to abandonment, 180 days (from diagnosis) could be an appropriate cut off to discriminate between the most intensive and less intensive phases. (Figure 7 A-B).

Figure 7. Overall Event-free survival of (A) GHS-2000 and (B) BFM-based protocols, with treatment abandonment as event, censored and "third way".



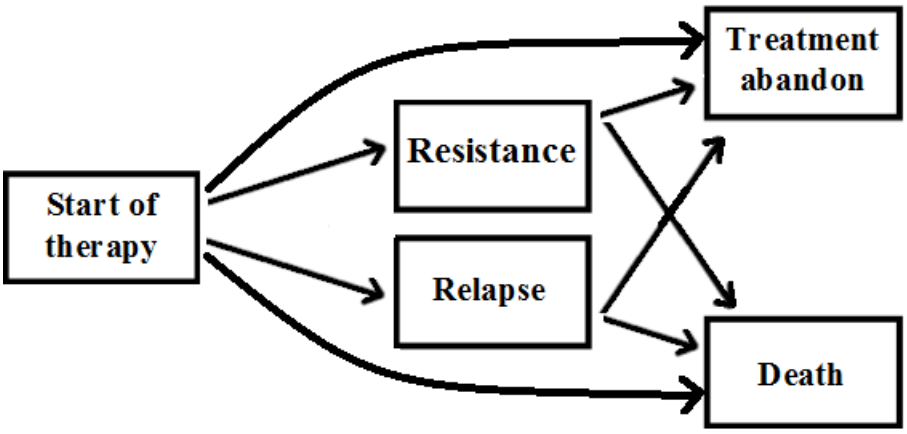
As a consequence, only abandonments before 180 days (6 months) were counted as events. With this proposed analysis, the GHS-2000 estimated survival is almost in the middle, instead the BFM-based gets closer to the "worst" scenario (abandon as event) estimation, because in the former 61.7% if the abandonments were before 180 days (events) while in the latter almost all (75.5%) abandonments occurred in the first 6 months. Of note that this estimation is based only on a cut-off for the timing of the event and it does not depend on other covariates.

5.4. COMPETING RISKS AND MULTISTATE ANALYSIS

To get insight into the dynamic of abandons patterns in time, one may consider the occurrence of abandon as a possible cause of failure in a competing risks setting. The cumulative incidences were estimated by Aalen-Johansen method (Figure 6 A-B).

Comparing the crude cumulative incidence curves of the two protocols, one may argue that relapse was more frequent among patients included in the BFM-based protocol. Nevertheless, it is important to notice that in the GHS-2000 protocol the abandonment was heavier. In the competing risk analysis, this higher probability of abandon leads to an artificial "protection" against relapse (as the first event). For a deeper description of the data, one should consider a model where patients are followed beyond the first event. This drives to the extension of the competing risks framework to the multistate model. In the case of our data, patients can move from the start of the therapy (initial state) to resistance or relapse (intermediate states). From both states, patients are allowed to further move to death or abandon of treatment (absorbing states). Obviously, the possibility of moving directly to death or abandon from the initial state is also accounted for (Figure 8). Abandonment of therapy was considered as an absorbing state, because patient status after abandon is not homogeneously available, so to avoid a possible bias, we stopped the observation of the patients after abandon. This model is more general than the one introduced in section 4.2.6, although estimation is based on the same approach.

Figure 8. Diagram of the states and possible transitions.



The transition hazards are estimated using the Cox model and then plugged into the Aalen-Johansen formula to obtain state probabilities. The results of the multistate model analysis, for each of the two protocols, are represented in figures 9 (overall) and 10 (by country).

Figure 9. State probabilities for GHS-2000 and BFM-based protocols.

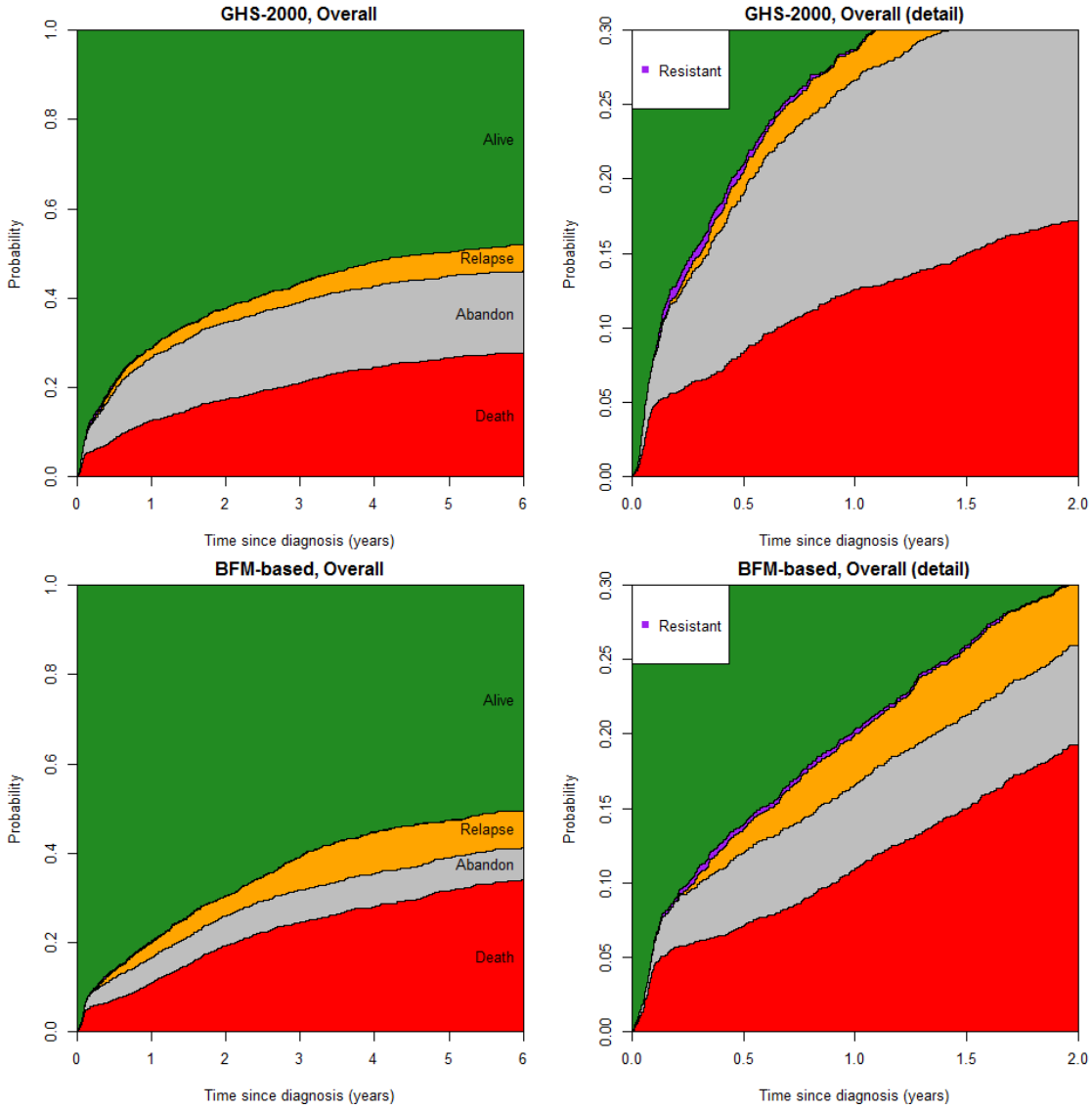


Figure 10A. State probabilities for GHS-2000 and BFM-based protocols - GUATEMALA.

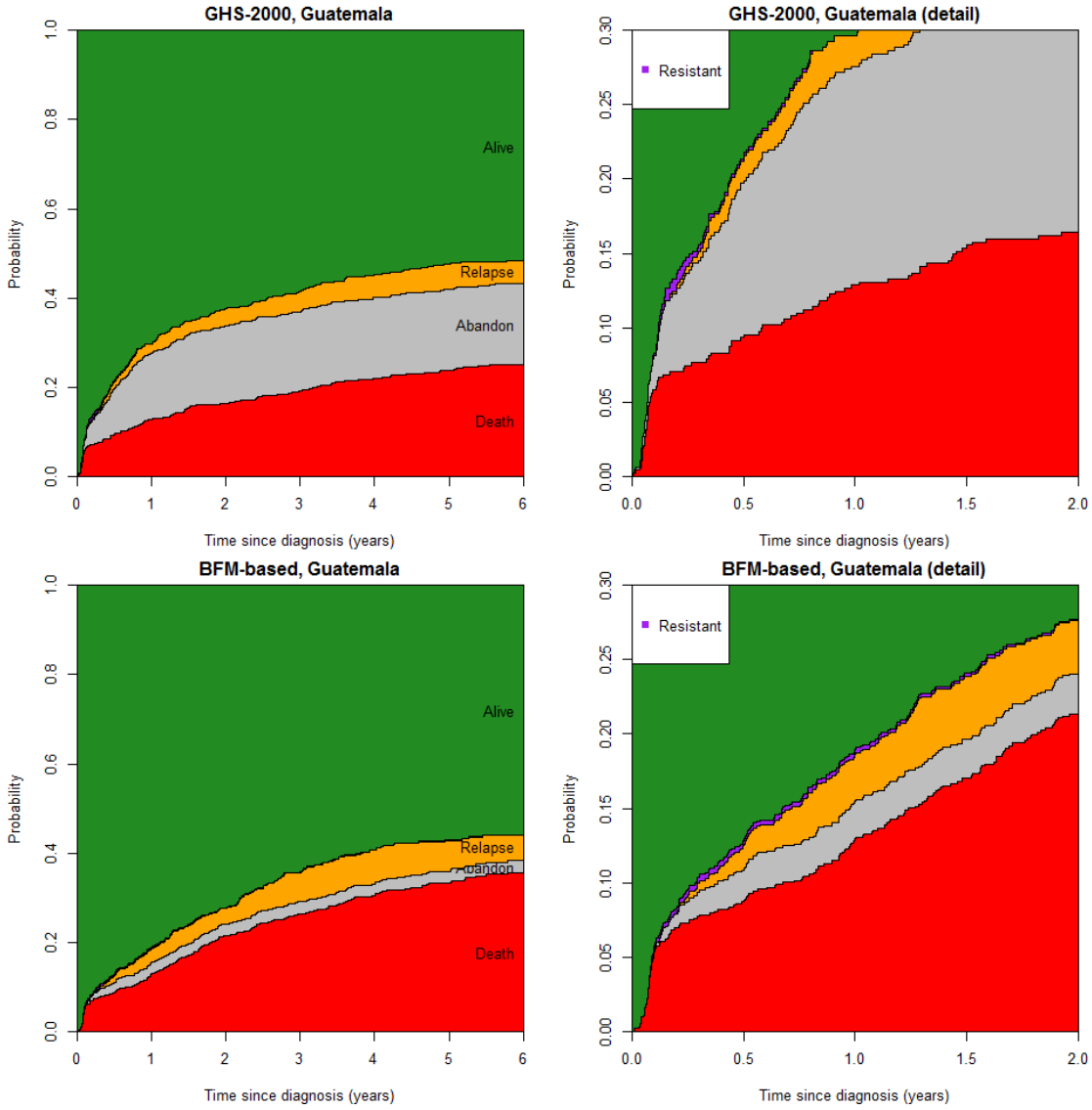


Figure 10B. State probabilities for GHS-2000 and BFM-based protocols - HONDURAS.

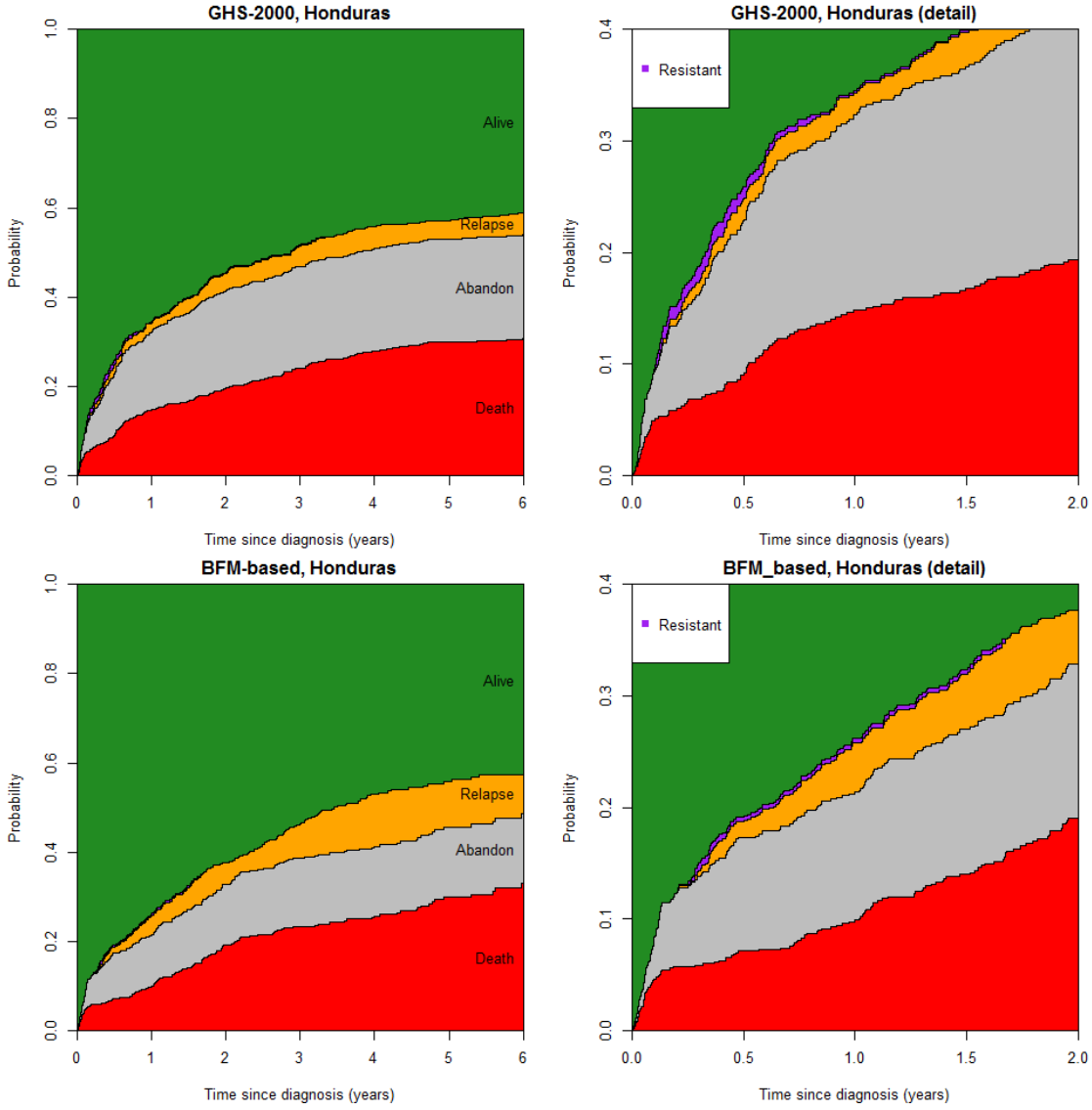
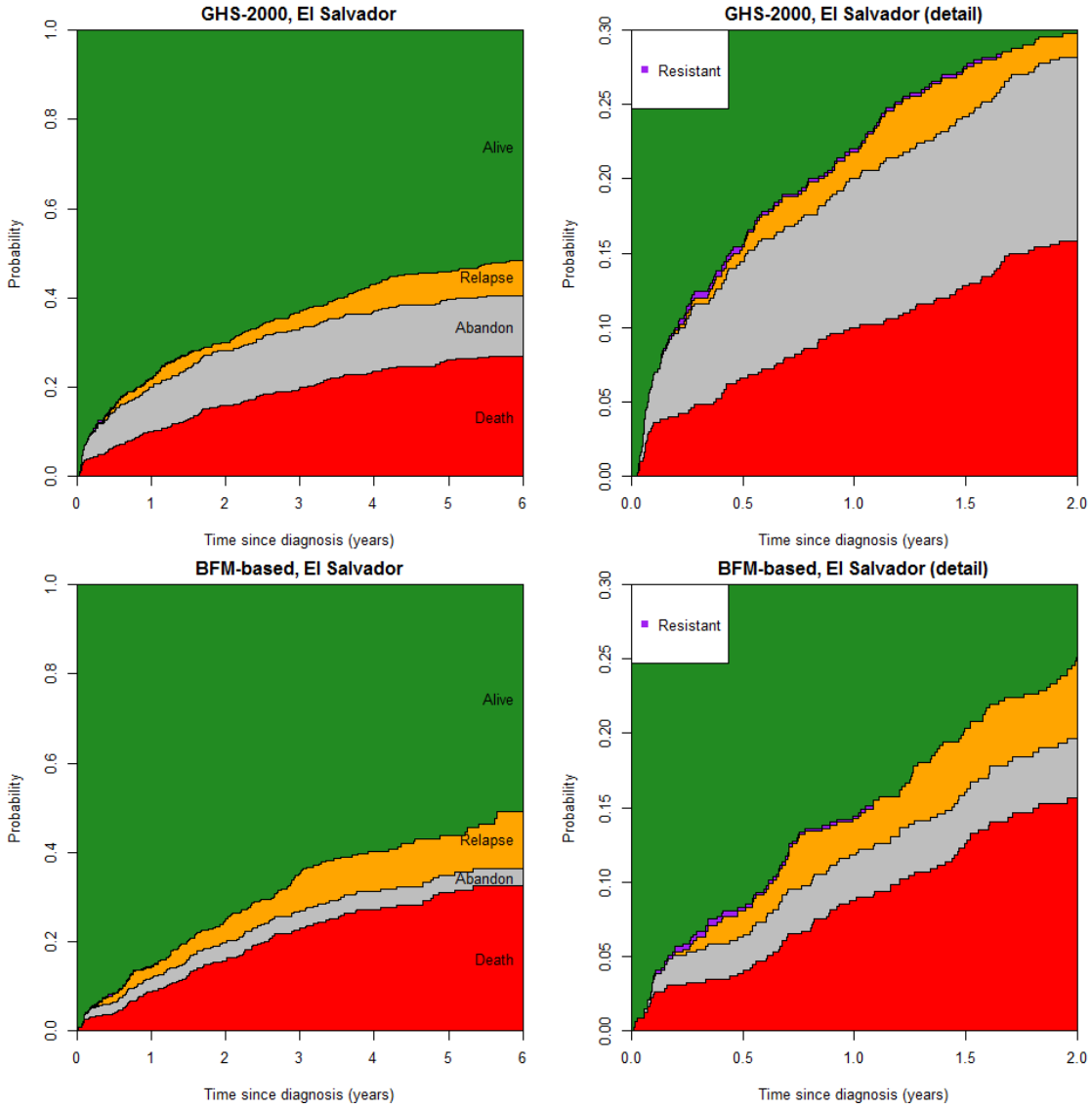


Figure 10C. State probabilities for GHS-2000 and BFM-based protocols – EL SALVADOR.



This method is very useful to describe the amount of patients moving from one state to another at each time. In these "stacked plots" the proportion of patients in one state at a particular time point is given by the vertical distance between the two following lines encompassing the state at that point. In particular, one can see explicitly the proportion of patients who abandon the treatment during follow-up. For instance looking at figure 9, about 15-20% of patients abandoned before 2 years in the GHS-2000 protocol, while about 5-10% of patients abandoned in the BFM-based protocol. In both protocols the rate of abandon does not increase furthermore after 2 years. Of note, relapse (orange area) and death (red area) are wider in BFM-based protocols, this is due to the fact that abandon is less frequent in the

recent protocols. From pictures 10 A, B, C it is also evident that the most recent protocol has a lower rate of abandon in all countries.

The plots on the right-hand side represent a detail of the left-hand side figures (the time scale is 2 years instead of 6 years and the vertical axis does not go to 1) to visualize the amount of patients developing a treatment resistance. The multistate analysis provides a clear picture of the observed outcomes in time. However, it does not allow to "remove" the effect of abandonment from survival. In order to focus on the potential outcomes we would observe under no abandonment, the IPCW method was applied.

5.5. RESULTS OF IPCW METHOD

As mentioned in section 4.3.1 a crucial assumption for the validity of the IPCW method is that the probability of abandon for a patient with a given prognosis depends only on the covariates included in the model for predicting abandon. Thus, these covariates must be related to both the outcome and to the propensity to abandon treatment.

First of all, we considered the following clinical and biological features: gender, age, WBC count at diagnosis, ALL-linage, CNS involvement, BM status on day 15 (was the common measure response for both protocols). These covariates are usually used as risk stratification criteria, because they identifies subgroups of patients at different prognosis.

In addition, the available information on socio-economic factors such as living conditions, time to arrive to the treatment center, the availability of phone at home, the type of family (this somehow represent a support system for the patient and its caregiver) and parental literacy was considered. We selected these factors based on the findings published in the literature on this topic. [16–19]

It was observed that the risk of abandonment was associated with different factors in each country, we decided to perform three different analyses, one for each country. Indeed, to perform a single overall analysis one would have included an interaction term between the country indicator and each other factor, increasing the complexity and possibly reducing the precision of the estimates.

The predictive performance of the model was evaluated with a bootstrap cross-validated C-index.

5.5.1. GUATEMALA

The covariates/predictors included in the models to estimate the risk of treatment abandonment are those reported in table 10.

Table 10. Guatemalan patients' characteristics by treatment protocol

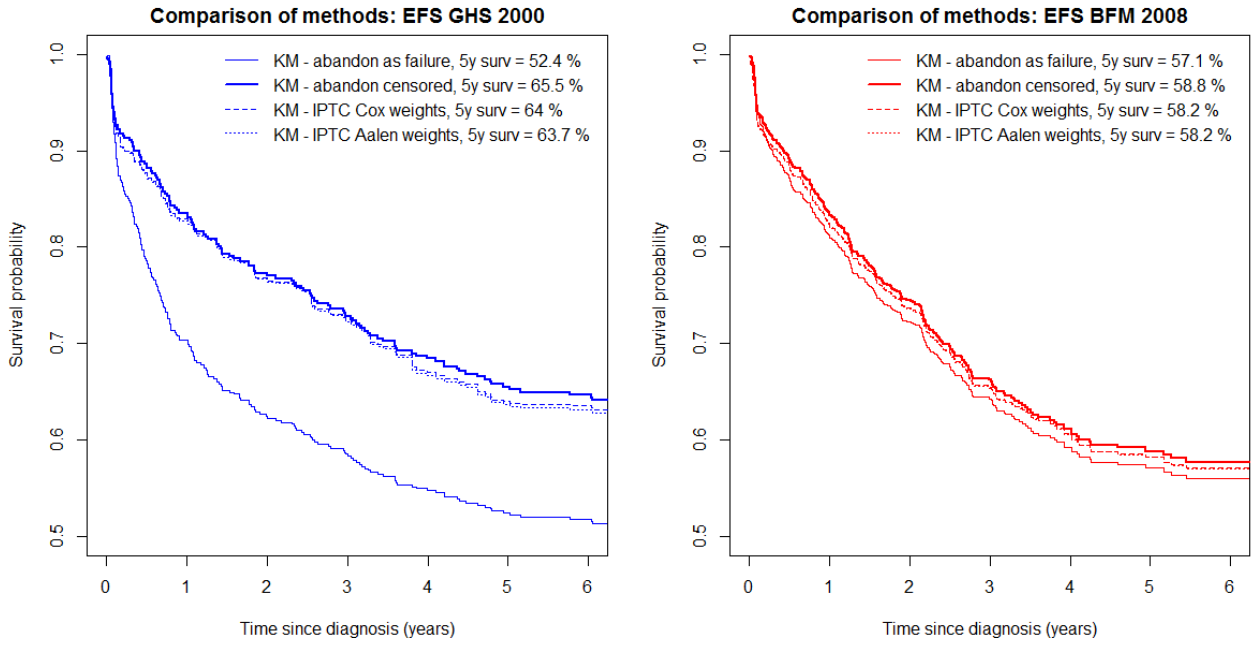
	GHS-2000		BFM-based	
	N	***%***	**N**	***%***
TOTAL	**482**		**936**	
Gender				
Male	**263**	*54.6*	**541**	*57.8*
Age (years)				
(median[IQR])	**7.11**	*[4.3-10.6]*	**7.09**	*[4.2-11.4]*
WBC count				
(median[IQR])	**9,000**	*[3,137-44,425]*	**11,630**	*[4,190-52,782]*
Immunophenotype				
T-Lineage	**37**	*7.7*	**59**	*6.3*
CNS involvement				
1	**386**	*80.1*	**544**	*58.1*
2	**54**	*11.2*	**276**	*29.5*
3	**42**	*8.7*	**116**	*12.4*
BM status d15				
M1	**364**	*75.5*	**452**	*48.3*
M2	**70**	*14.5*	**271**	*29.0*
M3	**10**	*2.1*	**121**	*12.9*
NP o NR [a]	**38**	*7.9*	**13**	*1.4*
Missing	**0**	*0*	**79**	*8.4*
Living conditions				
Urban	**97**	*20.1*	**238**	*25.4*
Suburbs	**186**	*38.6*	**200**	*21.4*
Rural	**199**	*41.3*	**498**	*53.2*
Time to reach the center (h)				
(median[IQR])	**3**	*[1-5]*	**3**	*[1-5]*
Phone at home				
Yes	**204**	*42.3*	**500**	*53.4*
Nutritional status				
Adequate	**204**	*42.3*	**442**	*47.2*
Undernourished	**171**	*35.5*	**492**	*52.6*
Missing	**107**	*22.2*	**2**	*0.2*
Family type				
Integrated	**285**	*59.1*	**771**	*82.4*
Parent literacy				
High*	**47**	*9.8*	**331**	*35.5*
Low*	**92**	*19.1*	**566**	*60.5*
Missing	**343**	*71.2*	**39**	*4.2*

[a] NP=Not Pertinent, NR=Not Realized.

** Educational systems are similar in the different countries. High: parents who received secondary or advanced education; Low: parents who did not receive any education or only received elementary school.*

The performance of both the Cox and Aalen model reported in appendix A, models was quite good, for given that the concordance index was close to 0.8 for both methods (a value of 1 would suggest perfect predictive ability while 0.5 would suggest no predictive ability).

Figure 11: Comparison (within protocol) of the EFS estimated by four methods



The protocol-specific EFS curves estimated with the standard and IPCW weighted methods, are shown in figure 11. In the GHS 2000 protocol, there is a strong difference in the EFS curves estimated considering abandon as an event with respect to the other methods. Both IPCW Cox and Aalen methods are very close to the estimate obtained after censoring the abandons. Given that the weighted curves represent what we would observe with no abandons, this means that patients who abandoned were very similar to those who did not abandon therapy and that had a good outcome. The expected outcome if no one would have abandoned therapy should be very similar to the curve censoring abandon.

In the BFM-based protocol, all the curves are very close one to each other, due to the strong reduction of abandons. The weighted curves seem to be in the middle between the two standard approaches (abandon as event and censored), probably because those who

abandoned therapy were equally distributed between those with good (typically late abandons) and bad (typically early abandons) prognosis.

5.5.2. HONDURAS

The covariates/predictors included in the models to estimate the risk of treatment abandonment are those reported in table 11.

Table 11. Honduran patients' characteristics by treatment protocol

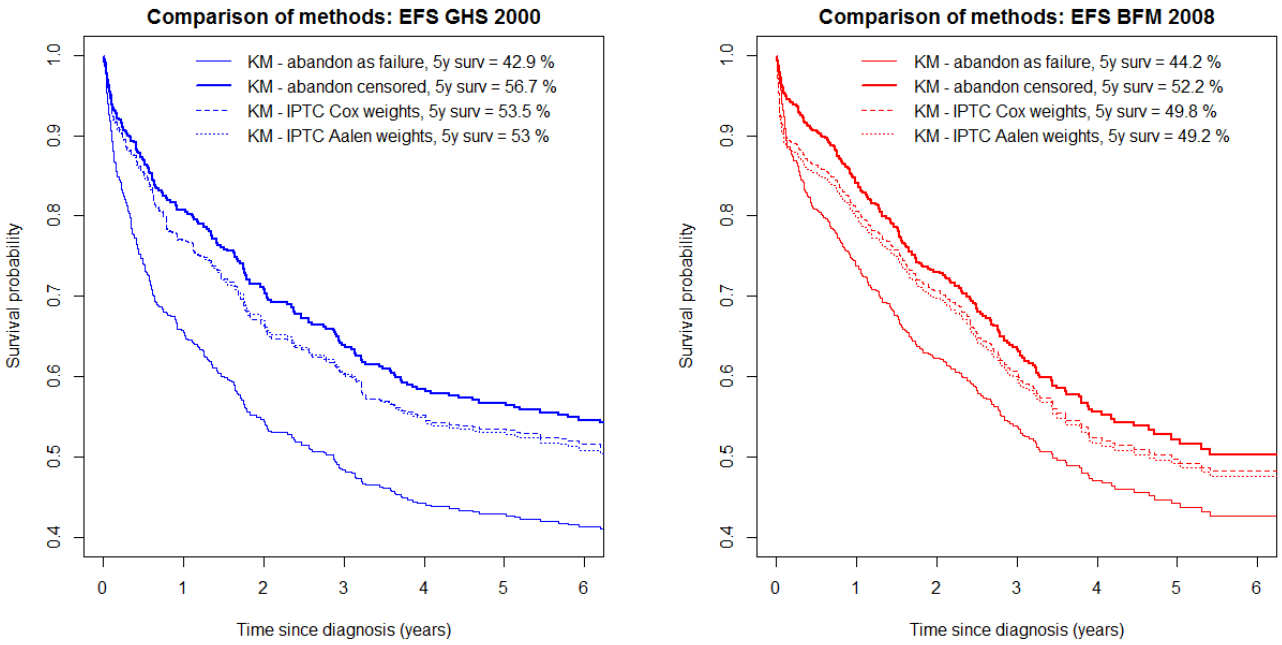
	GHS-2000		BFM-based	
	N	***%***	**N**	***%***
TOTAL	**514**		**717**	
Gender				
Male	**312**	*60.7*	**394**	*55.0*
Age (years)				
(median[IQR])	**6.79**	*[3.8, 11.3]*	**6.13**	*[3.4, 12.4]*
WBC count				
(median[IQR])	**11300**	*[4455, 42400]*	**11125**	*[5000, 41500]*
Immunophenotype				
T-Lineage	**41**	*8.0*	**55**	*7.7*
CNS involvement				
1	**484**	*94.2*	**612**	*85.4*
2	**9**	*1.8*	**73**	*10.2*
3	**21**	*4.1*	**32**	*4.5*
BM status d15				
M1	**431**	*83.9*	**509**	*71.0*
M2	**32**	*6.2*	**87**	*12.1*
M3	**12**	*2.3*	**83**	*11.6*
Missing	**39**	*7.6*	**38**	*5.3*
Living conditions				
Urban	**53**	*10.3*	**200**	*27.9*
Suburbs	**264**	*51.4*	**46**	*6.4*
Rural	**197**	*38.3*	**471**	*65.7*
Time to reach the center (h)				
(median[IQR])	**4**	*[1.5, 6]*	**3**	*[1, 4]*
Missing	**0**		**181**	*25%*
Phone at home				
Yes	**183**	*35.6*	**226**	*31.5*
Nutritional status				
Adequate	**179**	*34.8*	**0**	*0.0*
Undernourished	**71**	*13.8*	**0**	*0.0*
Missing	**264**	*51.4*	**717**	*100.0*
Family type				
Integrated	**232**	*45.1*	**75**	*10.5*
Parent literacy				
High*	**68**	*13.2*	**13**	*1.8*
Low*	**132**	*25.7*	**22**	*3.1*
Missing	**314**	*61.1*	**682**	*95.1*

[a] NP=Not Pertinent, NR=Not Realized.

** Educational systems are similar in the different countries. High: parents who received secondary or advanced education; Low: parents who did not receive any education or only received elementary school.*

The performance of the models was less good than the Guatemalan one; both Cox and Aalen model obtain a concordance index close to 0.7.

Figure 12: Comparison (within protocol) of the EFS estimated by four methods



In the GHS-2000 protocol we observe that, in case of no abandon, the survival is closer to the estimate obtained censoring abandon. In addition, in this case, it seems that the majority of those who abandoned would have had a good outcome in case they had continued treatment (Figure 12).

Looking at the results of the BFM-based protocol, in the first 2/3 months, the weighted curves are equal to the one considering abandon as an event. It means that those who early abandoned would have had a bad outcome, while those who abandoned after the consolidation phase would have had a better survival, although a little lower than the curves estimated under the best scenario (i.e. with censored abandon). This means that a portion of those who abandoned would have experienced a fatal event, even if they had continued treatment.

5.5.3. EL SALVADOR

The covariates/predictors included in the models to estimate the risk of treatment abandonment are those reported in table 12.

Table 12. Salvadoran patients' characteristics by treatment protocol

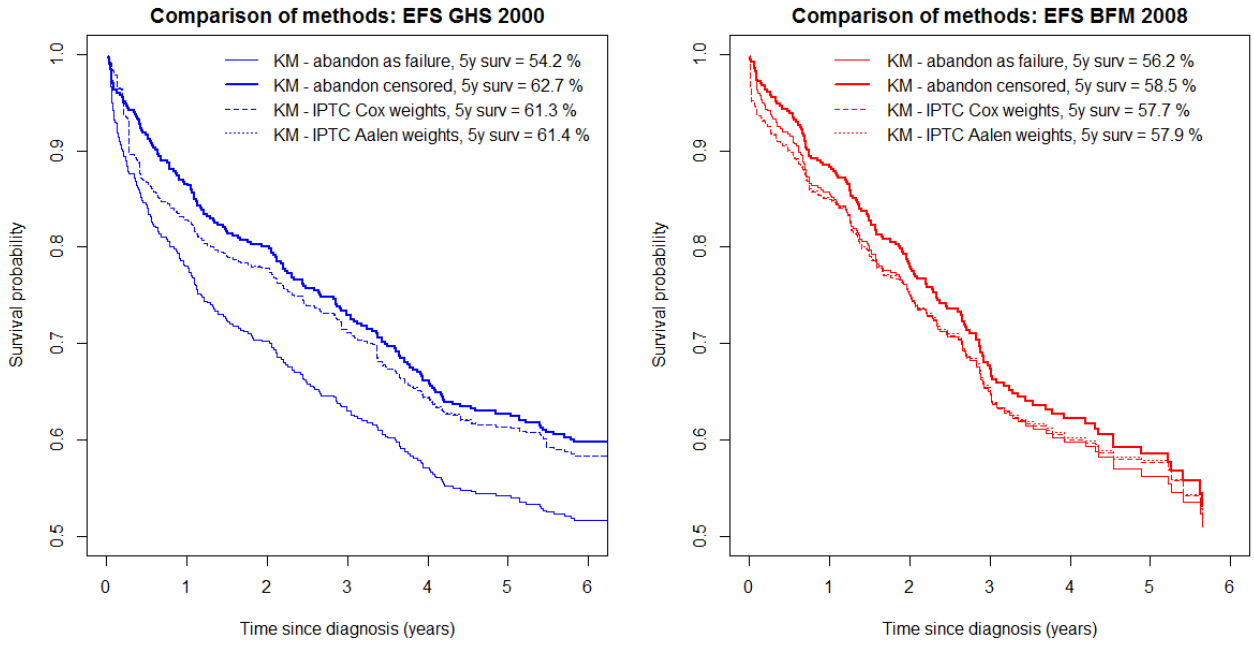
	GHS-2000		BFM-based	
	N	***%***	**N**	***%***
TOTAL	**500**		**495**	
Gender				
Male	**275**	*55.0*	**250**	*50.5*
Age (years)				
(median[IQR])	**5.2**	*[3.4, 8.4]*	**5.3**	*[3.2, 8.9]*
WBC count				
(median[IQR])	**11005**	*[4538, 36631]*	**11280**	*[4620, 39565]*
Immunophenotype				
T-Lineage	**39**	*7.8*	**29**	*5.9*
CNS involvement				
1	**481**	*96.2*	**397**	*80.2*
2	**10**	*2.0*	**66**	*13.3*
3	**9**	*1.8*	**32**	*6.5*
BM status d15				
M1	**354**	*70.8*	**278**	*56.2*
M2	**17**	*3.4*	**170**	*34.3*
M3	**4**	*0.8*	**37**	*7.5*
NP o NR [a]	**118**	*23.6*	**8**	*1.6*
Missing	**7**	*1.4*	**2**	*0.4*
Living conditions				
Urban	**67**	*13.4*	**91**	*18.4*
Suburbs	**311**	*62.2*	**120**	*24.2*
Rural	**122**	*24.4*	**284**	*56.2*
Time to reach the center (h)				
(median[IQR])	**2**	*[1.0, 3.0]*	**2**	*[1.5, 3.0]*
Missing	**0**		**1**	*0.0*
Phone at home				
Yes	**186**	*37.2*	**130**	*26.3*
Nutritional status				
Adequate	**180**	*36.0*	**0**	*0.0*
Undernourished	**215**	*43.0*	**0**	*0.0*
Missing	**105**	*21.0*	**495**	*100.0*
Family type				
Integrated	**473**	*94.6*	**2**	*0.4*
Parent literacy				
High*	**161**	*32.2*	**130**	*26.3*
Low*	**329**	*65.8*	**94**	*19.0*
Missing	**10**	*2.0*	**271**	*54.7*

[a] NP=Not Pertinent, NR=Not Realized.

** Educational systems are similar in the different countries. High: parents who received secondary or advanced education; Low: parents who did not receive any education or only received elementary school.*

The concordance index seems to be better for the Aalen method (0.7) than for the Cox method (0.65).

Figure 13: Comparison (within protocol) of the EFS estimated by four methods



In the GHS-2000 protocol, there is a strong different in the EFS curves estimated considering abandon as an event with respect to the other methods. Both IPCW by Cox and Aalen methods are very close to the estimate obtained censoring the abandons, especially after the first year of treatment, while before they are closer to the "worst scenario".

In the BFM-based protocol, all the curves are very close to each other, due to the strong reduction of abandons. The weighted curves seem to be overlapped with the curve where abandon was an event, probably because those who abandoned were those with bad prognosis.

Overall, when evaluation the risk of abandonment, the WBC count at diagnosis, modeled with a spline function, does not seem to impact on the risk of abandonment while the relationship to age tend to suggest a higher risk for older age. Of the socio-economic features, under nourish (Guatemala) and not availability of phone at home are related to higher risk of abandonment.

5.6 SURVIVAL AFTER TREATMENT ABANDONMENT

At this point, we wondered if the results obtained with the IPCW method are realistic. As mentioned before, only for a fraction of patients who abandoned treatment the next or final status is known as seen table 13.

Table 13. Overall survival of patients who abandoned treatment.

	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients who abandoned treatment	**256**		**139**		**395**	
The last status known						
Alive	**24**	*21.6*	**24**	*42.1*	**48**	*28.6*
Dead	**87**	*78.4*	**33**	*57.9*	**120**	*71.4*
Abandon *	**145**		**82**		**227**	

*Patients that abandoned and never returned to the center.

Table 13A. Next status of patients who abandoned treatment. (GUATEMALA)

	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients who abandoned treatment	**85**		**25**		**110**	
The last status known						
Alive	**8**	*19.0*	**1**	*16.7*	**9**	*18.7*
Dead	**34**	*81.0*	**5**	*83.3*	**39**	*81.3*
Abandon *	**43**		**19**		**62**	

*Patients that abandoned and never returned to the center.

Table 13B. Next status of patients who abandoned treatment. (HONDURAS)

	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients who abandoned treatment	**109**		**96**		**205**	
The last status known						
Alive	**8**	*23.5*	**14**	*40.0*	**22**	*31.9*
Dead	**26**	*76.5*	**21**	*60.0*	**47**	*68.1*
Abandon *	**75**		**61**		**136**	

*Patients that abandoned and never returned to the center.

Table 13C. Next status of patients who abandoned treatment. (EL SALVADOR)

	GHS-2000		BFM-based		TOTAL	
	N	%	N	%	N	%
Patients who abandoned treatment	**62**		**18**		**80**	
Last status kwon						
Alive	**8**	*22.9*	**9**	*56.2*	**17**	*33.3*
Dead	**27**	*77.1*	**7**	*43.8*	**34**	*66.7*
Abandon *	**27**		**2**		**29**	

*Patients that abandoned and never returned to the center.

These results show that patients who abandoned have a higher probability to die than remaining alive. Overall almost a 60% of the patients have an unknown outcome, then that 40% that returned for further treatment and clinical follow-up are already a selected group, thus these results are presumably biased.

6. DISCUSSION

A key characteristic of the collected survival data in the framework of the AHOPCA network is the presence of dropouts during treatment (treatment abandonment). In the past, treatment abandon reached 50% in some countries of AHOPCA like Nicaragua or Honduras[1]. Table 6 showed that in the last two decades in these three countries abandonment reached 17.1% of children under front line treatment for ALL protocols.

Children with ALL who abandon therapy are indeed likely to die for disease progression, especially when they abandon in an early phase of treatment and clinical centers are rarely able to retrace them and to update the information on their status. For this reason and for the fact that abandonment is, per se, a failure in treatment policy, abandon is considered an event in the estimation of survival or event free survival probability. This type of analysis is referred as "worst scenario" evaluation since abandonment is included in the definition of the survival time as cause of failure. Sometimes some selected cases come back to the hospital after dropout to seek for more care, thus it was proposed to censored abandonment, since in principle there is no certainty of death after treatment abandonment, especially if it occurs in the last phase of treatment (maintenance) and this type of analysis is referred as "best scenario" evaluation which gives a higher survival probability than the former scenario.

Of note, the "best scenario" approach assumes non-informative censoring due to abandonment, which cannot be formally validated. This assumption is obviously unrealistic and, is not even appropriate for describing the protocol outcome as it is likely to be biased. Abandonment (like noncompliance), is often related to the clinical condition of a patient, either very bad, such as to discourage the family in continuing treatment (typical of early dropouts) or quite good, such as to provide the illusion that therapy can be safely omitted (typical of late drop-outs).

The "worst" scenario does not require the assumption of non informative censoring and is also useful from a public health perspective since it considers abandonment as a failure of the clinical center and of the health system. However, these analyses cannot provide insights into the potential outcome in absence of abandonment. We can only consider that the "truth" on the clinical outcome of the front line treatment in the presence of abandonment is likely to lie between the two estimated curves (worst and best scenario).[15]

Another approach could be to estimate the outcome treating abandonment as event (failure) or censored observation according to the phase of treatment when it occurred: a failure for patients who abandoned treatment before starting maintenance and censored observation for those who abandoned treatment during maintenance phase. This approach makes a "deterministic" choice on how to handle drop-out and, although interesting to consider when no data are available on the type of patients who abandoned, is quite arbitrary (Figure 7).

The issue on how to handle informative censoring thus needs to be addressed with other statistical methods that take into account the covariates that characterize those patients who abandon with the aim to obtain a more accurate estimate of probability of survival. In particular, in our case example, treatment abandonment is not necessarily related to the disease or the treatment side effects, as we are used to assume in clinical trials conducted in Italy or richer countries. In the setting of low-income countries, poor socio-economic conditions, distance from the specialized treatment center, parental illiteracy, living conditions and cultural background are important factors shown to be related with a higher risk of treatment abandonment [16–18]. From a clinical perspective, however, the question of clinical interest is on the impact of the treatment protocol per se and the question is: *what would the unobserved event-free survival be in case no one would have abandoned the treatment?* The challenge posed by this question seems to be more evident when the rate of abandonment exceeds 5-10% level (as in our case of study).

In summary, we cannot assume that all patients that abandon will die (even when in some cases this could be realistic) neither that all patients that abandon will be alive free from disease progression, because both assumptions are unrealistic. The outcome estimates obtained with the current approaches (Figure 5, "best" and "worst" scenario) can be considered as the boundaries of the true outcome, and it is necessary to look for new methodologies to estimate the outcome as if no abandon occurred.

The competing risks approach could help in describing the impact of treatment abandonment on survival adjusting for the competing effect of the other events. Multistate models extend this framework allowing some states to be transitory (e.g. resistance, relapse). The multistate analysis is helpful to describe the proportion of patients in each state during time, as it become clear in the panels of figure 10. However, it does not allow to "remove" the effect of abandonment from survival and to answer the clinical question on treatment effect.

In order to focus on the potential outcomes we would observe under no abandonment, we propose to apply an IPCW approach to estimation. This approach estimates survival probabilities after weighting fully observed patients in order to artificially represent the censored outcome of patients with similar characteristics who abandoned. In this approach the observation of a patient who abandons can be represented, at each time *t*, by one belonging to a fully observed patient with similar characteristic profile.

To apply this method we assume that that the probability of abandon for a patient with a given prognosis depends only on the covariates included in the model for predicting abandonment. Thus, these covariates, such as clinical and biological features, and socio-economic factors must be related to both the outcome and to the propensity to abandon treatment (see appendices A1, B1 and C1). However other crucial factors that can help in predicting abandonment, especially those related to socio-economic and cultural variables, are difficult to collect and to measure especially in the setting we considered. Of the few variables we could consider, many for example had missing values because of the difficulties to collect and report them routinely. This acts as a limitation for the IPCW method, given that it is not possible to test for the presence of unmeasured confounding. To check the consistency of the methods, two different models for the estimation of the weights (Cox and Aalen additive model) were adopted. They provide similar results in terms of weighted curves.

The obtained results are very different among the three countries (figures 11-13), meaning that, in addition to the quantity, also the type of abandon and its effect on survival is country specific. The problem can be faced by performing separate analyses (as it was done here) or by carrying out a single overall analysis. In this case, however, countries specific effects of all the risk factors should be accounted for by including interaction terms.

As expected, the weighted curves lie as close to the "worst" scenario EFS as much as the patients who abandoned are similar to those with characteristics that are related to poor outcome among those at risk. In contrast, the weighted curve tends to overlap the "best" scenario estimate as much as the patients who abandoned are similar to those with better features and prognosis. In general, the results showed that the majority of patient who abandoned would have had high chances to

survive relapse-free if they did not abandon treatment. This is an important issue to consider in order to develop public health strategies to improve cancer survival for children in LIC/LMIC.

To directly evaluate the real impact of abandon on the survival outcome, a further analysis on the outcome after abandonment itself should be performed. Indeed, analysis based on the subgroup of patients who come back to the clinic after abandonment or are retraces is biased, since these patients represent a selected sample, both on the socio-economic level and on prognosis (patients who died at home after abandonment are obviously not retraces as well as patients who do not have a phone at home).

In conclusion, in this work we faced the problem of informative censoring in a particular case (abandon of therapy), but it is applicable also in other contexts, for instance to handle drop-outs in clinical trials, especially in situations where the phenomenon is not rare.

The "worst" scenario approach is useful form a public health perspective to evaluate the performance of the health system and of the treatment approach in LIC/LMIC in a rare but dismal disease such as childhood cancer. The "best" scenario approach provides an optimistic description of the potential outcome under no abandonment, where the upward bias derives from the implausible assumption of non informative censoring.

We investigate the use of a novel and more complex approach to estimate potential outcome as if no informative censoring occurs. Besides its limitations, this method can provide important information about the potential of a treatment approach and its ability to control relapse and toxic death when applied in full in the clinical practice. The comparison of the estimated potential effect with the estimate under a public health perspective gives a measure of how much the survival of children with cancer would be improved with the introduction of appropriate strategies aimed at avoiding treatment abandonment.

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APPENDIX A: RESULTS OF IPCW METHOD IN GUATEMALAN PATIENTS

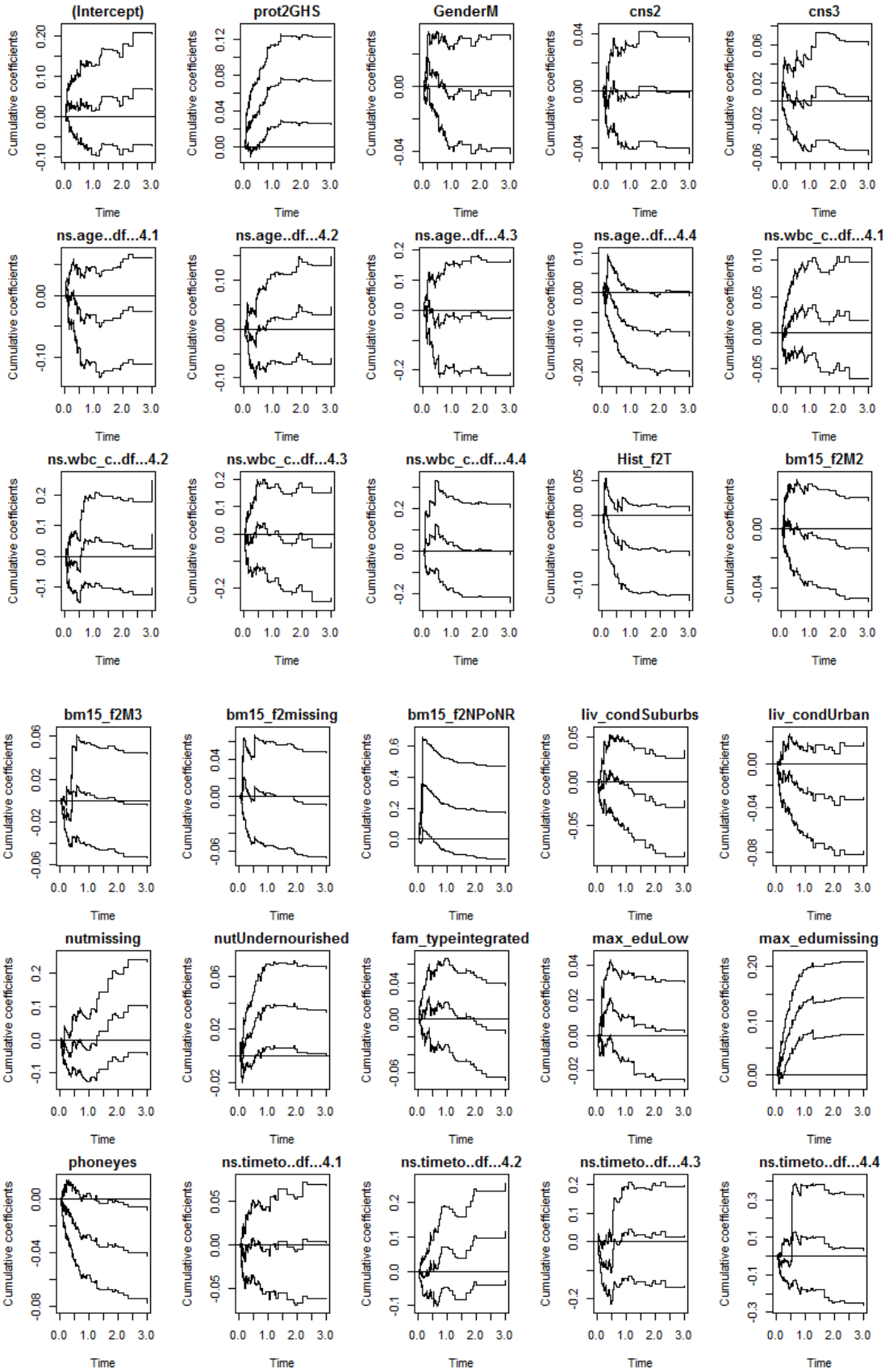
A1: Cox model coefficients for predicting abandon (and building IPC Weights)

	Coef	*exp (coef)*	*SE(coef)*	*Pr(>|z|)*
Protocol GHS	1.090539	2.975876	0.356834	0.00224**
Gender Male	0.009014	1.009055	0.196460	0.96340
CNS 2	-0.003455	0.996551	0.300791	0.99084
CNS 3	0.148490	1.160081	0.331217	0.65392
f(Age)[§]				0.2374
f(WBC)[§]				0.2566
Hist T	-0.649656	0.522225	0.486549	0.18180
BM15 M2	-0.154873	0.856524	0.309174	0.61642
BM15 M3	-0.119717	0.887171	0.491379	0.80751
BM15 Missing	0.107680	1.113691	0.634012	0.86514
BM15 NP-NR	0.959706	2.610928	0.407962	0.01865*
Liv_cond Suburbs	-0.087664	0.916069	0.247537	0.72323
Liv_cond Urban	-0.398401	0.671393	0.423210	0.34651
Nutri Missing	0.555659	1.743089	0.284277	0.05063
Nutri Undernourished	0.597099	1.816840	0.232333	0.01017*
Fam_type Integrated	-0.030121	0.970328	0.232489	0.89691
Max_edu Low	0.796961	2.218787	0.498396	0.10981
Max_edu Missing	1.661769	5.268621	0.543088	0.00221**
Phone yes	-0.471639	0.623979	0.227249	0.03795*
f(Time_to hospital)[§]				0.03985*

**** P highly significant (<0.01), * P significant (p<0.05)**

[§]Continuous variables are modeled with restricted cubic spline functions. The Likelihood Ratio Test P-values is reported

A2: Aalen model coefficients for predicting abandon (and building IPC Weights)



APPENDIX B: RESULTS OF IPCW METHOD IN HONDURAN PATIENTS

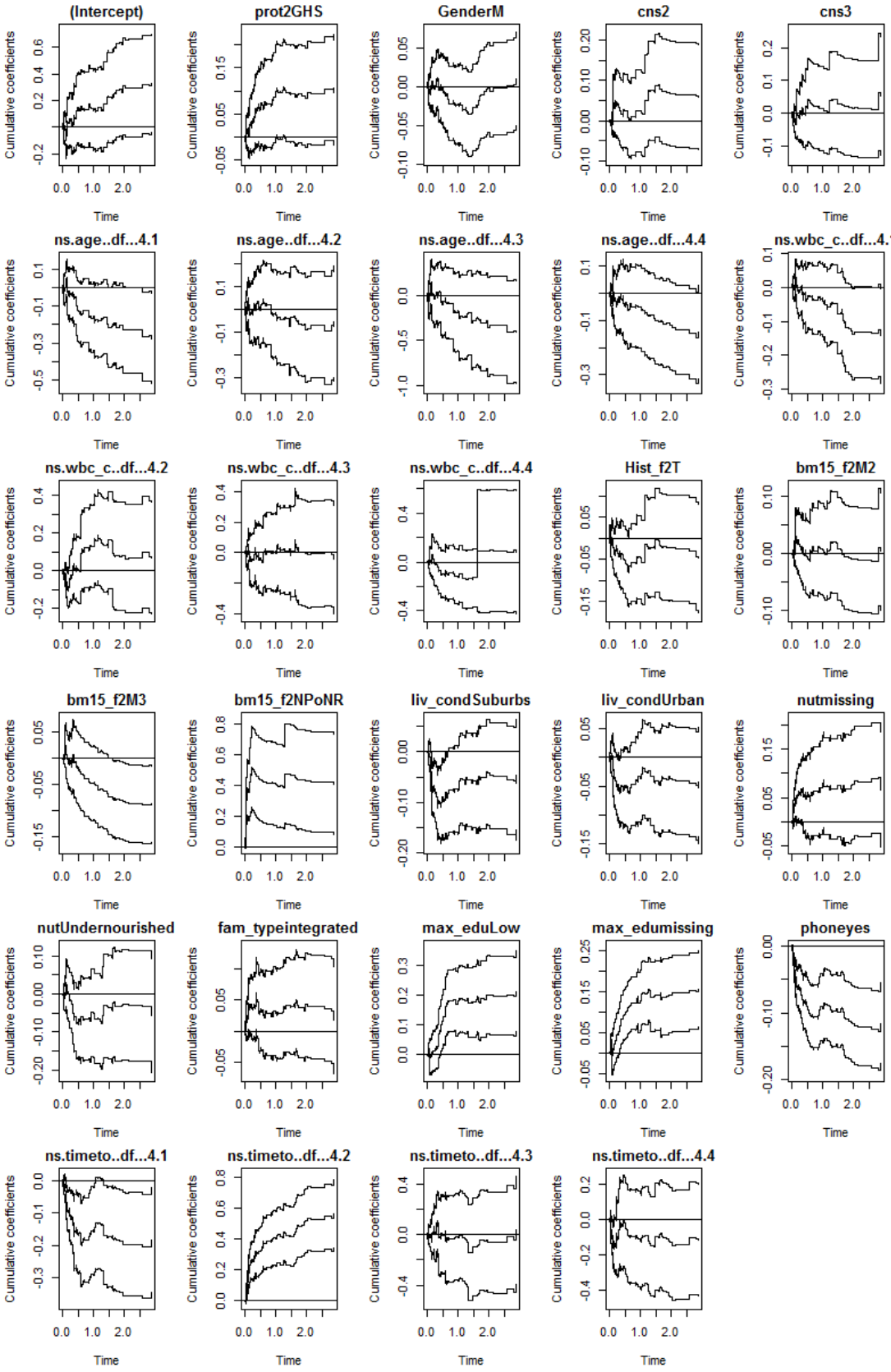
B1: Cox model coefficients for predicting abandon (and building IPC Weights)

	Coef	*exp (coef)*	*SE(coef)*	*Pr(>|z|)*
Protocol GHS	0.35825	1.43082	0.20926	0.08690
Gender Male	-0.06569	0.93642	0.14585	0.65241
CNS 2	0.22146	1.24789	0.28254	0.43316
CNS 3	0.10605	1.11188	0.35866	0.76746
f(Age)[§]				0.5057
f(WBC)[§]				0.5216
Hist T	-0.02811	0.97228	0.28917	0.92257
BM15 M2	-0.00392	0.99608	0.26347	0.98811
BM15 M3	-0.59774	0.55005	0.39698	0.13214
BM15 NP-NR	1.60468	4.97626	0.23160	0.00000**
Liv_cond Suburbs	-0.31915	0.72677	0.20041	0.11128
Liv_cond Urban	-0.24915	0.77947	0.28088	0.37507
Nutri Missing	0.34818	1.41649	0.22418	0.12040
Nutri Undernourished	-0.12413	0.88327	0.35361	0.72557
Fam_type Integrated	0.12772	1.13623	0.18125	0.48104
Max_edu Low	1.01987	2.77282	0.44740	0.02264*
Max_edu Missing	0.8737	2.39577	0.43119	0.04274*
Phone yes	-0.66108	0.51629	0.18619	0.00038**
f(Time to hospital)[§]				0.00000**

**** P highly significant (<0.01), * P significant (p<0.05)**

[§]Continuous variables are modeled with restricted cubic spline functions. The Likelihood Ratio Test P-values is reported

B2: Aalen model coefficients for predicting abandon (and building IPC Weights)



APPENDIX C: RESULTS OF IPCW METHOD IN SALVADORIAN PATIENTS

C1: Cox model coefficients for predicting abandon (and building IPC Weights)

	Coef	*exp (coef)*	*SE(coef)*	*Pr(>|z|)*
Protocol GHS	0.3882	1.474	8.44E-01	0.64567
Gender Male	0.1365	1.146	2.38E-01	0.56664
CNS 2	0.6412	1.899	4.68E-01	0.17019
CNS 3	-0.3593	0.6981	7.53E-01	0.63323
f(Age)§				0.04806*
f(WBC)§				0.862
Hist T	0.264	1.302	4.45E-01	0.55287
BM15 M2	0.123	1.131	4.37E-01	0.77814
BM15 M3	0.31	1.363	7.70E-01	0.68723
BM15 Missing	1.863	6.442	5.69E-01	0.00105**
BM15 NP-NR	1.252	3.498	2.87E-01	0.00001**
Liv_cond Suburbs	-0.2664	0.7661	2.94E-01	0.36469
Liv_cond Urban	-0.3765	0.6863	4.65E-01	0.41763
Nutri Missing	-0.07177	0.9307	3.73E-01	0.84747
Nutri Undernourished	0.2066	1.23	3.11E-01	0.50637
Fam_type Integrated	0.8362	2.308	7.03E-01	0.23432
Max_edu Low	0.4491	1.567	3.24E-01	0.1652
Max_edu Missing	0.6184	1.856	4.88E-01	0.20516
Phone yes	-0.9532	0.3855	3.29E-01	0.00379**
f(Time to hospital)§				0.1472

**** P highly significant (<0.01), * P significant (p<0.05)**

§Continuous variables are modeled with restricted cubic spline functions. The Likelihood Ratio Test P-values is reported

C2: Aalen model coefficients for predicting abandon (and building IPC Weights)