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Effect of transplant characteristics and of complications on survival outcomes of allogeneic stem cell transplantation: the experience of Stockholm center

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Abstract

Background and aims: Outcomes of haematopoietic stem cell transplantation (HSCT) are influenced by several factors; a key component is donor's selection, which might cause complications. Recently, treatments have improved, but finding a compatible donor remains a major issue.

Methods: Using data from the last transplant of patients treated in Stockholm's center, from 2015 to 2023, we compared the survival of recipients based on the donor from which they received healthy stem cells. Patients' characteristics, like gender, age and diagnosis, transplant information and follow-up results, such as Graft Versus Host Disease (GVHD) events and relapses, were considered. Cox proportional hazard regression models were fitted to estimate the hazard ratios of the different groups of donors. In addition, considering that a non-optimal selection might end up in worse complications, such as acute and/or chronic GVHD, the pathway between the exposure and the outcome includes a mediator. In order to evaluate the possible mediation of the effect of the donor, a causal mediation analysis was also performed.

Results: A total of 674 patients were identified and considered eligible for the retrospective study; of those, 142 experienced the outcome. The median age was 51 (IQR [27;63]), most of whom were men (59%), while the most frequent source of stem cells was peripheral blood. A total of 455 patients received stem cells from an unrelated donor, while 138 and 81 had an identical sibling and a mismatched relative as a donor, respectively. During the follow-up, 141 experienced acute GVHD, and 25 of these were life-threatening cases; in addition, 176 received a diagnosis for the chronic form of GVHD. Lastly, 124 experienced a recurrence of the disease. Compared to those who had an identical sibling as a donor, patients with a mismatched relative had lower survival and a higher hazard of experiencing the event (HR 2.24 [1.11,4.51]). The same analysis carried out in the subgroup of patients who received a diagnosis of a malignant disease pointed out the same result, confirming the crucial role of donor selection. At last, causal mediation analysis was carried out considering both chronic and acute forms of GVHD, but none of the two resulted in pointing out a moderate amount of mediated effect, as the mediated percentage was equal to 10% for the first and 3% for the second, but none of the two was statistically significant.

Conclusions: After this retrospective study, we confirmed the difference in survival for patients who are not able to find a compatible donor. Search for the best fit remains fundamental to maximise the recipient's likelihood of recovery and minimize the possibility of life-threatening complications such as Graft Versus Host Disease, even though relevant improvements have been accomplished lately in terms of drugs used to treat these specific events. An important limit of this process lies in the number of volunteers that are available for transplantation, as the probability of finding a matched donor varies and will lower in the future.

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Contents

Chapter 1

Introduction

Blood cancer is a family of tumoral diseases that affects blood cells. These diseases are caused by a change in the DNA sequence of the cells, which is the consequence of their abnormal behaviour [24]. Different types of blood cancer exist, and so symptoms and prognosis change. Patients who receive a diagnosis of blood cancer usually have a quality of life that changes drastically with the disease but can improve with treatment [1].

Treatment for blood cancer depends on the specific disease and can be identified as a single treatment or a combination of different ones. Possibilities include chemotherapy, immunotherapy, radiotherapy and stem cell transplantations. In particular, in the case of stem cell transplant, this is not directly used to fight cancer but to restore the body's ability to produce healthy blood cells[25].

Stem cell transplants are complex and are constituted of different phases, which go from the selection of the donor to the follow-up visits used to monitor patients' health status. A fundamental step is donor selection, and this can impact the outcome because of complications. The selection process is based on the Human Leukocyte Antigen (HLA) histocompatibility complex, which can trigger adverse events in recipients[2].

Annually, 1.28 million people receive a diagnosis of blood cancer, and it accounts for almost 6% of all cancer cases [26]. As synthesized in the 2021 European Group for Blood and Marrow Transplantation (EBMT) annual report, during that year, 47,412 HCTs in 43,109 patients were completed successfully by 694 European centres [3]. Karolinska Hospital in Huddinge is one of the accredited European centres that carry out cell therapy and stem cell transplantation and is known for its higher-than-average performances compared to other European countries.

With this study, we aim to provide a quality assessment of the transplants completed from 2015 to 2023 in this centre, to understand the overall performance of the treatments carried out here and to study the importance of the selection of the right donor.

1.1 Blood cancer

1.1.1 Origin of the disease and treatments

Blood Cancer is a family of neoplastic diseases that involves blood cells in various ways. The event that starts cells' abnormal and uncontrolled proliferation is a mutation inside their DNA, which causes the change in their behaviour. Usually, this process starts in the bone marrow, where blood cells are produced[4].

This kind of mutation happens because of events that cannot be controlled by patients and physicians. Theories that explain why this type of genomic change happens are emerging and are one of the areas of focus of researchers in the healthcare field. Possible causes are age, gender, ethnicity, familiarity with disease, chemical exposure and other health conditions or treatments[5].

Blood cancer can also be categorized in different categories, depending on the cell type it affects, symptoms and the length of the illness. A distinction that is widely used in this field is the categorization of myeloma and lymphoma, whether the change in behaviour starts from lymphocytes or from bone marrow cells[6].

In addition, there are also other blood disorders that are not referred to as cancer but can be a source of danger for the patient, such as non-malignant disease, which might transition to malignant ones and other disorders like hemoglobinopathies[7].

Each of these different diseases has its own prognosis and treatments [27]. Symptoms are different and can include unexplained weight loss, bleeding, bruising, lumps or swellings, persistent infections, fever and different others.

Different treatments for blood cancer exist, but those usually depend on the kind of cancer the patient is fighting and his/her biological characteristics like age, gender, the pace at which the cancer is spreading and other features that are used to make a decision.

Possibilities include[28]:

- **Chemotherapy** uses anticancer drugs to block the uncontrolled proliferation of abnormal cells in the body. This therapy might be carried out either through pills or in veins.
- **Radiation therapy** aims at destroying cancer cells or relieving pain.
- **CAR-T** is a treatment in which T lymphocytes are engineered to fight against cancer in order to take back normal order cells' proliferation.
- **Immunotherapy**, similarly to CAR-T, cells of the immune system are helped through this treatment to fight cancer.
- **Stem Cell Transplantation**, stem cells donated by a compatible donor are infused into the recipient to restore the abnormal cells that were causing the disease. Those might be collected from bone marrow, peripheral blood and umbilical cord blood.

Patients might also undergo combinations of the previously listed treatments. Guidelines that are periodically published by the competent authorities of this field are used by physicians to decide which treatment is the best for each specific patient. Those guidelines are based on the latest discoveries made by researchers[8].

1.2 Stem Cell Transplantation

Some treatments consist in fighting cancer, but others aim to destroy neoplastic cells and replace them with healthy ones which come from the bone marrow of a donor that is said to be the best fit. Transplants are composed of five stages[9]:

- **Tests and exams** aim to assess the level of health of the patient to determine how severe the illness is.
- **Harvesting**, is the process during which stem cells are collected from the donor, whether it is the patient or not.
- **Conditioning** can consist of chemotherapy or radiotherapy or a combination of the two and is used to fight abnormal cells and prepare your body for the transplant.
- **Transplanting** is a phase of the process in which healthy stem cells are transplanted into the body of the recipient.

- **Recovery**, the recipient has to stay under observation for a period of time, which goes from weeks to months, in order to monitor his/her health condition.

Transplantation is an important treatment for ill people who are affected by blood cancer, and sometimes, it might be able to bring back patients to their original health status. On the other hand, this treatment comes with side effects that can manifest during or after the transplant, such as[8]:

- **Graft versus host disease (GvHD)** is an event during which the recipient's immune system recognizes transplanted cells as foreign and attacks them. This reaction can happen a few weeks after the transplant or also months or years later. Usually, symptoms are mild, but in some cases, they might also be life-threatening. Those usually include itchy rash, diarrhoea, dry eyes, and shortness of breath, among others. Some treatments shut down the immune system to stop the transplanted stem cells from attacking the rest of the body.

- **Reduced number of blood cells**: the idea is that, before the receiving cells of the bone marrow of the donor, diseased blood cells have to be destroyed by one of the treatments used in the conditioning phase. Damaged cells will then be replaced by the product of transplanted cells, even if this stage can take several weeks or more. Until that moment, the patient might be at risk of iron deficiency anaemia, excessive bleeding or bruising and infections, which are caused by the lack of red and white cells and platelets, respectively. The follow-up period at the hospital is key in this phase of the process because it prevents infections, and possible side effects can be treated as they emerge.

- **Chemotherapy's side effects** include feeling and being sick, loss of appetite, tiredness, and hair loss. Those are usually temporary and only last a few weeks, but also depend on the dose of chemotherapy. All these collateral effects are clearly explained to the patient before undergoing the treatment.

1.3 Importance of donor's selection

Outcomes of transplant-related mortality are influenced by donor-related issues and stem cell sources[9]. In part the result depends on the matching of the donor for HLA, which is known as the most polymorphic genetic region known in the human genome. On the other hand other factors such as stem cell source, donor age and gender and ABO compatibility may play a role in transplant outcome.

The donor can be the patient himself, and in this case, the transplant is referred to as autologous or can be another person with specific characteristics, and in this case, the transplant is allogeneic. Most relevant genes for transplantation are divided into:

- Class I, HLA-A, HLA-B, and HLA-Cw;
- Class II, HLA-DR, HLA-DQ, and HLA-DP.

A set of HLA gene alleles, called haplotype, is inherited from each parent; therefore, the probability that a child inherits and shares both parental haplotypes with a full sibling is 25%, and it is considered the optimal donor.

When an HLA-identical sibling is not available, unrelated donor registries are consulted in order to find an HLA-10/10 matched unrelated donor. In addition, previously mentioned characteristics are also considered, such as matching conditions for CMV, gender and ABO. The probability of finding a fully Matched Unrelated Donor (MUD) varies on average between 16% and 75% and depends also on ethnicity[10]. An increase in donor-recipient HLA disparity in Class I genes of HLA is associated with poorer outcomes after unrelated donor transplantation[11].

Usually, the choice of the donor depends on the severity of the illness and the availability of the right donor, and as the risk profile of the patient worsens, a wider degree of HLA mismatching is considered acceptable for the procedure to be carried out.

Patients lacking an HLA-matched sibling or unrelated donor have 3 different options for graft sources[12]:

- Partially HLA-mismatched unrelated adult donors;
- Unrelated donor umbilical cord blood;
- Partially HLA-mismatched or HLA-haploidentical, related donors.

An HLA-haploidentical donor is a related donor who shares exactly one HLA haplotype and differs by a variable number of HLA genes on the unshared haplotype.

Chapter 2

Materials and methods

2.1 Data Source

The study is based on electronic records retrieved from Karolinska's hospital database, called TakeCare][29], which is used by healthcare professionals to store details about every single visit regarding patients who are treated only in this specific centre. This information is also shared with the European Bone Marrow Transplant (EBMT) registry, which stores similar data from all adhering centres in Europe. In addition, this centre also receives patients from other cities in Sweden, as those centres might not have the capability to carry out specific kinds of treatments.

The database contains information about each visit and can refer to first visits, diagnosis or follow up after transplants. The result of each visit is stored in an electronic medical report, which is then used by the data manager to enter information into the database. This can refer to the overall health status of the patient, specific symptoms after the transplants, treatment prescribed to cure those symptoms and much more information coming from medical tests that are considered to be useful when looking at the medical history of that patient. Specific focus is brought on Graft versus host disease and results from blood sample analysis, in order to test if the hematopoiesis process of the patient is improving. Other important sources of information come from infection indicators contained in blood samples, which indicate possible infections during the recovery.

Information regarding other diseases is not considered in this database, but physicians are in charge of a specific patient are notified in order to provide help regarding the previous disease or also to take new diagnoses into account in the next visits regarding the original diagnosis.

The health status of each specific patient, more specifically referring to death, is updated at each specific follow-up visit or through other registries.

2.2 Study design and patient selection

2.2.1 Eligibility criteria

As this study is focused on the importance of the donor, autologous transplants were excluded because the donor is the patient him/herself, and only allogeneic transplants after 2014 were considered, as 2015 is the year when haploidentical transplants started to be considered a valid alternative, in Huddinge's centre, for patients who did not have a compatible donor.

On the other hand, in terms of patients' characteristics, there were no eligibility criteria as this study is also considered a quality assessment of the centre, which has never carried out this kind of analysis before. The hospital is public and so it cannot refuse to treat patients in need. The only patients excluded were the ones who refused to sign the agreement for treatment of their sensitive data. As the status of each patient in terms of characteristics of the disease can change over time, the data management section will explain how useful information for this study will be used.

Each patient that has been treated has been asked to sign an ethical permission to store his/her information in order to share it with the scientific community as a useful contribution towards the progress of treatments in this field. Only electronic data of those who agreed will be used in this study.

2.2.2 Data management

For each patient, there could be several records in the registry because of the several visits that are planned to assess the recovery of the patients who underwent transplantations. We decided to keep only patients who agreed to give access to their data and who have had their transplant from 2015.

Some of them might have had more than one transplant, but we will consider only the last one, as the outcome that we observe is the consequence of the last treatment. This is justified by the fact that the previous transplants are the cause of the last one, as it has been performed because the previous ones were not successful.

Relapse and Graft Versus Host Disease, both acute and chronic, will be considered as time dependent variable and so the date in which the event has been experienced for the first time will be stored for each patient and used as the start of the period during which that individual was considered exposed to the effect of that disease. For aGVHD the maximum grade of the disease will be considered for that patient.

At the end of the data management, the final sheet will contain one observation for each patient, containing information about if and when the specific event of interest was experienced.

2.3 Study Exposure

The study exposure was considered to be the kind of donor from which the recipient received stem cell source from, according to the European Bone Marrow Transplant definitions, which contained: Identical siblings, mismatched relatives and unrelated donors.

2.4 Study Covariates

Variables that were considered important in the definition of the outcome, and so that were to enter the analysis in order to produce a correct interpretation of the exposure of interest, were obtained from the database and were containing information about demographic characteristics, treatments received from the patient and following results from each single follow up visit.

In particular:

- **Stem cell source**, binary variable that indicates the source of the stem cells used in the transplants, coded as:
  - *'BM'*, which stands for bone marrow. This is the reference category;
  - *'PB'*, which stands for peripheral blood.
- Gender, a binary variable that refers to the patient's gender, is coded as:
  - *'Male'*, which is the reference level;
  - *'Female'*.
- **Acute Graft Versus Host Disease (aGVHD)** is a categorical variable and is considered ordinal, as its values refer to the severity of the disease. This event is referred to as 'acute' because it is usually diagnosed within 100 days of the transplant. It is coded as:

  - *'Absence'*, the patient has not experienced this event. This is the reference category.
  - *'Grade I-II'*, the patient has experienced this event and was classified by the doctor as grade one or two. Usually, patients with this grade are treated, but this represents a minor issue.
  - *'Grade III-IV'*, the patient has experienced this event and was classified by the doctor as grade three or four. Patients with this grade of the complication are at life threat and are treated immediately as this might be fatal.

- **Chronic Graft Versus Host Disease (cGVHD)** is a binary variable and is recorded as:

  - *'Absence'*, which is the reference category.
  - *'Presence'*.

  In this case, the categorization is tricky and does not reflect the real severity of the disease. Doctors have been reporting problems in describing the levels of this disease as it is highly subjective, and sometimes the affected organs cannot be inspected.

- **Relapse** is a binary variable and is recorded as:

  - *'Absence'*, which is the reference category.
  - *'Presence'*. Represents the experience of recurrence of the disease.

- **Age** is a continuous variable and represents the age of the patient at the moment of the transplant. In the analysis it might be considered to use a categorical alternative of this variable to accommodate for non-linearity in terms of the effect. Important in this phase will be to clearly divide pediatric patients from adult ones, and levels will be organized as:

  - *Pediatric cases,* two categories, 0-9 and 10-19;
  - *Adult cases,* ten years categories starting from 20 years old to more than 70: 20-30,31-40, 41-50,51-60,61-70,70+.

- **Malignancy** represents the kind of disease the patient was diagnosed with. Is a binary variable and is recorded as:

  - 'Non-malignant', which is the reference category.
  - 'Malignant'.

- **Donor**, specifies the relationship between the donor and the recipients and also its characteristics in terms of histocompatibility. This is a categorical variable and is coded as follow:
  - *'Identical sibling'*, is the reference category and is also the one that is associated with better outcomes both in terms of time and event.
  - *'Mismatched relative'*, is the level of interest and represents haploidentical donors, which means that the patient received stem cells from one of his relatives who matched half of his/her histocompatibility genes.
  - *'Unrelated'*, refers to patients whose donor was found in the registry of donors and has no familiar relationship with the recipients.

2.5 Study Outcomes

The main objective of this study was to compare survival, in years, among the three different groups of recipients based on their donors. There were no competing risks as we considered all kinds of deaths equally and not as competing events. Also, relapse was used as a predictor and not an event that impedes the one of interest. One-year, two-year survival and relapse-free survival were used in comparison with the other European centers as quality assessments of performances.

2.6 Statistical Analysis

2.6.1 Descriptive statistics

Age, the only continuous independent variable, was described using mean and standard deviation. In addition, considering that it will also be used as a categorical variable, percentages of Age categories will be reported. The outcome variable, Time was described using years as a time unit.

Categorical variables were described with percentages. Variables containing information about patients' specific kind of blood disorder, source of stem cells, type of donor and further complications such as GVHD or relapse were reported. Levels of variables were then stratified within the event outcome and compared to test the possible relationship between those levels and the outcome of interest. Chi-square tests were carried out to test the relationship between variables such as GVHD and the specific type of donor; in addition, the same was done to explore independence between complications of transplantations and stem cell source or malignancies.

One year, two years and overall survival were used to compare the performances of the centre with those of the other European countries. Relapse-free survival was also used to highlight possible differences with other clinics performing similar methodologies.

2.6.2 Survival Analysis

Cox regression models were fitted to model the time-to-event outcomes, in order to estimate Hazard Ratios for each level of the variables of interest, keeping as reference categories the ones reported in the 'Covariates' section.

Survival analysis, also referred to as time-to-event analysis, is used when the outcome is bidimensional and the user is not only interested in knowing which event each statistical unit experiences, but also how long it takes the patient to experience it. There are several models useful for studying this kind of outcome, and they are mainly divided into parametric and semi-parametric. The first mentioned come with robust and efficient estimates but also with strong assumptions, which might not hold, and that would drive to wrong inference.

The Cox model, a semi-parametric approach, is widely used thanks to its assumption, which allows the hazard to vary along the time period, but has to be proportional over time. Formally, the assumption can be represented as:

$$\frac{\lambda_1(t)}{\lambda_0(t)} = \theta$$

Where is the Hazard Ratio, which is the parameter that synthesizes the magnitude and the direction of the impact of the factor, which is the object of study. The previous expression explains that the hazard of the two groups of interest can vary, but the Hazard Ratio does not depend on time. A Cox regression model can be represented as:

$$\lambda_1(t) = \lambda_0(t) e^{\sum_j \beta_j X_j}$$

Where β refers to the parameter estimated for the j-th variable X. When the proportional hazard assumption does not hold, there are two possibilities to tackle this problem. The first one is to allow for time dependence for the variable that does not fulfill the assumption, while the second one consists in dividing the follow up in two different periods, within each of them the assumption is satisfied. Different approaches can be used to test this assumption and consist of the graphical representation of the hazards, expected survival time and residuals.

Other approaches are based on statistical tests but do not provide high statistical power even with a large sample size.

Moreover, there are variables that might change their values during the follow-up, and so will their effect. In order to properly estimate the impact of this variable, the dataset has to be restructured in the counting process form, so that each individual has an observation for each of his/her comorbidity status. In this case, aGVHD, cGVHD and Relapse are all time varying variables and the number of observations for each individual increases based on which event has been experienced.

Usually, adjusting for age in the time-to-event analysis is important because of the key role that this variable plays in increasing or decreasing hazard for a specific event. Even though adjusting for this variable is an obvious choice, it is easy to miss specify the model and use Age in the wrong way. Continuous variables should have a linear effect in this model, and this assumption is often not valid. Solutions might require introducing a non-linear term or dependence with time, which solves the problem of linearity but leads to loss of interpretability. Another possibility, which is widely used in observational studies such as this, is to use Age as a time scale, as patients are enrolled along time and not at a specific moment before the study starts and also because Age might greatly influence the likelihood of experiencing the event. In addition, creating categorical variables for each class of age allows for non-linearity, even though it also leads to loss of information; on the other hand, it is easier to interpret clinically.

The Cox regression model was initially fitted for each single variable to study the survival of each different group within each factor. In order to assess differences in survival the log-rank test was performed, as well as the Hazard Ratio calculation, in order to quantify the importance of levels of variables and to identify possible risk factors for patients.

The effect of the specific group of donors on the recipients' survival was assessed using the classical Hazard Ratio parameter, typical for time-to-event outcomes.

In addition, as the frequency of Graft versus Host Disease can depend on the donor, a mediation analysis was used to quantify the causal effect of the variable on the outcome and the mediated effect through the GVHD events.

Multivariate models were then fitted, adjusting for Gender, Stem cell source, Malignancies, aGVHD, cGVHD, Relapse and type of donor. Time-dependent variables were considered as such, and so for each patient, there might have been more than one observation, one window of time for each health status of the individual. Age was used in different ways in the model. First, it entered the model as a continuous variable, then it was used as a categorical variable according to methods developed in this specific field of blood disorders[13], and, in the end, it was used as a time scale of the model. In literature, multivariable models containing both chronic GVHD and acute GVHD have been discussed for a long time because of the tricky relationship between the two events. In order to use information from both variables, different models were fitted, one with both variables and the other two with only one of the Graft Versus Host Disease events[9].

In addition, as the original analyses included all patients with a diagnosis for blood disorders, both malignant and non-malignant, we decided to carry out an additional subanalysis in which only malignant cases were considered in order to evaluate the impact of the donor only in that subgroup of patients.

2.7 Mediation Analysis

Even though all the variables that are to enter the model are well studied, and their inclusion in the study prevents possible miss specification, relationships between some of them are difficult to understand from the clinical point of view, and so the interpretation that comes with the model's hampers.

In particular, as widely discussed in the literature[14], there is still uncertainty about the differences between the acute and chronic forms of GVHD; some doctors use clear definitions of the two in terms of time periods, as the acute one cannot manifest after 100 days of the transplant, on the other hand, there are other physicians that talk about a possible transition from the acute form to the chronic one. In addition, a not optimal choice in terms of donor might have implications on the probability of experiencing GVHD, which, in statistical terms, means that Graft Versus Host Disease might act as a mediator of the type of donor. The situation can be explained with Figure 2.1.

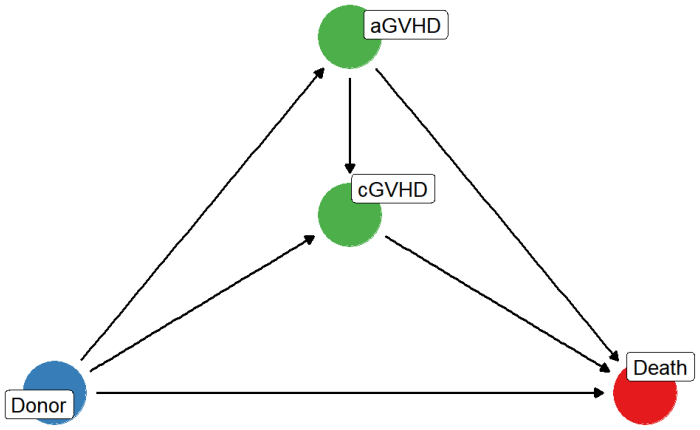

Figure 2.1, Relationship between Donor, GVHD complications and the outcome.

In this situation, in order to quantify the impact of the type of donor on the outcome of interest, a mediation analysis is required. A causal mediation analysis is a method that aims to decompose the effect of a factor into direct and indirect in order to draw conclusions about possible mediators in the model.

This analysis is characterized by the settings illustrated in Figure 2.2, a simplification of Figure 2.1, where there is a variable X, in this case, Donor, which relates with the outcome variable directly and through a mediator, which in this case is GVHD.

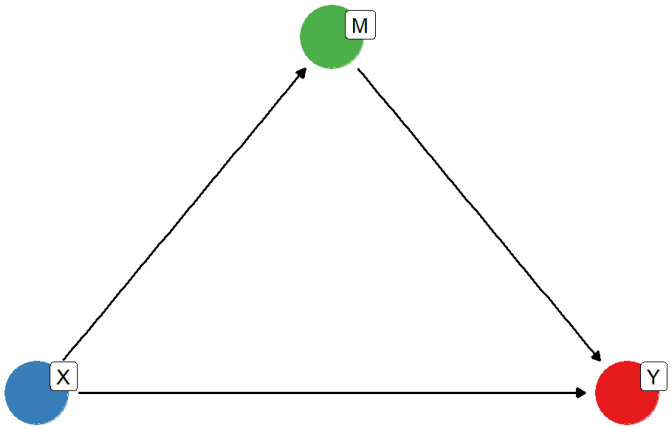

Figure 2.2,Typical settings of a mediation analysis.

In order for mediation analysis estimates to be interpreted as causal, and so not to be biased, the assumption of measured confounding with respect to the outcome, the mediator, and the treatment has to hold.

2.8 Diagnostics

The Cox regression model is based on the proportional hazard assumption, which, if it does not hold, drives estimates and conclusions that are wrong. In this case, as the model included time-varying covariates, this assumption does not hold, and the model is considered an extension of the original Cox regression. As a result, the validity does not need to be checked. On the other hand, as we are considering several models in which variables and their functional form are changing, it is useful to compare fitness indexes that are specifically thought of as tools for comparisons.

In this case, indexes of referral were log-likelihood and AIC index, which are obtained as follows. In addition, ROC curves at one and two years were obtained for each of the models and used as a measure for comparison[15].

Analyses were performed using R 4.3.0.

Chapter 3

Results

3.1 Descriptive Statistics

Between 1975 and 2023, more than two thousand patients received transplants at the Karolinska Institutet Hospital. From the first January of 2015, data chosen as the start of the study, 717 allogeneic treatments were carried out successfully on a total number of patients equal to 676. Some of these patients, as reported in Table 3.1, had one or more transplants in this period of time, but only the last one was selected for this study.

Characteristic	**N = 676**[1]
N.Transplants	
1	627 (93%)
2	45 (6.7%)
3	3 (0.4%)
4	1 (0.1%)

[1] n (%)

Table 3.1, Number of transplants that each patient has received.

Of those 676 patients, two died the day of the transplant and were deleted from the cohort of interest as their contribution to the analysis was null. The cohort used in the analysis was composed of 674 unique patients.

In Table 3.2, information about patients' characteristics and treatments is reported. Out of the 674 patients, 142 died, and 532 were alive at the time of the analysis.

Peripheral blood is used more frequently as a source of stem cells in allogeneic transplantations, even though there is not a clear association with the outcome[16]. Age had a median of 51 years (IQR [27;63]), while in the levels of Donor an important difference was reported among levels, as people in the 'Mismatched relative' group were younger than the other groups, with a median age of 20 [9;53]. This is explained by the fact that this procedure is usually carried out for pediatric cases. In the categorical form of the variable there is a clear increase in the number of people in the categories '51-60' and '61-70'.

The malignant disease with the highest number of patients was 'Acute Leukemia', with 276 patients, followed by MDS/MPN and Lymphoma, with 202 and 47, respectively. Within the Donor variable, unrelated donors were the ones present in higher measure, with 455 patients, nearly 68% of the total cohort, as expected because of the difficulties related to finding the right donor. On the other hand, the number of patients with an identical sibling donor was 138, while mismatched relatives were 81. Nearly 4% of the total experienced acute GVHD at 'Grade III-IV', while 17% were diagnosed with 'Grade I-II'. Indeed, patients with chronic GVHD were 176, 26% of the cohort. Lastly, 18% of the patients experienced relapse.

Variable	Overall, N = 674[1]	Identical Sibling, N = 138[1]	Mismatched Relative, N = 81[1]	Unrelated, N = 455[1]
Status				
Alive	532 (79%)	119 (86%)	62 (77%)	351 (77%)
Dead	142 (21%)	19 (14%)	19 (23%)	104 (23%)
Time	1.04 (0.29, 2.08)	1.10 (0.31, 2.97)	1.03 (0.28, 3.12)	1.04 (0.29, 2.05)
Age	51 (27, 63)	42 (15, 59)	20 (9, 53)	56 (38, 66)
Gender				
Female	274 (41%)	52 (38%)	29 (36%)	193 (42%)
Male	400 (59%)	86 (62%)	52 (64%)	262 (58%)
Malignant	546 (81%)	106 (77%)	56 (69%)	384 (84%)
Disease				
Acute Leukaemia	276 (41%)	66 (48%)	32 (40%)	178 (39%)
Bone Marrow Failure	32 (4.7%)	9 (6.5%)	5 (6.2%)	18 (4.0%)
Chronic Leukaemia	24 (3.6%)	5 (3.6%)	2 (2.5%)	17 (3.7%)
Hemoglobinopathies	14 (2.1%)	10 (7.2%)	4 (4.9%)	0 (0%)
Histiocytic Disorders	7 (1.0%)	1 (0.7%)	1 (1.2%)	5 (1.1%)
Inherited Disorders	39 (5.8%)	7 (5.1%)	12 (15%)	20 (4.4%)
Lymphoma	47 (7.0%)	10 (7.2%)	5 (6.2%)	32 (7.0%)
MDS/MPN	202 (30%)	25 (18%)	20 (25%)	157 (35%)
Plasma Cell Disorders	33 (4.9%)	5 (3.6%)	0 (0%)	28 (6.2%)
Stem Cell Source				
Bone Marrow	139 (21%)	41 (30%)	40 (49%)	58 (13%)
Peripheral Blood	535 (79%)	97 (70%)	41 (51%)	397 (87%)
aGVHD				
Absence	533 (79%)	112 (81%)	61 (75%)	360 (79%)
Grade I-II	116 (17%)	21 (15%)	13 (16%)	82 (18%)
Grade III-IV	25 (3.7%)	5 (3.6%)	7 (8.6%)	13 (2.9%)
cGVHD				
Absence	498 (74%)	85 (62%)	64 (79%)	349 (77%)
Presence	176 (26%)	53 (38%)	17 (21%)	106 (23%)
Relapse				
Absence	550 (82%)	116 (84%)	74 (91%)	360 (79%)
Presence	124 (18%)	22 (16%)	7 (8.6%)	95 (21%)
Age categories				
0-9	76 (11%)	19 (14%)	23 (28%)	34 (7.5%)
10-19	63 (9.3%)	21 (15%)	16 (20%)	26 (5.7%)
20-30	51 (7.6%)	15 (11%)	7 (8.6%)	29 (6.4%)
31-40	56 (8.3%)	12 (8.7%)	7 (8.6%)	37 (8.1%)
41-50	82 (12%)	17 (12%)	6 (7.4%)	59 (13%)
51-60	135 (20%)	30 (22%)	12 (15%)	93 (20%)
61-70	159 (24%)	21 (15%)	8 (9.9%)	130 (29%)
70+	52 (7.7%)	3 (2.2%)	2 (2.5%)	47 (10%)

[1] n (%); Median (IQR)

Table 3.2, Characteristics of patients, regarding variables of interest.

Overall survival and relapse-free survival were calculated using the Kaplan-Meier estimator and are plotted in Figure 3.1. One- and two-year survival were respectively equal to 0.83 (CI 95%: 0.80-0.86) and 0.73 (CI 95%: 0.69-0.77). These values are higher than the European mean of reference, reported by the EBMT, and this is explained by the quality of the treatments and the ratio of doctors and nurses that are available in this centre. The same is valid for the relapse-free survival, which has values of 0.75 (CI 95%: 0.72-0.79) and 0.67 (CI 95%: 0.63-0.72) after one and two years.

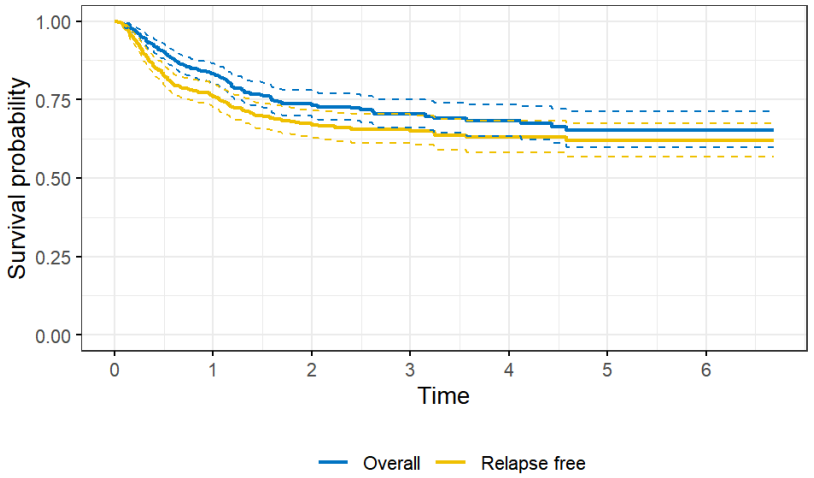

Figure 3.1, Overall and Relapse free survival during the six years follow-up.

3.1.1 Univariate analysis

Each variable that entered the model was fitted singularly against the bidimensional outcome and plotted using the Kaplan-Meier estimator to explore possible relationships and compare survival within levels of each factor. For aGVHD, cGVHD and Relapse, the dataset was restructured into the counting process format as these are time-dependent variables.

In Gender, no significant difference was found between survivals of 'Male' and 'Female', and the same was valid for Stem Cell Source, where the p-value from the log-rank test was not lower than 0.05, revealing no statistically significant difference between the 'Bone Marrow' and 'Peripheral Blood' group. On the other hand, as found in the literature[17] and illustrated in Figure 3.2, the specific source of stem cells chosen for the transplant might influence the outcome when specific conditions, such as having an 'Identical Sibling' as the donor in malignant cases, are met. In this specific case, there is a statistically significant difference between the two survival curves. The log-rank test provided a significant difference between the curves of malignancies against non-malignancies, with survival values lower for the first group mentioned, with a p-value of 0.01.

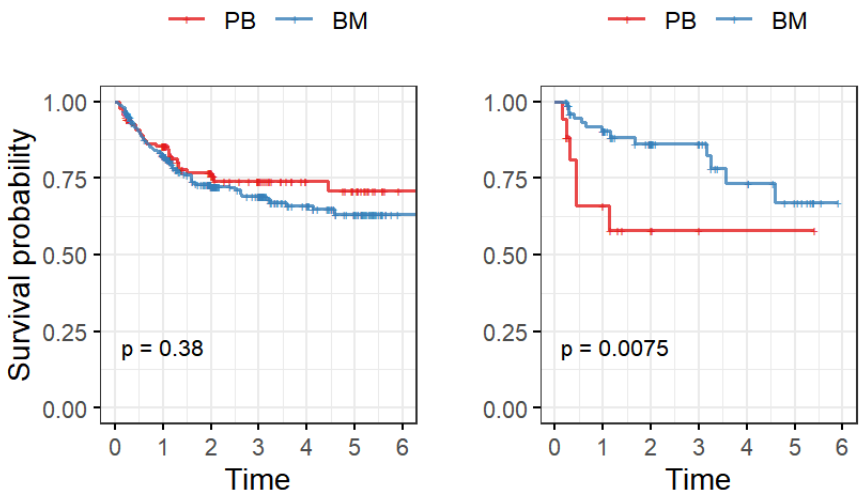

Figure 3.2, Importance of source of stem cells in two different scenarios.

The variable Age is continuous and cannot be fitted to a Kaplan-Meier curve if not categorized. In this study, it will be used both as a categorical and continuous variable, even though it might cause a loss of information; an univariate Cox model was fitted for the continuous form of the variable, and the Hazard Ratio, equal to 1.01 (CI 95% [1.003;1.019]), was found to be statistically significant, with a p-value lower than 0.01. Moreover, survival curves were fitted for the categorical form of Age.

The log-rank test gave a statistically significant result as older recipients had a lower survival than younger groups of patients. A curious result was found with chronic GVHD, where the presence appeared to be protective and statistically significant with respect to the outcome. This has been widely discussed in the HSCT field and is now accepted as it is a sign of a successful transplant[18]. On the contrary, this effect is no longer valid if only non-malignant cases are considered in the analysis, as Figure 3.3 shows.

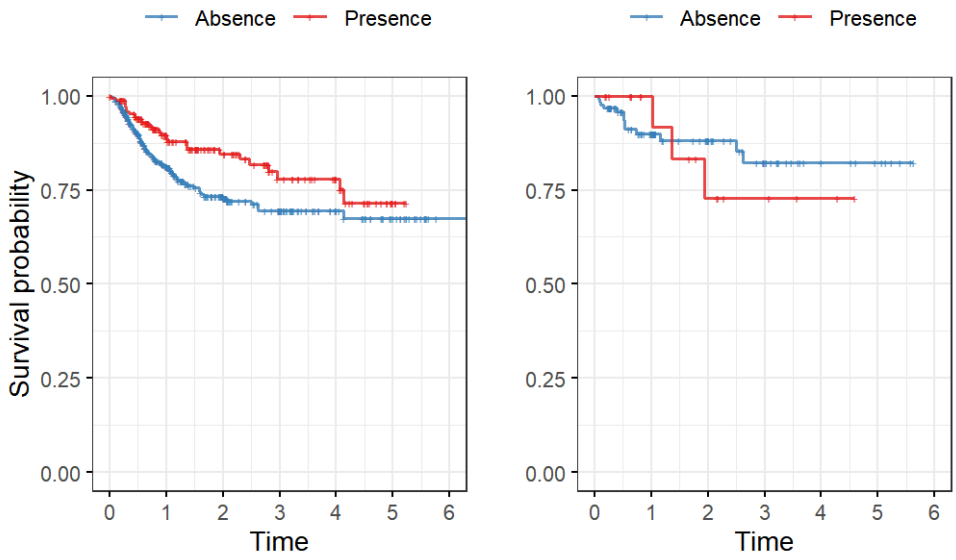

Figure 3.3, Effect of chronic GVHD on survival in pooled cases and in non-malignant ones.

A greater effect was found with the acute exacerbation of Graft Versus Host Disease, where different levels were grouped as suggested in literature into 'Grade I-II' and 'Grade III-IV'. 'Grade I-II' group did not show any difference with the curve that represents survival of patients who did not experience acute GVHD. On the contrary, the third level, the one that physicians use to treat and is considered life-threatening [19], is greatly associated with the outcome and has a lower survival curve, resulting in a p-value lower than 0.05. Similarly to aGVHD, but with a much greater negative effect, is Relapse, with a p-value lower than 0.001 and a survival curve that decreases quickly with the number of events, pointing out that people who have experienced a relapse are more likely to experience the outcome.

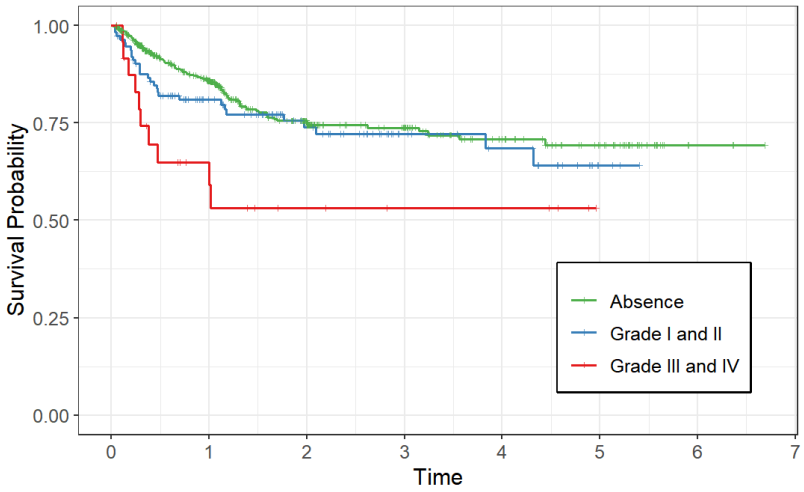

Figure 3.4, Survival stratified by aGVHD.

In conclusion, a survival curve for each of the three possible groups of donors, the variable of interest for this study, was obtained, and, as expected, identical siblings are the donors that have a higher survival compared to the other two groups, which are overlapping along the entire follow-up period. The log-rank test reported a p-value equal to 0.055.

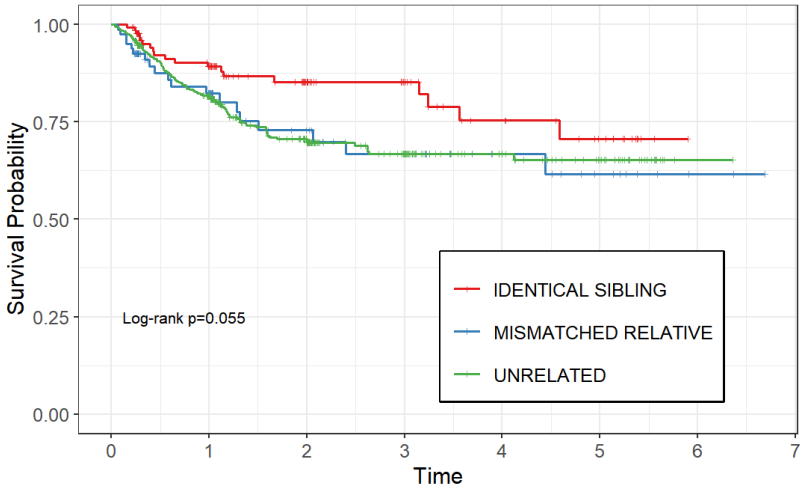

Figure 3.5, Survival stratified by type of donor.

3.2 Multivariate analysis

In this section are reported results of multivariate analysis with respect to the outcome, which intent is to determine the importance and the magnitude of each group of donors considered when a patient needs a transplant. Covariates and their relationships with the outcome and themselves are represented in Figure 3.6, where the Donor is considered to be the exposure, and all the other covariates are measured factors that might influence the event of interest. The gray node called U represents unmeasured variables, such as the genetic landscape and the availability of a specific donor or stem cell source, that might influence other factors in the analysis.

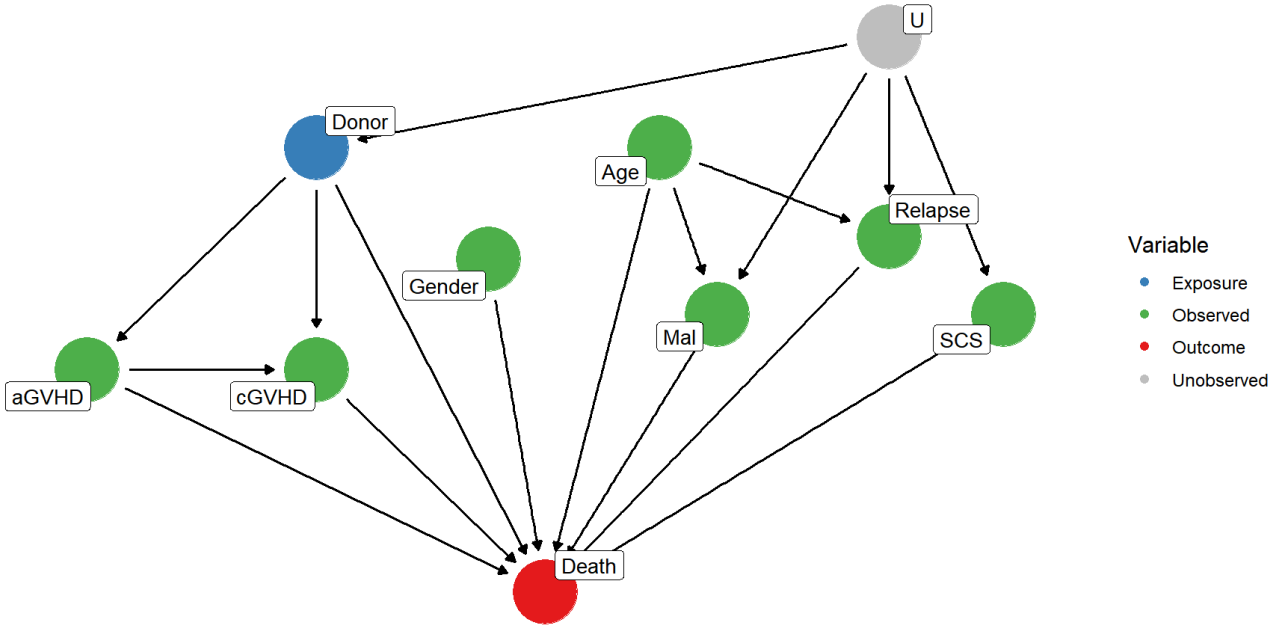

Figure 3.6, Relationship between variables that enter the model.

Attention should be focused on the complex relationship between Donor, aGVHD and cGVHD, which will be addressed later in this section in two separate analyses, in which these two complications will be considered first together and then separately. In conclusion, a mediation analysis will be carried out for each of the two events.

3.2.1 Adjusting for age

Age is a variable that has to be taken into account in this kind of analysis, in particular because, as shown in the univariate analysis, it becomes more likely to have such diseases as age increases and at the same time, because this is an observational study, in which such source of variability is difficult to control as patients enter the study at different time.

In order not to miss specify the model, there are different strategies that can be used to use Age properly. The first one is to adjust for it, entering that variable into the model, while the second one consists of using Age as a time scale; in conclusion, the third one consists in dividing Age into a categories. Figure 3.7 shows the difference in risk sets if we consider adjusting for age instead of using it as a time scale.

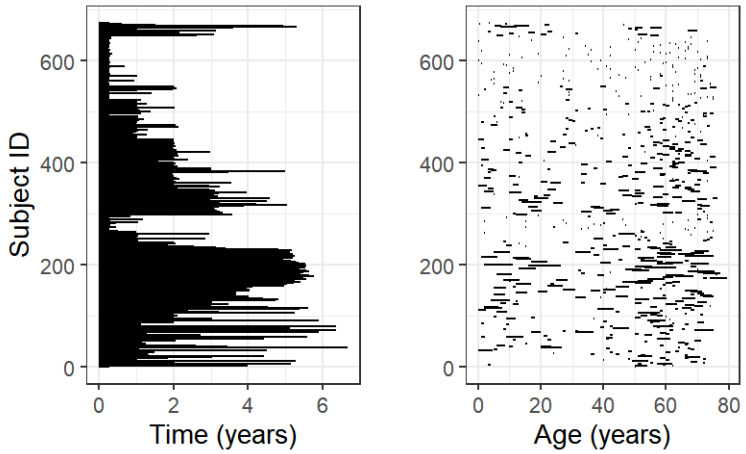

Figure 3.7, Difference in risk sets between adjusting for Age and using it as a time scale.

In order to evaluate the effect on the outcome of each variable and, in particular, the increase in hazard given by a specific kind of donor, all three models were carried out and are displayed in Table 3.3.

Results of the three models showed that there were no big differences between the three different specifications of the model. In all models, Relapse was the variable with the highest Hazard Ratio, with a value of 24.9 [17.1 ;36.3] in the model adjusted by Age and a value of 25.5 [17.6;37.2] in the one with Age as a time scale and p-value lower than 0.001, highly significant. This demonstrates that experiencing that event makes it more likely to experience the outcome of interest. The same can be said about aGVHD, where the Hazard Ratio for the 'Grade III-IV' level was equal to 5.82 [2.90;11.7] in the adjusted model and 5.81 [2.90;11.6] in the model

with a different time scale, and a p-value lower than 0.001. On the other hand, there was no statistical difference between the level 'Grade I-II' and the reference level 'Absence'. This confirms what was found in the univariate analysis, where the life-threatening level was the one with the lowest survival.

The other variables that entered the model, such as Gender, Age and Stem Cell Source, did not appear to influence the outcome in any of the three models, pointing out that these factors are not crucial in defining the outcome. Indeed, the variable Malignant, in the model where Age was specified as continuous, had a Hazard Ratio equal to 2.73 [1.52;4.89], and in all three models had an associated p-value lower than 0.001, indicating that a malignant diagnosis increases the hazard of experiencing death.

	Adj. Age			Age Time Scale			Age Categories		
Variable	**HR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**
Relapse									
Absence	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Presence	24.9	17.1, 36.3	**<0.001**	25.5	17.6, 37.2	**<0.001**	25.0	17.1, 36.7	**<0.001**
aGVHD									
Absence	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Grade I-II	1.32	0.85, 2.05	0.2	1.33	0.86, 2.07	0.2	1.40	0.90, 2.17	0.13
Grade III-IV	5.82	2.90, 11.7	**<0.001**	5.81	2.90, 11.6	**<0.001**	6.44	3.14, 13.2	**<0.001**
cGVHD									
Absence	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Presence	1.0	0.61, 1.63	>0.9	0.99	0.61, 1.62	>0.9	0.98	0.60, 1.61	>0.9
Malignant									
No	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Yes	2.73	1.52, 4.89	**<0.001**	3.00	1.73, 5.22	**<0.001**	2.81	1.52, 5.18	**<0.001**
Donor									
Identical Sibling	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Unrelated	1.62	0.96, 2.74	0.069	1.65	0.98, 2.76	0.059	1.58	0.92, 2.70	0.094
Mismatched Relative	2.01	1.00, 4.06	0.052	2.02	1.01, 4.02	**0.046**	2.24	1.11, 4.51	**0.024**
Stem Cell Source									
Peripheral Blood	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Bone Marrow	1.32	0.76, 2.30	0.3	1.21	0.72, 2.03	0.5	1.17	0.64, 2.12	0.6
Gender									
Male	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Female	1.07	0.76, 1.50	0.7	1.08	0.77, 1.52	0.6	1.11	0.79, 1.56	0.5
Age	1.00	0.99, 1.01	0.7						
Age categories									
0-9							1.00	Ref.	
10-19							1.23	0.52, 2.92	0.6
20-30							0.81	0.29, 2.29	0.7
31-40							0.95	0.34, 2.67	>0.9
41-50							0.86	0.35, 2.11	0.7
51-60							0.72	0.31, 1.67	0.4
61-70							1.23	0.52, 2.87	0.6
70+							1.32	0.52, 3.34	0.6

[1] HR = Hazard Ratio, CI = Confidence Interval

Table 3.3, Comparison of the three different models, where Age is specified in different forms.

In conclusion, the variable of interest Donor, showed that there was no clear difference between the reference level 'Identical sibling' and 'Unrelated', with a Hazard Ratio around 1.62 [0.96;2.74] in the model adjusted by Age and a p-value that higher equal to 0.069. On the other hand, the group 'Mismatched relative' had a Hazard Ratio equal to 2.01 [1.00;4.06], 2.02 [1.01;4.02] and 2.24 [1.11;4.51] in the three models. The three respective p-values are all near the significance level 0.05, and the parameter becomes lower than that value in the models where age is the time scale or is categorized, with a value of 0.046 and 0.024. Even though the p-value is not particularly lower than the significance level, this has to be seen as the confirmation that identical sibling or unrelated donors with a higher match should

always be preferred, as the estimate of the Hazard Ratio has a value of two in all three models, meaning that this might greatly influence the outcome.

Measure	Adj. Age	Age Time Scale	Age Categories
AIC	1,624	625	1,629
Log Likelihood	-803	-305	-800
AUC 1 year	0.86	0.86	0.87
AUC 2 years	0.83	0.83	0.84

Table 3.4, Measures of goodness of fit for the three different models.

In terms of goodness of fit, there is a clear improvement in AIC and log-likelihood for the model where Age is considered as the time scale, probably because there is one less variable to take into account, and so the model is more parsimonious. Indeed, in terms of AUC, there is no clear difference between the models.

3.2.2 GVHD

In the HSCT field, a valid and accepted motivation for the selection of the best donor is the likelihood of Graft Versus Host Disease that a patient might experience because of the mismatch between recipients and donors. On the contrary, the link between acute and chronic GVHD and a possible transition from the first to the second form is not clear in the literature. As a result, only one of the two usually enters the model, and this decision is based on clinical reasons. In order to explore the behaviour of the variables of interest, a model with both events, a model with only aGVHD, and, at last, a model with only cGVHD were carried out and compared. Results are shown in Table 3.5.

With respect to the model with both GVHDs, the one containing only cGVHD reports lower Hazard Ratios for age categories, while it increases for Gender, Stem Cell Source and Malignant. Whereas the HR for Relapse decreases. Moreover, the Hazard Ratio for the 'Presence' level of cGVHD increases from 0.98 [0.60;1.61] to 1.17 [0.73;1.90] but remains not statistically significant. On the contrary, the estimate of the 'Mismatched relative' level increases from 2.24 [1.11;4.51] to 2.35 [1.17;4.71], with a 13% increase of hazard, and so the p-value lowers to 0.016.

	Both GVHDs			aGVHD			cGVHD		
Variable	**HR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**
Relapse									
Absence	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Presence	25.0	17.1, 36.7	**<0.001**	25.1	17.1, 36.7	**<0.001**	23.5	16.1, 34.2	**<0.001**
aGVHD									
Absence	1.00	Ref.		1.00	Ref.				
Grade I-II	1.40	0.90, 2.17	0.13	1.40	0.90, 2.16	0.13			
Grade III-IV	6.44	3.14, 13.2	**<0.001**	6.40	3.17, 12.9	**<0.001**			
cGVHD									
Absence	1.00	Ref.					1.00	Ref.	
Presence	0.98	0.60, 1.61	>0.9				1.17	0.73, 1.90	0.5
Malignant									
No	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Yes	2.81	1.52, 5.18	**<0.001**	2.80	1.52, 5.16	**<0.001**	2.72	1.48, 5.01	**0.001**
Donor									
Identical Sibling	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Unrelated	1.58	0.92, 2.70	0.094	1.59	0.94, 2.67	0.083	1.70	0.99, 2.91	0.053
Mismatched Relative	2.24	1.11, 4.51	**0.024**	2.25	1.13, 4.49	**0.022**	2.35	1.17, 4.71	**0.016**
Stem Cell Source									
Peripheral Blood	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Bone Marrow	1.17	0.64, 2.12	0.6	1.17	0.65, 2.12	0.6	1.28	0.72, 2.30	0.4
Gender									
Female	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Male	0.90	0.64, 1.27	0.5	0.90	0.64, 1.27	0.5	0.93	0.66, 1.30	0.7
Age categories									
0-9	1.00	Ref.		1.00	Ref.		1.00	Ref.	
10-19	1.23	0.52, 2.92	0.6	1.23	0.52, 2.92	0.6	1.09	0.46, 2.59	0.8
20-30	0.81	0.29, 2.29	0.7	0.81	0.29, 2.29	0.7	0.82	0.29, 2.31	0.7
31-40	0.95	0.34, 2.67	>0.9	0.95	0.34, 2.67	>0.9	0.96	0.35, 2.66	>0.9
41-50	0.86	0.35, 2.11	0.7	0.86	0.35, 2.09	0.7	0.78	0.32, 1.89	0.6
51-60	0.72	0.31, 1.67	0.4	0.72	0.31, 1.67	0.4	0.74	0.32, 1.71	0.5
61-70	1.23	0.52, 2.87	0.6	1.22	0.52, 2.85	0.6	1.14	0.49, 2.64	0.8
70+	1.32	0.52, 3.34	0.6	1.31	0.52, 3.32	0.6	1.26	0.50, 3.19	0.6

[1] HR = Hazard Ratio, CI = Confidence Interval

Table 3.5, Comparisons of three different models where aGVHD and cGVHD are included and then used separately.

Lastly, in the model where only aGVHD is considered, the same conclusions drawn in the model where both GVHDs are present are valid, as all Hazard Ratios remain the same as before and so do all p-values and the respective choices in inferential terms.

Measure	Both GVHD	aGVHD	cGVHD
AIC	1,629	1,630	1,637
Log Likelihood	-800	-801	-804
AUC 1 year	0.87	0.84	0.84
AUC 2 years	0.84	0.81	0.82

Table 3.6, Measures of goodness of fit for the three different models, considering different GVHD events.

Table 3.6 shows that there is not an important difference between the three models considering different combinations of GVHD events, both in terms of AUC or goodness of fit of the two different indexes used.

3.2.3 Subanalysis within Malignant Cases

In the cohort used for the analysis, patients who have received a diagnosis for a disease belonging to the family of blood disorders are also comprehended, meaning that there might be patients who are not at life risk but need a transplant to prevent a possible progression of the disease. In order to explore variables' behaviour when only malignant cases are considered, the analyses were performed within malignancies. The same three models from the previous section were considered and repeated.

Restricted the cohort to those patients with malignancies (N=546), our results showed differences in the magnitude of variables but not in their direction. In particular, Relapse has seen its Hazard Ratio increase from 25 to almost 30 in all three models. The same happened to 'Grade III-IV' for aGVHD and for Age categories, where estimates increased significantly, even though no p-value reached a value lower than 0.05. On the contrary, Hazard Ratios of cGVHD, Stem Cell Source and Gender remained stable and did not reach statistical significance.

Indeed, Hazard Ratio for 'Mismatched relative' increased slightly even though its p-value lowered, while for 'Unrelated' estimates decreased remaining non significant. Results might be influenced by the statistical power which is lower than the previous analysis, considering there were one hundred less patients.

	Both GVHDs			aGVHD			cGVHD		
Variable	**HR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**
Relapse									
Absence	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Presence	30.2	20.0, 45.6	**<0.001**	30.3	20.1, 45.7	**<0.001**	27.4	18.4, 40.9	**<0.001**
cGVHD									
Absence	1.00	Ref.					1.00	Ref.	
Presence	0.94	0.56, 1.59	0.8				1.07	0.64, 1.78	0.8
aGVHD									
Absence	1.00	Ref.		1.00	Ref.				
Grade I-II	1.22	0.76, 1.96	0.4	1.21	0.76, 1.95	0.4			
Grade III-IV	8.40	3.91, 18.0	**<0.001**	8.28	3.89, 17.6	**<0.001**			
Donor									
Identical Sibling	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Unrelated	1.28	0.74, 2.21	0.4	1.30	0.77, 2.20	0.3	1.35	0.78, 2.33	0.3
Mismatched Relative	2.57	1.17, 5.61	**0.018**	2.60	1.20, 5.64	**0.016**	2.53	1.15, 5.54	**0.020**
Stem Cell Source									
Peripheral Blood	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Bone Marrow	1.02	0.52, 2.00	>0.9	1.03	0.53, 2.01	>0.9	1.11	0.57, 2.16	0.8
Gender									
Female	1.00	Ref.		1.00	Ref.		1.00	Ref.	
Male	0.98	0.68, 1.41	0.9	0.97	0.68, 1.40	0.9	1.00	0.69, 1.43	>0.9
Age categories									
0-9	1.00	Ref.		1.00	Ref.		1.00	Ref.	
10-19	3.13	0.86, 11.3	0.082	3.13	0.86, 11.3	0.082	2.61	0.73, 9.35	0.14
20-30	1.33	0.32, 5.48	0.7	1.34	0.33, 5.49	0.7	1.34	0.33, 5.47	0.7
31-40	1.75	0.43, 7.20	0.4	1.75	0.43, 7.19	0.4	1.76	0.43, 7.15	0.4
41-50	1.29	0.34, 4.97	0.7	1.28	0.33, 4.90	0.7	1.12	0.29, 4.28	0.9
51-60	1.12	0.31, 4.01	0.9	1.12	0.31, 4.00	0.9	1.15	0.32, 4.06	0.8
61-70	2.13	0.60, 7.61	0.2	2.12	0.59, 7.56	0.2	1.88	0.53, 6.66	0.3
70+	2.27	0.60, 8.52	0.2	2.25	0.60, 8.45	0.2	2.08	0.55, 7.81	0.3

[1] HR = Hazard Ratio, CI = Confidence Interval

Table 3.7, Comparison of the three different models containing different GVHDs, within malignancies.

3.3 Mediation Analysis

Selection of the best donors is key for this kind of disease, in particular, to prevent events such as Graft Versus Host Disease, and because of this, it is possible to see the effect of a non-optimal choice on recipients. As shown in Figure 3.6, a specific pattern relates Donor, the exposure of interest, to GVHD events of both kinds, whether acute or chronic. This relationship can be isolated as Figure 3.8 illustrates, where Donor is the variable of interest, and part of its effect on the outcome is mediated through GVHDs.

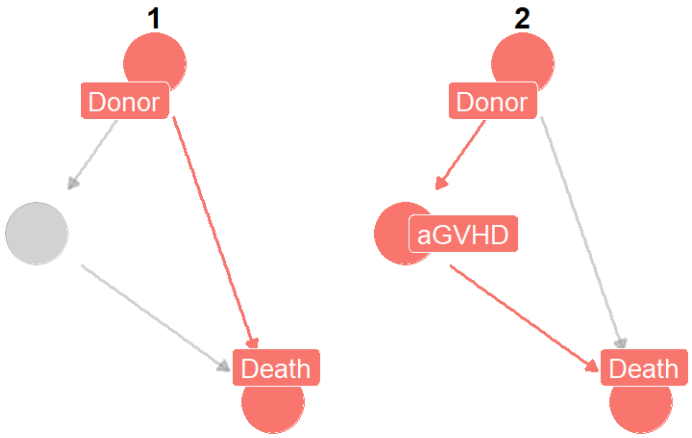

Figure 3.8, Mediation pathway between Donor, acute GVHD and Death.

In order to evaluate this effect, it is possible to perform a Mediation Analysis so that we can define if part of the effect of the exposure is expressed through the events that are caused by a mismatch between the donor and recipient. Two cases were evaluated, where each event was considered separately, as settings with multiple mediators are difficult to consider, both clinically and statistically speaking. Also, the relationship between the acute and chronic exacerbation of Graft Versus Host Disease is not well established, so it is difficult to study such cases.

In the first case, aGVHD had to be converted into a dichotomous variable because the first part of the mediation analysis, which consists of logistic regression, needs the mediator to be coded as a binary variable. As learnt from the previous analysis, as level 'Grade I-II' was not significantly different from 'Absence', these two levels were merged together. In this first part of the analysis the exposure does not provide a p-value lower than 0.05, which tells that the effect of Donor is not consistently mediated through aGVHD.

Table 3.8 shows the output of the first two parts of mediation analysis, where the survival output is almost identical to the one shown in the previous section; few differences are highlighted in the Hazard Ratios because aGVHD has no more than three levels.

	Logistic			Survival		
Variable	**OR**[1]	**95% CI**[1]	**p-value**	**HR**[1]	**95% CI**[1]	**p-value**
Mismatched Relative	2.02	0.59, 7.31	0.3	2.21	1.10, 4.43	**0.025**
Relapse	0.62	0.10, 2.20	0.5	15.0	10.5, 21.6	**<0.001**
Malignant	1.16	0.37, 4.26	0.8	2.78	1.53, 5.06	**<0.001**
Unrelated	0.92	0.33, 2.94	0.9	1.61	0.96, 2.69	0.070
Stem Cell Source	1.96	0.68, 5.61	0.2	1.15	0.64, 2.06	0.6
Gender	1.09	0.48, 2.43	0.8	1.13	0.80, 1.59	0.5
10-19	0.54	0.07, 3.02	0.5	1.14	0.48, 2.71	0.8
20-30	1.70	0.28, 9.58	0.5	0.74	0.26, 2.08	0.6
31-40	1.64	0.26, 9.74	0.6	0.86	0.31, 2.37	0.8
41-50	0.35	0.02, 2.85	0.4	0.74	0.30, 1.78	0.5
51-60	1.46	0.33, 7.33	0.6	0.64	0.28, 1.46	0.3
61-70	1.00	0.19, 5.53	>0.9	0.94	0.40, 2.16	0.9
70+	0.56	0.02, 5.21	0.6	1.25	0.50, 3.14	0.6
aGVHD				4.37	2.25, 8.47	**<0.001**

[1] OR = Odds Ratio, CI = Confidence Interval, HR = Hazard Ratio

Table 3.8, First two steps of the mediation analysis with aGVHD.

Specific to this kind of analysis is the division of the total effect into the direct and indirect part. In this case, Table 3.9 shows that the mediated percentage of the total effect of the exposure that is expressed through the mediator is equal to 0.10, which means that 10% of the total effect of Donor is mediated by aGVHD. However, this estimate is not statistically significant (p-value = 0.4), meaning that this 10% is not significantly different from zero. We can conclude that aGVHD is part of the pathway and behaves as a mediator, even though the mediated percentage of the effect is not consistent. As shown in the survival analysis output, the total direct effect is statistically significant, meaning that Donor has an influence on the outcome.

Variable	Est.[1]	95% CI[1]	p-value
CDE	0.79	0.1, 1.49	**0.025**
PNDE	0.79	0.1, 1.49	**0.025**
TNIE	0.06	-0.08, 0.2	0.4
TNDE	0.79	0.1, 1.49	**0.025**
PNIE	0.06	-0.08, 0.2	0.4
TE	0.85	0.14, 1.56	**0.019**
PM	0.10	-0.13, 0.32	0.4

[1] Estimate of mediation analysis, CI = Confidence Interval

Table 3.9, Decomposition of the total effect of Donor, considering aGVHD as the mediator.

Indeed, considering cGVHD instead, we can see that there is a relationship between unrelated donors and patients with chronic GVHD, as the p-value is lower than 0.002. In this case the output of the survival analysis is identical to the previous one as levels of the variables of interest remained the same. In addition, aGVHD was considered a binary variable, not time-varying, to adjust for its effect, considering the possible relationship between the two events.

	Logistic			Survival		
Variable	OR[1]	95% CI[1]	p-value	HR[1]	95% CI[1]	p-value
Mismatched Relative	0.64	0.33, 1.20	0.2	2.04	1.02, 4.08	**0.044**
Relapse	0.81	0.47, 1.34	0.4	15.0	10.5, 21.5	**<0.001**
aGVHD	2.49	1.21, 4.93	**0.010**	2.86	1.45, 5.63	**0.002**
Malignant	1.50	0.87, 2.71	0.2	2.75	1.50, 5.04	**0.001**
Unrelated	0.54	0.37, 0.80	**0.002**	1.48	0.87, 2.50	0.15
Stem Cell Source	0.90	0.49, 1.62	0.7	1.17	0.66, 2.09	0.6
Gender	0.99	0.70, 1.38	>0.9	1.08	0.77, 1.52	0.7
10-19	0.97	0.37, 2.55	>0.9	1.11	0.47, 2.63	0.8
20-30	1.48	0.56, 4.04	0.4	0.75	0.27, 2.09	0.6
31-40	2.03	0.81, 5.36	0.14	0.87	0.31, 2.38	0.8
41-50	1.96	0.82, 4.97	0.14	0.74	0.31, 1.79	0.5
51-60	1.80	0.77, 4.48	0.2	0.66	0.29, 1.52	0.3
61-70	1.74	0.74, 4.35	0.2	0.95	0.41, 2.20	>0.9
70+	0.81	0.25, 2.50	0.7	1.25	0.50, 3.15	0.6
cGVHD				0.67	0.43, 1.05	0.080

[1] OR = Odds Ratio, CI = Confidence Interval, HR = Hazard Ratio

Table 3.10, First two steps of the mediation analysis with cGVHD.

While in the second part of the mediation analysis, as obtained for aGVHD, the total direct effect is statistically significant and so significantly different from zero, but the mediated percentage of cGVHD is equal to 3% with a p-value of 0.3, meaning that the effect of Donor is not mediated through cGVHD.

Variable	Est.[1]	95% CI[1]	p-value
CDE	0.71	0.02, 1.41	**0.044**
PNDE	0.71	0.02, 1.41	**0.044**
TNIE	0.02	-0.01, 0.04	0.3
TNDE	0.71	0.02, 1.41	**0.044**
PNIE	0.02	-0.01, 0.04	0.3
TE	0.73	0.03, 1.42	**0.040**
PM	0.03	-0.03, 0.08	0.3

[1] Estimate of mediation analysis, CI = Confidence Interval

Table 3.11, Decomposition of the total effect of Donor, considering cGVHD as the mediator.

Chapter 4

Discussion

In this prospective study of a patient cohort (n=674) with a blood disorder diagnosis and a transplant received between 2015 and 2023, where both malignant and non-malignant cases were included, recipients with a Mismatched relative or Unrelated as a donor had lower survival than patients who received stem cells from an Identical Sibling. All patient included in the study signed an ethical agreement regarding the treatment of their sensitive data.

Overall survival and relapse-free survival rates were found to be higher than the other European centres, explained by the higher ratio of healthcare professionals that follow patients during the stages that are immediately after the transplant.

The multivariate analysis was adjusted for other factors considered important in the HSCT field, such as demographic characteristics, transplant characteristics and follow-up complications, like Graft Versus Host Disease events and recurrences of the neoplastic disease. Different approaches were performed and compared in order to take into account the right specification for Age, given the importance it has in retrospective studies in which it plays a key role in determining the hazard with respect to the outcome.

Results highlighted a statistically significant Hazard Ratio equal 2.24 [1.11;4.51] for patients belonging to the Mismatched Relative group, while the estimate for Unrelated recipients was equal to 1.58 [0.92;2.70], pointing out that the increase in hazard was not significantly different from zero. However, the European Group for Blood and Marrow Transplantation (EBMT), the organization where all researchers of the HSCT field share results of new groundbreaking studies, highlights the importance of finding the best fit for each patient to prevent life-threatening complications [9].

This result can be explained by the power of the study. Given that the aim of the study is to highlight possible differences in hazards between the best fit in terms of the Human leukocyte antigens (HLA), which lie in Identical Siblings, the two other groups were pooled together, and the statistical power was calculated. The total number of events registered, which was 142, ensured a power equal to 74%. On the other hand, in order to reach a satisfying statistical power (i.e. 80%), the number of events registered should have been at least 201, almost half more than the registered ones.

As widely explained in the EBMT handbook [9], the importance of donor selection is well established, and this study confirms it, but relationships with other factors that might influence the outcome of the transplantation are still the subject of debate.

Nevertheless, the best fit in terms of the donor is not only based on HLA but also on other biological characteristics of the donor. In particular, a study based on the National Marrow Donor Program (NMDP)[20], based on an unrelated registry, highlighted the importance of selecting a donor whose age is between 18 and 30 as recipients receiving stem cells from this group will have a survival that is significantly higher than those who received haematopoietic cells from older volunteers. In addition, from the same study emerged that selecting younger donors might lower the incidence of Graft Versus Host Disease. On the other hand, ethnicity matching was also tested but results did not provide differences that were statistically significant between the different groups.

In addition, in this study, diagnoses were divided into malignant and non-malignant, while specific diseases were not taken into account because of the sample size, which would have lowered dramatically. Other studies that focused on specific categories of diseases, such as acute leukaemia, showed different results regarding donor selection as the cause of complications compared to what was found in this study. First, a Japanese study[21] conducted on patients affected by acute leukaemia showed a protective effect of Grade I-II of acute GVHD. This result can be replicated in our cohort but does not reach a significant effect, probably due to the sample size, as one of the studies mentioned was nearly seven thousand. Positive effects of this grade of acute GVHD complication were highlighted on treatment failure, overall mortality, relapse and non-relapse mortality. Second, a retrospective study from the US[22] reported that, in patients with acute leukaemia, the type of donor was not influencing the effect and suggested performing transplantation as soon as any donor becomes available.

We conclude that findings might be difficult to compare because of the differences in the subject of the study, focusing on a specific disease instead of the entirety of malignant cases might cause results to differ.

In conclusion, this study confirms the importance of selecting the right donor but finds in the sample size the biggest limit, as the power of the statistical analysis results moderate to test our hypothesis. At the same time results would be difficult to interpret on specific types of diseases because of the low number of patients belonging to that group. On the other hand, the strengths of this study are the ones that reflect the points that make Huddinge's Centrum one of the best hospitals for the treatment of these disorders. As an example, the number of follow-up visits and their frequency help in conducting patients to their original health status, minimizing the possibility of experiencing death or complications attributable to poor care after receiving the treatment. Moreover, the availability of an electronic registry that is well updated, such as TakeCare, minimizes the differences between the real date of the emergence of complications and the one that is recorded, a factor that is crucial when using time-varying variables, as their estimated parameter might be underestimated. Furthermore, another strength is the fact that all variables that are considered to be time-dependent were considered as such, while in different studies, this has been debated for a long time since dates are not always recorded for this event, and the right importance of this specific topic was not seriously addressed, even though it consisted in miss specifying the model.

In the end, another possible limitation is the possible role that the genetic landscape of each neoplastic disease plays in determining the response to the treatment[23].

A suggestion for the future would be to include in the study more than one medical centre that carries out the same procedures, possibly with the same clinical rationale, in order to be comparable. This would also permit us to focus on specific groups of diseases which are the ones of more interest, such as acute leukaemia, in order to compare results and performances with other centres, with the aim of benchmarking.

Chapter 5

Conclusions

The study we conducted on 674 patients with a diagnosis of blood disorders highlighted the importance of selecting the donor that best matches HLA's characteristic of the recipients, even though performances of the centre are better when compared to other European units.

On the other hand, several other complications might greatly affect the outcome of the transplants, positively or negatively, and those are highly specific to the disease and might also be a consequence of the donor that was chosen. More factors related to the genetic landscape are also considered responsible for the progression of the disease, and these connections are not well established yet.

Chapter 6

Appendix

6.1 Figures

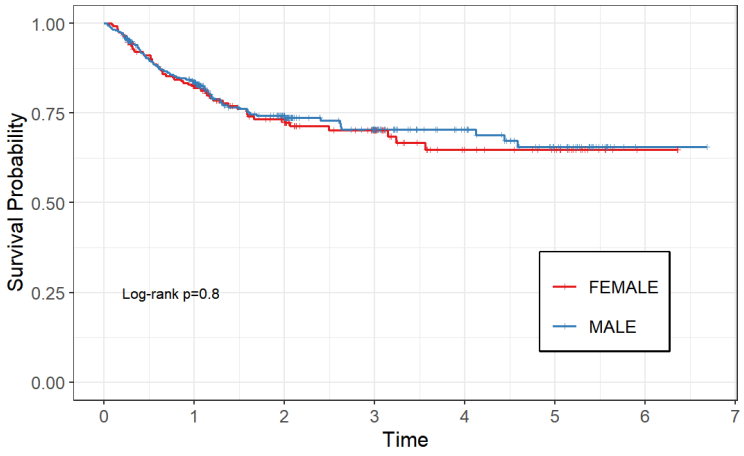

Figure A.1, Univariate Kaplan-Meier for Gender.

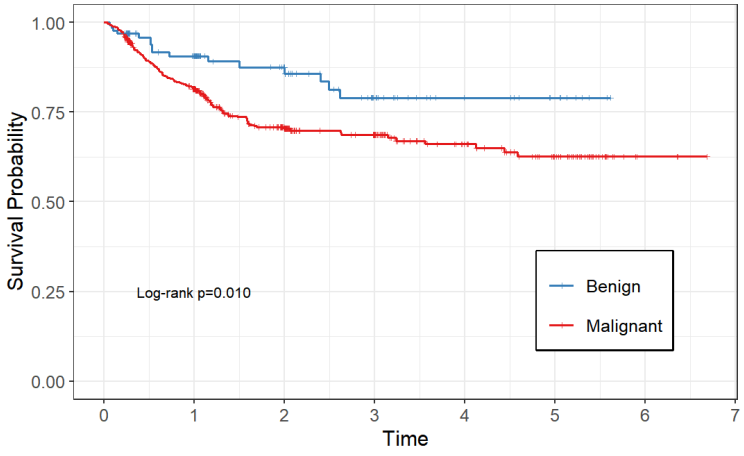

Figure A.2, Univariate Kaplan-Meier for Malignancy.

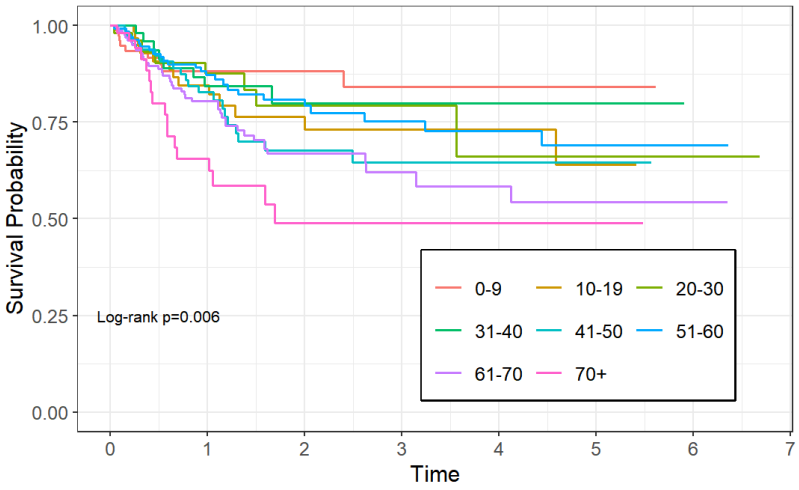

Figure A.3, Univariate Kaplan-Meier for Age categories.

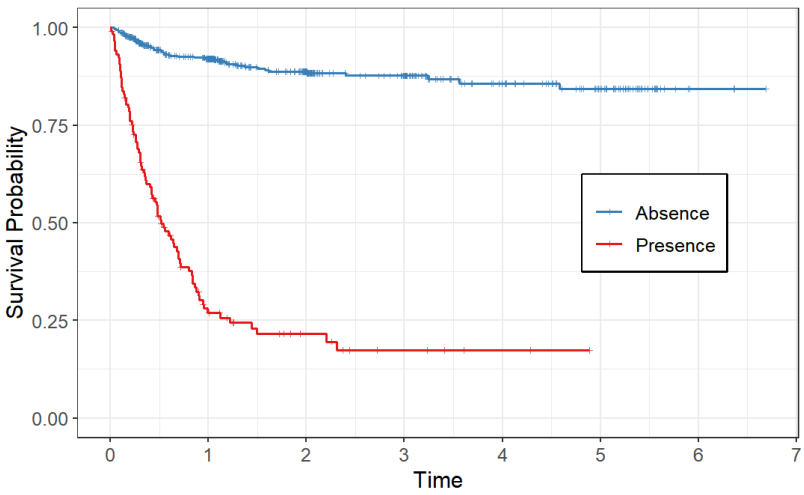

Figure A.4, Univariate Kaplan-Meier for Relapse.

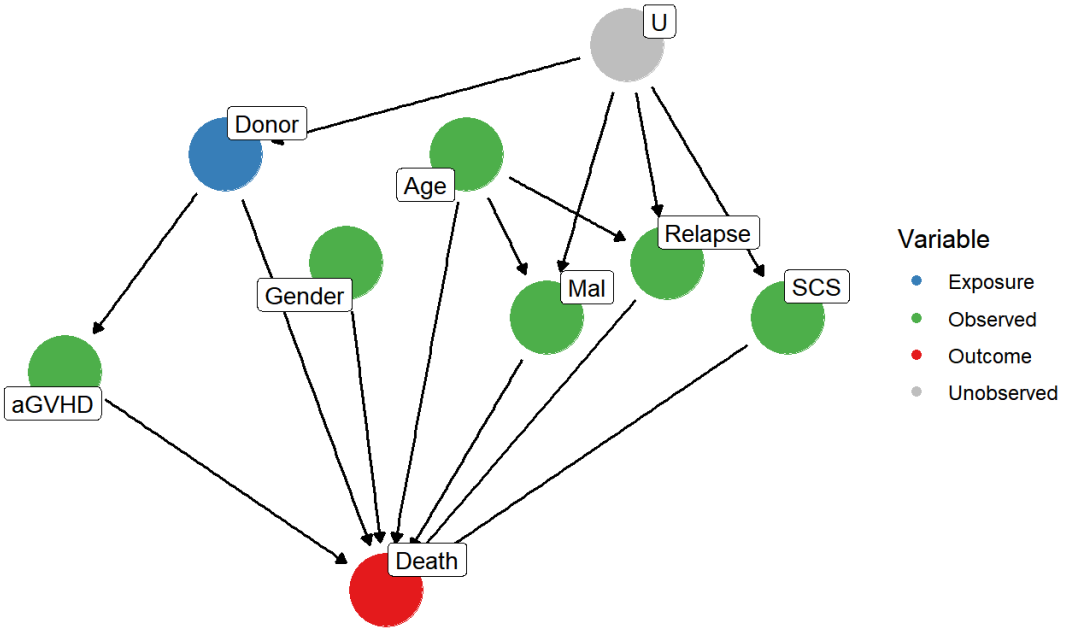

Figure A.5, Relationships between variables in the model including only aGVHD.

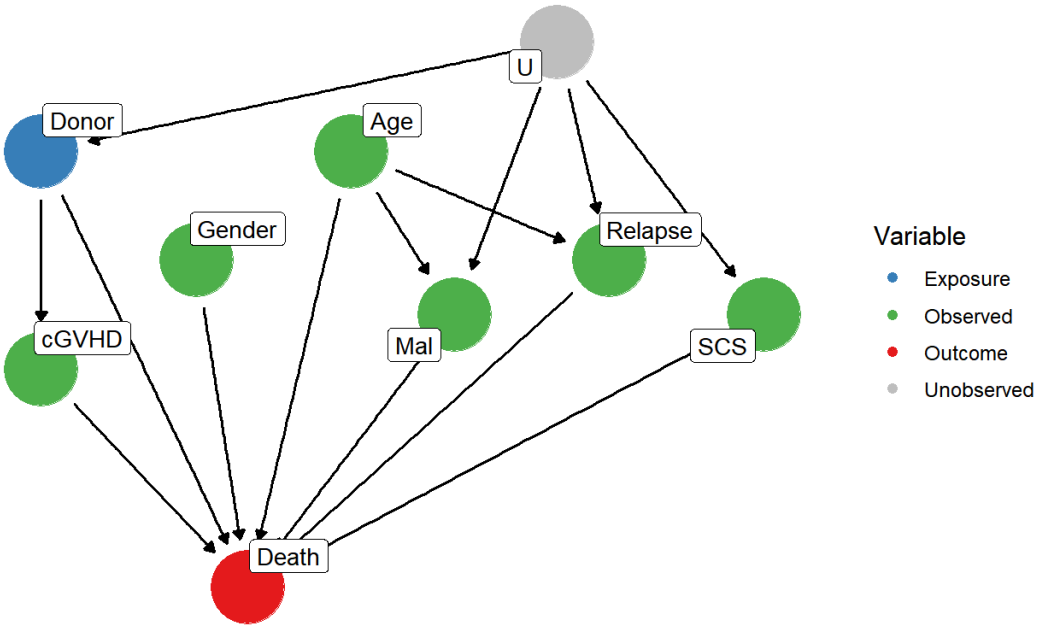

Figure A.6, Relationships between variables in the model including only cGVHD.

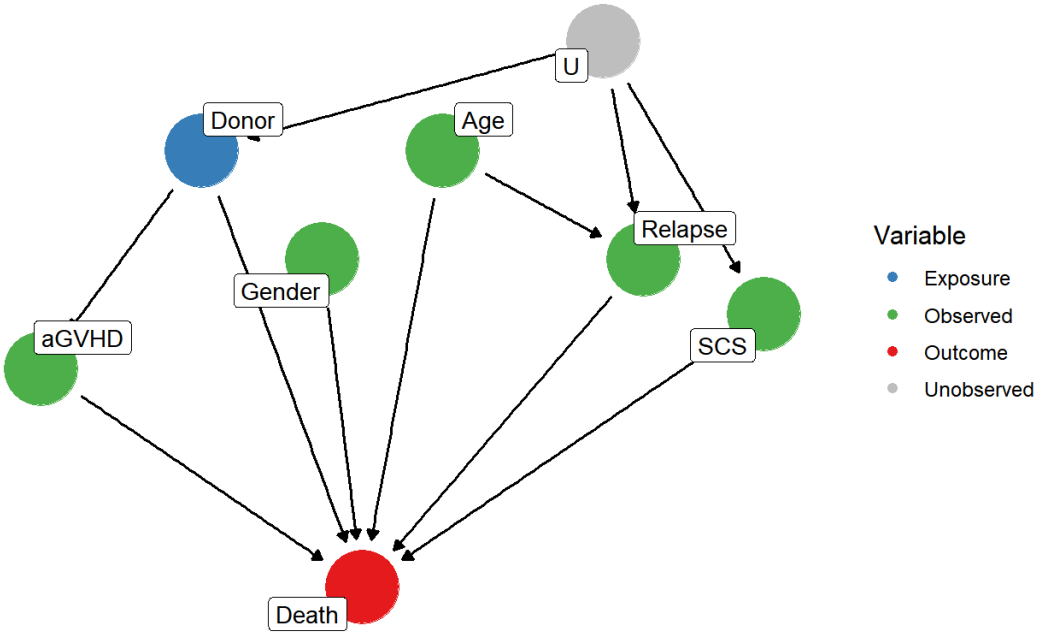

Figure A.7, Relationships between variables in the model including only aGVHD, within malignancies.

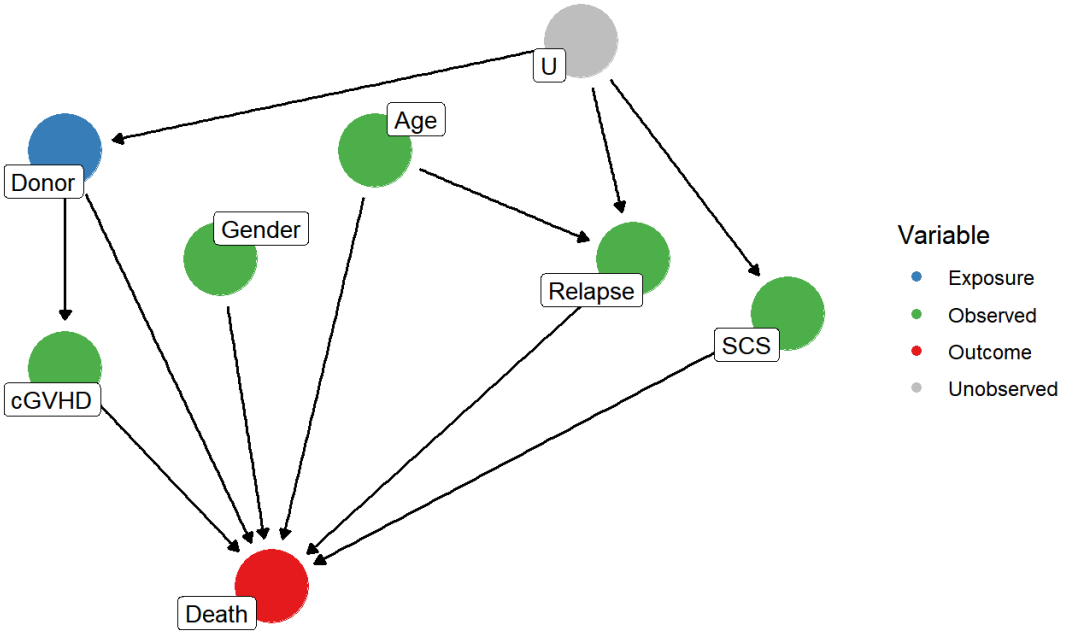

Figure A.8, Relationships between variables in the model including only cGVHD, within malignancies.

6.2 Tables

Variable	Identical Sibling, N = 138[1]	Mismatched Relative, N = 81[1]	Unrelated, N = 455[1]	p-value[2]
Status				0.062
Alive	119 (86%)	62 (77%)	351 (77%)	
Dead	19 (14%)	19 (23%)	104 (23%)	
Time	1.10 (0.31, 2.97)	1.03 (0.28, 3.12)	1.04 (0.29, 2.05)	0.5
Age	42 (15, 59)	20 (9, 53)	56 (38, 66)	**<0.001**

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test

Table A.1, Age and Time by Donor.

Variable	Absence, N = 498[1]	Presence, N = 176[1]	p-value[2]
aGVHD			**<0.001**
Absence	415 (83%)	118 (67%)	
Grade I-II	71 (14%)	45 (26%)	
Grade III-IV	12 (2.4%)	13 (7.4%)	

[1] n (%)

[2] Pearson's Chi-squared test

Table A.2, Contingency table of cGVHD and aGVHD.

Variable	Overall, N = 674[1]	Female, N = 274[1]	Male, N = 400[1]	p-value[2]
Status				0.8
Alive	532 (79%)	215 (78%)	317 (79%)	
Dead	142 (21%)	59 (22%)	83 (21%)	
Time	1.04 (0.29, 2.08)	1.03 (0.29, 2.06)	1.06 (0.28, 2.09)	0.7
Age	51 (27, 63)	52 (30, 62)	51 (22, 63)	0.4
Malignant	546 (81%)	226 (82%)	320 (80%)	0.4
Disease				
Acute Leukaemia	276 (41%)	112 (41%)	164 (41%)	
Bone Marrow Failure	32 (4.7%)	10 (3.6%)	22 (5.5%)	
Chronic Leukaemia	24 (3.6%)	9 (3.3%)	15 (3.8%)	
Hemoglobinopathies	14 (2.1%)	4 (1.5%)	10 (2.5%)	
Histiocytic Disorders	7 (1.0%)	2 (0.7%)	5 (1.3%)	
Inherited Disorders	39 (5.8%)	14 (5.1%)	25 (6.3%)	
Lymphoma	47 (7.0%)	18 (6.6%)	29 (7.3%)	
MDS/MPN	202 (30%)	89 (32%)	113 (28%)	
Plasma Cell Disorders	33 (4.9%)	16 (5.8%)	17 (4.3%)	
Stem Cell Source				>0.9
Bone Marrow	139 (21%)	56 (20%)	83 (21%)	
Peripheral Blood	535 (79%)	218 (80%)	317 (79%)	
aGVHD				>0.9
Absence	533 (79%)	216 (79%)	317 (79%)	
Grade I-II	116 (17%)	47 (17%)	69 (17%)	
Grade III-IV	25 (3.7%)	11 (4.0%)	14 (3.5%)	
cGVHD				>0.9
Absence	498 (74%)	203 (74%)	295 (74%)	
Presence	176 (26%)	71 (26%)	105 (26%)	
Relapse				0.7
Absence	550 (82%)	222 (81%)	328 (82%)	
Presence	124 (18%)	52 (19%)	72 (18%)	
Donor				0.4
Identical Sibling	138 (20%)	52 (19%)	86 (22%)	
Mismatched Relative	81 (12%)	29 (11%)	52 (13%)	
Unrelated	455 (68%)	193 (70%)	262 (66%)	
Age categories				0.2
0-9	76 (11%)	21 (7.7%)	55 (14%)	
10-19	63 (9.3%)	28 (10%)	35 (8.8%)	
20-30	51 (7.6%)	21 (7.7%)	30 (7.5%)	
31-40	56 (8.3%)	24 (8.8%)	32 (8.0%)	
41-50	82 (12%)	36 (13%)	46 (12%)	
51-60	135 (20%)	62 (23%)	73 (18%)	
61-70	159 (24%)	57 (21%)	102 (26%)	
70+	52 (7.7%)	25 (9.1%)	27 (6.8%)	

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test

Table A.3, Table 1. Stratified by gender.

Variable	Overall, N = 674[1]	Bone Marrow, N = 139[1]	Peripheral Blood, N = 535[1]	p-value[2]
Status				>0.9
Alive	532 (79%)	110 (79%)	422 (79%)	
Dead	142 (21%)	29 (21%)	113 (21%)	
Time	1.04 (0.29, 2.08)	1.40 (0.48, 3.10)	1.03 (0.28, 2.04)	**0.004**
Age	51 (27, 63)	12 (6, 30)	56 (40, 65)	**<0.001**
Gender				>0.9
Female	274 (41%)	56 (40%)	218 (41%)	
Male	400 (59%)	83 (60%)	317 (59%)	
Malignant	546 (81%)	71 (51%)	475 (89%)	**<0.001**
Disease				
Acute Leukaemia	276 (41%)	38 (27%)	238 (44%)	
Bone Marrow Failure	32 (4.7%)	26 (19%)	6 (1.1%)	
Chronic Leukaemia	24 (3.6%)	4 (2.9%)	20 (3.7%)	
Hemoglobinopathies	14 (2.1%)	14 (10%)	0 (0%)	
Histiocytic Disorders	7 (1.0%)	5 (3.6%)	2 (0.4%)	
Inherited Disorders	39 (5.8%)	20 (14%)	19 (3.6%)	
Lymphoma	47 (7.0%)	7 (5.0%)	40 (7.5%)	
MDS/MPN	202 (30%)	24 (17%)	178 (33%)	
Plasma Cell Disorders	33 (4.9%)	1 (0.7%)	32 (6.0%)	
aGVHD				0.10
Absence	533 (79%)	103 (74%)	430 (80%)	
Grade I-II	116 (17%)	27 (19%)	89 (17%)	
Grade III-IV	25 (3.7%)	9 (6.5%)	16 (3.0%)	
cGVHD				**0.044**
Absence	498 (74%)	112 (81%)	386 (72%)	
Presence	176 (26%)	27 (19%)	149 (28%)	
Relapse				0.063
Absence	550 (82%)	121 (87%)	429 (80%)	
Presence	124 (18%)	18 (13%)	106 (20%)	
Donor				**<0.001**
Identical Sibling	138 (20%)	41 (29%)	97 (18%)	
Mismatched Relative	81 (12%)	40 (29%)	41 (7.7%)	
Unrelated	455 (68%)	58 (42%)	397 (74%)	
Age categories				**<0.001**
0-9	76 (11%)	51 (37%)	25 (4.7%)	
10-19	63 (9.3%)	44 (32%)	19 (3.6%)	
20-30	51 (7.6%)	9 (6.5%)	42 (7.9%)	
31-40	56 (8.3%)	4 (2.9%)	52 (9.7%)	
41-50	82 (12%)	8 (5.8%)	74 (14%)	
51-60	135 (20%)	11 (7.9%)	124 (23%)	
61-70	159 (24%)	8 (5.8%)	151 (28%)	
70+	52 (7.7%)	4 (2.9%)	48 (9.0%)	

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test

Table A.4, Table 1. Stratified by stem cell source.

Variable	Absence, N = 533[1]	Grade I-II, N = 116[1]	Grade III-IV, N = 25[1]	p-value[2]
Status				**0.011**
Alive	432 (81%)	85 (73%)	15 (60%)	
Dead	101 (19%)	31 (27%)	10 (40%)	
Time	1.02 (0.27, 2.01)	2.03 (1.01, 3.12)	1.11 (0.60, 2.27)	**<0.001**
Age	51 (27, 63)	53 (24, 62)	42 (21, 60)	0.6

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test

Table A.5, Age and Time by aGVHD.

Variable	Absence, N = 498[1]	Presence, N = 176[1]	p-value[2]
Status			**0.030**
Alive	383 (77%)	149 (85%)	
Dead	115 (23%)	27 (15%)	
Time	0.99 (0.27, 2.00)	2.01 (1.12, 3.60)	**<0.001**
Age	51 (21, 63)	53 (36, 62)	0.3

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test

Table A.6, Age and Time by cGVHD.

Variable	Absence, N = 550[1]	Presence, N = 124[1]	p-value[2]
Status			**<0.001**
Alive	498 (91%)	34 (27%)	
Dead	52 (9.5%)	90 (73%)	
Time	1.04 (0.28, 2.36)	1.06 (0.54, 1.73)	0.4
Age	51 (23, 62)	55 (41, 65)	**0.006**

[1] n (%); Median (IQR)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test

Table A.7, Age and Time by Relapse.

Variable	HR[1]	95% CI[1]	p-value
Relapse			
Absence	1.00	Ref.	
Presence	21.5	14.9, 31.0	**<0.001**
aGVHD			
Absence	1.00	Ref.	
Grade I-II	1.56	1.01, 2.40	**0.044**
Grade III-IV	2.87	1.49, 5.53	**0.002**
cGVHD			
Absence	1.00	Ref.	
Presence	0.82	0.52, 1.28	0.38
Malignant			
No	1.00	Ref.	
Yes	2.07	1.21, 3.53	**0.008**
Donor			
Identical Sibling	1.00	Ref.	
Unrelated	1.78	1.09, 2.91	**0.020**
Mismatched Relative	1.84	0.98, 3.48	0.060
Stem Cell Source			
Peripheral Blood	1.00	Ref.	
Bone Marrow	0.83	0.55, 1.25	0.38
Gender			
Female	1.00	Ref.	
Male	0.95	0.68, 1.32	0.75
Age categories			
0-9	1.00	Ref.	
10-19	1.75	0.75, 4.10	0.20
20-30	1.46	0.56, 3.78	0.44
31-40	1.28	0.50, 3.33	0.61
41-50	2.12	0.97, 4.66	0.061
51-60	1.48	0.69, 3.17	0.31
61-70	2.44	1.19, 5.01	**0.015**
70+	3.93	1.75, 8.84	**<0.001**
Age	1.01	1.00, 1.02	**0.007**

[1] HR = Hazard Ratio, CI = Confidence Interval

Table A.7, Univariate analysis.

6.3 Code

The following lines of code were used to implement the counting process structure for one or more variable; in this case the three different complications were used. This function is also available on CRAN in the package mtvc.

```
mtvc=function(data,
              dates,
              origin='1970-01-01',
              start,
              stop,
              event,
              complications){
  #
  dtfrm=data %>%
    ungroup() %>%
    mutate(id=row_number()) %>%
    mutate_at(vars({{dates}}),~as.Date(ifelse(is.na(.),
                                              as.Date(origin),
                                              as.Date(.)
    )))
  # go from wide to long in order to order dates
  melted=gather(dtfrm,event,day,c({{start}},{{dates}})) %>%
    arrange(id,day) %>%
    group_by(id) %>%
    mutate(t=row_number()) %>%
    filter(day!=origin) %>%
    select(id,t,day) %>%
    mutate(tstart=day,
           tstop=lead(day))
  # merge with initial dataset
  merged=merge(dtfrm,melted,by='id',all.x = T) %>%
    group_by(id) %>%
    mutate(tstop=as.Date(ifelse(is.na(tstop),
                                as.Date({{stop}}),
                                as.Date(tstop))),
           time=as.numeric(tstop-tstart))%>%
    filter(time!=0) %>%
    mutate(tevent=case_when(row_number()==n()~{{event}},
                            T~0)) %>%
    ungroup(id)
  # dates list
  dat.list=merged %>%
    select({{dates}}) %>%
    list()
  # comp list
  comp.list=merged %>%
    select({{complications}}) %>%
    list()
  # create df that contains results
  complication=matrix(NA,
                      nrow=dim(merged)[1],
                      ncol=dim(comp.list[[1]])[2])
```

```
 # match
  for(j in 1:dim(comp.list[[1]])[2]){
    for(i in 1:dim(comp.list[[1]])[1]){
      datecomp=as.numeric(dat.list[[1]][i,j])
      datest=as.numeric(merged$tstart[i])
      value=as.numeric(comp.list[[1]][i,j])
      complication[i,j]=ifelse(datecomp==datest,
                               value,
                               0)
    }
  }
  baba=complication
  # lag
  for (j in 1:dim(complication)[2]){
    for(i in 2:dim(complication)[1])
      baba[i,j]=ifelse(merged$id[i]==merged$id[i-1] & baba[i-1,j]!=0,
                       baba[i-1,j],
                       baba[i,j])
  }
  # give names to variables
  baba=as.data.frame(baba)
  comp.names=c(names(comp.list[[1]]))
  for(i in 1:length(comp.names)){
    comp.names[i]=paste('tdep',tolower(comp.names[i]),sep='_')
  }
  names(baba)=comp.names
  # merge the two dataset
  example=as.data.frame(cbind(merged,baba))
  # add index for time
  example=example %>%
    group_by(id) %>%
    mutate(ind=row_number())
  # create start and stop from time
  for(i in 1:dim(example)[1]){
    # stop
    example[i,'stop']=ifelse(example[i,'ind']==1,
                             as.numeric(example[i,'tstop']-example[i,'tstart']),
                             example[i-1,'stop']+as.numeric(example[i,'tstop']-
                                 example[i,'tstart']))
    # start
    example[i,'start']=ifelse(example[i,'ind']==1,
                              0,
                              example[i-1,'stop'])

  }
  return(example)
}

    # example

data(simwide)
cp.dataframe=mtvc(data=simwide,
                  origin='1970-01-01',
                  dates=c(FIRST_CHRONIC,FIRST_ACUTE,FIRST_RELAPSE),
                  complications=c(CHRONIC,ACUTE,RELAPSE),
                  start=DATETRAN,
                  stop=DLASTSE,
                  event=EVENT)
```

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