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A multicenter retrospective study on the prognostic impact of pregnancy in women with history of *BRCA* mutated breast cancer

Protocol version 2.0: 10 January 2022

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Matteo Lambertini, MD PhD	January 3, 2022	
Michail Ignatiadis, MD PhD	January 10, 2022	
Hatem A. Azim Jr., MD PhD	January 4, 2022	

DOCUMENT HISTORY OF REVISIONS

Type of amendment	**Protocol version number**	**Final version date**	**Nature of amendments**
Not applicable	1.0	20 December 2016	Not applicable
Amendment 1	2.0	10 January 2022	1) Update of the study team and its affiliations with the addition of Elisa Agostinetto and Eva Blondeaux as study secretariat, as well as Marco Bruzzone and Marcello Ceppi among study statisticians. 2) Among primary objectives, revision of the definition of "incidence of pregnancy" and the way to analyze it; current definition is "rate of pregnancy" to be analyzed according to the Kaplan-Meier method. 3) Addition of the three following secondary objectives (and respective statistical analyses): - To describe baseline, tumor and treatment characteristics as well as patterns of care including access to risk-reducing surgeries in *BRCA* mutated patients. - To evaluate the prognostic impact of baseline, tumor and treatment characteristics as well as of risk-reducing surgeries in *BRCA* mutated patients. - To evaluate tumor infiltrating lymphocytes (TILs) in *BRCA* mutated patients. 4) Clarification and update of study eligibility criteria: accrual is allowed for patients diagnosed up to December 2020, while patients with invasive breast cancer and germline *BRCA* variants of unknown significance are excluded. 5) Inclusion of the following information for data collection: cause of death.

			6) Clarification of the priority for the analysis of survival endpoints: DFS is the primary endpoint and BCSS/OS are secondary endpoints. 7) Clarifications of the type of survival analyses to be conducted for reducing the impact of selection bias (i.e. guaranteed time bias). Two analyses are expected: the extended Cox model analysis with occurrence of pregnancy as a time-varying covariate and a "case-control" analysis. Type of matching for the "case-control" analysis has been clarified and will be performed according to the following factors: disease-free interval, year at diagnosis, nodal status, hormone receptor status and type of BRCA mutation are the matching criteria. 8) Addition of the ClinicalTrials.gov identifier of this study: NCT02308085. 9) Corrections of typos and inconsistencies within the text. 10) Addition of 2 columns in the excel file for data collection 11) Updated list of participating centers.

Contents

SYNOPSIS

Title of the study	A multicenter retrospective study on the prognostic impact of pregnancy in women with history of *BRCA* mutated breast cancer
Protocol version	2.0 – 10 January 2022
Sponsor	Institut Jules Bordet, Brussels, Belgium
Study chairs	• Matteo Lambertini, MD PhD, IRCCS Ospedale Policlinico San Martino – University of Genova • Michail Ignatiadis, MD PhD, Institut Jules Bordet • Hatem A. Azim Jr., MD PhD, Hospital Zambrano Hellion Tecnolgico de Monterrey
Study Objectives	**Primary objectives:** - To evaluate the prognostic impact of pregnancy following breast cancer diagnosis in *BRCA* mutated patients. - To evaluate the rate of pregnancy following breast cancer diagnosis in *BRCA* mutated patients. **Secondary objectives:** - To evaluate the prognostic impact of pregnancy following breast cancer diagnosis in various subgroups of *BRCA* mutated patients according to: • Type of *BRCA* mutation; • Hormone receptor status; • HER2 status; • Exposure to chemotherapy; • Exposure to endocrine therapy; • Interval between diagnosis and pregnancy; • Outcome of pregnancy; • Breastfeeding status. - To evaluate the pregnancy, fetal and obstetrical outcomes of the pregnancies following breast cancer diagnosis in *BRCA* mutated patients.

	- To evaluate the safety of assisted reproductive technology (ART) procedures following breast cancer diagnosis in *BRCA* mutated patients. - To evaluate the impact of anticancer therapies on patients' ovarian function. - To describe baseline, tumor and treatment characteristics as well as patterns of care including access to risk-reducing surgeries in *BRCA* mutated patients. - To evaluate the prognostic impact of baseline, tumor and treatment characteristics as well as of risk-reducing surgeries in *BRCA* mutated patients. - To evaluate tumor infiltrating lymphocytes (TILs) in *BRCA* mutated patients.
Number of patients	More than 2000 non-pregnant *BRCA* mutated breast cancer patients and more than 200 pregnant *BRCA* mutated breast cancer patients (estimated numbers, considering a rate of pregnancy of approximately 10%).
Eligibility criteria	**Inclusion criteria:** - Diagnosis of invasive breast cancer between January 2000 and December 2020; - Breast cancer diagnosis at the age of ≤ 40 years; - Known presence of germline *BRCA* pathogenic variant. **Exclusion criteria:** - Known *BRCA* mutation with no diagnosis of invasive breast cancer; - Diagnosis of ovarian cancer or other malignancies with no history of invasive breast cancer; - Diagnosis of hereditary or familiar invasive breast cancer without *BRCA* mutation or with *BRCA* genes not tested; - Diagnosis of invasive breast cancer with germline *BRCA* variants of unknown significance.
Design of the trial	Retrospective multicenter hospital-based study

Statistics	The achievement of pregnancy after diagnosis of breast cancer will be the criteria used to distinguish between two cohorts of patients: women with no subsequent pregnancies after breast cancer diagnosis ("non-pregnant cohort"), and women with one or more pregnancies any time after breast cancer diagnosis ("pregnant cohort"). To evaluate the prognostic impact of pregnancy following breast cancer diagnosis in *BRCA* mutated survivors, three survival endpoints will be considered in the two cohorts: disease-free survival (DFS, as primary endpoint) breast cancer specific survival (BCSS, as secondary endpoint) and overall survival (OS, as secondary endpoint). To reduce the impact of selection bias (i.e. guaranteed time bias), two analyses will be performed. In the extended Cox model analysis with occurrence of pregnancy as a time-varying covariate, all eligible patients included in the study will be considered. In the "case-control" analysis, each patient in the pregnant cohort (case) will be matched with 3 patients from the non-pregnant cohort (controls). Each nonpregnant control should have a disease-free interval equal to or longer than the time elapsing between breast cancer diagnosis and date of pregnancy of the matched pregnant case. Patients who became pregnant after the date of the DFS event will be dropped from this analysis. The other matching factors will be year at diagnosis (± 2.5 years), nodal status (negative vs. positive), hormone receptor status (positive vs. negative), and type of BRCA mutation (BRCA1 vs. BRCA2). Because all included patients are aged ≤ 40 years at the time of diagnosis, matching according to age will not be performed. The matching will be done centrally at the coordinating center. The prognostic impact (in terms of DFS, BCSS and OS) of pregnancy following breast cancer diagnosis will be then evaluated in various subgroups of *BRCA* mutated survivors. Pregnancy rate will be computed according to the Kaplan-Meier method.

1. BACKGROUND AND SCIENTIFIC RATIONALE

Breast cancer is the most common tumor type diagnosed in women of reproductive age [1]. The inheritance of a mutation in one of the breast cancer susceptibility genes (i.e. *BRCA1* or *BRCA2*) generates the hereditary breast and ovarian cancer syndrome [2]. For healthy women with *BRCA1* mutated gene (i.e. *BRCA1* carriers), the average lifetime risk of developing breast and ovarian cancer is approximately 67% and 45%, respectively; for *BRCA2* carriers, these average cumulative risks are 66% and 12%, respectively [3].

Since young women with breast cancer, including those with *BRCA* mutation, are more likely to develop biologically aggressive tumors [4], the majority of them are candidates to receive antineoplastic treatments that include chemotherapy. The use of cytotoxic therapy in premenopausal patients may result in the occurrence of treatment-related premature ovarian failure (POF) [5]. *BRCA* mutations seem to negatively affect ovarian reserve with consequent accelerated ovarian aging [6]. Although very limited data are available on this topic, it has been suggested that *BRCA* carriers may be more sensitive to the gonadotoxic effect of chemotherapy as compared to patients without mutations with subsequent higher risk of developing this side effect [6]. The impairment in ovarian function negatively impacts on global health of young breast cancer survivors being associated with several side effects including infertility [7].

Concerns about fertility preservation and future chance of achieving a pregnancy are prevalent issues affecting young breast cancer patients [8]. Hence, accordingly, major international guidelines recommend physicians to address with patients as part of informed consent before anticancer therapies the possibility of developing POF and infertility, and then to discuss fertility preservation options in interested patients [9–11]. Specific issues on this regard should be considered in patients with *BRCA* mutation in the context of their unique concerns [12]. For carriers, given the increasing risk of ovarian cancer with age, bilateral salpingo-oophorectomy is generally recommended for both *BRCA1* and *BRCA2* carriers between the ages of 35 and 40 years and upon completion of childbearing [13]. Moreover, some carriers wish to eliminate the mutation from future offspring through pre-implantation genetic diagnosis [14]. Finally, given the increasing risk of ovarian and breast cancer and the risk of developing long-term treatment-related POF [15], the window for fertility and pregnancy may be narrow in such young women. Of note, although, the last years have brought many safety and efficacy data on the available options for fertility preservation increasing the chances of

future offspring in cancer survivors, numerous challenges remain for young breast cancer patients considering fertility preservation and to have a pregnancy after the end of treatments, especially for those with *BRCA* mutations. Thus, further research is needed in the field; particularly, reproduction studies to address the specific issues of women with *BRCA* mutations are lacking and should be considered a research priority.

Approximately 40% to 50% of women in whom breast cancer is diagnosed during childbearing age wish to have a subsequent pregnancy [16]. However, the percentage of breast cancer patients reported in the literature that has at least one full-term pregnancy after breast cancer diagnosis is very low. Of note, among cancer survivors, breast cancer patients have the lowest pregnancy rate with an overall 67% reduction in the chance of giving birth after cancer treatment as compared to the general population [17]. This observation reflects not only the damage to ovarian reserve due to the gonadotoxic treatments required, but also patient and provider concerns related to a possible negative impact of pregnancy on the evolution of breast cancer [18].

Recent data support the statement that pregnancy in cancer survivors after adequate treatment and follow-up should not be discouraged including among patients with breast cancer [19]. As shown in a meta-analysis of 14 retrospective control-matched studies, breast cancer patients who became pregnant following diagnosis and treatment had a 41% reduced risk of death compared to women who did not get pregnant (pooled relative risk [PRR], 0.59; 95% confidence intervals [CI], 0.50-0.70) [20]. Furthermore, a more recent multicenter retrospective cohort study confirmed the safety of pregnancy after breast cancer even in patients with endocrine-sensitive disease [21]. Importantly, no impact of abortion on patient outcome was observed either [21]. Nevertheless, in the specific subgroup of patients with *BRCA* mutations very limited data consisting in one single study are available on the impact of pregnancy on breast cancer survival [22]. In this study, out of the 128 pregnant cases included, only 53 had a pregnancy following breast cancer; no difference in breast cancer specific mortality was observed between pregnant cases and matched non-pregnant control (adjusted hazard ratio [HR], 0.73; 95% CI, 0.21-2.68) [22]. However, due to the limited number of patients included in the analysis, no solid conclusion can be drawn from this study to counsel *BRCA* mutated survivors on the safety of having a pregnancy after breast cancer diagnosis. Furthermore, no information is available on the impact of breastfeeding in these patients, and it remains not clear yet the ideal interval to wait between the end of anticancer treatments and conception. Moreover, other

highly relevant issues remain elusive pertaining on subsequent pregnancy in *BRCA* mutated breast cancer patients, including the safety of assisted reproductive technology (ART), pregnancy outcomes, risk of congenital malformations, breastfeeding patterns and many more.

Thus, due to the lack of data on several issues on this regard, the present study aims at refining the understanding of the effect of pregnancy on breast cancer outcomes in the specific population of *BRCA* mutated patients with known history of breast cancer.

2. STUDY OBJECTIVES

Primary objectives:

- To evaluate the prognostic impact of pregnancy following breast cancer diagnosis in *BRCA* mutated patients. The following survival endpoints will be considered: disease-free survival (DFS, as primary endpoint), breast cancer specific survival (BCSS, as secondary endpoint) and overall survival (OS, as secondary endpoint).
- To evaluate the rate of pregnancy following breast cancer diagnosis in *BRCA* mutated patients.

Secondary objectives:

- To evaluate the prognostic impact (in terms of DFS, BCSS and OS) of pregnancy following breast cancer diagnosis in various subgroups of *BRCA* mutated patients according to:
    - Type of *BRCA* mutation: *BRCA1* vs. *BRCA2*;
    - Hormone receptor status: positive vs. negative;
    - HER2 status: positive vs. negative;
    - Exposure to chemotherapy: prior exposure vs. no prior exposure;
    - Exposure to endocrine therapy: prior exposure vs. no prior exposure;
    - Interval between diagnosis and pregnancy: ≤ 2 years vs. > 2 years after diagnosis;
    - Outcome of pregnancy: abortion vs. completed pregnancy;
    - Breastfeeding status: breastfeeding vs. no breastfeeding.
- To evaluate the pregnancy, fetal and obstetrical outcomes of the pregnancies following breast cancer diagnosis in *BRCA* mutated patients.
- To evaluate the safety of ART procedures following breast cancer diagnosis in *BRCA* mutated patients.

- To evaluate the impact of anticancer therapies on patients' ovarian function, in terms of incidence of treatment-induced POF and age at menopause.
- To describe baseline, tumor and treatment characteristics as well as patterns of care including access to risk-reducing surgeries in *BRCA* mutated patients.
- To evaluate the prognostic impact of baseline, tumor and treatment characteristics as well as of risk-reducing surgeries in *BRCA* mutated patients.
- To evaluate tumor infiltrating lymphocytes (TILs) in *BRCA* mutated patients.

3. METHODOLOGY

This is a multicenter hospital-based retrospective cohort study (ClinicalTrials.gov identifier: NCT02308085) conducted across several Institutions managing patients with hereditary breast cancer. The study aims to refine the understanding of the effect of pregnancy on breast cancer outcomes in *BRCA* mutated patients with known history of breast cancer.

3.1. Eligibility criteria

The eligibility criteria are intentionally broad for trying to exclude as few patients as possible so that true population-based data can be obtained. To be eligible for the present study, patients should fulfill the following inclusion and exclusion criteria.

Inclusion criteria:

- Diagnosis of invasive breast cancer between January 2000 and December 2020;
- Breast cancer diagnosis at the age of ≤ 40 years;
- Known presence of germline *BRCA* pathogenic variant.

Exclusion criteria:

- Known *BRCA* mutation with no diagnosis of invasive breast cancer;
- Diagnosis of ovarian cancer or other malignancies with no history of invasive breast cancer;
- Diagnosis of hereditary or familiar invasive breast cancer without *BRCA* mutation or with *BRCA* genes not tested;
- Diagnosis of invasive breast cancer with germline *BRCA* variants of unknown significance.

3.2. Contact with Investigators

The study chairs and secretariat of this project will invite investigators from different institutions worldwide to participate in this project.
Upon agreement, investigators will be requested to provide anonymous individual patient data. A principal contact will be requested per Institution in case of data clarification needs. A timeframe of 4 months is given for data collection from each Institution after a positive opinion is granted by the respective Independent Ethical Committee / Institutional Review Board.

3.3. Data collection

For all consecutive eligible patients included in the study from each participating Institution, their medical records will be retrieved and anonymized data entered into a database. The following information will be collected, if available:

1) Baseline characteristics: body mass index; smoking habit; age at menarche; age at menopause; menopausal status at the time of breast cancer diagnosis; use of birth control pills; prior children before breast cancer diagnosis; history of spontaneous abortion; history of induced abortion; prior treatment for infertility; prior gynecological surgery and/or medical history with possible impact on fertility.
2) Breast cancer history: date of diagnosis; age at diagnosis; disease stage; laterality of the primary tumor; type of breast and axillary surgery; use of breast reconstruction surgery; grade; histology; tumor size and nodal status (for patients who underwent primary systemic therapy, only clinical tumor size and nodal status will be collected); hormone receptor status (estrogen and progesterone receptors); HER2 status.
3) Treatment of the primary tumor: timing of systemic therapy administration; use of chemotherapy (type and number of cycles); use of endocrine therapy (type and duration); use of anti-HER2 targeted therapy (type and duration); use of radiotherapy.
4) Survival status: any recurrence of invasive breast cancer including date of the event; any second primary breast cancer including date of the event; any second primary malignancy including date of the event; date of last follow-up and/or death; cause of death (breast cancer-related or not).
5) Ovarian function after chemotherapy: treatment-induced POF; age at menopause.

6) Pregnancy after breast cancer: any pregnancy including date of the event; pregnancy outcome; gestational age at delivery; any pregnancy/fetal/birth complications; breastfeeding and its duration; use and type of ART.
7) *BRCA* mutation: date of *BRCA* testing; type of *BRCA* mutation; use and date of breast prophylactic surgery; use and date of gynecological prophylactic surgery.
8) Additional information: diagnosis of breast cancer during pregnancy, availability of digital scanned images for TILs analysis

Data can be sent in confidence to Matteo Lambertini (matteo.lambertini@unige.it) and Elisa Agostinetto (elisa.agostinetto@bordet.be) where they will be held secure and only used for the purposes of this study.Analyses will only be undertaken under the supervision of the study statisticians.

4. STATISTICS

The achievement of pregnancy after diagnosis of breast cancer will be the criteria used to distinguish between two cohorts of patients: women with no subsequent pregnancies after breast cancer diagnosis ("non-pregnant cohort"), and women with one or more pregnancies any time after breast cancer diagnosis ("pregnant cohort"). Pregnancies could have resulted in live births, therapeutic or spontaneous abortions.

More than 2000 non-pregnant *BRCA* mutated breast cancer patients and more than 200 pregnant *BRCA* mutated breast cancer patients are expected to be included in the present study (estimated numbers, considering an incidence of pregnancy of approximately 10%).

To evaluate the prognostic impact of pregnancy following breast cancer diagnosis in *BRCA* mutated survivors, three survival endpoints will be considered in the two cohorts: DFS (as primary endpoint) BCSS (as secondary endpoint) and OS (as secondary endpoint). DFS event is defined by the occurrence of one of the following invasive events: local recurrence, distant metastases, contralateral or ipsilateral breast tumor, second primary malignancy, or death from any cause. BCSS event is defined as death from breast cancer. OS event is defined as death from any cause. For DFS, observation times of patients without the event will be censored on the date of their last contact. Using individual patient data from all patients included in the study, survival outcomes of patients in the

pregnant cohort will be compared to those of patients in the non-pregnant cohort. To reduce the impact of selection bias (i.e. guaranteed time bias), two analyses will be performed. In the extended Cox model analysis with occurrence of pregnancy as a time-varying covariate, all eligible patients included in the study will be considered. In the "case-control" analysis, each patient in the pregnant cohort (case) will be matched with 3 patients from the non-pregnant cohort (controls). To adjust for guaranteed time bias, each patient in the non-pregnant cohort should have a disease-free interval equal to or longer than the time elapsing between breast cancer diagnosis and date of pregnancy of the matched patient in the pregnant cohort. Patients who became pregnant after the date of the DFS event will be dropped from this analysis. Patients will be also matched according to several variables including type of *BRCA* mutation (BRCA1 vs. BRCA2), hormone receptor status (positive vs. negative), nodal status (negative vs. positive), and year at diagnosis (± 2.5 years). Because all included patients are aged ≤ 40 years at the time of diagnosis, matching according to age will not be performed. The matching will be done centrally at the coordinating center. Two data sets will be constituted: one for the cases and one for the potential controls. Cases will be sorted randomly and matching will be done starting for the first case until the last one. For each case, all possible controls will be extracted from the data set of potential controls. If they are only 2 controls available, matching criteria will be relaxed one by one (starting from year of diagnosis, etc) until at least a third control is found. It is anticipated, given the expected low rate of cases in the full database, to reach matching for almost each case. In these analyses, all survival endpoints will be calculated from the date of pregnancy until the date of the event. In the non-pregnant cohort, survival endpoints will be calculated from the date of diagnosis, adding the time elapsing between diagnosis and pregnancy of the matched pregnant case.

The prognostic impact (in terms of DFS, BCSS and OS) of pregnancy following breast cancer diagnosis will be then evaluated in various subgroups of *BRCA* mutated survivors according to: type of *BRCA* mutation (*BRCA1* vs. *BRCA2*), hormone receptor status (positive vs. negative), HER2 status (positive vs. negative), exposure to chemotherapy (prior exposure vs. no prior exposure), exposure to endocrine therapy (prior exposure vs. no prior exposure), interval between diagnosis and pregnancy (≤ 2 years vs. > 2 years after diagnosis), outcome of pregnancy (abortion vs. completed pregnancy), and breastfeeding status (breastfeeding vs. no breastfeeding).

Considering a rate of pregnancy of approximately 10%, with 2000 non-pregnant and 200 pregnant *BRCA* mutated breast cancer patients, assuming a DFS rate of 65% at 5 years in the non-pregnant cohort [21], the study will have a power of 0.83 to detect a hazard ratio of 0.75 and a power of 0.62

to detect a hazard ratio of 0.80 in favor of the pregnant cohort, at a two-sided significance level 0.05 (accrual duration 2000-2020, total study duration 2000-2030).

The rate of pregnancy following breast cancer diagnosis will be computed according to the Kaplan-Meier method. A possible increasing or decreasing trend in rate of pregnancy following breast cancer over the years will be also assessed.

The statistical analysis to evaluate the pregnancy, fetal and obstetrical outcomes of the pregnancies following breast cancer diagnosis in *BRCA* mutated survivors will be mainly descriptive and will include the following parameters:

1. Gestational age at delivery;
2. Incidence of early pre-term (< 34 weeks), late-preterm (34-36 weeks) and full-term (≥ 37 weeks) pregnancies;
3. Number of pregnancies resulting in live birth and number of children born;
4. Incidence of induced and spontaneous abortion;
5. Incidence and nature of pregnancy complications (if any);
6. Incidence and nature of fetal complication and/or congenital malformations (if any);
7. Incidence and nature of obstetrical complications (if any).

To evaluate the safety of ART procedures following breast cancer diagnosis in *BRCA* mutated survivors, two groups of patients within the pregnant cohort will be considered: patients who achieved pregnancy with the use of any ART procedure (i.e. “ART group”) and patients who achieved pregnancy without the use of any ART procedure (i.e. “no ART group”). A subgroup analysis will be performed to evaluate the safety of controlled ovarian stimulation as part of ART within the ART group.

In patients who underwent chemotherapy as part of (neo)adjuvant systemic therapy, the impact of anticancer therapies (i.e. chemotherapy) on patients’ ovarian function, in terms of incidence of treatment-induced POF and age at menopause will be evaluated, if available. According to the World Health Organization definition of postmenopausal status [23], treatment-induced amenorrhea will be defined by the absence of menses for ≥ 12 months after the end of chemotherapy. Age at menopause will be defined as age in which patients had absence of menses for ≥ 12 months. For patients who

developed treatment-induced amenorrhea but then had a recovery of menses more than 12 months after the end of chemotherapy and with active menstrual function within 12 months prior to the last follow-up visit, they will not be considered as having developed menopause. Subgroup analyses will be performed according to age at diagnosis, type of chemotherapy used (anthracycline- and taxane-based regimens vs. anthracycline-based regimens vs. other regimens), and use of endocrine therapy (yes vs. no). These results will be compared to historical data in the *BRCA* wild-type breast cancer population.

Considering the sample size and the uniqueness of the young cohort of *BRCA* mutated patients expected to be included in the study, additional analyses will be conducted to describe baseline, tumor and treatment characteristics as well as patterns of care including access to risk-reducing surgeries. Possible changes over time will be also assessed. Moreover, similarly, the prognostic impact of baseline, tumor and treatment characteristics as well as of risk-reducing surgeries in *BRCA* mutated patients will be also evaluated.

In order to conduct the evaluation of TILs in this cohort, there will be no need for new cutting/preparation of tumor slides. The evaluation will be conducted on one archived diagnostic tumor-containing slide stained with hematoxylin and eosin representative of the primary tumor. The TILs scoring will be performed centrally. If available, anonymized digitally scanned images of the diagnostic hematoxylin and eosin-stained slide should be sent via email to Maria Vittoria Dieci (mariavittoria.dieci@unipd.it) at University of Padova-Istituto Oncologico Veneto (Padova, Italy). The University of Padova-Istituto Oncologico Veneto will take care of costs related to TILs scoring and storage support (hard disk). The evaluation will be performed according to the TILs working group recommendations [24].

Patients demographic, clinical and pathologic baseline characteristics will be compared by pregnancy status using chi-square test or Fisher Exact test for categorical variables and Wilcoxon rank-sum test or t-test for continuous variables, as appropriate.

Dichotomous outcomes will be compared using univariate and multivariate logistic regression models. Time-to-event outcomes will be estimated and plotted using Kaplan-Meier methods. Survival rates will be compared using log-rank test and Cox proportional hazards models for univariate and multivariate analysis, respectively.

All tests will be two-sided and p-values of < 0.05 will be considered statistically significant. All

analyses will be performed using standard available statistical programs.

5. ETHICAL ASPECTS

The study will be submitted by the investigator(s) or the Sponsor (or its legal representative) in accordance with local regulations to and approved by an appropriate Independent Ethical Review Committee / Institutional Review Board and a Regulatory Authority if required by the national laws of the countries where the study will be conducted.

The investigators and the Sponsor will ensure that the study is conducted in full conformance with the principles of the "Declaration of Helsinki" 1964, as revised from time to time or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. For the study, the principal investigators will ensure compliance with the latest European Union (EU) Data Protection Directive or with local law especially if it affords greater protection to the patient.

If required according to the national regulations and laws, it is the responsibility of the Investigator or a person designed by the Investigator (if acceptable by local regulations) to obtain written informed consent from each potential subject prior any study data collection being carried out. However, when allowed by the national regulations and laws, the investigators of the present study will apply to the Independent Ethical Review Committee / Institutional Review Board for a waiver of the need to obtain informed consent. The reasons are:
(i) it is a retrospective study that involves no interventions with human subjects. Medical records will be reviewed, but none of the patients will be contacted;
(ii) the research involves no more than minimal risk to the subjects;
(iii) the waiver will not adversely affect the rights and welfare of the subjects; moreover, the research could not practicably be carried out without the waiver;
(iv) the information collected does not include information that may be damaging to the individual should it be wrongfully disclosed; patients' privacy will be protected throughout the study;
(v) data will be anonymized and stored electronically in a restricted access network folder and will only be accessible to the research team.

6. DATA HANDLING AND RECORD KEEPING

The Patient's right to confidentiality is paramount. Collected data will be anonymized. All data will be kept confidential and will be used only for the purpose of this study. Local data privacy laws, rules and guidelines must be respected.

7. DATA CONFIDENTIALITY AND TIMELINE

Data will be provided by investigators of the participating Institutions in confidence to Institut Jules Bordet, where they will be kept secure. The data collection will be performed by Matteo Lambertini and Elisa Agostinetto, whereas the database set up, data cleaning and the statistical analysis will be performed by the study secretariat and statisticians in charge of the overall analysis once the database has been cleaned and locked.
The proposed timeline for project from the receipt of all data, database assembly and cleaning, statistical analysis to first results is 6 months.

8. PUBLICATION POLICY

A manuscript summarizing the results of the main analysis will be prepared for submission to and publication in a peer-reviewed scientific journal. Regular emails, teleconferences and/or face-to-face meetings will be organized to discuss progress of the data analysis. The manuscript will be prepared by one or more of the study chairs along with the study secretariat and statistical team and will be submitted to the all collaborators for review. Authorship will be based on number of included patients: the 3 Institutions with the highest number of included patients will be granted to up to two authors, while each of the collaborating Institutions will allocate one or no author.

Any other publication arising from this project will be made on behalf of the study chairs directly involved in that specific analysis and the principal investigators from the participating Institutions that actively contributed to that specific analysis.

All authors will be given sufficient time to provide comments and attempts will be made to come to mutual agreement. No financial benefits will be pursued or derived by the study and the corresponding results.

9. FUNDING

This project will be supported by Institut Jules Bordet. Unfortunately, it cannot be offered any financial reimbursement for involved investigators.

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