SEMEN QUALITY AND FATHERHOOD IN NON-HODGKIN LYMPHOMA SURVIVORS: A 15 YEAR MONOCENTRIC RETROSPECTIVE STUDY

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ABSTRACT

Study question: What is semen quality and fertility like in Non-Hodgkin Lymphoma (NHL) survivors?

Summary answer: Most NHL subjects are normozoospermic before cancer treatment, and the semen parameters of the survivors are comparable to the baseline 18 months after standard first-line treatments. Salvage therapy followed by hematopoietic stem cell transplant had the worst impact, with 66% azoospermic subjects in the long term. Overall, most patients can achieve fatherhood post-treatment through natural fertility or assisted reproductive technology.

What is known already: Testicular function has been widely investigated in relation to the most common malignancies in reproductive age, such as testicular cancer and Hodgkin lymphoma, but NHL has been somewhat under-investigated. The available reports generally show a post-treatment worsening of semen parameters in NHL survivors, but the only papers to have focused on the subject have non-comparable results from small caseloads or from a subgroup of broader caseloads.

Study design, size, duration: We conducted a retrospective analysis of 222 subjects who attended the Laboratory of Seminology – "Loredana Gandini" Sperm Bank of the Department of Experimental Medicine – "Sapienza" University of Rome between 2002-2017 for sperm cryopreservation after a diagnosis of NHL.

Participants/materials, setting, methods: We retrospectively selected 222 NHL patients who underwent sperm cryopreservation before any antineoplastic treatment between 2002 and 2017. Subjects with any comorbidity and/or other conditions interfering with sperm parameters were excluded. All patients underwent a careful medical history and physical examination at the time of sperm cryopreservation (T0) and had at least one follow-up visit at 6 (T6), 12 (T12), 18 (T18) and/or 24 months (T24) or more than 24 months (T>24), with a median follow-up of 47.5 months (range 28-140 months). Fertility information was collected through the administration of a questionnaire.

Main results and the role of chance: *Pre-treatment evaluation* – More than 80% of NHL patients were previously normozoospermic and in 16% of cases had already fathered a child. Aggressive lymphomas were associated with worse baseline semen volume and total sperm number compared to indolent subtypes ($p < 0.05$). *Post-treatment evaluation* – Total sperm number worsened at T6 and returned to near-baseline values only after a median of 47.5 months (T>24), although the mean value was still below the T0 mean value (T0: 209.2 ± 190.5 vs. T>24: 160.5 ± 137.8, $p = 0.118$). The percentage of progressive motility and of abnormal forms significantly worsened at T6 compared to

baseline (p <0.001), returning to near-baseline at T12. Two years post-treatment, 25.5% of NHL survivors showed persistent oligozoospermia and 14.1% azoospermia. Subgroup analyses showed that standard first-line treatments had a more favourable outcome for semen parameters, with total sperm number returning to near-baseline values at 18 months (T0: 195.0 ± 189.8 vs. T18: 113.4 ± 103.1, p = 0.278), and a lower prevalence of azoospermia at two years (7.7%). In this subgroup, radiotherapy of the pelvis vs. other "high" sites (mediastinum, laterocervical and axillary lymph nodes, etc.) was associated with an increased risk of developing post-treatment azoospermia (OR 4.29, 95% CI 1.81-10.14; p = 0.001). Two-thirds of subjects who had relapsed or had disease progression after first-line treatment and then underwent salvage treatment ± hematopoietic stem cell transplant became azoospermic. *Fertility questionnaire* – Fertility data was available for 176 patients: 15.9% already had at least one child prior to the NHL diagnosis and 12.5% (22 patients) desired children after treatment. Of these, 14 achieved fatherhood through natural fertility (2 patients) or ART (12 patients).

Limitations, reasons for caution: The main limitations of the study are the unavailability of blood hormones for evaluation of testicular function as a whole and the non-compliance of several patients in undergoing follow-up visits at all time points, resulting in a reduced sample size for treatment subgroup analyses. Furthermore, despite a good fertility questionnaire response rate (>80%), the low number of NHL survivors actively seeking fatherhood limits the generalization of results.

Wider implications of the findings: The increased survival of NHL patients of reproductive age makes it essential to focus on the testicular toxicity of its treatment. Sperm cryopreservation must be suggested before any treatment. It is expected that up to 30% of subjects will require treatment intensification, which could result in permanent testicular damage; in such cases the use of banked semen might represent the patient's best chance for future fertility.

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Introduction

The impact of cancer is a cause of great global concern. Italian cancer registers show that nearly 5% of the population has received a cancer diagnosis (AIRTUM, 2017). However, modern treatments grant a life expectancy comparable to the general population in about 60% of paediatric and young adult cancer survivors (Capocaccia et al., 2015). Oncofertility clinicians and researchers focus on subjects considered of reproductive age (18-49 years); men in this age group are mainly affected by testicular cancer (TC) and lymphomas. Despite the high incidence, these subjects have excellent 5-year survival rates, ranging from about 80% for non-Hodgkin lymphomas to above 90% for TC (Dal Maso et al., 2014; Capocaccia et al., 2015).

The scientific literature offers plenty of evidence on testicular function in relation to the most common malignancies in reproductive age, such as testicular cancer (Gandini et al 2006) and Hodgkin lymphoma (Paoli et al. 2016). However, information on other cancers is scanty and more inconsistent, to the point that non-Hodgkin Lymphoma (NHL) survivors cannot currently be offered adequate counselling in our oncofertility outpatient clinic. NHLs are lymphoid neoplasms that affect approximately 1.5 million people worldwide. They are classified as B cell lymphomas (B-NHLs), which account for more than 85% of cases, or T cell (natural killer cell) lymphomas (T-NHLs). Clinically, they are categorized as indolent or aggressive, with follicular lymphoma (FL) the most common indolent histotype and diffuse large B cell lymphoma (DLBCL) the most common aggressive histotype.

NHLs account for about 4.7% of all diagnosed cancers in Italy. While they are generally considered typical of older age groups, they are currently the third most common cancer in males aged 0-49 (~8% of diagnoses) (AIRTUM, 2017). Several industrialized countries have reported a slightly increasing incidence (Chiu and Hou, 2015), but in recent decades survival rates are also improving, with 5-year survival now above 60% (1999-2001 vs. 2005-2007: +9.7% northern Europe, +3.7% southern Europe), and this improvement seems more pronounced in patients aged under 55 years at diagnosis (75% 5-year survival) (AIRTUM, 2017). Early diagnosis and the availability of new treatments and regimens are paramount in these improved outcomes (Shankland et al., 2012), but the benefits are counteracted by the long-term side effects of the treatments, which affect both general health (cardiovascular side effects, secondary cancers, etc.) and reproductive/sexual health (Botchan et al., 1997, Hammond et al., 2008; Arden-Close et al., 2011; Di Bisceglie et al., 2013; Jensen et al., 2013; Kang et al., 2018). In fact, human spermatogenesis can be severely damaged by most chemotherapy drugs (nitrogen mustards, alkylating agents, etc.), with a dose-dependent relationship (Dohle, 2010). Direct and scattered radiation from radiotherapy can also affect the testis (Gandini et al., 2006), where even small doses (2-4 Gy) can cause irreversible damage to seminiferous tubules. Radioprotection

protocols limit this damage, but this does not apply to whole body irradiation prior to bone marrow transplant, with obvious consequences for future fertility (Dohle, 2010).

There is also abundant information in the literature about testicular function in relation to the most common malignancies in reproductive age, such as testicular cancer (Gandini et al 2006; Di Bisceglie et al., 2013) and Hodgkin lymphoma (Sieniawski et al., 2008; Paoli et al. 2016). However, there is little information on NHL, and the few studies that are available report small caseloads and often do not propose any follow-up (Botchan et al., 1997; Caponecchia et al., 2015). In relation to fertility, a recent systematic review (Ferrari et al., 2016) reported a low take-up rate of cryopreserved samples for ART (4-16%), but the review included patients who cryopreserved semen samples for a wide range of oncological diseases and may not be representative of NHL patients. In any case, very little is known about natural fertility in male NHL survivors. This article therefore aims to evaluate semen quality and fertility status before and after the treatment of NHL patients who underwent semen cryopreservation after their diagnosis.

Materials and methods

Subjects

The study was approved by the Policlinico Umberto I Ethics Committee. Initially, we retrospectively selected 227 consecutive patients who attended the Laboratory of Seminology – "*Loredana Gandini*" Sperm Bank of the Department of Experimental Medicine, "Sapienza" University of Rome between 2002 and 2017 for sperm cryopreservation after a diagnosis of NHL. The exclusion criteria were any comorbidity and any other factor known to affect sperm parameters (cryptorchidism, varicocele, testicular trauma or torsion, hypogonadism, urogenital surgery, history of cancer/previous cancer treatments, Klinefelter syndrome or other chromosomal/genetic abnormalities). All patients underwent a thorough medical history and physical examination at the baseline (T0) and had at least one follow-up visit at 6 (T6), 12 (T12), 18 (T18) and/or 24 months (T24) or more than 24 months (T>24), with a median follow-up of 47.5 months (range 28-140 months). Fertility information was collected through the administration of a questionnaire.

Semen analysis

Semen samples were collected by masturbation after 2-7 days' abstinence. All samples were allowed to liquefy at 37 °C for 60 minutes and were then assessed according to WHO (1999, 2010). The following variables were taken into consideration: volume (ml), total sperm number ($N \times 10^6$ per ejaculate), progressive motility (%), and morphology (% abnormal forms). Azoospermic semen samples were centrifuged at 4000 rpm and the entire pellet was examined.

Treatment modality

NHL treatment usually depends on both the subtype/stage of lymphoma and the prognostic factors of disease. For B-NHLs, clinical guidelines recommend the use of anti-CD20 immunotherapy, mainly rituximab, associated with chemotherapy agents, usually with the CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen as first-line therapy. Immunochemotherapy has improved the outcome of most subtypes of B-NHL, with high response rates (DLBCL 80%; FL 90%) and with a 3-year progression-free survival of around 70%, but many patients relapse.
The treatment of relapsed/refractory NHL is still challenging. It typically involves anti-CD20 monoclonal antibodies and different chemotherapy regimens, including platinum-based regimens (R-DHAP; R-ICE; R-DHAOX) or others without cisplatin (R-MAD, R-IEV). High-dose chemotherapy following autologous stem cell transplantation remains the best option to prolong remission in eligible patients.

T-cell lymphomas are rare aggressive diseases, with a very poor prognosis. For these reasons, current guidelines recommend chemotherapy with CHOP or CHOEP followed by high dose chemotherapy and autologous transplantation as first-line treatment in eligible patients.

Statistical analysis

Continuous variables are presented as means, medians and standard deviations. Differences between groups were evaluated by ANOVA or Kruskal-Wallis test, based on data distribution as evaluated by Kolmogorov-Smirnov test. Post-hoc results were corrected for multiple comparisons (Bonferroni). Categorical variables are presented as counts and percentages and were compared by χ^2 test. Statistically significant correlations among the variables examined were evaluated using Spearman's rank correlation test. The probability values are 2-sided and a p value <0.05 was considered statistically significant. All computations were carried out with Statistical Package for the Social Sciences (SPSS) 25.0 (SPSS Inc., Chicago, USA).

Results

Semen quality before cancer treatment

Five of the 227 selected NHL subjects were excluded for the following reasons: two could not collect the semen sample for cryopreservation and three had already started chemotherapy (two of these were azoospermic after 6 cycles of CHOP-R and one was normozoospermic after 1 cycle of CHOP-R). Therefore the analyses included 222 NHL patients (mean age at cryopreservation 32.6 ± 8.6 years). The most frequent histological diagnosis was DLBCL (53.0% of subjects), followed by FL (17.4%) and other B cell subtypes (22.6%). T cell lymphoma was found in 7.0%. Additional subject information (BMI, smoking status, occupation) is available in **Supplementary Table I.**

At the baseline, the mean semen parameters were within the WHO 2010 reference 5th percentile (total sperm number $\geq 39 \times 10^6$ /ejaculate), with 184 (82.9%) normozoospermic patients. No significant correlation was found between age at cryopreservation or specific histological subtype and sperm parameters. However, when stratifying subjects by clinical classification (aggressive, indolent or localized) we found that aggressive lymphomas were associated with a worse semen volume and total sperm number (21.2% oligozoospermia) at the baseline compared to indolent subtypes (5.1% oligozoospermia) and an increased percentage of abnormal forms compared to localized lymphomas (**Figure 1**).

Semen quality after cancer treatment

Table I shows the baseline and longitudinal evaluation of sperm parameters for the whole caseload. Total sperm number, percentage of progressive motility and abnormal forms significantly worsened at T6 compared to the ($p < 0.001$). At T12 the percentage of progressive motility and abnormal forms improved and returned to near-baseline values, while total sperm number remained significantly worse than the baseline at T12, T18 and T24, returning to a near-baseline value only at (T>24) after a median of 47.5 months, although it was still below the T0 mean value (T0: 209.2 ± 190.5 vs. T>24: 160.5 ± 137.8, $p = 0.118$). There was also an increased percentage of patients with a persistent change in total sperm number (below the WHO 2010 5th percentile) 2 years post- treatment, with 25.5% and 21.8% oligozoospermic subjects (χ^2 $p = 0.567$) and 17.0% and 14.1% azoospermic subjects (χ^2 $p = 0.600$) at T24 and T>24, respectively. Linear models were built to detect significant associations of semen parameters 2 years post-treatment, but none of the variables considered (age, BMI, treatment duration, number of chemotherapy cycles, total radiotherapy dose) was significantly associated with post-treatment sperm parameters (total sperm number, progressive motility, abnormal forms).

Type of treatment

Treatment details were available for 172 patients. Of these, 131 (mean age 32.8 ± 8.4 years) had undergone only *first-line treatment*, including a standard chemotherapy regimen (R-CHOP/MACOP-B) and field radiotherapy on the single PET-positive residual disease after the treatment or on the bulky disease presenting at the diagnosis. Following first-line treatment 18 patients (mean age 30.6 ± 7.2 years) had a relapse or disease progression and underwent *salvage treatments* followed by haematopoietic stem cell transplant (HSCT). Both groups were comparable for age, BMI and smoking status. Twenty-three patients who could not be included with either of the previous subgroups were further excluded from analyses as they had undergone different therapeutic regimes for indolent/localized pathology (monotherapy with rituximab; localized radiotherapy; mitoxantrone) or modified regimes due to significant adverse events.

First-line treatment - Subjects undergoing first-line treatment only (mean 6.0 ± 3.4 chemotherapy cycles) appeared to have a more favourable semen parameter outcome. While the progressive motility and abnormal forms worsened at T6 compared to baseline ($p < 0.001$) and subsequently recovered at T12, total sperm number worsened significantly at T6 (T0: 195.0 ± 189.8 vs. T6: 42.4 ± 70.2, p

<0.001) and returned to near-baseline values at T18 (T0: 195.0 ± 189.8 vs. T18: 113.4 ± 103.1, p = 0.278) (**Table II**).

The prevalence of azoospermia was 7.7% at T24 and 6.7% at prolonged follow-up (median 47.5 months). Although no significant association was found between azoospermia and number of cycles of chemotherapy or radiotherapy doses in this subgroup, patients who had undergone radiotherapy of the pelvis vs. other "high" sites (mediastinum, latero-cervical and axillary lymph nodes, etc.) had an increased risk of developing azoospermia after treatment (OR 4.29, 95% CI 1.81 – 10.14; p = 0.001).

Haematopoietic stem cell transplant – Severely impaired spermatogenesis was observed in subjects with NHL relapse or disease progression after first-line treatment who then underwent salvage therapy (R-DHAP, R-MAD etc.) followed by HSCT. Although they were reported as being in complete disease remission, after a follow-up of from 8 months to 7 years after HSCT, two-thirds of subjects (12/18) were azoospermic, three had severe oligoasthenoteratozoospermia and three more were normozoospermic at long term follow-up (7, 4.5, 4.2 years). **Table III** shows the treatments received by these subjects.

Fertility questionnaire

Fertility data was available for 176 patients. Forty-six patients were not included in this survey: 42 did not answer the questionnaire and four died due to cancer progression. Of those answering the questionnaire, 154 (87.5%) did not desire fatherhood: 28 (15.9%) already had at least one child prior to their cancer diagnosis and did not want any more, while 129 patients (73.3%) had no children but had no current desire for fatherhood, for various reasons (young age, financial reasons, marital status). Twenty-two patients (12.5%) wanted children, but only 14 of these succeeded in achieving fatherhood, through natural fertility (two patients) or ART (12 patients). The partners of the remaining eight patients underwent several cycles of ART but failed to carry any pregnancy to term, due to early miscarriage or embryo implantation failure (**Figure 2**).

Discussion

The improved survival rates of NHL patients, peaking at more than 80% in young males in reproductive age (AIRTUM 2017), highlights the need to investigate thoroughly the impact of cancer therapies on quality of life. While reproductive and sexual health are considered an important issue in oncofertility, they seem to be under-investigated in these patients (Greaves et al 2014). Moreover,

the scant literature data refers to small caseloads and must often be inferred from subgroups of different records.

The first published observations date back to the ’90s, but the information is inferred from observations of subgroups from larger caseloads. In 1994, Radford et al. reported that six of seven NHL patients had normal sperm concentration after a mean of 20 months after VAPEC-B and radiotherapy, but data on sperm parameters prior to treatment was not available for most patients. In the same year, Bokemeyer et al. (1994) stated that three of 14 men treated for high grade NHL showed signs of testicular dysfunction, probably due to higher cumulative doses of cyclophosphamide than in the other NHL patients. These results were similar to observations from Tal et al. (2000), who reported a post-treatment worsening of sperm parameters in four of eight NHL patients. Botchan et al. (1997) evaluated pre-treatment semen samples from 89 lymphoma patients (of whom only 18 had NHL), finding significantly worse sperm parameters compared to healthy sperm donors.

In 2012 Bizet et al. carried out an in-depth analysis of pre-treatment sperm parameters in a large caseload of cancer patients. This included 93 NHL patients, whose mean sperm parameters were reported to be within normal ranges for WHO 1999. This was in agreement with the observations of Di Bisceglie et al. (2013; Caponecchia et al. (2015) and Auger et al. (2016). Di Bisceglie et al. (2013) examined 94 NHL patients in a group of 480 patients with various cancers. They found a worsening of semen parameters at 6 and 12 months post-therapy (3-6 cycles of cyclophosphamide, doxorubicin, vincristine plus rituximab and radiotherapy for most patients) and a return to baseline values at 18 months. In contrast, Bujan et al. (2014) found worse pre-treatment sperm parameters in NHL patients compared to healthy controls and a significantly reduced rate of recovery at 24 months post-treatment compared to HL patients (75 lymphoma patients, of whom 18 with NHL).

To our knowledge, the present paper reports the longest follow-up of a large caseload of NHL patients to date. Our data clearly show that most NHL patients present with pre-treatment semen parameters within the WHO 2010 5^{th} percentile; only 17.1% were oligozoospermic. The clinical classification and symptoms of NHL also seem to be linked to semen quality, as indolent and localized NHL were associated with a lower prevalence of oligozoospermia (5.1% and 7.7%, respectively), while aggressive disease was associated with worse semen parameters as well as a 21.2% prevalence of oligozoospermia. After treatment, semen volume did not seem to be affected, probably because the male genital accessory glands are spared whatever the treatment modality and do not suffer permanent damage. However, we did see the expected post-treatment impairment in spermatogenesis, with all remaining semen parameters worsening 6 months post-treatment and a nearly 50% prevalence of azoospermia. This is consistent with previous observations from our group in different types of cancers (Gandini et al., 2004; Paoli et al., 2016), showing the deleterious effects of chemo- and

radiotherapy on spermatogenesis. The total sperm number seems more affected than progressive motility, with heavy damage at T6 and quicker recovery at T12 for motility and longer latency for total sperm number. The duration of the effect on abnormal forms seemed similar to motility, although an increase was seen at longer follow-up, probably associated with ageing. In general, an improvement in semen parameters was only seen with long-term follow-up, returning to near-pre-treatment values only in subjects undergoing first-line treatment.

More intensive regimens appeared to be incompatible with full recovery of spermatogenesis. The increased rate of persistent oligozoospermia and azoospermia at 2 years and longer (median 47.5 months) suggests that permanent quantitative and qualitative impairment of spermatogenesis should be expected for roughly one-third of patients. However, while we did see a slightly increased likelihood of worse outcome in more intensive treatments, we were unable to find any associations between permanent sperm damage and any specific drug, regimen or dose. Unsurprisingly, the only factor associated with a significantly increased risk of azoospermia was pelvic radiotherapy, irrespective of other chemotherapy or cumulative radiotherapy dose. This information is of exceptional value for newly diagnosed patients, as it shows that pre-treatment sperm cryopreservation is essential if the patient has any wish to have children.

The effects of chemo- and radiotherapy on spermatogenesis are well known. Even low doses of radiotherapy disrupt spermatogenesis by inducing both direct and indirect ionization of sperm DNA (Coogle, 1983). This can affect both proliferating spermatogonia, due to rapid mitotic activity, and spermatids, which are particularly vulnerable, due to the lack of any damage repair mechanism (Gandini, 2006). Chemotherapy drugs can cross the blood-testis barrier and actively damage proliferating type B spermatogonia by creating DNA adducts and breaks. However, type A spermatogonia, which possess minimal mitotic activity, are intrinsically more resilient and may survive polychemotherapy threshold doses (Trottmann et al. 2007). Recovery of testicular function after antineoplastic drugs is thus related to the class of drugs used and the cumulative doses.

Even with combination treatments, most subjects in our study showed only transient effects, with spermatogenesis within WHO 2010 normal ranges at long-term follow-up. This might be because most of our patients had more favourable clinical characteristics, such as localized disease, and did not need urgent treatment. Furthermore, patients with early stage NHL received fewer chemotherapy cycles, further minimizing disruption. However, some subjects did show a degree of persistent damage, but with the exception of pelvic/testicular radiotherapy, we could not find any clearly associated variables.

Literature reports concerning the fertility of NHL survivors mainly focus on women. Most analyse the management of NHL diagnosed during pregnancy, a controversial topic with implications for the

appropriate therapeutic approach and outcomes (Avivi et al., 2014; Pinnix et al., 2016). There is little data on post-treatment fertility in male NHL survivors. The previously cited work from Botchan et al. (1997) reported two post-treatment natural pregnancies in the NHL group and two more after intrauterine insemination, all resulting in the delivery of healthy babies. A survey by Meissener et al. (2014) showed that 16 of 23 and eight of 13 NHL survivors conceived naturally after CHOP and CHOEP therapy, respectively. In a large population study involving Norwegian male survivors of childhood cancers, Gunnes et al. (2016) observed a reduced probability of paternity (HR 0.66) in a subgroup of NHL survivors, as well as a nearly three-fold increased probability (RR 2.7) of achieving fatherhood through ART, compared to the general population. ART may in fact offer an important chance of fertility, especially in patients with persistent semen impairment or azoospermia. However, a recent systematic review by Ferrari et al. (2016) revealed that the use of semen cryopreserved prior to treatment is relatively low (about 8%), although nearly half of patients who did use it (49%) were able to achieve fatherhood. However, this review included studies of caseloads of a wide range of neoplastic diseases, and these probabilities cannot therefore be fully extrapolated to NHL patients. The results of our fertility survey are substantially in agreement with previous observations: several patients already had children prior to NHL diagnosis and, among those who wanted children (24 patients), fourteen (63.6%) achieved fatherhood through natural fertility or ART.

In conclusion, our paper reports one of the largest caseloads and the longest follow-ups of NHL patients published to date. The monocentric nature and the consequent standardization of the semen analysis adds strength to our results. However, they are limited by the unavailability of blood hormone tests and the limited compliance of several patients in performing follow-up visits at all time points, resulting in a reduced number of subjects for therapy subtype analyses, especially in the HSCT subgroup. The fertility survey had an acceptable response rate (nearly 80%), limiting the probability of patient selection bias.

The increased survival of NHL patients in reproductive age highlights the need to focus on the gonadal toxicity of its treatment. Although natural fertility in NHL survivors has been described, many patients, although cured, may experience long-term reproductive problems and more than one-third may suffer permanently impaired spermatogenesis or azoospermia. More than 90% of subjects undergoing standard first-line treatment (mostly R-CHOP) are likely to recover spermatogenesis, with pelvic radiotherapy as the only negative prognostic factor for permanent sperm damage. However, subjects who experience disease progression or relapse and have to undergo second-line cisplatin-based treatment with HSCT have a worse chance of spermatogenesis recovery compared to cisplatin-free regimens (experimental protocols). This is important, because is not possible to predict with certainty the evolution of the pathology at the time of diagnosis: the treatment might need to be

intensified, resulting in a higher probability of testicular damage. It is thus essential to suggest sperm cryopreservation before any treatment, as the use of banked semen might represent the patient's best chance for future fertility.

Author's roles

FP and DP conceived the study design and drafted the article; FP, MP, ADR and FF acquired the data; FP and SDC administered the fertility questionnaires; DP performed the semen analyses; FP analysed the data; and FL and AL assisted in data interpretation. All authors revised the manuscript critically.

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Conflict of interest

The authors have no conflicts of interest.

Figure captions

Figure 1 – Comparisons of baseline sperm parameters of the study group, stratified by clinical appearance of NHL.

Figure 2 – Flowchart of the fertility questionnaire study of NHL patients.

Tables legends

Table I - Semen parameters of NHL patients at baseline (T0) and follow up (T6-T24). Data are presented as mean, SD, medians (in brackets) and 25^{th}-75^{th} percentile distribution. Azoospermic patients are excluded from statistical analysis. (n.s.: not signficant) (Kruskal-Wallis test with Bonferroni correction for multiple comparisons)

[a] n.s. vs. T0

[b] $p < 0.001$ vs. T0

[c] $p < 0.01$ vs. T0

[d] $p < 0.05$ vs. T0

Table II – Semen parameters of NHL patient subgroup who underwent only a first-line treatment at baseline (T0) and follow up (T6-T24). Data are presented as mean, SD, medians (in brackets) and 25^{th}-75^{th} percentile distribution. Azoospermic patients are excluded from statistical analysis. (n.s.: not significant) (Kruskal-Wallis test with Bonferroni correction for multiple comparisons)

[a] n.s. vs. T0

[b] $p < 0.001$ vs. T0

Table III – Outcome of subjects who underwent further treatments ± HSCT after relapse and/or disease progression.

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