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ONCOFERTILITY AND ONCOSEXUALITY IN NON-HODGKIN LYMPHOMA AND TESTICULAR CANCER SURVIVORS

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ABSTRACT

Background – Recently great attention was brought on long-term consequences of antineoplastic treatments, with special focus to reproductive and sexual health. Human testis can be severely damaged by chemo and radiotherapy but, while literature offers plenty of data regarding common malignancies in reproductive age such as testicular cancer and Hodgkin lymphoma, we know little about other neoplasias. Similarly, knowledge of male sexological alterations in cancer derives mainly from prostate cancer, which can hardly be generalized to other pathologies. Since little is known about Non-Hodgkin Lymphoma survivors' fertility and there is incomplete information for Testicular Cancer survivors sexological counselling, the aim of this work was: (Study 1) to evaluate semen quality and fertility status before and after treatments of patients who underwent semen cryopreservation after the diagnosis of NHL; (Study 2) to evaluate the effect of TC after orchiectomy and provide a complete follow up in order to highlight possible post treatment short and long-term sexological alterations.

Material and Methods – Patients attending the Laboratory of Seminology – Sperm Bank "Loredana Gandini" for sperm cryopreservation were selected for both studies after a careful medical history collection. Exclusion criteria were any comorbidity and any other known factor interfering with sperm parameters. For Study 1, 224 patients (mean age 32.7 ± 8.6 years) with diagnosis of Non-Hodgkin Lymphoma were selected, and semen analysis was performed at baseline (T0) and at least one follow up visit at 12 (T1), 24 (T2) or more than 24 months (T3; median 52 months); fertility status was ascertained with a dedicated questionnaire. For Study 2, 241 sexually active consecutive patients (mean age 31.3 ± 6.9 years) with recent diagnosis of testicular cancer were selected. IIEF questionnaire was completed for sexual function evaluation at baseline post-orchiectomy (T0) and at least one follow up control at 6 (T1), 12 (T2), 18 (T3), 24 (T4), 48 months (T5) and after 5 years (T6, median 96 months) after chemotherapy; 223 healthy controls were also recruited for IIEF scores comparisons. Moreover, both TC patient and controls underwent blood hormones analysis (FSH, LH, total Testosterone).

Results – *Study 1* – Non-Hodgkin Lymphoma patients showed pre-therapy mean semen parameters within WHO 2010 normal range. Longitudinal post-therapy evaluation

showed that sperm parameters significantly worsened at T1 compared to T0 ($p < 0.001$). Total sperm number at T2 remained significantly worse than T0 ($p = 0.040$) whereas it returned to values comparable to baseline at T3. Progressive motility and abnormal forms returned comparable to baseline at T2. 13.7% of survivors were azoospermic at T3. Permanent spermatogenesis impairment was associated with pelvic radiotherapy (OR 14.54, 95% CI 1.90 – 111.28) and treatment intensification for bone marrow transplant (33% azoospermia at T3). Regarding fertility, 14/22 pts who desired children were able to achieve fatherhood either through natural fertility (2 pts) or through ART (12 pts). *Study 2* – Baseline prevalence of erectile dysfunction is 37.8% in TC pts vs 9.9% in healthy controls ($p < 0.001$). IIEF-15 baseline scores were significantly worse in TC group compared to controls ($p < 0.001$), with the exception of the orgasmic function domain ($p = 0.334$). Post-chemotherapy evaluation showed that erectile function improves significantly at T2 (T0 vs T2; $p < 0.001$) with further improvements at T3 and T4 compared to baseline (T0 vs T3: $p = 0.014$; T0 vs T4: $p = 0.002$). However, we detected an increase in erectile dysfunction prevalence at T5 with a significant reduction of erectile function domain scores, which seemed to persist at T6. Compared to controls, erectile function remains significantly worse at T1 then return comparable to healthy controls. Sexual desire, intercourse satisfaction and general satisfaction showed trends of improvement from baseline but remained significantly worse compared to controls for the whole duration of the study. No significant variation of the orgasmic function was detected against both baseline values and controls. The evaluation of sexual hormones revealed that prevalence of biochemical hypogonadism was 5.4% in the TC group. There were no hypogonadal patients in CTR group. Total testosterone in post-orchidectomy patients (T0) is significantly lower than controls ($p < 0.001$), but no significant variation was detected at T1 and T2. Finally, no significant correlation was detected between total testosterone levels and scores of any IIEF15 domain.

Discussion – Study 1 demonstrates that Non-Hodgkin Lymphoma survivors undergoing intensive treatments and pelvic radiotherapy risk severe and permanent impairment of spermatogenesis. However, routine NHL chemotherapy regimens are compatible with spermatogenesis recovery after 2 years from the end of treatments and, while average

sperm parameters may not fully return to pretreatment values, more than a half (63%) of patient who actively desire fatherhood can conceive either through natural conception or ART. Similarly, for Testicular Cancer survivors we confirm the presence of erectile dysfunction and impairment of sexual desire and satisfaction compared to a healthy population with improvements expected within one year from the end of the treatments. Absence of clear correlations with biochemical hypogonadism suggests that psychological burden following cancer diagnosis and treatments may play an important role. Information from these studies is of extreme importance since it will allow to increase the effectiveness of patients' counseling interventions in an oncofertility service.

BACKGROUND

Oncofertility is a relatively new field whose purpose is to preserve and investigate reproductive function in cancer patients (Trost and Brannigan, 2012) and current trends of improvement of cancer patients' survival amplified its importance. In fact, the impact of cancer on worldwide overall mortality is a great cause of 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 general population to about 60% of young and young adult cancer survivors. In particular, attention of clinicians and researchers has focused on men in reproductive age (18-49 years) who are mainly affected by testicular cancer (TC) and lymphomas. Despite a 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). Thus, these cancer survivors face long-term physical and psychological consequences of both treatments (surgical, chemical, radiant) and cancer diagnosis (Carpentier et al., 2010; Brand et al., 2014). Health, social and economic repercussions are relevant (Jönsson et al., 2016) since these long-term consequences will involve people during their working and reproductive life, influencing physical abilities as well as reproductive and sexual health. Thus, the consequences of antineoplastic treatments on sexuality and fertility, essential for the patient's successful return to family and social life, must be evaluated (Paoli et al., 2015). In fact, human testis and spermatogenesis can be severely damaged by chemo and radiotherapy (Gandini et al., 2006; Dohle, 2010; Meistrich, 2013; Paoli et al., 2018). Scientific literature offers plenty of data about gonadal function of the most common malignancies in reproductive age, such as testicular cancer (Gandini et al 2006) and Hodgkin Lymphoma (Paoli et al. 2015), but data regarding other cancers are scarce, report small caseloads and, often, do not constantly propose a follow up of these subjects. Similarly, knowledge of male sexological alterations following antineoplastic treatments and surgery derives mainly from prostate cancer, which presents peculiar differences because of more invasive surgical procedures and hormonal treatments. (Katz and Dizon, 2015). Thus, it is clear that we currently cannot offer adequate knowledge on Non-Hodgkin Lymphoma (NHL) survivors' fertility in an oncofertility

outpatient clinic. Moreover for Testicular Cancer (TC) patients sexological counselling we still rely on information which may not necessarily be representative of their condition. Hence, the aim of this work will be to bridge the gap in two important emerging issues in the clinical practice.

STUDY 1: EVALUATION OF SEMEN QUALITY AND FERTILITY BEFORE AND AFTER ANTINEOPLASTIC TREATMENT OF NON-HODGKIN LYMPHOMA SURVIVORS

Introduction

NHLs are a heterogeneous group of lymphatic cancers. In Italy, they represent about 4.7% of all diagnosed cancers and, while they are generally considered typical of older age classes, they are currently the third most common cancers in males aged 0-49 (~8% of diagnoses) (AIRTUM, 2017). Several industrialized countries reported a mildly increasing incidence of LNH (Chiu and Hou, 2015), but in the last decades survival rates are improving as well: 5-year survival is now above 60% (1999-2001 vs 2005-2007: +9,7% northern Europe, +3,7% southern Europe) and such improvement seems more pronounced in patients aged < 55 years at diagnosis (75% 5-year survival) (AIRTUM, 2017). Early diagnosis and the availability of new therapies and regimens were paramount in these improved outcomes (Shankland et al., 2012), but benefits are counterbalanced by long term side effect of the treatments affecting 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 drugs (nitrogen mustards, alkylating agents, etc.), with a dose-dependent relation (Dohle, 2010). Also, direct and scattered radiation from radiotherapy can 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 occur in whole body irradiation prior to bone marrow transplant, with obvious consequences on future fertility (Dohle, 2010). Furthermore, there is abundant literature data about gonadal function of 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), but data regarding NHL are scarce, report small caseloads and, often, do not propose a follow up of these subjects (Botchan et al., 1997; Caponecchia et al., 2015).

Regarding fertility, a recent systematic review (Ferrari et al., 2016) reported a low utilization rate of cryopreserved samples for ART (4-16%), but it refers to patients who cryopreserved semen samples for a wide range of oncological diseases and may not be representative of NHL patients. Moreover, very little is known about natural fertility in male NHL survivors. Thus, this study will aim to evaluate semen quality and fertility status before and after treatments of patients who underwent semen cryopreservation after the diagnosis of NHL.

Materials and methods

Subjects

We selected 224 consecutive patients who attended the Laboratory of Seminology – Sperm Bank "Loredana Gandini" of the Department of Experimental Medicine – "Sapienza" University of Rome between 2006-2015 for sperm cryopreservation after the diagnosis of NHL for baseline evaluation. Exclusion criteria were any comorbidity and any other known factor interfering with 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 careful medical history and semen analysis at baseline (T0) and had at least one follow up visit at 12 months (T1), 24 months (T2) or more than 24 months (T3) with a median of 52 months (range 28-140 months). Furthermore, information about fertility 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 × 10^6 per ejaculate), progressive motility (%), and morphology (% abnormal forms). Azoospermic semen samples were centrifuged at 4000 rpm and the entire pellet was examined.

Statistical analysis

Continuous variables are presented as mean, 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 by the Bonferroni method for multiple comparisons. 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

Pre-therapy - We evaluated 224 NHL patients (mean age at cryopreservation 32.7 ± 8.6 years). The most frequent histological diagnosis was a diffuse large cell B lymphoma (DLCBL) in 53.0% of subjects, followed by follicular lymphoma in 17.4% and other B cell subtypes were present in 22.6%. T cell lymphoma was present in 7.0%.

Table 1 summarises sperm parameters at baseline (T0). Mean semen parameters were normal according to WHO 2010 references; 188/224 (83.9%) had total sperm number ≥ 39 x 10^6 /ejaculate. No significant correlation was detected among age at cryopreservation or histological subtype and sperm parameters.

Post-therapy - Results from longitudinal evaluation of sperm parameters of the whole caseload is reported in Table 2. Total sperm number, percentage of progressive motility and abnormal forms significantly worsened at T1 compared to T0 ($p < 0.001$). Total sperm number at T2 remained significantly worse than T0 ($p = 0.040$) whereas at T3 it returned to values comparable to baseline (214.7 ± 212.7 vs 138.9 ± 120.8, T0 vs T3 respectively; $p = 0.107$) (Figure 1a). Progressive motility and abnormal forms, instead, returned to values comparable to baseline at T2, without significant variations at T3 (Figure 1b, c). It should be stressed, however that the percentage of patients with total sperm number below WHO 2010 5^{th} percentile increased significantly from 16.1% at T0 to 33.9% at T3 (χ^2 $p = 0.006$) with roughly one third of azoospermia (T3, 13.7%).

Data regarding therapies was available for 173/224 patients: 69 patients (mean age 33.7 ± 9.0 years) underwent chemotherapy alone (Group A); 55 patients (mean age 30.3 ± 7.5 years) underwent chemotherapy and radiotherapy (Group B); 48 patients (mean age 34.0 ± 7.9 years) underwent intensified therapy regimens followed by haemopoietic stem cell transplant (Group C). Table 3 reports a summary of the therapy regimens underwent by subjects from each group. All patients from these groups were comparable by age and pre-treatment sperm parameters, as shown in Table 4.

Longitudinal evaluation of sperm parameters of Group A, B and C is reported in Table 5 a, b, c. In particular:

- Group A - Total sperm number and progressive motility worsened significantly at T1 vs T0 ($p < 0.001$ and $p = 0.01$, respectively) and returned to values comparable to T0 at T2 and T3. Moreover, no significant differences regarding

semen volume and abnormal forms were detected at all time points compared to baseline, although we observed a trend of increase of percentage of abnormal forms T1 vs T0 ($p = 0.09$).

- Group B - Total sperm number and progressive motility worsened significantly at T1 vs T0 ($p < 0.001$ and $p = 0.017$, respectively) and percentage of abnormal forms increased significantly T1 vs T0 ($p = 0.011$). No significant variation of semen volume was detected. All parameters returned comparable to baseline values at T2 and at T3.
- Group C - Similarly, total sperm number and progressive motility worsened significantly at T1 vs T0 ($p = 0.004$ and $p = 0.050$, respectively) and percentage of abnormal forms increased significantly T1 vs T0 ($p = 0.027$). However, total sperm number remains significantly worse compared to T0 at T2 ($p = 0.029$) and T3 ($p = 0.011$). Progressive motility and abnormal forms, instead, showed no significant differences at T2 and T3 compared to T0. Semen volume did not differ significantly at all time points.

Also, it is noteworthy that percentages of azoospermic patients at long term follow up differed for each treatment group: 33.3% of patients from Group C had persistent azoospermia at T3, while in Group B and A these percentages were 12.0% and 0%, respectively (χ^2 $p = 0.021$). Logistic binary regression models showed no significant association between sperm parameters and number of cycles of chemotherapy and radiotherapy doses. However, we detected a significantly increased risk of azoospermia in patients who underwent radiotherapy localized at the pelvis vs other "high" sites (mediastinum, latero-cervical and axillary lymph nodes, etc.) (83.3% azoospermia vs 11.6%, respectively; $p = 0.003$; OR 14.54, 95% CI 1.90 – 111.28).

Finally, data on fertility was available for 178/224 patients. Sixty-six patients were interviewed personally, while for 158 data was retrieved by telephone interview. Forty-six patients were not included in this survey: 42 were untraceable and 4 deceased for cancer progression. A total of 156 patients (87.7%) revealed not to desire fatherhood: 28 patients (15.6% of total sample) had already at least one child prior to cancer diagnosis and did not desire more, while 128 patients (72.1%) had no children but had no desire for fatherhood yet for various reasons (young age, economical reasons, marital status).

Twenty-two patients (12.3%) desired children, but only 14/22 were able to achieve fatherhood either through natural fertility (2 patients) or through ART (12 patients). The remaining 8 patients underwent several cycles of ART but were unable to carry out pregnancy due to early miscarriage or embryo implantation failure.

	Age (Years)	**Volume (ml)**	**Total Sperm Number (n x 10^6/ejac.)**	**Progressive Motility (%)**	**Abnormal Forms (%)**
T0 *224 patients*	**32.7 ± 8.6 (32.5)** *26.0 – 39.0*	**3.3 ± 1.6 (3.0)** *2.1 – 4.2*	**214.7 ± 212.7 (173.2)** *61.2 – 303.2*	**38.8 ± 17.3 (45.0)** *30.0 - 50.0*	**80.3 ± 12.6 (80.0)** *72.8 – 88.0*

Table 1 – Mean, SD, medians (in brackets) and 25^{th}-75^{th} percentile distribution of NHL patients age and baseline sperm parameters.

	Volume (ml)	Total Sperm Number (n x 10^6/ejac.)	Progressive Motility (%)	Abnormal Forms (%)	Azoospermia (%)
T0 *224 pts*	**3.3 ± 1.6 (3.0)** *2.1 – 4.2*	**214.7 ± 212.7 (173.2)** *61.2 – 303.2*	**38.8 ± 17.3 (45.0)** *30.0 - 50.0*	**80.3 ± 12.6 (80.0)** *72.8 – 88.0*	**0% (0/224)**
T1 *86 pts*	**3.3 ± 1.9[a] (3.0)** *2.0 – 4.0*	**69.3 ± 91.4[b] (37.0)** *2.4 - 100*	**27.0 ± 20.2[b] (25.0)** *5.0 – 45.0*	**87.0 ± 10.3[b] (87.5)** *78.0 – 97.0*	**32.8% (42/128)**
T2 *49 pts*	**3.4 ± 1.8[a] (3.0)** *2.0 – 4.2*	**129.0 ± 131.1[c] (99.0)** *41.6 – 174.0*	**39.8 ± 17.2[a] (45.0)** *25.0 – 55.0*	**82.9 ± 9.3[a] (82.0)** *77.0 – 89.0*	**22.2% (14/63)**
T3 *51 pts*	**3.3 ± 1.6[a] (3.0)** *2.0 – 4.2*	**138.9 ± 120.8[a] (108.0)** *50.0 – 216.0*	**38.1 ± 19.9[a] (45.0)** *25.0 – 55.0*	**84.1 ± 14.7[a] (88.0)** *78.0 – 90.0*	**13.3% (8/59)**
P value	***n.s.***	***<0.001***	***<0.001***	***<0.001***	*//*

Table 2 – Semen parameters of NHL patients at baseline and follow up. Data are presented as mean, SD, medians (in brackets) and 25th-75th 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*

[c] *$p < 0.05$ vs T0*

Study group (224 subjects)	
Age at diagnosis (years)	**32.7 ± 8.6 (32.5)** *26.0 – 39.0*
BMI (kg/m^2)	**24.6 ± 3.3 (24.0)** *22.6 – 25.9*
Smokers	**37 (16.8%)**
Cigarettes/day [a]	**13.2 ± 18.3 (10.0)** *5.0 – 15.0*
Years of smoking [a]	**13.3 ± 6.9 (15.0)** *9.3 – 20.0*
Chemotherapy Regimens (Group A and B)	**80.1% R-CHOP/CHOEP 19.9% MACOP-B/BEACOPP**
No. Cycles of Chemotherapy	**6.9 ± 2.9 (6.0)** *6.0 – 8.0*
Radiotherapy Dose (Gy) [b]	**33.8 ± 8.6 (30.0)** *30.0 – 36.0*
Chemotherapy Regimens (Group C)	**4 cycles CHOP-R + 2-3 cycles RMAD/IEV + BEAM/FEAM or 6-8 cycles CHOEP-R + 2-3 cycles DHAO/IEV + BEAM/FEAM**

Table 3 – Patients demographics.

[a] smokers only
[b] group b only

	Age (years)	Volume (ml)	Total Sperm Number (n x 10^6/ejac.)	Progressive Motility (%)	Abnormal Forms (%)
Group A *67 pts*	**33.7 ± 9.1 (33.0)** *26.0 – 40.0*	**3.4 ± 1.4 (3.4)** *2.5 – 4.5*	**206.7 ± 166.2 (168.0)** *52.5 – 337.5*	**38.6 ± 19.3 (45.0)** *30.0 – 55.0*	**80.8 ± 12.9 (82.0)** *70.0 – 90.0*
Group B *58 pts*	**30.2 ± 7.5 (29.0)** *25.0 – 38.0*	**3.2 ± 1.6 (3.0)** *2.0 – 4.0*	**210.4 ± 273.1 (125.0)** *51.1 – 294.0*	**36.7 ± 17.9 (40.0)** *30.0 – 50.0*	**79.3 ± 15.0 (80.0)** *74.0 – 88.0*
Group C *48 pts*	**34.0 ± 7.9 (34.0)** *29.0 – 39.0*	**3.3 ± 1.8 (3.0)** *2.0 – 4.0*	**235.8 ± 240.4 (187.0)** *69.0 – 300.0*	**39.6 ± 15.7 (42.5)** *30.0 – 50.0*	**80.3 ± 9.5 (80.0)** *73.5 – 87.5*
p-value	**0.058**	**0.450**	**0.646**	**0.510**	**0.961**

Table 4 - Semen parameters of NHL patients at baseline and follow up. Data are presented as mean, SD, medians (in brackets) and 25^{th}-75^{th} percentile distribution. (Kruskal-Wallis test with Bonferroni correction for multiple comparisons)

	Volume (ml)	Total Sperm Number (n x 10^6/ejac.)	Progressive Motility (%)	Abnormal Forms (%)	Azoospermia (%)
T0 *67 pts*	**3.4 ± 1.4** **(3.4)** *2.5 – 4.5*	**206.7 ± 166.2** **(168.0)** *52.5 – 337.5*	**38.6 ± 19.3** **(45.0)** *30.0 – 55.0*	**80.8 ± 12.9** **(82.0)** *70.0 – 90.0*	**0%** **(0/67)**
T1 *35 pts*	**3.4 ± 1.9**[a] **(2.8)** *1.8 – 4.0*	**69.9 ± 72.3**[b] **(42.0)** *5.4 – 120.0*	**28.1 ± 19.5**[c] **(35.0)** *0.0 – 45.0*	**86.1 ± 11.5**[a] **(87.0)** *76.0 – 100.0*	**23.9%** **(11/46)**
T2 *21 pts*	**3.0 ± 1.5**[a] **(2.8)** *1.9 – 4.0*	**149.0 ± 158.5**[a] **(99.0)** *48.4 – 216.0*	**41.0 ± 17.3**[a] **(50.0)** *25.0 – 55.0*	**83.3 ± 9.7**[a] **(82.0)** *77.0 – 89.0*	**4.5%** **(1/22)**
T3 *18 pts*	**3.6 ± 1.3**[a] **(3.0)** *3.0 – 4.5*	**179.6 ± 149.0**[a] **(141.3)** *63.0 – 280.0*	**41.4 ± 19.0**[a] **(47.5)** *30.0 – 55.0*	**86.3 ± 9.1**[a] **(88.0)** *80.0 – 93.0*	**0.0%** **(0/18)**
P value	***0.439***	***<0.001***	***0.005***	***0.121***	*//*

Table 5 a - Semen parameters of Group A patients at baseline and follow up. Data are presented as mean, SD, medians (in brackets) and 25th-75th 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*

[c] *p = 0.010 vs T0*

	Volume (ml)	Total Sperm Number (n x 10^6/ejac.)	Progressive Motility (%)	Abnormal Forms (%)	Azoospermia (%)
T0 *58 pts*	**3.2 ± 1.6** **(3.0)** *2.0 – 4.0*	**210.4 ± 273.1** **(125.0)** *51.1 – 294.0*	**36.7 ± 17.9** **(40.0)** *30.0 – 50.0*	**79.3 ± 15.0** **(80.0)** *74.0 – 88.0*	**0%** **(0/58)**
T1 *30 pts*	**3.0 ± 1.4**[a] **(3.0)** *2.0 – 4.0*	**59.4 ± 79.0**[b] **(37.0)** *2.3 – 89.6*	**26.4 ± 20.3**[c] **(25.0)** *7.5 – 45.0*	**87.3 ± 9.8**[d] **(87.0)** *80.0 – 95.0*	**33.3%** **(15/45)**
T2 *20 pts*	**3.3 ± 1.5**[a] **(3.0)** *2.4 – 4.2*	**139.5 ± 110.6**[a] **(115.0)** *48.8 – 198.0*	**44.8 ± 13.7**[a] **(47.5)** *42.5 – 55.0*	**80.1 ± 8.5**[a] **(81.0)** *74.0 – 87.5*	**20.0%** **(5/25)**
T3 *22 pts*	**3.1 ± 1.3**[a] **(2.8)** *3.0 – 3.8*	**137.3 ± 94.3**[a] **(120.5)** *64.0 – 190.0*	**40.9 ± 16.9**[a] **(50.0)** *35.0 – 50.0*	**85.1 ± 7.5**[a] **(87.5)** *78.0 – 89.0*	**12.0%** **(3/25)**
P value	***0.867***	***<0.001***	***0.003***	***0.006***	*//*

Table 5 b - Semen parameters of Group B patients at baseline and follow up. Data are presented as mean, SD, medians (in brackets) and 25th-75th 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*

[c] *p = 0.017 vs T0*

[d] *p = 0.011 vs T0*

	Volume (ml)	Total Sperm Number (n x 10^6/ejac.)	Progressive Motility (%)	Abnormal Forms (%)	Azoospermia (%)
T0 *48 pts*	**3.3 ± 1.8** **(3.0)** *2.0 – 4.0*	**235.8 ± 240.4** **(187.0)** *69.0 – 300.0*	**39.6 ± 15.7** **(42.5)** *30.0 – 50.0*	**80.3 ± 9.5** **(80.0)** *73.5 – 87.5*	**0%** **(0/48)**
T1 *10 pts*	**4.5 ± 3.3**[a] **(3.0)** *2.4 – 5.8*	**44.5 ± 72.5**[b] **(23.0)** *0.6 – 45.0*	**19.4 ± 20.8**[e] **(25.0)** *0.0 – 25.0*	**90.6 ± 8.4**[f] **(90.0)** *88.0 – 100.0*	**61.5%** **(16/26)**
T2 *8 pts*	**4.3 ± 3.1**[a] **(3.0)** *2.3 – 5.5*	**50.7 ± 66.2**[c] **(33.5)** *6.9 – 60.0*	**24.4 ± 17.6**[a] **(33.5)** *12.5 – 37.5*	**88.6 ± 8.1**[a] **(88.5)** *85.5 – 95.0*	**50.0%** **(8/16)**
T3 *10 pts*	**3.0 ± 2.5**[a] **(2.0)** *1.2 – 3.8*	**54.1 ± 67.8**[d] **(40.0)** *0.3 – 72.0*	**23.6 ± 23.1**[a] **(50.0)** *35.0 – 50.0*	**77.5 ± 29.1**[a] **(84.5)** *75.0 – 95.0*	**33.3%** **(5/15)**
P value	***0.867***	***<0.001***	***0.008***	***0.011***	*//*

Table 5 c - Semen parameters of Group C patients at baseline and follow up. Data are presented as mean, SD, medians (in brackets) and 25th-75th 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 = 0.004 vs T0*

[c] *p = 0.029 vs T0*

[d] *p = 0.011 vs T0*

[e] *p = 0.050 vs T0*

[f] *p = 0.027 vs T0*

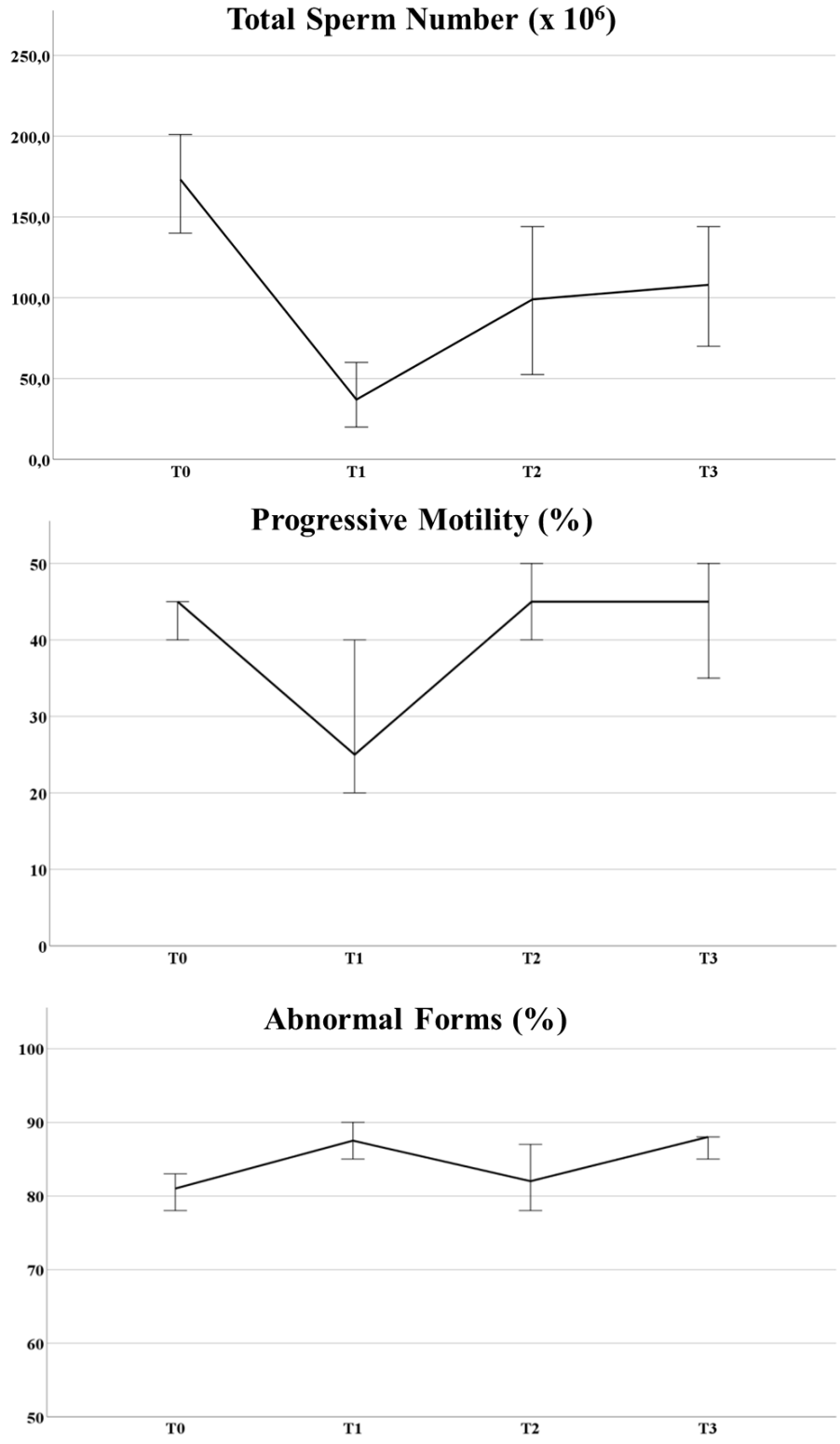

Figure 1 - Semen parameters of NHL patients at baseline and follow up.

Discussion

The improvement of the survival rates of NHL patients, with peaks >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 setting, these seem to be often overlooked in these patients (Greaves et al 2014). Moreover, literature data is scarce, refers to small caseloads, and often must be inferred from subgroups of different records. The first observations published, date back to the '90s but mention observations of subgroup of patients from larger caseloads of lymphomas. Earlier observations date back to 1994, when Radford et al. reported that 6/7 NHL patients had normal sperm concentration after a mean of 20 months from VAPEC-B and radiotherapy, but data on sperm parameter prior to treatment was not available for most patients; the same year, Bokemeyer et al. (1994) stated that 3/14 men treated for high grade NHL showed signs of gonadal dysfunction, probably due to higher cumulative dose of cyclophosphamide than the remaining NHL patients. These results were similar to further observations from Tal et al. (2000) who reported the post therapy worsening of sperm parameters of 4/8 NHL patients.

Pre-therapy- These observations were enriched by comparison with a control group in 1997, when Botchan et al. evaluated pre-treatment semen samples from 89 lymphoma patients (of whom 18 NHL), finding significantly worse sperm parameters than those from healthy sperm donors. The first in-depth analysis of pre-therapy sperm parameters of NHL patients, however, dates to 2012, when Bizet et al. reported a wide caseload of cancer patients, including 93 NHL patients whose mean sperm parameters were reported to be within normal range values for WHO 1999. This was in agreement with following observations from Di Bisceglie et al. (2013), who reported NHL to have better baseline semen parameters than testicular cancers and comparable to other hematological malignancies, Caponecchia et al. (2015), who observed that 25 NHL referring for sperm cryopreservation had baseline sperm parameter comparable to a group of fertile controls, and Auger et al. 2016, who reported pre-therapy sperm parameters of 439 NHL patients compared to other pretherapy cancer groups.

Post-therapy- Di Bisceglie et al. (2013), in particular, examined 94 NHL patients, among a group of 480 patients with various malignancies, showing a worsening of semen parameters at 6 and 12 months from therapy (3-6 cycles of cyclophosphamide, doxorubicin, vincristine plus rituximab and radiotherapy for most patients) and return to baseline values from 18 months. Other Authors, instead, published a caseload of 75 lymphoma patients, of whom 18 NHL reporting different results (Bujan et al., 2014). In fact, these Authors showed worse NHL patients pre-treatment sperm parameters compared to healthy controls and a significantly reduced rate of recovery after 24 months from therapy compared to HL patients. In our knowledge, our paper reports the larger caseload of NHL patients to date. Our overall data clearly show that most NHL patients present with pre-therapy semen parameters within WHO 2010 5^{th} percentile, with only 16.1% of oligozoospermic patients. Moreover, we confirm previous observations of recovery of spermatogenesis after therapy. All semen parameters, but semen volume, significantly worsen after therapy with more than 30% showing azoospermia at 12 months after the end of antineoplastic treatment. After 24 months average semen parameters show improvements, returning comparable to pre-treatment values. However, the increased rate of persistent oligoozoospermia and azoospermia (cumulatively 33.9%) at longer follow up (median 52 months) demonstrate permanent qualitative alterations of spermatogenesis for roughly one-third of patients. To investigate the role of different treatments in NHL survivors' gonadal function, patients were divided in three groups: chemotherapy only (Group A), combined chemo and radiotherapy (Group B) and intensive treatments followed by hematopoietic stem cell transplant (Group C). Subgroup analysis revealed that no treatment significantly affected semen volume, probably since male genital accessory glands are spared by each treatment modality and do not suffer permanent damage. Total sperm number and progressive motility are affected in a similar way in group A and B, with transient damage at T1 and at least partial recovery from the second year after treatments. Abnormal forms instead seem to be more affected by radiotherapy (Group B), although a trend of increase is also detected in subjects treated by chemotherapy only. These alterations all together are coherent with our previous observations in other cancer groups (Gandini et al., 2006; Paoli et al., 2016). Moreover, persistent azoospermia was detected in 12.0% of patients from Group B and in no patients from Group A at T3. It

should be noticed that almost all azoospermic patients underwent radiotherapy localized at the pelvic region. Further, results from group C show that total sperm number, progressive motility and abnormal forms are all affected by the intensive multimodal treatment preceding bone marrow transplant and long term follow up revealed persistent alterations of TSN (median 52 months of follow up) and up to 33.3% patients with azoospermia at the end of follow up. In all groups, we were unable to detect significant predictors of permanent damage and found only a significantly increased risk of azoospermia for patients who underwent radiotherapy to the pelvic region, irrespective of other chemical treatment or cumulative radiotherapy dose. Taken together, these are relevant informations for a newly diagnosed patient, as indicates that sperm cryopreservation in mandatory before the start of any therapy if there is or there may be desire for fatherhood. In fact, the effects of radiotherapy on spermatogenesis are known. Radiations, even at low doses, disrupt spermatogenesis by inducing both direct and indirect ionization of sperm DNA (Coogle, 1983). This can negatively affect both proliferating spermatogonia, due to rapid mitotic activity, and spermatids, whose are particularly vulnerable due to the lack of damage repair mechanism (Gandini, 2006). The effects of chemotherapy alone seem short-termed in our study, as most patients at longer follow up show spermatogenesis within WHO 2010 normal ranges and no cases of permanent azoospermia have been detected. One possible explanation is that our group of patients treated with chemotherapy alone is made up of good-prognosis subjects who underwent only the minimal number of cycles necessary for treatment and, thus, their spermatogenesis received minimal disruption compared to the other groups. In fact, chemotherapy drugs are capable to cross the blood-testis barrier and to damage actively proliferating type B spermatogonia by creating DNA adducts and breaks. However, type A spermatogonia which possess minor mitotic activity are intrinsically more resilient and, provided that polychemotherapy threshold doses are not surpassed, may survive the chemical insult (Trottmann et al. 2007). Thus, gonadal function recovery after antineoplastic drugs is related to the class of drugs used and cumulative doses. Regarding fertility of NHL survivors, literature data focuses mostly on female survivors. Most data analyses the management of NHL diagnosed during pregnancy, which is a rather controversial with implication on the appropriate therapeutic approach and outcomes (Avivi et al., 2014; Pinnix et al., 2016). Data on post therapy fertility

outcomes is scarce. Dann et al. (2005), observed that of 13 consecutive women aged ≤ 40 years treated for NHL after a follow up of 70 months, only one patient had iatrogenic ovarian failure and eight were able to conceive spontaneously with the delivery of 12 healthy babies. Anderson et al. (2017) in a large population studies of childhood and young adult female cancer survivors, found that obstetric complications, such as pre-term birth and low birth weight, were also associated with a diagnosis of NHL and, possibly, its treatments. Very little is known about male NHL survivors. The previously cited work from Botchan et al. (1997) reported two post treatment natural pregnancies in the NHL group and the achievement of two more after IUI, all with delivery of healthy babies. A survey from Meissener et al. (2014) showed that 16/23 and 8/13 NHL survivors were able to conceive naturally after CHOP and CHOEP therapy, respectively. Gunnes et al. (2016) in a large population studies involving Norwegian males survived to childhood cancers, 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 general population. In fact, ART can represent an important chance for fertility, especially in patients with persistent semen alteration or azoospermia. However, a recent systematic review from Ferrari et al. (2016) pointed out that the utilization rate of pre-treatment cryopreserved semen is relatively low (about 8%), but nearly half of these patients (49%) can achieve fatherhood. However, the systematic review included papers which reported patients who cryopreserved their semen for a wide range of neoplastic diseases. Thus, these probabilities cannot be fully generalized for NHL patients. The results of our fertility survey are somehow in agreement with previous observations: several patients already had children prior to NHL diagnosis and among those who wanted to conceive (24 pts), fourteen (63.6%) achieved fatherhood either by natural fertility or ART. In our knowledge our paper has one of the largest caseloads and the longest follow up of NHL patients published to date. The monocentric nature of the study increases standardization of semen analysis results, adding strength to our results. Limits of this study are the unavailability of blood hormones and a reduced compliance of several patients to perform control visits at all time points, resulting in a reduced number of subjects for therapy subtypes analyses. The survey had an acceptable response rate (nearly 80%), limiting the probability of patient selection bias.

In conclusion, the increased survival of NHL patients in reproductive age highlights the need to focus on the treatments gonadotoxicity. Despite natural fertility in NHL survival has been described, many patients, although cured, may suffer from long term reproductive problems and up to about one third, depending on treatment modality, may suffer permanent azoospermia. This is relevant, because at diagnosis is not possible to predict with certainty the evolution of the pathology: treatment might be intensified, resulting in higher probability of gonadal damage. Moreover, apart from pelvic radiotherapy, no risk factor is strongly associated with permanent spermatogenesis damage or recovery. It is thus mandatory to suggest sperm cryopreservation before any treatment, because the use of banked semen might represent the patient's best chance for future fertility.

STUDY 2: LONG TERM FOLLOW UP OF SEXUAL FUNCTION OF TESTICULAR CANCER SURVIVORS

Introduction

Cancer, together with cardiovascular disease, is currently the main cause of mortality across the world. 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 general population to about 60% of young and young adult cancer survivors. Men in reproductive age are mainly affected by testicular cancer (TC) and lymphomas, but despite a high incidence their 5-year survival rates are above 80-90% (Dal Maso et al., 2014; Capocaccia et al., 2015). Thus, these cancer survivors will have to live with long-term physical and psychological consequences of both treatments (surgical, chemical, radiant) and cancer diagnosis (Carpentier et al., 2010; Brand et al., 2014). Health, social and economic repercussions are relevant since these long-term consequences will involve people during their working and reproductive life, influencing physical abilities as well as reproductive and sexual health. These are intended as a state of psychic and physical well-being related to sexuality and not as mere absence of pathology: in fact, sexual health should be considered as a complex interaction of multiple factors such as social, cultural, individual experiences and self-image. Thus, cancer and its treatments should indeed be considered capable of disrupting sexual life, but the difficulty of many patients in disclosing these problems and the lack of consensus regarding valid outcome measures for assessing sexual functioning in cancer patients on the basis of a broader definition of sexual health (Nagele et al., 2015) are still issues to be faced both in common practice and in research. Most knowledge regarding male sexual dysfunction after cancer focuses on prostate cancer, which however present peculiar differences because of more invasive surgical procedures and hormonal treatments (Katz and Dizon, 2015). Sexological features of testicular cancer have been investigated by several Authors, revealing associations with perception of loss of masculinity and of sexual function, paving the way for psycho-organic sexual dysfunctions (Carpentier et al., 2010). Orchiectomy itself can cause alteration of body image perception, which can manifest with reduced

libido and sexual gratification linked to psychological stress for not “being normal” (Gilbert et al., 2013). Also linked to psychological stress, cancer diagnosis represents a moment of intense distress and, despite large variability in literature, about one third of patients with testicular cancer complain erectile dysfunction and/or ejaculation disorders (Rossen et al., 2012). Invasive and demolishing surgery, such as retroperitoneal lymph node dissection, increases the frequency of these dysfunctions (Phuse et al., 2012; Rossen et al., 2012; Dimitropulos et al., 2016). However, most data focus on either short or long-term consequences of therapy and reports of thorough longitudinal follow up from diagnosis to long-term survivorship are scarce. The aim of this study is to evaluate the effect of TC after orchiectomy and provide a complete follow up in order to highlight possible post treatment short and long-term sexological alterations.

Materials and Methods

Patients

We recruited two-hundreds and forty-one sexually active consecutive patients (mean age 31.3 ± 6.9 years, range 18-52) with recent diagnosis of testicular cancer who referred to the Laboratory of Seminology - Sperm Bank "Loredana Gandini" between 2013 and 2018 for sperm cryopreservation before any antineoplastic treatment. All patients underwent orchidectomy within the previous 30 days. As control group, we recruited two-hundreds and twenty-three healthy subjects (mean age 32.0 ± 7.7 years, range 18-55) who attended the Ambulatory of Endocrinology and Andrology of the Department of Experimental Medicine in the same years for idiopathic primary infertility. Subjects with hypogonadism and other endocrine disorders, diabetes, hypertension, cryptorchidism, history of neoplasia and/or previous treatment with chemio/radiotherapy, history of urogenital surgery, Klinefelter syndrome and other chromosomal abnormalities or any genetic disease, were excluded. Both subjects and controls underwent a careful medical history, general and andrological physical examination and were administered the International Index of Erectile Function 15 questionnaire (IIEF15) to evaluate the sexual function. IIEF15 questionnaire was administered to TC patients at baseline post orchiectomy but before chemotherapy (T0) and at 6 (T1), 12 (T2), 18 (T3), 24 (T4), 48 months (T5) and after 5 years (T6, median 96 months) from chemotherapy. Each patient performed baseline evaluation and at least one follow up control. Moreover, a subgroup of TC patients volunteered for the evaluation of blood hormones (FSH, LH, total Testosterone) for later comparisons with healthy controls correlations with IIEF scores. This subgroup of TC patients underwent blood hormone evaluation after orchidectomy (T0) and after chemotherapy at six (T1) and twelve months (T2).

Hormone analysis

Blood samples were collected at 8.00 am after at least 8 hours of overnight fasting for measurement of FSH, luteinizing hormone (LH) and total testosterone. Serum FSH, LH, and testosterone were measured by chemiluminescent microparticle immunoassay (CMIA, Architect System; Abbott Laboratories, Abbott Park, IL, USA), with detection

limits of 0.05 mIU/ml, 0.07 mIU/ml, and 0.28 nmol/l, respectively. Intra- and interassay coefficients of variation were 3.1% and 7.0% at 3.2 mIU/ml (FSH), 3.6% and 5.1% at 3.3 mIU/ml (LH), and 2.1% and 3.6% at 10.08 nmol (total testosterone). Normal ranges for adults were 1.38–9.58 mIUml (FSH), 1.80–8.16 mIU/ml (LH), and 9.4–33.5 nmol/l (total testosterone).

IIEF-15

Patient's sexual function can be evaluated in a reassuring and comfortable setting with self-administrated questionnaires. One of the most widely used for both clinical practice and research is the IIEF-15 questionnaire. It is a multidimensional tool offering a rapid, reliable and reproducible measurement of several domains of patient's sexual function (Rosen et al., 1997). It was developed to provide evaluation of patient's sexuality in clinical trials for erectile dysfunction with high sensitivity and specificity. The advantage of the self-administration is that it is felt less invasive and burdensome from the patient than a direct interview. The classical form has 15 items grouped into five domains: erectile function (EF), questions from 1-5 and 15; orgasmic function (OF), questions 9-10; sexual desire (SD), questions 11-12; intercourse satisfaction (IS), questions 6-8; general satisfaction (GS), questions 13-14. Generally, a score in the EF domain below 26 is considered diagnostic for erectile dysfunction.

Statistical Analysis

Continuous variables are presented as mean, 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 using the Bonferroni method for multiple comparisons. 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

Pre-therapy - Table 6 describes demographics from the recruited TC patients and control subjects. Both TC cancer and control groups were comparable in relation to age, BMI and percentage of smokers. Baseline prevalence of erectile dysfunction as reported from IIEF questionnaires (EF domain score < 26) is 37.8% (91/241) in TC pts compared to 9.9% (22/223) in our control group (χ^2 $p < 0.001$). In particular, erectile disfunction in TC pts was severe in 23.2% (56/241), moderate in 4.1% (10/241) and mild in 10.4% (25/241) subjects while all cases from control group were mild. Baseline comparison of TC and CTR groups are shown in Table 7: all IIEF-15 domain scores are significantly worse in TC group compared to controls (all $p < 0.001$), with the exception of the orgasmic function domain ($p = 0.334$). No significant correlations were found between IIEF scores and age, BMI, smoking status, cigarettes smoked/day and years of smoking in both groups.

Post-therapy - All patients underwent only a chemotherapy regimen as indicated in Table 6. IIEF scores from longitudinal follow up, in comparison with healthy controls, are shown in Table 8. Kruskal Wallis test with post-hoc corrections for multiple comparisons (Bonferroni) showed:

- ED domain scores improves significantly only after one year from the end of antineoplastic treatments (T0 vs T2: $p = 0.001$) and further improve at T3 and T4 compared to baseline (T0 vs T3: $p = 0.014$; T0 vs T4: $p = 0.002$) (Figure 2) as is also demonstrated by the lower prevalence of erectile dysfunction up to two years from the end of therapies (Table 8). However, the prosecution of the follow up detected an increase in erectile dysfunction prevalence after 48 months from the end of therapy with significant reduction of ED domain scores, which seemed to persist at T6. Compared to controls, ED scores remain significantly worse at T1 then return comparable to healthy controls.
- OF domain scores show a trend of improvement from baseline, but pairwise comparisons against both baseline and controls do not reach statistical significance.
- SD, IS and GS domain scores, although showing a trend of improvement, do not differ significantly from baseline at all time points, but TC patients scores are

significantly worse compared to controls for the whole duration of the study (Table 8).

Finally, for a subgroup of TC patients the evaluation of sexual hormones was available. The prevalence of biochemical hypogonadism (total testosterone < 10.4 nmol/l) was 5.4% in the TC group. There were no hypogonadal patients in the control group. Kruskal Wallis test with post-hoc corrections for multiple comparisons (Bonferroni) revealed that gonadotropin (both FSH and LH) levels are increased both in post orchiectomy patients (T0) and in post chemotherapy (T1, T2) when compared to controls (all $p < 0.001$). Moreover, total testosterone in post orchiectomy patients (T0) is significantly lower than controls ($p < 0.001$), but no difference was detected between T1, T2 and CTR group (Table 9). Finally, no significant correlation was detected between total testosterone levels and scores of any IIEF15 domain.

Table 6 – Testicular cancer and Control groups demographics: continuous data are presented as means ± SD, medians (in brackets) and 25th-75th percentile of data distribution; categorical data as percentages and counts.

	Testicular Cancer (241 pts)	**Controls (223 pts)**
Age at diagnosis (years)	**31.3 ± 6.9 (31.0)** *26.0 – 36.0*	**32.0 ± 7.7 (32.0)** *26.0 – 37.0*
BMI (kg/m^2)	**24.9 ± 3.0 (24.5)** *23.0 – 26.7*	**24.6 ± 2.7 (24.1)** *22.7 – 25.9*
Smokers	**19.5% 47 pts**	**23.3% 52 pts**
Cigarettes/day [a]	**11.4 ± 8.5 (10.0)** *5.0 – 15.0*	**11.4 ± 8.5 (10.0)** *5.0 – 15.0*
Years of smoking [a]	**12.3 ± 6.6 (10.0)** *7.0 – 16.0*	**10.6 ± 6.2 (10.0)** *6.0 – 15.0*
Histological Diagnosis	**58.5% Seminoma pT1-pT2 30.7% Mixed germ cell tumour pT1-pT2 8.0% Embryonal Carcinoma pT1-pT2 2.8% Yolk Sac Tumour**	/
Chemotherapy Regimens	**BEP 1-3 Cycles Cysplatin 1 cycle**	/

Table 7 – Baseline Testicular cancer IIEF scores vs Control group: continuous data are presented as means ± SD, medians (in brackets) and 25th-75th percentile of data distribution. (Mann Whitney U test)

	ED domain	OF domain	SD domain	IS domain	GS domain
TC *241 pts*	**22.7 ± 9.1 (27.0)** *20.0 – 29.0*	**8.2 ± 1.9 (10.0)** *8.0 – 10.0*	**7.5 ± 1.9 (8.0)** *6.0 – 9.0*	**8.3 ± 4.7 (10.0)** *7.0 - 12.0*	**7.4 ± 2.6 (8.0)** *6.0 – 10.0*
CTR *223 pts*	**27.9 ± 2.6 (28.5)** *27.0 – 30.0*	**8.9 ± 1.2 (10.0)** *8.0 – 10.0*	**8.9 ± 1.2 (9.0)** *8.0 – 10.0*	**12.6 ± 1.9 (13.0)** *11.5 – 14.0*	**9.0 ± 1.3 (9.0)** *8.0 – 10.0*
P value	***<0.001***	***0.334***	***<0.001***	***<0.001***	***<0.001***

Table 8 – IIEF scores of TC cancer and Control group: continuous data are presented as means ± SD, medians (in brackets) and 25th-75th percentile of data distribution. (Kruskal Wallis test with Bonferroni correction for multiple comparisons)

[a] p < 0.001 vs Controls
[b] p < 0.01 vs Controls
[c] p < 0.05 vs Controls
[c] χ^2 p < 0.001 vs Controls

	ED domain	OF domain	SD domain	IS domain	GS domain	Erectile Disfunction (%)
T0 *241 pts*	**22.7 ± 9.1[a] (27.0)** *20.0 – 29.0*	**8.2 ± 1.9 (10.0)** *8.0 – 10.0*	**7.5 ± 1.9[a] (8.0)** *6.0 – 9.0*	**8.3 ± 4.7[a] (10.0)** *7.0 - 12.0*	**7.4 ± 2.6[a] (8.0)** *6.0 – 10.0*	**37.8%[c] (91/241)**
T1 *74 pts*	**24.1 ± 8.6[c] (28.0)** *24.0 – 30.0*	**8.8 ± 2.4 (10.0)** *9.0 – 10.0*	**8.0 ± 1.7[b] (8.0)** *7.0 – 9.0*	**8.7 ± 4.2[b] (10.0)** *8.0 – 11.0*	**7.9 ± 2.3[a] (8.0)** *8.0 – 10.0*	**28.4%[c] (21/74)**
T2 *110 pts*	**25.8 ± 7.1 (29.0)** *26.0 – 30.0*	**8.8 ± 2.3 (10.0)** *9.0 – 10.0*	**7.6 ± 1.7[b] (8.0)** *7.0 – 9.0*	**9.4 ± 3.6[b] (10.0)** *8.0 – 12.0*	**7.9 ± 2.2[a] (8.0)** *7.0 – 10.0*	**23.6%[c] (26/110)**
T3 *60 pts*	**26.5 ± 6.3 (29.0)** *27.0 – 30.0*	**8.7 ± 2.3 (10.0)** *8.0 – 10.0*	**7.6 ± 1.6[b] (8.0)** *7.0 – 9.0*	**9.7 ± 3.7[b] (10.0)** *9.0 – 12.0*	**8.0 ± 2.2[b] (8.0)** *7.0 – 10.0*	**18.3%[c] (11/60)**
T4 *75 pts*	**26.9 ± 5.7 (29.0)** *27.0 – 30.0*	**9.2 ± 1.7 (10.0)** *9.0 – 10.0*	**8.0 ± 1.5[b] (8.0)** *7.0 – 9.0*	**10.0 ± 3.3[b] (11.0)** *9.0 – 12.0*	**8.5 ± 1.7[b] (9.0)** *8.0 – 10.0*	**16.0%[c] (12/75)**
T5 *67 pts*	**24.9 ± 8.0 (28.0)** *25.0 – 30.0*	**8.0 ± 2.9 (10.0)** *6.0 – 10.0*	**7.5 ± 1.8[b] (8.0)** *6.0 – 9.0*	**8.9 ± 4.0[b] (9.0)** *8.0 – 12.0*	**7.8 ± 2.3[a] (8.0)** *7.0 – 10.0*	**25.4%[c] (17/67)**
T6 *36 pts*	**25.2 ± 7.3 (28.0)** *24.0 – 30.0*	**9.0 ± 2.0 (10.0)** *8.0 – 10.0*	**7.7 ± 1.6[b] (8.0)** *7.0 – 9.0*	**9.8 ± 3.6[b] (10.0)** *9.0 – 12.0*	**8.2 ± 2.0[c] (8.0)** *8.0 – 10.0*	**30.5%[c] (11/36)**
CTR *223 pts*	**27.9 ± 2.6 (28.5)** *27.0 – 30.0*	**8.9 ± 1.2 (10.0)** *8.0 – 10.0*	**8.9 ± 1.2 (9.0)** *8.0 – 10.0*	**12.6 ± 1.9 (13.0)** *11.5 – 14.0*	**9.0 ± 1.3 (9.0)** *8.0 – 10.0*	**9.9% (22/223)**
P value	***<0.001***	***0.068***	***<0.001***	***<0.001***	***<0.001***	*//*

Figure 2 – Variation of the mean EF domain score and statistical significance comparison vs T0 (Bonferroni correction for multiple comparisons).

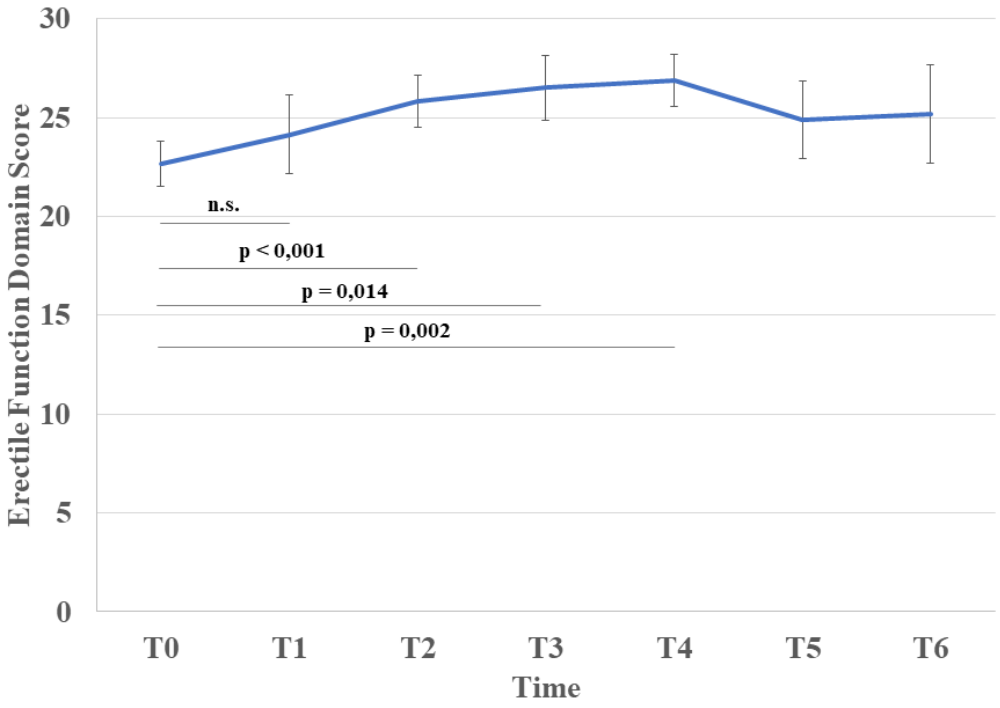

Table 9 – FSH, LH and total testosterone of TC cancer and Control group: continuous data are presented as means ± SD, medians (in brackets) and 25th-75th percentile of data distribution. (Kruskal Wallis test with Bonferroni correction for multiple comparisons)

[a] p < 0.001 vs CTR

	FSH (mUI/ml)	**LH (mUI/ml)**	**Total Testosterone (nmol/l)**	**Biochemical Hypogonadism (%)**
T0 *194 pts*	**7.6 ± 6.3[a] (6.1)** *4.0 – 9.8*	**4.6 ± 4.1[a] (4.0)** *2.7 – 5.6*	**17.9 ± 6.1[a] (17.0)** *13.3 – 20.9*	**5.4% (13/241)**
T1 *68 pts*	**14.3 ± 8.9[a] (12.3)** *7.3 – 19.7*	**6.6 ± 3.5[a] (5.6)** *3.7 – 8.2*	**19.2 ± 6.7 (19.0)** *15.0 – 21.6*	**5.8% (4/68)**
T2 *71 pts*	**13.0 ± 8.5[a] (10.0)** *7.1 – 18.5*	**6.8 ± 6.1[a] (5.3)** *3.8 – 7.4*	**17.9 ± 5.7 (17.9)** *13.5 – 20.5*	**7.0% (5/71)**
CTR *223 pts*	**4.7 ± 4.5 (3.5)** *2.3 – 5.4*	**3.7 ± 1.8 (3.3)** *2.4 – 4.6*	**20.2 ± 7.1 (19.4)** *15.1 – 24.4*	**0.0% (0/223)**
P value	***<0.001***	***<0.001***	***0.002***	*//*

Discussion

Trends of mortality reduction for various neoplastic diseases, such as the case of testicular cancer, increased clinicians' awareness towards long term quality of life after surgical, chemical and radiant treatments. Recent literature especially focuses on cancers involving directly gonads and genitalia (Katz and Dizon, 2016; Capogrosso et al., 2016; Dimitropulos et al., 2016; Bandak et al., 2018), but sexual functioning in male survivors from other frequent cancers have also been investigated (Arden-Close et al., 2011; Haavisto et al., 2016). In general, while TC survivors maintain and/or retrieve a good quality of life, the investigations of patient's sexual life reveal marked changes (Joly et al. 2002; Mykletun et al., 2005; Huddart et al., 2005). Carpentier et al. highlight that the diagnosis and therapy stages are associated with peak levels of anxiety and concerns, with consequent decrease in the post treatment period. Similarly, due to stress-related central inhibition of sexual function, disorders of libido, erection and ejaculation increase during antineoplastic treatments (Carpentier et al., 2010). In fact, sexual dysfunctions in TC cancer patients may rise from a combination of treatment-related physical side effect (genital mutilation, reduced testosterone levels, chronic pain and other residual organic side effects from antineoplastic treatments) and psychological vulnerabilities (anxiety, fear, mood disorders, etc.) (Jonker-Pool et al., 2001). A possible underlying cause may be the induction of iatrogenic hypogonadism: in fact, orchidectomy, chemo and radiotherapy may all induce gonadal dysfunction. Recently, Petrozzi et al. showed that a cohort of orchiectomized TC patient before any antineoplastic treatment had demonstrated by increased levels of gonadotropins and reduced testosterone than healthy controls, although still within normal range values (Petrozzi et al., 2018). Some Authors demonstrated the persistence of gonadotropin alterations after chemotherapy and identifying that about 10% of patients who may suffer from low total testosterone levels after treatments or have higher risk of late onset hypogonadism (Berger et al., 1996; Bokemeyer et al., 1996; Brennemann et al., 1997; Nord et al., 2003; Huddart et al., 2005). However, other Authors observed only mild effects on hormone levels from chemotherapy (Lackner et al., 2005; Tasdemir et al., 2012). Radiotherapy, instead, may impact testosterone levels for up to 5 years in TC patients who received testicular radiation for contralateral carcinoma in situ (Petersen et

al., 2002), but direct testicular irradiation is not a standard treatment for TC patients and current radiation treatment protocols probably have only minor effect on testicular function (Huddart et al., 2005). However, the impact of altered gonadotropin and testosterone levels as the only determinant of sexual impairment in TC patients is debated. The already cited work form Huddart et al. detected about 10% of post therapy TC patients affected by biochemical hypogonadism and worse sexual functioning compared to non-hypogonadal TC survivors (Huddart et al., 2005). However, a following work from Lackner et al. (2007) found a higher percentage of post treatment hypogonadal patients (26%). The Authors could not identify an unambiguous threshold level for testosterone associated with the onset of sexological symptoms, thus hypothesizing that each patient could have an individual threshold (Lackner et al. 2007). In 2009, Eberard et al. published a caseload of 129 TC survivors from 3 to 5 years post therapy compared to an age-matched group of men without cancer, observing that TC survivors had a higher likelihood of presenting low sexual desire (OR 6.7) and erectile disfunction (OR 3.8) compared to controls, but none of these conditions could be predicted based upon the presence of hypogonadism (Eberard et al., 2009). However, the lack of the pre-treatment status of the TC patients limits these results. Following papers further failed to find a clear association between sexual dysfunctions and biochemical hypogonadism (Tasdemir et al., 2012; Kim et al., 2012; Tal et al, 2014; Kurobe et al., 2018). In conclusion, evidence from most of the papers suggest that the high prevalence of sexual dysfunctions cannot be justified by a relatively low prevalence of biochemically detected hypogonadism. Another hypothesis could link sexual dysfunctions to specific treatment modalities. Side effect of several chemotherapy drugs include endothelial damage, angiopathy and peripheral neuropathy which may be linked to erectile and ejaculatory disorders (Van Basten et al. 1997; Van Basten et al., 2000). Radiotherapy can induce sexual dysfunctions by inducing damage of the cavernous nerve and/or progressive fibrosis of cavernous tissue and endothelial damage, which can become clinically evident with the onset of erectile dysfunction even after several years from treatment (Mahmood et al., 2016). Literature data however is inconsistent as several papers report no significant associations between sexual dysfunctions and specific treatment modalities (Eberard et al., 2009), while others report relevant influence of either chemo or radiotherapy (Jonker-Pool et al., 1997; Kim et al.,

2012; Capogrosso et al., 2016; Bandak et al., 2018). The reason of this variability may be that various combination of these therapies with different surgical procedures (tailored on the patient considering various clinical parameters as stage disease, etc.) as well as individual variability may induce different outcomes. Kim et al., in particular, reported that surgery combined with chemotherapy has higher incidence of libido reduction and ejaculatory disorders, while surgery combined with radiotherapy is followed by greater incidence of erectile dysfunction (Kim et al., 2012). More recently, Bandak et al. observed that each antineoplastic treatment modality has an increased risk of erectile and orgasmic dysfunctions, with multimodal treatment associated with the highest risk (Bandak et al., 2018). Invasive surgical procedures, such as retroperitoneal lymph node dissection, are known to have stronger impact on sexual functioning (especially ejaculatory and orgasm disorders and impaired satisfaction) (Dimitropulos et al., 2016). Several studies confirm a worse sexological outcome resulting from retroperitoneal surgery (lymph node dissection and/or re-surgery for relapse after chemotherapy) as consequence of ejaculatory nerve damage during the procedures (Aass et al., 1993; Hartmann et al., 1999; Jonker-Pool et al. 2001; Rossen et al., 2012; Dimitropulos et al., 2016).

Another issue about SDs in TC cancer survivors is their trend over time as most studies in literature are cross-sectional and only a few longitudinal studies are available. Currently, we expect a higher incidence of SDs close to orchidectomy and the end of antineoplastic treatment. Tuinman et al. (2010) show low IIEF scores close to orchidectomy and three months after treatments, with significant improvements after one year of follow up (Tuinman et al., 2010), results comparable to other studies focusing on SD within the first year from treatments (Brand et al., 2015; Wortel et al., 2015; Catanzariti et al., 2016). Data on long term comparisons pre vs post therapy are scarce. Aass et al. the persistence of sexual problems in about 30% of TC survivors after 36 months from the end of treatments (Aass et al., 1993) while Böhlen et al. reported no significant pre vs post therapy differences in sexual functioning after at least 32 months of follow up (Böhlen et al., 2001). Despite wide agreement on the presence of sexual dysfunctions in TC survivors, the absence of a longitudinal long term follow up and the lack of standardization in sexual functioning measurement limit the assessment of their effective burden on patients. In fact, data comparison and generalization are difficult as

different papers utilize a variety tools and methods to evaluate sexual functioning. Moreover, questionnaires are not efficient in discriminating if sexual dysfunctions are secondary to organic sequalae of antineoplastic treatments (Deveci et al., 2008).

In accordance to most literature data available, our data clearly show that TC patients undergoing orchiectomy and chemotherapy suffer from a higher degree of sexual dysfunctions compared to a control non-neoplastic population: mainly erectile dysfunctions, but also impaired sexual desire and satisfaction. The incidence of orgasmic dysfunctions seemed not to differ significantly from controls. It is noteworthy that the presence of these sexual dysfunctions at baseline suggests that they might be induced by orchiectomy. However, it is difficult to find a biological relationship. Our data indicate that the incidence of biochemical hypogonadism at baseline (total testosterone < 10.4 nmol/l) is lower compared to other reports (about 5%), but like them no significant correlation has been detected with sexual function domains (Huddart et al., 2005; Lackner et al., 2007; Eberard et al., 2009). This finding suggests that sexual dysfunctions are not explained by abnormal hormone levels consequent to orchiectomy and they might be associated more closely with a psychological burden in these patients, which eventually may coexist and be synergistic with therapy induced hypogonadism. Indeed, testis are associated with masculinity and surgery of TC might induce changes in body perception. Rossen et al. in a caseload of 407 TC patients observed that about 17% had a reduced perception of masculinity induced by orchiectomy. This was associated with a 9-fold increased risk of erectile dysfunction and a 15-fold increased risk of sexual discomfort (Rossen et al., 2012). Wortel et al. indicated that after orchiectomy up to 50% of patients might complain a body image perception distortion (Wortel et al., 2015). Furthermore, all of our patients had the insertion of a testicular prosthesis. This may have positively influenced their body perception, as suggested by a study from Catanzariti et al. (Catanzariti et al., 2016), but whether this may have contributed to the improvements of the IIEF15 scores we detected and to which extent is unknown and should be investigated in further studies.

Furthermore, through the investigation of the IIEF15 scores, we detected a significant improvement of erectile dysfunction post therapy. Despite the incidence of some degree of erectile dysfunction remains high at all time points, it constantly decreases up to two years from the end of treatments (T4). Similarly, IIEF15 erectile dysfunctions scores

improve and return comparable to controls within one year from treatments (T2). Also, sexual desire and both general and intercourse satisfaction show some trend of improvement after chemotherapy, but trough follow up they remain significantly worse compared to healthy controls.

In our knowledge, our data currently represents the longest monocentric follow up available for sexological evaluation of TC patients. A relevant study population of 241 patients, a control group comparable by age and the use of a validated psychometric tool, add strength to the study. Unfortunately, generalizability of data comparison against healthy controls might be reduced for long term follow up as the increased percentage of patients with erectile dysfunction at T5 and T6 may be due to the increased age of patients with the consequent possible onset of other factors (hypertension and other cardiovascular diseases, drug assumptions, etc.) that might have increased the incidence of sexual dysfunctions independently from cancer and its therapies.

In conclusion, data indicate that TC patients need adequate sexological counseling already at the stage of diagnosis/orchiectomy and prior to chemotherapy. Discussing these aspects with the patients might help him to cope with the disease and to understand that an improvement of erectile function is expected within one year from therapy. Further studies would need to identify those subjects who are more likely to suffer from SDs might allow better a follow up of these subjects and to offer all necessary support for maintenance of a satisfactory sexual life and, consequently, a good quality of life.

Concluding remarks

Fertility in NHL patients has been overlooked for many years since the fear of an unfavorable prognosis urged clinicians and oncologists to focus more on treatments than to their consequences. Recent reports of improved survival have made mandatory to consider long term effect of NHL treatments with particular attention to survivors' reproductive health. This data show that intensive treatments and pelvic radiotherapy are indeed associated with severe and permanent impairment of spermatogenesis with about 30% of bone marrow transplant recipients that will suffer from permanent azoospermia. However, milder treatments are compatible with spermatogenesis recovery within 2 years from the end of treatments and, while average sperm parameters may not fully return to pretreatment values, more than a half (63%) of patient who actively desire fatherhood can conceive either through natural conception or ART. Similarly, sexological evaluation of TC patients have often been under-investigated. While we confirm the presence of erectile dysfunction and impairment of sexual desire and satisfaction compared to a healthy population, our observations show that sexological dysfunctions, especially erection quality, are expected to improve within one year from the end of the treatment. Moreover, the absence of clear correlations with hormonal levels, and with biochemical hypogonadism in particular, suggests that psychological burden following cancer diagnosis and treatments may play an important role. Information from these studies is of extreme translational importance since they improve our knowledge of a neglected clinical area and it will allow to increase the effectiveness of patients' counseling interventions in an oncofertility service.

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