OBJECTIVES: To determine the frequency of spermatogenesis in testicular cancer patients and to assess for any predictors of spermatogenesis.

PATIENTS AND METHODS: Retrospective review of 103 TGCTs orchiectomy specimens conducted at Guy’s Hospital London between 2011 to 2015. Primary outcome measures included: the presence and characteristics of spermatogenesis (widespread/focal/proximity to tumour). Secondary outcome measures included: the presence of TML, tumour characteristics (size, stage and type) and tumour markers. Secondary outcome measures as potential predictors of spermatogenesis were assessed using univariate and multivariate logistic regression analyses (significance p<0.05).

RESULTS Spermatogenesis was present in 70% (72/103); it was widespread in 63% (45/72), focal in 38% (27/72). Neither tumor type, stage, presence of microcalcification, nor tumor markers predicted spermatogenesis. Men with a percentage testis tumor occupation (PTTO) >50% of their testis were 82% (95% CI: 73.2-98.4) less likely to have spermatogenesis than a PTTO <50%.

CONCLUSIONS: Spermatogenesis is present in the majority of testes affected by TGCTs. It is widespread in two thirds of cases, and located away from the tumour in 50%. These findings can help predict and guide successful surgical sperm retrieval in testes with germ cell tumours. The finding of focal spermatogenesis in a third of cases would support a microsurgical approach to at the time of orchidectomy to maximise success.
INTRODUCTION

Testicular germ cell tumours (TGCTs) are the most common malignancy in young men in their peak fertile years(1). The inherent subfertility risk(2) and treatment induced (often permanent) infertility(3,4), quantified as an average 30% decrease in fertility(5,6), in testicular cancer patients warrants significant interest in predicting and managing these patients’ infertility. At first presentation, over 50% of patients with Testicular cancer (TC) are oligozoospermic(7), while up to 24% may be azoospermic(8). After combination therapy of surgery, and chemotherapy, 10 year post-treatment natural paternity rates have been reported at 50%(9). Pre-treatment, but optimally pre-surgery(10), semen cryopreservation is strongly recommended for all testicular cancer patients(11,12). However, this is invariably unsuccessful for the azoospermic patient and this sub-group of patients present a unique challenge.

Microsurgical testicular sperm extraction (microTESE) has been shown to successfully retrieve sperm in azoospermic men in 54% of cases(13). In the setting of a surgical sperm retrieval (SSR) in a non-malignant testis, the underlying pathological changes in the testis correlate to the successful chance of SSR. In patients with hypospermatogenesis (HS), maturational arrest (MA) and sertoli cell only (SCO), retrieval rates of 85%, 49%, and 37% have been reported respectively using microTESE(13). MicroTESE may also be used to extract sperm from the ipsilateral testis at the time of an orchidectomy, a technique known as an onco-microTESE. At present, there is no clear way to predict which testicular units affected by cancer may yield sperm at the time of SSR. The purpose of this study was to investigate potential markers of spermatogenesis by assessing the relationship of tumor characteristics (type, grade), tumor size, presence of testicular microlithiasis (TML) and
tumor markers with the presence of spermatogenesis in the affected testis in patients with a testicular germ cell tumor.
METHODS

Study Design

This is a retrospective cohort study conducted at Guy’s Hospital, London in 2016.

Patients

All testicular specimens for men who underwent radical orchiectomy at Guy’s Hospital, from February 2011 to December 2015, were retrospectively reviewed (n=109).

Men above the age of 16 at the time of orchiectomy with testicular germ cell tumours and spermatogenesis data were included. Patients were excluded if they had a testicular neoplasm other than a germ cell tumor (n=3), or if they had no available data on the presence of spermatogenesis (n=3), leaving a final cohort of 103 patients.

Outcome measures

Our primary outcome measures included the presence of spermatogenesis, identified as ‘present’ or ‘absent’. Secondary outcome measures included the nature of the spermatogenesis: whether it was focal or widespread, and its relation to the tumor, whether it was within the tumor, adjacent to the tumor, or distant from the tumor. Other outcome measures included: the size of the tumor, measured as the percentage tumor testis occupation (PTTO), the subtype of tumor, the presence of microcalcification and the levels of tumor markers (LDH, βHCG, αFP) pre-orchiectomy. All secondary outcome measures were assessed in their ability to predict spermatogenesis.

Data Collection

All specimens had been initially reported by local pathologists. Slide review of all cases with evaluation of spermatogenesis, and confirmation of identification of tumor type, staging and
size in relation to testis were then conducted by one expert pathologist (CH).

Spermatogenesis was defined as the presence of fully formed, mature spermatozoa, which would be suitable for IVF/ICSI. Spermatids alone were not considered as indicative of spermatogenesis. A Johnson score was not used in this series as this scoring system was not developed for whole orchidectomy specimens. The Johnson score is calculated by grading the most advanced level of sperm maturation on a scale of 1 to 10 in at least 100 different seminiferous tubules from a testis biopsy, and then calculating the mean score in that small sample by dividing the total score by the number of tubules in the biopsy (14).

The presence of spermatogenesis was determined as being either focal or widespread throughout the testis and its distance from the tumor was described as within tumor, next to the tumour, or away from tumour. Next to tumour was defined as the presence of spermatogenesis abutting the tumour. ‘Away from the tumour’ was defined as the presence of spermatogenesis occurring at any distance from the tumour where there was normal testicular parenchyma separating the tumour and the presence of spermatogenesis.

The pathological presence of microcalcification, typical and indicative of testicular microlithiasis (TML), was reported in the histopathological reviews, and its presence correlated to spermatogenesis.

Macroscopic planar measurements were taken from the pathological specimen. Tumour diameter was recorded and testes were measured in three planes. From these dimensions, the percentage tumor testis occupation (PTTO) was calculated as a ratio after calculating the volumes of the testis, and tumour.

Tumor markers (αFP, βHCG and LDH) pre-orchiectomy were identified from patient records and correspondence documents. Only αFP and βHCG data was used in the analysis. Raised
αFP was defined as >10ng/l, and βHCG≥1ng/l. Testicular function markers including FSH, LH, testosterone and pre-operative semen analysis were also sought from the patient records.

Patient age was defined as age at the time of orchiectomy.

**Statistical analysis**

Descriptive statistics were conducted for patient age.

To evaluate how patient and or tumour characteristics were predictive for spermatogenesis a univariate logistic regression was conducted for each hypothesized predictor of spermatogenesis, including: age, tumor stage, type, tumor size (PTTO), presence of microcalcification and raised tumor markers. Multivariable logistic regression with backward stepwise elimination (α=0.20) was then used to identify the best predictors of spermatogenesis. Statistical significance was defined as p<0.05.

In 2013 LDH assays were changed, which shifted the numerical upper and lower normal boundaries, rendering the statistical analysis of the association of LDH with spermatogenesis implausible. The relationship between FSH and LH levels, and testosterone with the presence of spermatogenesis could not be conducted due to insufficient data.

Data were compiled and analyzed using *Microsoft Excel* (15) and statistical analysis was conducted using *SPSS* software, version 24.0 (16). The logistic regression probability graph was plotted using the statistical software *R* (17).

**Ethics**

The study was not defined as a research study according to the National Health Service and no requirement for ethic committee approval was necessary.
RESULTS

Demographics

A total of 103 cases were included in the study, and all patients underwent radical orchiectomy from February 2011 to December 2015. The mean age of the cohort was 35 years (SD 11) and the range was 17-77 years; 62 patients were aged 35 or less and 41 patients were older than 35 years. Table 1 summarises the patient characteristics.

Presence and characteristics of Spermatogenesis

Overall, 70% (72/103) of cases had spermatogenesis present. Of these, spermatogenesis was widespread in 63% (45/72) and focal in 38% (27/72). Spermatogenesis occurred away from the tumour in 50% (36/72) and in 44% (32/72) it occurred both near to and away from the tumour. Only 1% (1/72) of spermatogenesis found occurred within the tumour alone and 4% (3/72) occurred adjacent to the tumour alone, see figure 1.

Predictors of Spermatogenesis

Table 2 summarises the results of the multivariate analysis.

Tumour Type and Stage

Spermatogenesis was present in seminomas, non-seminomas and mixed tumours in 70% (44/63), 58% (11/19) and 81% (17/21) respectively. Spermatogenesis was widespread in the majority of all patients with all tumour subtypes; widespread spermatogenesis was present in 59% of seminomas (26/44), 73% of NSGCT (8/11) and 65% (11/17) of mixed tumours. Regarding tumour stage, 75% (46/61) of T1 tumours had spermatogenesis, as did 85% (11/13) of T2 tumours and 52% (15/29) of T3 tumours.

Neither tumour type nor tumour stage were found to be predictors of spermatogenesis on univariate or multivariate analysis.
**Tumour size (percentage testis tumour occupation (PTTO))**

It was possible to measure the PTTO in 99 of the 103 cases. The average PTTO was 34%: 25 men had <10%, 39 had 10-49%, 33 had 50-75% and 2% had greater than 75%. The average PTTO in those with spermatogenesis was 28%, and in those without spermatogenesis it was 48%. Figure 2 illustrates the frequency of spermatogenesis with increasing PTTO. A discrepancy exists between the presence of spermatogenesis in patients whose PTTO was <50% and in those whose PTTO was >50%.

An age-adjusted logistic regression model found that for every 1% increase in PTTO, the chance of the presence of spermatogenesis decreased by 4% (OR: 0.96 95% CI: 0.95-0.98, p<0.001). When categorising PTTO based on the 50% cut-off, it was found that if the patient had a PTTO > 50% they were 82% (OR: 0.19 95% CI: 0.07-0.48, p<0.001) less likely to have spermatogenesis than those whose PTTO was <50%. Figure 1 illustrates the presence of spermatogenesis in tumours of varying percentages of testis volume. In order to use age-adjusted PTTO as a means to predict an individual’s likelihood of spermatogenesis, the logistic regression model was plot (Figure 3) to show the negative relationship between PTTO and probability of spermatogenesis.

**Testicular microcalcification**

Microcalcification was identified on the pathological review in 43/103 specimens. In those with microcalcification, 9 (21%) did not have spermatogenesis. Microcalcification was not significantly related to spermatogenesis on univariate or multivariate analysis. However, spermatogenesis was 7.25 times more likely to occur in areas away from microcalcification.
than where microcalcification was present; spermatogenesis only occurred within the area of microcalcification in 4 patients (9%).

**Age**

Age was not found to be a predictor of spermatogenesis on univariate nor multivariate analysis. Although a decline in the rate of spermatogenesis occurred after the age of 50, the number of men in this age group in this series were too small make any meaningful conclusion (see Figure 4).

**Tumor markers**

97/103 (94%) patients had pre-orchiectomy βHCG levels measured and 48/97 (49%) had an elevated βHCG value. Spermatogenesis was not present in 17/48 (35%) of those with a raised βHCG compared to 14/49 (29%) in those with a normal value. 94/103 (91%) patients had pre-orchiectomy αFP levels measured. 23/94 (24%) had an elevated αFP, of which 7/23 (30%) did not have spermatogenesis. Neither elevated βHCG nor αFP were statistically related to presence of spermatogenesis on univariate nor multivariate analysis.
DISCUSSION

These results demonstrate that increasing size of tumour relative to testis size (or PTTO) is associated with reduced likelihood of spermatogenesis. Our findings can be summarised into a rule of 50s: men with PTTO >50% have <50% chance of spermatogenesis in their affected testis. Neither tumour type, TMN stage, presence of microcalcification, nor raised tumour markers were found to predict spermatogenesis on either univariate or multivariate analysis.

Recently, several other studies have also investigated spermatogenesis predictors. Our overall rate of spermatogenesis (70%) is comparable to previous findings of 68%(18), 62%(19) and 79%(20). These studies have also found a statistically significant negative relationship between testicular tumour size and presence of spermatogenesis. Choy et al using their logistic model estimated the probability of spermatogenesis in a tumor of 1 cm was 86% and for a tumour of 5 cm it was 57%(19). One of the strengths of our study is that we took into account the testis size when comparing tumour size, which is more clinically relevant than tumour diameter alone for two reasons: 1) we know that spermatogenesis is more likely to occur away from the tumour, therefore smaller testis may be more impacted by the same tumour size than someone with a larger testis; 2) tumours are not symmetrical, so an estimate of testis occupation is more representative of tumour bulk compared to a single measurement. One other study took testis size into consideration when measuring tumour size: Suzuki et al found that patients with non-cancerous testicular tissue width of <7.5mm had a significantly lower retrieval rate (41%) compared to those whose width was >7.5mm (93%)(21). However, the measurement we used (PTTO) is more likely to be reproducible on ultrasound, as tumour free width will vary depending on the side of the tumour.
The relationship between microcalcification and spermatogenesis is intriguing. While there was no clear association between the presence of microcalcification and spermatogenesis on univariate or multivariate analysis, spermatozoa where 7 times more likely to be found away from areas of microcalcification, and were only seen in areas of microcalcification in 9% of cases. This certainly warrants further investigation, as presence of widespread microcalcification or TML on pre-operative USS may have a negative predictive impact on the chance of subsequent successful sperm retrieval.

The relationship between age and presence of spermatogenesis is less consistent in the literature. Our results support Suzuki et al, who did not find age to be associated with the presence of spermatogenesis, in a similar cohort size of 104 specimens (21). However, in a cohort of 145 patients, Shoshany et al found on univariate analysis that older age was a negative predictor of spermatogenesis (p=0.05) (18). Our results show that men ≥50 years were much less likely to have spermatogenesis (45%) compared to men <50 years (75%), although this data was only based on 11 patients; a larger cohort is required to strengthen this finding. Indeed, in the background population, increasing age has been shown to be strongly associated with decreased semen quality, including decreases in sperm motility, normal morphology and increased genetic malformations (22).

With regards to other markers, Delouya et al (20) and Shoshany et al (18) found that raised tumour markers and more advanced tumour stage were negatively associated with presence of spermatogenesis respectively, but neither of these results have been reproduced in our or other studies (19,21).
There was a significant paucity of testicular functional markers such FSH and LH, testosterone and semen analysis measurements taken prior to orchiectomy, so fertility markers could not be assessed as predictors of spermatogenesis as it was intended. This lack of data demonstrates an endemic lack of focus on determining patient testis function and fertility status pre-operatively. In this respect, this study’s retrospective design therefore is a significant limitation. On extensive review of patients notes it was clear there was little information available about patient’s desires for parenthood, or known risk factors for infertility. Since 2016, our team has changed our approach to proactively assess patients’ fertility status pre-op with assessment of the pre-op FSH, LH and analysis of semen parameters prior to any planned orchiectomy to be able to prospectively assess this relationship. In other surgical disciplines, base line organ function is a mandatory assessment prior to the removal of a paired organ, such as a kidney; it seems anomalous therefore that testis are removed without measuring testicular function first.

Assessing TC patients’ desires for future paternity, measuring their fertility markers (FSH, LH and testosterone), determining patient age and PTTO at diagnosis, and conducting semen analysis are important steps in fertility planning and preservation. Identification of the severely oligozoospermic or azoospermic patient prior to orchiectomy can allow referral for onco-TESE, and PTTO measurements can help determine sperm retrieval success.

Based on this study’s findings, the following fertility planning in men with testicular cancer is recommended (figure 2).

The findings of this study are limited by its retrospective design and sample size. Future directions would be to expand the dataset in a prospective manner with baseline testicular
functional data to correlate to the histopathological findings and pre-operative ultrasound assessment. A closer assessment of testicular microlithiasis (TML) on pre-operative imaging with the final histopathological pattern of spermatogenesis would clarify the potential impact and predictive role of TML in determining the chances of finding sperm in testicular cancer.

CONCLUSIONS AND RECOMMENDATION FOR PRACTICE

Our results show that men with a tumour occupying greater than 50% of their testis, are less than 50% likely to have spermatogenesis in their affected testis. Spermatogenesis is widespread in two thirds of cases, and located away from the tumour in 50%. In approximately a third of cases, spermatogenesis is focal only. These findings can predict and guide successful surgical sperm retrieval in testes with germ cell tumours, and would support a microsurgical approach to at the time of orchidectomy if required.

This information is useful for the patient found to be azoospermic at diagnosis in order to aid fertility planning and adequately identify those men who have a good chance of onco-TESE success.

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