Impacts of platinum-based chemotherapy on subsequent testicular function and fertility in boys with cancer

Lim Tian En1, Mark F.H. Brougham2, William Hamish B. Wallace2, and Rod T. Mitchell1,3,*

1MRC Centre for Reproductive Health, The Queen’s Medical Research Institute, The University of Edinburgh, Edinburgh, UK
2Department of Paediatric Oncology, Royal Hospital for Sick Children, Edinburgh, UK
3Department of Paediatric Endocrinology, Royal Hospital for Sick Children, Edinburgh, UK

*Correspondence address. MRC Centre for Reproductive Health, The University of Edinburgh, The Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK. Tel: +44-131-242-6470; Fax: +44-131-242-6197; E-mail: rod.mitchell@ed.ac.uk

https://orcid.org/0000-0003-4650-3765

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BACKGROUND: Children with cancer often face infertility as a long-term complication of their treatment. For boys, compromised testicular function is common after chemotherapy and currently there are no well-established options to prevent this damage. Platinum-based agents are used to treat a wide variety of childhood cancers. However, platinum agents are not currently included in the cyclophosphamide equivalent dose (CED), which is used clinically to assess the risks to fertility posed by combination chemotherapy in children with cancer.

OBJECTIVE AND RATIONALE: This was a systematic search of the literature designed to determine the evidence for effects of platinum-based cancer treatment on the prepubertal human testis in relation to subsequent testicular function and fertility.

SEARCH METHODS: PubMed and EMBASE were searched for articles published in English between 01 January 1966 and 05 April 2020 using search terms including ‘cancer treatment’, ‘chemotherapy’, ‘human’, ‘prepubertal’, ‘testis’, ‘germ cells’, ‘testosterone’ and related...
Introduction

Childhood cancer rates have increased dramatically over recent decades and it is estimated that one in 530 young adults is a survivor of childhood cancer (Ward et al., 2014). The increasing incidence, coupled with remarkable improvements in cure rates (>80% 5-year survival), have resulted in an increase in young adults experiencing treatment-related morbidity (Anderson et al., 2015). Infertility is a well-recognized complication of cancer treatment, although the relative contribution of individual agents to fertility risk in childhood cancer survivors has not been fully elucidated. Amongst chemotherapeutics, alkylating agents (e.g. cyclophosphamide) are known to affect fertility in males and an objective measure of relative gonadotoxicity between chemotherapeutics, the cyclophosphamide equivalent dose (CED), has been established (Green et al., 2014b). This calculation can be used to estimate cumulative gonadotoxicity of multiple agents for a specific regimen.

Platinum-based chemotherapy (cisplatin or carboplatin) agents are used in treatment of a variety of childhood cancers including brain tumours, osteosarcoma, neuroblastoma, hepatoblastoma and germ cell tumours (GCTs). Whilst most of the commonly used chemotherapy drugs are included in the CED calculator, platinum-based chemotherapy is a notable exception. Therefore, the present study aimed to review the literature relating to effects of exposure to platinum-based chemotherapy during childhood on gonadal function and future fertility.

Methods

We performed a systematic search of the existing literature reporting the effects of platinum-based chemotherapy for childhood cancer on testicular development and function. We followed PRISMA guidelines for reporting systematic reviews (Moher et al., 2009). Given that the search was conducted to identify clinical and laboratory experimental data, the study was not registered with PROSPERO.

Information source

PubMed and EMBASE were searched (to 05 April 2020) to identify original clinical and experimental studies describing the testicular effects of platinum-based chemotherapy on prepubertal human testis. Additional studies were identified from a bibliographic screen of reference lists of included articles.

Inclusion criteria

The inclusion criteria for study selection were:
- articles published between 01 January 1966 and 05 April 2020 and written in English;
- clinical and experimental studies; and
- outcomes relating to reproductive function including fatherhood, sperm counts, reproductive hormones (LH, FSH, testosterone, inhibin B and anti-Müllerian hormone, testicular volume and puberty).

Exclusion criteria

The exclusion criteria for the review were:
- exposure to cancer treatment during (peri)puberty (>12 years) and adulthood;
- studies that did not include human data or experimental studies involving human tissue/cells; and
- review publications.

Search and study selection

The search terms and strategy (Supplementary Table S1) used for the systematic review were adapted from a previous study (Skinner et al., 2017). After removing duplicates and screening by titles, we identified 1449 studies. R.T.M. and L.T.E. screened abstracts independently to determine eligibility. For abstracts that were not selected by both authors, these were discussed, and a joint decision made regarding...
inclusion for full-text screening. Full texts were obtained for 624 studies that met the criteria for inclusion. An initial screen of full texts identified 588 studies that were excluded for not meeting at least one aspect of the inclusion criteria (Supplementary Table SII). Of the 36 remaining papers that were subjected to a detailed assessment, a further 16 papers were excluded for a variety of reasons (Supplementary Table SIII). In total, 20 papers were included in the final analysis. A PRISMA flowchart for the search and study selection is presented in Fig. 1.

Summary measures

Data were extracted in relation to four categories: pregnancy and fertility; semen analysis; hormones and testicular volume; and experimental studies (tissue and cells). Risk of bias within and between studies was assessed using the principles described for the assessment of risk of bias in non-randomized studies (Sterne et al., 2019). This included an assessment of risk of confounding and bias in selection, information and reporting for each study (Supplementary Table SIV). For each category, risk of bias was assessed as ‘low’, ‘moderate’, ‘serious’, ‘critical’ or ‘no information’. Given the heterogeneity of reported summary measures, all methods of reporting were included.

Results

Studies assessing testicular function and fertility after treatment with platinum agents

Overall, 20 studies reported testicular function and fertility following exposure to platinum-based agents (Table I). The risk of bias for each
outcome measures of fertility (Table II). Several papers reported

We identified eight papers which included pregnancy and fatherhood

A detailed description of the data extracted from these studies is pro-

Fertility and fatherhood

We identified eight papers which included pregnancy and fatherhood

study is presented in Supplementary Table SIV. Overall risk of bias

was lower in the studies that included a sub-analysis of platinum expo-

sure, compared with cohort studies that did not include a sub-analysis. A detailed description of the data extracted from these studies is pro-

vided in Supplementary Table SV.

Table I Summary of 20 studies including use of platinum agents, prepubertal study participants and an outcome measure relating to fertility.

| Study | Study design | Level | Control group | Fertility outcome |
|-------|--------------|-------|---------------|------------------|
| Flamant et al. (1984) | Multi-centre cohort | 3 | No | Gonadotropins |
| Wallace et al. (1989) | Single-centre cohort | 3 | No | Gonadotropins |
| Kiltie and Gattamaneni (1995) | Single-centre cohort | 3 | No | Gonadotropins |
| Muller et al. (1996) | Single-centre cohort | 3 | Yes* | Semen analysis; testicular volume |
| Hale et al. (1999) | Single-centre cohort | 3 | No | Gonadotropins |
| Relander et al. (2000) | Single-centre cohort | 3 | No | Semen analysis; gonadotrophins; testicular volume |
| Longhi et al. (2003) | Single-centre cohort | 3 | No | Semen analysis |
| Ridola et al. (2009) | Multi-centre cohort | 2 | No | Pregnancy and fertility; gonadotropins |
| Romerus et al. (2011) | Single-centre cohort | 3 | No | Semen analysis |
| Tromp et al. (2011) | Single-centre cohort | 3 | No | Gonadotrophins |
| Odagiri et al. (2012) | Single-centre cohort | 3 | No | Gonadotrophins |
| Reinmuth et al. (2013) | Multi-centre cohort | 3 | No | Pregnancy and fertility |
| Green et al. (2014a) | Multi-centre cohort | 3 | No | Semen analysis |
| Green et al. (2014b) | Multi-centre cohort | 3 | No | Pregnancy and fertility |
| Wasilewski-Masker et al. (2014) | Multi-centre cohort | 3 | Yes | Pregnancy and fertility |
| Brignardello et al. (2016) | Single-centre cohort | 3 | No | Gonadotrophins |
| Chemaitilly et al. (2016) | Case report | 5 | No | Gonadotrophins; testicular volume |
| Chow et al. (2016) | Multi-centre cohort | 3 | Yes* | Pregnancy and fertility |
| Isaksson et al. (2018) | Multi-centre cohort | 2 | Yes* | Gonadotrophins |
| Utriainen et al. (2019) | Multi-centre cohort | 2 | Yes* | Pregnancy and fertility; gonadotrophins |

*Level of Evidence (adapted from https://www.cebm.net/2016/05/cebms-levels-of-evidence/).
1—Properly powered and conducted randomized clinical trial; systematic review with meta-analysis.
2—Well-designed controlled study without randomization; prospective comparative cohort study.
3—Case–control studies; retrospective cohort study.
4—Case series with or without intervention; cross-sectional study.
5—Opinion of respected authorities; case reports.
*Healthy age-matched controls.
*Healthy siblings.

associated with a lower likelihood of siring pregnancies and livebirths

(Chow et al., 2016). Multivariable models found that the upper tertile of
cisplatin dose ($\geq 488 \text{mg/m^2}$) was associated with a reduced likelihood
of siring pregnancies (hazard ratio (HR) = 0.56, \(P=0.0023\)), while
lower doses of cisplatin (355–487 mg/m2) were associated with a re-
duced likelihood of siring livebirths (HR = 0.64, \(P=0.071\)). Another
study reported a lower percentage of fatherhood among patients who
received cisplatin (10.9%) compared to patients who did not (14.9%), al-
though no statistical data was provided (Green et al., 2014b).

Other studies have drawn conflicting conclusions on the impact of
platinum agents on fertility. A study of 159 males ranging from 0 to 21
years at diagnosis using fatherhood and hormone measurements as
outcome measures reported that addition of platinum agents to
cyclophosphamide or ifosfamide did not increase the risk of gonadal
dysfunction (statistical data not shown) (Ridola et al., 2009).

A study of 234 males aged 0–15 years reported an increased likeli-
hood of infertility (unsuccessful at fathering a child or abnormal fertility
test) among patients receiving cisplatin or carboplatin (Reinmuth et al.,
2013). However, this increased risk was not statistically significant
under both univariate and multivariate analysis, even at high doses (≥500 mg/m² for cisplatin, ≥2000 mg/m² for carboplatin). In a small study involving nine males with neuroblastoma ranging from 0.2 to 3.6 years of age at diagnosis, only one male patient was observed to have fathered a child (Utriainen et al., 2019). This patient had received cisplatin and etoposide as induction chemotherapy, followed by high-dose melphalan. However, participants had also received total body irradiation (TBI) and haematopoietic stem cell transplants (HSCTs), which are known to be sterilizing treatments (Freycon et al., 2019).

A study of a cisplatin-including regimen for GCT in children also reported a single case of sterility, which was attributed to whole abdominal irradiation (Flamant et al., 1984). In a larger study of 565 males, aged 0–17.8 years at diagnosis, 73 of the participants sired pregnancies and 56 of these men conceived naturally (Tromp et al., 2011). A total of 120 conceptions were reported with 103 livebirths and 4 miscarriages. However, the study did not include an individual analysis of platinum agents with regards to pregnancy and fertility.

### Table II Summary of studies involving pregnancy and fertility outcome measures and reported to include platinum-including treatment regimens.

| Study                  | Diagnoses       | Males (n) | Age at diagnosis (years) | Chemotherapy                                      | Platinum analysis | Summary finding                                                                 |
|------------------------|-----------------|-----------|--------------------------|--------------------------------------------------|-------------------|--------------------------------------------------------------------------------|
| Flamant et al. (1984)  | Nonseminomatous GCT | 13        | 1–14                     | Actinomycin D, Cyclophosphamide, Vincristine, Doxorubicin, Bleomycin, Cisplatin | No                 | One case of sterility attributed to whole abdominal irradiation.               |
| Ridola et al. (2009)   | Various         | 159       | 9–19                     | Ifosfamide versus Cyclophosphamide protocols     | Yes               | Platinum agents reported to have no impact on testicular function but data not shown. |
| Tromp et al. (2011)    | Various         | 565       | 0–17.8                   | Various                                          | Yes               | 73 partner pregnancies (56 natural conception). Platinum sub-analysis for hormones only. |
| Reinmuth et al. (2013) | Various         | 234       | 0–15                     | Cyclophosphamide, Ifosfamide, Cis/Carboplatin, Etoposide | Yes<sup>c</sup>    | Higher rate of infertility for platinum-based agents (carboplatin or cisplatin). |
| Green et al. (2014b)   | Various         | 4579      | 5–20                     | Various                                          | Yes               | Lower rate of fatherhood after cisplatin compared to no cisplatin. Statistical data not shown. |
| Wasilewski-Masker et al. (2014) | Various         | 1622      | 9<sup>a</sup>          | Various                                          | Yes               | Platinum agents did not have independent association with fertility—preliminary modelling. |
| Chow et al. (2016)     | Various         | 5640      | ≤20                      | Various                                          | Yes<sup>b</sup>   | Cisplatin associated with reduced pregnancy (≥488 mg/m²) and livebirth (≥355 mg/m²) rates. |
| Utriainen et al. (2019) | Neuroblastoma   | 9         | 0.2–3.6<sup>b</sup>     | Various, HSCT                                      | Yes               | Cisplatin cumulative dose did not correlate with testicular size.              |

Note that summary findings relate to patients receiving treatment when <12 years of age.

GCT, germ cell tumour; HSCT, haematopoietic stem cell transplant.

<sup>a</sup>Mean age.

<sup>b</sup>Interquartile range.

<sup>c</sup>Multivariate and univariate regression analysis.

<sup>d</sup>One patient fathered a child (patient received cisplatin).

### Semen analysis

Semen analysis represents a reliable indicator of fertility status in adult men. We identified five studies reporting the impact of platinum-based chemotherapy regimens for childhood cancer treatment on sperm count in adulthood (Table III). Methods and cut-offs for semen analysis varied between studies (Supplementary Table SV) and 3/5 studies reported the use of World Health Organization standards relevant to the period of study (Table III). All studies combined semen analysis with assessment of gonadotrophins, testicular volume or pubertal staging. A study involving 129 males diagnosed between the ages of 0–17 years reported azoospermia in 4/5 (80%) of those who had received what the authors considered a ‘sterilizing’ dose (≥500 mg/m²) of cisplatin (without radiotherapy), compared with 0/8 (0%) of those who had received <500 mg/m² of cisplatin (Romerius et al., 2011). Two studies included a control group of age-matched healthy participants (Muller et al., 1996; Utriainen et al., 2019). A study involving 33 males aged 0–19 years with various cancers showed azoospermia in 2/14 (14%) and oligozoospermia in 3/14 (21%) of long-term survivors compared with normospermia in 8/14 (100%) of healthy age-matched controls (Muller et al., 1996). Patients with azoospermia were significantly more likely to have been exposed to alkylating agents than those with normospermia. However, the contribution of cisplatin to the semen parameters was not reported (Muller et al., 1996). The second study involving a control group investigated nine male childhood cancer patients...
Hormone analysis and puberty

Gonadotrophin measurements can be used to identify hypogonadism in adulthood. We identified 13 studies of gonadotrophins, testicular volume and puberty in childhood cancer survivors exposed to treatment regimens that are reported to include platinum-based chemotherapy (Table IV). A study including 33 pubertal males (aged 7–17 years at diagnosis) with a range of malignancies demonstrated a significant increase in basal FSH and LH when compared with healthy age-matched controls, indicating impaired spermatogenesis in the cancer survivor group. Of these, 29 males had received chemotherapy, which included alkylating agents for 24 patients (Muller et al., 1996). FSH levels correlated with testicular volumes and sperm counts (see above), providing further evidence to indicate impaired spermatogenesis. Despite the higher LH, suggesting a degree of Leydig cell failure, no differences in testosterone were identified between cancer survivors and controls (Muller et al., 1996). Whilst some patients had received cisplatin, the relationship between cumulative cisplatin dose and testicular function was not reported.

Assessment of the hypothalamic–pituitary–gonadal (HPG) axis was conducted on a cohort of 14 young males (aged 8–19 years) with intracranial GCT (IGCT), of whom six were < 12 years at diagnosis (Odagiri et al., 2012). All patients were treated with carboplatin and etoposide. However, patients also received cranial irradiation, which can impair pituitary gonadotrophin secretion. None of the six boys had impairment of gonadotrophin secretion by last assessment, which was 2–11 years after diagnosis (Odagiri et al., 2012). Whether this related only to hypogonadotropic hypogonadism is not clear as no definition of what constituted abnormal gonadotrophin production was given.

Another study of patients with IGCT, including 18 boys aged 3–15 years, reported pubertal timing in four boys treated before the age of 12 years (Klitoe and Gattamaneni, 1995). Of these, two had precocious puberty prior to therapy, whilst two entered puberty at a normal age (11–12 years). Some patients in this study received platinum-based treatment; however, specific treatment regimens for individual patients were not described.

Table III Summary of studies involving semen analysis in childhood cancer survivors exposed to treatment regimens that are reported to include platinum-based chemotherapy.

| Study                  | Diagnoses     | Age at diagnosis (years) | Males (n) | Chemotherapy treatment | Platinum analysis | Summary finding |
|------------------------|---------------|--------------------------|-----------|------------------------|-------------------|-----------------|
| Muller et al. (1996)   | Various       | 7–17                     | 33        | Various                | No                | Differences between azoospermic and normospermic survivors were not detected for non-alkylating agents. |
| Longhi et al. (2003)   | Osteosarcoma  | 10–42                    | 96        | Methotrexate, Cisplatin, Doxorubicin ± Ifosfamide ± Etoposide | No                | None of the patients < 12-years-old at diagnosis had a sperm count performed. |
| Romerius et al. (2011) | Various       | 0–17                     | 129       | Alkylating agents/Cisplatin | No                | Azoospermia in 4/5 (80%) versus 0/8 (0%) based on a threshold dose of > 500 mg/m² cisplatin. |
| Green et al. (2014a)   | Various       | 0–19                     | 214       | Various                | Yesb              | No increase in azoospermia in subgroup receiving cisplatin treatment for neuroblastoma/osteosarcoma. |
| Utriainen et al. (2019)| Neuroblastoma | 0–3                      | 9         | Various                | Yes               | Sperm count performed in one patient who had received cisplatin—oligozoospermia although reported to have fathered a child. |

Note that summary findings relate to patients receiving treatment when < 12 years of age.

Sub-group analysis for cisplatin in osteosarcoma and neuroblastoma survivors.
| Study                      | Diagnoses | Males (n) | Age at diagnosis (years) | Chemotherapy | Cranial irradiation | Platinum analysis | Summary finding                                                                 |
|---------------------------|-----------|-----------|--------------------------|--------------|---------------------|-------------------|-------------------------------------------------------------------|
| Wallace et al. (1989)     | Various   | 8         | 7–14                     | Multiple regimens | No                  | No                | Gonadotrophins remained low at follow-up (<3 years post-diagnosis) in keeping with retained prepubertal status. |
| Kiltie and Gattamaneni (1995) | IGCT      | 18        | 3–15                     | Multiple regimens | Yes (n = 18)          | No                | Precocious puberty in two boys prior to treatment and normal puberty in another two. Specific impact of cisplatin not investigated. |
| Muller et al. (1996)      | Various   | 33        | 7–17                     | Multiple regimens | Yes (n = 6)           | No                | Overall increase in basal FSH and LH compared with healthy controls. Specific impact of cisplatin not investigated. |
| Hale et al. (1999)        | GCT       | 26        | 0–16                     | Multiple regimens | No                  | No                | Puberty normal in all patients. Specific impact of cisplatin not investigated. |
| Relander et al. (2000)    | Various   | 77        | 0–17                     | Multiple regimens | Yes (n = 28)          | No                | Puberty and testosterone normal in 98% and 95%, respectively. Raised FSH in 14% of entire cohort. Only one patient received cisplatin. |
| Ridola et al. (2009)      | Various   | 159       | 0–20                     | Cyclophosphamide versus Ifosfamide | No                  | Yes               | Inclusion of platinum-based agents reported to have no effect on testicular function but data not shown. |
| Romerius et al. (2011)    | Various   | 129       | 0–17                     | Multiple regimens | Yes (n = 61)          | No                | Elevated FSH in 32.5% of the entire cohort. Specific impact of cisplatin not investigated. |
| Tromp et al. (2011)       | Various   | 565       | 0–17                     | Multiple regimens | Yes (n = 135)         | Yes               | Raised FSH in 33% and low testosterone in 12%. No association between cisplatin and FSH. |
| Odagiri et al. (2012)     | IGCT      | 14        | 8–19                     | Carboplatin and Etoposide | Yes (n = 14)          | No                | No impairment of gonadotrophic secretion at 2–11 years post-diagnosis. |
| Brignardello et al. (2016)| Various   | 199       | 0–18                     | Various         | Yes (n = 71)          | Yes               | FSH or low Inhibin B in 35% (alkylators) versus 60% (alkylators + platinum agent). Compared with 12% (no alkylators or platinum agents). |
| Chemaitilly et al. (2016) | Glioma    | 1         | 2                        | Carboplatin, Vincristine, Temozolomide, Lomustine, Thioguanine, Procarbazine | No                  | No                | Case report aged 11 years. Tanner stage 4 with testicular volumes 4.5 ml indicating impaired spermatogenesis. |
| Isaksson et al. (2018)    | Various   | 121       | 5–15                     | Multiple regimens | Yes (n = 12)          | No                | Raised FSH in 5.8% of childhood cancer survivors compared with 1% of healthy age-matched controls. |

Continued
A case report of an 11-year-old boy treated with alkylating agents and carboplatin for a glioma at the age of 2 years reported puberty with evidence of impaired spermatogenesis (Chemaitilly et al., 2016). He was Tanner stage 4 with a testosterone of 8.9 nmol/L, although his testicular volumes were consistent with early puberty (4.5 ml). Subsequent follow-up into adulthood was not reported.

Normal puberty has been reported in 26 males (aged 0–18 years) treated for GCT (Hale et al., 1999). Treatment regimens were varied in terms of exposure to radiotherapy and/or platinum-based chemotherapy and the relative contribution of cisplatin cannot be concluded (Hale et al., 1999).

In a study involving 129 childhood cancer survivors (Romerius et al., 2011), gonadotrophins correlated with risk of azoospermia. Elevated FSH levels (>10.9 IU/l) were identified in 42 (32.5%) of patients of whom 22 were azoospermic giving a positive predictive value of 50% and a negative predictive value of 99%. Similar associations were seen between combined testicular volume ≤24 ml and azoospermia (Romerius et al., 2011). However, the study did not distinguish between impacts attributed to cisplatin and alkylating agents.

Gonadotrophins were evaluated in 159 boys (aged 4–20 years) treated for various malignancies (Ridola et al., 2009). Patients were grouped according to whether they received ifosfamide or cyclophosphamide as their sole alkylating agent in the treatment regimen and 42 had also received a platinum-based agent (cisplatin or carboplatin). Only two patients had a low testosterone within this cohort. Higher doses of cyclophosphamide (>12 g/m²) were associated with an increased risk of elevated FSH compared with lower doses and with all doses of ifosfamide. The inclusion of platinum-based agents to regimens was reported to have no impact on testicular function, although the data to support this conclusion was not shown (Ridola et al., 2009).

A study of 77 men who had been treated for a variety of malignancies included gonadotrophin and testosterone measurements in 66 (Relander et al., 2000). Testosterone was in the normal adult range for 65/66 and normal puberty was reported for 62/66 men. FSH was normal in 42/66 (63%) and in nine patients FSH was raised, indicating impaired spermatogenesis. Of these nine patients, seven had a sperm count performed and this was abnormal (five azoospermia and two oligozoospermia) in all cases. However, few patients in this study had received cisplatin. One patient with Ewing Sarcoma received cisplatin and cyclophosphamide and he was found to have testicular volumes <15 ml, whilst two patients with testicular GCT had received cisplatin but these patients were aged >14 years at diagnosis (Relander et al., 2000).

Long-term follow-up of gonadotrophin levels was conducted in a large cohort of 565 survivors of childhood cancer diagnosed between the ages 0–17 years and surviving for a minimum of 5 years (Tromp et al., 2011). The majority (90%) had received chemotherapy. FSH levels were obtained in 488 (86%) patients and were raised (>10 IU/l) in 121 (33%) cases. Raised LH and low testosterone, indicating primary Leydig cell failure, were reported in 2.9% and 12.4% of cases, respectively. The impact of platinum agents on FSH level was assessed using linear regression analysis and no significant association was identified either by univariate or multivariate analysis with odds ratios of 0.68 (0.35–1.32) and 2.29 (0.89–5.89), respectively. However, the number of patients receiving platinum-based chemotherapy represented a very small proportion of the cohort.

Cumulative exposure to cisplatin has been reported in relation to gonadotrophin data in a cohort of 20 patients (nine males) diagnosed with high-risk neuroblastoma at between 0 and 3 years of age (Utriainen et al., 2019). Cisplatin was part of their initial treatment and carboplatin was used for conditioning for HSCT, which included TBI in 10 (50%) cases. After a median follow-up time of 15 years, male survivors (n = 6) had significantly higher FSH (26.3 vs 3.6 IU, respectively; P < 0.001) and lower cumulative testicular volumes (8.5 vs 39 ml, respectively; P < 0.001) compared with healthy controls. Despite the high FSH, 3/5 patients in the non-TBI group had testicular volumes >15 ml, whereas all (4/4) patients receiving TBI had testicular volumes <10 ml. No correlation between cumulative cisplatin dose and testicular volume was identified, which may reflect the fact that these individuals also had HSCT and in many cases TBI. Of the nine patients, eight entered puberty spontaneously (three early), whilst one patient in the TBI group required pubertal induction. All four patients in the TBI group required testosterone replacement, whilst 0/5 in the non-TBI group required testosterone.

A study of 199 childhood cancer survivors aged <18 years at diagnosis (minimum follow-up of 5 years) had their HPG axis assessed (Brignardello et al., 2016). Impaired spermatogenesis, defined as FSH

| Study | Diagnoses | Males (n) | Age at diagnosis (years) | Chemotherapy | Cranial irradiation | Platinum analysis | Summary finding |
|-------|------------|-----------|--------------------------|--------------|--------------------|------------------|----------------|
| Utriainen et al. (2019) | Neuroblastoma | 9 | 0–3 | Multiple regimens | Yes (n = 4) | Yes | *Significantly raised FSH and reduced TV compared with healthy controls. No correlation between cumulative cisplatin dose and TV. |

IGCT, intracranial germ cell tumour; TV, testicular volume.

*Includes all non-testicular irradiation.

**Multivariate and univariate regression analysis.

Comparisons of alkylators ± platinum agent.

Summary includes only patients receiving treatment <12 years of age.

**Significantly raised FSH and reduced TV compared with healthy controls. No correlation between cumulative cisplatin dose and TV.
>10 IU/l or inhibin B <100 pg/ml, was identified in 52/130 (35%) patients treated with alkylating agents, compared to 14/23 (60%) patients treated with a combination of alkylating and platinum-based chemotherapy. For those treated with chemotherapy other than alkylating or platinum-based agents, impaired spermatogenesis only occurred in 2/17 (11.8%). Biochemical evidence for impaired spermatogenesis was confirmed by semen analysis in all patients (41/68) who provided a sample; however, the proportion that had received platinum-based chemotherapy was not described. In addition to impairment of spermatogenesis, Leydig cell failure (defined as raised LH and total testosterone <3 ng/dl) occurred in 13/147 (8.8%) of patients receiving alkylating chemotherapy, compared with 2/23 (8.7%) of those receiving alkylating agents in combination with platinum-based chemotherapy (Brignardello et al., 2016).

In a study of 14 male patients treated for CNS GCT, four patients were aged <12 years at diagnosis (Buckner et al., 1999). All four patients received chemotherapy, including etoposide and cisplatin, and localized radiotherapy. The study reported no post-treatment effects on growth and development; however, hormone levels were not reported.

Whilst not specifically reporting effects of cisplatin treatment, impaired spermatogenesis has also been shown in a cohort of 121 male childhood cancer survivors (Isaksson et al., 2018). Patients were diagnosed before the age of 18 years and were a minimum of 3 years post-treatment. Primary hypogonadism (definition includes FSH >10 IU/l) was reported in 7/121 (5.8%), compared to 1/122 (0.8%) of the control patients.

A study by Wallace et al. (1989) included eight male patients aged 7–12 years at diagnosis, who had received cisplatin treatment. Three were described as prepubertal at diagnosis. In the prepubertal patients, serum levels of gonadotrophins were low at follow-up, in keeping with their prepubertal status. However, no further follow-up data were available for these patients.

**Experimental evidence for effects on testicular development and function**

No laboratory studies were identified that investigated the effect of platinum-based chemotherapy on human testicular tissue or cells.

**Discussion**

The data on the impact of platinum-based chemotherapy in males on partner pregnancy rates provide conflicting results. Whilst one large study reported no effect of platinum-based chemotherapy on pregnancy rates (Waslewski-Masker et al., 2014), the CCSS demonstrated a significant reduction in pregnancy rates based on a dose threshold of ~500 mg/m² (Green et al., 2014b) and another study involving the CCSS cohort reported similar findings with a lower threshold of ~350 mg/m² (Chow et al., 2016). A major limitation of the studies is the use of self-reporting of fertility using questionnaires. These may be prone to selection bias and variations in interpretation of the terms used to define infertility. In addition, it does not account for co-existing morbidities that may impact fertility e.g. obesity, or individual wish to father a child.

Semen analysis can provide an accurate objective measure of fertility potential in males and may be less prone to bias. However, obtaining a semen sample is challenging from a practical perspective and such studies are prone to participation bias. The majority of the studies that include sperm counts are limited by low numbers of patients providing a sample or by lack of sub-analysis to determine the contribution of platinum-based chemotherapy to azoospermia. The only study to specifically assess this did not find an association, although this may be confounded by the concomitant use of alkylating agents and low numbers of patients receiving platinum-based treatments (Green et al., 2014a).

Whilst the study of Isaksson et al. (2018) did not specifically report on cisplatin exposure in the childhood cancer survivor cohort, it did report a cohort of adult testicular cancer patients in whom there was a significantly increased risk of hypogonadism in those who received >4 cycles of cisplatin-based chemotherapy. This is in keeping with the studies reporting cisplatin-induced fertility impacts on men with testicular GCT (Lampe et al., 1997; Brydoy et al., 2010). The finding of persistent azoospermia in men after platinum-based chemotherapy suggests damage to the spermatogonial stem cell (SSC) population, which is also the likely target for damage by platinum-based chemotherapy during childhood, leading to impairment of fertility in adulthood. A reduced number of spermatogonia, including SSCs, has been reported in several recent studies involving histological examination of testicular biopsies in boys with cancer (Pogantis-T Korhonen et al., 2017; Stukenborg et al., 2018; Vally-Pulaski et al., 2019; Portela et al., 2020). However, these studies did not include data on the contribution of cisplatin to the germ cell loss.

Gonadotrophins provide an indirect assessment of testicular function and spermatogenesis. Raised gonadotrophins in the context of chemotherapy or radiotherapy indicate primary gonadal failure and an FSH threshold can be applied to identify azoospermia in adults with good sensitivity and specificity (Kelsey et al., 2017). However, the use of gonadotrophins in determining impairment to spermatogenesis and fertility in childhood cancer survivors is limited because these need to be measured post-puberty due to the quiescence of the HPG axis during prepuberty. The specific contribution of cisplatin was only assessed in 2/12 studies reporting gonadotrophin data, with conflicting results. One study showing no association was limited by the low numbers receiving cisplatin (Tromp et al., 2011). The other study did report an increased frequency of raised FSH in those receiving a combination of alkylators and cisplatin, compared to alkylators alone (Brignardello et al., 2016); however, the specific regimens and cumulative doses are not reported.

The major limitations of clinical studies involving childhood cancer survivors relate to the number of patients receiving specific regimens and the challenges of identifying the relative contribution of an individual agent to infertility in adulthood. Experimental studies can contribute to the understanding of the impact of platinum-based chemotherapy by exposing human testis tissues to single agent chemotherapy in human-relevant model systems. Such systems have recently been developed utilizing in vitro and xenograft approaches and can be applied to the study of exposure to pharmacological agents on germ and somatic cells of the testis (van den Driesche et al., 2015; Jorgensen et al., 2018). The in vitro approach has recently been used to expose prepubertal rodent testis tissue to a variety of chemotherapy drugs, including cisplatin (Smart et al., 2018; Allen et al., 2020). These studies report significant reductions in total germ cell and putative SSCs following exposure to concentrations of platinum-based
chemotherapy that reflect serum levels in patients treated with these agents. To date, such experimental studies have not been replicated using human prepubertal testicular tissues from patients who are having tissue stored for fertility preservation. In addition to conducting such studies, retrospective examination of archived testicular tissues that have already been exposed to platinum-based chemotherapy may be performed, similar to previous studies investigating the effects of exposure to alkylating agents (Poganitsch-Korhonen et al., 2017).

Overall, the results of this study indicate that platinum-based chemotherapy should be taken into consideration by clinicians when counselling patients and their carer(s) on the fertility risk associated with cancer treatment. Platinum-exposure should also be factored into discussions regarding fertility preservation options (e.g. testicular tissue cryopreservation) in prepubertal boys. However, it should be emphasized that identifying clear thresholds for testicular dysfunction following exposure to platinum-based chemotherapy in childhood requires further study. Children receiving treatment for osteosarcoma, hepatoblastoma and extracranial GCT may represent appropriate groups to determine the individual contribution of platinum agents, as these patients will often receive platinum-based chemotherapy without concomitant exposure to alkylating agents. These studies should include detailed clinical histories and drug exposures (including cumulative doses) for all patients. Regular follow-up of reproductive function, including pubertal assessment, hormone measurements and semen analysis, should also be performed.

Conclusion

Whilst many studies report the reproductive outcomes of childhood cancer survivors who have received platinum-based chemotherapy, relatively few have investigated the individual contribution of these agents to gonadal function, especially fertility. In addition, human-relevant experimental model systems in which to test the impact of specific chemotherapeutics on testicular development and function are lacking. Whilst this study indicates that platinum-based agents should be included in fertility risk assessments in prepubertal boys, it also highlights the importance of conducting large-scale prospective follow-up studies in these patients.

Supplementary data

Supplementary data are available at Human Reproduction Update online.

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Authors’ roles

R.T.M. conceived and designed the study. R.T.M. and L.T.E. conducted the literature search and data collection. R.T.M. wrote the manuscript. W.H.B.W. and M.B. provided critical discussion of the data. All authors edited the manuscript and agreed upon the submitted manuscript.

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Conflict of interest

The authors have no conflict of interest to declare.

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