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Male Gonadal Function After Pediatric Hematopoietic Stem Cell Transplantation: A Systematic Review

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A B S T R A C T

Male gonadal dysfunction is a frequent late effect after pediatric hematopoietic stem cell transplantation (HSCT) that can lead to disturbances in pubertal development, sexual dysfunction, and infertility. However, no systematic review exists regarding prevalence and risk factors in relation to different treatment regimens. We aimed to systematically evaluate the current evidence regarding the prevalence of male gonadal dysfunction after pediatric HSCT, related risk factors, and the diagnostic value of surrogate markers of spermatogenesis in this patient group. We searched PubMed and Embase using a combination of text words and subject terms. The eligibility screening was conducted using predefined criteria. Data were extracted corresponding to the Leydig cell compartment involved in testosterone production and the germ cell compartment involved in spermatogenesis, respectively. Subsequently, data synthesis was performed. Of 2369 identified records, 25 studies were eligible. The studies were heterogeneous in terms of included diagnoses, gonadotoxic therapy, follow-up time, and definitions of gonadal dysfunction. The data synthesis revealed a preserved Leydig cell function in patients treated with non-total body irradiation (TBI) regimens, whereas the evidence regarding the impact of TBI conditioning on Leydig cell function was conflicting. Based on surrogate markers of spermatogenesis and only limited data on semen quality, the germ cell compartment was affected in half of the patients treated with non-TBI regimens and in nearly all patients treated with TBI conditioning. Testicular irradiation as part of front-line therapy before referral to HSCT led to complete Leydig cell failure and germ cell failure. Evidence regarding the impact of diagnosis, pubertal stage at HSCT, and chronic graft-versus-host disease is limited, as is the evidence of the diagnostic value of surrogate markers of spermatogenesis. Testicular irradiation as part of front-line therapy and TBI conditioning are the main risk factors associated with male gonadal dysfunction after pediatric HSCT; however, impaired spermatogenesis is also observed in half of the patients treated with non-TBI regimens. Methodological shortcomings limit existing evidence, and future studies should include semen quality analyses, follow-up into late adulthood, and evaluation of the cumulative exposure to gonadotoxic therapy.

Hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for children with high-risk hematological cancers, severe immunodeficiencies, and other nonmalignant diseases. Improved survival and increasing numbers of transplantations performed result in growing numbers of long-term survivors [1], thus calling for a focus on late effects to facilitate a reduction in long-term morbidity and improved quality of life [2,3].

Male gonadal dysfunction, including testosterone deficiency and impaired spermatogenesis, is a frequent late effect following pediatric HSCT, potentially affecting pubertal development, sexual health, and fertility [4]. Identification of underlying risk patterns associated with gonadal dysfunction is paramount for accurate patient information before HSCT, targeted fertility preservation among the patients at risk, and adequate follow-up strategies [5–7].

Evaluation of male gonadal dysfunction reflects the two compartments of the male reproductive axis: the Leydig

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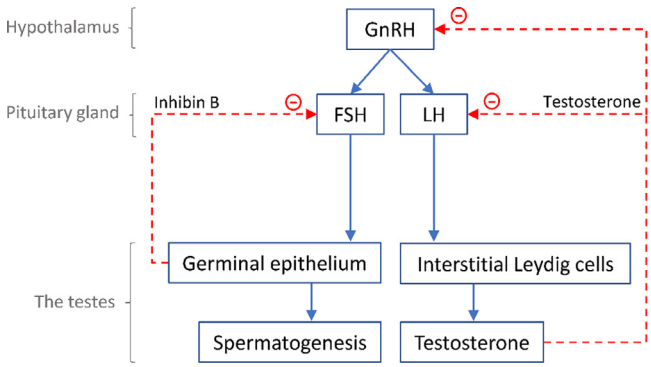

Figure 1. Hormonal regulation of the male hypothalamic-pituitary-gonadal axis. *Dashed red lines* indicate negative feedback. The germinal epithelium includes germ cells at all spermatogenic stages and the supportive Sertoli cells. Inhibin B is produced by the Sertoli cells in the prepubertal period, whereas partly by Sertoli cells and partly by germ cells after the onset of puberty. GnRH indicates gonadotropin-releasing hormone.

compartment and the germ cell compartment (Figure 1). Interstitial Leydig cells are stimulated by luteinizing hormone (LH) to produce testosterone, the primary male reproductive hormone [8]. Leydig cell function is therefore evaluated by pubertal development, serum LH levels, and serum testosterone levels (preferably morning levels due to diurnal variation) [9]. The germinal epithelium, including Sertoli cells and germ cells, is stimulated by follicle-stimulating hormone (FSH) to induce spermatogenesis [8]. Semen analysis is the current gold standard for evaluating the overall germ cell function; however, surrogate markers of spermatogenesis, such as testicular volumes, FSH, and inhibin B, are often applied in clinical studies, although the diagnostic value of these markers is questionable [10,11].

Chemotherapy and irradiation are generally considered the primary risk factors for development of gonadal dysfunction after pediatric HSCT [12–14]. High-risk chemotherapeutic drugs include alkylating agents such as cyclophosphamide, busulfan, and melphalan, all of which are frequently used in front-line therapy for malignant diseases, as well as in HSCT conditioning regimens [13]. Likewise, both cranial and testicular irradiation—targeted, as part of TBI, or as scatter doses from thoraco-abdominal irradiation (TAI) or total lymphoid irradiation (TLI)—affect the male reproductive axis by direct damage to the testicular tissue and by interfering with hormonal regulation. Although germs cells are very sensitive to chemotherapy and irradiation, the Leydig cells appear more resistant [14–18]. Nevertheless, safe threshold doses have yet to be identified [11].

During the last decade, focus has moved from traditional myeloablative conditioning (e.g., 10-12 Gy TBI plus high-dose cyclophosphamide or high-dose busulfan plus cyclophosphamide) to reduced intensity conditioning (RIC) to reduce toxicity and non-relapse mortality. The RIC regimens typically consist of an anti-metabolite such as fludarabine combined with a lower dose of alkylating chemotherapy such as melphalan, thiotepa, busulfan, or treosulfan [19–21]. The gonadotoxic effects of these newer regimens remain uncertain at present [22,23].

Numerous studies have addressed male gonadal function after pediatric HSCT; however, overall conclusions are still lacking because of essential methodological differences across studies. Consequently, we aimed to systematically review the literature with a focus on (1) the prevalence of testosterone deficiency and impaired spermatogenesis in relation to different treatment regimens, (2) risk factors associated with testosterone deficiency and impaired spermatogenesis, and (3) the diagnostic value of surrogate markers of spermatogenesis in this specific patient population.

METHODS

Protocol and registration

This systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement [24,25], and the protocol was specified and registered at International Prospective Register of Systematic Reviews in June 2019 (registration number CRD42019140150) [26].

Eligibility criteria

The population of interest included male patients treated with allogeneic or autologous HSCT before the age of 18 years, regardless of conditioning regimen. Because signs of gonadal dysfunction are rarely evident before pubertal age, eligible patients were required to be pubertal/postpubertal (as defined by the authors) or at least 14 years of age at last evaluation [27].

The main outcomes were (1) impaired spermatogenesis evaluated by semen sample analyses (detectable sperm versus azoospermia) and (2) testosterone deficiency defined as the use of testosterone replacement therapy (TRT) or reduced serum testosterone levels. Secondary outcomes included surrogate markers of spermatogenesis (testicular volumes, FSH levels, inhibin B levels) and paternity, in addition to indicators of Leydig cell function, specifically onset of puberty (spontaneous or induced), timing of puberty, and serum LH levels. Reporting of at least one of these primary or secondary outcomes was required for inclusion. Only studies which reported the applied definition of gonadal dysfunction (e.g., by cutoff levels for hormone measurements) were included.

Conference abstracts, case reports, case series (eligible population $n < 10$), reviews, comments not including original data, and studies not available in English were excluded. Detailed eligibility criteria are provided in Supplemental Material 1.

Search strategy

We searched Medline via PubMed and Embase via Ovid on June 21, 2019. Two supplementary searches were conducted to update the review with records published from June 21, 2019 to April 9, 2021. The bibliographies of reviews addressing gonadal function or late effects after pediatric HSCT (identified in the primary search) and of all included studies were screened for additional eligible studies. The systematic search string (organized according to participants, intervention, and outcomes) included a combination of text words and subject terms, as provided in Supplemental Material 2.

Study selection

After removing duplicates, two authors (S.M. and L.A.J.) independently screened titles and abstracts for eligibility. Conflicts were resolved by discussion and by consulting a third author (K.M.). Subsequently, a full text screening of studies included from the title and abstract screening was conducted by the same method. Excluded studies were labeled with the reason for exclusion.

Study quality assessment and risk of bias

Evaluation of study quality and risk of bias for each included study was performed using an adapted version of the Newcastle-Ottawa Quality Assessment Scale developed to fit the content and purpose of this systematic review. To evaluate both cohort and cross-sectional studies by one uniform scale, we merged the official Newcastle-Ottawa Quality Assessment Scale for Cohort

Studies [28] and the un-official Newcastle-Ottawa Quality Assessment Scale for Cross-sectional Studies developed by Herzog et al. [29], with few modifications. The adapted scale is provided in Supplemental Material 3. Three study domains were evaluated (selection, comparability, and outcome). Two authors (S.M. and L.A.J.) independently reviewed the quality and risk of bias of the eligible studies, and conflicts were resolved by discussion.

Data items and data extraction

Data regarding study characteristics, Leydig cell outcomes, and germ cell outcomes were extracted from included studies according to pre-specified data extraction items (Supplemental Material 4). One author (S.M.) independently extracted the data, subsequently validated by a second author (L.A.J.). Discrepancies were resolved by discussion and by consulting a third author (K.M.). All included studies were assessed for duplicate publication of results by comparing author names, institutions, and study populations (diagnoses, transplantation period, transplant type, and conditioning regimens).

Data regarding pubertal onset (spontaneous or medically induced) were extracted only for patients who were prepubertal at time of HSCT. Data regarding pubertal timing were extracted for those with spontaneous onset of puberty. Leydig cell function was categorized as (1) testosterone deficiency (use of TRT or low testosterone levels), (2) compensated Leydig cell dysfunction (high LH levels combined with normal testosterone levels), and (3) normal Leydig cell function (normal LH levels combined with normal testosterone levels). Hormone cut-off levels were as defined by the study authors. Data regarding LH, FSH, testosterone levels, testicular volumes, and semen quality were extracted for patients without TRT, whenever possible, to exclude the effects of TRT (i.e., lower gonadotropin levels, higher testosterone levels, smaller testicular volumes, and risk of azoospermia).

Synthesis of results

Because of marked heterogeneity of the included studies, no meta-analyses could be performed. Instead, syntheses of results were conducted to address the three specific questions posed in this review. Regarding the prevalence of gonadal dysfunction in relation to different treatment regimens, data were extracted across studies according to the following treatment groups, taking the conditioning regimen and testicular irradiation as part of front-line treatment into account: (1) chemotherapy only; (2) chemotherapy and low-dose testicular irradiation (single-dose TBI 2-3 Gy, TLI, TAI with gonadal shielding, or full-dose TBI with gonadal shielding); (3) chemotherapy and TBI (single dose or fractionated) *without* additional testicular irradiation; (4) chemotherapy and TBI *plus* a testicular boost at HSCT; and (5) chemotherapy and TBI *plus* additional testicular irradiation as part of front-line therapy. Several studies included patients treated with cranial irradiation as part of pre-HSCT front-line treatment, but because data generally could not be extracted separately for these patients, cranial irradiation before conditioning was not accounted for in the treatment groups.

Regarding investigated risk factors associated with gonadal dysfunction, results were summarized across studies. Finally, regarding the diagnostic value of surrogate markers of spermatogenesis, we compared results from studies reporting receiver operating characteristics curves to describe area under the curve, sensitivity, and specificity.

RESULTS

Study selection

We identified 3288 records of which 919 were duplicates. The titles and abstracts of 2369 studies were screened, after which 278 were examined in full text, and 25 studies were found eligible (Figure 2) [30–54].

Study characteristics

Study characteristics are summarized in Table 1. All 25 studies were observational in design, primarily retrospective (n = 22) and single-center studies (n = 23). Most study populations were small (median 28 patients, range 10-106) and several studies appeared to include overlapping patient populations. Three studies from the same center included patients with the same diagnoses and from the same transplantation period [33,34,54]; three studies from the same authors and centers included patients with partly overlapping

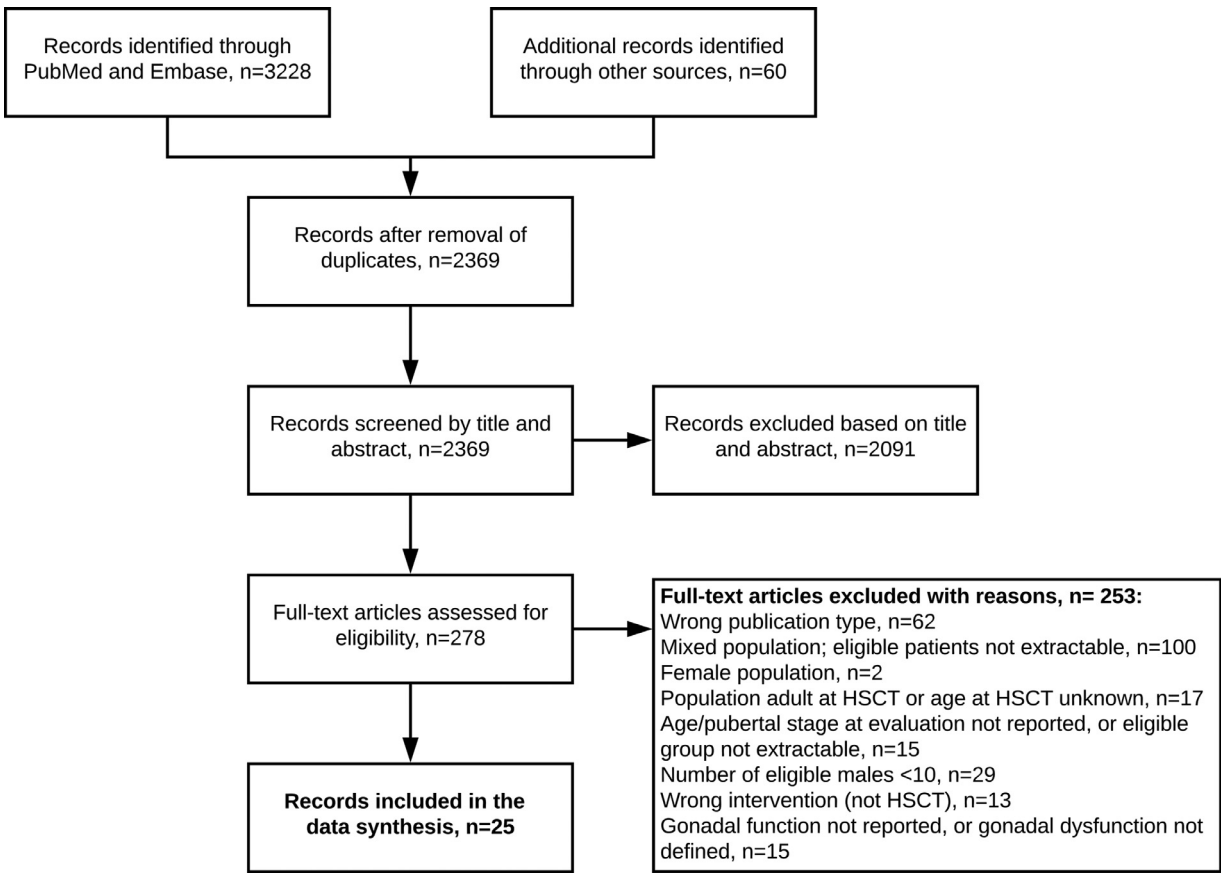

Figure 2. Flowchart of study selection process.

Table 1
Study Characteristics of the 25 Included Studies

Included studies	25
Total number of eligible males	869
Number of eligible* males per study, median (range)	28 (10-106)
Publication period across studies (y)	1991-2020
Study designs, number of studies	
Retrospective, cross-sectionally reported	17
Retrospective, longitudinally reported	5
Cross-sectional	2
Prospective	1
Single versus multicenter study, number of studies	
Single center	23
Multicenter (>1 center)	2
Diagnoses included, number of studies	
Malignant only	9
Non-malignant only	2
Malignant and nonmalignant	14
Transplantation period across studies (year)	1970-2017
Type of transplantation, number of studies	
Only autologous	1
Only allogeneic	11
Both autologous and allogeneic	11
Not available	2
Conditioning regimens included, number of studies	
Chemotherapy-based only	4
TBI-based only	8
Different regimens included	13
Time from transplant across studies, range in years	0.3-34.6
Outcomes reported, number of studies	
Germ cell compartment	
Semen samples	8 (case reports in 7 studies)
Paternity	7 (case reports in 6 studies)
Testicular volume	10
FSH	23
Inhibin B	4
Leydig cell compartment	
Pubertal onset (spontaneous versus induced)	16
Pubertal timing	10
Testosterone substitution	19
Testosterone	20
LH	23

* Eligible according to the inclusion criteria in this review

diagnoses and conditioning regimens; however, transplantation periods were not reported [36,48,49]; and two studies included Finnish HSCT survivors from the same centers and with partly overlapping transplantation periods [43,45].

Quality assessment and risk of bias

Results from the quality assessments of each individual study are presented in Supplemental Material 5. Only eight of 25 studies reported a study population representative of the total HSCT population, whereas the risk of selection bias was high in the remaining studies. No studies described the origin of the reference material used for reproductive hormone levels or testicular volumes. No studies demonstrated the absence of gonadal dysfunction before HSCT; nevertheless, because gonadal dysfunction is rarely evident before pubertal age and most patients were prepubertal at HSCT, meeting this criterion was considered impossible.

Regarding comparability, 12 of 25 studies evaluated gonadal function according to a reference material matched on age or pubertal stage, whereas 13 of 25 studies applied uniform cutoff levels regarding reproductive hormone levels and testicular volumes, although some of the included patients were at peripubertal age when examined. Concerning exposure to gonadotoxic therapy prior to HSCT (front-line therapy), only one of 25 studies included pre-HSCT exposure to testicular irradiation, cranial irradiation, and alkylating chemotherapy in the reporting and analyses of results.

All studies assessed the outcomes directly or through information from medical records. All studies demonstrated "long enough follow-up for outcomes to occur," because all patients included in this review were required to be pubertal/postpubertal or at least 14 years of age at last evaluation. None of the studies were suspected of bias due to missing data or subjects lost to follow-up. Nearly half of the studies (12 of 25) were merely descriptive, and two of 25 studies reported inadequate statistical information (no estimates or *P* values).

Comparability of studies and individual results

Results from the individual studies regarding study characteristics and gonadal dysfunction are summarized in Table 2. The included studies were heterogeneous in terms of included diagnoses, pre-HSCT exposure to gonadotoxic therapy, type of transplant (autologous, allogeneic, or both), and conditioning regimens. Studies generally included patients at peripubertal ages at last follow-up; however, only six studies specifically reported the pubertal stage of the patients at last follow-up [31,34,45,46,51-54]. Data on pubertal timing were sparse, as were data on spermatogenic capacity evaluated by semen samples and data on paternity. Only one study systematically investigated semen samples in all patients [45], whereas seven other studies reported spermatogenic status in only some of the patients (case reports) [32,40,42,43,46,51,52]. Likewise, only one study systematically investigated paternity [45], whereas five studies reported cases of paternity [32,33,37,43,51]. The applied cut-off levels for definition of abnormal serum levels of gonadotropins, and for definition of abnormal testicular volumes, differed substantially between studies (Table 2).

PREVALENCE OF GONADAL DYSFUNCTION ACCORDING TO TREATMENT GROUPS

Data extraction across studies for each of the treatment groups specified above are presented in Supplemental Material 6 and summarized below. The available data did not allow comparisons of the gonadotoxic effects of RIC versus myeloablative conditioning or effects of malignant versus non-malignant diagnosis, as these data were too few or could not be extracted.

Chemotherapy only

Pubertal development was reported for 71 patients, of which only five cases needed induction of puberty [30,33,37,41,46]. Regarding Leydig cell function at last follow-up, reported for 118 patients, a total of 19 patients were treated with TRT, of which 11 were treated for β-thalassemia [30,37,41,45,46,50]. Only five cases of compensated Leydig cell dysfunction were reported [30,41,46]. One study compared

Table 2
Summary of Study Design, Leydig Cell Compartment Outcomes, and Germ Cell Compartment Outcomes in the 25 Included Studies

Author Study design	Eligible males, N	Diagnoses	Autologous/allogeneic HSCT	Pre-HSCT therapy reported	Types of conditioning regimens included in the study	Age at follow-up	Induced puberty, n/N	Precocious or delayed puberty, n/N	Testosterone replacement therapy, n/N	Elevated LH, n/N	Azoospermia, n/N	Elevated FSH, low inhibin B, small testis vol., n/N
Studies only including patients treated with chemotherapy-only conditioning												
De Sanctis et al. [50] Retrospective, longitudinal Single-center	12	Thalassemia	Allogeneic	No	Chemotherapy only (BuCy)	12.6-18 y *	—	—	TRT: 9/12	—	—	—
Afify et al. [30] Retrospective, longitudinal Single-center	10	AML	Autologous and allogeneic	Partially	Chemotherapy only (BuCy)	13.2-22.6 y	Induced: 1/9	—	TRT: 1/10	LH: 2/7 (2 missing data)	—	FSH: 6/7 (2 missing data)
Vlachopapadopoulou et al. [41] Retrospective Single-center	11	Thalassemia	Allogeneic	No	Chemotherapy only (BuCy)	14.2-20.9 y	Induced: 1/6	Delayed: 1/6	TRT: 2/11	LH: 1/9	—	FSH: 2/9
Panasiuk et al. [37] Retrospective Single-center	47	Malignant and non-malignant	Autologous and allogeneic	Partially	Chemotherapy only (BuCy and FluMel)	11-20 y †	Induced: 3/47	—	TRT: 3/47 (temporary use)	—	—	FSH: 17/47
Studies only including patients treated with TBI-based conditioning regimens												
Sarafoglou et al. [38] Retrospective, longitudinal Single-center	15	AML ALL	Autologous and allogeneic	Partially	TBI-based	10.4-17.1 y *	Induced: 1/15	Precocious: 1/14	TRT: 2/15	LH: 5/14 (min. 1 occasion)	—	FSH: 9/14 (min. 1 occasion)
Bakker et al. [31] Retrospective, longitudinal Single-center	21	Malignant	Allogeneic	Partially	TBI-based	16.1-23.3 y	Induced: 2/15	Delayed: 1/13	TRT: 2/21	LH: 10/19	—	FSH: 18/19 Testis vol.: 7/19
Frisk et al. [53] Retrospective Single-center	11	ALL	Autologous Syngeneic	Partially	TBI-based	15.9-22.1 y *	Induced: 2/9	—	TRT: 9/11	LH: 0/2	—	—
Faraci et al. [52] Retrospective Single-center	21	Malignant	Autologous and allogeneic	Partially	TBI-based	14.6-25.1 y	—	—	TRT: 6/21	—	Azoospermia: 2/2	FSH: 18/21 (incl. 6 TRT)
Couto-Silva et al. [49] Retrospective Single-center	32	Malignant	—	Partially	TBI-based	12-22 y *†	—	—	TRT: 2/32	LH: 17/30	—	FSH or testis vol.: 26/30

(*continued*)

Steffens et al. [40] Cross-sectional Single-center	12	ALL NHL	Autologous and allogeneic	Partially	TBI-based	17.0-28.9 y *	Induced: 6/12	—	TRT: 10/12	LH: 0/2	Azoospermia: 1/1	FSH: 2/2
Inagaki et al. [32] Retrospective Single-center	12	SAA Refractory cytopenia	Allogeneic	No	TBI-based (3 Gy)	14-31 y	—	—	TRT: 0/12	LH: 0/11 (1 missing data)	Azoospermia: 0/1	FSH: 3/11 (1 missing data)
Taneja et al. [44] Retrospective, longitudinal Single-center	42	Leukemia MDS	Autologous and allogeneic	Partially	TBI-based	14.5-47.7 y	Induced: 5/42	Precocious: 0/37 Delayed: 3/37	TRT: 27/42	LH: 27/42 (min. 1 occasion)	—	—
Studies including patients treated with different kinds of conditioning regimens (chemotherapy only, low-dose irradiation, TBI-based)												
Cohen et al. [42] Retrospective Single-center	15	Malignant and non-malignant	Allogeneic	Partially	-Chemotherapy only - TBI-based	—	—	—	—	—	Azoospermia: 9/15	—
Sanders et al. [51] Retrospective Single-center	66	Malignant	—	Partially	-Chemotherapy only - TBI-based	—	—	—	—	—	Azoospermia: 11/13	—
Couto-Silva et al. [48] Retrospective Single-center	28	Malignant and non-malignant	Autologous and allogeneic	Partially	- Low-dose irradiation -TBI based	14-20.6 y	—	—	TRT: 3/28	LH: 7/25	—	FSH or testis vol.: 17/25
Ishiguro et al. [33] Retrospective Single-center	30	Malignant and non-malignant	Allogeneic	No	-Chemotherapy only - Low-dose irradiation - TBI-based	15.8-29.6 y	Induced: 0/30	—	—	LH: 7/30	—	FSH: 18/30 Testis vol.: 21/30
Ishiguro et al. [34] Retrospective Single-center	39	Malignant and non-malignant	Allogeneic	Partially	- Chemotherapy only - Low-dose irradiation - TBI-based	>15 y	Induced: 0/39	—	—	—	—	—
Jung et al. [35] Retrospective Single-center	28	Malignant and non-malignant	Autologous and allogeneic	No	- Non-TBI regimen - TBI-based	≥ 14 y	—	Precocious: 0/28	—	LH: 5/28 ‡	—	FSH: 16/28 ‡
Laporte et al. [36] Retrospective Single-center	38	Malignant and non-malignant	Autologous and allogeneic	Partially	- Chemotherapy only - Low-dose irradiation - TBI-based	13.2-21.3 y	—	—	TRT: 3/38	LH: 9/35	—	FSH: 25/35 Inhibin B: 28/38 (incl. 3 TRT)
Hyodo et al. [54] Retrospective Single-center	34	Malignant and non-malignant	Allogeneic	Partially	- Chemotherapy only - Low-dose irradiation - TBI-based	18.0-36.0 y	Induced: 0/34	—	—	—	—	—

(*continued*)

Wilhelmsson et al. [43] Retrospective Multicenter	106	Malignant and non-malignant	Allogeneic	Partially	- Chemotherapy only - Low-dose irradiation - TBI-based	12-42 y	Induced: 14/82	Precocious: 0/82	TRT: 28/106	—	Azoospermia: 21/31	—
Shalitin et al. [39] Retrospective Single-center	79	Malignant and non-malignant	Autologous and allogeneic	Partially	- Chemotherapy only - Low-dose irradiation - TBI-based	Pubertal or ≥ 14 y	—	Precocious: 4/79	TRT: 10/79	—	—	—
Mathiesen et al. [45] Cross-sectional Multicenter	98	Malignant and non-malignant	Allogeneic	Yes	- Chemotherapy only - Low-dose irradiation - TBI-based	18.5-47.0 y	Induced: 15/71	—	TRT: 24/98	LH: 27/74	Azoospermia: 65/95 (±TRT)	FSH: 33/74 Inhibin B: 44/74 Testis vol.: 28/74
Borgström et al. [46] Prospective Single-center	16	Malignant and non-malignant	Autologous and allogeneic	Partially	- Chemotherapy only - TBI-based	12-25 y	Induced: 0/9	Precocious: 0/9 Delayed: 0/9	TRT: 2/16	LH: 2/14	Azoospermia: 4/6	FSH: 7/14 Inhibin B: 3/9 Testis vol.: 8/13
Weinhard et al. [47] Retrospective Single-center	46	Malignant and non-malignant	Allogeneic	No	- Chemotherapy only - TBI-based	17-47	Induced: 5/46	Delayed: 6/41	TRT: 11/46	LH: 2/35	—	FSH or testis vol.: 21/35

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; fTBI, fractionated TBI; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; SAA, severe aplastic anemia; SD, standard deviation; T, testosterone; vol., volume.

Studies are sorted according to conditioning regimens included in the study. Induction of puberty is reported for patients being prepubertal at HSCT. Timing of puberty (precocious/delayed) is reported for patients with spontaneous onset of puberty. LH, FSH, and inhibin B levels as well as testicular volumes are reported for patients without TRT, unless otherwise specified. Accordingly, n/N refers to number of patients with an abnormal outcome out of number of patients eligible for evaluation. FSH cut-off values defined by the authors of the studies varied between 6 and 20 IU/L, LH cut-off levels varied between 5 and 15 IU/L, and testicular volume cut-off levels varied between 10 and 15 mL.

* Age range at follow-up is reported for the total study population, as age range for the eligible patient was not extractable.

† Age range at follow-up is estimated as mean age at follow-up ± 2*SD.

‡ Elevated LH and FSH levels were reported as 17.9% and 57.1% of 28 males, respectively.

levels of LH and testosterone (at peripubertal ages) between patients treated with busulfan-cyclophosphamide (BuCy) and fludarabine-melphalan (FluMel) conditioning and found no difference [37].

Detectable sperm was reported in 27 of 41 patients [42,43,45,46]. Regarding surrogate markers of spermatogenesis (reported for 78 patients), elevated FSH levels were common, but the proportion of patients with elevated FSH levels varied across studies (20%-85%), probably due to small sample sizes (range 5-47) and differences in FSH cut-off levels (range 6-20 IU/L), Supplemental Material 6 [30,33,36,37,41,46]. The largest study (n = 47) found elevated FSH levels in one third of patients and no differences in FSH levels or testicular volumes (at peripubertal ages) between patients treated with BuCy compared with FluMel conditioning [37]. A total of four males were reported to have fathered children [37,43,45].

Chemotherapy and low-dose testicular irradiation (TBI 2-3 Gy, TLI 5-6 Gy, TAI 3-10 Gy with gonadal shielding, or TBI 10-12 Gy with gonadal shielding)

Pubertal development was addressed in one study only, reporting spontaneous onset of puberty in 9/9 patients.[33] Leydig cell function at last follow-up was reported for 42 patients, of which only one case of TRT and two cases of elevated LH were reported [32,33,48].

Detectable sperm was reported in 10 of 13 patients in this treatment group [32,45]. Regarding surrogate markers of spermatogenesis (reported for 31 patients), elevated FSH levels, small testicular volumes, or both were found in one fourth to two thirds of these patients, depending on the chosen FSH cut-off level (range 9-20 IU/L); however, sample sizes were very small (3-11 patients; Supplemental Material 6) [32,33,36,48]. A total of five males were reported to have fathered children [32,33,45].

Chemotherapy and TBI (7-15 Gy) without additional testicular irradiation

Onset of puberty was reported for 40 patients with spontaneous onset in all [31,33,46,53]. Regarding Leydig cell function at last follow-up (reported for 168 patients), the proportion of patients treated with TRT varied substantially between studies from less than 10% to two-thirds of patients, based on small sample sizes (6-38 patients; Supplemental Material 6) [31,33,40,44-46,48,49,53]. One study found an increasing proportion of patients treated with TRT with time from onset of puberty, reaching 50% at last-follow-up (median 19 years after HSCT) [44], whereas another study with the similar follow-up time reported TRT in only 13% of the patients [45]. Studies with much shorter follow-up time (age range 14-23 years at last follow-up) reported TRT in few patients only [31,48,49]. Compensated Leydig cell dysfunction was found in more than one third of patients in this treatment group (Supplemental Material 6) [31,33,48,49].

Azoospermia was reported in 63 of 75 patients [40,42,43,45,46,52]. Regarding surrogate markers of spermatogenesis (reported for 151 patients), most patients had elevated FSH levels (above 9-20 IU/L) [31,33,36,40,46]. Reduced testicular volumes (< 10 mL) were reported in two studies with very different results (37% versus 94% of patients), even though TBI doses, TBI fractions, and ages at follow-up, were comparable [31,33]. Two studies (same author) combined increased FSH levels and low testicular volumes to identify “tubular failure,” which was reported in more than 75% of the patients [48,49]. A total of six males were reported to have fathered children [43,45,51].

Chemotherapy and TBI (9-15 Gy) plus a testicular boost (3-4 Gy) at HSCT

Only one study addressed onset of puberty and found spontaneous onset in all 14 patients [38]. Regarding Leydig cell function at last follow-up (reported for 41 patients), the proportion of patients treated with TRT differed substantially between studies, with the lowest proportion (7%) reported in the study with short follow-up (maximum age 17 years at last follow-up) [38], and the highest proportions (54% and 93%, respectively) reported in the studies with longer follow-up (median 18 and 19 years after HSCT) [44,45].

Azoospermia was reported in 13/13 patients [45]. Regarding surrogate markers of spermatogenesis (reported for 28 patients), one study found increased FSH levels (>8 IU/L) among two thirds (9/14) of males [38], whereas the other study with longer follow-up found high FSH levels (range 24.3-60.6 IU/L) among all males (n = 14) [44]. No data on paternity were available.

Chemotherapy and TBI conditioning (7.5-15 Gy) plus testicular irradiation as part of front-line treatment (10-32 Gy)

Pubertal development, reported for six patients, was medically induced in all but one patient [31,38,53]. Regarding Leydig cell function at last follow-up (reported for 26 patients), all but one eventually needed TRT [31,38,40,45,53]. Azoospermia was reported for 10 of 10 patients [45]. No additional data regarding germ cell function were reported.

RISK FACTORS FOR GONADAL DYSFUNCTION

An overview of the risk factors investigated in the eligible studies and related results is presented in Table 3.

Risk factors associated with Leydig cell dysfunction

Risk factors associated with Leydig cell dysfunction were investigated in eight studies [33,37,38,43-45,48,54] Regarding gonadotoxic therapy, the cumulative cyclophosphamide equivalent dose (CED), representing total exposure to alkylating chemotherapy, was not associated with risk of TRT [45]. Likewise, BuCy and FluMel conditioning did not appear to differ in their impact on levels of LH and testosterone [37]. Evidence regarding the impact of TBI conditioning on Leydig cell function was conflicting [33,43,45,48,54]. Testicular irradiation in addition to TBI appeared to be associated with an increased risk of TRT [43,45] and risk of no spontaneous puberty [43]. One study investigated the cumulative testicular irradiation dose (pre-HSCT plus conditioning doses) and found an increased risk of TRT with increasing doses [45]. Evidence regarding the impact of cranial irradiation in addition to TBI was limited and furthermore conflicting [43,45]. Of patient-related factors, prepubertal stage at HSCT was associated with increased risk of TRT in two studies [43,45], whereas the evidence was conflicting regarding the impact of age at HSCT [33,38,45,48]. Diagnosis, age at assessment, and time from HSCT did not seem to influence Leydig cell function [33,43,45], nor did the presence of chronic graft-versus-host disease (GvHD) [43,45].

Risk factors associated with germ cell failure

Risk factors associated with impaired spermatogenesis (evaluated by semen samples or surrogate markers) were investigated in eight studies [33,36,37,43-45,48,54]. Higher cumulative CED was associated with increased risk of azoospermia in patients treated with chemotherapy only [45], but no difference in FSH and testicular volumes was found in

Table 3
Potential Risk Factors for Male Gonadal Dysfunction After Pediatric HSCT Investigated in the Included Studies

	Confirmed	Not confirmed
Leydig cell compartment		
Gonadotoxic therapy		
Alkylating agents	–	No association with risk of testosterone substitution (cumulative cyclophosphamide equivalent dose) (Mathiesen [45])
BuCy versus FluMel	–	No difference in LH or testosterone levels between groups (Panasiuk [37])
TBI conditioning (single dose or fractionated)	Associated with increased risk of testosterone substitution; adjusted for pubertal stage at HSCT, testicular and cranial irradiation for leukemia, diagnosis, and no spontaneous puberty (Wilhelmsson [43]) Associated with reduced testosterone/LH ratio compared to TAI conditioning (Ishiguro [33]) Associated with higher LH levels compared to TAI or chemotherapy only conditioning (Hyodo [54])	No association with risk of testosterone substitution (Mathiesen [45]) No difference in testosterone levels compared to TAI or chemotherapy only conditioning (Hyodo [54]) No difference in testosterone/LH ratio compared to chemotherapy only conditioning (Ishiguro [33]) No difference in LH levels compared to TLI conditioning (Couto-Silva [48]) No difference in LH levels compared to non-TBI conditioning (Wilhelmsson [43])
Testicular irradiation in addition to TBI	Associated with increased risk of testosterone substitution; adjusted for TBI, pubertal stage at HSCT, cranial irradiation for leukemia, diagnosis, and no spontaneous puberty (Wilhelmsson [43]) Associated with increased risk of testosterone substitution (Mathiesen [45]) Associated with risk of non-spontaneous puberty (Wilhelmsson [43]) Associated with higher LH levels (Taneja [44])	No differences in LH levels (Wilhelmsson [43])
Cumulative testicular irradiation dose (pre-HSCT plus HSCT doses)	Associated with increased risk of testosterone substitution; adjusted for time from HSCT and pubertal stage at HSCT (Mathiesen [45])	–
Cranial irradiation in addition to TBI	Associated with increased risk of testosterone substitution (Wilhelmsson [43])	No association with risk of testosterone substitution (Mathiesen [45]) No difference in LH levels compared to no additional cranial irradiation (Wilhelmsson [43])
Patient-related factors		
Leukemia diagnosis	–	No associations with risk of testosterone; adjusted for the cumulative testicular irradiation dose (Mathiesen [45]) No association between primary disease and testosterone levels (Ishiguro [33]). No differences in LH levels according to diagnosis (Wilhelmsson [43])
Prepubertal stage at HSCT	Associated with increased risk of testosterone substitution; adjusted for TBI, testicular and CNS irradiation for leukemia, diagnosis, and no spontaneous puberty (Wilhelmsson [43]) Associated with increased risk of testosterone substitution; adjusted for cumulative testicular irradiation dose and time from HSCT (Mathiesen [45])	No differences in LH levels according to pubertal stage at HSCT (Wilhelmsson [43])
Younger age at HSCT	Associated with higher LH levels (Sarafoglou [38])	No association with risk of testosterone substitution (Mathiesen [45]) No association with testosterone levels (Ishiguro [33]) No correlation with LH or testosterone levels (Couto-Silva [48])
Age at evaluation	–	No association with risk of testosterone substitution (Mathiesen [45])
Longer time from HSCT	–	No association with risk of testosterone substitution; adjusted for cumulative testicular irradiation dose and pubertal stage at HSCT (Mathiesen [45])
Chronic GvHD	–	No association with risk of testosterone substitution (Mathiesen [45]) No difference in LH levels according to chronic GvHD (yes/no) (Wilhelmsson [43])
Fatty liver	Associated with lower testosterone levels (Hyodo [54])	–
Ferritin level	–	No association with risk of testosterone substitution (Mathiesen [45])

(*continued*)

Germ cell compartment		
Gonadotoxic therapy		
Alkylating agents	Cumulative cyclophosphamide equivalent dose associated with increased risk of azoospermia in patients treated with chemotherapy only (Mathiesen [45])	No association with risk of azoospermia in the total population (Mathiesen [45])
BuCy versus FluMel	—	No difference in FSH levels or testicular volumes between groups (Panasiuk [37])
TBI conditioning (single dose or fractionated)	Associated with increased risk of azoospermia (Mathiesen [45]) Associated with smaller testicular volume; adjusted for pubertal stage at HSCT, and single versus fractionated doses (Wilhelmsson [43]) Associated with smaller testicular volumes compared to TLI conditioning (Couto-Silva [48]). Associated with smaller testicular volumes and higher FSH levels compared to TAI and chemotherapy only conditioning (Ishiguro [33]) Associated with higher FSH levels compared with non-TBI conditioning (Wilhelmsson [43]) Associated with higher FSH levels compared to TAI or chemotherapy only conditioning (Hyodo [54])	No difference in percentage of patients with elevated FSH levels compare to TLI conditioning (Couto-Silva [48])
Testicular irradiation in addition to TBI	Associated with increased risk of azoospermia (all patients had azoospermia) (Mathiesen [45])	No differences in testicular volume compared to no add. testicular irradiation (Wilhelmsson [43]) No difference in FSH levels compared to TBI without testicular boost (Taneja [44]) No differences in FSH levels compared to no add. testicular irradiation (Wilhelmsson [43])
Cumulative testicular irradiation dose (pre-HSCT plus HSCT doses)	Associated with increased risk of azoospermia; adjusted for time from HSCT, pubertal stage at HSCT, and ferritin level (Mathiesen [45])	—
Cranial irradiation in addition to TBI	—	No difference in testicular volume compared to no add. cranial irradiation (Wilhelmsson [43]) No differences in FSH levels compared to no add. cranial irradiation (Wilhelmsson [43])
Patient related factors		
Leukemia diagnosis	Associated with increased risk of azoospermia; adjusted for testicular volume, TBI and FSH (Wilhelmsson [43]) Associated with smaller testicular volume (Wilhelmsson [43]) Associated with higher FSH levels (Wilhelmsson [43])	No associations with risk of azoospermia; adjusted for the cumulative testicular irradiation dose (Mathiesen [45])
Prepubertal stage at HSCT	Associated with smaller testicular volume; adjusted for TBI and single versus fractionated dose (Wilhelmsson [43])	No association with risk of azoospermia; adjusted for cumulative testicular irradiation dose, time from HSCT and ferritin level (Mathiesen [45]) No association with spermatogenic status (detectable sperm versus azoospermia) (Wilhelmsson [43]) No difference in FSH level in relation to pubertal status at HSCT (Wilhelmsson [43])
Younger age at HSCT	—	No association with risk of azoospermia (Mathiesen [45]) No association with spermatogenic status (detectable sperm versus azoospermia) (Wilhelmsson [43]) No correlation with FSH levels (Couto-Silva [48]) No correlation with inhibin B levels (Laporte [36])
Age at evaluation	—	No association with risk of azoospermia (Mathiesen [45]) No association with spermatogenic status (detectable sperm versus azoospermia) (Wilhelmsson [43])
Longer time from HSCT	—	No association with risk of azoospermia; adjusted for cumulative testicular irradiation dose, pubertal stage at HSCT and ferritin level (Mathiesen [45]) No association with spermatogenic status (detectable sperm versus azoospermia) (Wilhelmsson [43]) No correlation with inhibin B levels (Laporte [36])
Chronic GvHD	—	No association with risk of azoospermia (Mathiesen [45]) No association with spermatogenic status (detectable sperm versus azoospermia) (Wilhelmsson [43]) No difference in testicular volume according to chronic GvHD (yes/no) (Wilhelmsson [43]) No difference in FSH levels according to chronic GvHD (yes/no) (Wilhelmsson [43])
Ferritin level	Associated with increased risk of azoospermia; adjusted for cumulative testicular irradiation dose, time from HSCT and pubertal stage at HSCT (Mathiesen [45])	—

add. indicates additional (to TBI).
—Not available
Multivariable analyses are indicated by “adjusted for.”

patients treated with BuCy compared with FluMel conditioning [37]. TBI conditioning was consistently associated with increased risk of azoospermia [45], smaller testicular volumes [33,43,48], and higher FSH levels [33,43,54] compared with non-TBI regimens. Testicular irradiation in addition to TBI led to azoospermia in all patients [45], but no further impact on FSH levels or testicular volumes was found when compared with patients treated with TBI *without* additional testicular irradiation [43,44]. One study investigated the cumulative testicular irradiation dose (pre-HSCT plus conditioning doses) and reported an increased risk of azoospermia with increasing doses of testicular irradiation [45]. Cranial irradiation in addition to TBI did not seem to exacerbate the impact on germ cell function, although only investigated in one study [43].

Regarding patient-related factors, prepubertal stage at HSCT was not associated with increased risk of azoospermia [43,45] but with smaller testicular volumes at follow-up [43]. Leukemic disease was associated with an increased risk of azoospermia when adjusted for TBI (yes/no) in one study [43], whereas another study found no increased risk of azoospermia in relation to diagnosis when adjusting for the cumulative testicular irradiation dose [45].

One study found higher ferritin levels to be associated with increased risk of azoospermia after pediatric HSCT [45]. Age at HSCT [36,43,45,48], age at assessment [43,45], or time from HSCT [36,43,45] was not associated with germ cell function, nor was presence of chronic GvHD [43,45].

DIAGNOSTIC VALUE OF SURROGATE MARKERS OF SPERMATOGENESIS

Two studies addressed the diagnostic value of surrogate markers of spermatogenesis in patients who were postpubertal at follow-up [43,45]. Both studies found testicular volume to have reliable diagnostic value (Table 4). Regarding FSH, one study reported a low sensitivity (56%) for identification of patients with active spermatogenesis [43], whereas the other study reported high sensitivity and specificity (>80% for both) for identifying patients with azoospermia [45]. Inhibin B was evaluated in one study only and was found to be the strongest marker of azoospermia when compared with FSH and testicular volume [45] (Table 4).

DISCUSSION

In this first study to systematically review the literature regarding gonadal dysfunction in males treated with HSCT during childhood, some common finding were identified across studies despite substantial heterogeneity regarding design (diagnoses, type of transplant, age/pubertal stage at follow-up), gonadotoxic exposure (front-line treatment and conditioning), and definitions of gonadal dysfunction. Among patients treated with chemotherapy only or low-dose irradiation regimens, the Leydig cell function was usually preserved, whereas the germ cell compartment appeared affected in about half of these patients [30,32,33,36,37,41-43,45,46,48]. Among patients receiving full-dose TBI conditioning, signs of germ cell failure were found in most [31,33,36,40,42-44,46,48,49,52]; nevertheless, one study reported detectable sperm in one fifth of these patients at long-term follow-up and cases of paternity were reported [45]. The impact of full-dose TBI conditioning on Leydig cell function appears more complex as most patients experienced spontaneous onset of puberty, and only few patients needed TRT in adolescence [31,33,40,46,48,49,53], whereas evidence regarding need for TRT in adulthood was conflicting [44,45]. In contrast, complete Leydig cell failure and germ cell failure appear to be almost inevitable in patients treated with testicular irradiation as part of front-line therapy before HSCT [31,38,40,44,45,53]. In summary, these findings support the general understanding that the risk of gonadal dysfunction increases with increasing treatment intensity and that the germ cell compartment is more sensitive to gonadotoxic therapy than the Leydig cell compartment.

Studies investigating patients conditioned with chemotherapy only were sparse and did not allow for conclusions regarding the gonadotoxic effects of different types of regimens. Likewise, only one of the included studies reported specifically on the gonadotoxic effects of RIC regimens [37]. Thus gonadotoxic effects of chemotherapy only regimens, as well as RIC regimens, should be a focus in future studies. Furthermore, TBI conditioning continues to play a key role in successful transplantation of children with acute lymphoblastic leukemia, as implied by the recent results from the FORUM study [55]. Currently available evidence regarding the impact of TBI on Leydig cell function in adulthood was conflicting, and we were not able to conclude on the gonadotoxic effects of different TBI doses, fractions, or effects of testicular shielding; thus these aspects merit further investigation in future studies.

Several potential confounders need consideration when evaluating gonadal dysfunction after pediatric HSCT; however, it is evident that our understanding of these remains limited. Some evidence suggested pubertal stage at HSCT to be an important factor [43,45]. Although the male hypothalamic-pituitary-gonadal axis is quiescent in the prepubertal years, the testicular tissue is highly vulnerable to gonadotoxic therapy [56–58]. Studies of childhood leukemia survivors have indicated that prepubertal Leydig cells are more sensitive to irradiation than mature Leydig cells [59,60], supporting the finding that prepubertal stage at HSCT is associated with increased risk of testosterone substitution in adulthood [43,45]. Similarly, a study of non-human primates reported that testicular irradiation was more detrimental to the germinal epithelium if performed before rather than during puberty [61], in line with the finding of smaller testicular volumes in patients transplanted at prepubertal stage compared with pubertal/postpubertal stage [43]. Furthermore, a large study (n = 206 males) of pediatric and adult HSCT survivors (not

Table 4
Diagnostic Value of Surrogate Markers of Spermatogenesis

Predictor	Study	Population	Outcome	AUC	Cut-off level	Sensitivity	Specificity
FSH	Wilhelmsson et al. [43]	Postpubertal, n = 31	Detectable spermatozoa	0.79	10 IU/L	56%	81%
FSH	Mathiesen et al. [45]	Postpubertal, n = 72	Azoospermia	0.88	9.8 IU/L	83%	83%
Mean testicular volume	Wilhelmsson et al. [43]	Postpubertal, n = 30	Detectable spermatozoa	0.89	15 mL	80%	91%
Mean testicular volume	Mathiesen et al. [45]	Postpubertal, n = 72	Azoospermia	0.83	15 mL	79%	80%
Inhibin B	Mathiesen et al. [45]	Postpubertal, n = 72	Azoospermia	0.91	51 ng/l	90%	83%

AUC indicates area under the curve.

eligible for this review) suggested age less than 13 years at HSCT to increase risk of infertility [62]. Taken together, this evidence emphasizes the need for fertility preservation methods available for prepubertal males, as well as close clinical follow-up during puberty and adulthood.

Besides pubertal stage at HSCT, the underlying disease may also be an important confounder. In clinical studies the effect of diagnosis is investigated retrospectively, thereby confounded by the treatment given, particularly in patients with leukemia treated with gonadotoxic chemoradiotherapy at front-line and during conditioning. In addition, some nonmalignant diseases, such as thalassemia, require frequent blood transfusions, which comprises a risk of transfusional hemosiderosis, potentially affecting the pituitary gland and the testicular tissue [63,64]. Ideally, to explore effect of diagnosis, gonadal function should be evaluated at time of diagnosis. In prepubertal boys, an option could be to investigate testicular biopsies undertaken for fertility preservation, although these should be taken before any gonadotoxic therapy [65].

Last, chronic GvHD may exert an impact on gonadal function. The studies included in this review found no such effect [43,45], whereas studies among mixed pediatric-adult HSCT cohorts have reported conflicting results [66–68]. A possible pathogenic link between chronic GvHD and testicular dysfunction needs further investigation, including effects of immunosuppressive therapy in addition to potential direct impact by inflammatory and fibrotic processes.

As for the third focus of this review, the diagnostic value of surrogate markers of spermatogenesis, two studies evaluated this during adulthood and found a reliable diagnostic value of testicular volumes, conflicting results regarding FSH, and high diagnostic value for inhibin B [43,45]. In these studies, several patients had been exposed to cranial irradiation, which may lead to hypothalamic-pituitary dysfunction and a blunted gonadotropin response [69] (i.e., the germinal epithelium may be severely affected despite normal/subnormal FSH levels), thus theoretically compromising the use of gonadotropins as surrogate markers of testicular function in these specific patients. Furthermore, a larger study of male childhood cancer survivors (n = 275) have reported poor specificity and positive predictive value of both FSH and inhibin B for identifying patients with azoospermia [10]. Finally, none of the studies included in this review addressed the diagnostic or predictive value of these markers when evaluated during the pubertal years, although these markers were frequently applied in peripubertal patients Taken together, we conclude that semen sample analysis is needed to sufficiently evaluate the patients' fertility potential in future studies and at clinical follow-up.

The systematic approach in this literature review revealed three main areas of methodological shortcomings that need to be considered in future studies. First, lack of information on pre-HSCT exposure to gonadotoxic therapy was a main area of concern. Information on dose-specific exposure to chemotherapy, gonadal irradiation, and cranial irradiation is necessary to identify and compare gonadotoxic effects of different conditioning regimens and to identify gonadotoxic threshold doses. For instance, alkylating chemotherapy is highly gonadotoxic and widely used in the front-line treatment of hematological cancers, as well as in the conditioning before HSCT [13,58]. The cumulative exposure to alkylating agents can be quantified by the CED, enabling comparisons across different chemotherapy regimens [70]. One of the studies included in this review revealed an increased risk of azoospermia with increasing doses of cumulative CED in patients treated with chemotherapy only [45], as previously shown in non-HSCT populations of childhood cancer survivors [71–73]. In contrast, the only study included that compared the gonadotoxic effects of different chemotherapy conditioning (BuCy versus FluMel) could not reveal any differences between the two regimens [37]. The cumulative CED for each of the two regimens was, however, not reported, and a significantly higher proportion of patients in the BuCy group were treated for acute myeloid leukemia (as opposed to nonmalignant diagnoses); that is, these patients had most likely been exposed to potentially gonadotoxic chemotherapy before conditioning, which was not accounted for in the analyses.

Second, direct evaluation of spermatogenic capacity was generally lacking. Only one study systematically evaluated semen samples [45], and only two studies had enough data on semen quality to statistically investigate risk factors for azoospermia [43,45]. The remaining studies relied on surrogate markers of spermatogenesis, including FSH, inhibin B, and testicular volume, although the diagnostic value of these markers is not sufficiently documented in this specific patient population, as discussed above. In addition, the cut-off levels for testicular volume and reproductive hormone levels differed substantially between studies, compromising direct comparison across studies. This challenge could be solved if standard deviation scores are reported. Furthermore, future studies should be aware of the effects of TRT on markers of gonadal function (i.e., higher testosterone levels, suppression of gonadotropin levels, risk of azoospermia, and smaller testicular volumes) [74,75].

Last, several studies included a mix of peripubertal and postpubertal patients at follow-up without taking pubertal stage into account when evaluating gonadal function. During puberty, gonadotropin levels and testicular volumes are still developing with large individual variation in timing [27,76], thus possibly limiting the conclusions regarding gonadal function when simple (adult) cut-off levels of gonadotropins and testicular volumes are applied. Accordingly, the inclusion of peripubertal patients requires a suitable reference population and preferably also longitudinal assessment to be able to determine whether pubertal development differs from healthy boys or between treatment regimens. For instance, in the study comparing gonadal function after BuCy versus FluMel conditioning, the patients were at peripubertal ages at last follow-up, and the patients treated with FluMel were significantly younger at evaluation than patients treated with BuCy, thereby hindering direct comparison of the reproductive hormone levels between the two groups [37]. Based on these observations, we present suggestions for methodological considerations (Table 5) and study reporting items (Table 6) for future use to improve comparability across studies and thereby facilitate more robust evidence that ultimately can guide novel treatment strategies.

To our knowledge, this is the first systematic review to address male gonadal function after pediatric HSCT. By specifying the scientific questions in advance and applying a systematic approach by explicit methods, the evidence was presented in a transparent manner, thereby minimizing the risk of study selection bias and reporting bias (as opposed to narrative reviews). Nevertheless, there are several limitations. The heterogeneity and quality of the included studies limits the conclusions in this review, as discussed above. A narrowing of the inclusion criteria could have enhanced comparability between studies; however, such an approach would have resulted in very few eligible studies. Instead, the broad design revealed some methodological shortcomings

Table 5
Methodological Considerations for Future Studies of Male Gonadal Function After Pediatric HSCT

Considerations regarding study design	
Sample size	Consider multicenter studies to increase study power
HSCT population	Consider homogeneous subpopulation according to study aim (diagnosis, pre-HSCT treatment, conditioning regimen, pubertal stage at HSCT, pubertal stage at follow-up, etc.)
Pre-HSCT exposure and conditioning regimens	Consider using cumulative doses of: - Testicular irradiation - CNS irradiation - Chemotherapy (especially alkylating agents and preferable by equivalent doses)
Pubertal stage at follow-up	Consider including patients who are postpubertal at last follow-up, otherwise adjust for pubertal stage at follow-up in the analyses
Considerations regarding outcomes	
Pubertal development	Report separately for patients who are prepubertal, peripubertal and postpubertal at HSCT Onset of puberty: Evaluate by Tanner stage and reproductive hormones (not testicular volumes because of risk of persistent testis atrophy) Pubertal progression: Evaluate by Tanner stage and conclude only when puberty is completed
Testicular volumes	Measure by orchidometer or ultrasound (be aware of interobservational reliability) Reference material should match pubertal stage Report separately for patients on TRT*
Reproductive hormone measurements	Morning levels preferred and preferably repeated Reference material should match pubertal stage Consider reporting standard deviation scores Report separately for patients on TRT*
Semen samples	Sampling after sexual abstinence of 2-7 days Preferably repeated as sperm counts vary Consider using WHO reference limits to enhance comparability between studies Report separately for patients on TRT*
Surrogate markers of spermatogenesis	Diagnostic value may be limited in: - Peripubertal patients - Patient treated with TRT - Patients exposed to cranial irradiation prior to HSCT
Paternity	Include information on: - Wish of parenthood - Attempts to conceive - Natural versus assisted reproduction

CNS indicates central nervous system; WHO, World Health Organization.
* Because of effects on testosterone levels, gonadotropins, testicular volume, and risk of azoospermia.

Table 6
Suggested Reporting Items for Studies of Male Gonadal Function After Pediatric HSCT

	Reporting items
Study design	Retrospective, cross-sectional, prospective, etc.
	Single-center/multicenter
	Derivation of study cohort (representativeness)
	Transplantation period
Study population	Diagnoses
	Complete remissions status at HSCT
	Age at HSCT
	Pubertal stage at HSCT
	Age at time of study
	Pubertal stage at time of study
	Time from HSCT
Treatment characteristics	Allogeneic and/or autologous HSCT
	Pre-HSCT therapy: - Chemotherapy (doses) - Irradiation (type, dose, and fractions) If no pre-HSCT therapy was given, then state this.
	Donor match
	Stem cell source
	Conditioning regimens: - Chemotherapy (doses) - Irradiation (type, dose, and fractions)
	Orchiectomy (at any timepoint)
	Acute and chronic GvHD (and the methods of assessment)
Outcomes	Definition of each outcome
	Definition of abnormal results for each outcome
	Ascertainment of each outcome (e.g., directly measured, medical records, self-report)
	Methods of assessments for each outcome
	Reference material for each outcome
Statistics	In statistical analyses, consider adjustment for/stratification by: - Diagnosis - Pubertal stage at HSCT - Pubertal stage at time of study - Age at time of study - Time from HSCT - Cumulative gonadotoxic exposure/treatment groups
Results	Comparison of participants and non-participants regarding key patient and transplant characteristics (representativeness)
	Report outcomes separately for patients receiving TRT (hormone levels, testicular volumes, and semen samples)

that need to be considered when performing research in this specific field.

To conclude, studies of male gonadal function after pediatric HSCT are numerous, but the evidence is limited by heterogeneity across designs, as well as by methodological shortcomings. However, the current evidence indicates an increased risk of gonadal dysfunction with increasing intensity of treatment, providing an opportunity for more differentiated information to the patients and their parents prior to HSCT. Furthermore, the evidence indicates a need for fertility preservation, as well as systematic and prolonged follow-up of gonadal function after pediatric HSCT. Future studies should prioritize multicenter design, include the cumulative exposure to gonadotoxic therapy, extend follow-up into late adulthood, and include semen quality analyses.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.05.036.

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