Evidence tables from the systematic literature search for gonadotoxicity surveillance for male CAYA cancer survivors

Impaired spermatogenesis – Who needs surveillance?				
Lopez Andreu et al. Persistent altered spermatogenesis in long-term childhood cancer survivors. Pediatr Hematol Oncol 2000;17:21-30				
Study design Treatment era Years of follow-up	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
Single-centre cohort study Treatment era not mentioned Follow-up: Mean 13.6 (3.9-25.2) years after treatment	43 male CCS >16 years of age at follow-up Diagnoses: ALL (n=21), AML (n=1), NB (n=8), GNB (n=1), GN (n=2), Wilms' tumour (n=9), mesoblastic nephroma (n=1) Age at diagnosis: Range 0.0-9.4 years Age at follow-up: Mean 20.2 (16.1-30.6) years Controls: 15 healthy volunteers aged ≤30 years; 373 patients aged ≤30 years consulting for infertility	Cranial irradiation: 14 (32.6%); 18-46 Gy Spinal irradiation: 3 (7.0%); 20-24 Gy (also treated with cranial radiation) Testicular irradiation: 1 (2.3%); 30 Gy (also treated with cranial and spinal radiation) Abdominal irradiation: 2 (4.7%); 30 Gy Cyclophosphamide: 9 (20.9%); 1200-27,200 mg/m^2	Outcome definitions: - Moderate oligozoospermia: sperm cell density 10-19 x 10^6/mL - Severe oligozoospermia: sperm cell density <10 x 10^6/mL - Moderate asthenozoospermia: 20-39% progressive motility - Severe asthenozoospermia: <20% progressive motility - Infertile: azoospermia or severe oligo-asthenozoospermia Spermatogenesis: - Azoospermia: 8 (18.6%) - Moderate oligozoospermia: 1 (2.3%) - Severe oligozoospermia: 0 (0.0%) - Moderate asthenozoospermia: 8 (18.6%) - Severe asthenozoospermia: 3 (7.0%) - Moderate oligo-asthenozoospermia: 2 (4.6%) - Severe oligo-asthenozoospermia: 2 (4.6%) - Moderate oligozoospermia + severe asthenozoospermia: 3 (7.0%) - Infertile: 10 (23.2 %) Risk factors for infertility in multivariable analyses: - Cumulative cyclophosphamide dose significant (no effect measure reported) - FSH level significant (no effect measure and p-value reported)	90% of eligible survivors completed follow-up assessment. A forward-stepping logistic regression analysis was performed. In addition to cumulative cyclophosphamide dose, FSH level was included as an independent factor. Testicular volume was excluded from the model. Radiotherapy and follow-up duration were not included in the model. 1 survivor was on replacement therapy after 30 Gy testicular radiation and high-dose cyclophosphamide.

			Univariable results cyclophosphamide: - 5/9 (55.6%) treated with cyclophosphamide azoospermic and 1/9 (11.1%) severe oligozoospermic - Cumulative cyclophosphamide dose negatively correlated with sperm count: r=-0.43 (p=0.004) - Cumulative cyclophosphamide dose negatively correlated with sperm motility: r=-0.45 (p=0.002) Univariable results cranial radiotherapy: - No correlation with sperm count, sperm motility, testicular volume, FSH level	

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; FSH, follicle-stimulating hormone; GNB, ganglioneuroblastoma; GN, ganglioneuroma; NB, neuroblastoma.

Impaired spermatogenesis – Who needs surveillance?				
van Beek et al. Inhibin B is superior to FSH as a serum marker for spermatogenesis in men treated for Hodgkin lymphoma with chemotherapy during childhood. Human Reprod 2007;22:3215-3222				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
Multi-centre cohort study 1974-1998 <u>Follow-up:</u> Median 15.5 (5.6-30.2) years after treatment	56 male survivors of childhood Hodgkin lymphoma <u>Age at diagnosis:</u> Median 11.4 (3.7-15.9) years <u>Age at follow-up:</u> Median 27.0 (17.7-42.6) years	<u>ABVD or EBVD:</u> 56 (100%) Adriamycin 25mg/m^2 or epirubicin 30 mg/m^2, bleomycin 10 mg/m^2, vinblastine 6 mg/m^2, dacarbazine 250 mg/m^2 (days 1 and 8) <u>MOPP:</u> 40 (71.4%) of whom 14 (25.0%) 3-4 cycles 26 (46.4%) ≥6 cycles Mechlorethamine 6mg/m^2(days 1 and 8), vincristine 2 mg/m^2 (days 1 and 8), prednisone 40 mg/m^2/day (days 1 – 14), procarbazine 100 mg/m^2/day (days 1 – 14) <u>Involved field irradiation:</u> 7 (12.5%) <u>Abdominal irradiation:</u> 0 (0.0%) <u>Pelvic irradiation:</u> 0 (0.0%)	<u>Outcome definitions:</u> - Oligozoospermia: sperm cell density <20 x 10^6/mL - Severe oligozoospermia: sperm cell density <5 x 10^6/mL - Decreased inhibin B: <150 ng/L - Increased FSH: >7.0 U/L <u>Sperm concentration (n=21 assessed):</u> - MOPP$^-$: 49.1 (28-63) x 10^6/mL (n=4) - MOPP$^+$: 1.1 (0-72) x 10^6/mL (n=17) p<0.05 - MOPP$^-$: 0/4 (0.0%) azoospermia or oligozoospermia (all 4 normospermia) - MOPP$^+$: 9/17 (52.9%) azoospermia; 1/17 (5.9%) oligozoospermia; 3/17; (17.6%) severe oligozoospermia <u>Inhibin B (n=38 assessed):</u> - MOPP$^-$: 144.0 (93.0-274.0) ng/L (n=12) - MOPP$^+$: 16.5 (0.0-173.0) ng/L (n=26) p<0.01 <u>FSH (n=56 assessed):</u> - MOPP$^-$: 3.0 (1.7-6.0) U/L (n=16) - MOPP$^+$: 16.8 (1.3-51.0) U/L (n=40) p<0.01 <u>Risk factors for decreased sperm</u>	56 out of 100 (56%) eligible patients included in this study. There were no differences in age, disease characteristics and treatment between the included 56 male survivors and the 44 not included. Multiple linear regression analyses were performed, including number of MOPP cycles, age at diagnosis, number of EBVD/ABVD cycles, radiotherapy (mantle or mediastinal), puberty at diagnosis, presence of B-symptoms and follow-up duration. 3 men reported 5 spontaneously conceived pregnancies. 2 men treated without MOPP each fathered 1 child. 1 man treated with 6 MOPP cycles fathered 1 child and reported 2 spontaneous abortions.

			concentration in multivariable analysis: - Number of MOPP cycles: beta -6.25 ($p<0.05$) - Age at diagnosis: beta -6.18 ($p<0.05$) - Number of EBVD/ABVD cycles, radiotherapy (mantle or mediastinal), puberty at diagnosis, presence of B-symptoms and follow-up duration ($p>0.05$) (no effect measures reported) Risk factors for decreased inhibin B in multivariable analysis: - Number of MOPP cycles: beta -21.59 ($p<0.05$) - Other factors not significant Risk factors for increased FSH in multivariable analysis: - Age at diagnosis: beta 1.4 ($p<0.05$) - Number of MOPP cycles: beta 2.57 ($p<0.01$) - Other variables not significant	

Abbreviations: FSH, follicle-stimulating hormone.

Impaired spermatogenesis – Who needs surveillance?				
Green et al. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. Lancet Oncol 2014;15:1215-1223				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
Single-centre cohort study 1970-2002 Follow-up: Median 21.0 (10.5-41.6) years since diagnosis	214 adult male survivors of childhood cancer Diagnoses: ALL (n=70), AML (n=5), NHL (n=53), HL (n=2), NB (n=26), osteosarcoma (n=32), other (n=26) Age at diagnosis: Median 7.7 (0.01-20.3) years Age at follow-up: Median 29.0 (18.4-56.1) years	Alkylating agent chemotherapy: 214/214 (100%) Cyclophosphamide: 195/214 (91.1%) Ifosfamide: 26/214 (12.1%) Procarbazine: 2/214 (0.9%) Chlormethine (mechlorethamine): 1/214 (0.5%) Chlorambucil: 0/214 (0%) Busulfan: 3/214 (1.4%) Cyclophosphamide equivalent dose (CED): Median 7,400 (1,000-41,311) mg/m^2 Cisplatin/carboplatin: 44/214 (20.6%) Dacarbazine: 3/214 (1.4%) Radiotherapy: 0/214 (0%) Bilateral orchiectomy: 0/214 (0%)	Outcome definitions: - Oligospermia: sperm cell density >0 - <15 x 10^6/mL - Normospermia: sperm cell density ≥15 x 10^6/mL Spermatogenesis and CED: - Azoospermia: 53 (24.7%); mean CED 10,830 mg/m^2 - Oligospermia: 59 (27.6%); mean CED 8,480 mg/m^2 - Normospermia: 102 (47.7%); mean CED 6,626 mg/m^2 - Correlation CED and sperm concentration: r=-0.37 (p<0.0001) Risk factors for azoospermia as compared to normospermia in multinomial logistic regression analysis: - CED per 1,000 mg/m^2: OR 1.22; 95% CI 1.11-1.34 (p<0.0001) - Age at diagnosis per years: OR 0.97; 95% CI 0.91-1.05 (p=0.45) - Age at assessment per years: OR 0.99, 95% CI 0.94-1.05 (p=0.8) Risk factors for oligospermia as compared to normospermia in multinomial logistic regression analysis: - CED per 1,000 mg/m^2:	Of the 549 men eligible for the semen analysis project, 226 (41%) participated in a SJLIFE on-campus assessment and agreed to semen analysis; 12 were unable to produce a semen specimen, resulting in 214 assessable participants. The multinomial logistic regression analysis included age at diagnosis, age at assessment and CED. Unable to identify threshold dose (substantial overlap). Impaired spermatogenesis unlikely when CED <4000 mg/m^2.

			OR 1.14; 95% CI 1.04-1.25 (p=0.006) - Age at diagnosis per years: OR 0.95; 95% CI 0.89-1.02 (p=0.13) - Age at assessment per years: OR 0.97, 95% CI 0.92-1.03 (p=0.28)	

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CED, cyclophosphamide equivalent dose; HL, Hodgkin lymphoma; NB, neuroblastoma; NHL, non-Hodgkin lymphoma; OR, odds ratio; 95% CI, 95% confidence interval.

Impaired spermatogenesis – Who needs surveillance?				
Wilhelmsson et al. Adult testicular volume predicts spermatogenetic recovery after allogeneic HSCT in childhood and adolescence. Pediatr Blood Cancer 2014;61:1094-1100				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
Two-centre cohort study 1978-2000 Follow-up: Mean 13 (4-28) years since HSCT	106 male CCS who received allogeneic HSCT >5 years after treatment and reached puberty at follow-up Diagnoses: ALL (n=44), AML (n=20), SAA (n=17), other (n=25) Analysed as: Leukaemia (n=64) SAA (n=17) Others (n=25) Age at diagnosis: Mean 8.0 (1-17) years Age at follow-up: Mean 22 (12-42) years	sTBI + cyclophosphamide: 30 (28%) fTBI + cyclophosphamide: 20 (19%) fTBI + cyclophosphamide + etoposide: 2 (2%) fTBI + cytarabine: 16 (15%) fTBI + melphalan: 3 (3%) Busulfan: 3 (3%) Busulfan + cyclophosphamide: 15 (14%) Cyclophosphamide only: 12 (11%) Cyclophosphamide + total nodal irradiation (TNI): 5 (5%) CRT for leukaemia: 14 (22%) Testicular irradiation for leukaemia: 8 (12%) Dose: Cyclophosphamide 120 mg/kg (except SAA who had 200 mg/kg) TBI 10-12 Gy	Outcome definition: Azoospermia - no spermatozoa detected in any field of a double-chambered haemacytometer before or after centrifugation. Spermatogenesis (n=31 assessed): - Azoospermia: 21 (67.7%) - TBI: 20/24 (83.3%) azoospermia, 4/24 (16.7%) non-azoospermia - Cyclophosphamide-only: 0/3 (0%) azoospermia, 3/3 (100%) non-azoospermia - Busulfan-based: 1/4 (25%) azoospermia, 3/4 (75%) non-azoospermia - Leukaemia: 17/19 (89.5%) azoospermia, 2/19 non-azoospermia (10.5%) Predictors for active sperm production in multivariable logistic regression analysis: - No leukaemia diagnosis vs. leukaemia diagnosis: OR 19.8; 95% CI 1.9-210.3 (p<0.01) - Testicular volume ≥15 ml vs. <15 ml: OR 17.1; 95% CI 1.4-215.8 (p<0.03) - No TBI vs. TBI: p>0.05 (OR not mentioned) - FSH <10 IU vs. ≥10 IU: p>0.05 (OR not mentioned) Predictors for active sperm production in	106 out of 123 (86.2%) eligible survivors participated in this study; 31 out of 106 (29.2%) semen analysis. Testicular volume measured with orchidometer or ruler & formula. If both testes measured, mean of both used. Adult testicular volume (n=74) documented by Tanner stage 5, reached final height, or age >18 years. In the multivariable analysis the effect of TBI was evaluated. However, 5 patients treated in the no TBI group had received TNI, hence potentially exposing the testes to radiotherapy (no details were provided to indicate if testicular shielding was used). In addition, the no TBI group was also treated with cyclophosphamide, busulfan, or both. Leukaemia was associated with azoospermia. This might be due to CRT and/or testicular

		TNI (in SAA) 6 Gy <u>Overall:</u> TBI-based: 71 (67.0%) Busulfan-based (no TBI or TNI): 18 (17.0%) Cyclophosphamide only: 12 (11.3%) Cyclophosphamide + TNI (no TBI): 5 (4.7%)	<u>bivariate logistic regression analysis:</u> - No leukaemia diagnosis vs. leukaemia diagnosis: OR 17.0; 95% CI 2.6-113.0 (p<0.003) - Testicular volume ≥15 ml vs. <15 ml: OR 14.2; 95% CI 2.1-98.1 (p<0.007) - No TBI vs. TBI: OR 30.0; 95% CI 2.8-322.1 (p<0.005) - FSH <10 IU/L vs. ≥10 IU/L: OR 0.8; 95% CI 0.7-1.0 (p<0.047)	irradiation. It is, however, unclear how many patients with sperm samples were treated with CRT and/or testicular irradiation. 2 out of 106 fathered a child.

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CRT, cranial radiotherapy; FSH, follicle stimulating hormone; fTBI, fractionated TBI; HSCT, haematopoietic stem cell transplantation; OR, odds ratio; SAA, severe aplastic anaemia; sTBI, single fraction TBI; TBI, total body irradiation; TNI, total nodal irradiation; 95% CI, 95% confidence interval.

Testosterone deficiency – Who needs surveillance?				
Mackie et al. Gonadal function following chemotherapy for childhood Hodgkin's disease. Med Pediatr Oncol 1996;27:74-78				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
National multi-centre trial cohort study Treatment era not reported <u>Follow-up:</u> Median 6 (2.5-11.1) years from diagnosis	58 postpubertal male survivors of childhood Hodgkin disease treated according to UKCCSG HD Trial 8201 <u>Age at diagnosis:</u> Mean 12.2 (8.2-15.3) years <u>Age at follow-up</u> not reported	<u>ChlVPP:</u> 58 (100%); 6-8 cycles Chlorambucil 504-672 mg/m^2, vinblastine, prednisolone, procarbazine 8,400-11,200 mg/m^2 <u>Radiotherapy below diaphragm:</u> 0 (0.0%)	<u>Outcome definitions:</u> - Leydig cell dysfunction: LH >10 IU/L Testosterone <10 nmol/L - Abnormal gonadotropin levels: FSH >10 IU/L <u>Leydig cell dysfunction (↑ LH) (n=41 assessed):</u> 10 increased (24.4%); range of these increased LHs, 10.3-18 IU/L <u>↓ testosterone levels (n=37 assessed):</u> 5 decreased (13.5%); range of all testosterones, 4.3-37.8 nmol/L <u>↑ FSH levels (n=46 assessed):</u> 41 increased (89.1%); range of increased FSHs, 10.8-40.7 IU/L <u>Risk factors for Leydig cell dysfunction in multivariable regression analyses:</u> - Amount of chemotherapy, NS - Age at treatment, NS - Follow-up duration, NS (no effect measures reported) <u>Risk factors for ↑ FSH in multiple regression analyses:</u> - Age or pubertal status at time of treatment, NS	101 out of 168 (60.1%) eligible male and female CCS included in this study. 46 out of 58 (79.3%) males had gonadotropin levels obtained. Lower level of evidence – no control group, relatively narrow range of cumulative doses Note potential bias - LH and testosterone only described clearly in survivors with increased FSH levels. Note concerns about outcome definitions: • Leydig cell dysfunction based on high LH rather than low testosterone ▪ Cutoff of 10 may be too high ▪ High LH definition not appropriate if patient received cranial RT (not specified in this paper albeit unlikely) Azoospermia present in 7 survivors in whom semen analyses were performed. All progressed spontaneously through puberty. Unclear how "amount of chemotherapy" is defined.

			- Follow-up duration, NS (no effect measures reported)	Multiple regression performed for gonadotropins and Leydig cell dysfunction. However, methodology of testosterone analysis not clear.

Abbreviations: FSH, follicle-stimulation hormone; LH, luteinising hormone; NS, not significant.

Testosterone deficiency – Who needs surveillance?				
Tromp et al. Reproductive status in adult male long-term survivors of childhood cancer. Hum Reprod 2011;26:1775-1783				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
Single-centre cohort study 1966-2003 <u>Follow-up:</u> Median 15 (5.0-39.0) years from diagnosis	565 male CSS who survived ≥5 years from diagnosis and were ≥18 years at follow-up <u>Diagnoses:</u> Leukaemia (22.1%), brain tumour (8.3%), lymphoma (27.3%), testicular tumour (21.9%), kidney tumour (11.3%), bone tumour (9.4%), soft tissue sarcoma (12.3%), neuroblastoma (3.3%), other (4.3%) <u>Age at diagnosis:</u> Median 7.8 (range 0-17.8) years <u>Age at follow-up:</u> Median 21.0 (18.0-46.0) years	<u>Alkylating agents:</u> 336 (59.5%) <u>Pelvic/abdominal irradiation only:</u> 51 (9.0%) <u>Cranial irradiation only:</u> 120 (21.2%) <u>Cranial + pelvic/abdominal irradiation:</u> 4 (0.7%) <u>Radiation to fields including testes:</u> Not reported <u>TBI:</u> 11 (1.9%) <u>Surgery testicular region:</u> 38 (6.7%)	<u>Outcome definitions:</u> - ↓ testosterone levels: <11 nmol/L - ↑ LH levels: >15 U/L <u>↓ testosterone levels (n=460 assessed):</u> 57 (12.4%) Mean 17.2 nmol/L, SD 5.5 for total group <u>↑ LH levels (n=489 assessed):</u> 14 (2.9%) Median 6.0 U/L, range 1.0-40.0 for total group <u>Risk factors for lower (but not necessarily abnormal) testosterone:</u> Model 1 – univariable linear regression analysis - Adjusted for age at diagnosis and follow-up duration - TBI yes vs. no: beta -3.53 (p=0.036) Model 2 – multivariable linear regression analysis (adjustment for all treatments) - All treatment variables NS (procarbazine, cyclophosphamide, other alkylating agents (busulfan, carmustine, mechlorethamine, ifosfamide, lomustine, melphalan, temozolamide – not evaluated separately), cisplatin/carboplatin, antimetabolites, vinca alkaloids, antimetabolites, anthracyclines, other chemotherapeutic agents, cranial irradiation, pelvic/abdominal radiation, other irradiation, TBI, surgery testicular	565 out of 796 (71.0%) eligible CCS included in this study. However, missing endocrine measurements were randomly distributed among different treatment groups (data not shown). NB 1) Higher incidence of decreased testosterone levels compared to elevated LH levels (unclear what time of day samples were taken). Note higher LH threshold in this study. 2) Normal LH levels do not exclude testosterone deficiency in this group – clinically, testosterone and LH need be assessed together, especially in patients who received cranial irradiation (in whom LH response may be blunted by central hypogonadism), but data not reported 3) Difficult to interpret clinical relevance of testosterone risk factor analyses since most of the testosterones were in the normal range 73 men reported that their

			region)	partner had become pregnant: 120 conceptions resulted in 103 live births and 14 miscarriages. 56 (77%) natural conception. No data presented to prove paternity in these pregnancies.

Abbreviations: LH, luteinising hormone; NS, not significant; OR, odds ratio; TBI, total body irradiation; 95% CI, 95% confidence interval.

Testosterone deficiency – Who needs surveillance?				
Siimes et al. Prophylactic cranial irradiation increases the risk of testicular damage in adult males surviving ALL in childhood. Med Pediatr Oncol 1993;21:117-121				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Treatment**	**Main outcomes**	**Additional remarks**
Multi-centre cohort study 1966-2003 Follow-up: Mean 15.2 (4.0-25.0) years from diagnosis	41 male childhood ALL survivors at least 1 year off chemotherapy Age at diagnosis: Median 7.5 (range 1-16) years Age at follow-up: Median 21.0 (18.0-27.0) years	Cyclophosphamide: 23 (56.1%) Cytosine arabinoside: 9 (22.0%) Vincristine, prednisone, 6-mercaptopurine, methotrexate: 41 (100%) Adriamycin: 21 (51.2%) Asparaginase: 33 (80.5%) CRT 20-24 Gy: 17 (41.5%) Radiation to fields including testes: 0 (0%) Both cyclophosphamide and cranial radiotherapy: 12 (29%)	Outcome definitions: - ↓ testosterone levels: threshold level not reported - ↑ LH levels: threshold level not reported Testosterone levels: - Cranial radiotherapy: Mean 17.0 (±7.5) - No cranial radiotherapy: Mean 20.2 (±6.7) p=0.242 LH levels: - Cranial radiotherapy: Mean 8.2 (±8.1) - No cranial radiotherapy: Mean 6.0 (±3.4) p=0.456 Risk factors for lower (but not necessarily abnormal) testosterone in multivariable analysis: - Chemotherapy NS - Cranial radiotherapy NS - Age at diagnosis NS	3 patients treated with CRT had been started on testosterone supplementation from 4 to 9 years earlier. Their mean testosterone concentration was lower (9.8 U/L at 2 weeks after the preceding testosterone injection) than that of the other patients at time of the study. A forward-stepping linear regression analysis was used to identify factors independently associated with testosterone deficiency. 3 patients with testosterone supplementation excluded from analysis. Note limitations in interpreting LH level in patients who have received cranial radiotherapy (LH response may be blunted by central hypogonadism.

Abbreviations: CRT, cranial radiotherapy; LH, luteinising hormone; NS, not significant.

Impaired spermatogenesis – What surveillance modality should be used?				
Green et al. Lack of specificity of plasma concentrations of inhibin B and follicle-stimulating hormone for identification of azoospermic survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. J Clin Oncol 2013;31:1324-1328				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Diagnostic tests**	**Main outcomes**	**Additional remarks**
Single-centre cohort study Treatment period not reported <u>Follow-up:</u> ≥10 years from diagnosis	275 male CCS who received gonadotoxic treatment, were not receiving exogenous androgens, had received <40 Gy hypothalamic/pituitary irradiation and were ≥18 years of age at follow-up <u>Age at diagnosis:</u> Range 0-21 years <u>Age at follow-up:</u> Median 30.5 (19.7-59.1) years <u>Gonadotoxic treatment:</u> 275 (100%); Alkylating agents, direct testicular irradiation or <40 Gy hypothalamic/pituitary irradiation (≥40 Gy hypothalamic/pituitary irradiation specifically excluded from analysis) <u>Prevalence azoospermia:</u> 105 (38.2%)	Inhibin B, FSH and inhibin B:FSH ratio <u>Cut-off levels for azoospermia:</u> - Inhibin B ≤31 ng/L - FSH >11.5 IU/L - Inhibin B:FSH ratio ≤2.52 pg/mIU ROC analysis determined the optimal cut-off levels	**Azoospermia** <u>Sensitivity</u> - Inhibin B: 100% - FSH: 78.1% - Inhibin B:FSH ratio: 75.3% <u>Specificity</u> - Inhibin B: 45.0% - FSH: 74.1% - Inhibin B:FSH ratio: 74.5% <u>Negative predictive value</u> - Inhibin B: 100% - FSH: 84.6% - Inhibin B:FSH ratio: 83.5% <u>Positive predictive value</u> - Inhibin B: 52.1% - FSH: 65.1% - Inhibin B:FSH ratio: 63.8% <u>Area under the ROC curve</u> - Inhibin B: 0.72 - FSH: 0.83 - Inhibin B:FSH ratio: 0.83	298 out of 485 (61.4%) eligible survivors participated in this study; 23 treated with ≥40 Gy hypothalamic/pituitary irradiation and/or tumour in hypothalamic/pituitary region excluded. Inhibin B was measured in 238 patients and FSH was measured in 275 patients. Patient sample divided into a learning set (n=140) and a validation set (n=135) by random assignment: diagnostic values were similar. Results are shown for the combined data sets.

Abbreviations: FSH, follicle-stimulating hormone; ROC, receiver operating characteristics.

Impaired spermatogenesis – What surveillance modality should be used?				
Romerius et al. High risk of azoospermia in men treated for childhood cancer. Int J Androl 2010;34:69-76				
Study design Treatment era Years of follow-up	**Participants**	**Diagnostic tests**	**Main outcomes**	**Additional remarks**
Single-centre cohort study 1970-2002 <u>Follow-up:</u> Median 19 (4-36) years after treatment	129 male CCS >18 years of age at follow-up <u>Age at diagnosis:</u> Median 10 (0.1-17) years <u>Age at follow-up:</u> Median 29 (20-46) years <u>Treatment:</u> - Chemotherapy only: 35 (27.1%); carmustine, lomustine, chlorambucil, cisplatin, cyclophosphamide, melphalan, procarbazine - Radiation involving testes: 1 (0.8%) - Other radiation: 13 (10.2%) - Chemotherapy and radiation: 48 (37.2%) - Brain surgery: 16 (12.4%) - Other surgery: 16 (12.4%) <u>Prevalence azoospermia:</u> 23 (17.8%)	Inhibin B, FSH and testicular volume <u>Cut-off levels for azoospermia:</u> - Inhibin B <50 ng/L - FSH >10.9 IU/L - Testicular volume <24 mL ROC analysis determined the optimal cut-off levels	**Azoospermia** <u>Sensitivity</u> - Inhibin B: 91% - FSH: 96% - Testicular volume: 70% <u>Specificity</u> - Inhibin B: 90% - FSH: 96% - Testicular volume: 93% <u>Negative predictive value</u> - Inhibin B: 98% (95% CI 93-100%) - FSH: 99% (95% CI 94-100%) - Testicular volume: 92% (95% CI 84-96%) <u>Positive predictive value</u> - Inhibin B: 66% (95% CI 47-81%) - FSH: 50% (95% CI 35-67%) - Testicular volume: 61% (95% CI 39-80%) Combining hormone values with testicular volume did not result in any increase in positive or negative predictive value.	151 out of 397 (38.0%) eligible survivors participated in this study; 8 survivors on testosterone replacement therapy and 14 men who could not ejaculate were excluded from the study.

Abbreviations: FSH, follicle-stimulating hormone; 95% CI, 95% confidence interval

Impaired spermatogenesis – What surveillance modality should be used?				
Rendtorff et al. Low inhibin B levels alone are not a reliable marker of dysfunctional spermatogenesis in childhood cancer survivors. Andrologia 2012;44:219-225				
Study design Treatment era Years of follow-up	**Participants**	**Diagnostic tests**	**Main outcomes**	**Additional remarks**
Single-centre cohort study 1980 onwards <u>Follow-up:</u> Mean 13.6 (1-28) years since diagnosis	73 male CCS >18 years of age at follow-up, of whom 42 underwent semen analysis <u>Age at diagnosis:</u> Mean 10 (1-18) years <u>Age at follow-up:</u> Mean 24 (19-43) years <u>Treatment</u> not reported <u>Prevalence azoospermia:</u> 13 (31.0%)	Inhibin B and FSH <u>Cut-off levels for azoospermia:</u> - Inhibin B <80 pg/mL - FSH >10 IU/L	**Azoospermia** <u>Positive predictive value</u> - Inhibin B: 42.3% - FSH: 61.5% - Inhibin B + FSH: 66.7%	Unclear how many patients were eligible for the study; only stated that 77 patients returned the questionnaire and consent.

Abbreviations: FSH, follicle-stimulating hormone; ROC, receiver operating characteristics

Impaired spermatogenesis – What surveillance modality should be used?				
Lähteenmäki et al. Male reproductive health after childhood cancer. Acta Paediatr 2008;97:935-942				
Study design Treatment era Years of follow-up	**Participants**	**Diagnostic tests**	**Main outcomes**	**Additional remarks**
Multi-centre cohort study Treatment period not reported Follow-up: Median 14.5 (2.1-26.1) years since diagnosis	25 male CCS, of whom 23 underwent semen analysis Age at diagnosis: Median 8.5 (0.9-15.9) years Age at follow-up: Median 20.5 (15.6-31.2) years Treatment: - Cyclophosphamide: 11 (44.0%) - Cisplatin: 1 (4.0%) - MOPP: 1 (4.0%) - ABVD: 1 (4.0%) - MOPP / ABVD: 1 (4.0%) - CNS radiation: 8 (32.0%) - Testicular radiation: 1 (4.0%) - TBI: 1 (4.0%) - Abdominal radiation: 3 (12.0%) Prevalence azoospermia: 3 (13.0%) Prevalence low sperm concentration (<20 x10^6/mL): 8 (34.8%)	Inhibin B, FSH and testicular volume Cut-off levels for low sperm concentration (<20 x10^6/mL): - Inhibin B: not reported - FSH >10.5 IU/L - Testicular volume: not reported	**Low sperm concentration (<20 x10^6/mL)** Area under the ROC curve - Inhibin B: 0.83 (95% CI 0.67-0.99) - FSH: 0.73 (95% CI 0.49-0.96) - Inhibin B + FSH: 0.78 (95% CI 0.56-0.99) - Testicular volume: 0.79 (95% CI 0.59-0.99) Multiple regression analysis showed that inhibin B, testicular volume and FSH together explained 44% of the variance in sperm concentration (p=0.003)	Unclear how many patients were eligible for the study.

Abbreviations: ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; CNS, central nervous system; FSH, follicle-stimulating hormone; MOPP, nitrogen mustard, vincristine, prednisone, and procarbazine; ROC, receiver operating characteristics; TBI, total body irradiation; 95% CI, 95% confidence interval

Impaired spermatogenesis – What surveillance modality should be used?				
Jahnukainen et al. Semen quality and fertility in adult long-term survivors of childhood acute lymphoblastic leukaemia. Fertil Steril 2011;96:837-42				
Study design Treatment era Years of follow-up	**Participants**	**Diagnostic tests**	**Main outcomes**	**Additional remarks**
Single-centre cohort study 1970-1995 <u>Follow-up:</u> Median 20 (11-30) years after treatment	51 male ALL survivors, of whom 47 underwent semen analysis <u>Age at diagnosis:</u> Median 5 (1-15) years <u>Age at follow-up:</u> Median 29 (26-38) years <u>Treatment:</u> - Cyclophosphamide: 26 (51.0%) - Other chemotherapy: prednisolone, vincristine, doxorubicin, asparaginase, methotrexate, 6-mercaptopurine - Cranial radiation: 38 (74.5%) - Testicular radiation: 18 (35.3%) - Spinal radiation: 1 (2.0%) <u>Prevalence azoospermia:</u> 17 (36.2%)	Inhibin B, FSH and testicular size <u>Cut-off levels for non-azoospermia:</u> - Inhibin B >122 ng/L - FSH <5 IU/L - Testicular size >17 mL <u>Cut-off levels for fertility:</u> - Inhibin B >180 ng/L - FSH <2.5 IU/L - Testicular size >23 mL	**Non-azoospermia** <u>Sensitivity for predicting non-azoospermia</u> - Inhibin B: 80% - FSH: 80% - Testicular size: 98% <u>Specificity for predicting non-azoospermia</u> - Inhibin B: 100% - FSH: 80% - Testicular size: 100% <u>Area under the ROC curve</u> - Inhibin B: 0.74 - FSH: 0.80 - Testicular size: 0.99 **Fertility (fathering a child)** <u>Sensitivity for identifying patients who fathered a child</u> - Inhibin B: 80% - FSH: 80% - Testicular size: 80% <u>Specificity for identifying patients who fathered a child</u> - Inhibin B: 60% - FSH: 70% - Testicular size: 70% <u>Area under the of ROC curve</u> - Inhibin B: 0.63	51 out of 77 (66.2%) eligible survivors participated in this study.

			- FSH: 0.70 - Testicular size: 0.77	

Abbreviations: ALL, acute lymphoblastic leukaemia; FSH, follicle-stimulating hormone; ROC, receiver operating characteristics

Impaired spermatogenesis – What surveillance modality should be used?				
Wilhelmsson et al. Adult testicular volume predicts spermatogenetic recovery after allogeneic HSCT in childhood and adolescence. Pediatr Blood Cancer 2014;61:1094-1100				
Study design **Treatment era** **Years of follow-up**	**Participants**	**Diagnostic tests**	**Main outcomes**	**Additional remarks**
Two-centre cohort study 1978-2000 <u>Follow-up:</u> Mean 13 (4-28) years	106 male CCS who received allogeneic HSCT >5 years after treatment and reached puberty at follow-up; 31 semen analysis <u>Age at diagnosis:</u> Mean 8.0 (1-17) years <u>Age at follow-up:</u> Mean 22 (12-42) years <u>Gonadotoxic treatment:</u> sTBI + cyclophosphamide: 30 (28%) fTBI + cyclophosphamide: 20 (19%) fTBI + cyclophosphamide + etoposide: 2 (2%) fTBI + cytarabine: 16 (15%) fTBI + melphalan: 3 (3%) Busulfan: 3 (3%) Busulfan + cyclophosphamide: 15 (14%) Cyclophosphamide only: 12 (11%) Cyclophosphamide + TNI: 5 (5%) <u>Prevalence azoospermia:</u> 21/31 (68%)	FSH and testicular volume <u>Cut-off level for non-azoospermia:</u> - FSH <10 IU/mL - Testicular volume ≥15 mL ROC analysis determined the optimal cut-off levels	**Non-azoospermia** <u>Sensitivity for predicting non-azoospermia</u> - FSH: 56% - Testicular volume: 80% <u>Specificity for predicting non-azoospermia</u> - FSH: 81% - Testicular volume: 91% <u>Area under the ROC curve</u> - FSH: 0.79 - Testicular volume: 0.89	106 out of 123 (86.2%) eligible survivors participated in this study; 31 out of 106 (29.2%) semen analysis.

Abbreviations: FSH, follicle-stimulating hormone; fTBI, fractionated TBI; ROC, receiver operating characteristics; sTBI, single fraction TBI; TBI, total body irradiation; TNI, total nodal irradiation