A prospective study on the long-term outcome of prepubertal and pubertal boys undergoing testicular biopsy for fertility preservation prior to hematologic stem cell transplantation

Birgit Borgström¹,² | Margareta Fridström³ | Britt Gustafsson² | Per Ljungman⁴ | Kenny A. Rodriguez-Wallberg³,⁵

¹ Department of Pediatric Endocrinology, Karolinska University Hospital, Stockholm, Sweden
² Division of Pediatrics, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden
³ Division of Gynecology and Reproduction, Department of Reproductive Medicine, Karolinska University Hospital, Stockholm, Sweden
⁴ Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital Huddinge and Division of Hematology, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden
⁵ Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

Correspondence
Kenny A. Rodriguez-Wallberg, Section of Reproductive Medicine, Karolinska University Hospital, Novumhuset Plan 4, SE-141 86 Stockholm, Sweden.
Email: kenny.rodriguez-wallberg@ki.se

Funding information
Swedish Childhood Cancer Foundation; Swedish Cancer Society; Radiumhemmet's Research Grants; Karolinska Institutet; Stockholm County Council

Abstract

Background: Few studies have reported the long-term outcomes of prepubertal and pubertal boys undergoing testicular biopsy for fertility preservation (FP).

Procedure: This prospective longitudinal study examined 21 boys (aged 1.5-14.5 years) who underwent testicular biopsy for FP prior to allogeneic (n = 20) or autologous (n = 1) hematological stem cell transplantation (HSCT) between 2003 and 2010. During counseling, pubertal boys were encouraged to produce a sperm sample by masturbation, while prepubertal boys were presented with surgical testicular tissue retrieval as an option for experimental FP. Clinical outcomes included postoperative complications, pubertal development, and sex-hormone levels. Survivors approaching adulthood were encouraged to provide semen samples.

Results: Twenty boys, including 14 in prepuberty and six in early puberty (Tanner stage 2-3), underwent open testicular biopsies. Two pubertal biopsies contained mature sperms, which were cryopreserved. Testicular tissue was vitrified in the remaining 18 cases. One pubertal boy (Tanner stage 4) underwent percutaneous testicular sperm aspiration and sperms obtained were cryopreserved. Postoperative complications (hematoma or infection) were rare. Overall, 14 boys survived > 5 years (mean follow-up after HSCT, 7.2 years) and 11 showed advanced puberty. Semen samples were provided by five boys and obtained sperm were cryopreserved from two. Individuals at
INTRODUCTION

The survival of childhood cancer has improved during the last decades, in particular due to the introduction of hematological stem cell transplantation (HSCT) in the 1980s for patients with relapsed or high-risk leukemia.1 HSCT is now a routine procedure for treatment of certain malignant or benign hematological diseases or for hematological rescue during chemotherapy treatment of other cancers.2 The conditioning for HSCT with chemotherapy, sometimes in combination with radiation therapy, depends on the diagnosis, the disease stage, and the donor cell origin.3

Despite its benefits, HSCT has a number of severe long-term side effects in many organs. Endocrine dysfunction, including gonadal insufficiency, is frequent in young adults and children after HCST. Girls often develop ovarian failure, with impairment or absence of pubertal development.4,5 By contrast, boys typically show spontaneous puberty and pubertal progression due to sustained testosterone secretion, but testis growth is often impaired, indicating a risk for future absence of sperm production. The serious damage to the gonads in both sexes leads to a high risk of permanent infertility.6-8 A study from 199 European transplantation centers reported only a few conceptions in patients that had undergone HSCT (232/37362, 0.6%), with the highest probability of achieving pregnancy occurring in the patient group conditioned with cyclophosphamide only, mostly for treatment of nonmalignant diseases.9 The frequency of most endocrine deficits is reduced if conditioning does not include total body irradiation (TBI); however, the infertility risk remains due to the high doses of alkylating drugs used for conditioning. Loss of fertility is recognized as a severe side effect, with a negative impact on the quality of life.5 However, advances in reproductive medicine and in techniques for cryopreservation of reproductive cells and tissues now hold promise for fertility preservation (FP).10-12

In selected cases, certain FP techniques can be applicable to children.8,10-12 For postpubertal boys, the established method for FP is the cryopreservation of mature spermatozoa from semen samples. For over three decades, postpubertal adolescent patients aged 14 or older at our center have provided semen samples to bank sperms before gonadotoxic treatments.13 For prepubertal patients, testicular tissue cryopreservation has been proposed,12,14-18 aimed at preserving spermatogonic stem cells present in the tissue. Recent research has demonstrated the integrity, survival, and proliferative ability of cryopreserved spermatogonia.14 However, the cryopreservation of human prepubertal testicular tissue is still under development and functional mature sperm have not been reported, making this an experimental method of FP.10-12,19

This study was a prospective follow-up of a cohort of prepubertal and peripubertal boys who underwent testicular tissue cryopreservation at our center in 2003-2010 prior to HCST. The aim was to determine the patient acceptability of the procedure and the potential long-term follow-up risks.

1.1 Patients and methods

The patients were referred to our center for HSCT 2 weeks before the start of conditioning. Eligibility criteria included the planning of a treatment with an inherent high risk (>80%) of future infertility, prepubertal stage or peripubertal up to Tanner stage 3-4, but unable to provide a sperm sample for cryopreservation. In total, 26 boys were evaluated from 2003 to 2010. One patient declined due to concern about possible surgical pain and one patient had an increased risk of bleeding. Another two patients could finally provide a semen sample, but sperms were not found and the patients decided not to proceed to testicular biopsy, and one other did not undergo HSCT, leaving the 21 cases reported here.

1.1.1 Informed consent

Written and oral information about the study were provided to the boys and their families by pediatric endocrinologists or reproductive medicine specialists. Information was provided about FP and the current experimental stage of testicular tissue cryopreservation in prepubertal boys. Pubertal boys with Tanner stage 3 or above were encouraged to produce a sperm sample by masturbation. If that was not possible, invasive methods, such testicular sperm aspiration (TESA) or a testicular biopsy, were presented, depending on the boy’s age and pubertal development. For pubertal boys, the possibility of isolating sperms from the biopsy and their cryopreservation was also discussed. Patients aged 10 years or older were provided specific age-adapted written information to allow discussion about future fertility. Written
informed consent was obtained from the parents and verbal assent from boys younger than 10 years, when applicable. Boys aged 10 years and older signed a consent form, as this is the age recommended for this in Sweden.

The current lack of methods for obtaining mature sperm from prepubertal tissue was explained during counseling, as well as the plausible development of those methods in the future. Patients were informed that the tissue collected would be entirely cryopreserved for the patient’s potential use in a reproductive treatment in the future and that only a minimal tissue piece would undergo histopathological analysis. Information was provided on short-term surgical complications including pain, bleeding, or infection. Upon receipt of informed consent from the patient and/or his family, the surgical procedure to retrieve testicular tissue was planned. Low blood counts, thrombocytopenia, or any other vulnerabilities posing an increased surgery risk were considered contraindications for the invasive FP procedures in this study.

1.1.2 Testicular biopsy

Testicular tissue retrieval was conducted under general anesthesia by a urologist during the planned placement of the central venous line installation and routine bone marrow aspiration performed prior to HSCT. The biopsy usually did not delay the transplantation procedure and required no additional anesthesia or surgical intervention. The amount of tissue removed was at the discretion of the surgeon, as was the decision to take unilateral or bilateral biopsies.

1.1.3 Histopathology

A small fraction of the testicular biopsy was prepared for clinical histopathological examination at the Department of Clinical Pathology and Cytology, Karolinska University Hospital. This included an overall evaluation of pubertal status according to histology, presence of Sertoli and Leydig cells, spermatogonial quantification, and spermatogenesis maturation. Hematoxylin-eosin and immunohistochemistry (IHC) stainings were requested. IHC included inhibin, vimentin, Cam 5.2, CD30, CK19, PLAP, and CD34. The investigation of presence of malignant cells was also requested.

1.2 Patient follow-up

After HSCT, patients underwent regular checkups at the Pediatric Oncology and Pediatric Endocrine units. The boys were followed yearly by the same pediatric endocrinologist, and pubertal progression and hormone tests were documented. Measurements of testosterone, LH, follicle stimulating hormone (FSH), anti-Müllerian hormone (AMH), and inhibin B were performed at the Central Laboratory for Clinical Chemistry, Karolinska University Hospital. The boys were also encouraged to provide a sperm sample for analysis during the consultations when they reached a pubertal stage Tanner 4 or 5. The nine patients who reached adulthood continued their checkups in adult care from 18 years of age. They were contacted by post and requested to provide a voluntary blood sample for measurement of hormones indicative of testicular function (sex hormones, inhibin B, and AMH); five patients agreed. These hormone determinations had become routine tests during follow-up at our center but were not available at the time of study inclusion in 2003.

1.3 Ethical approval

Ethical approval for this study and for the follow-up of patients up to an adult age were granted by the Ethical Review Board of Karolinska Hospital and the Regional Ethics Committee (Dnr 427/03 and 2016/2530-32, respectively).

2 RESULTS

2.1 Patient demographics and HSCT

Table 1 shows demographic data and HSCT details of the 21 included patients. The age range at inclusion was 1.5–14.5 years. The HSCT procedures were indicated by malignant disease in 20 cases, usually following cytotoxic treatment. Ten boys suffered from ALL and were in second remission or had a high risk disease; they had completed cytotoxic courses 2 months to 1 week before referral. Figure 1 shows the history of the ALL disease in patients such as those included in the study, according to data of the Nordic Society of Pediatric Hematology and Oncology (NOPHO). In our study, children presenting with ALL were all in position C (gray square). The boys with other malignant diagnoses had similar, but usually shorter, medical histories of diseases such lymphoma and myelodysplastic syndrome (MDS). One boy presenting with thalassemia had a long disease history but no previous cytotoxic treatment.

The HSCTs were allogeneic in 20 cases. Ten boys were conditioned with TBI (4 fractions × 3 Gy, 12 Gy in 1 week), and the other 10 received high-dose busulfan (BU), usually in combination with high-dose cyclophosphamide. The choice of conditioning depended on the disease, disease stage, and the type of HSCT donor. One boy was planned for high-dose chemotherapy followed by autologous HSCT for hematologic rescue for neuroblastoma treatment.

2.2 Prepubertal/pubertal status

Pubertal status was classified according to Tanner stage and a Prader orchidometer for testicular size. Most boys were prepubertal (N = 14), with a testicular size ≤3 mL. Two boys were pubertal Tanner stage 2 with testicular volumes of 4–6 mL. Four boys had reached Tanner 3–4 with testes size ≥8 mL, and one boy was at stage 5 but unable to produce a semen sample with spermatozoa. Table 1 summarizes clinical data at the time of inclusion.
### TABLE 1 Clinical data of the patients at the time of inclusion in the study

| Patient | Diagnosis  | Remission | Age (years) | Tanner stage | Testicular size (mL) R/L | Conditioning before HSCT | Donor | Biopsy Uni/Bi R/L | Other preservation | Histology normal for age |
|---------|------------|-----------|-------------|-------------|-------------------------|--------------------------|-------|------------------|----------------------|--------------------------|
| 1       | CML        | -         | 1.5         | 1           | 2/2                     | BU+Cy                   | MUD   | Uni/L            |                      | Yes, but leukemia cells |
| 2       | AML        | 1         | 2           | 1           | 2/2                     | BU+Cy                   | MUD   | Uni/L            |                      | Yes                     |
| 3       | ALL        | 1         | 4           | 1           | 2/2                     | TBI                     | MUD   | Bil              |                      | ND                      |
| 4       | ALL        | 2         | 6           | 1           | 2/2                     | TBI                     | Cord blood | Bil          |                      | Yes                     |
| 5       | ALL        | 1         | 6.3         | 1           | 2/2                     | TBI                     | MUD   | Bil              |                      | ND                      |
| 6       | ALL        | 1         | 6.8         | 1           | 2/2                     | TBI                     | MUD   | Bil              |                      | Yes                     |
| 7       | Lymphoma   | ALL       | 2           | 8           | 1                       | TBI                     | MUD   | Bil              |                      | ND                      |
| 8       | Neuroblastoma | -      | 9.7         | 1           | 3/3                     | spec                    | Autologous | Uni/R         |                      | Yes                     |
| 9       | ALL        | 2         | 9.8         | 1           | 3/3                     | TBI                     | Sibling | Uni/R         |                      | Yes                     |
| 10      | ALL        | 2         | 10          | 1           | 2/2                     | TBI                     | MUD   | Uni/L            |                      | No                      |
| 11      | ALL        | 2         | 10.7        | 1           | 3/3                     | TBI                     | MUD   | Uni/R            |                      | Yes                     |
| 12      | MDS        | 1         | 11          | 2           | 4/4                     | BU+Cy                   | Sibling | Bil            |                      | ND                      |
| 13      | MDS        | 1         | 12.2        | 3           | 6/6                     | BU+Cy                   | MUD   | Bil              |                      | Yes                     |
| 14      | ALL        | 1         | 12.2        | 1           | 3/3                     | TBI                     | Sibling | Bil            |                      | ND                      |
| 15      | AML        | 2         | 12.7        | 2           | 4/5                     | BU+Cy                   | MUD   | Bil              |                      | Yes                     |
| 16      | MDS        | 1         | 13.1        | 5           | 14/18                   | BU+Cy                   | MUD   | ND               |                      | Yes, semen sample provided but no sperm found. Sperm obtained by TESA |
| 17      | SAA/MDS    | 1         | 13.3        | 1           | 3/3                     | BU/Cy                   | MUD   | Bil              |                      | Yes                     |
| 18      | MDS        | 1         | 13.4        | 3           | 7/8                     | BU+Cy                   | MUD   | Uni/L            |                      | Yes, semen sample provided but no sperm found. Sperm obtained through biopsy |
| 19      | Thalasemia | -         | 14.1        | 1           | 3/3                     | BU+Cy                   | Sibling | Uni/R         |                      | Yes                     |
| 20      | Lymphoma   | 1         | 14.3        | 3           | 8/8                     | TBI                     | MUD   | Uni/R            |                      | Yes                     |
| 21      | MDS        | 1         | 14.5        | 3           | 8/8                     | BU+Cy                   | MUD   | Uni/L            |                      | Yes, semen sample provided but no sperm found. Sperm obtained through biopsy |

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BU, busulfan; CML, chronic myeloid leukemia; Cy, cyclophosphamide; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; MUD, matched unrelated donor; ND, not done; SAA, severe aplastic anemia; TBI, total body irradiation; TESA, testicular sperm aspiration; Uni/Bi, unilateral/bilateral.
2.3 | FP procedures

Data on the FP procedures are presented in Table 1. Three pubertal boys (nos. 16, 18, and 21) attempted to provide a semen sample; one (no. 16) succeeded but no motile sperms were found. That boy had testes of pubertal size and underwent percutaneous TESA instead of open biopsy. Sperms were found and cryopreserved. The other two boys (nos. 18 and 21) agreed to testicular biopsy. Motile sperms were identified in the biopsies and were cryopreserved for future fertility treatment. The tissues from these two biopsies were not cryopreserved or analyzed because all tissue material was used to obtain cryopreservable sperm.

Testicular biopsies were also performed on the 14 prepubertal, two in early puberty, and four mid-pubertal boys for a total of 20 biopsies. These were bilateral in 10 cases and unilateral in 10, with sizes varying from 1–2 x 2–3 mm in the prepubertal to 5 x 5 mm in pubertal boys. Cryopreservation was performed by vitrification, with tissue pieces per straw varying from 2 to 24 for 1–10 straws, depending on the size of the biopsies.

Few postoperative complications were associated with the testicular biopsies. One boy developed an epididymal infection that was treated with antibiotics, and one boy developed a local hematoma. The testicular biopsy was done together with the venous line installation and bone marrow aspiration procedures, so the nurses were asked to estimate if the biopsy caused additional pain. Most boys required extra doses of pain-relieving drugs after surgery on the first day, whereas boys who underwent only venous line installation and bone marrow aspiration did not. Pain was estimated with a VAS scale. Most boys had paracetamol at maximal doses for their weights, and two also needed a small dose of codeine. Only the boy with hematoma required morphine.

2.3.1 | Histopathological analysis

A sample of testicular tissue was analyzed by histopathology in 15 cases. Detailed information is presented in Table S1. Histopathology findings were normal in most cases, indicating prepubertal testes with the presence of the expected number of spermatogonia per seminiferous tubule and Sertoli cells. One boy (no. 10) had no identifiable spermatogonia by age 10, and another boy (no. 1) showed malignant cells in the testicular tissue.

2.4 | Long-term follow-up

2.4.1 | Survival and mortality

Fourteen of the 21 boys were alive in July 2019 (mean follow-up at the pediatric unit 7.2 years, range: 5-13.7). Seven died at 3 months to 4 years after treatment, all following allogeneic HSCT for malignant disease. Leukemia relapse and severe graft versus host disease were the most frequent reasons for fatal outcomes (Table 2). Follow-up data on the survivors are presented in Table 3.
TABLE 2  Clinical and follow-up data in the group of patients who did not survive to adult age after HSCT

| Patient # | Diagnosis | Years after HSCT | Age, years | Tanner stage | Testicular size (mL) | Right/left | FSH, U/L | LH, U/L | Testosterone, Nmol/L | Cause of death |
|-----------|-----------|-----------------|-----------|-------------|---------------------|------------|---------|---------|---------------------|----------------|
| 1         | CML       | 4               | 5.6       | 1           | 2/2                 | ND         | ND      | ND      | ND                  | GVHD           |
| 4         | ALL       | 1.1             | 7.1       | 1           | 2/2                 | 1.4        | 0.1     | <0.4    | Relapse, multiorgan failure |
| 6         | ALL       | 1               | 7.7       | 1           | 2/2                 | 0.6        | 0.05    | <0.4    | Relapse, multiorgan failure |
| 9         | ALL       | 1               | 10.8      | 1           | 3/3                 | ND         | ND      | ND      | Relapse             |
| 11        | ALL       | 2.6             | 13.3      | 1           | 3/3                 | 1.2        | 0.3     | <0.4    | GVHD, respiratory failure |
| 16        | MDS       | 0.4             | 13.5      | 4           | 14/18               | 3.8        | 2.3     | 7.7     | EBV, lymphoma        |
| 18        | MDS       | 0.3             | 13.7      | 3           | 7/8                 | 2.3        | 1.5     | 2.3     | GVHD, multiorgan failure |

Abbreviations: ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; EBV, Epstein Barr virus; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; ND, not done.

2.5  Pubertal development

Of the 14 surviving boys, 10 were prepubertal or in very early puberty (Tanner 1-2) at the time of HSCT. All survivors had initiated or completed pubertal development within the normal range regarding genital growth and pubertal hair. At the last follow-up in the pediatric clinic, two boys had progressed to Tanner 2 and one to Tanner 3. The remaining 11 had completed their pubertal development to Tanner 4-5 according to genital stage and pubertal hair (Table 3). After HSCT, testicular volume remained subnormal in most boys; six had a volume <10 mL, three 10-15 mL, and two >15 mL.

Of the 14 surviving boys, 13 had bilateral (N = 7) or unilateral (N = 7) testicular biopsies. Most who underwent unilateral testicular biopsy had a similar testicular size to that of the contralateral testis at the last follow-up. In general, a small variation was noted in testicular size during follow-up after testicular biopsy. Four boys presented with a similar size on both sides, two had a slightly larger biopsied testis, and one (patient no. 2) presented with biopsied testes 5 mL smaller than the contralateral one. The seven boys who had bilateral biopsies had equal testicular size in one case, a small difference of 1 mL in four individuals, and a 2 mL difference in the remaining two.

2.6  Hormonal determinations

FSH, LH, and testosterone hormones were repeatedly sampled during follow-up in the 14 surviving patients. The measurements were within normal limits for age and pubertal stage, so only the results from the last visit are presented in Table 3. A few boys also had AMH and inhibin B analyzed at the last visit. Three boys had not yet passed puberty (still at Tanner stage 2-3). All boys had spontaneous pubertal development with increasing levels of testosterone. Eight of 11 boys who were prepubertal at time of HSCT and three boys who were in the early stage showed spontaneous and normal puberty to Tanner 4-5 and the levels of testosterone as adults were normal (>10 mmol/L). However, nine of the 14 boys had FSH levels above the reference levels and three also had LH levels above the reference levels (upper limits 12.5 U/L and 9.6 U/L, respectively).

AMH levels and inhibin were obtained for nine boys. The levels were normal for age and stage of puberty in two patients (nos. 2 and 8) and subnormal in the remaining seven, according to reference values. Inhibin B levels, measured in eight of 14 boys at the last follow-up, were normal according to the laboratory reference (5-355 ng/L for prepubertal boys and 25-325 ng/L for adult men). In five boys, the levels were within the reference range; one boy (no. 12) had lower limit levels and two (nos. 13 and 14) were below the normal range (Table 3).

2.7  Follow-up of boys transferred to adult care

Eight boys reached adulthood (Table 4); five of them agreed to provide new blood samples. Two boys (nos. 20 and 21), who both reached the age of 25 years more than 10 years after HSCT, are currently treated with testosterone supplementation. Three boys (nos. 10, 15, and 17) maintained normal testosterone levels without treatment. Inhibin B was measured in four boys: three had a very low levels, and one was within the normal range.

2.8  Sperm samples

Four of six boys who had a sperm test performed 4-9 years after HSCT presented with azoospermia (nos. 8, 10, 12, and 13). Two boys (nos. 2 and 15) showed a few mobile sperms that were cryopreserved for
| Patient# | Diagnosis       | Years after HSCT | Age, years | Tanner stage | Testicular size (mL) R/L | FSH, U/L | LH, U/L | Testosterone, AMH, Inhibin B, ng/L | Sperm analysis and new specimen for cryopreservation |
|----------|-----------------|------------------|------------|-------------|--------------------------|----------|---------|------------------------------------|------------------------------------------------------|
| 2        | AML             | 13.7             | 15.7       | 5           | 25/20                    | 6.5      | 5.8     | 18.0, 16.8, 98                     | Few sperm found and cryopreserved 13.7               |
| 3        | ALL             | 12               | 15         | 2           | 4/4                      | 1.4      | 0.9     | 1.1, 102, 150                      | ND                                                  |
| 5        | ALL             | 6.2              | 12.7       | 2           | 3/4                      | 1.7      | 0.6     | 0.5, 38.1, 91                      | ND                                                  |
| 7        | Lymphoma ALL    | 6                | 15         | 3           | 5/6                      | 13       | 6.8     | 14, 5.7, 46                        | ND                                                  |
| 8        | Neuroblastoma   | 6.3              | 16         | 4           | 12/10                    | 12       | 6       | 16, 11.7, 35                      | Azoospermia 10.7                                   |
| 10       | ALL             | 11.2             | 21         | 5           | 5/5                      | 58       | 23      | 18, ND, ND                         | Azoospermia 14.4                                   |
| 12       | MDS             | 7.3              | 18.4       | 5           | 5/5                      | 23       | 8       | 10, 1.3, 25                       | Azoospermia 11.3                                   |
| 13       | MDS             | 6                | 18.3       | 5           | 7/8                      | 17       | 11      | 16, 1.6, 21                       | Azoospermia 9.0                                     |
| 14       | ALL             | 6                | 18.2       | 4           | 5/6                      | 15       | 8.7     | 13, 5.9, <10                      | ND                                                  |
| 15       | AML             | 5                | 17.8       | 5           | 6/10                     | 7.7      | 3.9     | 17, ND, ND                         | Few sperm found and cryopreserved 10.0               |
| 17       | SAA/mDS         | 6.5              | 18.7       | 5           | 12/10                    | 19       | 3.6     | 11, 14.4, ND                       | ND                                                  |
| 19       | Thalassemia     | 5                | 19         | 5           | 9/8                      | 13       | 4.4     | 13, ND, ND                         | ND                                                  |
| 20       | Lymphoma        | 5                | 19         | 5           | 8/8                      | 12       | 12      | 8.7, ND, ND                        | ND                                                  |
| 21       | MDS             | 5                | 19         | 5           | 15/15                    | 12       | 4.8     | 17, ND, ND                         | ND                                                  |

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; ND, not done; SAA, severe aplastic anemia. Six children provided semen samples. In four cases, azoospermia was confirmed. In two cases, sperms were found and cryopreserved. Nine patients have reached adult age.
TABLE 4

| Background | Adult follow-up | Patient # | Diagnosis | Years after HSCT | Age, years | Tanner stage | Testosterone replacement | FSH, U/L | LH, U/L | AMH, µg/L | Inhibin B, ng/L | New sperm analysis | ND sperm cryopreserved at the last pediatric visit | ND sperm cryopreserved at the last pediatric visit |
|------------|----------------|-----------|-----------|-----------------|------------|--------------|--------------------------|----------|--------|-----------|----------------|------------------|----------------------|----------------------|
|            |                | 10        | Neuroblastoma | 7.8             | 18.5       | 5            | No                       | 16       | 12     | 6.4       | 12             | ND               | ND                   | ND                   |
|            |                | 15        | AML       | 8.3             | 18.5       | 5            | No                       | 16       | 7.7    | 3.9       | 17             | ND               | ND                   | ND                   |
|            |                | 17        | SAA/MDS  | 10.8            | 21.7       | 5            | No                       | 16       | 5.7    | 14        | 14             | ND               | ND                   | ND                   |
|            |                | 20        | Lymphoma | 10.5            | 25         | 5            | Yes                      | 14       | 1.7    | 1.4       | 1.7            | 32               | 16                   | 8.0                  |
|            |                | 21        | MDS       | 10.5            | 25         | 5            | Yes                      | 10       | 4.6    | 10        | 4.6            | 27               | 28.1                 | 13                   |

Abbreviations: HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; ND, not done; SAA, severe aplastic anemia.

In 2002, at the time of planning this study, only a few reports had appeared concerning FP in prepubertal or pubertal children and the overall experience worldwide was very limited. Our FP program had been running for over 10 years, and postpubertal adolescents with cancer were offered the possibility to bank sperms from semen samples.13 This had been an established option for several decades for FP of patients with cancer.8,10,11 However, broad inclusion of very sick children of prepubertal or pubertal age in studies offering an eventual option of achieving reproduction at adult age is difficult, as well as ethically debatable.8,11,12,20-22 These facts are probably reflected in the low number of patients included in this study over the years. However, that only one patient among the 26 approached patients and families declined participation is remarkable and reflects a high interest. Unfortunately, we did not keep a record of reasons why healthcare professionals did not approach boys and their families to join our study; nevertheless, the abundant literature identifies common barriers in discussing fertility issues with children with cancer.24-26 A number of boys also did not fulfill the inclusion criteria of having a high (>80%) risk for future infertility or they were too sick when they arrived to our unit for the extra planning needed.

Several centers have reported early experiences with testicular biopsies for boys before gonadotoxic treatment aiming at FP,12,27-30 as well as a similar high interest from patients and families for participation.24-26 Gupta et al.28 showed that participants considered a potential 25-30% risk for infertility as sufficient reason to undergo testicular biopsy. Our experience indicates that the patients and families considered the potential risks and benefits and generally wished to undergo an experimental procedure, despite the lack of methods to produce mature spermatozoa from prepubertal testicular tissue. However, the future risk for infertility was very high in our study (>80%, in our patients), and possibly even higher in relapsed patients with ALL planned for HSCT with TBI (see Figure 1). These factors likely increased the interest in FP despite the experimental nature of our study.

Figure 1 shows schematically the proportion of patients treated for ALL who attained a first remission (green box, approximately 75%). That group’s risk for infertility was no higher than that of the general population, at about 10-15% (yellow oval).31,32 Considering this low future risk, testicular biopsy may not be appropriate in these children. If these children are sufficiently mature to produce sperm samples during adolescence or early adulthood, cryopreservation may be feasible. Approximately 15% of ALL patients have a high-risk disease (red “high risk” box). After primary treatment, about 8% will require additional treatment due to relapse (red “relapse” box). This group of children has a higher risk for future infertility than the standard risk group (green box 74.5%). FP could be considered at time point A or B for the high risk group, but there is a risk, of unknown magnitude, to spread the circulating blasts into testes with the surgical intervention, potential future use. The remaining patients expressed anxiety for a negative result and preferred to delay the test.

3 | DISCUSSION

In 2002, at the time of planning this study, only a few reports had appeared concerning FP in prepubertal or pubertal children and the overall experience worldwide was very limited. Our FP program had been running for over 10 years, and postpubertal adolescents with cancer were offered the possibility to bank sperms from semen samples.13 This had been an established option for several decades for FP of patients with cancer.8,10,11 However, broad inclusion of very sick children of prepubertal or pubertal age in studies offering an eventual option of achieving reproduction at adult age is difficult, as well as ethically debatable.8,11,12,20-22 These facts are probably reflected in the low number of patients included in this study over the years. However, that only one patient among the 26 approached patients and families declined participation is remarkable and reflects a high interest. Unfortunately, we did not keep a record of reasons why healthcare professionals did not approach boys and their families to join our study; nevertheless, the abundant literature identifies common barriers in discussing fertility issues with children with cancer.24-26 A number of boys also did not fulfill the inclusion criteria of having a high (>80%) risk for future infertility or they were too sick when they arrived to our unit for the extra planning needed.

Several centers have reported early experiences with testicular biopsies for boys before gonadotoxic treatment aiming at FP,12,27-30 as well as a similar high interest from patients and families for participation.24-26 Gupta et al.28 showed that participants considered a potential 25-30% risk for infertility as sufficient reason to undergo testicular biopsy. Our experience indicates that the patients and families considered the potential risks and benefits and generally wished to undergo an experimental procedure, despite the lack of methods to produce mature spermatozoa from prepubertal testicular tissue. However, the future risk for infertility was very high in our study (>80%, in our patients), and possibly even higher in relapsed patients with ALL planned for HSCT with TBI (see Figure 1). These factors likely increased the interest in FP despite the experimental nature of our study.

Figure 1 shows schematically the proportion of patients treated for ALL who attained a first remission (green box, approximately 75%). That group’s risk for infertility was no higher than that of the general population, at about 10-15% (yellow oval).31,32 Considering this low future risk, testicular biopsy may not be appropriate in these children. If these children are sufficiently mature to produce sperm samples during adolescence or early adulthood, cryopreservation may be feasible. Approximately 15% of ALL patients have a high-risk disease (red “high risk” box). After primary treatment, about 8% will require additional treatment due to relapse (red “relapse” box). This group of children has a higher risk for future infertility than the standard risk group (green box 74.5%). FP could be considered at time point A or B for the high risk group, but there is a risk, of unknown magnitude, to spread the circulating blasts into testes with the surgical intervention, potential future use. The remaining patients expressed anxiety for a negative result and preferred to delay the test.
thus increasing the risk of future testicular recurrence. Considering the present level of knowledge, FP by invasive methods may not be safe in that stage of treatment and could not be recommended. At time point C, immediately before HSCT is therefore the time of choice for FP using testicular aspiration or a biopsy. The conditioning before HSCT will promptly eliminate circulating blasts. The calculation of infertility risk in different stages of the disease illustrated in Figure 1 would be helpful for planning invasive and experimental methods for FP.

The biopsy itself should also not increase the risk for infertility in young boys. A recent study of boys (n = 64) followed with repeated ultrasound examinations for 1 year after unilateral testicular biopsy showed no significant difference in testicular size between the biopsied and the intact testis.33 Very few patients in that study had cancer that required HSCT. Another study30 described the procedures as safe and the biopsies (N = 52) were all unilateral to further decrease the potential risk of tissue damage. The authors recommend this strategy to others. Unilateral biopsies seem to be a safe procedure and should be preferred. In our study, the amount of tissue obtained and the choice of unilateral or bilateral biopsy was left to the discretion of the urologist performing the biopsy. Our study population was small so we could not further investigate differences among unilateral or bilateral procedures.

No clinically established treatment exists for using male prepubertal tissue for fertility treatment. The most promising recovery of fertility in the future seems to be retransplantation of the gonadal tissue obtained at a prepubertal age, similar to what is reported in women.34,35 However, in females, prepubertal ovarian tissue contains the immature oocytes enclosed in follicle structures that ensure production of mature oocytes. By contrast, sperms have not yet been formed in the prepubertal testis, although some recent success in mature sperm production, fertilization, and offspring has been reported following retransplantation of prepubertal testicular tissue in an experimental primate model.36 An additional challenge for young patients treated for cancer is that retransplantation of the tissue may introduce the risk of malignant cells hiding in the testicular tissue.37,38 Avoiding reintroduction of malignant cells would require development of in vitro methods for culture of prepubertal tissue to obtain mature spermatozoa; this has been successful in experimental xenotransplantation models.39 Reports on possible DNA damage and impaired imprinting due to methylation changes might represent risks to the offspring. Three boys in our study had cryopreserved mature spermatozoa obtained from the testicular biopsies in two and from sperm aspiration in one.

Some very difficult questions remain in this field, including the choice of diagnoses and the timing at which FP should be discussed and offered. Sadri-Ardekaniet al.,29 Wynset al.,30 and Ginsberget al.40 proposed inclusion only of boys who have not yet started cancer treatments. Our strategy was different, as we included patients in remission after treatment for leukemia and their biopsies were taken just before the planned HSCT. This means that most of our study subjects had previously undergone treatment with various chemotherapy combinations. Nevertheless, most biopsies showed a normal histology and the presence of spermatogonial cells, similar to previous findings even in advanced stages of cancer.41,42

Unfortunately, the current diagnostic methods available preclude an accurate prediction of which boys will relapse and need more treatment and possibly even HSCT. Those who reach the time point C in Figure 1 face a highly gonadotoxic treatment. FP should be recommended for these patients, even by methods still under development.43,44 After HSCT, very few patients remain fertile.5,9 In our study, only two boys (nos. 2 and 17) had a low number of live sperms after HSCT at time point D.

In the future, we want to cure our cancer patients while simultaneously overcoming serious long-term effects that impair quality of survival, such as infertility. To reach this goal, we need to continue to develop FP programs in close collaboration with pediatric hematologists, oncologists, endocrinologists, reproductive medicine specialists, embryologists, and other researchers. The expectations from patients, parents, and society are high.

ACKNOWLEDGMENTS

The authors thank the personnel of the Department of Pediatric Oncology of Karolinska University Hospital, particularly Anki Hjelt and Jacek Winiarski, and of the Department of Reproductive Medicine, especially Outi Hovatta, Victoria Keros, and Julius Hreinsson. This work was supported by grants from the Swedish Childhood Cancer Foundation, the Swedish Cancer Society, Radiumhemmet’s Research Grants, Stockholm County Council, and the Karolinska Institutet (to K.A.R.W.). Dr Rodriguez-Wallberg is supported by a Clinical Investigator Grant from Stockholm County Council and The Swedish Cancer Society.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, study design, inclusion of study subjects, acquisition and analysis of data, interpretation of data, writing initial draft, manuscript revision, and approval of the final draft: Borgström. Inclusion of study subjects, interpretation of data, manuscript revision, and approval of the final draft: Fridström. Inclusion of study subjects, interpretation of data, manuscript revision and approval of the final draft: Gustafsson. Interpretation of data, manuscript revision and approval of the final draft: Ljungman. Conceptualization, study design, inclusion of study subjects, acquisition and analysis of data, interpretation of data, writing initial draft, manuscript revision, and approval of the final draft: Rodriguez-Wallberg.

ORCID

Kenny A. Rodriguez-Wallberg https://orcid.org/0000-0003-4378-6181

REFERENCES

1. Ringden O, Bolme P, Borgstrom B, et al. The Stockholm experience with allogeneic bone marrow transplantation. Transplant Proc. 1986;18(1):119-122.
2. London WB, Bagattelli R, Weigel BJ, et al. Historical time to disease progression and progression-free survival in patients with recurrent/refractory neuroblastoma treated in the modern era on Children’s Oncology Group early-phase trials. Cancer. 2017;123(24):4914-4923.

3. Svenberg P, Remberger M, Uzunel M, et al. Improved overall survival for pediatric patients undergoing allogeneic hematopoietic stem cell transplantation—a comparison of the last two decades. Pediatr Transplant. 2016;20(5):667-674.

4. Cohen A, Bekassy AN, Gaiero A, et al. Endocrinological late complications after hematopoietic SCT in children. Bone Marrow Transplant. 2008;41(Suppl 2):S43-S48.

5. Thibaud E, Rodriguez-Macias K, Trivin C, Esperou H, Michon J, Brauner R. Ovarian function after bone marrow transplantation during childhood. Bone Marrow Transplant. 1998;21(3):287-290.

6. Tonorezos ES, Hudson MM, Edgar AB, et al. Screening and management of adverse endocrine outcomes in adult survivors of childhood and adolescent cancer. Lancet Diabetes Endocrinol. 2015;3(7):545-555.

7. Pfitzer C, Orawa H, Balcerek M, et al. Dynamics of fertility impairment and recovery after allogeneic hematopoietic stem cell transplantation in childhood and adolescence: results from a longitudinal study. J Cancer Res Clin Oncol. 2015;141(1):135-142.

8. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917-2931.

9. Salooja N, Szydlow RM, Socie G, et al. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. Lancet. 2001;358(9278):271-276.

10. Rodriguez-Wallberg KA, Oktay K. Fertility preservation during cancer treatment: clinical guidelines. Cancer Manag Res. 2014;6:105-117.

11. Rodriguez-Wallberg KA, Oktay K. Fertility preservation medicine: options for young adults and children with cancer. J Pediatr Hema tol/Oncol. 2010;32(5):390-396.

12. Picton HM, Wynn C, Anderson RA, et al. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys. Hum Reprod. 2015;30(11):2463-2475.

13. Rodriguez-Wallberg KA, Anastacio A, Vonhime E, Deen S, Malmros J, Borgström B. Fertility preservation for young adults, adolescents, and children with cancer. Ups J Med Sci. 2020;1:9. Epub ahead print.

14. Baert Y, Van Saen D, Haentjens P, In’t Veld P, Tournaye H, Goossens E. What is the best cryopreservation protocol for human testicular tissue banking? Hum Reprod. 2013;28(7):1816-1826.

15. Grundy R, Wyns C, Van Langendonckt A, et al. Management of fertility preservation for children treated for cancer (1): scientific advances and research dilemmas. Arch Dis Child. 2001;84(4):355-359.

16. Armuang GM, Nilsson J, Rodriguez-Wallberg KA, et al. Physicians’ self-reported practice behaviour regarding fertility-related discussions in paediatric oncology in Sweden. Psychooncology. 2017;26(10):1684-1690.

17. Grundy R, Larcher V, Gosden RG, et al. Fertility preservation for children treated for cancer (2): ethics of consent for gamete storage and experimentation. Arch Dis Child. 2001;84(4):360-362.

18. Lee SJ, Schover LR, Partridge AH, et al. American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol. 2016;34(12):1889-1895.

19. Sadri-Ardekani H, Akhondi MM, Vossough P, et al. Parental attitudes toward fertility preservation in boys with cancer: context of different risk levels of infertility and success rates of fertility restoration. Fertil Steril. 2013;99(3):796-802.

20. Fulbright JM, Raman S, McClellan WS, August KJ. Late effects of childhood acute lymphoblastic leukemia therapy. Curr Hematol Malig Rep. 2011;6(3):195-205.

21. Fallat ME, Hutter J. American Academy of Pediatrics Committee on Bioethics. Committee on Bioethics. Children’s Oncology Group Early-Phase Trials. Cancer. 2017;123(24):4914-4923.

22. Matthews SJ, Picton H, Ernst E, Andersen CY. Successful pregnancy in children with dipsogenic hypogonadotropic hyponadism and testicular tissue cryopreservation in children undergoing gonadotoxic therapy. J Clin Endocrinol Metab. 2011;96(6):2107-2109.

23. Nurmio M, Keros V, Lahteenmaki P, Salmi T, Kallajoki M, Jahnukainen K. Effect of childhood acute lymphoblastic leukemia therapy on spermatogonia populations and future fertility. J Clin Endocrinol Metab. 2009;94(6):2119-2122.

24. Yukihiro S, Ueno H, Iwai T, et al. Development of a pediatric fertility preservation program: a report From the Pediatric Initiative Network of the Oncofertility Consortium. J Adolesc Health. 2019;64(5):563-573.

25. Jurewicz M, Hiller B, Mehta S, Gilbert BR. Fertility preservation in pubertal and pre-pubertal boys with cancer. Pediatr Endocrinol Rev. 2018;15(3):234-243.

26. Moravek MB, Apiah LC, Anzado A, et al. Development of a pediatric fertility preservation program: a report From the Pediatric Initiative Network of the Oncofertility Consortium. J Adolesc Health. 2019;64(5):563-573.

27. Ginsberg JP, Carlson CA, Lin K, et al. An experimental protocol for fertility preservation in pre-pubertal boys recently diagnosed with cancer: a report of acceptability and safety. Hum Reprod. 2010;25(1):37-41.

28. Gupta AA, Donen RM, Sung L, et al. Testicular biopsy for fertility preservation in pre-pubertal boys with cancer: identifying preferences for procedure and reactions to disclosure practices. J Urol. 2016;196(1):219-224.

29. Jurewicz M, Hiller B, Mehta S, Gilbert BR. Fertility preservation in pubertal and pre-pubertal boys with cancer. Pediatr Endocrinol Rev. 2018;15(3):234-243.

30. Keros V, Hultenby K, Borgstrom B, Fridstrom M, Jahnukainen K, Hovatta O. Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. Hum Reprod. 2007;22(5):1384-1395.

31. Keros V, Rosenlund B, Hultenby K, Aghajanova L, Levkov L, Hovatta O. Optimizing cryopreservation of human testicular tissue: comparison of protocols with glycerol, propanediol and dimethylsulphoxide as cryoprotectants. Hum Reprod. 2005;20(6):1676-1687.

32. Wyns C, Van Langendonckt A, Donnez J, Wynn C. Can pre-pubertal human testicular tissue be cryopreserved by vitrification? Fertil Steril. 2011;95(6):2123.e9-e12.

33. Svenberg P, Remberger M, Uzunel M, et al. Improved overall survival for pediatric patients undergoing allogeneic hematopoietic stem cell transplantation—a comparison of the last two decades. Pediatr Transplant. 2016;20(5):667-674.

34. Rabo J, Sorensen K, Boas M, et al. Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. J Clin Endocrinol Metab. 2010;95(12):5357-5364.
40. Ginsberg JP, Li Y, Carlson CA, et al. Testicular tissue cryopreservation in pre-pubertal male children: an analysis of parental decision-making. Pediatr Blood Cancer. 2014;61(9):1673-1678.

41. Jahnukainen K, Stukenborg JB. Clinical review: present and future prospects of male fertility preservation for children and adolescents. J Clin Endocrinol Metab. 2012;97(12):4341-4351.

42. Pietzak EJ, 3rd, Tasian GE, Tasian SK, et al. Histology of testicular biopsies obtained for experimental fertility preservation protocol in boys with cancer. J Urol. 2015;194(5):1420-1424.

43. Joshi S, Savani BN, Chow EJ, et al. Clinical guide to fertility preservation in hematopoietic cell transplant recipients. Bone Marrow Transplant. 2014;49(4):477-484.

44. Rodriguez-Wallberg KA, Borgström B, Petersen C, et al. National guidelines and multilingual age-adapted patient brochures and videos as decision aids for fertility preservation (FP) of children and teenagers with cancer—A multidisciplinary effort to improve children’s information and access to FP in Sweden. Acta Obstet Gynecol Scand. 2019;98:679-680.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Borgström B, Fridström M, Gustafsson B, Ljungman P, Rodriguez-Wallberg KA. A prospective study on the long-term outcome of prepubertal and pubertal boys undergoing testicular biopsy for fertility preservation prior to hematologic stem cell transplantation. Pediatr Blood Cancer. 2020;67:e28507.
https://doi.org/10.1002/pbc.28507