Gonadal function in pediatric Fanconi anemia patients treated with hematopoietic stem cell transplant

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Abstract

Gonadal dysfunction and reduced fertility are clinical manifestations well described in patients with Fanconi anemia (FA) and following hematopoietic stem cell transplantation (HSCT). It is difficult to differentiate gonadal dysfunction from the primary disease itself or from HSCT procedures. Therefore, it is important to manage expectations about gonadal failure and infertility for all patients with FA, regardless of the HSCT status. We performed a retrospective analysis of 98 pediatric patients with FA who were transplanted between July 1990 and June 2020 to evaluate the incidence of gonadal dysfunction in female and male patients with FA. New-onset premature ovarian insufficiency (POI) was diagnosed in a total of 30 (52.6%) patients. Follicle-stimulating hormone and luteinizing hormone levels were increased in patients diagnosed with POI. Anti-Mullerian hormone levels declined in POI patients after HSCT (r^2=0.21; *P*=0.001). Twenty (48.8%) male patients were diagnosed with testicular failure. Follicle-stimulating hormone levels increased after HSCT even in patients without testicular failure (r^2=0.17; *P*=0.005). Inhibin B levels decreased over time after HSCT in patients with testicular failure (r^2=0.14; *P*=0.001). These data indicate brisk decline in already impaired gonadal function in transplanted children with FA.

Introduction

Fanconi anemia (FA) is an inherited DNA repair disorder with heterogeneous clinical manifestations including bone marrow failure, cancer predisposition and other congenital anomalies. Gonadal dysfunction with infertility is commonly described in patients with FA.[1-3] Previous reports estimate that up to 65% of patients with FA have gonadal dysfunction.[1,2] Cytopenias and progressive bone marrow failure occur early in life during childhood in patients with FA.[4] Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative option for bone marrow failure in patients with FA and is typically required during the first two decades of life. Gonadal dysfunction, manifest as premature ovarian insufficiency (POI) or testicular failure, is also a frequent endocrinologic complication in long-term survivors after HSCT.[5-10] Few studies have evaluated gonadal dysfunction and fertility outcomes in both females and males with FA who have completed HSCT.[11-14] Here, we report our observations of pubertal development, POI and testicular failure in female and male patients with FA who were treated with HSCT.

Methods

Patient demographics and data collection

We performed a retrospective cohort analysis of all female and male patients with FA who completed HSCT between July 1990 and June 2020 either at Cincinnati Children's Hospital Medical Center (CCHMC) or at outside centers who had longitudinal pubertal and reproductive hormonal data from follow-up visits conducted at CCHMC. Patients with at least 1 year of follow-up data and were evaluated at CCHMC at our Fanconi Anemia Comprehensive Care Center within the last 5 years at the time of data analysis were included. This study was approved by our Institutional Review Board. Patients with more than one transplant episode were included. Patients were evaluated by physical exam and laboratory evaluation at each visit by a medical provider, including at least one annual assess-

ment by an endocrinologist. Records for each patient were reviewed for demographic and transplant-related clinical information.

Hematopoietic stem cell transplantation procedures

Transplant procedures were chosen according to the treating physician per institution practices. Prior to 2009, CCHMC used a low-dose radiation (450 cGy) based HSCT preparative regimen for all patients with FA. Starting in 2009, CCHMC eliminated radiation and transitioned to a busulfan-based preparative regimen for patients with FA. Further information on fertility preservation service practices are outlined in the *Online Supplementary Appendix*.

Pubertal development

Puberty stage was defined by clinical examination and pubertal hormone lab values for females and males. Puberty onset was described as either spontaneous or induced by exogenous estrogen and testosterone supplementation. Patients were classified as prepubertal if there were no secondary sexual features (breast buds for girls and testis volume ≤3 mL for boys), nor evidence of luteinizing hormone (LH) activation, which is characterized by pulsatile LH secretion at the onset of puberty. Delayed puberty was defined as the absence of any evidence of LH activation nor secondary sexual features after the age of 13 years for girls and 14 years for boys. Patients with missing data and patients who were also already pubescent at the time of transplantation were excluded from the analysis to determine if HSCT affected progression into puberty.

Diagnosis of premature ovarian insufficiency and testicular failure

POI in prepubertal females was defined as those with undetectable anti-Mullerian hormone (AMH) and in pubertal females as those with follicle-stimulating hormone (FSH) >20 mIU/L, or undetectable serum AMH with absent menstrual periods. Testicular failure was defined in pubertal males as serum FSH >20 mIU/L and/or serum LH >10 mIU/L. The diagnosis of POI or testicular failure was made for each patient at the first instance any patient met these diagnostic criteria. Further details on reproductive hormone assessments are outlined in the *Online Supplementary Appendix*.

Statistical analysis

Median and range, or number (n) and percentage are reported for continuous or categorical patient characteristic variables. Median and range are reported for numerical parametric data. Differences in frequencies between groups were determined using the X^2 test or Fisher's exact tests when the sample size was small. A simple regression model was used to quantify the relationship between serum hormone levels and time from HSCT. Differences were considered significant when *P* value was <0.05.

Results

Cohort description

Demographic and transplant data for all patients are shown in Table 1. Data in the electronic medical record were available for a total of 98 female (n=57) and male (n=41) patients with FA. We excluded patients who had no clinical or hormonal data available for analysis (n=7). All patients had a diagnosis of FA confirmed by increased chromosomal breakage following exposure to DNA cross-linking agents such as mitomycin C or diepoxybutane (DEB). Sequencing of FA complementation group (*FANC*) genes was available in 42.9% (n=42) of patients. Median age at time of HSCT was 8.2 years (range, 4.8-24.9 years) in female patients and 8.8 years (range, 3.7-18.7 years) in male patients. Two female patients were diagnosed with congenital urogenital anomalies including obliteration of the clitoral hood (n=1) and vulvar lesions with anorectal malformation (n=1). Six male patients were diagnosed with cryoptorchidism (n=4), hypospadias (n=1) and microphallus (n=1).

Female patients with Fanconi anemia and premature ovarian insufficiency

The majority of data was collected at the first visit to CCHMC before HSCT (n=91). The entire cohort of patients were followed for a median of 7.9 years (range, 0.11-31.1 years). The median follow-up time for female patients was 7.7 years (range, 0.1-24.7 years). The median follow-up time for male patients was 9.0 years (range, 0.6-31.3 years). Hormonal data after HSCT were available for a median of 5.8 years after HSCT (range, 0.1-20.0 years). New onset POI was diagnosed in 30 (52.6%) female patients during the course of the study (Table 2). Three patients (10%) were diagnosed with POI prior to HSCT and 27 (90%) after HSCT. Median age at POI diagnosis pre-HSCT was 10.6 years old (range, 5.8-12.6 years, n=3). Complementation group data were available only for 14 (46.7%) of patients diagnosed with POI. Eight (57.1%) of these patients had mutations in the *FANCA* complementation group. The remaining patients had mutations in the *FANCC* (n=1), *FANCF* (n=1), *FANCG* (n=1), *FANCI* (n=1), *FANCL* (n=1) and *FANCP* (n=1). For the three patients diagnosed with POI prior to HSCT, all were transplanted between the age of 8 and 11 years old after the year 2013. Additionally, only two patients had known *FANC* complementation data and were confirmed to have mutations in the *FANCA* complementation group. None of these patients had any congenital urogenital abnormalities. The diagnosis of POI was made at a median of 1 year (range, -4.4 to -0.2 years) prior to HSCT. Median

age at POI diagnosis after HSCT was 13.6 years (range, 8.2-26.9 years), at a median of 4.6 years (range, 0.6-15.4 years) after HSCT. Eight (26.7%) of patients diagnosed with POI had radiation as part of their conditioning regimen, while six (22.2%) of the patients who were not diagnosed with POI received radiation as part of their conditioning regimen. Additionally, only two (6.7%) patients diagnosed with POI were diagnosed with graft-*versus*-host disease (GvHD) at day 100 from HSCT.

Nineteen female patients (33.3%) required HRT with estrogen, four of whom were already on estrogen replacement therapy prior to HSCT. Nine patients with POI started HRT with estrogen at a median of 7.2 years (range, 0.3-15.9 years) after HSCT. Fertility preservation was offered to 28 female patients in this cohort. Serum AMH levels are measured in each patient at the time of consultation to determine ovarian reserve for potential ovarian tissue cryopreservation. Ovarian tissue cryopreservation services were accepted by 14 female patients (50%). There have been no attempts to use any cryopreserved ovarian tissue for pregnancy at the time of data analysis.

Table 1. Patient demographics and transplant characteristics.

Demographics	Females (N=57)	Males (N=41)
Race, N (%)		
Caucasian	48 (84.2)	34 (83.0)
African-American	5 (8.7)	4 (9.8)
Asian	2 (3.5)	1 (2.4)
Hispanic	0	1 (2.4)
Mixed/Other	2 (3.6)	1 (2.4)
Fanconi anemia complementation group, N (%)		
FANCA	10 (17.5)	5 (12.2)
Non-*FANCA*	11 (19.3)	16 (39.0)
Unknown	36 (63.2)	20 (48.8)
Number of transplant episodes, N (%)		
1	57 (91.9)	41 (97.6)
2	5 (8.1)	1 (2.4)
Median age at transplant, years (range)	8.2 (4.8-24.9)	8.8 (3.72-18.7)
Median age at last follow-up, years (range)	17.1 (6.5-32.1)	9.0 (0.6-31.3)
Stem cell donor type, N (%)		
Related	12 (21.1)	9 (22.0)
Unrelated	45 (78.9)	32 (78.0)
Stem cell source, N (%)		
Bone marrow	10 (17.5)	10 (24.4)
Peripheral blood	44 (77.2)	26 (63.4)
Cord blood	3 (5.3)	5 (12.2)
Conditioning regimen, N (%)		
Radiation-based	15 (26.3)	12 (29.3)
Busulfan-based	42 (73.7)	29 (70.7)
HLA match, N (%)		
Matched	32 (56.1)	27 (65.9)
Mismatched	25 (43.9)	14 (34.1)
GvHD prophylaxis, N (%)		
Calcineurin inhibitor-based	20 (35.1)	24 (58.5)
Steroids	20 (35.1)	18 (43.9)
T-cell depletion	32 (56.1)	20 (48.8)
Other	5 (8.8)	4 (9.8)
GvHD grade at day 100, N (%)		
0	47 (82.5)	28 (68.2)
1	4 (7.0)	2 (4.9)
2	1 (1.7)	1 (2.4)
3	3 (5.3)	3 (7.3)
4	0	0
Unknown	2 (3.5)	5 (12.2)
Congenital urogenital anomalies, N (%)	2 (3.5)	6 (14.6)

GvHD: graft-*versus*-host disease.

Male patients with Fanconi anemia and testicular failure

New-onset testicular failure was diagnosed in 20 (48.8.%) male patients during the course of the study (Table 2). Complementation group data were available only for ten (50%) of patients diagnosed with testicular failure. Four (40%) of these patients had mutations in the *FANCA* complementation group. The remaining patients had mutations in the *FANCB* (n=1), *FANCC* (n=3), *FANCD2* (n=1) and one patient had homozygous mutations in the *FANCE* complementation group and a heterozygous mutation in the *FANCF* complementation group. Three patients (15%) were diagnosed with testicular failure a median of -0.1 years (range, -0.13 to -0.7 years) before HSCT at a median age of 17.8 years old (range, 7.3-18.5 years). These three patients were transplanted at 7 years (n=1) and 18 years (n=2) of age. We only had complementation group data available for two of the patients. One patient had a mutation in the *FANCB* complementation group, the other had homozygous mutations in the *FANCE* complementation group and a heterozygous mutation in the *FANCF* complementation group. None of these patients had any congenital urogenital anomalies. Seventeen patients (85%) were diagnosed with testicular failure after HSCT at a median age of 15.5 years (range, 9.5-34.6 years), a median of 5.4 years (range, 0.7-30.4 years) after HSCT. While no statistical significance or direct correlation can be provided, four (20%) of the patients diagnosed with testicular failure received radiation therapy as part of their conditioning regimen. Six (28.6%) of the patients who were not diagnosed with testicular failure received radiation therapy as part of their conditioning regimen. Additionally, two (10%) of patients diagnosed with testicular failure were diagnosed with GvHD at day 100 from HSCT.

Three patients required HRT with testosterone. One patient

Table 2. Gonadal failure and pubertal outcomes in female and male patients with Fanconi anemia.

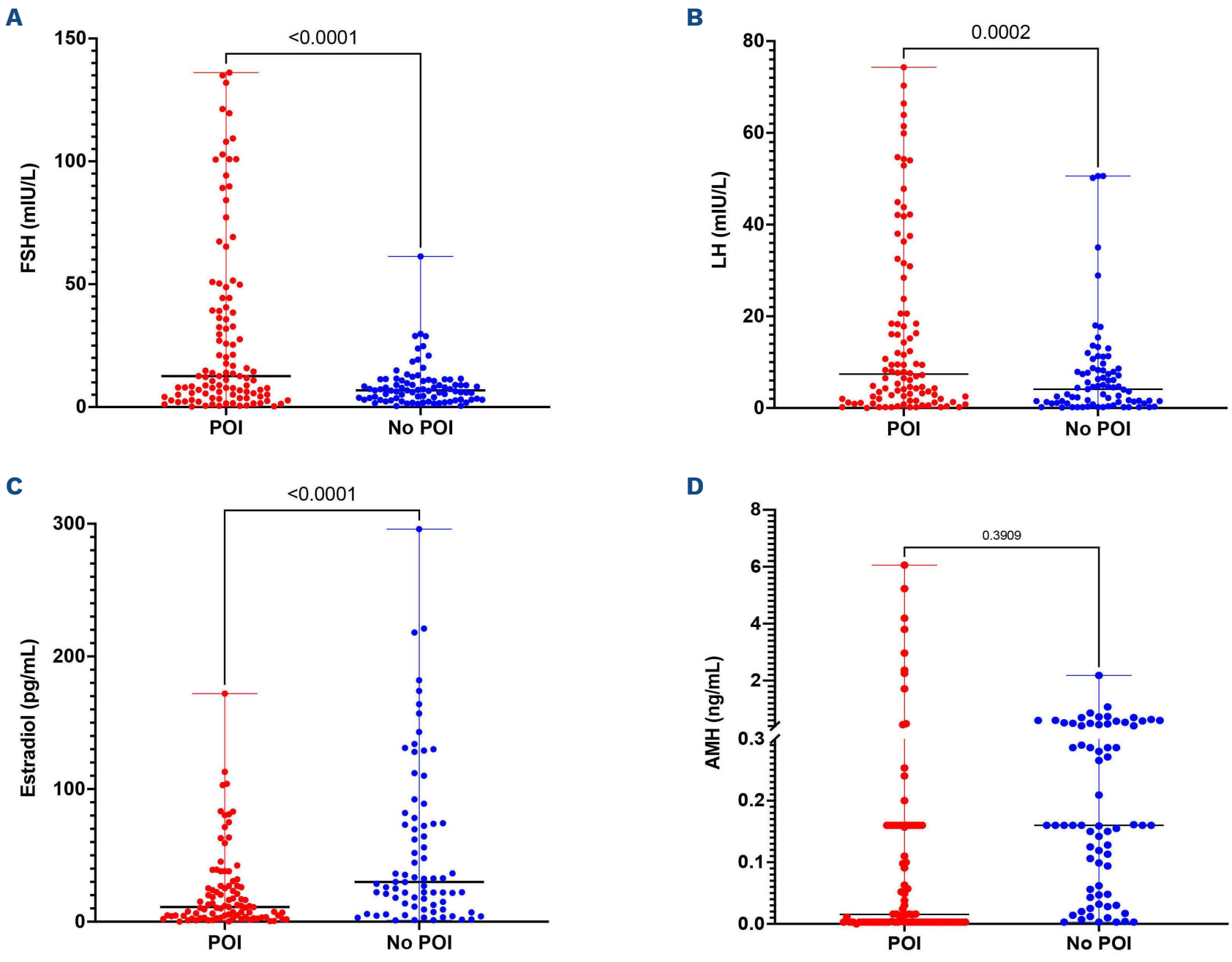
	Females (N=57)	Males (N=41)
Spontaneous puberty, N (%)	24 (82.8)	21 (77.8)
Delayed puberty, N (%) Pre-HSCT Post-HSCT	5 (17.2) 0 5 (100)	6 (22.2) 2 (33.3) 4 (66.7)
POI diagnosis, N (%) Pre-HSCT Post-HSCT	30 (52.6) 3 (10) 27 (90)	-
Age at POI diagnosis in years, median (range) Pre-HSCT Post-HSCT	 10.6 (5.8-12.6) 13.6 (8.2-26.9)	-
Time to diagnosis from HSCT in years, median (range) Pre-HSCT Post-HSCT	 -1 (-4.4 to -0.2) 4.6 (0.6-15.4)	-
Testicular failure, N (%) Pre-HSCT Post-HSCT	- - -	20 (48.8) 3 (15) 17 (85)
Age at testicular failure diagnosis in years, median (range) Pre-HSCT Post-HSCT	 - -	 17.8 (7.3-18.5) 15.5 (9.5-34.6)
Time to diagnosis from HSCT in years, median (range) Pre-HSCT Post-HSCT	 - -	 -0.1 (-0.7 to -0.14) 5.4 (0.7-30.4)
Hormone replacement therapy, N (%) Total Pre-HSCT Post-HSCT	 19 (33.3) 4 (21.1) 15 (78.9)	 3 (7.3) 1 (33.3) 2 (66.7)
Time from HSCT that HRT was started in years, median (range) Pre-HSCT Post-HSCT	 -2.4 (-4,1 to -0,8) 7.1 (0.3-15.9)	 -5.6 5.6 (0.3-11)
Fertility preservation services accepted, N (%) Total offered Accepted	 28 14 (50)	 14 1 (7.1)
Successful pregnancy, N (%)	0	0

Pre-HSCT: before HSCT; Post-HSCT: after HSCT; POI: premature ovarian insufficiency; HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation; HRT: hormone replacement therapy.

was started on hormone replacement therapy (HRT) with testosterone -5.6 year prior to HSCT. The other two patients were started on testosterone 0.3 and 11 years after HSCT. Fertility preservation services were offered to 14 patients. Testicular tissue (n=1) and sperm cryopreservation (n=1) were accepted by only two patients (14.2%). The quality of the sperm are unknown. There was no report of a pregnancy in the partners of the male FA patients.

Hormonal data in female Fanconi anemia patients with and without premature ovarian insufficiency

Female reproductive hormone levels (FSH, LH, oestradiol and AMH) in patients diagnosed with POI and without POI are presented in Figure 1. As expected, serum FSH and LH levels were increased in patients with POI compared to patients without POI (Figures 1A, B). Median FSH levels in patients with POI were 12.6 mIU/L (range, 0.2-136.1 mIU/L)



	POI	No POI	*P*
Reproductive hormones, median (range)			
FSH, mIU/L	12.6 (0.2-136.1)	6.8 (0.4-61.3)	<0.0001
LH, mIU/L	7.4 (0.02-74.3)	4.1 (0.1-50.6)	0.0002
Estradiol, pg/mL	11.1 (0.15-116)	29.95 (1-296)	<0.0001
AMH, ng/mL	0.0155 (0.003-6.057)	0.16 (0.003-2.182)	0.39

Figure 1. Hormonal data analysis for female Fanconi anemia patients with premature ovarian insufficiency and without premature ovarian insufficiency. Hormonal data analysis of female Fanconi anemia (FA) patients with premature ovarian insufficiency (POI) or no POI. These hormone levels were measured pre- and post-hematopoietic stem cell transplantation (HSCT) (A) The median serum follicle-stimulating hormone (FSH) hormone level is 12.6 mIU/L in POI patients and 6.8 mIU/L in patients without POI; *P*<0.0001. (B) The median serum luteinizing hormone (LH) hormone level is 7.4 mIU/L in POI patients compared to 4.1 mIU/L in patients without POI; *P*=0.0002. (C) The median serum oestradiol level is 11.1 pg/mL in POI patients compared to 29.95 pg/mL in patients without POI; *P*<0.0001. (D) The median anti-Mullerian hormone (AMH) is 0.0155 ng/mL in POI patients compared to 0.16 ng/mL in patients without POI; *P*=0.39.

compared to 6.8 mIU/L (range, 0.4-61.3 mIU/L) in patients without POI (P<0.0001). Median LH levels were 7.4 mIU/L (range, 0.02-74.3 mIU/L) in patients with POI which was significantly higher compared to patients without POI (4.1 mIU/L; range, 0.1-50.6; P=0.0002). Seven female patients with FA had FSH >20 mIU/L but were prepubertal at the time of hormone analysis. Estradiol and AMH levels were higher in patients without POI (Figures 1C, D). Median estradiol levels were lower in patients with POI, 11.1 pg/mL (range, 0.15-116 pg/mL) compared to patients without POI (30 pg/mL; range 1-296 pg/mL; P<0.0001). AMH levels were not significantly different in patients diagnosed with POI (median 0.0156 ng/mL; range, 0.003-6.057 ng/mL) and patients without POI (median 0.16 ng/mL; range, 0.003-2.182 ng/mL; P=0.39). Simple regression analysis showed rising FSH levels in patients with POI after HSCT (r^2=0.01; P=26). There was no correlation of FSH levels in patients without POI (r^2=0.0001; P=0.92) after HSCT (Figure 2A). AMH levels decreased significantly over time from HSCT in patients diagnosed with POI (r^2=0.21; P=0.001). AMH levels did not decrease significantly in those without POI (r^2=0.02; P=0.27) (Figure 2B).

Hormonal data analysis for male patients with and without testicular failure

Data on male reproductive hormone levels (FSH, LH, testosterone and inhibin B) in patients diagnosed with and without testicular failure are presented in Figure 3. Levels of serum FSH and LH were significantly higher in patients with testicular failure compared to patients without (Figures 3A, B). FSH levels in patients with testicular failure were 20.5 mIU/L (range, 0.2-243 mIU/L) compared to 4.1 mIU/L (range, 0.5-16.9 mIU/L) in patients without testicular failure (P<0.0001). Median LH levels were 6.1 mIU/L (range, 0.2-60.3 mIU/L) in patients with testicular failure which was significantly higher compared to patients without testicular failure (1.4 mIU/L; range, 0.2-15.4 mIU/L; P<0.0001). One patient had LH >10 mIU/L but was pre-pubertal at the time of hormone analysis and was not diagnosed with testicular failure. Interestingly, testosterone levels were higher in patients with testicular failure compared to patients without testicular failure (Figure 3C). Inhibin B levels were significantly decreased in patients with testicular failure (Figure 3D). FSH levels increased with time after HSCT (r^2=0.17; P=0.005; Figure 4A). Median testosterone levels were higher in patients with testicular failure, 348.4 ng/dL (range, 0.1-951.2 ng/dL) compared to patients without testicular failure (58.8 ng/mL; range 1-652 ng/dL; P<0.0001). Inhibin B levels were significantly lower in patients with testicular failure (median 44 pg/mL; range, 3-304 pg/mL) and patients without testicular failure (median 111 pg/mL; range 59-304 pg/mL; P=0.003). Inhibin B levels declined significantly after HSCT in patients diagnosed with testicular failure (r^2=0.14; P=0.001) (Figure 4B).

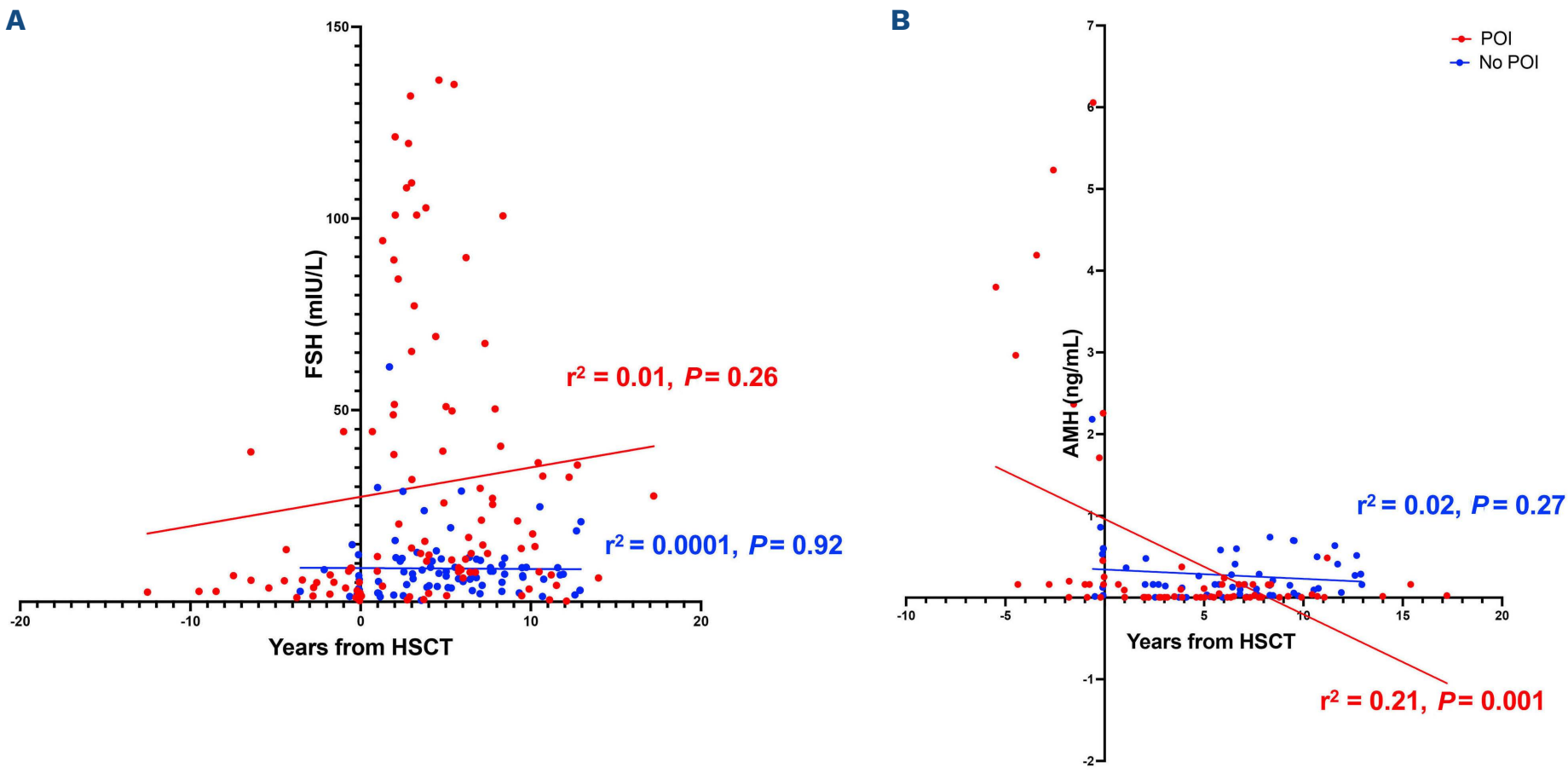
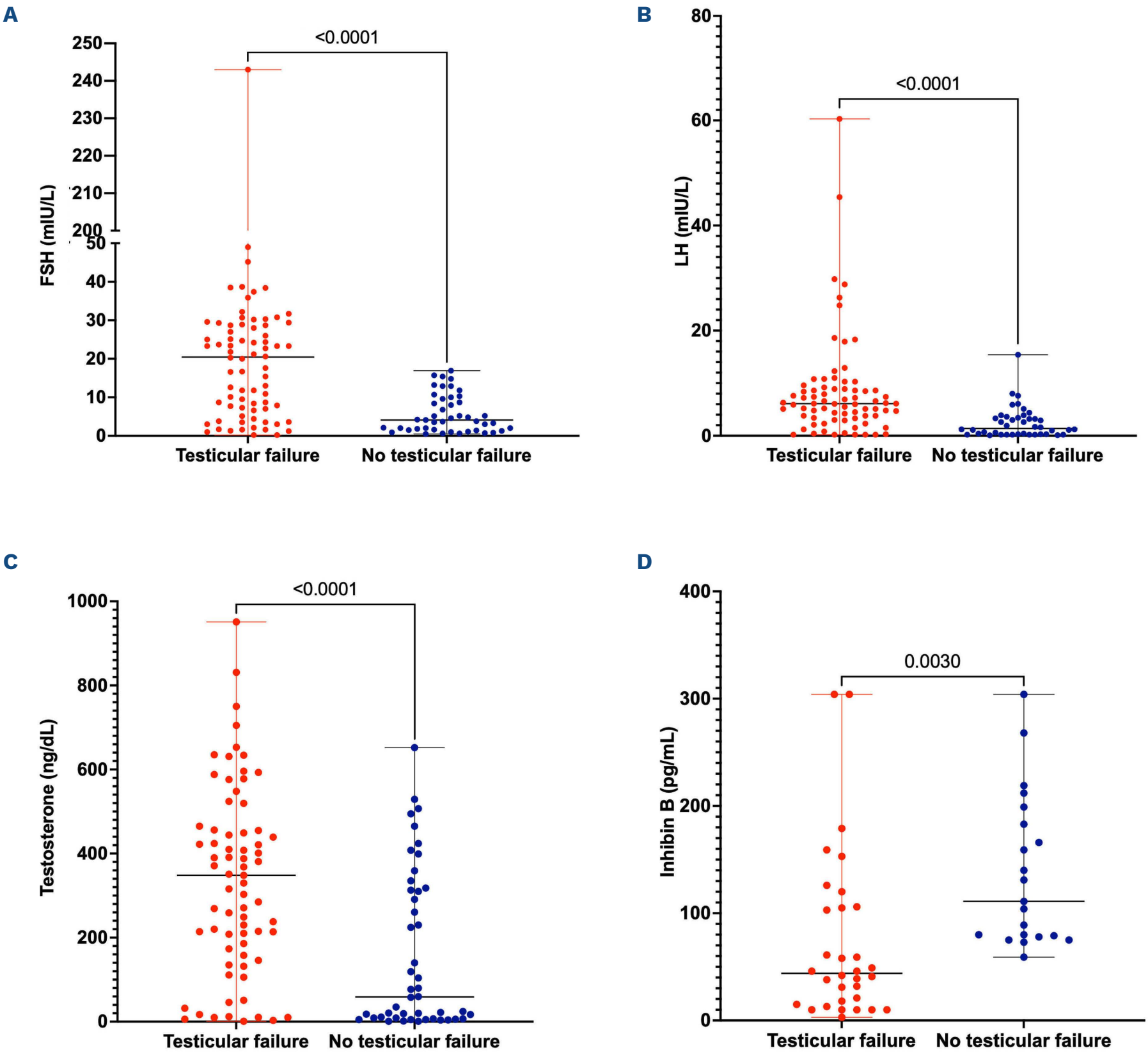


Figure 2. Longitudinal analysis of follicle stimulating hormone and anti-Mullerian hormone levels in transplanted female Fanconi anemia patients. Simple linear regression analysis of serum (A) follicle-stimulating hormone (FSH) and (B) anti-Mullerian hormone (AMH) levels in patients with primary ovarian insufficiency (POI) compared to female patients without POI over time in years from hematopoietic stem cell transplant (HSCT). The overall regression was not statistically significant for serum FSH levels in patients diagnosed with POI (r^2=0.01; P=0.26). The overall regression was statistically significant for serum AMH levels in patients with POI (r^2= 0.21; P=0.001).



	Testicular failure	No testicular failure	*P*
Reproductive hormones, median (range)			
FSH, mIU/L	20.45 (0.2-243)	4.1 (0.5-16.9)	<0.0001
LH, mIU/L	6.1 (0.2-60.3)	1.4 (0.2-15.4)	<0.0001
Testosterone, ng/dL	348.4 (1-951.2)	58.8 (1-652)	<0.0001
Inhibin B, pg/mL	44 (3-304)	111 (59-304)	0.003

Figure 3. Reproductive hormonal data analysis for male Fanconi anemia patients with testicular failure and without testicular failure. Hormonal data analysis of male Fanconi anemia (FA) patients with testicular failure or no testicular failure. (A) The median serum follicle-stimulating hormone (FSH) hormone level is 20.45 mIU/L in testicular failure patients and 4.1 mIU/L in patients without gonadal insufficiency; *P*<0.0001. (B) The median serum luteinizing hormone (LH) hormone level is 6.1 mIU/L in testicular failure patients compared to 1.4 mIU/L in patients without testicular failure; *P*<0.0001. (C) The median serum testosterone level is 348.4 ng/mL testicular failure patients compared to 58.8 ng/dL in patients without testicular failure; *P*<0.0001. (D) The median inhibin B 44 pg/mL in testicular failure patients compared to 111 pg/mL in patients without testicular failure; *P*=0.003.

Pubertal outcomes in female and male patients

Pubertal data were available for a total of 45 female and male patients with FA. Twenty-four female patients (88.9%) entered spontaneous puberty as defined in the methods. Puberty was delayed in five (20.8%) female patients. Three female patients (11.1%) required estrogen replacement therapy to induce pubertal onset. Spontaneous puberty was achieved in 21 (95.5%) male patients, and was delayed in six patients (27.3%).

Discussion

In this retrospective single-center study, we evaluated longitudinal reproductive and pubertal outcomes of 98 female and male patients with FA treated with HSCT. To our knowledge, this is the largest retrospective study evaluating reproductive outcomes in both pediatric and young adult female and male patients with FA after HSCT. Overall, we observed a high proportion of patients with POI or testicular failure. Prior studies have analyzed fertility and pregnancy outcomes in transplanted patients with FA but focused only on adolescent or adult female patients, excluding a large proportion of this population.[12,15-17] In addition, available data include only very small number of male patients with FA,[3,18] or only a focus on patients with a primary malignancy diagnosis and include little semen data.[19,20]

We initially anticipated that HSCT overall would contribute to a higher incidence of POI and testicular failure because chemotherapy and radiation administered prior to HSCT are known to lead to gonadal failure.[15] However, our rates of POI and testicular failure were lower than previously reported incidences in smaller cohorts.[21] Furthermore, patients who have not yet been diagnosed with POI or testicular failure completed HSCT at much younger ages and had shorter follow-up times at the time of analysis compared to the rest of the cohort. These patients may represent patients who have yet to be diagnosed with POI or testicular failure, and in fact our rates of POI and testicular failure may be underrepresented. Additionally, in our both female and male patients, there were clear differences in serum FSH and LH levels with POI or testicular failure. FSH and LH levels are typically used to diagnose POI or testicular failure.[22,23] However, our data show FSH levels do not significantly increase with time after HSCT in patients diagnosed with POI or testicular failure. This suggests FSH levels may not be the best hormone to follow longitudinally in patients diagnosed with POI or testicular failure after HSCT. We also expected that the majority of patients with testicular failure would have lower levels of testosterone compared to patients without testicular failure. A majority of these patients with higher testosterone levels were pubertal at the time of analysis. This is normal physiologically, based on known mechanisms of pubertal

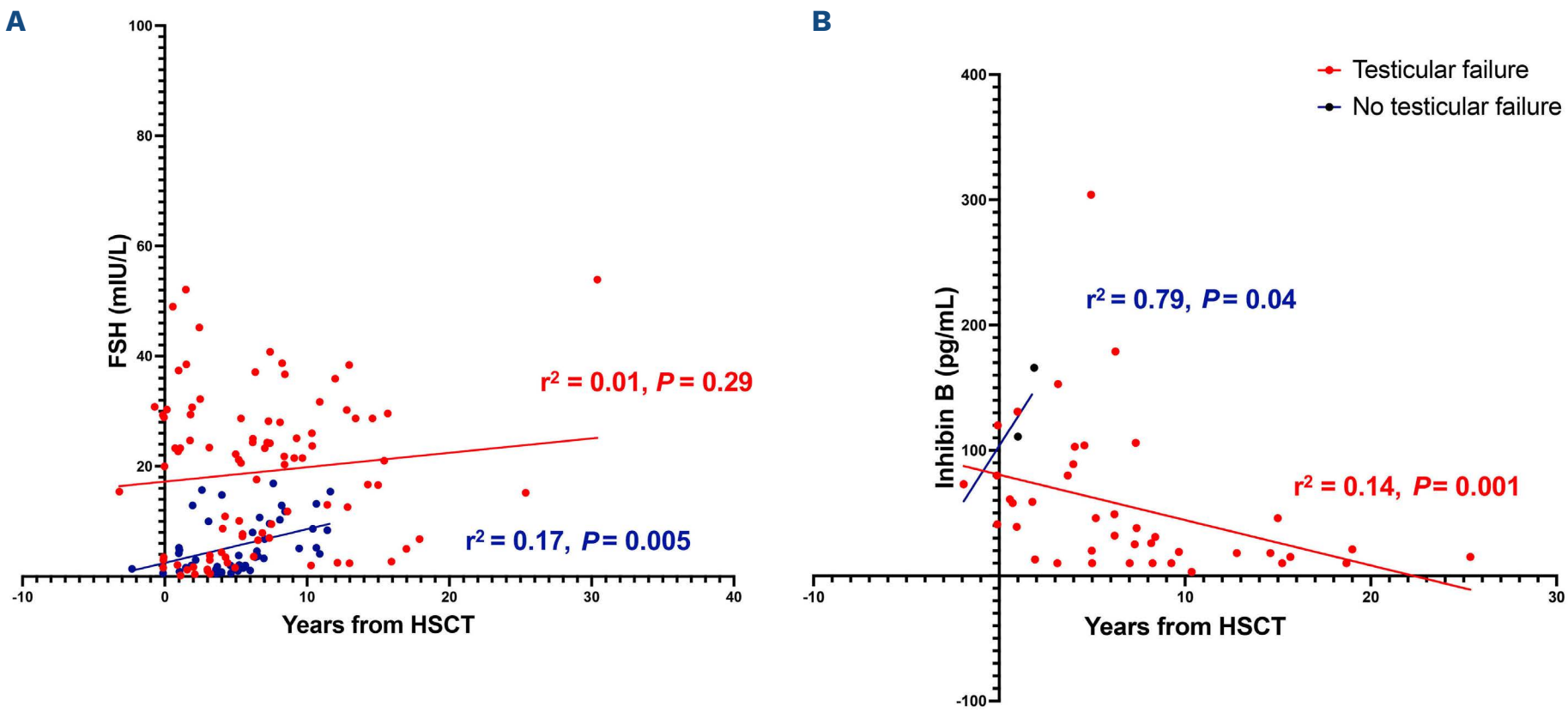


Figure 4. Longitudinal analysis of follicle stimulating hormone and inhibin B levels in transplanted male Fanconi anemia patients. Simple linear regression analysis of serum (A) follicle-stimulating hormone (FSH) and (B) inhibin B levels in patients with testicular failure compared to male patients without testicular failure over time in years from hematopoietic stem cell transplantation (HSCT). The overall regression was not statistically significant for serum FSH levels (r^2=0.01; *P*=0.29) in patients diagnosed with testicular failure. The overall regression was statistically significant for serum inhibin B levels (r^2=0.14; *P*=0.001) in patients diagnosed with testicular failure.

changes that occur where increases in gonadotropin releasing hormone (GnRH) cause changes in testosterone levels. No patients on HRT were included in this analysis. Additionally, testosterone can be created in the peripheral tissue from dehydroepiandrosterone and andreostenedione precursors, which may contribute to higher levels of testosterone in the testicular failure cohort.[24] These data indicate that testosterone may not be the best marker to use to diagnose testicular failure in male patients with FA.

Previous reports have shown AMH levels tend to be low in women with FA, regardless of the HSCT status.[12,25] Our data support this trend as we observed decreased AMH levels in female patients with FA after HSCT. However, there was no statistically significant difference between AMH levels in patients with POI compared to patients without POI. Levels of AMH were measured longitudinally before and after HSCT. Interestingly, our data demonstrate that AMH levels continue to decline many years after HSCT in patients with POI. Conversely, AMH levels are largely unchanged after HSCT in patients without POI. We add to the evidence that AMH levels may be a more suitable longitudinal hormonal marker to follow after HSCT in patients diagnosed with POI. In addition, AMH levels should be followed for extended periods of time after HSCT as these levels continue to decline without a plateau.

Inhibin B levels are reported to be lower in male patients with FA.[12,26] We also observed lower inhibin B levels in patients diagnosed with testicular failure. FSH levels did not reliably increase after HSCT in patients with testicular failure. Conversely, serum inhibin B levels did decline after HSCT in patients with testicular failure. This decline continued for decades after HSCT. If patients are being followed for testicular failure after HSCT, they should have regular inhibin B levels checked long-term as this is a more reliable marker for testicular function after HSCT.

We saw only a few cases of pubertal delay in this study. The actual incidence of pubertal delay in patients with FA is unknown. Chemotherapy and radiation regimens administered prior to HSCT are known to cause reproductive disturbances leading to incomplete or absent puberty and gonadal failure in other clinical settings.[27] However, the vast majority of patients who had complete pubertal clinical and laboratory data entered spontaneous puberty.

Fertility is often impaired in female patients with FA due to premature menopause and shortened fertility windows. Similarly, poor or absent spermatogenesis in male patients with FA leads to impaired fertility.[1,15] It is our institution's policy to offer fertility preservation services to all HSCT recipients. In order to preserve patient autonomy, the patient and their families ultimately decide whether they choose to pursue and accept fertility preservation prior to HSCT. Each family and patient receives an individualized consultation with extensive counseling by our fertility preservation team before HSCT procedures begin. A modest proportion of patients with FA consented to fertility preservation services prior to HSCT. The rates of fertility preservation in female patients was higher, although significantly lower in males, when both were compared to previously reported rates of fertility preservation services being accepted.[28,29] Fertility preservation in prepubertal males is currently in preclinical experimental stages and may be an explanation for decreased uptake in male patients.

Our work has strengths and limitations. The strengths include the large number of patients with FA treated with HSCT at a single institution. An additional strength of our study includes the length of follow-up we had for patients compared to prior studies.[15] The retrospective nature of the study is a limitation and data are necessarily incomplete. We are unable to make a direct comparison of gonadal dysfunction outcomes in patients who were transplanted using a radiation-based or busulfan-based preparative regimen because this data is confounded by changes in institutional transplant-related practices over time. And while outside the scope of this paper, we were not able to make the connection between gonadal failure and disease severity of FA. This information may be important for prompting earlier and timely assessment of fertility function before and after transplant in patients with certain physical manifestations of FA.

Also, serum AMH levels are not a widely accepted marker for POI diagnosis, however, they serve as a better option in prepubertal patients. However, these data highlight the importance that prolonged, consistent, regular follow-up with experienced endocrinologists are needed to make sure these complications are being identified appropriately. Complications after HSCT that may perplex the diagnosis of POI include GvHD that affects the genitourinary tract, especially in female patients. This makes it especially important for patients with POI to be followed closely by an experienced endocrinologist who is astute in diagnosing and monitoring these endocrinologic complications after HSCT. These patients would need to be followed through time with regular, annual visits with an endocrinologist to closely monitor pubertal transitions and to make the diagnoses of POI or testicular failure. Additionally, AMH levels may be affected by unilateral oophorectomy. This analysis would be deeply confounded by differences in the time period HSCT was completed for the patient and that our institution changed our way of practice to eliminate radiation in place of busulfan. Our data show that long-term follow-up of patients with FA who have completed HSCT should always include regular physical exams and hormonal as-

sessments. Prospective studies in this area would be of value.

Disclosures

No conflicts of interest to disclose.

Contributions

JK is the principal investigator and KCM the senior author. JK, JCH, JMR, PAM, PAM, SMD and KCM developed the Concept and designed the study. JK and IGM collected and assembled data. JK, JCH, JMR and KCM analyzed and interpreted data. JK, IGM, JCH, JMR, PAM, SMD and KCM prepared and wrote the manuscript. All authors gave their final approval of the manuscript.

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Data-sharing statement

All data presented in this manuscript will be shared upon e-mail request.

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