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Bicalutamide does not raise transaminases clinically significantly compared to alternative anti-androgen regimens among transfeminine adolescents and young adults: a retrospective cohort study

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ABSTRACT

***Background*:** Bicalutamide is a potential anti-androgen for transgender individuals with feminizing embodiment goals, but use is limited because of hepatotoxicity in cisgender men with prostate cancer. This study compared transaminase changes in transfeminine adolescents and young adults (AYA) using low dose bicalutamide with individuals using another androgen blockade.

***Methods*:** A retrospective analysis was conducted using electronic health record data for patients starting gender affirming hormone therapy with at least 10 months of follow-up between 2015 and 2023. The primary outcome was change in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) from baseline. Linear mixed models compared change in ALT and AST from baseline and maximum ALT and AST values in bicalutamide and comparison groups. Secondary outcomes included % individuals with ALT and AST elevation more than 1, 2, or 3 times the upper limit of normal (ULN) (Fisher's exact test), standardized mean estradiol dose by group (t test), and Tanner staging of breast tissue by group (Fisher's exact test).

***Results*:** Eighty-four transfeminine AYA (median age 18) taking bicalutamide were compared to 69 transfeminine AYA (median age 19) taking GnRH agonists, spironolactone or no agent in addition to estradiol. In linear mixed models adjusted for baseline age, race, BMI, baseline ALT or AST, and alcohol use, there were no clinically significant differences in delta or maximum ALT or AST in bicalutamide and comparison groups. No individuals had AST or ALT levels > 3x ULN though % with AST > ULN was higher for bicalutamide (10.7 v 1.5%, $p = 0.02$). Estradiol doses and Tanner stages were similar between groups among individuals receiving pediatric care.

***Conclusion*:** Bicalutamide was not associated with a clinically significant change in transaminases as compared with other anti-androgen regimens over one year. Bicalutamide appears to be a safe anti-androgen for transfeminine individuals at low dose with close monitoring and deserves further study.

KEYWORDS
Adolescent and young adult (AYA); anti-androgen; bicalutamide; hepatotoxicity; transgender

Introduction

Transgender people have various options available to align their physical attributes with their gender. For transgender people seeking feminine embodiment goals (i.e., transfeminine people), medical recommendations from the World Professional Association of Transgender Health (WPATH) and the Endocrine Society include estradiol with or without use of anti-androgens such as GnRH agonists (leuprolide, histrelin), cyproterone acetate, 5-alpha reductase inhibitors (e.g. finasteride) or spironolactone (Coleman et al., 2022; Hembree et al., 2017).

Bicalutamide is an anti-androgen that blocks the binding of dihydrotestosterone and testosterone to the androgen receptor. With this blockade,

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testosterone levels naturally rise with the loss of negative feedback of androgens on the hypothalamic-pituitary-gonadal access. Separately, due to the effects of aromatase, testosterone is aromatized to estradiol. Bicalutamide, and other members of the same drug class, are traditionally used in the treatment of prostate cancer with a known side effect of gynecomastia (Wirth et al., 2004). In transfeminine individuals, blocking testosterone action and promoting breast development could be a desired effect. Additionally, given the high cost and variable insurance coverage of GnRH agonists in the United States, additional anti-androgen options are needed. Spironolactone is the currently recommended oral alternative in the US, although this medication is associated with some adverse effects, including hyperkalemia especially in individuals >45 years of age or with underlying renal disease (Hayes et al., 2022). Other potential concerns associated with spironolactone include polyuria, orthostasis, drowsiness, and premature breast bud fusion (Patibandla et al., 2023; Wierckx et al., 2014).

Bicalutamide use has been associated with a rare but serious side effect of hepatotoxicity, which has been reported in several case reports and studies of bicalutamide use in prostate cancer (Blackledge et al., 1997; Dawson et al., 1997; Hussain et al., 2014; Kolvenbag & Blackledge, 1996; McLeod et al., 2006). The risk is typically highest in the first three months of therapy and likely affected by dosage, age, and patient comorbidities; flutamide seems to confer the highest risk in this drug class (Bruni et al., 2012). Bicalutamide doses for prostate cancer can reach 150 mg daily which is substantially higher than the 25–50 mg dose used in transgender individuals.

Small studies, including no more than 40 individuals, without a comparison group have been published on the use of bicalutamide in transgender care finding no evidence of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) elevation in individuals on bicalutamide (Fuqua et al., 2024; Karakılıç Özturan et al., 2023; Neyman et al., 2019). There is a single current report in the literature of proposed bicalutamide-induced hepatotoxicity in a transfeminine adolescent on bicalutamide 50 mg daily that occurred within three months on therapy (Wilde et al., 2024). Given the potential benefits of bicalutamide, it was incorporated as an option for AMAB transfeminine adolescents and young adults (AYA) seeking gender affirming care at our center beginning in 2017. Patients and their guardians were carefully counseled about the risks and benefits of different anti-androgen options and selected their treatment plan through shared decision making with the center's endocrinologist.

To date, no studies specifically compare AST and ALT over time among individuals taking bicalutamide and individuals receiving other feminizing therapies. Given the paucity of literature on bicalutamide use in gender affirmation, and the proposed safety concerns based on data from older cisgender men with prostate cancer, the primary objective of this study was to identify any significant differences in transaminase levels over one year among individuals on bicalutamide compared with individuals on other feminizing regimens.

Methods

A retrospective cohort study was conducted utilizing data extracted from the electronic health record. This study was conducted among individuals seeking care at a tertiary medical center in St. Louis, MO. Approximately half of individuals presenting to the center live in the St. Louis metropolitan area, ~25% come from other areas of southeast Missouri and ~25% come from southern IL. Transfeminine individuals ≤ age 30 newly starting anti-androgen and feminizing hormone therapy with our transgender care team were eligible for inclusion. Depending upon their age at presentation, some individuals were on bicalutamide or GnRH agonist alone for a variable length of time prior to estradiol initiation. Additional inclusion criteria were: on anti-androgen and/or estradiol for at least 1 year with at least 10 months of laboratory data after medication start from January 1, 2015 to February 1, 2023 and prescription of bicalutamide, GnRH agonist or spironolactone and estradiol or estradiol alone. Individuals were excluded if they were assigned female at birth or if they were assigned male at birth but not seeking feminizing therapy. Additional exclusion criteria were: absence of baseline or follow-up lab data to at least 10 months

after medication start, change in anti-androgen therapy during the follow-up period, or initiation of puberty blockers or gender-affirming hormone therapy (GAHT) at an outside health facility prior to transferring care to our clinic. Given the time constraints of manual data extraction, we limited follow-up data collection to one year post medication start. However, as these were clinical data and not everyone had data at exactly one year, all AST and ALT data were collected from baseline to as close to the one year follow-up as possible. For individuals whose last data point before one year of follow-up was earlier than 10 months, the first data point after one year post medication start was used for the one year time point. Otherwise, data between 10 months and one year were used for the one year time point.

Demographic information (age, gender identity, race, ethnicity and insurance status) and medical and social history relevant to liver dysfunction, (obesity, alcohol or other substance use) were collected. Additionally, exam data (BMI, blood pressure and Tanner staging if documented by the clinician performing the visit), and laboratory data (ALT, AST) and estradiol routes and doses were extracted from the medical record. Individuals were assigned a study number and all data were stored in a de-identified REDCap database.

Individuals were included in the bicalutamide group if they remained on bicalutamide for at least one year and on no other anti-androgen in conjunction with estradiol during that follow-up period. Individuals were included in the comparison group if they were on a GnRH agonist, spironolactone, or no anti-androgen in conjunction with estradiol during the follow-up. It was estimated that a sample size of 64 individuals per group would provide 80% power to detect a mean group difference in ALT and AST of 0.5 standard deviation units using a two-sample t-test.

Baseline values in the bicalutamide and comparison groups were compared using t tests, Mann Whitney U test, chi square, or Fisher's exact test depending on variable type and distribution. ALT and AST differences between groups throughout the follow-up period were assessed in multiple ways. The primary outcomes of ALT or AST change from baseline and maximum ALT and AST were analyzed with a multivariable linear mixed-effect model, including the treatment group as the fixed effect term and participant as a random intercept term with variance components covariance structure to account for repeated measurements on participants. Three covariates that were significantly different between groups (baseline ALT or AST, age, and BMI), were included in the models. The model also included reported alcohol use as it could have a potentially important effect on liver function and key demographic characteristics (race) as covariates. The estimated means of the treatment group, mean difference between the groups, and 95% confidence intervals with p-values from our linear mixed model were reported. Model assumptions were checked with residual plots. Missing data were not imputed. A sensitivity analysis including four individuals that had been excluded for switching anti-androgen therapy within the year follow-up was also performed with their treatment group assigned by their initial therapy.

Additionally, the percentage of individuals in the bicalutamide and comparison groups with an ALT or AST value after baseline that was more than the upper limit of normal (ULN), 2x ULN, and 3x ULN was determined. These outcomes were compared by group utilizing a chi square or Fisher's exact test as appropriate.

Maximal estradiol dose was evaluated to determine if the effect of testosterone aromatization to estradiol in the bicalutamide group vs the comparison group led to lower requirements for prescribed estradiol. To compare maximal estradiol dose required over the follow-up period, given that different estradiol routes were utilized, we calculated a mean standardized dose for each route of administration (sublingual, injection, transdermal patch) and then utilized the mean standardized variable to compare doses across routes. A t test was used to compare the mean standardized maximum estradiol dose between bicalutamide and comparison groups. A Fisher's exact test was used to compare maximal Tanner stage (for those in whom it was documented) over the one-year follow-up between groups.

The study was approved by the Washington University Human Research Protection Office on December 15, 2021 (202112098) with a full

HIPAA waiver. Statistical analysis was performed using SAS v 9.4 (Cary, NC).

Results

Three hundred and seventy-eight transfeminine individuals ≤ 30 years of age that had received gender affirming hormone therapy in our transgender center were initially identified using the Slicer Dicer tool on our Epic electronic health record. After exclusions for lack of baseline labwork, inadequate monitoring of labs during the one-year follow up period, treatment use for < 1 year at time of data extraction, initiation of GAHT prior to presentation to our center, or transitioning to another treatment regimen during the one year follow up, 164 individuals had data extracted to the REDCap database. After data quality assessment, an additional 11 individuals were excluded (3 for initiation of GAHT prior to establishing with the center, 4 for inadequate baseline labs, and 4 for switching anti-androgen regimens during the one-year follow-up). Of note, individuals who switched anti-androgen therapy did not do so because of AST or ALT abnormalities. After these exclusions, 84 individuals in the bicalutamide group and 69 individuals in the comparison group were included in the analyses (Figure 1). The mean (SD) follow-up time from medication start to last ALT and AST extracted in the cohort was 394 (113) days.

Baseline demographic, medical history, exam, and lab data are shown in Table 1. Median age of the total population was 18 years (IQR 16, 20), with the majority identifying as binary transgender females. A majority of individuals identified as white (79.7%) and had private insurance (78.4%). Individuals in the bicalutamide group were younger than the comparison group (median age 18 (IQR 16,18) v. 19 (IQR 17,24) years, $p < 0.0001$). There were no statistically significant differences between groups for gender identity, race/ethnicity, or insurance carrier. There were also no significant differences between groups in the prevalence of other comorbidities. Median BMI was significantly different between groups (bicalutamide, 21.6 kg/m^2 (IQR 18.9, 25.3) v. comparison, 25.5 kg/m^2 (IQR 21.0, 30.4), $p = 0.001$). Baseline median ALT and AST were also significantly different between groups [median ALT: bicalutamide,14 U/L (IQR 11, 22) v comparison, 21 U/L (IQR 13, 32), $p = 0.002$; median AST bicalutamide, 19 U/L (IQR 16, 23) v comparison, 21 U/L (IQR 18, 29), $p = 0.008$].

Tables 2 and 3 summarize linear mixed models with outcomes of change in ALT and AST over baseline and maximum ALT and AST levels during the follow-up period. Adjusted ALT and AST declined in both bicalutamide and comparison groups over the follow-up period and adjusted maximum ALT and AST were not elevated in either group. There was no significant

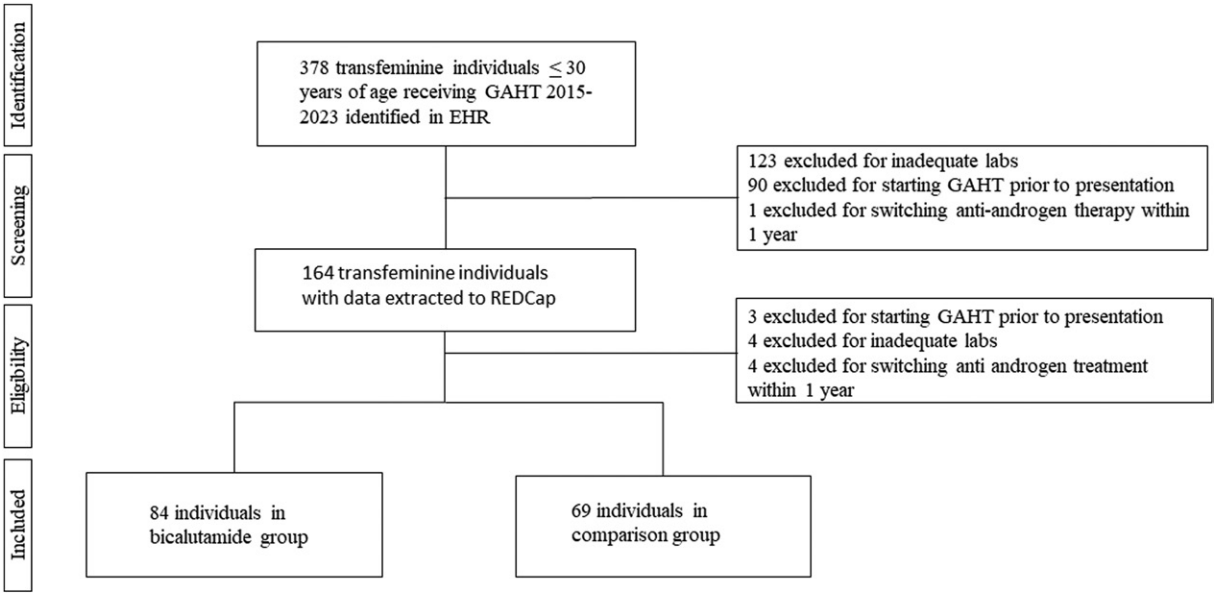

Figure 1. Flow diagram for inclusion of AMAB transfeminine individuals in retrospective cohort definition.

Table 1. Baseline characteristics of AMAB transfeminine retrospective cohort by treatment group.

Variable	Total population $n=153$	Bicalutamide $n=84$	Comparison $n=69$	P-value
Age (at first encounter), Median (IQR)	18 (16, 20) Range: 12–30	18 (16, 18)	19 (17, 24)	<0.0001[a]
Gender identity, n (%)				0.86
Binary transgender female	139 (90.8%)	76 (90.5%)	63 (91.3%)	
Another gender identity with feminine embodiment goals (nonbinary, agender, genderqueer)	14 (9.2%)	8 (9.5%)	6 (8.7%)	
Race/Ethnicity, n (%)				0.59[b]
Asian, non-Hispanic	3 (2%)	1 (1.2%)	2 (2.9%)	
Black, non-Hispanic	18 (11.8%)	7 (8.3%)	11 (15.9%)	
White, non-Hispanic	122 (79.7%)	70 (83.3%)	52 (75.4%)	
Hispanic	7 (4.6%)	4 (4.8%)	3 (4.3%)	
Another race/ethnicity or multiple race/ethnicities	3 (2%)	2 (2.4%)	1 (1.4%)	
Primary insurance, n(%)				0.10[b]
Private	120 (78.4%)	64 (76.2%)	56 (81.2%)	
Medicaid	26 (17%)	18 (21.4%)	8 (11.6%)	
Other government insurance	4 (2.6%)	2 (2.4%)	2 (2.9%)	
Uninsured	3 (2%)	0 (0%)	3 (4.3%)	
Medical History, n (%)				
No medical history	35 (22.9%)	19 (22.6%)	16 (23.2%)	0.93
Type 1 diabetes	1 (0.7%)	1 (1.2%)	0 (0%)	>0.99[b]
Type 2 diabetes	0 (0%)	0 (0%)	0 (0%)	NA
Hyperlipidemia	2 (1.3%)	2 (2.4%)	0 (0%)	0.50[b]
Hypertension	2 (1.3%)	1 (1.2%)	1 (1.4%)	>0.99[b]
Liver disease	0 (0%)	0 (0%)	0 (0%)	NA
HIV	4 (2.6%)	1 (1.2%)	3 (4.3%)	0.33[b]
Medical problem not listed	106 (69.3%)	61 (72.6%)	45 (65.2%)	0.32
Reported alcohol use, n (%)	13 (8.5%)	10 (11.9%)	3 (4.3%)	0.10
Reported substance use, n (%)	18 (11.8%)	13 (15.5%)	5 (7.2%)	0.12
BMI, Median (IQR)	22.4 (19.7, 28.3), $n=152$	21.6 (18.9, 25.3)	25.5 (21.0, 30.4), $n=68$	0.001[a]
Systolic blood pressure, mean (SD)	125.2 (13.0), $n=149$	122 (11.2), $n=82$	129.2 (14), $n=67$	0.0006
Diastolic blood pressure, mean (SD)	74 (9.7), $n=149$	70.4 (8.3), $n=82$	78.5 (9.5), $n=67$	<.0001
Baseline ALT				
ALT (Units/L), median (IQR)	16 (12, 28)	14 (11, 22)	21 (13, 32)	0.002[a]
ALT > 2x ULN	2 (1.3%)	0 (0%)	2 (2.9%)	0.20[b]
ALT > 3x ULN	0 (0%)	0 (0%)	0 (0%)	NA
Baseline AST	$n=152$		$n=68$	
AST (Units/L), median (IQR)	19 (16, 24)	19 (16, 23)	21 (18, 29)	0.008[a]
AST > 2x ULN	0 (0%)	0 (0%)	0 (0%)	NA
AST > 3x ULN	0 (0%)	0 (0%)	0 (0%)	NA

Abbreviations: n = sample size; SD = standard deviation; IQR = Interquartile Range; NA = not applicable.
When less than the entire cohort provided data, sample size (n) or the denominator is also reported.
[a]P-value is from Wilcoxon Rank Sum Test due to the small sample size and skewness of data. Median (IQR) are reported.
[b]P-value are from Fishers' exact test because ≥ 25% of the cells have expected counts less than 5.
Abbreviations: n = sample size; ALT = alanine transaminase; BMI = body mass index; CI = confidence interval.

between-group difference in adjusted delta ALT or maximum ALT (3.4 U/L, 95% CI −0.6, 7.5; $p=0.10$). No significant differences in delta AST or maximum AST were noted between the bicalutamide and comparison group (1.1 U/L, 95% CI −1.1, 3.2, $p=0.32$). Sensitivity analyses including 4 individuals excluded for changing anti-androgen therapy during the one year follow-up showed similar results (Supplemental Tables 2 and 3).

Table 4 demonstrates individuals in the bicalutamide and comparison groups with any ALT or AST levels > ULN, > 2x ULN, and > 3x ULN during the 1-year follow-up period. There were no significant differences in the % of individuals

Table 2a. Linear mixed model examining fixed effect of medication group on delta ALT, adjusting for age, race, BMI, alcohol use, and baseline ALT ($n=152$).

	Least squares means test		
	Delta ALT estimate	95% CI	p value
Bicalutamide ($n=84$)	−3.8	−7.6, 0.06	0.05
Comparison ($n=68$)	−7.2	−11.7, −2.7	0.0018
	Difference in least squares means test		
	Delta ALT estimate	95% CI	p value
Group difference: Bicalutamide-Comparison	3.4	−0.6, 7.5	0.10
Covariates	Coefficient estimate	95% CI	P value
Baseline ALT	−0.6	−0.6, −0.5	<0.0001
Alcohol use (No)	5.9	−0.3, 12.1	0.06
Age	−0.1	−0.7, 0.5	0.73
BMI	0.2	−0.2, 0.5	0.33
Race (White)	−0.1	−5.3, 5.1	0.97

Table 2b. Linear mixed model examining fixed effect of medication group on maximum ALT, adjusting for age, race, BMI, alcohol use, and baseline ALT (n = 152).

	Least squares means test		
	Maximum ALT estimate	95% CI	p value
Bicalutamide ($n = 84$)	22.2	18.3, 26.0	< 0.0001
Comparison ($n = 68$)	18.7	14.2, 23.2	< 0.0001
	Difference in least squares means test		
	Maximum ALT estimate	95% CI	p value
Group difference: Bicalutamide-Comparison	3.4	−0.6, 7.5	0.10
Covariates	Coefficient estimate	95% CI	p value
Baseline ALT	0.5	0.4, 0.6	<0.0001
Alcohol use (No)	5.9	−0.3, 12.1	0.06
Age	−0.1	−0.7, 0.5	0.73
BMI	0.2	−0.2, 0.5	0.33
Race (White)	−0.1	−5.3, 5.1	0.97

Abbreviations: n = sample size; ALT = alanine transaminase; BMI = body mass index; CI = confidence interval.
One individual in comparison group was missing baseline BMI.

Table 3a. Linear mixed model examining fixed effect on medication group on delta AST, adjusting for age, race, BMI, alcohol use, and baseline AST ($n = 151$).

	Least squares means test		
	Delta AST estimate	95% CI	p value
Bicalutamide ($n = 84$)	−1.5	−3.5, 0.5	0.14
Comparison ($n = 67$)	−2.6	−5.0, −0.2	0.03
	Differences in least squares means test		
	Delta AST estimate	95% CI	p value
Group difference: Bicalutamide-Comparison	1.1	−1.1, 3.2	0.32
Covariates	Coefficient estimate	95% CI	p value
Baseline AST	−0.5	−0.7, −0.4	<0.0001
Alcohol use (No)	2.1	−1.1, 5.3	0.20
Age	−0.1	−0.4, 0.2	0.59
BMI	0.0	−0.2, 0.1	0.82
Race (White)	−0.2	−2.9, 2.5	0.89

Table 3b. Linear mixed model examining fixed effect of medication group on maximum AST, adjusting for age, BMI, alcohol use, and baseline AST (n = 151)

	Least squares means test		
	Maximum AST estimate	95% CI	p value
Bicalutamide ($n = 84$)	20.2	18.2, 22.2	< 0.0001
Comparison ($n = 67$)	19.1	16.7, 21.5	< 0.0001
	Differences in least squares means test		
	Maximum AST estimate	95% CI	p value
Group difference: Bicalutamide-Comparison	1.1	−1.1, 3.2	0.32
Covariates	Coefficient estimate	95% CI	p value
Baseline AST	0.5	0.3, 0.6	<0.0001
Alcohol use (No)	2.1	−1.1, 5.3	0.20
Age	−0.1	−0.4, 0.2	0.59
BMI	0.0	−0.2, 0.1	0.82
Race (White)	−0.2	−2.9, 2.5	0.89

Abbreviations: n = sample size; AST = aspartate transaminase; BMI = body mass index; CI = confidence interval.
One individual in comparison group was missing baseline BMI.
One individual in comparison group was missing baseline AST related to hemolyzed specimen.

Table 4. Comparison of maximum ALT and AST elevations in relation to upper limit of normal by medication group ($n = 153$).

	ALT n (%)	P value	AST n (%)	P value
	> ULN		**> ULN**	
Bicalutamide	14 (16.7)	0.37	9 (10.7)	0.02
Comparison	8 (11.6)		1 (1.5)	
	> 2x ULN		**>2x ULN**	
Bicalutamide	2 (2.4)	> 0.99	0 (0)	0.45
Comparison	1 (1.5)		1 (1.5)	
	> 3x ULN		**> 3x ULN**	
Bicalutamide	0 (0)		0 (0)	
Comparison	0 (0)		0 (0)	

Abbreviations: n = sample size; ALT = alanine transaminase; AST = aspartate transaminase; ULN = upper limit of normal.
*P value calculated using chi square or Fisher's exact test.

with any ALT > ULN in each group (16.7% bicalutamide v. 11.6% comparison; $p = 0.37$). There was a significant difference in percentage of individuals with any AST > ULN (10.7% bicalutamide v. 1.5% comparison; $p = 0.02$). However, few individuals had AST or ALT > 2x ULN and no individuals in the bicalutamide or comparison groups had any ALT or AST > 3x ULN (typically considered clinically significant transaminitis). For individuals with either baseline elevations in ALT or AST or individuals who developed elevations during the follow up period, 64% of those in the bicalutamide group and 63% of those in the comparison group had normalization of their ALT levels over 1 year. Similarly, 56% in the bicalutamide group and 100% in the comparison group had normalization of their AST by the 1-year follow up laboratory assessment (Supplemental Table 1).

Estrogen dosing and Tanner staging data are shown in Tables 5 and 6. The mean standardized maximum daily estradiol dose [mean (SD)] was −0.43 (0.68) in the bicalutamide group v. 0.51 (1.05) in the comparison group, which was statistically significant ($p < 0.001$) (Table 5a). However, given practice differences in the typical starting estradiol dose and titration among pediatric and adult- trained endocrinologists, a sub-analysis of individuals who were treated only by pediatrics-trained practitioners was completed. In this subgroup, there was no significant difference between standardized maximum daily estradiol dose in the bicalutamide and comparison groups (bicalutamide 0.04 (0.96) v. comparison −0.10 (1.16), $p = 0.57$) (Table 5b). Among individuals seen by pediatric-trained providers, there was also no significant difference in maximum daily estradiol

Table 5a. Maximum daily estradiol dose in bicalutamide vs comparison groups for entire study population ($n = 151$).

	Bicalutamide n, mean (SD)	Comparison n, mean (SD)	P-value[a]
All estradiol routes[b]	82, −0.43 (0.68)	69, 0.51 (1.05)	< 0.0001
Total daily dose by route[c]			
Sublingual	66, 2.61 (1.38)	58, 4.53 (2.04)	
Patch	15, 0.02 (0.01)	10, 0.03 (0.02)	
Injection	1, 0.29	1, 1.43	

Table 5b. Maximum daily estradiol dose in bicalutamide vs comparison groups, for individuals treated by pediatric providers (n= 102).

	Bicalutamide n, mean (SD)	Comparison n, mean (SD)	P-value[a]
All estradiol routes[b]	79, 0.04 (0.96)	23, −0.10 (1.16)	0.57
Total daily dose by route[c]			
Sublingual	64, 2.54 (1.22)	15, 1.98 (1.19)	
Patch	15, 0.02 (0.01)	8, 0.02 (0.02)	

Abbreviations: n = sample size; SD = standard deviation.
[a]P value calculated using student's t test.
[b]Reported dose is mean standardized to allow comparison across routes.
[c]Reported dose is raw dose in mg for each route (weekly dose for injection is divided by 7 to approximate daily dose).

dose for either sublingual or transdermal routes of administration.

Tanner staging data were missing among a large number of individuals in the comparison group, again related to differences in practice and documentation between pediatric and adult-trained endocrinologists. A majority of patients in the bicalutamide group had at least Tanner stage 3 breast development after a minimum of 6 months of bicalutamide treatment, and there was no difference in Tanner stage between groups (Table 6).

Discussion

This retrospective cohort study demonstrates no clinically significant differences in ALT and AST values in transfeminine individuals taking bicalutamide when compared with a group of individuals on GnRH agonists, spironolactone or estradiol alone. There have only been three published studies evaluating the use of bicalutamide in transfeminine individuals which did not show clinically significant ALT and AST elevation (Fuqua et al., 2024; Karakılıç Özturan et al., 2023; Neyman et al., 2019) and one case report of potential bicalutamide induced hepatotoxicity in a transgender woman (Wilde et al., 2024). Our study is the largest study to date in this population and the first study in transfeminine individuals to systematically evaluate liver function changes on bicalutamide

Table 6. Maximum achieved breast Tanner stage at end of year follow-up by group ($n = 100$).

	Bicalutamide ($n = 77$) n (%)	Comparison ($n = 23$) n (%)	P value[a]
Tanner 1	0 (0)	1 (4.3)	0.30
Tanner 2	5 (6.5)	2 (8.7)	
Tanner 3	49 (63.6)	14 (60.9)	
Tanner 4	20 (26.0)	4 (17.4)	
Tanner 5	3 (3.9)	2 (8.7)	

[a]P value calculated using Fisher's exact test because ≥25% of cells have expected counts < 5.

with respect to a comparison group. Additional exploratory findings from our study suggest that estradiol dose requirements are likely similar on bicalutamide and other anti-androgen regimens after accounting for different prescribing practices. Individuals on bicalutamide also appear to reach appropriate Tanner stage 3 breast development in the first year on therapy, similar to those on other anti-androgens.

Bicalutamide has been used for female pattern hair loss, PCOS, and testotoxicosis at doses close to those used for gender affirmation without hepatotoxicity. A retrospective review of 316 cisgender females, mean 49 years of age (range 15–85), prescribed bicalutamide (dose range 5 mg- 50 mg daily; standard dose 10 mg daily) for female pattern hair loss demonstrated 2.9% of individuals on bicalutamide having mild elevation in liver transaminases, no greater than 2x ULN (Ismail et al., 2020). Additionally, there was subsequent normalization of transaminases in about half of the patients without a dose reduction, consistent with the findings of our study. In a double blind randomized controlled trial of 88 cisgender women, a dose of bicalutamide 50 mg daily was evaluated in combination with oral contraceptives in the treatment of hirsutism in PCOS. No significant changes in ALT and AST were documented in the year the randomized study was conducted (Moretti et al., 2018). Finally, bicalutamide has also been used for the treatment of testotoxicosis, and case reports and one phase 2 trial do not document clinically significant ALT or AST elevation (Lenz et al., 2010; Reiter et al., 2010; Tessaris et al., 2012).

In transfeminine individuals seeking gender affirming hormone therapy, our study demonstrated no difference in change in ALT or AST from baseline, maximum ALT or AST values or clinically significant transaminitis (defined as

ALT or AST > 3x ULN) between individuals using bicalutamide (25 mg daily) and individuals in a comparison group on GnRH agonists, spironolactone or estradiol alone. In fact, ALT and AST declined in both groups over the study period, and among individuals with elevations in ALT and AST at baseline or at follow-up, the majority normalized over one year follow-up with no change in therapy in both the bicalutamide and comparison groups. Among individuals without normalization of ALT and AST, these elevations were not considered clinically significant because definitions of drug-induced liver injury (DILI) indicate that ALT < 2x the ULN does not meet DILI criteria, and that ALT < 5x the ULN is very unlikely to represent clinically significant liver injury (Aithal et al., 2011). Additionally, AST is a nonspecific enzyme due to expression not just in the liver but also in muscle, brain, pancreas, lung, kidney, erythrocytes, and leukocytes (Xu et al., 2015). We therefore postulate that the modest AST elevations observed are unlikely to represent clinically significant liver injury because (i) the degree of elevation falls below the consensus threshold definition for drug-induced liver injury, and (ii) AST elevations, particularly in the absence of ALT elevations, are more likely to be nonspecific to liver.

Bicalutamide use in this study population was associated with lower to no significant difference in mean standardized estradiol doses with the comparison group and demonstrated promising results for breast development based on limited Tanner staging data. There was one individual in whom bicalutamide was stopped after the follow-up period designated for the study. This individual developed ALT and AST >2x ULN after an episode of COVID and had a thorough hepatology evaluation. As ALT and AST were never > 3x ULN, it was not recommended that bicalutamide be stopped; however, ultimately a clinical decision was made to stop the medication and ALT and AST normalized. Based on these findings, bicalutamide appears to be a safe anti-androgen adjunct to estradiol therapy in transfeminine individuals. In our experience, it is often covered by insurance carriers and is another oral option for patients seeking an alternative to injectable or implantable GnRH agonists.

This study has several limitations. First, this is a retrospective study with data collected *via* electronic health record chart review. Data were fully extracted and reviewed by one author (KB). A second reviewer performed quality assurance and data validation on > 50% of charts to mitigate risk of human error in data extraction. Data extractors were not blinded to treatment assignment; however, individuals extracting data and performing quality assurance were not the same individuals who were prescribing the medications. Retrospective data collection is limited by availability in existing records. Some data, particularly Tanner staging, were missing in patient notes.

ALT is an incompletely sensitive marker of liver injury, with one study demonstrating that an elevated ALT can occur in biopsy-proven normal liver, but ALT can also be normal in those with biopsy-proven liver injury (Bedogni et al., 2005). However, ALT screening is still arguably the best noninvasive test currently available to initially screen for drug- induced hepatocellular liver dysfunction (European Association for the Study of the Liver, 2019). Additionally, while labs were scheduled every three months, some patients did not get labs completed on schedule. Moreover, given that individuals were not prospectively assigned or randomized to bicalutamide or comparison groups, there were differences in some baseline characteristics between groups. These limitations in retrospective study design were mitigated by the use of linear mixed models in statistical analysis. Finally, the study was completed using data from a single center and patients in their teens and twenties, which could limit the generalizability of findings to other centers and older patients.

While our sample size was larger than previous studies examining bicalutamide in transfeminine individuals, the study was powered to detect a difference in AST and ALT of 0.5 standard deviation units. Hence, although the study was not powered to detect a smaller difference in these laboratory values, smaller differences are unlikely to be clinically significant. The small size of the study would also not have detected rare adverse events occurring in less than one percent of the exposed population.

Nonetheless, despite these limitations, this study provides reassuring data as to the safety of

low dose bicalutamide as an anti-androgen option in feminizing regimens for AMAB transfeminine individuals. It also provides preliminary data to support additional larger, prospective studies comparing bicalutamide to other anti-androgens (spironolactone), GnRH agonists, or estradiol use alone, examining outcomes such as patient satisfaction with their regimen, liver function abnormalities, estradiol dose and level attainment, fertility, and breast development.

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Authors' contributions

KB designed the study, performed data collection and quality assurance and wrote and edited the manuscript. BD contributed to study design, provided pediatric hepatology expertise, and reviewed and edited the manuscript. JW performed data analysis and reviewed and edited the manuscript. CL contributed to study conceptualization and design, provided pediatric endocrinology expertise, and reviewed and edited the manuscript. CH contributed to study conceptualization and design, performed data validation, reviewed and edited the manuscript and supervised all aspects of the study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

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Data availability statement

Given vulnerability of the population and conduct of the study under a HIPAA waiver, our IRB will not allow individual level data sharing even with a restricted access limited data set.

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