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Article type : 5 Unsolicited Review

Title page

Title: A systematic review of anti-androgens and feminisation in transgender women

Short running title: Anti-androgens in transgender women

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Key words: Anti-androgen, transgender, feminisation, testosterone, spironolactone, cyproterone acetate

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Summary

Objective: Anti-androgens are frequently used with estradiol in transgender women seeking feminisation. Anti-androgens act by various mechanisms to decrease the production or effects of testosterone, but it is unclear which anti-androgen is most effective at feminisation.

Design: A systematic review was performed using PRISMA guidelines. We searched online databases (Medline, Embase and PsycINFO) and references of relevant articles for studies of anti-androgens in transgender women aged 16+ years to achieve feminisation (namely changes in breast size, body composition, facial or body hair) or changes in serum total testosterone concentration when compared to placebo, estradiol alone or an alternative anti-androgen.

Results: Four studies fulfilled eligibility criteria and were included in a narrative review. The addition of cyproterone acetate, leuprolide and medroxyprogesterone acetate may be more effective than spironolactone or estradiol alone at suppressing the serum total testosterone concentration. Body composition changes appear similar in transgender women treated with estradiol and additional cyproterone acetate or leuprolide. No eligible studies adequately evaluated the effects of anti-androgens on breast development or facial and body hair reduction.

Conclusion: It remains unclear which anti-androgen is most effective at achieving feminisation. Cyproterone acetate, medroxyprogesterone acetate and leuprolide may be more effective than spironolactone at suppressing the serum total testosterone concentration. However, due to spironolactone's antagonism of the androgen receptor, it is unclear whether this results in clinically meaningful differences in feminisation. Further research with clinically meaningful endpoints is needed to optimise the use of anti-androgens in transgender women.

(241 words)

Introduction

Trans, gender diverse and non-binary individuals desiring feminisation (herein referred to as transgender women) frequently seek medical care to achieve physical changes such as breast development, body fat redistribution and a reduction in facial and body hair. (1) Given estrogen monotherapy at physiological doses is not typically able to suppress serum total testosterone concentrations to the normal female range (2-4), treatment guidelines recommend the addition of an anti-androgen to assist with feminisation. (1, 5, 6)

For the purposes of this review, anti-androgens are defined as medications other than estradiol which are used to decrease the synthesis of or actions of androgens. Broadly speaking, mechanisms

involve suppression of gonadotrophin secretion, inhibition of key enzymes in androgen biosynthesis and antagonism of the androgen receptor. This expanded definition includes gonadotrophin releasing hormone (GnRH) analogues, progestogens, 5α-reductase inhibitors and androgen receptor antagonists.

The prescription of anti-androgens is highly variable throughout the world, reflecting differences in access and the cost of medications, prescriber familiarity and preference as well as the absence of rigorous data. In the United States, spironolactone is commonly prescribed as cyproterone acetate (CPA) is not licensed for use whereas CPA appears to be favoured in many European countries and forms standard care as part of the European Network for the Investigation of Gender Incongruence (ENIGI) treatment protocol. (6) In the United Kingdom, the high cost of GnRH analogues is heavily subsidised, facilitating first line use in combination with estradiol. (7) In Australia both spironolactone and CPA are subsidised by the Pharmaceutical Benefits Scheme (PBS), while the use of GnRH analogues is not PBS subsidised for transgender people and is funded instead by individual hospitals for the purpose of puberty suppression.

The mechanisms of action of the available anti-androgen agents is summarised in ***Table 1***. Androgen receptor antagonists include the steroid medications spironolactone and CPA, and non-steroid medications such as bicalutamide. While generally used for its mineralocorticoid antagonist properties, spironolactone exerts anti-androgen effects which have been exploited for the purposes of feminisation since the 1980s. (4) Spironolactone is a moderate androgen receptor antagonist (8, 9), which also partially inhibits 17α-hydroxylase/17,20 lyase, enzymes involved in testosterone synthesis. (10) Interestingly, even at high doses spironolactone treatment was not associated with a significant reduction in serum total testosterone concentration and actually caused a transient increase in luteinising hormone in a small pharmacodynamic study of five healthy men. (11) However, another study demonstrated that the administration of canrenone, a metabolite of spironolactone, at high doses caused a significant reduction in the total serum testosterone concentration (12) and the addition of spironolactone to estradiol appears to assist with suppression of testosterone to female concentrations in transgender women. (4) An observed increase in serum estradiol and estrone concentrations (13) as well as interaction with the estrogen receptor with spironolactone therapy (14) may also contribute to feminisation. Due to structural similarity to progesterone, spironolactone also possesses partial progesterone receptor agonist activity (9), though the relevance of this to feminisation is unclear. In comparison, CPA has also been used as part of feminising therapy since the 1980s and is a potent progestogen which exerts negative feedback on the hypothalamic pituitary gonadal axis to decrease gonadotrophin secretion and testosterone levels as well as moderate androgen receptor antagonism. (15)

Non-steroid androgen receptor antagonists such as bicalutamide are highly potent and as monotherapy do not cause a reduction in gonadotrophins or testosterone levels in contrast to CPA. Aromatisation of testosterone to estradiol is hypothesised to contribute to increased feminisation which was observed in transgender girls treated with bicalutamide without estradiol. (16) Other anti-androgens include GnRH analogues and progestogens which suppress the hypothalamic pituitary gonadal axis to decrease testosterone levels and 5α-reductase inhibitors, which decrease the conversion of testosterone to the more potent androgen dihydrotestosterone.

While there are numerous anti-androgens available to augment estradiol therapy in transgender women, it remains unclear which anti-androgen is the most effective at inducing changes of feminisation including breast growth, body fat redistribution and reduction of facial and body hair. As such, the aim of this systematic review was to synthesise available evidence to determine the comparative efficacy of anti-androgens to cause clinically meaningful feminisation – the ultimate objective of feminising hormone therapy. While the comparative safety of anti-androgen medications is also an important consideration, it is not the focus of this review.

Methods

Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) reporting guidelines were used in the development of this systematic review. (17)

Eligibility criteria

Study types

Given the paucity of randomised controlled trials evaluating the efficacy of gender-affirming hormone therapy, we considered the following types of studies for inclusion if published in English in a peer-reviewed journal: randomised controlled trials, prospective non-randomised cohort studies, retrospective cohort studies, retrospective case-control studies.

Participants

We included studies with transgender women aged 16 years and over, the age at which gender-affirming hormone therapy is commonly commenced.

Interventions

Anti-androgen medications including steroid and non-steroid androgen receptor antagonists, 5α-reductase inhibitors, progestogens and GnRH analogues.

Comparators

Comparators including placebo, estradiol therapy alone or an alternative anti-androgen. We chose not to include observational studies of estradiol with an anti-androgen in a single treatment cohort due to the inability to distinguish whether the observed effects were related to estradiol or anti-androgen therapy.

Outcomes

Clinical outcomes of interest included clinical features of feminisation (breast growth, body composition, suppression of facial and body hair). Serum total testosterone concentration was also examined as a surrogate marker of feminisation.

Information sources & search strategy

A search of online databases (MEDLINE, Embase and PsycINFO) was performed independently by the first two authors using the Ovid platform including records from inception to 16 April 2020. The search strategy used was: "transgender" OR "transsexualism" OR "gender dysphoria" OR "gender identity" OR "transfeminine" OR "transfemale" OR "MtF" OR "trans wom*" OR "transwom*" AND "androgen antagonist" OR "antiandrogen" OR "spironolactone" OR "cyproterone" OR "bicalutamide" OR "flutamide" OR "finasteride" OR "dutasteride" OR "progest*" OR "gonadorelin" AND "femini*" OR "body composition" OR "hair" OR "breast" OR "testosterone". Additional records were identified from the reference lists of relevant articles. Grey literature sources were not searched.

Study selection

Following the removal of duplicates, two authors (LMA and BJN) independently screened the titles and abstracts of records for relevance against eligibility criteria. Review articles, conference abstracts, case reports, articles not published in English and irrelevant articles were removed. The full text of remaining articles was assessed for eligibility, with data recorded including author, year of publication, study design, country of origin, study population, intervention, comparator and outcomes measured. Authors of studies were not contacted for additional unpublished data. Any discrepancies between the two review authors was resolved by consensus or arbitration by the senior author (ASC) in the event of disagreement.

Results

Search results

The literature search yielded 886 articles and 20 additional articles were identified from the reference of relevant articles. After duplicates were removed, 680 records were subjected to title

and abstract screening. The full text of the remaining 32 records was reviewed and 4 articles fulfilled eligibility criteria for inclusion. See ***Figure 1*** for full details of the review process.

Included studies

There were four studies deemed eligible for inclusion. All included studies were retrospective analyses of transgender women treated with an estrogen (estradiol or conjugated equine estrogens) with or without an anti-androgen. ***Table 2*** details the characteristics of the included studies. Given the small number and heterogeneity of studies, meta-analysis was not performed and a narrative summary is provided.

Serum total testosterone concentration

Serum total testosterone concentration was the most frequently reported outcome of interest in included studies and is commonly used as a surrogate for the efficacy of feminising therapy. Gava et al. (18) compared the efficacy of GnRH analogues or CPA in addition to estradiol in a retrospective study. Forty transgender women were randomised to treatment with leuprolide 3.75mg intramuscular injection monthly or CPA 50mg daily, in addition to standard estradiol therapy for 12 months. The serum total testosterone concentration decreased from 16.3±8.3 nmol/L at baseline to 0.7±1.0 nmol/L at 12 months in the CPA group ($P<0.05$) and from 22.2±7.6 nmol/L at baseline to 0.7 ± 0.3 nmol/L at 12 months in the leuprolide group ($P<0.05$), representing significant changes from baseline but with no significant difference between groups.

The addition of medroxyprogesterone (MPA) to estradiol was explored in a retrospective study performed by Jain et al. (19) Data was recorded from 290 follow up visits of 92 transgender women treated with estradiol and spironolactone 100-200mg, with or without MPA (5-10mg oral daily or 150mg intramuscular injection 3 monthly). Serum total testosterone concentration was significantly lower in the MPA group (79±18 ng/dL (2.74±0.62 nmol/L)) than the non-MPA group (215±29 ng/dL (7.45±1.01 nmol/L)) ($P<0.001$).

A retrospective analysis compared the serum testosterone concentration in 80 transgender women treated with estradiol alone (n=21), estradiol plus spironolactone (median dose 100mg daily) (n=38) or estradiol plus CPA (median dose 50mg daily) (n=21) (20). This showed a significantly lower median serum total testosterone concentration in those treated with CPA (0.8 nmol/L), compared to spironolactone (2.0 nmol/L) and estradiol alone (10.5 nmol/L) ($P=0.005$ after adjustment for serum estradiol concentration, estradiol dose, spironolactone dose, CPA dose and age). In contrast, Cunha et al. (21) observed a significant reduction in serum total testosterone concentrations at 6 months compared to baseline in a retrospective analysis of 51 transgender women treated with conjugated equine estrogens (CEE) alone or with CPA 50-100mg daily, but no significant between-group

difference (median serum total testosterone concentration at 6 months 21 ng/dL (0.73 nmol/L) in the CPA group versus 18.0 ng/dL (0.62 nmol/L) in the CEE alone group, P=0.217).

Body fat redistribution

Gava et al. (18) compared body composition, assessed by anthropometry and dual x-ray absorptiometry (DXA), in those treated with estradiol plus CPA versus estradiol plus leuprolide over a 12 month period. Notably, there was a significant increase in total body fat at 12 months in both the CPA group (19.3±4.7kg vs. 14.9±5.6kg at baseline, P<0.05) and the leuprolide group (19.9±6.8kg vs. 15.2±5.6 kg at baseline, P<0.05) but no significant between-group difference. Additionally, there was a significant decrease in lean mass in both the CPA group (49.9±7.8kg at 12 months vs. 51.7±8.3kg at baseline, P<0.05) and the leuprolide group (49.8±6.7kg at 12months vs. 50.2±7.0kg at baseline, P<0.05), but no significant between-group difference. There was no significant change in total body weight or waist-to-hip ratio throughout the study period.

Breast development

Limited studies have been performed to systematically examine breast development in transgender women, and none have provided a comparison of different anti-androgens.

Facial and body hair reduction

Limited studies have been performed to systematically examine reductions in facial and body hair in transgender women and none have provided a comparison of different anti-androgens.

Discussion

Summary of evidence

Despite anti-androgens being prescribed to most transgender women, there is a profound lack of research to guide choice of therapy. No available studies assessed breast development or reduction in facial and body hair in a way that allows meaningful comparison of different anti-androgens. There was one study comparing body composition changes, which found no difference in body composition between GnRH analogues and CPA. Due to difficulty in measuring feminisation, there is a reliance on the total testosterone concentration as a surrogate marker and evidence to date suggests that CPA, GnRH analogues and MPA are more effective than spironolactone at suppressing testosterone. However, serum total testosterone is an imperfect marker of treatment given androgen receptor antagonism is the predominant mechanism of action for many anti-androgens.

Serum total testosterone concentration

Serum total testosterone concentration is frequently used as a surrogate marker of feminising therapy and may be used for the titration of medication. However, there is a lack of data to support a clear relationship between suppression of serum total testosterone concentration and improved clinical feminisation, especially given some anti-androgens work predominantly via antagonism of the androgen receptor rather than by decreasing testosterone levels. Indeed, use of non-steroid androgen receptor antagonists (for example, bicalutamide) may cause feminisation with an increase in total testosterone concentrations due to potent androgen receptor antagonism without negative feedback of the hypothalamic pituitary gonadal axis. (16) In terms of serum total testosterone concentration suppression, the included four studies in suggest that CPA, GnRH analogues and progestins may be more effective at suppressing serum total testosterone concentrations than spironolactone when combined with an estrogen. The lack of between-group difference found by Cunha et al. (21) may reflect the small number of participants treated with CEE alone (n=8 in the CEE group) or perhaps differential ability of CEE to suppress testosterone compared to estradiol. All included studies were retrospective, may have been underpowered to detect a difference between groups and not all accounted for estradiol dose and estradiol concentrations when performing statistical comparison between groups.

Body fat redistribution

Body composition is readily measurable by anthropometry and whole body DXA in the research setting. A large prospective observational study described the changes in body fat distribution that occur with the commencement of feminising therapy (predominantly with estradiol and cyproterone acetate) with no comparator group. (22) In this cohort, there was an increase of 18% in the android region, 42% in the leg region and 34% in the gynoid region and a -0.03 decrease in waist-to-hip ratio due to an increase in hip circumference. (22) The study by Gava et al. included in this review showed no difference in body composition changes in those treated with estradiol plus either CPA or leuprolide. (18) However, the study may have been underpowered to detect such a difference and did not describe body fat redistribution by body region (android or gynoid). While CPA has additional androgen receptor antagonism compared to GnRH analogues, it is possible that the androgen receptor modulation is less important at the low serum testosterone concentrations achieved in both treatment groups.

Breast development

Breast development, a predominant desire of many transgender women, is not measured in a standardised, objective and reproducible manner making data comparison difficult between studies.

Additionally, breast development may not be routinely recorded at follow up clinical visits due to the sensitive and intimate nature of physical examination, limiting the utility of retrospective case review studies. Some transgender women may also have breast augmentation surgery, limiting the ability to discern the effects of estrogens and anti-androgen therapy. Various methods have been used in available studies to assess breast development, including self-assessed and clinician assessed Tanner stage, calculation of cup size using measurements of chest and breast circumference and qualitative assessment with photography.

No eligible studies assessed breast development in a manner that allowed robust comparison between different anti-androgens. However, De Blok et al. (23) provided insight into timing of breast development in a retrospective study of 229 transgender women taking estradiol plus CPA 50-100mg daily or spironolactone 100-150mg daily. Breast development (measured breast circumference and calculated cup size) was evaluated over a 12-month period following initiation of estradiol and anti-androgen therapy. This study did not stratify breast development by anti-androgen, though it is likely that most participants received CPA given it forms standard care in the ENIGI treatment protocol. (6) Nonetheless, results showed that breast development predominantly occurred within the first 6 months of therapy, with an average increase in breast circumference of 1.8cm (1.4 – 2.3) over the first 3 months, and 1.3cm (0.9 – 1.8) over the following 3 months. At 12 months, 48.7% of participants had a cup size less than AAA (<8cm) and only 7 participants (3.6%) had a cup larger than A (12-14cm). Additionally, Prior et al. (4) used self-reported cup size and clinical photography to document breast development with estradiol, MPA and spironolactone therapy over 12 months. An A cup size was reported in “most subjects”, though detailed data was not published. Difficulties in analysing photographic data in a quantitative way limited statistical comparison, though images provided a qualitative depiction of the potential effects of feminising therapy.

Breast development in cis- and transgender women was recently reviewed by Reisman et al. (24) The significant ductal and lobuloalveolar growth and fat deposition that occurs during puberty is regulated by local growth factors and hormones. Estradiol is principally responsible, with lesser contributions from growth hormone and glucocorticoids needed for normal breast development. (25, 26) Progesterone and prolactin play additional roles in the alveolar branching and proliferation of breast tissue that occurs during pregnancy in preparation for lactation. (26) Gynaecomastia occurs commonly in cisgender boys during puberty and may occur in cisgender men due to endocrinopathies or androgen deprivation therapy and is attributed to a relative increase in the estrogen to androgen ratio. (26) Interestingly, the histological changes observed in cis-gender men with gynaecomastia differ from transgender women treated with ethinylestradiol plus either CPA or orchiectomy in a small case series. (27) The authors suggest that the use of exogenous estradiol and

progestogens may be required to achieve complete acinar and lobular formation, though there is limited high-quality data to support this assertion. (27) Given the perceived importance of increasing the estrogen to androgen ratio, it is plausible that an anti-androgen causing more potent antagonism of the androgen receptor, or more significantly lower testosterone levels may contribute to enhanced breast development in transgender women.

Facial and body hair reduction

Similarly, changes in facial and body hair are not measured in a consistent manner to allow comparison across studies, and those that use techniques with high fidelity are highly labour intensive. Self-reported changes in facial and body hair, or clinical tools such as the modified Ferriman-Gallwey score are used in some studies but are limited by the subjective nature of responses and removal of unwanted facial and body hair by transgender women. No eligible studies assessed changes in facial and body hair adequately to allow comparison of anti-androgens in transgender women.

Notably, Giltay & Gooren (28) performed a prospective study of 21 transgender women treated with estradiol plus CPA 100mg daily for 12 months, examining changes in facial and body hair. Body hair growth and distribution was assessed using a modified Ferriman-Gallwey score of androgen-dependent areas. Clinical photography images taken with a macro lens of the face and peri-umbilical region were analysed to calculate hair growth per day, hair diameter and hair density. The modified Ferriman-Gallwey score significantly decreased from baseline (21/36) to 12 months (10/36) ($P<0.001$). The hair growth rate, diameter and density were significantly lower in the periumbilical region ($P<0.001$) and facial region ($P=0.009$, $P=0.049$ & $P=<0.001$ for hair growth rate, diameter and density respectively) over a 12-month period. The lack of a comparator group limited the ability to discern the effects of anti-androgen therapy from estradiol. Prior et al. (4) attempted to document changes in facial hair with estradiol, MPA and spironolactone therapy in 50 transgender women with clinical photography. However, difficulties analysing images in a quantitative manner limited interpretation of results, as did confounding effects of high dose estradiol therapy in many participants prior to enrolment and co-administration of MPA.

The interaction between androgens (particularly testosterone and dihydrotestosterone) and the androgen receptor present in some pilosebaceous units promotes differentiation into pigmented terminal hair follicles. (29) This results in the typical male pattern of facial and body hair. Paradoxically, androgenetic alopecia or male pattern baldness is also androgen-dependent, attributed to the miniaturisation of terminal hair follicles and suppression of scalp hair growth in genetically predisposed individuals. (29, 30) By reducing levels of the more potent androgen

dihydrotestosterone and therefore reducing interaction with the androgen receptor in hair follicles, 5-alpha reductase inhibitors are effective in the treatment of androgenic alopecia in cisgender men. (31) While 5-alpha reductase inhibitors are recommended by some clinicians for transgender women with pre-existing male pattern baldness (32), there is no high quality evidence in this population to suggest superiority of 5-alpha reductase inhibitors in achieving regrowth of scalp hair or reductions in facial and body hair compared to other anti-androgens. Given standard feminising therapy is able to achieve substantial reductions in androgen activity and/or androgen levels, there may be limited added benefit in further reducing production of dihydrotestosterone with 5-alpha reductase inhibition. In contrast, 5-alpha reductase inhibitors may be effective in treating androgenetic alopecia in transgender men treated with testosterone, though it is unclear whether this may decrease other masculinising effects of testosterone therapy such as the growth of facial and body hair. (33)

Extrapolation of anti-androgen use in other patient populations

Insights may be gained from the extrapolation of evidence related to the use of anti-androgens in women with hirsutism/polycystic ovarian syndrome (PCOS) and men with prostate cancer. Like transgender women, anti-androgens may be used together with estrogen for the treatment of excess facial and body hair in cisgender women. Guidelines for the treatment of PCOS recommend the use of an anti-androgen as second line treatment in combination with the oral contraceptive pill (OCP) if there has been an inadequate cosmetic response after six months of treatment, or as monotherapy in the presence of significant contraindications or intolerance to the OCP. (34, 35) Small randomised controlled trials have shown that spironolactone, flutamide and finasteride are more effective than placebo at reducing the modified Ferriman-Gallwey score and hair shaft diameter in women with moderate to severe hirsutism. (35, 36) CPA use has also been associated with significant reductions in hirsutism, when used at low doses (ethinylestradiol/CPA 2mg daily) and high doses in combination with the OCP. (37) Recently, the addition of bicalutamide 50mg daily to the OCP did not significantly decrease the modified Ferriman-Gallwey score compared to placebo in women with PCOS but did significantly decrease the hair density assessed by videodermoscopy. (38) Currently, available evidence does not support the use of one anti-androgen over another for the treatment of hirsutism. (35, 37) Additionally, women with hirsutism/PCOS are typically treated with synthetic estrogens (principally ethinylestradiol) and progestins as part of the OCP and have lower baseline serum total testosterone concentrations than transgender women, limiting the generalisability of findings.

Androgen deprivation therapy is commonly used for the treatment of prostate cancer. Use of GnRH agonists/antagonists to decrease testosterone synthesis form standard care for advanced prostate

cancer and may be combined with non-steroidal androgen receptor antagonists to inhibit interaction with the androgen receptor. (39) A review of men treated for prostate cancer showed much higher rates of gynaecomastia in men treated with non-steroidal androgen receptor antagonists (flutamide 30-79%, nilutamide 79%) compared to treatment with GnRH analogues (goserelin 1-5%, leuprolide 13-16%), combined androgen blockade (flutamide plus GnRH agonist 13-22.8%) or CPA (6 – 7.2%). These findings are consistent with current understandings of the pathophysiology of gynaecomastia, attributed to a relative increase in estrogenic activity and decrease in androgenic activity which is amplified by the aromatisation of increased testosterone to estradiol with use of non-steroidal androgen receptor antagonists. A reduction in lean body mass and increase in fat mass was observed following initiation of androgen deprivation therapy with GnRH analogues, like changes described in transgender women. Given treatment recommendations for anti-androgens in prostate cancer are guided by improved progression free survival rather than side effects of feminisation, and that estrogen therapy is used concurrently in transgender women, extrapolation of these findings is limited.

Safety considerations

While detailed discussion of the relative safety of anti-androgens is beyond the scope of this review, this will of course also influence anti-androgen prescribing practices. Severe and fatal hepatotoxicity has been reported in patients treated with flutamide, CPA, and rarely bicalutamide in the prostate cancer literature. (40) However, reported cases of severe hepatoxicity with CPA have occurred at doses of at least 100mg daily (40), which is higher than the doses typically used for transgender women. Additionally, use of CPA in transgender women has been associated with a four times higher incidence rate of meningioma when compared to a female reference population, thought to be related to the expression of progesterone receptors in human meningiomas and the potent progestogenic activity of CPA. (41) This risk appears to be associated with cumulative dose exposures greater than 3g. (42) While meningiomas are rare, both the European Medicines Agency (43) and the United Kingdom Medicines and Healthcare Products Regulatory Agency (44) have issued statements this year advising against use of CPA at doses of 10mg daily or greater unless there are no other treatment options. CPA use has been associated with hyperprolactinaemia of uncertain clinical significance, which is typically reversible following discontinuation. (45) A four-fold increase in the incidence of prolactinomas was also been observed in transgender women compared to female reference populations, most of whom were taking CPA. However, it is unclear whether this represents a true increase in incidence or if it is reflective of increased prolactin monitoring in this population as the incidence of symptomatic prolactinomas was similar. (41)

Strengths and limitations

The main outcome of this review is to highlight the lack of high-quality studies in the transgender health literature, particularly in relation to the optimal use of anti-androgens in transgender women. Indeed, there were no randomised controlled trials, perhaps reflecting the relative infancy of the transgender health literature. Existing studies are mostly retrospective analyses of clinic data, with a small number of study participants, lacking clinically relevant endpoints and without adequate comparison to different treatment groups. Instead, the serum total testosterone concentration is typically reported as a surrogate marker of therapy, a significant flaw given some commonly prescribed anti-androgens work predominantly via androgen receptor antagonism rather than decreasing testosterone levels. The results of this review emphasise the need for prospective randomised controlled studies to optimise the effective and safe delivery of gender-affirming care using clinically meaningful endpoints.

Conclusion

Anti-androgens are frequently added to estradiol to assist with feminisation and suppression of testosterone. Spironolactone, CPA, GnRH analogues and MPA all have anti-androgenic effects and despite less suppression of total testosterone with spironolactone, there is inadequate data to support enhanced feminisation with any particular anti-androgen. Serum total testosterone is a flawed surrogate marker of anti-androgen therapy given some medications work predominantly through androgen receptor antagonism rather than by decreasing testosterone levels. The comparative effects on breast development, body fat redistribution and reduction in facial and body hair are unclear. Further research is needed with clinically relevant endpoints to optimise the care of transgender women.

Funding

LMA is supported by the Research Training Program Scholarship from the Australian Commonwealth Government. BJN is supported by the Royal Australasian College of Physicians Fellows Research Entry Scholarship. ASC is supported by an Australian Government National Health and Medical Research Council Early Career Fellowship (#1143333) and receives research support from the Viertel Charitable Foundation Clinical Investigator Award, Endocrine Society of Australia Postdoctoral Award and the Royal Australasian College of Physicians Foundation.

Disclosure of interest

The authors have nothing to disclose

Author contributions

LMA conceptualised scope of systematic review, developed the search strategy, performed the search literature search, screening and full text review of records, discussed studies for inclusion and drafted the manuscript.

BJN performed an independent literature search, screening and full text review of records, discussed studies for inclusion and assisted with editing of the manuscript.

JDZ assisted with manuscript editing and preparation.

ASC assisted with conceptualisation of the systematic review, provided guidance on the search strategy, arbitrated in the event of disagreement between LMA and BJN on studies for inclusion and assisted with editing of the manuscript.

References

1. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al. Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2017;102(11):3869-903.
2. Gooren LJ, Giltay EJ, Bunck MC. Long-term treatment of transsexuals with cross-sex hormones: extensive personal experience. J Clin Endocrinol Metab. 2008;93(1):19-25.
3. Angus L, Leemaqz S, Ooi O, Cundill P, Silberstein N, Locke P, et al. Cyproterone acetate or spironolactone in lowering testosterone concentrations for transgender individuals receiving oestradiol therapy. Endocr Connect. 2019;8(7):935-40.
4. Prior JC, Vigna YM, Watson D. Spironolactone with physiological female steroids for presurgical therapy of male-to-female transsexualism. Archives of Sexual Behavior. 1989;18(1):49-57.
5. Cheung AS, Wynne K, Erasmus J, Murray S, Zajac JD. Position statement on the hormonal management of adult transgender and gender diverse individuals. Med J Aust. 2019;211(3):127-33.
6. Dekker MJ, Wierckx K, Van Caenegem E, Klaver M, Kreukels BP, Elaut E, et al. A European Network for the Investigation of Gender Incongruence: Endocrine Part. J Sex Med. 2016;13(6):994-9.
7. Mamoojee Y, Seal LJ, Quinton R. Transgender hormone therapy: understanding international variation in practice. Lancet Diabetes Endocrinol. 2017;5(4):243-6.
8. Bonne C, Raynaud JP. Mode of spironolactone anti-androgenic action: inhibition of androstanolone binding to rat prostate androgen receptor. Mol Cell Endocrinol. 1974;2(1):59-67.

9. Fagart J, Hillisch A, Huyet J, Bärfacker L, Fay M, Pleiss U, et al. A new mode of mineralocorticoid receptor antagonism by a potent and selective nonsteroidal molecule. J Biol Chem. 2010;285(39):29932-40.
10. Kossor DC, Kominami S, Takemori S, Colby HD. Role of the steroid 17 alpha-hydroxylase in spironolactone-mediated destruction of adrenal cytochrome P-450. Mol Pharmacol. 1991;40(2):321-5.
11. Stripp B, Taylor AA, Bartter FC, Gillette JR, Loriaux DL, Easley R, et al. Effect of spironolactone on sex hormones in man. J Clin Endocrinol Metab. 1975;41(4):777-81.
12. Erbler HC. Suppression by the spironolactone metabolite canrenone of plasma testosterone in man. Naunyn Schmiedebergs Arch Pharmacol. 1974;285(4):403-6.
13. Miyatake A, Noma K, Nakao K, Morimoto Y, Yamamura Y. Increased serum oestrone and oestradiol following spironolactone administration in hypertensive men. Clin Endocrinol (Oxf). 1978;9(6):523-33.
14. Levy J, Burshell A, Marbach M, Afllalo L, Glick SM. Interaction of spironolactone with oestradiol receptors in cytosol. J Endocrinol. 1980;84(3):371-9.
15. Raudrant D, Rabe T. Progestogens with antiandrogenic properties. Drugs. 2003;63(5):463-92.
16. Neyman A, Fuqua JS, Eugster EA. Bicalutamide as an androgen blocker with secondary effect of promoting feminization in male-to-female transgender adolescents. Journal of Adolescent Health. 2019;64(4):544-6.
17. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4:1.
18. Gava G, Cerpolini S, Martelli V, Battista G, Seracchioli R, Meriggiola MC. Cyproterone acetate vs leuprolide acetate in combination with transdermal oestradiol in transwomen: a comparison of safety and effectiveness. Clinical Endocrinology. 2016;85(2):239-46.
19. Jain J, Kwan D, Forcier M. Medroxyprogesterone Acetate in Gender-Affirming Therapy for Transwomen: Results From a Retrospective Study. The Journal of clinical endocrinology and metabolism. 2019;104(11):5148-56.
20. Angus L, Leemaqz S, Ooi O, Cundill P, Silberstein N, Locke P, et al. Cyproterone acetate or spironolactone in lowering testosterone concentrations for transgender individuals receiving oestradiol therapy. Endocrine Connections. 2019;8(7):935-40.
21. Cunha FS, Domenice S, Sircili MHP, Mendonca BB, Costa EMF. Low estrogen doses normalize testosterone and estradiol levels to the female range in transgender women. Clinics (Sao Paulo, Brazil). 2018;73:e86.

22. Klaver M, Blok CJMd, Wiepjes CM, Nota NM, Dekker MJHJ, Mutsert Rd, et al. Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: results from a multicenter prospective study. 2018;178(2):163.
23. De Blok CJM, Klaver M, Wiepjes CM, Nota NM, Heijboer AC, Fisher AD, et al. Breast development in transwomen after 1 year of cross-sex hormone therapy: Results of a prospective multicenter study. Journal of Clinical Endocrinology and Metabolism. 2018;103(2):532-8.
24. Reisman T, Goldstein Z, Safer JD. A Review of Breast Development in Cisgender Women and Implications for Transgender Women. Endocr Pract. 2019;25(12):1338-45.
25. Lyons WR, Li CH, Johnson RE. The hormonal control of mammary growth and lactation. Recent Prog Horm Res. 1958;14:219-48; discussion 48-54.
26. Sun SX, Bostanci Z, Kass RB, Mancino AT, Rosenbloom AL, Klimberg VS, et al. Breast Physiology: Normal and Abnormal Development and Function. The Breast: Comprehensive Management of Benign and Malignant Diseases. 6 ed: Elsevier Inc; 2018.
27. Kanhai RC, Hage JJ, van Diest PJ, Bloemena E, Mulder JW. Short-term and long-term histologic effects of castration and estrogen treatment on breast tissue of 14 male-to-female transsexuals in comparison with two chemically castrated men. Am J Surg Pathol. 2000;24(1):74-80.
28. Giltay EJ, Gooren LJ. Effects of sex steroid deprivation/administration on hair growth and skin sebum production in transsexual males and females. Journal of Clinical Endocrinology & Metabolism. 2000;85(8):2913-21.
29. Deplewski D, Rosenfield RL. Role of hormones in pilosebaceous unit development. Endocr Rev. 2000;21(4):363-92.
30. Lolli F, Pallotti F, Rossi A, Fortuna MC, Caro G, Lenzi A, et al. Androgenetic alopecia: a review. Endocrine. 2017;57(1):9-17.
31. Mella JM, Perret MC, Manzotti M, Catalano HN, Guyatt G. Efficacy and Safety of Finasteride Therapy for Androgenetic Alopecia: A Systematic Review. Archives of Dermatology. 2010;146(10):1141-50.
32. Spack NP. Management of Transgenderism. JAMA. 2013;309(5):478-84.
33. Moreno-Arrones OM, Becerra A, Vano-Galvan S. Therapeutic experience with oral finasteride for androgenetic alopecia in female-to-male transgender patients. Clinical and Experimental Dermatology. 2017;42(7):743-8.
34. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018;110(3):364-79.

35. Martin KA, Anderson RR, Chang RJ, Ehrmann DA, Lobo RA, Murad MH, et al. Evaluation and Treatment of Hirsutism in Premenopausal Women: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2018;103(4):1233-57.
36. Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, et al. Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. J Clin Endocrinol Metab. 2000;85(1):89-94.
37. van Zuuren EJ, Fedorowicz Z. Interventions for hirsutism excluding laser and photoepilation therapy alone: abridged Cochrane systematic review including GRADE assessments. Br J Dermatol. 2016;175(1):45-61.
38. Moretti C, Guccione L, Di Giacinto P, Simonelli I, Exacoustos C, Toscano V, et al. Combined Oral Contraception and Bicalutamide in Polycystic Ovary Syndrome and Severe Hirsutism: A Double-Blind Randomized Controlled Trial. J Clin Endocrinol Metab. 2018;103(3):824-38.
39. Dellis A, Zagouri F, Liontos M, Mitropoulos D, Bamias A, Papatsoris AG. Management of advanced prostate cancer: A systematic review of existing guidelines and recommendations. Cancer Treat Rev. 2019;73:54-61.
40. Thole Z, Manso G, Salgueiro E, Revuelta P, Hidalgo A. Hepatotoxicity Induced by Antiandrogens: A Review of the Literature. Urologia Internationalis. 2004;73(4):289-95.
41. Nota NM, Wiepjes CM, de Blok CJM, Gooren LJG, Peerdeman SM, Kreukels BPC, et al. The occurrence of benign brain tumours in transgender individuals during cross-sex hormone treatment. Brain. 2018;141(7):2047-54.
42. Weill A, Cadier B, Nguyen P, Coste J. Exposition prolongée à de fortes doses d'acétate de cyprotérone et risque de méningiome chez la femme. Paris: Agence Nationale de Sécurité du Médicament et des Produits de Santé; 2019.
43. European Medicines Agency. Restrictions in use of cyproterone due to meningioma risk 2020 [cited 2020 Aug 7]. Available from: https://www.ema.europa.eu/en/medicines/human/referrals/cyproterone-containing-medicinal-products.
44. Medicines and Healthcare Products Regulatory Agency. Cyproterone acetate: new advice to minimise risk of meningioma 2020 [cited 2020 Aug 7]. Available from: https://www.gov.uk/drug-safety-update/cyproterone-acetate-new-advice-to-minimise-risk-of-meningioma.
45. Defreyne J, Nota N, Pereira C, Schreiner T, Fisher AD, den Heijer M, et al. Transient Elevated Serum Prolactin in Trans Women Is Caused by Cyproterone Acetate Treatment. LGBT Health. 2017;4(5):328-36.

***Figure 1**: Flow diagram detailing systematic review process including identification and screening of records, assessment for eligibility and inclusion in review*

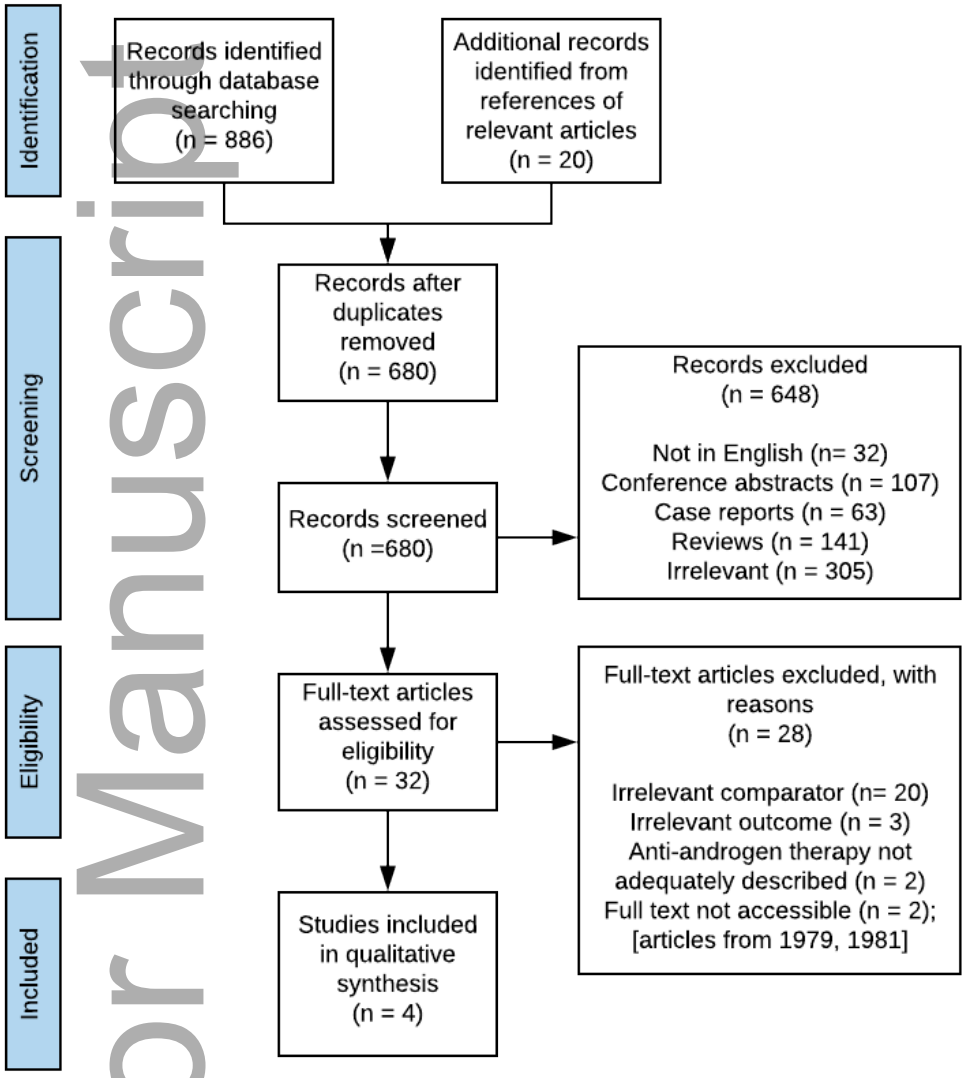

***Table 1**: Anti-androgen mechanisms of action*

Anti-androgen drugs	AR antagonist	PR agonist	ER agonist	Suppression of HPG axis
Spironolactone	Yes (weak)	Yes (weak)[#]	Yes (weak)[#]	No*
Cyproterone acetate	Yes (moderate)	Yes (strong)	No	Yes
Non-steroid anti-androgens (e.g. bicalutamide)	Yes (strong)	No	No	No*
GnRH analogues (e.g. leuprolide, triptorelin)	No	No	No	Yes
5—alpha reductase inhibitors	No	No	No	No*

(e.g. finasteride)				

[#] Clinical significance uncertain

* When used as monotherapy, reduced stimulation of the androgen receptor would be expected to stimulate the HPG axis to increase testosterone production. When combined with estradiol at sufficient doses, suppression of the HPG axis may occur resulting in decreased testosterone levels

AR, androgen receptor, ER, estrogen receptor, HPG axis, hypothalamic pituitary gonadal axis, GnRH, gonadotrophin releasing hormone, PR, progesterone receptor

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546 ***Table 2**: Characteristics of included studies*

Author	Sample size	Age (mean ±SD)	Intervention	Duration of intervention	Clinical outcomes	Change in serum total testosterone concentration
Gava et al. (2016) (18)	40	CPA group 32.9±9.4 Leu group 29.4±10.2	CPA 50mg daily + E2 **vs.** Leu 3.75mg monthly + E2	12 months	**Body composition**: No significant between-group difference. Total body fat increased at 12 months in both the CPA group (19.3±4.7kg vs. 14.9±5.6kg at baseline, P<0.05) and the leuprolide group (19.9±6.8kg vs. 15.2±5.6 kg at baseline, P<0.05) but there was no significant between-group difference. Lean mass decreased in both the CPA group (49.9±7.8kg at 12 months vs. 51.7±8.3kg at baseline, P<0.05) and the leuprolide group (49.8±6.7kg at 12months vs. 50.2±7.0kg at baseline, P<0.05), but no significant between-group difference	No significant between-group difference. Testosterone decreased from 16.3±8.3 nmol/L at baseline to 0.7±1.0 nmol/L at 12 months in the CPA group (P<0.05) and from 22.2±7.6 nmol/L at baseline to 0.7 ± 0.3 nmol/L at 12 months in the leuprolide group (P<0.05), representing significant changes from baseline but with no significant difference between groups.
Cunha et al. (2018) (21)	51	38.3±7.4	CPA 50-100mg + CEE **vs.** CEE alone	6 months	Nil relevant	No significant between-group difference. Testosterone was 21 ng/dL (0.73 nmol/L) in the CPA group and 18.0 ng/dL (0.62 nmol/L) in the CEE alone group, with no significant between-group difference (P=0.217).
Jain et al. (2019) (19)	92	31.0±7.1	E2 + SPL 100-200mg + MPA 5-10mg daily or MPA 150mg IM 3 monthly **vs.** E2 + SPL 100-200mg	Variable	**Breast growth**: 26 of 39 participants taking MPA self-reported improvement in breast development, with no comparison to those not taking MPA **Facial and body hair**: 11 of 39 participants taking MPA self-reported a decrease in facial and body hair, with no comparison to those not taking MPA	Testosterone was significantly lower in the MPA group (79±18 ng/dL (2.74±0.62 nmol/L)) than the non-MPA group (215±29 ng/dL (7.45±1.01 nmol/L)) (P<0.001).
Angus et al. (2019) (20)	80	27	CPA 25-50mg + E2 **vs.**	Variable	Nil relevant	Testosterone was significantly lower in the CPA

			SPL 87.5 - 200mg + E2 **vs.** E2 alone			group (0.8 nmol/L) than the spironolactone group (2.0 nmol/L) and estradiol alone group (10.5 nmol/L) (P=0.005)
Abbreviations: CEE, conjugated equine estrogens; CPA, cyproterone acetate, E2, estradiol; Leu, leuprolide; MPA, medroxyprogesterone acetate; SPL, spironolactone;						

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