Venous thrombotic Risk in Transgender Women Undergoing Estrogen Therapy: A Systematic Review and Meta-Analysis

Jenna Khan MD[1], Robert L. Schmidt MD, PhD, MBA[2], Matthew J. Spittal, PhD[3], Kristi J. Smock MD[2], Dina N. Greene PhD[1*]

[1]Department of Laboratory Medicine, University of Washington, Seattle, WA

[2]Department of Pathology and ARUP Laboratories, University of Utah, Salt Lake City, UT

[3]Center for Mental Health, Melbourne School of Population and Global Health, The University of Melbourne, Victoria, Australia

*Correspondence:

Dina N. Greene

1959 NE Pacific St, NW120, Box 357110

Seattle, WA 98195

Dngreene@uw.edu

Keywords:

Transgender, Transsexual, Trans women, Male to female, Cross-sex hormone therapy, Hormones, Estrogen, Thrombosis, Thromboembolism, Embolism

ABSTRACT (250 Words)

Background

Transgender women are individuals who were assigned as male at birth based on their biological sex, but who identify as female. Supporting a transgender person's gender identity can improve their psychological health, preventing gender dysphoria and its associated morbidities. One important component of medical care is estrogen administration for feminization. Previous reviews have reported conflicting literature on the thrombotic risk of estrogen therapy in transgender women and have highlighted the need for more high quality research.

Content

To help address the gap in understanding of thrombotic risk in transgender women on estrogen therapy, we performed a systematic review and meta-analysis of the literature. Two evaluators independently assessed study quality using the Ottawa Scale for Cohort Studies. The Poisson-normal model was used to estimate the study-specific incidence rates and the pooled incidence rate. Heterogeneity was measured using Higgins I^2 statistic. The overall estimate of the incidence rate was 0.0023 (95% CI:0.0008 – 0.0069). The heterogeneity was significant (I^2 = 74%, p =0.0039).

Summary

Our data suggest that the overall risk of thrombotic events in transgender women taking estrogen therapy is slightly higher than in the general population and comparable to the risk of thrombotic events associated with oral contraceptives in premenopausal women, making them a clinically acceptable therapy. There was insufficient data for subgroup analysis. Additional studies of current estrogen formulations, modes of administration, and combination therapies, as well as studies in the aging transgender population, are needed to confirm thrombotic risk and clarify optimal therapy regimes.

INTRODUCTION

Biological sex is defined at birth, typically by the visual appearance of the infant genitalia indicating the reproductive organs specific to the chromosomal make up. In contrast, gender is a psychosocial characteristic that develops with a sense of self. Social norms are generally based on the assumption that sex and gender are synonymous, but this is an unfortunate overgeneralization that leads to stereotypes and biases against those who experience gender/sex incongruence.

People who are transgender do not experience their gender as defined by their sex. This experience is not pathological, but the distress compounded from daily encounters of identity misrepresentation can lead to gender dysphoria, which is associated with depression, anxiety, and suicidal ideation(1). A national survey that solicited feedback from over 6,000 transgender people indicated that the suicide attempt rate for the transgender population is approximately 9 times that of the general population (40% vs 4.6%)(2). Standards of care endorse that supporting a transgender person's gender identity, rather than trying to identify the etiology or impose the natal sex as gender, significantly improves their psychosocial health(3). Not all transgender people seek medical interventions to affirm their gender but, for those who do, hormone therapy and/or gender affirmative surgeries are common.

Transgender women (also referred to as trans women or MtF) were assigned male at birth, but identify as female. Trans feminine individuals will also have been assigned male at birth, but identify on a gender spectrum rather than a distinct binary. Medical management of these populations includes estrogen administration, the goal of which is to feminize by softening skin, altering hair growth patterns,

redistributing body fat, altering mood, and decreasing erections (4). Estrogen can be administered orally, topically, or intramuscularly and is most often co-administered with androgen inhibitors such as spironolactone or cyproterone. Although guidelines have been published, there is international variation in prescribing practices, as well as variability in individual compliance and self-medication (3, 5, 6).

Although hormone therapy is considered a vital component of care by clinicians familiar with treating transgender individuals, many primary care physicians are not familiar with management and may be hesitant to prescribe hormone therapy (7). Lack of access to physicians competent in prescribing gender-affirming hormones has been shown to play a role in unprescribed hormone use (8). Documenting the risks associated with hormone treatment may allow for physicians to feel more comfortable with prescribing practices, allowing for better overall management of transgender people (9).

Previous studies on the clinical effects of combined oral contraceptives or hormone replacement therapy indicate that exogenous estrogen and/or progesterone is associated with an increased risk of thrombotic events (10, 11). This is not unexpected, as both of these hormones are responsible for hemostasis during pregnancy and delivery, and result in increased thrombotic risk during this time. Given this background, it was generally assumed that hormone therapies would also increase the risk of thrombotic events in trans women. While this is a reasonable hypothesis, it is not clear that studies focused on hormone replacement therapy in peri/post menopausal women or on oral contraceptives in premenopausal women can be generalized to predict the risk of hormone therapy in transgender women. Transgender women differ in important ways. For example, transgender women use different

estrogen formulations, different doses, differ in age, and, of course, differ with respect to biological sex. For that reason, studies specific to populations of trans women are necessary.

There have been several previous reviews describing the potential for estrogen to increase thrombotic risk in transgender women (4, 12, 13). These reviews have highlighted the conflicting literature, but no one has attempted to combine the results of individual studies to estimate the risk of estrogen therapy in transgender women. This is problematic because most studies in this area tend to be small and estimates from individual studies will have low reliability. A recent review highlighted thrombosis as a priority in outcomes-based research for transgender women because understanding risk can allow physician and patient to make informed decisions (14). Meta-analysis can combine the results of previous studies to provide better risk estimates. The objective of this study was to conduct a systematic review and meta-analysis to provide an estimate of the risk of venous thrombotic events associated with estrogen therapy in transgender women based on all available evidence.

METHODS

This systematic review and meta-analysis was conducted according the Cochrane Guidelines for studies on interventions(15). We also followed Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines(16). A protocol was registered in the Prospero database.

Literature Search: Search strategies were developed in consultation with a medical reference librarian (see Supplementary Materials) and executed on April 11, 2018. In brief, we searched PubMed and Embase for studies that included the incidence of thrombotic events in MtF transsexuals receiving

estrogen therapy. There were no language or date restrictions. Two additional articles were identified by hand searching the references of the included articles.

Study Selection: References were stored and reviewed using Covidence software for systematic reviews (https://www.covidence.org). Titles and abstracts were independently evaluated for inclusion by two authors (DG, JK) and discrepancies were resolved by discussion. Full-text review of the potentially relevant articles was independently performed by (DG, JK) and discrepancies were resolved by discussion and third-party review (RS). Each article had data extraction performed independently by two authors (either RS, JK or DG, KS). Studies were included if they had extractable data on the number of thrombotic events per formulation and mode of administration of estrogen therapy. Authors were contacted for clarification if studies lacked data components necessary for analysis.

Quality Appraisal: Study quality (risk of bias, lack of generalizability) was independently evaluated by two authors (DG, RS) using the Ottawa Scale for Cohort Studies(17). Discordant results were resolved by discussion.

Data Extraction: We used a data collection form to extract data from included articles. We extracted data on the following items: author, date, and language of publication; study design and control group; location and timeframe of the treatment cohort; age range and underlying conditions of the patient population; and doses and treatment duration of estrogen therapy. We created a tabulation of the number of treated patients with and without a thrombotic event by mode and formulation of estrogen therapy.

Statistical Methods: We evaluated thrombotic risk by calculating the incidence rate of venous thrombotic events (events per person year). Most studies did not categorize thrombotic events as provoked (e.g. post-surgery, related to trauma) or unprovoked. Thus, the incidence rate includes all thrombotic events (provoked and unprovoked).

Meta-analysis was performed using the *metafor* package in R (R Foundation for Statistical Computing)(18). We used the Poisson-normal model to estimate the study-specific incidence rates and the pooled incidence rate. We used this model because in 7 of our 12 studies there were zero events meaning that the estimates could not be calculated using standard meta-analysis methods. The Poisson-normal has been shown previously to estimate these parameters without bias in studies with structural zeros (19). Heterogeneity was measured using Higgins I^2 statistic(20).

RESULTS

Literature Search: Initial search results selected 2,032 references for abstract screening (Figure 1). Of these, 1,080 duplicates were removed and the remaining 952 abstracts were screened. Abstract screening indicated that 868 of the references were irrelevant; 84 references proceeded to full text review. Case reports and review articles were the most common exclusion (n=18 each), while wrong outcome (n=10) and wrong study design (n=11) also excluded a significant percent. After excluding commentaries (n=9), duplicates (n=4), and wrong patient population or wrong setting (n=1 each) 12 articles/abstracts remained for data extraction(21-32).

Characteristics of included studies: We identified twelve studies with data that allowed us to make quantitative estimates of the risk of thrombotic events in MtF transsexuals receiving estrogen therapy (Table 1). All of the studies were single arm (no controls) cohort studies. Ten of the studies (83%) were conducted in Europe, mainly in the Netherlands and Belgium; one study was conducted in the US and one in Canada. The crude incidence rate varied from 0 to 0.009 in individual studies (Figure 2).

Earlier studies (before approximately year 2000) administered oral conjugated equine estrogen or ethinyl oestradiol treatment; those after were more likely to administer oral, topical, or intramuscular estradiol valerate. Studies that spanned the time periods included a mixed cohort. Anti-androgen

administration remained consistent, with some European countries preferring cyproterone and others preferring GnRH agonists; spironolactone is the commonly prescribed anti-androgen in the US.

Duration of monitoring for thrombotic events ranged from 1-10 years, with 7 of the studies having a duration less than or equal to 2 years and 6 of the studies having a duration of greater than or equal to 3.8 years.

Sample sizes were variable across the different studies and ranged from 32-816 individuals, 7 of the studies included less than or equal to 60 participants.

Venous thrombotic risk: The overall estimate of the incidence rate was 0.0023 (95% CI:0.0008 – 0.0069) (Table 1, Figure 2). The heterogeneity was significant (I^2 = 74%, p =0.0039).

Quality Appraisal: Studies had variable quality, depending on the metric. All studies had excellent rankings for representativeness of the exposed cohort, ascertainment of exposure, demonstrating that the outcome of interest was not present at the start of the study, assessment of outcome and adequacy of follow-up. Studies had moderate ratings for length of follow-up being sufficient for outcomes to occur (assuming one event/500 patient-years). The most apparent quality limitation of the studies was their ability to utilize control groups, which was indicated by the poor scores for selection of exposed cohort and comparability of cohorts.

DISCUSSION

This review provides an estimate of the crude incidence rate for venous thrombotic events in transgender women treated with estrogen therapy. We used meta-analysis to combine the results from 12 studies and estimated that the incidence rate was 0.0023 (95% CI:0.0008 – 0.0069) . The estimated incidence rate of thrombotic events in the general population is between 0.00104 and 0.00183(33). The

estimated relative risk of thrombotic events in pre-menopausal women prescribed combined oral contraceptives is 3.5 (95% CI: 2.9-4.3) (10). Our data suggest that the risk of thrombotic events in trans women taking estrogen therapy is slightly higher than the risk of thrombotic events in the general population and comparable to the risk of thrombotic risks associated with oral contraceptives in premenopausal women.

We found significant heterogeneity in the estimates of thrombotic risk between studies. This is consistent with the informal findings of previous narrative reviews. Unfortunately, the number of studies was insufficient to perform subgroup analyses to explore the sources of heterogeneity. There are many potential sources of heterogeneity. These include mode of administration (oral vs transdermal), dose, type of androgen blocker (spironolactone vs cyproterone acetate). Given the variation in these factors, it is not surprising that we found significant heterogeneity in the estimated incidence rates. Although the heterogeneity is statistically significant, it is unlikely that it is clinically significant. Oral contraception is widely accepted and, as noted above, is about 3.5 times greater than the background risk in the general population. By comparison, our results suggest that the risk of therapy in trans women is approximately 50 to 80% greater than the background risk. When compared to the risk associated with oral contraception, the heterogeneity in results in thrombotic risk to trans women has little practical impact. Thus, despite the presence of statistically significant heterogeneity, our results suggest the risk of thrombosis associated with estrogen treatment in transgender women is clinically acceptable.

Our study may overestimate the risk of thrombosis because we included several older studies (i.e,. pre ~2000) that included data prior to the introduction of estrogen valerate. The conjugated and synthetic estrogens previously prescribed are thought to pose more of a thrombotic risk. There is controversy regarding if this increased risk is due to the type of estrogen or the route of administration (34). The earlier estrogens were administered orally only; estrogen valerate is administered orally,

intramuscularly, and topically. The existing data isn't partitioned in a way that allows us to discriminate between modes of estrogen valerate administration. Additionally, we wanted to include all available studies from the literature. Thus, our meta-analysis most likely over-estimates thrombotic risk of contemporary prescribing practices.

A gap in the literature is that the majority of studies were performed in Europe, which has different prescribing practices compared to the US. In general, European countries will have nationalized healthcare that allows for the standardized treatment of trans feminine gender incongruence. In contrast, the US has a diverse range of patients with differing access to care (9). Additionally, the anti-androgens commonly used in Europe are not FDA cleared for use in the US (cyproterone acetate) or are cost prohibitive (GnRH agonists). This distinction is not only relevant for calculating thrombotic risk, but also relevant to almost any study that seeks to investigate the influence of hormonal treatment on transgender women. Of the 12 articles included in our study, 10 were from European countries (and one was from Canada), which did not allow for sufficient data to perform a sub-group analysis. Determining whether there is a difference in rate of thrombotic events between the European and American therapeutic regimens should be an area of future study. In general, there is a need for more clinical data particular to the transgender population in the US.

There are several limitations to our analysis. First, there was insufficient data to compare risk associated with different therapies. We could not perform subgroup analysis to investigate sources of heterogeneity. Second, the studies did not include control groups, which limited our analysis to reporting the crude incidence rate rather than the incidence rate ratio. Finally, the incidence rate includes the background risk.

Additional studies are needed to address the relative contribution of estrogen formulation, mode of administration, and co-administered anti-androgen medications to thrombotic risk. Some researchers have speculated that the increased thrombotic risk is a function of first pass liver metabolism of oral estrogens. This may not be true, as oral estradiol valerate may impose little risk relative to topical. A recent study demonstrated an eight-fold higher incidence of VTE with CEE compared to oral estradiol suggesting that the formulation of estrogen used rather than the route of delivery may be more important for determining thrombotic risk (Seal 2012). Cyproterone acetate is a highly potent anti-androgen typically used in Europe and has been reported to have procoagulant effects. As it is not available in the USA and spironolactone is the more commonly used anti-androgen, current literature may overestimate the thrombosis risk in the USA.

Future studies should also address the overall risk in the aging population. The average age for participants in these studies was 30 years old. This average age is representative of many trans women, but may not adequately predict risk in those who are aging or have underlying co-morbidities, particularly smoking and obesity.

Conclusion: The risk of venous thrombotic events associated with estrogen therapy for trans women is less/comparable to the risk associated with oral contraceptives in premenopausal women. There is no evidence to suggest that there is a difference in the response to estrogen (thrombotic risk) in sex-assigned men and women.

REFERENCES

1. Bruessow D, Poteat T. Primary care providers' role in transgender healthcare. JAAPA 2018;31:8-11.
2. JM G, LA M, J T. Injustice at every turn: A report of the national transgender discrimination survey. . Washington, DC: National Center for Transgender Equality and National Gay and Lesbian Task Force 2011.
3. 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;102:3869-903.
4. Tangpricha V, den Heijer M. Oestrogen and anti-androgen therapy for transgender women. Lancet Diabetes Endocrinol 2017;5:291-300.
5. Mamoojee Y, Seal LJ, Quinton R. Transgender hormone therapy: Understanding international variation in practice. Lancet Diabetes Endocrinol 2017;5:243-6.
6. Royal College of Psychiatrists. Good practice guidelines for the assessment and treatment of adults with gender dysphoria. 2013;CR181.
7. Chipkin SR, Kim F. Ten most important things to know about caring for transgender patients. Am J Med 2017;130:1238-45.
8. de Haan G SG, Arayasirikul S, Raymond HF. Non-prescribed hormone use and barriers to care for transgender women in san francisco. LGBT Health 2015;4:313-23.
9. Safer JD, Coleman E, Feldman J, Garofalo R, Hembree W, Radix A, Sevelius J. Barriers to healthcare for transgender individuals. Curr Opin Endocrinol Diabetes Obes 2016;23:168-71.
10. de Bastos M, Stegeman BH, Rosendaal FR, Van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, Dekkers OM. Combined oral contraceptives: Venous thrombosis. Cochrane Database Syst Rev 2014:Cd010813.
11. Canonico M, Oger E, Plu-Bureau G, Conard J, Meyer G, Lévesque H, et al. Hormone therapy and venous thromboembolism among postmenopausal women: Impact of the route of estrogen administration and progestogens: The esther study. Circulation 2007;115:840-5.
12. Maraka S, Singh Ospina N, Rodriguez-Gutierrez R, Davidge-Pitts CJ, Nippoldt TB, Prokop LJ, Murad MH. Sex steroids and cardiovascular outcomes in transgender individuals: A systematic review and meta-analysis. J Clin Endocrinol Metab 2017;102:3914-23.
13. Shatzel JJ, Connelly KJ, DeLoughery TG. Thrombotic issues in transgender medicine: A review. Am J Hematol 2017;92:204-8.
14. Feldman J, Brown GR, Deutsch MB, Hembree W, Meyer W, Meyer-Bahlburg HF, et al. Priorities for transgender medical and healthcare research. Curr Opin Endocrinol Diabetes Obes 2016;23:180-7.
15. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions: John Wiley & Sons, 2011.
16. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The prisma statement. PLoS Medicine 2009;6.
17. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. Ottawa hospital research institute. The newcastle-ottawa scale (nos) for assessing the quality of nonrandomized studies in meta-analyses. Vol. Ottawa, Canada: The Ottawa Hospital, 2009.
18. Viechtbauer W. Conducting meta-analyses in r with the metafor package. J Stat Softw 2010;36:1-48.

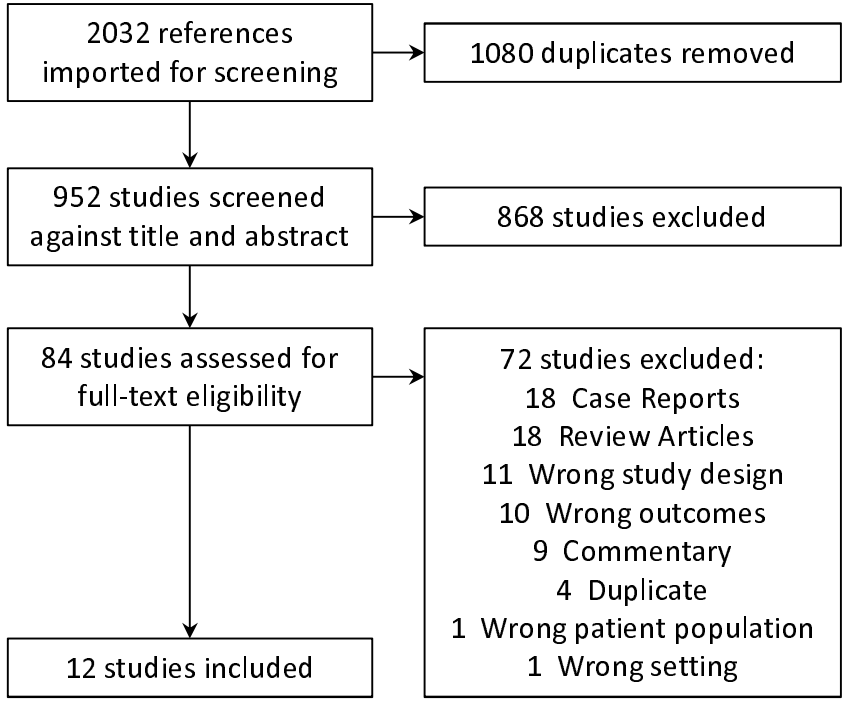
19. Spittal MJ, Pirkis J, Gurrin LC. Meta-analysis of incidence rate data in the presence of zero events. BMC medical research methodology 2015;15:42.
20. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58.
21. Van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. Clinical endocrinology 1997;47:337-43.
22. Prior JC, Vigna YM, Watson D. Spironolactone with physiological female steroids for presurgical therapy of male-to-female transsexualism. Archives of Sexual Behavior 1989;18:49-57.
23. Wierckx K, Elaut E, Declercq E, Heylens G, De Cuypere G, Taes Y, et al. Prevalence of cardiovascular disease and cancer during cross-sex hormone therapy in a large cohort of trans persons: A case–control study. European journal of endocrinology 2013;169:471-8.
24. Arnold JD, Sarkodie EP, Coleman ME, Goldstein DA. Incidence of venous thromboembolism in transgender women receiving oral estradiol. The journal of sexual medicine 2016;13:1773-7.
25. Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher A, Toye K, et al. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: Results from the european network for the investigation of gender incongruence. The journal of sexual medicine 2014;11:1999-2011.
26. 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;85:239-46.
27. Ott J, Kaufmann U, Bentz E-K, Huber JC, Tempfer CB. Incidence of thrombophilia and venous thrombosis in transsexuals under cross-sex hormone therapy. Fertility and sterility 2010;93:1267-72.
28. Dittrich R, Binder H, Cupisti S, Hoffmann I, Beckmann M, Mueller A. Endocrine treatment of male-to-female transsexuals using gonadotropin-releasing hormone agonist. Experimental and clinical endocrinology & diabetes 2005;113:586-92.
29. Wierckx K, Van Caenegem E, Taes Y, Kaufman JM, Schreiner T, T'Sjoen G. Endocrine treatment of transexual persons: A multicenter prospective study using a standardized treatment protocol. Endocrine Reviews 2013;34.
30. Seal L, Franklin S, Richards C, Shishkareva A, Sinclaire C, Barrett J. Predictive markers for mammoplasty and a comparison of side effect profiles in transwomen taking various hormonal regimens. The Journal of Clinical Endocrinology & Metabolism 2012;97:4422-8.
31. De Cuypere G, TSjoen G, Beerten R, Selvaggi G, De Sutter P, Hoebeke P, et al. Sexual and physical health after sex reassignment surgery. Archives of sexual behavior 2005;34:679-90.
32. Hohl CA, Camara VA, Zanardi UV, Inacio M, Verduguez EU, Sircili MHP, et al. Efficacy and safety of low doses of estrogens assciated with anitandrogens in hormal treatment of male-to-female transsexual patients. (Accessed May 10 2018).
33. Heit JA. Epidemiology of venous thromboembolism. Nature Reviews Cardiology 2015;12:464.
34. Seal LJ. A review of the physical and metabolic effects of cross-sex hormonal therapy in the treatment of gender dysphoria. Ann Clin Biochem 2016;53:10-20.

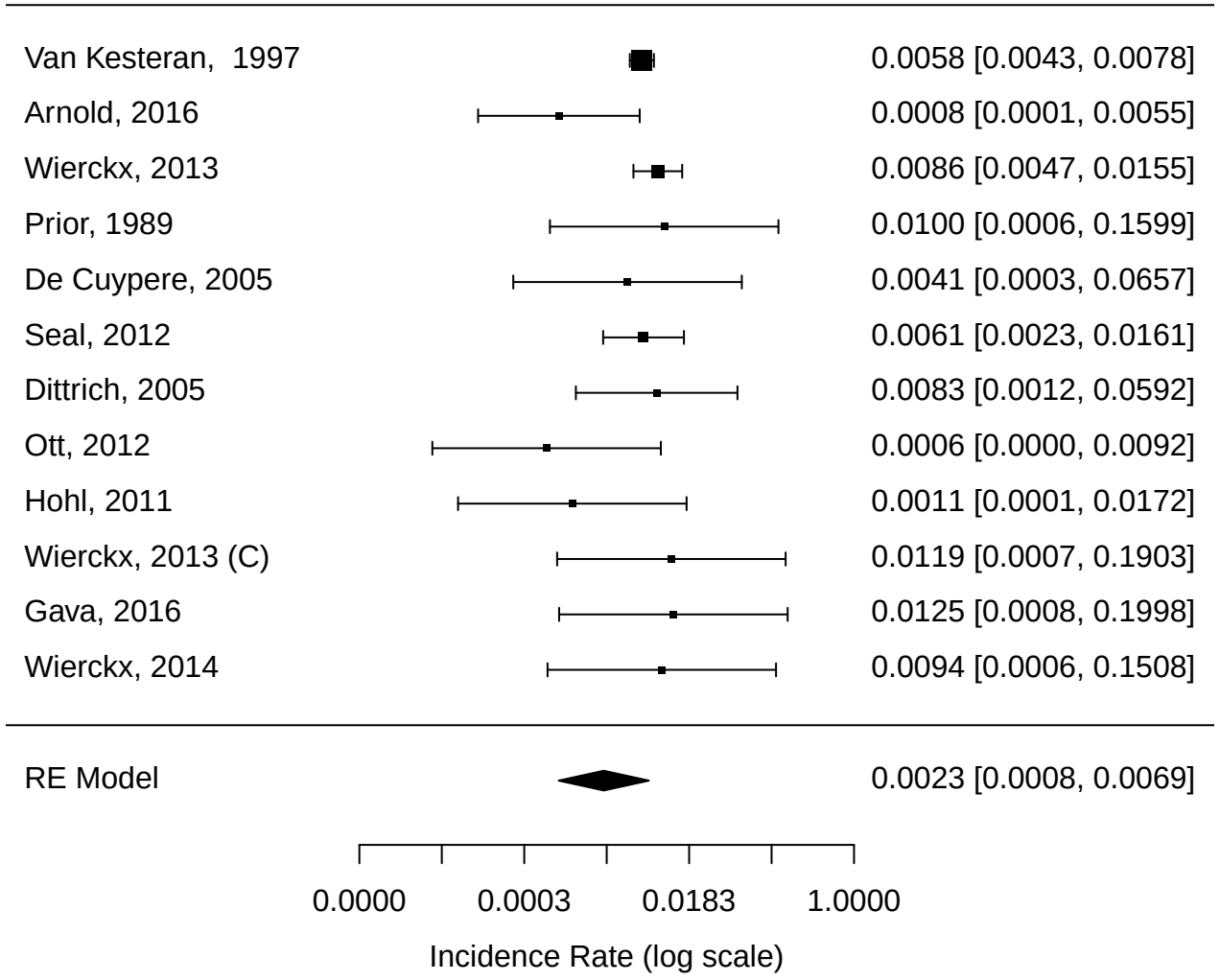
Table 1: Characteristics of Included Studies (Submitted as supplemental table due to formatting issues inserting such a large document into word document)

Figure Legends

Figure 1: PRISMA Diagram illustrating literature search results

Figure 2: Forest plot of included studies. The squares indicate the incidence rate (IR) of each individual study. The size of the square is proportional to the weight given each study in the meta-analysis. The whiskers indicate the 95% confidence interval for the IR for each individual study. The diamond at the bottom is the overall estimate based on all studies. The width of the diamond is the 95% confidence interval of the overall estimate. The numbers follow each study are the individual study estimate and the 95% confidence interval. RE = random effects.


2032 references imported for screening
1080 duplicates removed
952 studies screened against title and abstract
868 studies excluded
84 studies assessed for full-text eligibility
72 studies excluded:
18 Case Reports
18 Review Articles
11 Wrong study design
10 Wrong outcomes
9 Commentary
4 Duplicate
1 Wrong patient population
1 Wrong setting
12 studies included


Van Kesteran, 1997
0.0058 [0.0043, 0.0078]
Arnold, 2016
0.0008 [0.0001, 0.0055]
Wierckx, 2013
0.0086 [0.0047, 0.0155]
Prior, 1989
0.0100 [0.0006, 0.1599]
De Cuypere, 2005
0.0041 [0.0003, 0.0657]
Seal, 2012
0.0061 [0.0023, 0.0161]
Dittrich, 2005
0.0083 [0.0012, 0.0592]
Ott, 2012
0.0006 [0.0000, 0.0092]
Hohl, 2011
0.0011 [0.0001, 0.0172]
Wierckx, 2013 (C)
0.0119 [0.0007, 0.1903]
Gava, 2016
0.0125 [0.0008, 0.1998]
Wierckx, 2014
0.0094 [0.0006, 0.1508]
RE Model
0.0023 [0.0008, 0.0069]
0.0000
0.0003
0.0183
1.0000
Incidence Rate (log scale)

Author/s:
Khan, J; Schmidt, RL; Spittal, MJ; Goldstein, Z; Smock, KJ; Greene, DN

Title:
Venous Thrombotic Risk in Transgender Women Undergoing Estrogen Therapy: A Systematic Review and Metaanalysis

Date:
2019-01-01

Citation:
Khan, J., Schmidt, R. L., Spittal, M. J., Goldstein, Z., Smock, K. J. & Greene, D. N. (2019). Venous Thrombotic Risk in Transgender Women Undergoing Estrogen Therapy: A Systematic Review and Metaanalysis. CLINICAL CHEMISTRY, 65 (1), pp.57-66. https://doi.org/10.1373/clinchem.2018.288316.