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Effects of Tibolone on Fibrinogen and Anti-Thrombin III: A Systematic Review and Meta-Analysis of Controlled Trials

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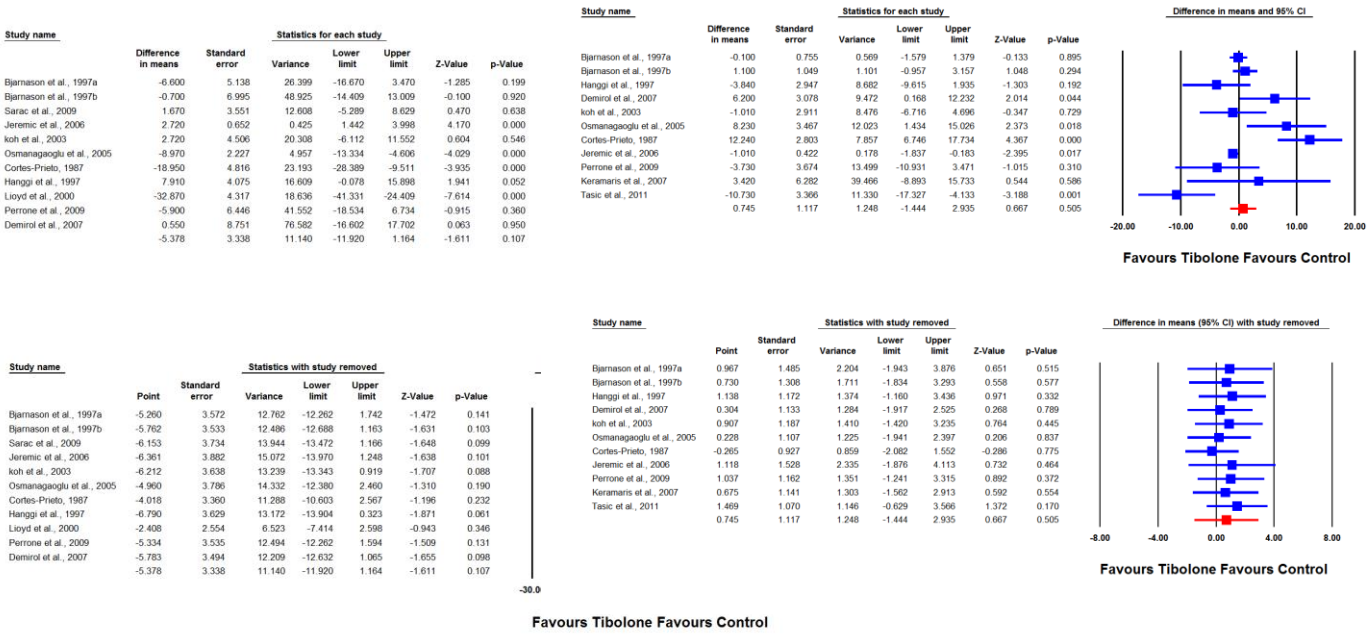
Graphical abstract

Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of tibolone on plasma fibrinogen (left panel) and ATP III (right panel) concentrations. Lower plot shows leave-one-out sensitivity analysis.



ABSTRACT:

Tibolone is a synthetic steroid with estrogenic, androgenic and progestogenic activity, but the evidence regarding its effects on fibrinogen and anti-thrombin III (ATIII) has not been conclusive. We assessed the impact of tibolone on fibrinogen and ATIII through a systematic review and meta-analysis of available randomized controlled trials (RCTs). The search included PUBMED, Web of Science, Scopus, and Google Scholar (up to January 31st, 2016) to identify controlled clinical studies investigating the effects of oral tibolone treatment on fibrinogen and ATIII. Overall, the impact of tibolone on plasma fibrinogen concentrations was reported in 10 trials comprising 11 treatment arms. Meta-analysis did not suggest a significant reduction of

fibrinogen levels following treatment with tibolone (WMD: -5.38%, 95% CI: -11.92, +1.16, p = 0.107). This result was robust in the sensitivity analysis and not influenced after omitting each of the included studies from meta-analysis. When the studies were categorized according to the duration of treatment, there was no effect in the subsets of trials lasting either < 12 months (WMD: -7.64%, 95% CI: -16.58, +1.29, p = 0.094) or ≥ 12 months (WMD: -0.62%, 95% CI: -8.40, +7.17, p = 0.876). With regard to ATIII, there was no change following treatment with tibolone (WMD: +0.74%, 95% CI: -1.44, +2.93, p = 0.505) and this effect was robust in sensitivity analysis. There was no differential effect of tibolone on plasma ATIII concentrations in trials with either < 12 months (WMD: +2.26%, 95% CI: -3.14, +7.66, p = 0.411) or ≥ 12 months (WMD: +0.06%, 95% CI: -1.16, +1.28, p = 0.926) duration. Consistent with the results of subgroup analysis, meta-regression did not suggest any significant association between the changes in plasma concentrations of fibrinogen (slope: +0.40; 95% CI: -0.39, +1.19; p = 0.317) and ATIII (slope: -0.17; 95% CI: -0.54, +0.20; p = 0.374) with duration of treatment. In conclusion, meta-analysis did not suggest a significant reduction of fibrinogen and ATIII levels following treatment with tibolone.

Key words: anti-thrombin III, fibrinogen, postmenopausal women, tibolone.

No of words: 323

INTRODUCTION

Several studies found association between elevated plasma fibrinogen levels and cardiovascular disease (CVD) (1-3). Fibrinogen is one of the most important nontraditional CV risk factors (3-8). Associations of fibrinogen levels and coronary artery disease (CAD) or stroke were not dependent on sex, smoking, blood pressure or blood lipid levels (2). However, measurement of plasma fibrinogen to improve the prediction of CV risk is not recommended (7). Anti-thrombin III (ATIII) represents a major physiological inhibitor of thrombin activity in circulating blood (9). Low ATIII activity levels have been linked to elevated CV risk (10-12), and its deficiency, a genetically determined severe thrombophilic state, is associated with

markedly increased risk of venous thromboembolism (13,14), however this abnormality may also predispose to arterial thromboembolic events, including stroke, at young age (15).

The incidence of CVD increases in women after menopause being a leading cause of mortality in postmenopausal women (16, 17). Menopause is associated with sex hormone deficiency and pro-atherogenic lipid profile changes including increase in total cholesterol and low density lipoprotein cholesterol (LDL-C) (18). Although observational studies had suggested some benefits with hormone replacement therapy (HRT) (19-21), randomized controlled trials (RCTs) failed to confirm it in terms of CV and thromboembolic risks (22, 23).

Tibolone (Livial©, Tibofem©) is a synthetic steroid exhibiting estrogenic, progestogenic and androgenic activity. Its two major active metabolites - 3α- and 3β-hydroxytibolone - act as potent, fully activating agonists of the estrogen receptor, and its metabolite Δ^4-tibolone act as agonist of the progesterone and androgen receptors (3α- and 3β hydroxytibolone conversely, act as antagonists of the same receptors). Moreover, tibolone acts as an antagonist of the glucocorticoid and mineralocorticoid receptors (24-27). Tibolone represents an attractive treatment option in postmenopausal women (24, 25) that effectively can reduce menopausal symptoms (24, 26, 27). At present tibolone is recommended as an alternative to menopausal hormone therapy in women with climacteric symptoms and no history of breast cancer and no other contraindications (28) especially in women with mood disorders and sexual dysfunction (29) and those with a history of endometriosis (30). A systematic review by Formoso *et al.* did not yield conclusive results regarding effect of tibolone on CV events (26). In the analyses of surrogate endpoints tibolone was reported to have various CV effects, including favourable effect on acute myocardial infarction and thromboembolism (24, 31, 32). Available data on tibolone-induced alterations to coagulation parameters were inconsistent (27, 31, 33). Previous studies reported reductions of fibrinogen level (8) or increase in ATIII level (27), while other investigators suggested procoagulant effects through lower ATIII levels (8, 34) or no change (31, 35). Therefore the aim of the present study was to assess the impact of tibolone on fibrinogen and ATIII levels.

METHODS

Search Strategy

This study was designed according to the guidelines of the 2009 preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement (36). SCOPUS (http://www.scopus.com), Medline (http://www.ncbi.nlm.nih.gov/pubmed) AND Google Scholar (http://www.scholar.google.com) databases were searched using the following search terms in titles and abstracts (also in combination with MESH terms): (tibolone OR OrgOD14 OR "Org OD14" OR livial OR livial®) AND (fibrinogen OR antithrombin OR antithrombin III or "antithrombin III" OR ATIII OR "AT III")). The wild-card term ''*'' was used to increase the sensitivity of the search strategy. No language restriction was used in the literature search. The search was limited to studies in human. The literature was searched from inception to January 31st, 2016.

Study Selection

Original studies were included if they met the following inclusion criteria: (i) being a controlled clinical trial with either parallel or cross-over design, (ii) investigating the impact of tibolone on plasma concentrations of fibrinogen and/or ATIII, (iii) presentation of sufficient data on fibrinogen and/or ATIII concentrations at baseline and at the end of follow-up in each group or providing the net change values. Exclusion criteria were (i) lack of a an appropriate control group in the study design, (ii)observational studies with case-control, cross-sectional or cohort design, and (iii) lack of sufficient information on baseline or follow-up fibrinogen and/or ATIII concentrations.

Data extraction

Eligible studies were reviewed and the following data were abstracted: 1) first author's name; 2) year of publication; 3) study location; 4) study design; 5) number of participants in the tibolone and control groups; 5) age and body mass index (BMI) of study participants; 6) prevalence of diabetes mellitus; and 7) baseline and follow-up plasma concentrations of fibrinogen and/or ATIII.

Quality assessment

A systematic assessment of bias in the included studies was performed using the Cochrane criteria (37). The items used for the assessment of each study were as follows: adequacy of sequence generation, allocation concealment, blinding of subjects and personnel, blinding of

outcome assessment, addressing of dropouts (incomplete outcome data), selective outcome reporting, and other potential sources of bias. According to the recommendations of the Cochrane Handbook, a judgment of "yes" indicated low risk of bias, while "no" indicated high risk of bias. Labeling an item as "unclear" indicated an unclear or unknown risk of bias.

Quantitative Data Synthesis

Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) (38). Net changes in measurements (change scores) were calculated as follows: measure at end of follow-up − measure at baseline. For cross-over trials, net change in plasma concentrations of fibrinogen and/or ATIII were calculated by subtracting the value after control intervention from that reported after treatment. All values were collated in percentage changes from baseline levels. Standard deviations (SDs) of the mean difference were calculated using the following formula: SD = square root $[(SD_{pre\text{-}treatment})^2 + (SD_{post\text{-}treatment})^2 - (2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]$, assuming a correlation coefficient (R) = 0.5. If the outcome measures were reported in median and inter-quartile range, mean and standard SD values were estimated using the method described by Hozo *et al.* (39). Where standard error of the mean (SEM) was only reported, standard deviation (SD) was estimated using the following formula: SD = SEM × sqrt (*n*), where *n* is the number of subjects. When the results were presented in multiple time points, only data relating to the longest duration of treatment were considered.

A random-effects model (using DerSimonian-Laird method) and the generic inverse variance method were used to compensate for the heterogeneity of studies in terms of demographic characteristics of populations being studied and also differences in study design. Heterogeneity was quantitatively assessed using I^2 index. Effect sizes were expressed as weighted mean difference (WMD) and 95% confidence interval (CI). In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using leave-one-out method, i.e. removing one study each time and repeating the analysis.

Meta-regression

Random-effects meta-regression was performed using unrestricted maximum likelihood method to evaluate the association between calculated WMD and duration of treatment as a potential moderator.

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, and Begg's rank correlation and Egger's weighted regression tests. Duval &Tweedie "trim and fill"method was used to adjust the analysis for the effects of publication bias (40).

RESULTS

Multidatabase search provided 64 articles. Of those, 51 were excluded after initial screening and the remaining 13 full text papers were reviewed. Finally, 12 were scrutinized as full texts and were selected to be included in the analysis (32, 35, 41-50). One article was excluded because of incomplete data **(Figure 1).**

In total, 459 participants were allocated to tibolone supplementation group and 382 to control group in the selected studies. The number of participants in the analyzed studies ranged from 19 to 90 in the tibolone group and from 11 to 88 in the control group. They were published between 1987 and 2011, and were conducted in Turkey (3), Serbia (2), Greece, Italy, Korea, the Netherlands, Spain, Switzerland and UK. A range of tibolone doses from 1.25 to 2.5 mg/day was administered in the included trials. Duration of supplementation with tibolone ranged between 2 and 24 months. Tibolone was safe and well-tolerated in all included studies with no report of any drug-related adverse events. Demographic and baseline parameters of the included studies are shown in **Table 1**.

Risk of bias assessment

In the majority of studies an unclear or high risk of bias with respect to sequence generation, allocation concealment and selective outcome reporting. Some studies were not randomized, nor blinded. The systematic assessment of bias in the included studies is presented in **Table 2**.

Effect of tibolone on plasma fibrinogen concentrations

Overall, the impact of tibolone on plasma fibrinogen concentrations was reported in 10 trials comprising 11 treatment arms. Meta-analysis did not suggest a significant reduction of fibrinogen levels following treatment with tibolone (WMD: -5.38%, 95% CI: -11.92, +1.16, p= 0.107; I^2=91.08%). This result was robust in the sensitivity analysis and not influenced after

omitting each of the included studies from meta-analysis (**Figure 2**). When the studies were categorized according to the duration of treatment, there was no effect in the subsets of trials lasting either < 12 months (WMD: -7.64%, 95% CI: -16.58, +1.29, p= 0.094; I^2=94.33%) or ≥ 12 months (WMD: -0.62%, 95% CI: -8.40, +7.17, p= 0.876; I^2=51.88%) (**Figure 3**).

There was no change in plasma ATIII following treatment with tibolone (WMD: +0.74%, 95% CI: -1.44, +2.93, p= 0.505; I^2=78.92%) and this effect was robust in sensitivity analysis (**Figure 4**). There was no differential effect of tibolone on plasma ATIII concentrations in trials with either < 12 months (WMD: +2.26%, 95% CI: -3.14, +7.66, p= 0.411; I^2=88.20%) or ≥ 12 months (WMD: +0.06%, 95% CI: -1.16, +1.28, p= 0.926; I^2=3.13%) duration (**Figure 5**).

Meta-regression

Random-effects meta-regression was performed to assess if the changes in fibrinogen and ATIII are associated with duration of treatment. Consistent with the results of subgroup analysis, meta-regression did not suggest any significant association between the changes in plasma concentrations of fibrinogen (slope: +0.40; 95% CI: -0.39, +1.19; p = 0.317) and ATIII (slope: -0.17; 95% CI: -0.54, +0.20; p = 0.374) with duration of treatment (**Figure 6**).

Publication bias

The funnel plot of the study standard error by effect size (WMD) was symmetric, suggesting lack of publication bias in the analysis of tibolone's effect on plasma ATIII concentrations. This finding was confirmed by the results of Egger's linear regression (intercept = 0.87, standard error = 0.93; 95% CI = -1.24, 2.98, t = 0.94, df = 9, two-tailed p = 0.374) and Begg's rank correlation tests (Kendall's Tau with continuity correction = 0.04, z = 0.16, two-tailed p-value = 0.876) (**Figure 7**). With regard to fibrinogen, visual inspection of funnel plot suggested a slight asymmetry that was imputed by two potentially missing studies on the left side of plot using "trim and fill "method (imputed WMD: -7.88%; 95% CI = -14.53, -1.23). In spite of this asymmetry, there was no sign of publication bias according to Egger's linear regression (intercept = -2.23, standard error = 1.22; 95% CI = -4.98, 0.53, t = 1.83, df = 9, two-tailed p = 0.101) and Begg's rank correlation tests (Kendall's Tau with continuity correction = -0.04, z = 0.16, two-tailed p-value = 0.876).

DISCUSSION

The present meta-analysis of the data from 12 controlled trials including 841 patients has not revealed any significant alterations in the levels of two key coagulation proteins, i.e. fibrinogen and ATIII following treatment with tibolone in postmenopausal women. Our meta-analysis provided new information on the effects of tibolone on fibrinogen and ATIII, indicating that these two variables did not contribute to cardiovascular and thrombotic risks in women receiving tibolone. This observation might suggest that this form of therapy is neutral in terms of the occurrence of thromboembolic events, which are increasingly observed after menopause especially during combined hormone replacement therapy.

Tibolone was reported to have various CV effects (31). So far beneficial effect of tibolone was shown with respect to vasodilation, due to its estrogenic activity (24), and blood pressure (32). However it has also been shown to change lipid profile – due to its androgenic activity (24) – reductions of HDL cholesterol level were observed (27, 33, 51), lower triglyceride levels were also reported (27, 51). On the other hand tibolone was also shown to reduce lipoprotein(a) levels, an independent risk factor for CVD in postmenopausal women (52).

In the Osteoporosis Prevention and Arterial effects of tiboLone (OPAL) trial tibolone effect on progression of atherosclerosis evaluated with carotid intima media thickness was assessed against continuous combined conjugated equine oestrogens plus medoxyprogesterone acetate and it was found that both active treatments were associated with increased progression as compared with placebo (53). Studies with clinical endpoint brought concern regarding tibolone use in older postmenopausal women. LIFT trial, which evaluated tibolone against placebo in older postmenopausal women was stopped early due to observed increased risk of stroke (6), but this finding was not confirmed in randomized controlled trial including younger women (54). Cochrane systematic review found higher risk of cerebrovascular events mainly due to results of abovementioned LIFT study and have not found significant differences in the risk of coronary heart disease, but since more cases were observed in tibolone than placebo group, it concluded that there was a trend toward increase of risk with the drug (26).

There is evidence of beneficial effect of tibolone on bone loss and risk of fractures (26, 55), incidence of vaginal bleeding in comparison with traditional hormone replacement therapy (26) and risk of invasive breast cancer in women without breast cancer history (5, 6), but not in women with breast cancer history (54). Therefore it is currently recommended by the Endocrine

Society as an alternative to menopausal hormone therapy in women with climacteric symptoms and no history of breast cancer and no other contraindications (28).

Our meta-analysis has strengths and limitations. Strength of present meta-analysis include extensive literature search including three databases without any language restrictions. The aim of nine of the studies was to measure the effect of tibolone on several markers, including two hemostasis markers assessed in present meta-analysis, so their aims were consistent with aims of our analysis. The risk of bias was performed in all included studies in accordance with Cochrane Risk of Bias Tool (37). The robustness of our results was checked using leave-one-out sensitivity analyses and heterogeneity was explored using both subgroup analyses and meta-regression.

The present meta-analysis also has several limitations. All the included studies had surrogate endpoints and assessed the effect of tibolone in comparison with placebo or no treatment; they also included estrogen only or estrogen/progestogen therapy group. Included studies used different assays for determination of fibrinogen and ATIII levels. None of the studies assessed any long-term cardiovascular or thrombotic outcomes. Most of the studies were of unclear or high risk of bias in more than one domain, especially with regard to randomization, which was not done or not properly reported in most of the studies. Unclear or inadequate sequence generation; allocation concealment is associated with overestimation of treatment effect (37, 56). Lack of blinding may also have an effect on the estimation of treatment effect (56), however it is believed to be less evident in case of objective outcomes (17, 57), such as laboratory measures, which were used in all the studies included in the meta-analysis. Regarding the influence of commercial funding bias (34) only four studies provided information about funding. So, overall quality of evidence on the effect of tibolone on fibrinogen and ATIII is low. Another limitation of our study is that we have not searched for unpublished studies, which may have introduced publication bias (8), however analyses of publication bias suggested no presence of such bias for ATIII level and slight asymmetry for fibrinogen level analyses.

Observed non-significant difference in fibrinogen and ATIII level changes after tibolone treatment as compared with placebo suggest no increase or decrease in cardiovascular risk attributed to those factors. In order to find out what is overall tibolone effect it would be necessary to evaluate its effect on all cardiovascular risk factors and on cardiovascular events.

In conclusion present meta-analysis revealed no consistent increase or decrease of fibrinogen or ATIII levels, however those results should be viewed cautiously because of high or

unclear risk of bias in the included studies. Use of tibolone requires careful assessment of risks and benefits of this therapy.

CONFLICT OF INTEREST:

Funding. This meta-analysis was written independently; no company or institution supported it financially.

***Declaration of interest*:** All the authors have nothing to declare. No professional writer was involved in the preparation of this meta-analysis. The meta-analysis has been prepared within *the Lipid and Blood Pressure Meta-analysis Collaboration* (LBPMC) Group (www.lbpmcgroup.umed.pl).

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FIGURE LEGENDS

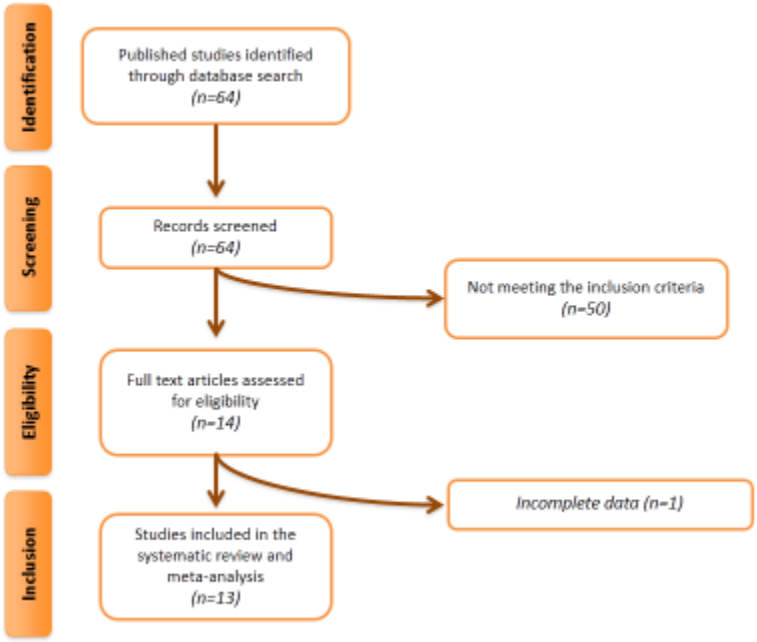

Figure 1. Flow chart of the number of studies identified and included into the meta-analysis.

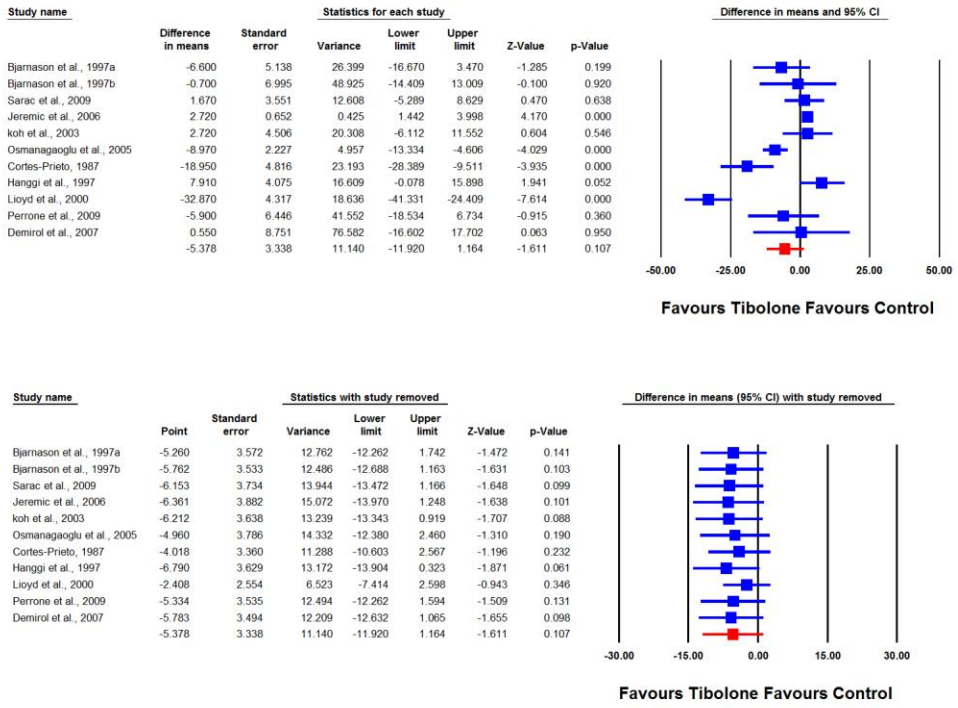

Figure 2. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of tibolone on plasma fibrinogen concentrations. Lower plot shows leave-one-out sensitivity analysis.

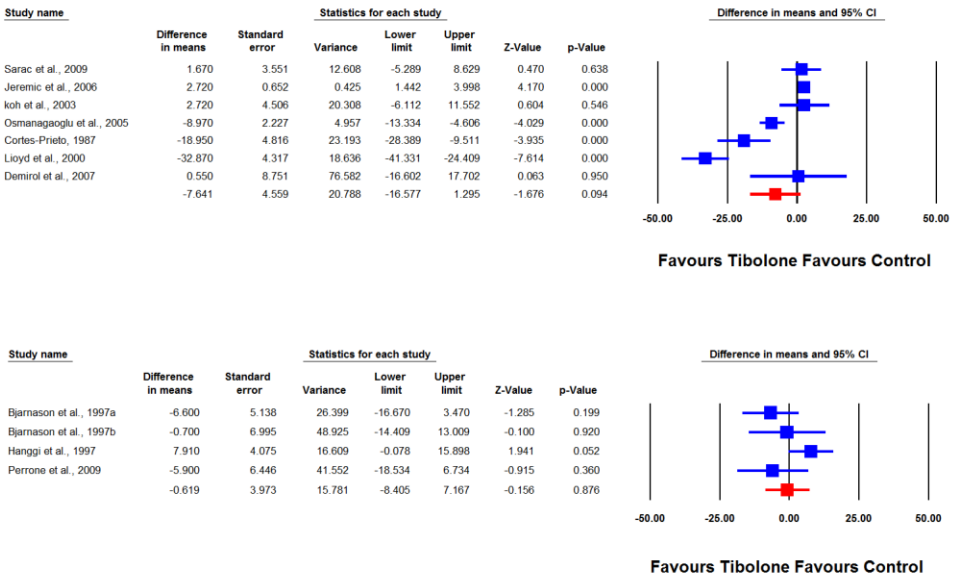

Figure 3. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of tibolone on plasma fibrinogen concentrations in trials lasting < 12 months (upper plot) and ≥ 12 months (lower plot).

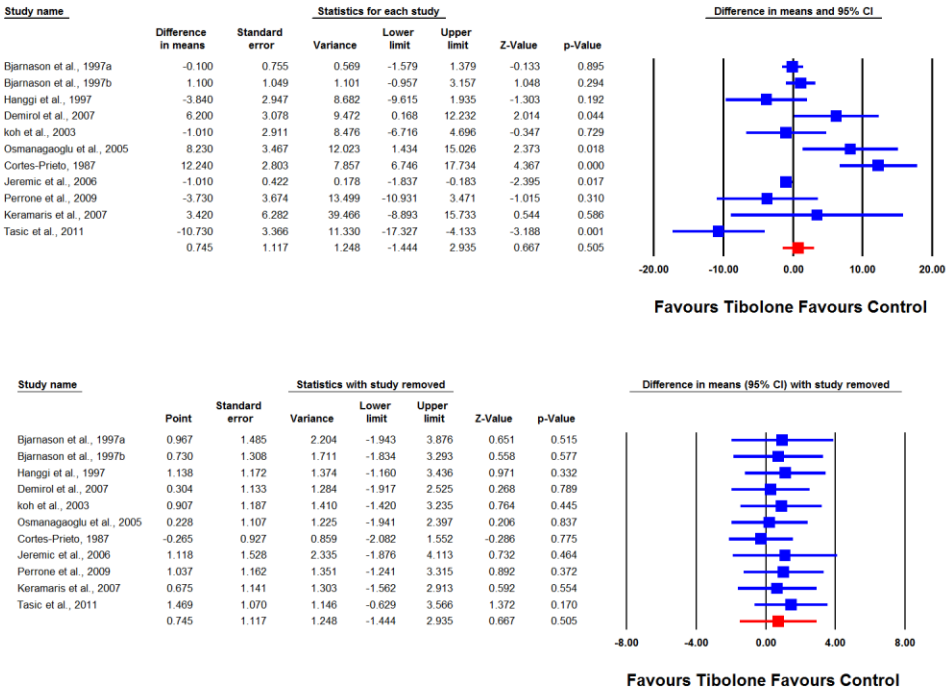

Figure 4. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of tibolone on plasma ATIII concentrations. Lower plot shows leave-one-out sensitivity analysis.

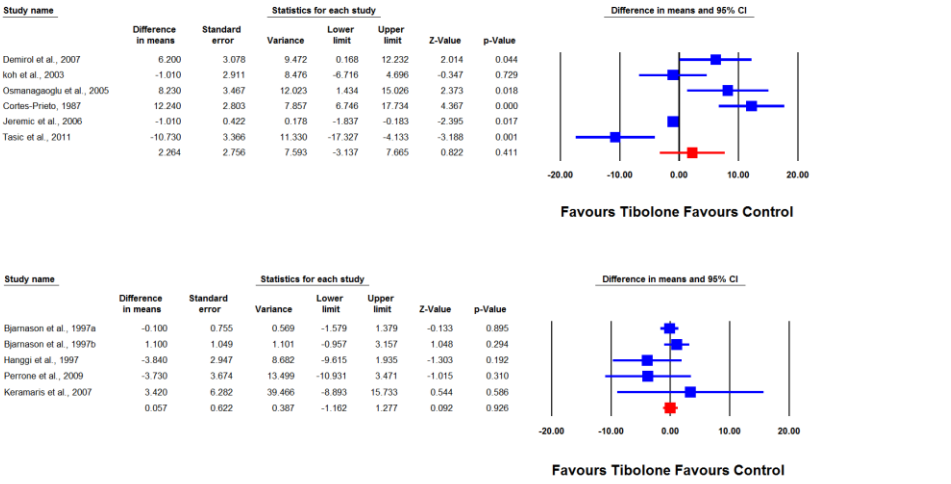

Figure 5. Forest plot displaying weighted mean difference and 95% confidence intervals for the impact of tibolone on plasma ATIII concentrations in trials lasting < 12 months (upper plot) and ≥ 12 months (lower plot).

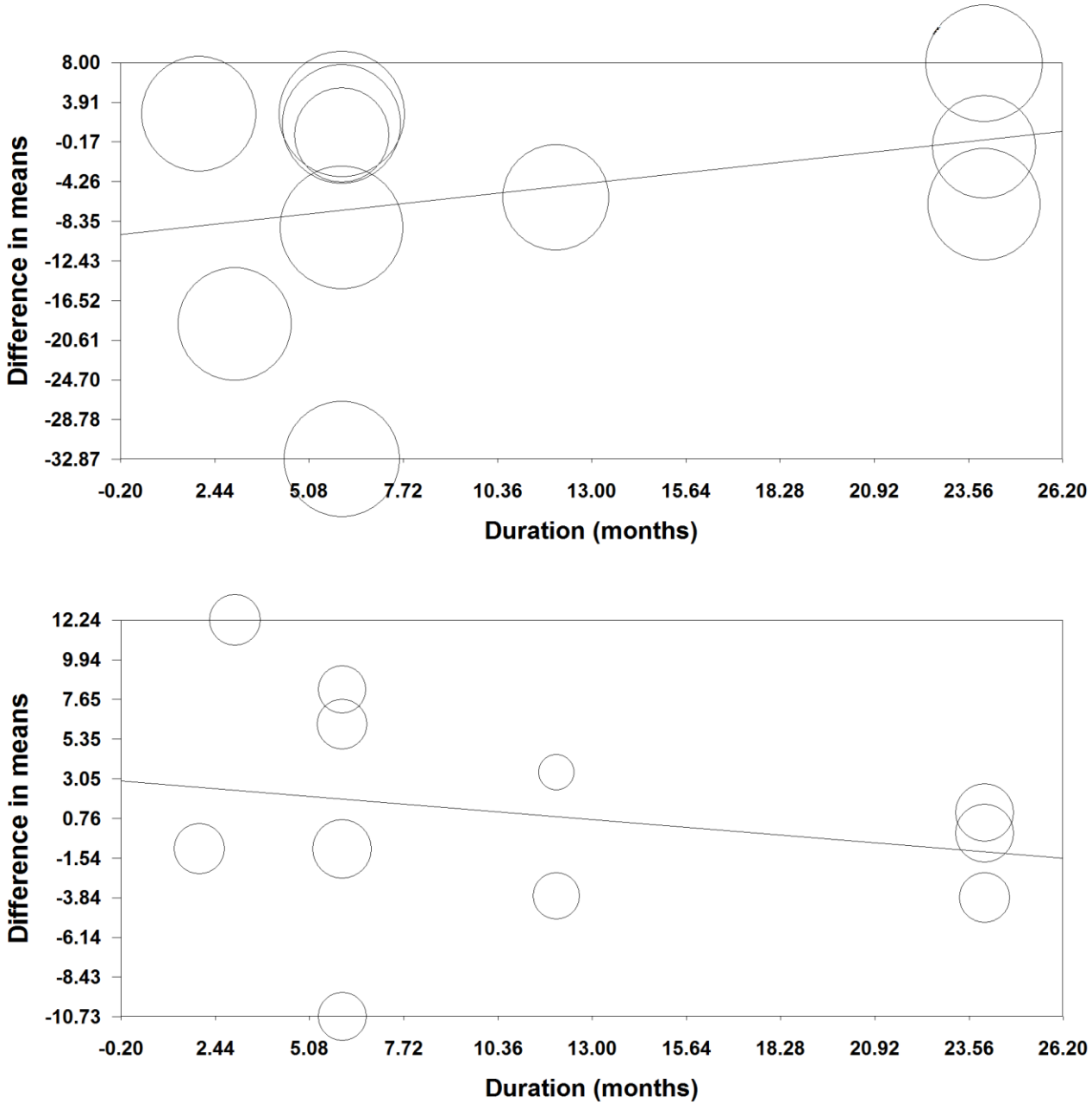

Figure 6. Meta-regression plots of the association between mean changes in plasma fibrinogen (upper plot) and ATIII (lower plot) concentrations with duration of treatment with tibolone. The size of each circle is inversely proportional to the variance of change.

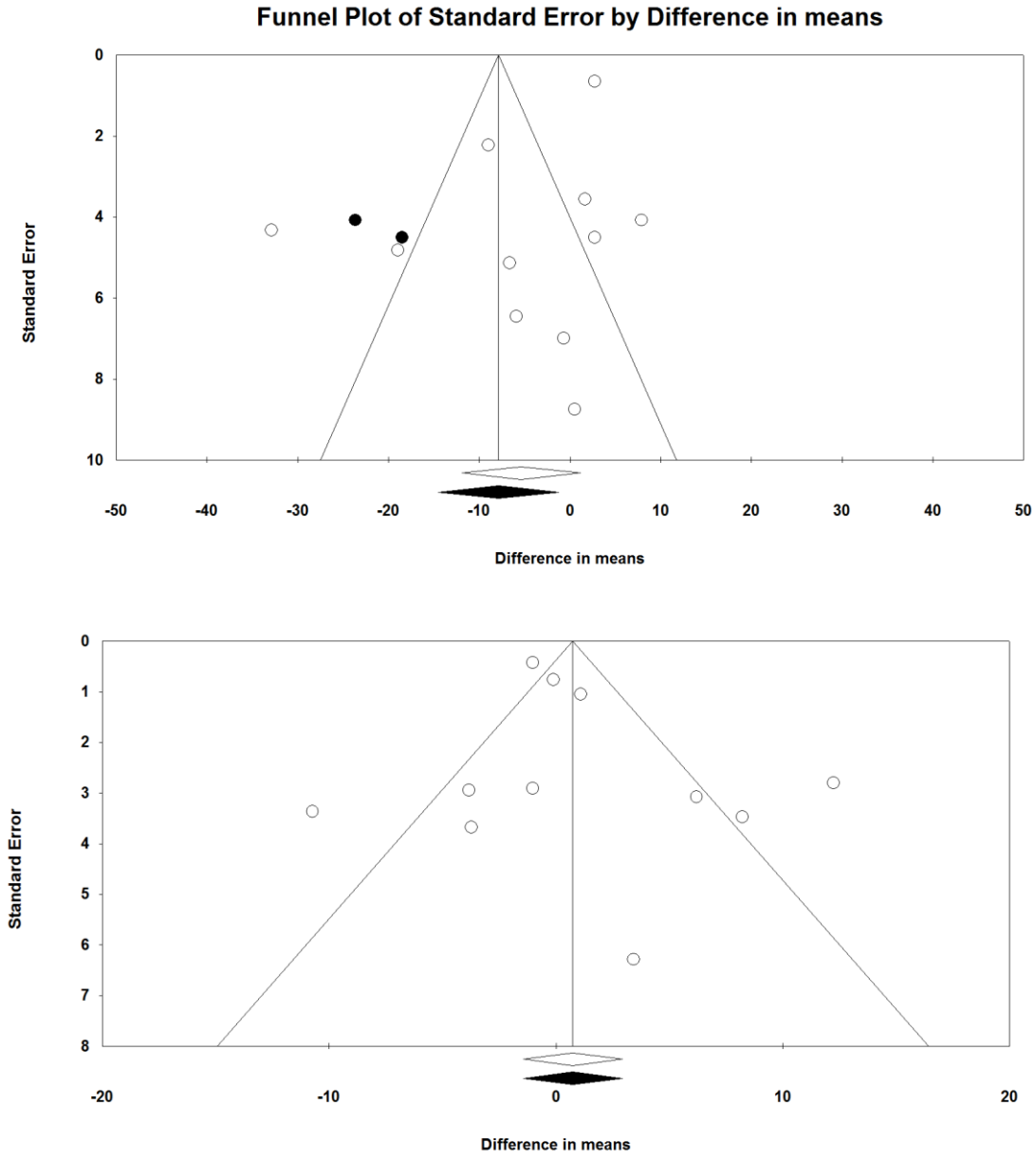

Figure 7. Funnel plot detailing publication bias in the studies reporting the impact of tibolone on plasma fibrinogen (upper plot) and ATIII (lower plot) concentrations. Open diamond represents observed effect size; closed diamond represents imputed effect size.

Table 1. Demographic characteristics and biological parameters of the included studies

Study		**Bjarnason *et al.* (41)**	**Sarac *et al.* (42)**	**Jeremic *et al.* (43)**	**Koh *et al.* (35)**	**Osmanagaoglu *et al.*(44)**	**Cortes-Prieto *et al.*(45)**	**Hänggi *et al.*(46)**	**Lloyd *et al.(32)***	**Perrone *et al.*(47)**	**Demirol *et al.*(48)**	**Keramaris *et al.* (49)**	**Tasic *et al.* (50)**
Year		1997	2009	2006	2003	2005	1987	1997	2000	2009	2007	2007	2011
Location		Netherlands	Turkey	Serbia	Korea	Turkey	Spain	Switzerland	UK	Italy	Turkey	Greece	Serbia
Design		Randomized double-blind, placebo controlled study	Prospective controlled study	Prospective controlled study	Randomized, double-blind, placebo-controlled, study	Randomized non-blind controlled study	Randomized, double-blind, placebo-controlled, study	Open, randomized, controlled study	Randomized double-blind, placebo-controlled study	Prospective controlled study	Randomized double-blind, placebo-controlled study	Prospective controlled study	Randomized, placebo controlled study
Duration of study		24 months	6 months	6 months	2 months	6 months	12 weeks	24 months	6 months	12 months	6 months	12 months	6 months
Inclusion criteria		Healthy women not being treated with any medication kno	Postmenopausal normotensive women amenorrheic for 12 months to 5 yrs	Women who had the concentration of l7ß estradiol <50 pg/ml, and in	Postmenopausal women with plasma l7ß estradiol <50 pg/mL and reported cessation of	Women, aged 44 to 65 years, who were overweight and obese (BMI 25 kg/m^2)	Post-menopausal women aged 47-55 yrs	Post-menopausal women, confirmed by two serum follicle stimulating hormone values > 30 IU/L, or an interval	Hypertensive patients on or off treatment with antihypertensive, with 1 or more years	No natural menstruation for at least 12 months, FSH >60 pg/ml,	Healthy menopausal women 45–50 years of age who had undergone hysterectom	Post-menopausal women with endometrial thickness≤5 mm, follicle	Healthy women in early postmenopause (had been postmenopausal for less

		wn to affect coagulation fibrinolysis lipid or bone metabolism and with more than 10 years of spontaneous menopause		menopause for more than 1 year	menses for at least 1 year	and had been postmenopausal for at least 1 year		of amenorrhoea of at least 12 months (nonhysterectomised women)	of postmenopausal on clinical grounds	estradiol <20 µg/ml and the presence of menopausal symptoms	y and bilateral salpingo-oophorec-tomy as a result of benign diseases	stimulating hormone>420 mIU/m, serum estradiol<l550 pg/ml	than 5 yrs)
Route of administration		oral	oral	oral	oral	oral	oral	oral	oral	oral	oral	oral	oral
Tibolone dose		1.25 mg/day or 2.5 mg/day	NS	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day	2.5 mg/day
Partic	Ca	29[a]	20	63	53	90	30	29	19	21	28	24	25

ipants	se	28[b]											
	Control	13	20	31	26	88	30	29	11	19	27	68	20
Age (years)	Case	66.4 ±7.0[a]	52.7±1.6	56.7±3.4	59.0±1.0*	50±3.91	NS	51.8±3.1	60.11±1.28*	53.4 ±3.6	46.0±3.0	52.2±1.7	52.5±4.5
		65.5 ±7.5[b]											
	Control	68.4 ±6.1	50.6±3.6	58.1±3.2	60.0±1.0*	51±5.43	NS	51.8±3.4	62.73±1.79*	52.7 ±3.3	47.0±0.6	51.4±4.2	52.5±4.5
BMI (kg/m^2)	Case	24.5 ±3.4[a]	24.32±4.9	25.1±3.8	24.7±0.4*	28±1.25	NS	23.7±2.8	33.90±5.99*	25.2 ±2.6	24.4±0.7	25.0±2.2	24.4±3.5
		23.1 ±2.8[b]											
	Control	24.7 ±3.2	25.30±2.5	25.6±3.6	24.5±0.6*	28±1.24	NS	25.9±4.5	29.60±1.30*	23.2 ±2.6	25.1±0.1	25.4±1.9	24.8±4.6
Prevalence of diabetes mellitus (%)	Case	NS[a]	0	NS	0	0	NS	NS	NS	NS[b]	0	0	0
		NS[b]											
	Control	NS	0	NS	0	0	NS	NS	NS	NS	0	0	0
Plasma fibrinogen (mg/dL)	Case	281.0±45.0 [a]	375.4±49.6	983.0±27.0	289.0±8.0*	339±46.08	260.0±40.0	244.0±43.0	337.0±20.0*	375.1 ±70.0	342.83±109.12	NS	NS
		276.0±57.0[b]											
	Control	295.0±48.0	304.0±35.5	993.0±34.0	292.0±10.0*	332±52.34	280±60.0	244.0±48.0	349.0±43.0*	360.5 ±78.0	391.90±116.82	NS	NS
Plasma anti-thrombin III (mg/d	Case	97.6 ±10[a#]	NS	29.6±0.5	29.6±0.5*	21±4.02	31.0±3.2	100.9±15.8[#]	NS	114.4 ±15.5[#]	23.67 ±2.67	25.26 **±68.4	96.0±9.8[#]
		96.9 ±12[b#]											
	Co	98.9	NS	30.1±	30.1±0	23±6.1	31.7±	101.7±7.	NS	110.7	23.83	23.04	97.2±9

L)	ntr ol	±7.5[#]		0.7	.7*	3	3.6	8[#]		±12. 4[#]	±1.59	**±5 0.9	.6[#]

Values are expressed as mean ± SD or median (25–75 percentiles). *Data are expressed as mean ± SEM, ** Due to deviation from normal distribution, geometric means are presented [#]Antithrombin factor III activity (%)

Abbreviations: BMI: body mass index; NS: not stated; [a]denotes 1.25 mg/day tibolone; [b]denotes 2.5 mg/day tibolone

Table 2. Risk of bias assessment in the studies considered for meta-analysis.

Study	Ref	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other potential threats to validity
Bjarnason *et al.*	**(41)**	**U**	**U**	**L**	**L**	**L**	**L**	**U**
Sarac *et al.*	**(42)**	**H**	**H**	**U**	**U**	**L**	**L**	**L**
Jeremic *et al.*	**(43)**	**H**	**H**	**U**	**U**	**L**	**L**	**L**
Koh *et al.*	**(35)**	**U**	**U**	**U**	**L**	**L**	**L**	**L**
Osmanagaoglu *et al.*	**(44)**	**L**	**U**	**U**	**U**	**L**	**L**	**L**
Cortes-Prieto *et al.*	**(45)**	**U**	**U**	**U**	**U**	**L**	**L**	**L**
Hänggi *et al.*	**(46)**	**H**	**H**	**L**	**L**	**L**	**L**	**L**
Lloyd *et al.*	**(32)**	**U**	**U**	**L**	**L**	**L**	**L**	**H**
Perrone *et al.*	**(47)**	**U**	**U**	**U**	**U**	**H**	**L**	**L**
Demirol *et al.*	**(48)**	**U**	**U**	**L**	**L**	**L**	**L**	**L**
Keramaris *et al.*	**(49)**	**H**	**H**	**U**	**U**	**H**	**L**	**L**
Tasic *et al.*	**(50)**	**U**	**U**	**U**	**U**	**L**	**L**	**L**

L: low risk of bias; H: high risk of bias; U: unclear risk of bias