Supplemental Appendix

TITLE: Use of menopausal hormone therapy before and after diagnosis and ovarian cancer survival – a prospective cohort study in Australia

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Supplementary Methods

Statistical analysis

Multiple imputation

Under the assumption of missing at random, we conducted multiple imputation by chained equations with 20 iterations using the MICE package in R. The variables we used in the imputation were confounders, particularly age and hysterectomy, which were strong predictors of missing data (Supplementary Table 1). We also included auxiliary variables including age at last birth, oral contraceptive use, comorbidities and associated medication use (non-steroidal anti-inflammatory medications), family history of ovarian cancer, stage, histotypes and residual disease, as well as the cumulative baseline cause-specific hazard and binary indicator variables for the outcome.[5,6] We imputed 20 datasets and the resulting estimates and standard errors were combined using Rubin's rules.[7]

Propensity scores

We applied propensity score (PS) approach to reduce or minimise the effects of confounding by indication and by histotype.[8] A propensity score is defined as the probability of an individual receiving one of the treatment categories of interest conditional on measured pre-treatment covariates. In this study, we applied inverse probability of treatment weighting (IPTW) approach using PS, focusing on the average treatment effect which denotes the average effect of MHT use among all women when every woman is moved from control to treated group. Specifically, we first used generalised boosted modelling (GBM) logistic regression models taking into account of interactions between any variables to calculate PS using TWANG package in R, which was then used to generate weights for both MHT users (1/PS) and non-uses (1/[1 – PS]). We included variables listed in Table 1 or 3 that were related to the outcome [9], except post-diagnosis variables in the pre-diagnosis GBM models. We assessed the distribution of weights among users and non-users and excluded women with extreme value of weights as extremely weights may increase bias and yield estimates with high variance.[10,11] We used standardised effect size to assess the balance of PS among MHT users and non-users; there was good balance across all covariates with <0.15 absolute standardised mean difference not only for covariates included in the GBM models but also stage, histotypes and residual disease for pre-diagnosis analysis. In the post-diagnosis analysis, while the absolute standardised mean difference was >0.25 for some variables (e.g. education, hypertension and diabetes) that were not confounders, additionally adjusting for these variables did not make any appreciable differences. Supplementary Figure 1 and 2 depict the absolute standardised mean difference for each variable before and after IPTW for pre- and post-diagnosis analysis respectively. Finally, we applied doubly robust estimation method by including confounders in the weighted Cox proportional hazard regression model; this method has been demonstrated to perform better as it is robust to misspecification of one (but not both) of PS and regression models.[12,13] We used bootstrapping to calculate 95% CIs using 2000 replications.

Supplementary Table 1. Characteristics of women with and without missing data for MHT use prior to diagnosis among peri-/postmenopausal women.

Variable[1]	Total (n = 690)	Complete data (n = 580)	Missing data[1] (n = 110)
Age at diagnosis, years (Mean, SD)	63 (8)	63 (8)	68 (6)
	N (%)	**N (%)**	**N (%)**
Menopause status			
Perimenopause[2]	44 (6)	42 (7)	2 (2)
Postmenopause	646 (94)	538 (93)	108 (98)
FIGO Stage			
I & II	168 (24)	150 (26)	18 (16)
III & IV	522 (76)	430 (74)	92 (84)
Histotype			
High-grade serous carcinoma	499 (77)	433 (75)	92 (84)
Non high-grade serous carcinoma	147 (23)	147 (25)	18 (16)
Residual disease			
None	384 (55)	327 (56)	57 (53)
Any	283 (45)	253 (44)	50 (47)
BMI 5 years before diagnosis (kg/m^2)			
<25	287 (42)	245 (42%)	42 (38%)
25-29.9	234 (34)	186 (32%)	48 (44%)
30+	169 (24)	149 (26%)	20 (18%)
Smoking status 1 year before diagnosis			
Never	363 (53)	303 (52)	60 (55)
Former	247 (36)	202 (35)	45 (41)
Current	80 (11)	75 (13)	5 (5)
Highest level of education			
High school	349 (51)	283 (49)	66 (60)
Technical college	171 (25)	142 (25)	29 (26)
University	167 (24)	152 (26)	15 (14)
Age at menarche, years (Mean, SD)	13 (4)	13 (4)	13 (2)
Hysterectomy prior to diagnosis			
No	531 (77)	459 (79)	72 (68)
Yes	158 (23)	124 (21)	34 (32)
Charlson Comorbidity Score			
0	505 (73)	422 (73)	83 (76)
1	119 (17)	99 (17)	20 (18)
2+	66 (10)	59 (10)	7 (6)
Medical history			
Coronary heart disease	22 (3)	20 (3)	2 (2)
Hypertension	266 (39)	215 (37)	51 (46)
Diabetes	52 (8)	44 (8)	8 (7)
Family history of breast or ovarian cancer			
No	559 (81)	471 (82)	878 (81)
Yes	127 (19)	106 (18)	21 (19)

[1] The type of MHT was unknown for 104 women (15%), including six women also missing duration of use, and one missing data on the recency of use. An additional six women were missing other variables: MHT duration (n=2), residual disease (n=3), and hysterectomy status (n=1).

Supplementary Table 2. MHT use prior to diagnosis and ovarian cancer-specific survival among peri-/post-menopausal women – multiple imputation (n = 690).

Variable[1]	All		HGSC		Non-HGSC	
	HR	**95%CI**	**HR**	**95%CI**	**HR**	**95%CI**
MHT use						
Never	1.0		1.0		1.0	
Ever	0.74	0.60-0.93	0.69	0.55-0.88	1.35	0.70-2.62
Recency of use						
Former	0.74	0.58-0.96	0.68	0.52-0.89	1.41	0.68-2.93
Current/recent	0.76	0.55-1.05	0.73	0.52-1.02	1.25	0.46-3.40
MHT type						
ET	0.85	0.62-1.18	0.82	0.57-1.17	1.12	0.49-2.56
E-P/P	0.68	0.51-0.90	0.62	0.46-0.85	1.75	0.64-4.77
MHT duration						
< 5 years	0.70	0.53-0.94	0.63	0.46-0.87	1.34	0.61-2.92
5+ years	0.78	0.60-1.02	0.74	0.55-0.98	1.27	0.50-3.21

Abbreviations: CI: confidence interval; E-P/P: oestrogen plus progestin or progestogen-only therapy; ET: oestrogen-alone hormone therapy; HGSC: high-grade serous carcinoma; HR: hazard ratio; MHT: menopausal hormone therapy.

[1] Variables with missing data that were imputed include MHT type, duration and recency of MHT use (total n=106), hysterectomy status (n = 1). All models adjusted for age (<55, 55-59, 60-64, 65-69, 70+), body mass index (< 25, 25-29, 30+ kg/m^2) smoking (never, former, current), history of hysterectomy prior to diagnosis (yes, no) and stratified by FIGO Stage (I & II, III & IV). Further adjustment for any other variables did not make any difference.

Supplementary Table 3. MHT use before diagnosis and progression-free survival among peri-/postmenopausal women

Variable[1]	All		HGSC		Non-HGSC	
	HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
MHT use						
Never	1.0		1.0		1.0	
Ever	0.76	0.62-0.93	0.67	0.54-0.83	1.55	0.86-2.80
Recency of use						
Former	0.73	0.58-0.92	0.63	0.49-0.81	1.43	0.72-2.84
Current/recent	0.82	0.62-1.08	0.74	0.55-1.00	1.78	0.79-4.02
MHT type						
ET	0.80	0.58-1.10	0.78	0.55-1.11	1.28	0.55-2.99
E-P/P	0.77	0.58-1.02	0.69	0.52-0.93	1.63	0.62-4.26
MHT duration						
< 5years	0.72	0.55-0.93	0.62	0.47-0.82	1.50	0.75-3.02
5+ years	0.80	0.62-1.02	0.72	0.55-0.93	1.57	0.68-3.60

Abbreviation**:** CI: confidence interval; E-P/P: oestrogen plus progestin or progestogen-only therapy. ET: oestrogen-alone hormone therapy; HGSC: high-grade serous carcinoma; HR: hazard ratio; MHT: menopausal hormone therapy; OS: overall survival; OVS: ovarian cancer-specific survival; PFS: progression-free survival.

[1] All models adjusted for age (<55, 55-59, 60-64, 65-69, 70+), body mass index (< 25, 25-29, 30+ kg/m^2) smoking (never, former, current), history of hysterectomy prior to diagnosis (yes, no) and stratified by FIGO Stage (I & II, III & IV). Further adjustment for any other variables did not make any difference.

Supplementary Table 4. MHT use before diagnosis and ovarian cancer specific survival among peri-/postmenopausal women stratified by stage

Variable[1]	Stage I & II							Stage III & IV						
	N (%)	All (n = 168)		HGSC (N = 66)		Non-HGSC (n = 102)		N (%)	All (n = 522)		HGSC (n = 459)		Non-HGSC (n = 63)	
		HR	95% CI	HR	95% CI	HR	95% CI		HR	95% CI	HR	95% CI	HR	95% CI
MHT use														
Never	118 (70)	1.0		1.0		1.0		299 (57)	1.0		1.0		1.0	
Ever	50 (30)	0.46	0.16-1.33	0.39	0.07-2.21	0.62	0.12-3.13	223 (43)	0.75	0.60-0.93	0.69	0.54-0.87	1.61	0.74-3.47
Recency of use														
Former	33 (20)	0.64	0.22-1.88	0.41	0.07-2.49	1.47	0.26-8.25	145 (28)	0.73	0.56-0.95	0.67	0.51-0.88	1.54	0.66-3.59
Current/recent	17 (10)	0.14	0.02-1.26	0.33	0.02-4.63	NA[2]	NA[2]	77 (15)	0.78	0.57-1.08	0.74	0.53-1.04	1.78	0.56-5.73
MHT type														
ET	22 (13)	0.07	0.01-0.69	0.08	0.01-1.58	NA[2]	NA[2]	65 (12)	0.92	0.64-1.34	0.96	0.65-1.43	1.37	0.41-4.56
E-P/P	11 (7)	0.42	0.08-2.29	0.17	0.01-3.59	1.69	0.06-51.7	71 (14)	0.67	0.48-0.93	0.62	0.44-0.88	1.71	0.47-6.20
MHT duration														
< 5years	31 (19)	0.54	0.17-1.69	0.41	0.06-3.00	0.60	0.11-3.26	97 (19)	0.70	0.52-0.95	0.63	0.46-0.86	1.81	0.72-4.59
5+ years	18 (11)	0.28	0.05-1.67	0.30	0.02-3.86	0.76	0.03-17.1	119 (23)	0.78	0.59-1.03	0.74	0.56-0.99	1.33	0.47-3.74

Abbreviation**:** CI: confidence interval; E-P/P: oestrogen plus progestin or progestogen-only therapy. HGSC: high-grade serous carcinoma; ET: oestrogen-alone hormone therapy; HR: hazard ratio; MHT: menopausal hormone therapy.

[1] All models adjusted for age (<55, 55-59, 60-64, 65-69, 70+), body mass index (< 25, 25-29, 30+ kg/m^2) smoking (never, former, current), history of hysterectomy prior to diagnosis (yes, no) and stratified by FIGO Stage (I & II, III & IV). Further adjustment for any other variables did not make any difference. Numbers may not add up to total due to missing data.

[2] The models did not converge due to an insufficient number of samples.

Supplementary Table 5. Total, direct, and indirect effects (mediated by residual disease) of ever use of MHT before diagnosis on ovarian cancer specific survival among women with advanced high-grade serous carcinoma (n = 457)

Effects	HR (95% CI)	Proportion (%)
Total effect	0.69 (0.55-0.87)	100
Direct effect	0.72 (0.60-0.85)	89
Indirect effect, through residual disease	0.96 (0.83-1.13)	11

Abbreviation**:** HR: hazard ratio; CI: confidence interval;

[1] All models adjusted for age (<55, 55-59, 60-64, 65-69, 70+), body mass index (< 25, 25-29, 30+ kg/m^2) smoking (never, former, current), history of hysterectomy prior to diagnosis (yes, no). Further adjustment for any other variables did not make any difference.

Supplementary Table 6. Use of MHT after diagnosis and progression-free survival among women ≤55 years at diagnosis (n = 229)[1].

MHT use	All (n = 229) HR (95%CI)	HGSC (n = 123) HR (95%CI)	LGSC & END (n=55) HR (95%CI)	Other histotypes (n=51) HR (95%CI)
MHT use time-varying				
No	1.0	1.0	1.0	1.0
Yes	0.67 (0.36-1.25)	0.86 (0.46-1.58)	NA[3]	0.07 (0.02-0.35)
MHT use during the first 12 months[2]				
No	1.0		1.0	1.0
Yes	0.78 (0.45-1.35)	1.29 (0.69-2.43)	0.32 (0.01-7.29)	0.03 (0.001-0.80)
MHT change[2]				
Never	1.0	1.0	1.0	1.0
Use prior to diagnosis only	0.28 (0.08-0.91)	0.37 (0.11-1.26)	NA[3]	NA[3]
Continuous use	1.16 (0.46-2.93)	1.14 (0.45-2.93)	NA[3]	NA[3]
New use	0.58 (0.30-1.10)	1.00 (0.46-2.18)	0.32 (0.01-7.29)	0.03 (0.001-0.80)

Abbreviations: HR: hazard ratio; CI: confidence internal; HGSC: high-grade serous carcinoma; MHT: menopausal hormone therapy;

Models adjusted for age (continuous), body mass index (<25, 25-29, 30+ kg/m^2), smoking (never, former, current), FIGO Stage (I & II, III & IV), residual disease (none, any) and further adjusted for pre-diagnosis MHT use (yes, no) except for the analysis of MHT change.

[1] 29 women progressed within the first 12 months were excluded, further excluded one women with missing data on progression status.

[2] MHT use during the first year after diagnosis.

[3] The models did not converge due to an insufficient number of samples.

Supplementary Table 7. Use of MHT after diagnosis and ovarian cancer-specific survival among women ≤55 years with complete or partial response (n = 245).

MHT use	All (n = 245)	HGSC (n = 138)	LGSC & END (n=53)	Other histotypes (n=54)
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
MHT use time-varying				
No	1.0	1.0	1.0	1.0
Yes	0.65 (0.34-1.25)	0.85 (0.41-1.77)	0.98 (0.09-10.3)	NA
MHT use during the first 12 months[1]				
No	1.0	1.0	1.0	1.0
Yes	0.86 (0.44-1.67)	0.99 (0.47-2.10)	0.90 (0.07-12.1)	0.24 (0.03-1.87)
MHT change[1]				
Never	1.0	1.0	1.0	1.0
Use prior to diagnosis only	0.49 (0.15-1.62)	0.32 (0.07-1.41)	NA[2]	NA[2]
Continuous use	0.58 (0.18-1.88)	0.52 (0.16-1.72)	NA[2]	NA[2]
New use	0.80 (0.38-1.69)	0.88 (0.37-2.12)	1.05 (0.07-14.8)	NA[2]

Abbreviations: END: endometrioid carcinoma; CI: confidence internal; HGSC: high-grade serous carcinoma; HR: hazard ratio; MHT: menopausal hormone therapy;

Models adjusted for age (continuous), body mass index (<25, 25-29, 30+ kg/m^2), smoking (never, former, current), FIGO Stage (I & II, III & IV), residual disease (none, any) and further adjusted for pre-diagnosis MHT use (yes, no) except for the analysis of MHT change.

[1] MHT use during the first year after diagnosis.

[2] The models did not converge due to an insufficient number of samples.

Supplementary Table 8. MHT use after treatment for ovarian cancer among women ≤55 years and quality of life measurements among women with data at all 3 time-points.

Health-related quality of life measurement1	**Time 0 – before MHT initiation[2]**		***P*[3]**	**Time 1 – 1-3 months after MHT initiation[2]**		***P*[3]**	**Time 2 – subsequent questionnaire[2]**		***P*[3]**
	Non-users (n = 168)	**MHT initiators (n = 12)**		**Non-users (n = 168)**	**MHT use (n = 12)**		**Non-users (n = 168)**	**MHT use (n = 12)**	
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Wellbeing (FACT-G)	85 (73-96)	73 (67-81)	0.1	89 (79-98)	77 (67-88)	**0.04**	88 (72-99)	75 (63-93)	0.1
Functional (max 28)	20 (16-25)	15 (11-21)	0.1	23 (18-26)	18 (14-20)	**0.04**	22 (17-26)	18 (14-22)	0.1
Emotional (max 24)	20 (18-23)	20 (17-21)	0.9	20 (18-22)	18 (16-20)	0.1	20 (16-22)	19 (16-21)	0.3
Social/family (max 28)	23 (19-26)	20 (17-24)	0.2	23 (19-27)	21 (17-24)	0.3	23 (18-26)	20 (18-25)	0.5
Physical (max 28)	24 (19-26)	19 (17-24)	0.1	25 (21-27)	21 (17-24)	**0.02**	24 (21-27)	23 (17-26)	0.2
Fatigue (max 52)	40 (30-46)	30 (21-38)	0.05	43 (35-48)	32 (22-41)	**0.01**	42 (32-48)	28 (25-45)	0.1
Insomnia (ISI, max 28)	6 (0-11)	6 (0-11)	1.0	6 (0-11)	9 (3-11)	0.6	1 (0-11)	10 (0-13)	0.5
Insomnia[4]	**N (%)**	**N (%)**		**N (%)**	**N (%)**		**N (%)**	**N (%)**	

No insomnia	98 (61)	5 (63)	1.0	100 (62)	3 (37)	0.3	96 (60)	3 (37)	0.3
Subthreshold/clinical	63 (39)	3 (37)		61 (38)	5 (63)		65 (40)	5 (63)	
FACT-GS7[5]			0.05			0.3			0.8
Not satisfied	57 (44)	8 (80)		50 (39)	6 (60)		62 (49)	6 (54)	
Somewhat/satisfied	71 (56)	2 (20)		78 (61)	4 (40)		64 (51)	5 (45)	

Abbreviations: FACIT-fatigue: Functional Assessment of Chronic Illness Therapy – fatigue (range 0-52); FACT: Functional Assessment of Cancer Therapy. IQR, inter-quartile range; ISI: Insomnia Severity Index. MHT: menopausal hormone therapy.

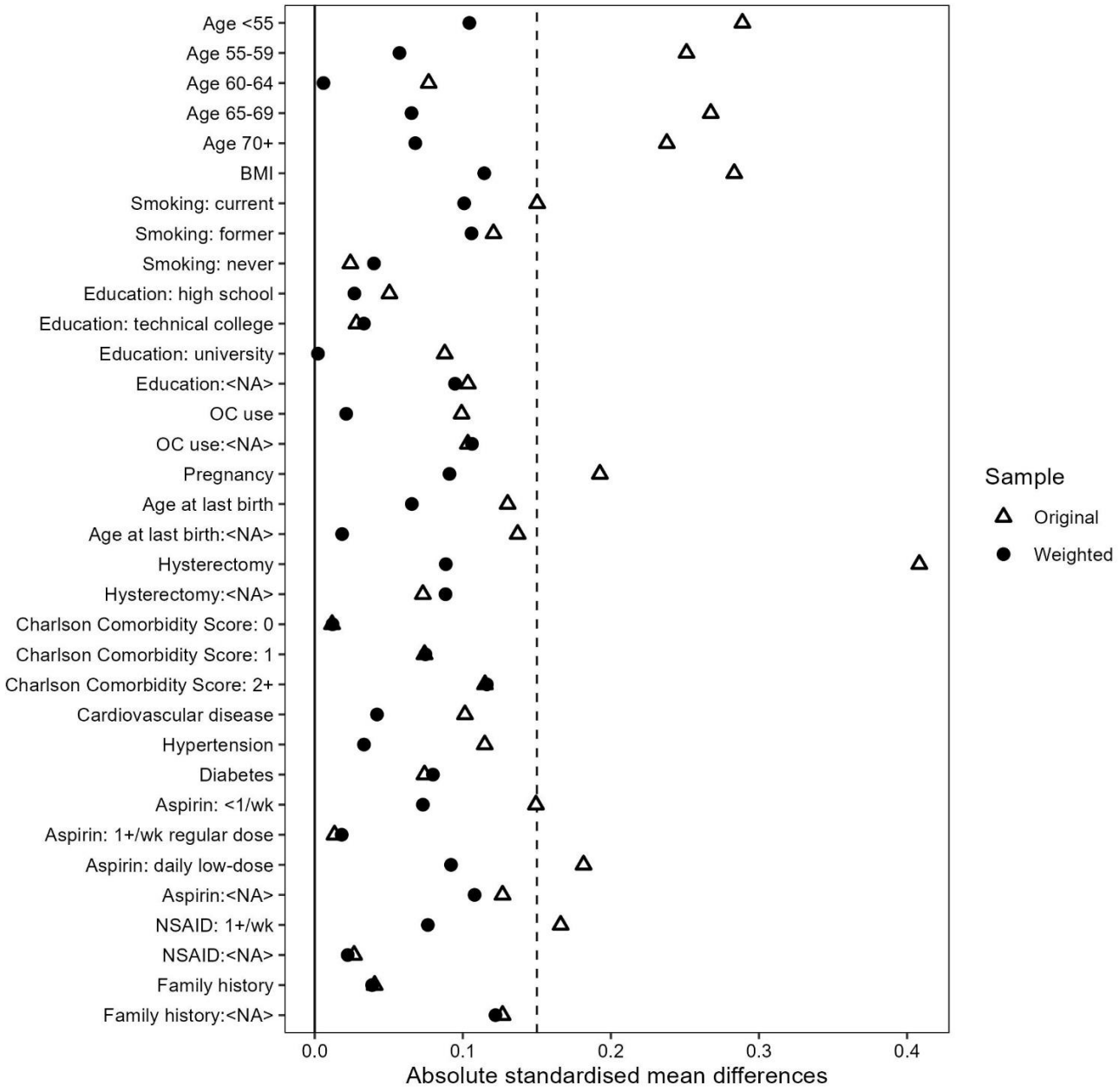
[1] The counts in sub-categories may not add up to the total number due to missing data. The FACT-G includes 27 items in 4 sub-scales providing a total quality of life (QOL) score (0-108), higher scores indicate better QOL. Fatigue was measured using FACIT-fatigue with higher scores indicating less fatigue.

[2] Time-0 was the first questionnaire after treatment; this had to be before starting MHT for users. Time-1 was the second questionnaire after treatment (never users) or the first after starting MHT (users). Time-2 was the questionnaire after Time 1.

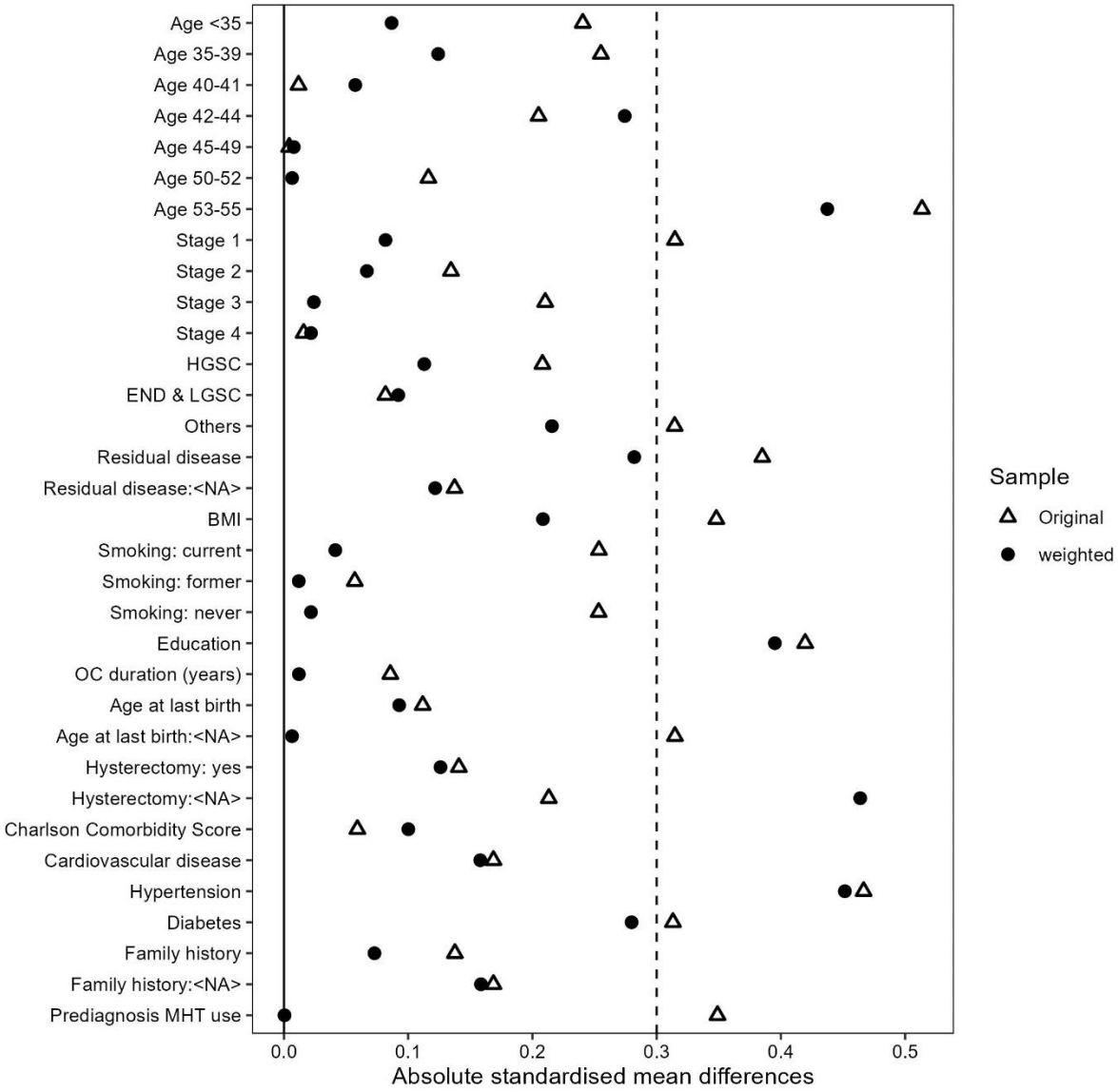
[3] Kruskal–Wallis non-parametric test, Pearson chi-square test or Fisher exact test as appropriate.

[4] ISI scores 8-14 (considered mild insomnia) and 15+ (moderate to severe insomnia) were combined as sub-clinical/clinical insomnia.

[5] The question regarding sex life: “I am satisfied with my sex life”; “not at all” and “a little bit” were combined as “not satisfied”; “somewhat, quite a bit and very much” were combined as “somewhat/satisfied”.



Supplementary Figure 1. Absolute standardized differences for baseline covariates comparing pre-diagnosis MHT use vs no use in the original and the propensity score weighted sample.



Supplementary Figure 2. Absolute standardized differences for covariates comparing MHT use vs no use during the first 12 months after diagnosis in the original and the propensity score weighted sample.

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