Appendix 1: Study reviewing association of cytokines level and prognosis of ovarian cancer

No	Author	Region	Measurement methods	Specimens type	Number of cases	No of control	Stage	Cytokines	Scoring measurement	Outcome assessed	Study design	Duration of follow up
1	Chen et al., 2013	Taiwan	Cytokine profiling kit	ascites	144		I-IV	IFN-γ	The IFN-γ expression levels were divided into tertiles (low, b1.4 pg/mL; medium, 1.4–16.0 pg/mL; and high: N16.0 pg/mL) for analysis.	OS, DFS, stage, grade, tumor histology, optimal surgery	Cohort retropective	80 months
2	Chen et al., 2012	China	IHC	tissue	124 M	40 Normal	I-IV	VEGF (41.9% positive)	The percentage of positive cells was rated on the following point scale: no points (negative), ≤10% positive cells, regardless of staining intensity; 2 points, 11%–50% positive cells; 3 points, 51%–80% positive cells; and 4 points, ≥81% positive cells. The staining intensity was rated as follows: 1 point, weak intensity; 2 points, moderate intensity; and 3 points, strong intensity. Points for the percentage of positive cells and staining intensity were added, and specimens were attributed to two groups according to their overall score. Finally, specimens of	Response to chemotherapy, sensitivity to chemotherapy, OS, PFS, histologic grade, pathologic type, stage, LN metastasis, residual disease	cohort	6-8 years

									≤3 points were rated as negative, or as positive			
3	Williams et al., 2012	China	IHC	tissue	97	Negative control slides in which the primary antibody was omitted were included in all the assays.	I-IV	VEGF	The H score value, for each marker, ranged from 0–300. The H score cut-off points between high and low expression groups were 100 for VEGF	OS, PFS, response to chemoterapy	Cohort retrospective	2-18tahun
4	Huang et al., 2011	China	IHC	tissue	136	borderline ovarian tumor (39 cases), benign ovarian tumor (45 cases), and normal ovary (7 cases)	i-iv	VEGF-C	The findings were scored according to the numbers of positive cells: (-) no or less than 5% cells stained, (?) 6–25% of cells stained, (??) 26–50% of cells stained, and (???) more than 50% of cells stained.	OS, EOC vs benign vs borderline	cohort prospective	The survival time of epithelial ovarian cancer patients ranged from 2 to 67 months, and the median survival time was 25.5 months

5	van der Bilt et al., 2012	Netherlands	Tissue microarrays, IHC	tissue	270 primary cancers; 112 omental metastases	control cores were present on each array containing tissue from various ovarian cancers (derived from a serous, endometrioid, mucinous, clear cell and undifferentiated tumor) as well as a benign ovarian cyst and normal endometrial and cervical tissue.	I-IV	VEGF A, VEGF B, VEGF C, VEGF D	The cytoplasmic staining intensity of cancer cells was catego- rized as absent (-), weak (-/+), moderate (+) or strong (++).	age, stage, grade, ascites, tumor histology, OS, PFS	cohort prospect ive	5 year survival
6	Engels et al., 2009	Germany	IHC	tissue	112	-	I-IV	VEGF	scored semiquanti- tatively based on staining intensity and percentage of stained tumour cells using the immunoreactive score (IRS) as described. Briefly, IRS = SI (staining intensity) 6 PP (percentage of positive cells). SI was assigned as: 0, negative; 1, weak; 2, moderate; 3, strong. PP was defined as: 0, negative; 1, ,10%; 2, 11–50%; 3, 51–80%;	stage, grade, OS, PFS	cohort prospect ive	NA

									and 4, .80% positive cells.			
7	Smerdel et al., 2009	Denmark	IHC	tissue	159	10 normal tissue	II-IV	VEGF	The intensity of positive staining was scored 0 for absent staining, 1+ for weak staining, 2+ for moderate staining, and 3+ for strong staining. In addition, the percentage of positive tumor cells was evaluated: 0 for less than 1%, 1 for 1% to 10%, 2 for 11% to 50%, and 3 for 51% to 100%. Subsequently, we categorized the results into 2 groups: high VEGF expression (3+ in more than 10% of the tumor cells) and low expression (all tumors with a 0, 1+, and 2+ reactions and tumors with 3+ in 10% or less of the tumor cells).	OS	cohort prospect ive	5-10 year

8	Li et al., 2009	China	IHC	tissue	78	Paraffin sections of normal human placenta were used as a positive control. Slides incu-bated without primary antibody were used as negative controls for VEGF-D staining.	I-IV	VEGF-D	staining was assessed by estimating the percentage of tumor cells and divided tumors into four groups: (0%), negative; (<10%), mild; (10-50%), moderate; (>50%), strong; tumors were considered positive when they showed moderate or strong staining for VEGF-D and mild staining was regarded as negative expression	age, grade, stage, metastasis limfatik, limfatik intratumo r, residual, LVD, tumor size, invasi vasa limfatik	cohort prospect ive	5 year survival
9	Duncan et al., 2008	United Kingdom	IHC	tissue	320	Negative control sections were incubated with normal swine serum under the same conditions	I-IV	VEGF	The intensity of the staining was estimated on a four-tiered scale, encoded as 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). cases were categorized as either ‘‘high’’ expressers (represented by the strongly stained group) or ‘‘low’’ expressers (composed of the negative, weak, and moderate groups).	cumulativ e survival 12 mo, 24 mo survival	Cohort prospect ive	14 year
10	Hefler et al., 2006	Austria	ELISA	serum	314	0	I-IV	VEGF	median preoperative serum VEGF in patients with ovarian cancer was 407 (238–746)pg/mL	OS, grade, stage, histologic type	Cohort prospect ive	5 year survival

11	Kuerti et al., 2017	Germany	Western blot	tissue	100	1 cell line breast cancer	pT1c pT2b pT2c pT3a pT3b pT3c	VEGF A, VEGF-D VEGF-C	were standardised with the positive controls, which were defined as 100 percent	OS, PFS, residual, metastase	Cohort retrospective	2-9 years
12	Dalal et al., 2018	India	ELISA	ascites	30 EOC	15 benign	I-IV	VEGF A and IL-6	The AUC of IL-6 and VEGF-A was found to be 1.0 and 1.0 respectively. Further, at a cut-off value of 204.9pg/ml, IL-6 displayed absolute combination of sensitivity and specificity (to determine difference malignant and benign)	age, stage, grade, histology, OS, PFS, preoperative ca-125, ascites, residual tumor size, tumor size, presentation	Cohort prospective	NA
13	Skirnisdottir et al., 2016	Sweden	IHC	tissue	98	-	I-II	VEGF A	A semi-quantitative analysis (15) was used and the stains were graded as negative, +, ++ and +++	DFS, recurrent diseases	Cohort prospective	the mean follow-up time was 65 months (range, 5-110 months)

14	Harlozinska et al., 2004	Poland	ELISA	serum	86 malignan	53 benign	I-IV	VEGF	The cut-off values, calculated as 95th percentile concentration based on our reference group of benign ovarian neoplasms, appeared to be much higher, at 758 pg/mL for serum and 19058 pg/mL for cyst fluid. ROC analysis resulted in an elevation of serum VEGF cut- off (372 pg/mL) and this higher cut-off point gave a sensitivity and specificity of 55.8% and 76.5%, respectively	OS, DFS, hist ological type	Cohort prospect ive	>5 years
15	Nishida et al., 2004	Japan	IHC	tissue	80	10 tumors with low potential malignancy (LPM) and 22 benign	I-IV	VEGF A and VEGF C	The staining results in tumor cells were classified into 3 levels: negative expression when im-munostain-positive tumor cells accounted for 10% of the tumor area on the section, low expression when the positive cells accounted for from 10% to 50% of the tumor area, and high expression when the positive cells accounted for 50% of the tumor area.	OS, CSF, 5 years survival	Cohort retrospe ctive	ranged from 11 weeks to 359 weeks (mean, 132.8 weeks)

16	Raspollini et al., 2004	Italy	IHC	tissue	83	-	Stage III G 3	VEGF	The intensity of the VEGF immunostaining of mem- brane and cytoplasm was classified into four grades: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. When the percentage of stain- ing cells with grade 2 or 3 was 30% or more, it was judged as positive and <30% as negative.	OS, DFI, respon kemotera pi, stage, relapse, death	Cohort retrospe ctive	The average follow-up was 31 months and the mean value was 44.8 months, with observed values ranging 3—204 months.
17	Dobrzycka et al., 2015	Poland	ELISA	serum	186	94	I-IV	VEGF-D	Different subgroups were plotted according to the cutoff value of VEGF 345 pg/ml	age,stage, grade, ASCITES , cytoreduc tion , OS, DFS	cohort prospect ive	5 year survival

18	Masoumi-Moghaddam et al., 2015	Australia	IHC	tissue	100	Prostate and breast cancer tissues were included as positive controls for VEGF	I-IV	VEGF	The percentage of positive cells was scored as: no positive cells (0); 1-25% (1); 26-50% (2); and 50% > (3). The intensity of the staining was scored as: no staining (0); weak (1); moderate (2); strong (3). immunohistochemical score = staining positivity score × staining intensity score	chemotherapy respons, ascites formation, OS	Cohort	3,5,8 years
19	Yokoyama et al., 2003	Japan	IHC	tissue	59 EOC	11 borderline	I-IV	VEGF-D and VEGF-C	VEGF-C and VEGF-D staining were assessed by estimating the percentage of tumour cells in which staining was as intense as that of positive control cells or more intense than it and placing tumours into four groups: (0%), negative; 7(o10%), weak; +(10–50%), moderate; ++(450%), strong.	age, grade, stage, histologic type, distant metastasis, lymphnode metastasis, peritoneal metastasis, CSF	cohort retrospective	follow-up ranged from 8 to 156 months (median, 54 months)

20	Cooper et al., 2002	USA	ELISA	serum	101 EOC	16 low malignant potential (LMP) ovarian tumors, 34 benign ovarian tumors	I-IV	VEGF	A VEGF cutoff level of 380 pg/ml was used for this analysis, as this was the level that maximized the estimated hazard ratio between the two groups in exploratory statistics.	age, grade, stage, cytoreductive, ascites, OS	Cohort retrospective	NA
21	Chen et al., 1999	Taiwan	ELISA	serum	56 EOC	20 benign	I-IV	VEGF	values are given with median and 25th and 75th quartiles	age, stage, grade, histology type, OS, DFS, residual tumor	cohort prospective	Median duration of follow-up was 34 months (range 9–78)
22	Cheng et al., 2014	China	sandwich enzyme immunoassay technique (Quantikine, R & D systems, China)	serum	109 ovarian cancer	76 benign 50 healthy	I-IV	VEGF-C	Cut off presented as median values 10200 Pg/mL	grade, residual tumor size, stage, resectability tumor, histology, OS	cohort prospective	a median duration of 49 months (range: 1–108 months)

23	Liang et al., 2013	China	sandwich enzyme immunoassay technique (Quantikine, R & D systems, China)	serum and ascites	118 Maligna	24Benign	I-IV	VEGF-C (cut off serum: 9470 pg/mL; ascites 10.250 pg/mL	Cut off are presented as median values of VEGF-C together with interquartile ranges (Q1 and Q3)	OS, stage, grade, lymph node metastasis	cohort prospective	median duration of 33.5 months (range: 1–60 months)
24	Sallinen et al., 2014	Finland	ELISA	serum	75 EOC	37 benign	I-IV	VEGF-A, C, D	using the median value as a cutoff value (30.8 ng/mL for Ang-1, 2.7 ng/mL for Ang-2, 0.43 ng/mL for VEGF-A, 7.04 ng/mL for VEGF-C, 0.46 ng/mL for VEGF-D, 0.13 ng/mL	OS, RFS, normal vs benign vs borderline vs carcinoma	cohort prospective	The median follow-up time was 63 months (range 0–162 months).
25	Shen et al., 2000	Japan	IHC, RT-PCR	tissue	94 (64 M, 13 Bor, 17 Bn)	13 borderline and 17 benign ovarian tumours	I-IV	VEGF	VEGF expression was determined and was assigned an arbitrary numerical score as: 0% = 0; 1–25% = 1; 26–50% = 2; 51–75% = 3; and 76–100% = 4. In addition, the mean intensity of immunostained areas based on the arbitrary numerical scores of none = 0, weak = 1, moderate = 2, and strong = 3 was recorded.	OS, stage, histologic grade, histologic type, tumor size	cohort retrospective	140 months

26	Ogawa et al., 2001	Japan	IHC	tissue	105	Placenta at 39 week gestation was used as a positive control	I-IV	VEGF	Immunoreactivity for VEGF was graded as follows: negative (2), less than 5% of cancer cells were stained; weakly positive (1), 5–49% of cancer cells were stained; strongly positive (11), more than 50% of cancer cells were stained.	PFS, tpe histologi	cohort prospect ive	140 months
27	Zhang et al., 2003	Italy	RT-PCR	tissue	50	4 benign 4 low malignant potential	I-III	VEGF	Patients were stratified based on relative expression of EG- VEGF or VEGF mRNA below or above the median.	DFI	cohort prospect ive	30 week
28	Brustmann et al., 2003	Austria	IHC	tissue	51	For negative controls, serial sections of the same specimens were used, omitting the primary antibody from the staining protocol and substituting it by a nonimmune serum.	I-III	VEGF	Intensity of the staining was scored as weak (+) and strong (2+). Strong staining was defined as equivalent to or more intense than the staining qualities of the theca cells of the cystic ovarian follicle, which served as positive control.	age, grade, stage, residual tumor	cohort prospect ive	median 15 months
29	Rudlowski et al., 2006	Germany	ELISA(serum and ascites)	serum, ascites, and tissue	65	8 Borderline	I-IV	VEGF	Ascites: The cutoff level was defined by the median.	grade, residual tumpr,	cohort	The median duration of

			IHC(tissue)			16 Benign			Tissue: The ovarian tumors were subdivided into strong (more than 50% marked cells), moderate (25–49%), weak (10–24%), and not expressing for VEGF (,10%).	subtype tumor, ascites, OS		follow-up was 56 months (range 6–68 months)
30	Mahner et al., 2010	Germany	ELISA	serum	37	90 control. 25 benign	I-IV	VEGF	patients or healthy individuals having>100 pg/ml serum VEGF levels are considered as positive, while those having<100 pg/ml serum VEGF levels are considered as negative.	time points during surgery,age, grade ascites, residual tumor, lymphnode status, PFS, OS	cohort prospective	median 29 months
31	O'Toole et al., 2006	Ireland	IHC	tissue	79	Positive control slides for CD31 were from an appendix section and from a gastric tumour for VEGF and MDR. Negative controls were incubated with the antibody diluent only.	I-IV	VEGF	Sections were scored from 0 to 2 based on the degree of staining: 0- no staining, 1- low degree of staining (<50% of section), 2- high degree of staining (>50% of section stained).	Chemotherapy respons, PFS, OS	Cohort retrospective	80 months

32	Siddiqui et al., 2011	United Kingdom	IHC	tissue	66	Negative controls were prepared by omitting the primary antibody (to VEGF) from the pro- cedure and substituting with mouse immunoglobulin (class IgG2b).	advance stage (III-IV)	VEGF	A cut-off point for scoring of VEGF as, high expression ([3) and low expression (B3) was used to statistically analyse the data. A P (probability) value of <0.05 was considered to be significant for all statistical tests.	OS, platinum sensitivity	Cohort retropec tive	100 months
33	Matte et al., 2012	Canada	ELISA and cytokine multiplex assay	ascites	38	0	I-II vs III-IV	IL-10	The validation of cytokine expression was performed for selected cytokines that give a strong signal (IL-6), a moderate signal (IL-10, leptin) or a low signal (osteoprotegerin (OPG), IL-8)	PFS, stage (I/II vs III/IV)	cohort prospect ive	All patients had a follow-up > 24 months (range, 26 to 120 months), with a median PFS of 16 months.
34	Chen et al., 2015	Taiwan	Cytokine profiling kit	ascites	144	0	Early (I-II), advanced (III-IV)	IL 4(2.5 pg/mL), IL 6(942.5 pg/mL), IL 10(5.8 pg/mL), IL-17a(16.9 pg/mL), IL 21(135.2 pg/mL)	a univariate Cox proportional hazard model was used to select the significant risk predictors for the OS of the derivation group. The expression levels of all cytokines were divided into high and low groups by median levels in order to facilitate statistical analysis.	OS, PFS	Cohort prospect ive	The mean follow-up duration was 29.3 months in the NTUH set and 33.7 months in the NCKUH set.

35	Lane et al., 2015	Canada	ELISA	ascites	53 M,	10 B	III-IV	IL 6 (median 1820 pg/mL) IL 10 (median 97,5 pg/mL	Receiver- operator curves (ROC) were created to determine the predictive value of the cytokines to distinguish between EOC patients and control, and between clinically resist- ant and sensitive patients	PFS, chemothe rapy response	Cohort	range, 12 to 108 months
36	Aune et al., 2012	Norway	Multiplex immunoassay Cytokine Human 25-plex panel (Luminex Corpora-tion, Austin, TX, USA)	serum	57 carcinoma	23 borderline, and 33 benign ovarian tumors	I-IV	IL1B, IL1 Ra, IL-2, IL-2 R, IL4, IL5, IL6, IL7, IL 8, IL10, IL12, IL13, IL15, IL17, TNF-α, IFN-α, INFg, GM-CSF, MIP-1a, MIP-1b, IP-10, HUMIG, eotaxin, rantes, MCP-1	cut-off value of IL-8 was determined at 2SD (59 pg/mL). below these cut-offs were defined as normal, and levels above were defined as elevated.	OS, FIGO stage	Cohort prospect ive	5 year survival
37	Kolomeyevskaya et al., 2015	USA	luminex multiplex asssay	asites	70	0	IIIB-IV	IL 8, IL 6, TNF-α	Stratifying patients into high and low groups based on the median of each of IL-6, IL-8, and TNF-α, we considered the combined levels of IL-6 and IL-8, of IL-8 and TNF-α, and of IL-6 and TNF-α.	age, grade, serosa, <1cm residual disease, OS, PFS	cohort retrospe ctive	5 year survival

38	Yigit et al., 2011	Netherlands	low Cyto- mix Multiplex (Bender MedSystems)	cyst fluid	20 malignant	19 benign	I-IV	IL 8	Values are considered positive if greater than 0 pg/mL for TGF-α, greater than 270 pg/mL for CCL22, and greater than 20 pg/mL for the other cytokines.	OS, benign vs malignant vs malignant (miscella neous)	cohort prospect ive	40 months
39	Matsuo et al., 2015	Multicenter (USA, Japan, England)	ELISA	plasma	200	0	I-IV	IL-6	IL-6 levels were grouped into IL-6>= 10 versus<10pg/mL	PFS, VTE	Cohort retro and prospect ive	5 year survival
40	Kumar et al., 2017	USA	quantitative two-site enzyme immunoassay	serum	48	0	I-IV	IL 6	A cutoff value of 24 pg/ml was selected according to the median serum levels	PFS, OS, age, grade, stage, tipe histopatol ogi, asites, residual tumor	cohort retrospe ctive	6 months and 12 months
41	Tempfer et al., 1996	Austria	Human IL-6 Immunoassay (Quan- tikine, R&D Systems, Inc., Minneapolis, MN)	serum	73	50	I-IV	IL 6	A cutoff value of 0.78 pg/ml was selected according to the 95th per- centile of serum concentrations measured in 50 healthy con- trols.	OS, PFS	Cohort retrospe ctive	100 months

42	Masoumi-Moghaddam et al., 2015 (IL-6)	Australia	IHC	tissue	98	Tonsil tissue was used as the positive control. As regards the negative control, the same tissue as our positive control was used but the primary antibodies were replaced with the primary antibody diluents.	I-IV	IL 6	The binary cut-off point was identified using the Classification and Regression Tree (CART) algorithm. According to the cut-off point determined, the immuno-histochemical scores were classified as either low (score ≤ 3.5) or high (score > 3.5).	DFS, OS, respons of chemothe rapy	cohort	50 months
43	D'Antonio et al., 2002	USA	IHC	tissue	50	Appropriate controls using either rabbit or mouse irrelevant IgG were used.0	I-IV	TGF-α	Both intensity of staining and percentage of immuno- positive cells were scored. Specific staining was semi- quantitated by assigning a score of 1 to 3 based on color intensity of the brown diaminobenzidine precipitate, with 1 representing light brown staining; 2 a moderately brown color; and 3, an intense brown color. The slides were evaluated by two investigators. The specimens were	PFS, grading, ekspresi MIB, tipe histopatol ogi, spesimen (ovary vs extraovar y)	cohort	60 months

									judged positive when at least 10% of the cells were stained.			
44	Lan et al., 2013	China	IHC	tissue	104	30	III-IV	IL-17	the positively stained cells were counted manually. The results were expressed as the mean number of cells per HPF for every specimen.	OS, PFS, grade, stage, tipe histopatologi, ekspresi CD-163 M2 macrophage	cohort retrospective	4-12 year
45	Chambers et al., 1997	USA	IHC	tissue	130	-	I-IV	CSF-1	Using a modified H score, with score of 0 implying no staining and 400 reflecting intense staining over whole slide	OS, DFS	cohort	1-136 month

46	Kassim et al., 2003	Egypt	IL-8 (IHC, RT PCR) VEGF (quantitative sandwich enzyme immunoassay technique, Western blot	tissue	24 maliganant	20 benign	I-IV	VEGF IL-8	determined the cutoff for VEGF that maximizes the sum of sensitivity (5/6, 83.3%) and specificity (15/18, 83.3%) in discriminating good from poor outcome of the disease. This cutoff was 120 pg/mg protein.	tipe (benign vs malignant), grade, stage, tipe histopatologi, survival	cohort prospective	total follow-up period of 36 months
47	Liu et al., 2012	China	IHC	tissue	79 malignant		I-IV	VEGF, IL 10, TGF-β1	The mean percentage of positive cells was determined in at least five areas at ×200 magnification and assigned to one of the following categories (P value): 0=<5%; 1=5%–25%; 3=50%–75%; 4=>75%. The immunostaining intensity (I value) was scored as 1 (yellow), 2 (buffy) or 3 (brown). The PI value was determined by the cross product of P value and I value: 0 (negative), 1–2 (weak), 3–4 (moderate), and 6–9 (intense).	OS	cohort	120 months

48	Droeser et al., 2013	Switzerland	IHC	tissue	47		I-IV	IL 17	The majority of all biopsies (n = 94) were found to be completely negative for IL-17 (n = 55). In the positive biopsies, the number of IL-17 protein expressing cells ranged from 1 to 93.	OS, PFS, chemothe rapy respons	cohort	not stated
49	Lambeck et al., 2007	Netherlands	LINCOplex kit	serum	187	45 benign 50 healthy	I-IV	IL-6 , IL-7, IL-8, dan IL-10	Median concentration (pg/ml) IL 6=15.1; IL-7=5.3; IL8=13.2; IL-10=8.2	DFS, OS	Cohort retrospe ctive	2-11 year
50	Scambia et al., 1995	Italy	specific enzymatic imunoassay	serum	114	74	I-IV	IL-6	A cut off value of 6 pg/ml based on >95 percentile value	OS	cohort	not stated