

Biomarkers for endometrial cancer

This application claims the benefit of European Patent Application EP21382680 filed the 23rd of July of 2021.

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Technical Field

The invention relates to the diagnosis and prognosis of endometrial cancer.

Background Art

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Endometrial cancer (EC) is the most frequently observed invasive tumor of the female genital tract and the fourth most common cancer in women in developed countries, accounting for 66,570 diagnosed cases and 12,940 estimated deaths in 2021 in the United States. Its early diagnosis is associated with 95% of 5-year survival rate. However, 15 when it is diagnosed at advanced stages, the 5-year survival rate decreases dramatically to 69% in case of local metastasis and 16% in those cases with distant metastasis. Currently, there are no screening tools for its early diagnosis, and the diagnostic process starts with the observation of related symptoms, being abnormal vaginal bleeding (AVB) the most common. Even though 90% of EC patients will experience AVB, this symptom is 20 not specific of the disease and only a 9% of the studied patients will finally present EC. The first diagnostic step is to perform the pathological evaluation of an endometrial pipelle biopsy. However, this procedure is associated to 22% of failure and these patients will undergo additional invasive procedures such as hysteroscopy to be diagnosed. Yearly, ~7M women experience AVB in Europe and begin this diagnostic process, causing 25 morbidity to patients and a big burden to the healthcare systems. Improving early diagnosis is hence a major issue to appropriately manage EC and decrease mortality associated to the disease. Discrimination of patients with benign endometrial pathologies and with EC is only achieved after a tedious diagnostic process consisting of a pelvic examination and transvaginal ultrasonography followed by a confirmatory 30 histopathological examination of an endometrial biopsy. The preferable biopsy used in this procedure is named uterine aspirate and/or pipelle biopsy and is obtained by a minimally invasive aspiration of endometrial fluid from inside the uterine cavity. Because the current diagnostic procedures on uterine aspirates rely on the presence of cellular material, this process has unfortunately a diagnostic failure and an associated inadequate sampling 35 rate of 8% and 15%, respectively. This is increased in postmenopausal women up to 12% and 22%. In those cases, a biopsy guided by hysteroscopy needs to be performed, where this invasive technique presents an increased risk of complications, including uterine perforation, hemorrhage and possible harm to other organs.

The European patent EP3452829B1, and the European patent application EP3655778A1 disclose useful markers that can be determined in the uterine aspirate and that provide good specificities and sensitivities for the differential diagnosis of EC from other endometrial conditions. The document EP3655778A1 provides a method for the prognosis of EC, for being able to distinguish among two of the subtypes of EC; endometrioid endometrial cancer (EEC) from non-endometrioid EC cases (NEEC).

To date, many studies have also been conducted to identify EC protein biomarkers, mainly in tissue and serum samples (see for example DeSouza LV, et al, "Endometrial cancer biomarker discovery and verification using differentially tagged clinical samples with multidimensional liquid chromatography and tandem mass spectrometry", Mol Cell Proteomics MCP- 2007, vol. no.6, pp.:1170–8, or Kemik P, et al. "Diagnostic and prognostic values of preoperative serum levels of YKL-40, HE-4 and DKK-3 in endometrial cancer", Gynecol Oncol- 2016; vol. no.140, pp.:64–9). None of them have been translated into clinical utility.

Other documents disclosing plasma or serum samples for retrieving important information about EC lead to controversial or contradictory conclusions. For example, whilst the document of Tanable et al., "Midkine and its clinical significance in endometrial carcinoma", cancer Sci-2008, vol. no. 99(6), pp.: 1125-1130, proposes midkine (MDK), a secreted heparin-binding growth factor, as useful serum biomarker for identifying high risk patients of EC; the document of Torres et al., "CD44, TGM2 and EpCAM as novel plasma markers in endometrial cancer diagnosis", BMC Cancer-2019 19:401 <https://doi.org/10.1186/s12885-019-5556-x>. (see Fig. 2 (f)), identified that MDK is not able to distinguish EC from healthy controls if the endometriosis patients are taken out of the endometrial cancer subgroup. Indeed, Torres et al. conclude that plasma markers like TGM2 can accurately diagnose EC, but others, such as MDK, might be altered in EC studies by the inclusion of endometriosis cases, and this need to be taken in consideration in future research design. Finally, MDK has also been identified in tissue biopsies from the cervix and giving information about cervical cancer in the document of Moon et al., "Immunohistochemical and quantitative competitive PCR analyses of midkine and pleiotrophin expression in cervical cancer", Gynecologic Oncology-2003, vol. no. 88, pp.: 289-297.

In any case, of note is that some of the studied samples (tissue biopsies, plasma and serum) are non-routine gynecological samples, and all of them (including uterine aspirates) are minimally-invasive, which precludes their use as an easy-access screening and/or diagnostic tool.

Concluding, despite the efforts made, there is still the need of a trustable rule out method to reduce the current burden of women entering through the diagnostic process, as well as highly accurate biomarkers that can be assessed easily in a clinical environment obtained from non-invasive samples to improve the diagnosis, and even the prognosis of
5 endometrial cancer.

Summary of Invention

Inventors have determined that certain protein markers detectable in isolated samples
10 obtainable from methods used in the regular or routine controls of gynecology, give valuable diagnostic information in endometrial cancer (EC). The proteins were first analysed in a retrospective way from a cohort of 60 patients, including control, EC and patients with cervical pathology. Then, the group of informative proteins was retrospectively validated in a larger cohort of 242 patients (106 non-EC, 129 EC, and 7
15 premalignant lesions of EC, i.e. hyperplasias).

In addition, inventors have determined that some proteins also detectable in these types of samples, are meaningful prognostic biomarkers of endometrial cancer (EC). These proteins allow discrimination between EC presenting different prognosis, including
20 different histological subtypes and grades and different molecular features, with high sensitivity and high specificity, and thus they allow minimizing the risk of false positive and false negative classification among these subtypes.

Thus, in a first aspect, the invention relates to a method of diagnosis and/or for the
25 prognosis of EC, the method comprising determining the presence and/or the level of expression of midkine (MDK) in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including or selected from a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations
30 thereof.

MDK is (MK or MDK), also known as neurite growth-promoting factor 2 (NEGF2). It is a protein that in humans is encoded by the MDK gene. Midkine is a basic heparin-binding growth factor of low molecular weight, and forms a family with pleiotrophin (NEGF1, 46%
35 homologous with MK). It is a nonglycosylated protein, composed of two domains held by disulfide bridges. It is a developmentally important retinoic acid-responsive gene product strongly induced during mid-gestation, hence the name midkine. Restricted mainly to certain tissues in the normal adult, it is strongly induced during oncogenesis, inflammation and tissue repair. The canonical amino acid sequence (Isoform 1) has a length of 143

amino acids, at it is identified on the UniProtKB database with the accession number P21741 (version of May 1, 1991 of the sequence and release 189 of the UniProtKB/Swiss-Prot database in April 7, 2021).

- 5 Another aspect of the invention is the use of MDK, as *in vitro* marker for the diagnosis and/or for the prognosis of endometrial cancer in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear like sample containing cervical fluid, a pap-smear sample, an endometrial
10 biopsy, a uterine fluid, uterine washings and combinations thereof

- In a third aspect, the invention proposes new kits comprising a solid support and means for detecting the presence and/or for determining the level of expression of MDK and optionally means for detecting the presence and/or for determining the level of expression
- 15 of one or more proteins selected from the group consisting of Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2
20 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG
25 Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Periplakin (PPL), Stathmin 1
30 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1),
35 Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and Exportin-2 (XPO2).

- In other words, this aspect can also be defined as new kits comprising a solid support and means for detecting the presence and/or for determining the level of expression of MDK and optionally means for detecting the presence and/or for determining the level of expression of one or more proteins selected from the group listed in Table 1 below, indicating the entry (accession number) in the UniProtKB/Swiss-Prot database of the release number accessible the 23th of July of 2021 in <https://www.uniprot.org/help/uniprotkb>:

10 Table 1. Informative proteins for EC.

Gene names	Entry	Entry name	Protein names
MDK MK1 NEGF2	P21741	MK_HUMAN	Midkine (MK) (Amphiregulin-associated protein) (ARAP) (Midgestation and kidney protein) (Neurite outgrowth-promoting factor 2) (Neurite outgrowth-promoting protein)
COL1A1	P02452	CO1A1_HUMAN	Collagen alpha-1(I) chain (Alpha-1 type I collagen)
VWF F8VWF	P04275	VWF_HUMAN	von Willebrand factor (vWF) [Cleaved into: von Willebrand antigen 2 (von Willebrand antigen II)]
APOB	P04114	APOB_HUMAN	Apolipoprotein B-100 (Apo B-100) [Cleaved into: Apolipoprotein B-48 (Apo B-48)]
C1QA	P02745	C1QA_HUMAN	Complement C1q subcomponent subunit A
FN1 FN	P02751	FN1_HUMAN	Fibronectin (FN) (Cold-insoluble globulin) (CIG) [Cleaved into: Anastellin; Ugl-Y1; Ugl-Y2; Ugl-Y3]
SERPIND1 HCF2	P05546	HEP2_HUMAN	Heparin cofactor 2 (Heparin cofactor II) (HC-II) (Protease inhibitor leuserpin-2) (HLS2) (Serp D1)
SPP1 BNSP OPN PSEC0156	P10451	OSTP_HUMAN	Osteopontin (Bone sialoprotein 1) (Nephropontin) (Secreted phosphoprotein 1) (SPP-1) (Urinary stone protein) (Uropontin)
APP A4 AD1	P05067	A4_HUMAN	Amyloid-beta precursor protein (APP) (ABPP) (APPI) (Alzheimer disease amyloid protein) (Amyloid precursor protein) (Amyloid-beta A4 protein) (Cerebral vascular amyloid peptide) (CVAP) (PreA4) (Protease nexin-II) (PN-II) [Cleaved into: N-APP; Soluble APP-alpha (S-APP-alpha); Soluble APP-beta (S-APP-beta); C99 (Beta-secretase C-terminal fragment) (Beta-CTF); Amyloid-beta protein 42 (Aβ42) (Beta-APP42); Amyloid-beta protein 40 (Aβ40) (Beta-APP40); C83 (Alpha-secretase C-terminal fragment) (Alpha-CTF); P3(42); P3(40); C80; Gamma-secretase C-terminal fragment 59 (Amyloid intracellular domain 59) (AICD-59) (AID(59)) (Gamma-CTF(59)); Gamma-secretase C-terminal fragment 57 (Amyloid intracellular domain 57) (AICD-57) (AID(57)) (Gamma-CTF(57)); Gamma-secretase C-terminal fragment 50 (Amyloid intracellular domain 50) (AICD-50) (AID(50)) (Gamma-CTF(50)); C31]

Gene names	Entry	Entry name	Protein names
FGFBP1 FGFBP HBP17	Q14512	FGFP1_HUMAN	Fibroblast growth factor-binding protein 1 (FGF-BP) (FGF-BP1) (FGF-binding protein 1) (FGFBP-1) (17 kDa heparin-binding growth factor-binding protein) (17 kDa HBGF-binding protein) (HBp17)
TCP1 CCT1 CCTA	P17987	TCPA_HUMAN	T-complex protein 1 subunit alpha (TCP-1-alpha) (CCT-alpha)
APOC1	P02654	APOC1_HUMAN	Apolipoprotein C-I (Apo-CI) (ApoC-I) (Apolipoprotein C1) [Cleaved into: Truncated apolipoprotein C-I]
APOF	Q13790	APOF_HUMAN	Apolipoprotein F (Apo-F) (Lipid transfer inhibitor protein) (LTIP)
CCT6A CCT6 CCTZ	P40227	TCPZ_HUMAN	T-complex protein 1 subunit zeta (TCP-1-zeta) (Acute morphine dependence-related protein 2) (CCT-zeta-1) (HTR3) (Tc20)
LBP	P18428	LBP_HUMAN	Lipopolysaccharide-binding protein (LBP)
COL12A1 COL12A1L	Q99715	COCA1_HUMAN	Collagen alpha-1(XII) chain
SAA4 CSAA	P35542	SAA4_HUMAN	Serum amyloid A-4 protein (Constitutively expressed serum amyloid A protein) (C-SAA)
ITIH2 IGHEP2	P19823	ITIH2_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H2 (ITI heavy chain H2) (ITI-HC2) (Inter-alpha-inhibitor heavy chain 2) (Inter-alpha-trypsin inhibitor complex component II) (Serum-derived hyaluronan-associated protein) (SHAP)
LCAT	P04180	LCAT_HUMAN	Phosphatidylcholine-sterol acyltransferase (EC 2.3.1.43) (1-alkyl-2-acetylgllycerophosphocholine esterase) (EC 3.1.1.47) (Lecithin-cholesterol acyltransferase) (Phospholipid-cholesterol acyltransferase) (Platelet-activating factor acetylhydrolase) (PAF acetylhydrolase)
LCN2 HNL NGAL	P80188	NGAL_HUMAN	Neutrophil gelatinase-associated lipocalin (NGAL) (25 kDa alpha-2-microglobulin-related subunit of MMP-9) (Lipocalin-2) (Oncogene 24p3) (Siderocalin) (p25)
MUC4	Q99102	MUC4_HUMAN	Mucin-4 (MUC-4) (Ascites sialoglycoprotein) (ASGP) (Pancreatic adenocarcinoma mucin) (Testis mucin) (Tracheobronchial mucin) [Cleaved into: Mucin-4 alpha chain (Ascites sialoglycoprotein 1) (ASGP-1); Mucin-4 beta chain (Ascites sialoglycoprotein 2) (ASGP-2)]
C1R	P00736	C1R_HUMAN	Complement C1r subcomponent (EC 3.4.21.41) (Complement component 1 subcomponent r) [Cleaved into: Complement C1r subcomponent heavy chain; Complement C1r subcomponent light chain]
C4BPA C4BP	P04003	C4BPA_HUMAN	C4b-binding protein alpha chain (C4bp) (Proline-rich protein) (PRP)
SPRR1B	P22528	SPR1B_HUMAN	Cornifin-B (14.9 kDa pancomulin) (Small proline-rich protein IB) (SPR-IB)
SERPINH1 CBP1 CBP2 HSP47 SERPINH2 PIG14	P50454	SERPH_HUMAN	Serpin H1 (47 kDa heat shock protein) (Arsenic-transactivated protein 3) (AsTP3) (Cell proliferation-inducing gene 14 protein) (Collagen-binding protein) (Colligin) (Rheumatoid arthritis-related antigen RA-A47)

Gene names	Entry	Entry name	Protein names
LMO7 FBX20 FBXO20 KIAA0858	Q8WWI1	LMO7_HUMAN	LIM domain only protein 7 (LMO-7) (F-box only protein 20) (LOMP)
S100A9 CAGB CFAG MRP14	P06702	S10A9_HUMAN	Protein S100-A9 (Calgranulin-B) (Calprotectin L1H subunit) (Leukocyte L1 complex heavy chain) (Migration inhibitory factor-related protein 14) (MRP-14) (p14) (S100 calcium-binding protein A9)
SPRR1A	P35321	SPR1A_HUMAN	Cornifin-A (19 kDa pancornulin) (SPRK) (Small proline-rich protein IA) (SPR-IA)
AGRN AGRIN	O00468	AGRN_HUMAN	Agrin [Cleaved into: Agrin N-terminal 110 kDa subunit; Agrin C-terminal 110 kDa subunit; Agrin C-terminal 90 kDa fragment (C90); Agrin C-terminal 22 kDa fragment (C22)]
TIMP2	P16035	TIMP2_HUMAN	Metalloproteinase inhibitor 2 (CSC-21K) (Tissue inhibitor of metalloproteinases 2) (TIMP-2)
COL3A1	P02461	CO3A1_HUMAN	Collagen alpha-1(III) chain
ANXA7 ANX7 SNX OK/SW-cl.95	P20073	ANXA7_HUMAN	Annexin A7 (Annexin VII) (Annexin-7) (Synexin)
PPL KIAA0568	O60437	PEPL_HUMAN	Periplakin (190 kDa paraneoplastic pemphigus antigen) (195 kDa cornified envelope precursor protein)
SCEL	O95171	SCEL_HUMAN	Sciellin
IGFALS ALS	P35858	ALS_HUMAN	Insulin-like growth factor-binding protein complex acid labile subunit (ALS)
GPLD1 PIGPLD1	P80108	PHLD_HUMAN	Phosphatidylinositol-glycan-specific phospholipase D (PI-G PLD) (EC 3.1.4.50) (Glycoprotein phospholipase D) (Glycosyl-phosphatidylinositol-specific phospholipase D) (GPI-PLD) (GPI-specific phospholipase D)
TMPRSS11E DESC1 TMPRSS11E2 UNQ742/PRO1461	Q9UL52	TM11E_HUMAN	Transmembrane protease serine 11E (EC 3.4.21.-) (Serine protease DESC1) (Transmembrane protease serine 11E2) [Cleaved into: Transmembrane protease serine 11E non-catalytic chain; Transmembrane protease serine 11E catalytic chain]
BTD	P43251	BTD_HUMAN	Biotinidase (Biotinase) (EC 3.5.1.12)
ANXA3 ANX3	P12429	ANXA3_HUMAN	Annexin A3 (35-alpha calcimedin) (Annexin III) (Annexin-3) (Inositol 1,2-cyclic phosphate 2-phosphohydrolase) (Lipocortin III) (Placental anticoagulant protein III) (PAP-III)
SERPINA3 AACT GIG24 GIG25	P01011	AACT_HUMAN	Alpha-1-antichymotrypsin (ACT) (Cell growth-inhibiting gene 24/25 protein) (Serp A3) [Cleaved into: Alpha-1-antichymotrypsin His-Pro-less]
FCGBP	Q9Y6R7	FCGBP_HUMAN	IgGFc-binding protein (Fcgamma-binding protein antigen) (FcgammaBP)
FAM107B C10orf45	Q9H098	F107B_HUMAN	Protein FAM107B
STX7	O15400	STX7_HUMAN	Syntaxin-7
PLBD1	Q6P4A8	PLBL1_HUMAN	Phospholipase B-like 1 (EC 3.1.1.-) (LAMA-like protein 1) (Lamina ancestor homolog 1) (Phospholipase B domain-containing protein 1) [Cleaved into: Phospholipase B-like 1

Gene names	Entry	Entry name	Protein names
			chain A; Phospholipase B-like 1 chain B; Phospholipase B-like 1 chain C]
GRN	P28799	GRN_HUMAN	Progranulin (PGRN) (Acrogranin) (Epithelin precursor) (Glycoprotein of 88 Kda) (GP88) (Glycoprotein 88) (Granulin precursor) (PC cell-derived growth factor) (PCDGF) (Proepithelin) (PEPI) [Cleaved into: Paragranulin; Granulin-1 (Granulin G); Granulin-2 (Granulin F); Granulin-3 (Epithelin-2) (Granulin B); Granulin-4 (Epithelin-1) (Granulin A); Granulin-5 (Granulin C); Granulin-6 (Granulin D); Granulin-7 (Granulin E)]
IMPA1 IMPA	P29218	IMPA1_HUMAN	Inositol monophosphatase 1 (IMP 1) (IMPase 1) (EC 3.1.3.25) (D-galactose 1-phosphate phosphatase) (EC 3.1.3.94) (Inositol-1(or 4)-monophosphatase 1) (Lithium-sensitive myo-inositol monophosphatase A1)
A2ML1 CPAMD9	A8K2U0	A2ML1_HUMAN	Alpha-2-macroglobulin-like protein 1 (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 9)
NEDD8	Q15843	NEDD8_HUMAN	NEDD8 (Neddylin) (Neural precursor cell expressed developmentally down-regulated protein 8) (NEDD-8) (Ubiquitin-like protein Nedd8)
CSE1L CAS XPO2	P55060	XPO2_HUMAN	Exportin-2 (Exp2) (Cellular apoptosis susceptibility protein) (Chromosome segregation 1-like protein) (Importin-alpha re-exporter)
EPS8L1 DRC3 EPS8R1 PP10566	Q8TE68	ES8L1_HUMAN	Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8-like protein 1) (Epidermal growth factor receptor pathway substrate 8-related protein 1) (EPS8-related protein 1)
VIM	P08670	VIME_HUMAN	Vimentin
CAPS	Q13938	CAYP1_HUMAN	Calcyphosin (Calcyphosine)
CA1	P00915	CAH1_HUMAN	Carbonic anhydrase 1 (EC 4.2.1.1) (Carbonate dehydratase I) (Carbonic anhydrase B) (CAB) (Carbonic anhydrase I) (CA-I)
KRT8 CYK8	P05787	K2C8_HUMAN	Keratin, type II cytoskeletal 8 (Cytokeratin-8) (CK-8) (Keratin-8) (K8) (Type-II keratin Kb8)
PEA15	Q15121	PEA15_HUMAN	Astrocytic phosphoprotein PEA-15 (15 kDa phosphoprotein enriched in astrocytes) (Phosphoprotein enriched in diabetes) (PED)
CHMP4B C20orf178 SHAX1	Q9H444	CHM4B_HUMAN	Charged multivesicular body protein 4b (Chromatin-modifying protein 4b) (CHMP4b) (SNF7 homolog associated with Alix 1) (SNF7-2) (hSnf7-2) (Vacuolar protein sorting-associated protein 32-2) (Vps32-2) (hVps32-2)
CTNNB1 CTNNB OK/SW-cl.35 PRO2286	P35222	CTNB1_HUMAN	Catenin beta-1 (Beta-catenin)
CALU	O43852	CALU_HUMAN	Calumenin (Crocalbin) (IEF SSP 9302)
GYG1 GYG	P46976	GLYG_HUMAN	Glycogenin-1 (GN-1) (GN1) (EC 2.4.1.186)

Gene names	Entry	Entry name	Protein names
LGALS1	P09382	LEG1_HUMAN	Galectin-1 (Gal-1) (14 kDa laminin-binding protein) (HLBP14) (14 kDa lectin) (Beta-galactoside-binding lectin L-14-I) (Galaptin) (HBL) (HPL) (Lactose-binding lectin 1) (Lectin galactoside-binding soluble 1) (Putative MAPK-activating protein PM12) (S-Lac lectin 1)
PKM OIP3 PK2 PK3 PKM2	P14618	KPYM_HUMAN	Pyruvate kinase PKM (EC 2.7.1.40) (Cytosolic thyroid hormone-binding protein) (CTHBP) (Opa-interacting protein 3) (OIP-3) (Pyruvate kinase 2/3) (Pyruvate kinase muscle isozyme) (Thyroid hormone-binding protein 1) (THBP1) (Tumor M2-PK) (p58)
HCLS1 HS1	P14317	HCLS1_HUMAN	Hematopoietic lineage cell-specific protein (Hematopoietic cell-specific LYN substrate 1) (LckBP1) (p75)
RAB21 KIAA0118	Q9UL25	RAB21_HUMAN	Ras-related protein Rab-21
LSP1 WP34	P33241	LSP1_HUMAN	Lymphocyte-specific protein 1 (47 kDa actin-binding protein) (52 kDa phosphoprotein) (pp52) (Lymphocyte-specific antigen WP34)
CD44 LHR MDU2 MDU3 MIC4	P16070	CD44_HUMAN	CD44 antigen (CDw44) (Epican) (Extracellular matrix receptor III) (ECMR-III) (GP90 lymphocyte homing/adhesion receptor) (HUTCH-I) (Heparan sulfate proteoglycan) (Hermes antigen) (Hyaluronate receptor) (Phagocytic glycoprotein 1) (PGP-1) (Phagocytic glycoprotein I) (PGP-I) (CD antigen CD44)
HSPE1	P61604	CH10_HUMAN	10 kDa heat shock protein, mitochondrial (Hsp10) (10 kDa chaperonin) (Chaperonin 10) (CPN10) (Early-pregnancy factor) (EPF)
MMP8 CLG1	P22894	MMP8_HUMAN	Neutrophil collagenase (EC 3.4.24.34) (Matrix metalloproteinase-8) (MMP-8) (PMNL collagenase) (PMNL-CL)
PIGR	P01833	PIGR_HUMAN	Polymeric immunoglobulin receptor (PIgR) (Poly-Ig receptor) (Hepatocellular carcinoma-associated protein TB6) [Cleaved into: Secretory component]
ATP6V1G1 ATP6G ATP6G1 ATP6J	O75348	VATG1_HUMAN	V-type proton ATPase subunit G 1 (V-ATPase subunit G 1) (V-ATPase 13 kDa subunit 1) (Vacuolar proton pump subunit G 1) (Vacuolar proton pump subunit M16)
ITGB2 CD18 MFI7	P05107	ITB2_HUMAN	Integrin beta-2 (Cell surface adhesion glycoproteins LFA-1/CR3/p150,95 subunit beta) (Complement receptor C3 subunit beta) (CD antigen CD18)
LDHA PIG19	P00338	LDHA_HUMAN	L-lactate dehydrogenase A chain (LDH-A) (EC 1.1.1.27) (Cell proliferation-inducing gene 19 protein) (LDH muscle subunit) (LDH-M) (Renal carcinoma antigen NY-REN-59)
CHAMP1 C13orf8 CAMP CHAMP KIAA1802 ZNF828	Q96JM3	CHAP1_HUMAN	Chromosome alignment-maintaining phosphoprotein 1 (Zinc finger protein 828)
LMNB1 LMN2 LMNB	P20700	LMNB1_HUMAN	Lamin-B1

Gene names	Entry	Entry name	Protein names
STMN1 C1orf215 LAP18 OP18	P16949	STMN1_HUMAN	Stathmin (Leukemia-associated phosphoprotein p18) (Metablastin) (Oncoprotein 18) (Op18) (Phosphoprotein p19) (pp19) (Prosolin) (Protein Pr22) (pp17)
NAMPT PBEF PBEF1	P43490	NAMPT_HUMAN	Nicotinamide phosphoribosyltransferase (NAMPTase) (Nampt) (EC 2.4.2.12) (Pre-B-cell colony-enhancing factor 1) (Pre-B cell-enhancing factor) (Visfatin)

Indeed, herewith disclosed are also the use of kits comprising the means for detecting and/or determining the expression of the one or more of the above-listed proteins in the indicated sample, as tools for the diagnosis and/or for the prognosis of EC.

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Finally, another aspect of the invention is a computer-implemented method for carrying out the in vitro method as defined in the first aspect, in which after the determination of the level of expression of MDK, and optionally of one or more of the proteins for the diagnosis and/or for the prognosis of endometrial cancer, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and/or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC presenting different prognosis, including different histological subtypes and grades and different molecular features. In other words, a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC subtypes, and/or between the options of suffering among different EC grades.

This aspect results from the algorithm for carrying out any of the methods as defined in this description. In the sense of the invention, the term "algorithm" is also synonymous of pannel or decision diagrams, predictors and combinatory of data to correctly categorize an individual sample.

Herewith disclosed is also a method for the diagnosis and/or for the prognosis of EC, the method comprising determining, in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof, the presence and/or the level of expression of one or more of the following proteins: Midkine (MDK), Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum

Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and
 5 Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1),
 10 Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Perioplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H+
 15 Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and Exportin-2 (XPO2). In some examples, the method further comprises the step of determining one or more clinical or featuring parameters of the subject, in particular selected from the group consisting of blood pressure, glycemia, age, scores for grading or staging (i.e., grading/staging system of the Federation of Gynecology and
 20 Obstetrics (FIGO)), thickness of the endometrium, CA125 and HE4 molecular markers, AVB, and combinations thereof.

It is also disclosed a method for the diagnosis and/or for the prognosis of EC, the method comprising determining, in a sample from the female genital tract part including one or
 30 more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear like sample, an endometrial biopsy, an uterine fluid, uterine washings and combinations thereof, the presence and/or the level of expression of one or more of the proteins from the group listed in Table 1.

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Also disclosed herewith is a particular prognosis method for the detection of histological type of EC, which comprises determining in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-

smear sample, a pap-smear like sample, an endometrial biopsy, an uterine fluid, uterine washings and combinations thereof, the presence and/or the level of expression of one or more of PIGR, PKM, optionally in combination with MDK or any other of the proteins in Table 1. In particular, the combination of PIGR with MDK provides AUC values near 0.846
5 for the correct determination (i.e., diagnosis) of the EC hystology. Of note also the combination of PIGR; RAB2; and MDK which gave an AUC value of 0.89.

Also disclosed herewith is a particular prognosis method for the grading of EC, which comprises determining in a sample from the female genital tract part including one or
10 more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, an uterine fluid, uterine washings and combinations thereof, the presence and/or the level of expression of one or more of PIGR, HSPE1, optionally in combination with MDK or any other of the proteins in Table 1. In particular, the
15 combination of PIGR with MDK provides AUC values near 0.920 for the correct determination (i.e., diagnosis) of the EC grade.

An additional aspect related with the diagnosis of EC, the invention provides a method for identifying a subject suspicious of suffering from EC, the method comprising:

20 a) determining, *in vitro*, the presence and/or the level of expression of midkine (MDK) in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, a pap-smear like sample, an
25 endometrial biopsy, a uterine fluid, uterine washings and combinations thereof; and
b) comparing the level of MDK of step (a) with a corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer, and/or comparing with a cut-off value discriminating among endometrial cancer and other gynecological
30 disorders or conditions, and wherein the subject is diagnosed of endometrial cancer if the level of MDK is within the value or interval of values from a subject suffering from this cancer, and/or if the level of MDK in relation to the cut-off value is classified to the endometrial cancer group.

35 In a further aspect, the present invention provides a method of deciding or recommending whether to initiate a medical regimen of a subject suspicious of suffering endometrial carcinoma, which method comprises the steps of:

a) determining, *in vitro*, the presence and/or the level of expression of midkine (MDK) in a

- sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof; and
- 5 b) comparing the level of MDK of step (a) with a corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer, and/or comparing with a cut-off value discriminating among endometrial cancer and other gynecological disorders or conditions, and wherein the subject is diagnosed of endometrial cancer if the
- 10 level of MDK is within the value or interval of values from a subject suffering from this cancer, and/or if the level of MDK in relation to the cut-off value is classified to the endometrial cancer group.

wherein:

- 15 i) if the subject is diagnosed of suffering from endometrial carcinoma, or of being suspicious of suffering from endometrial carcinoma, then the initiation of the medical regimen is recommended, and
- 20 ii) if the patient is diagnosed of not suffering from endometrial carcinoma, the follow-up is performed optionally in consideration of the result of an examination of the patient by a physician.

By determining the marker level in a test sample, the skilled person can establish,

25 additionally, which is the most suitable treatment that can be recommended, because the level detected in the sample may reflect the extension (i.e., severity) of the disease.

Brief description of the drawings.

- 30 Figure 1 shows the results obtained using an ELISA assay for determining MDK, tested in samples of a cohort of subjects and comparing EC vs non-EC patients (cervical sample, also called cervical fluid OR pap-smear like sample). In (A) Dotplot of the distribution of protein concentration between EC and non-EC patients. In (B) the a curve of an ROC analysis (Receiver Operating Characteristic analysis) of MDK assessed by ELISA.

35 Figure 2 shows the results obtained using (A) mass spectrometry (LC-MS/MS PRM) or (B) an ELISA assay for determining MDK, tested in samples of a cohort of subjects and comparing EC vs non-EC patients. Dotplots illustrate the distribution of protein concentration between EC and non-EC patients. Also a ROC analysis (Receiver Operating

Characteristic analysis) of MDK assessed by either LC-MS/MS or ELISA is depicted. In (C) correlation between the measurements of both approaches.

Figure 3 shows the results obtained using an ELISA assay for determining MDK in uterine aspirates/pipelle biopsies/uterine fluids, tested in samples of a cohort of subjects and comparing EC vs non-EC patients. In (A) Dotplot of the distribution of protein concentration between EC and non-EC patients. In (B) the a curve of an ROC analysis (Receiver Operating Characteristic analysis) of MDK assessed by ELISA.

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Detailed description of the invention

All terms as used herein in this application, unless otherwise stated, shall be understood in their ordinary meaning as known in the art. Other more specific definitions for certain terms as used in the present application are as set forth below and are intended to apply uniformly through-out the specification and claims unless an otherwise expressly set out definition provides a broader definition.

The present invention provides new biomarkers for the diagnosis and prognosis of endometrial cancer in a sample of the female genital tract (i.e., including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries), said sample selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, a pap-smear like sample containing fluids, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.

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The term "diagnosis" is known to the person skilled in the art. As used herein "diagnosis" is understood as becoming aware of a particular medical condition complication or risk in a subject; the determination of the nature of the disease or condition; or the distinguishing of one disease or condition from another. It refers both to the process of attempting to determine or identify the possible disease or disorder, and to the opinion reached by this process. A diagnosis, in the sense of diagnostic procedure, can be regarded as an attempt at classification of an individual's condition into separate and distinct categories that allow medical decisions about treatment and prognosis to be made. Subsequently, a diagnostic opinion is often described in terms of a disease or other condition. However, a diagnosis can take many forms. It might be a matter of detecting the presence and naming the disease, lesion, dysfunction or disability. It might be an exercise to attribute a category for management or for prognosis. It may indicate either degree of abnormality on a continuum or kind of abnormality in a classification. Thus, the term "diagnosis" also

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encompasses the "screening" or "differential diagnosis" of the subjects in order to classify the same in several differentiated groups including, in particular, asymptomatic subjects, a subject with risk of suffering from EC, a subject already diagnosed of suffering EC, the classification of subjects suffering from EC and presenting different prognosis, including
5 different histological subtypes (in particular EEC vs NEEC) and grades and different molecular features, etc. The methods of the invention are, therefore, powerful screening tools for the correct classification of all the analyzed samples from the subjects.

In general terms, diagnostic markers listed in this description are those protein
10 differentially detected at level expression in isolated samples of controls (non-cancer individuals) versus endometrial cancer samples (including characterization of the tumor, and/or several types of EC).

The term "patient" (or subject), as used herein, refers to any subject which show one or
15 more signs or symptoms typically associated with EC. The term "patient", as used herein, refers also to all animals classified as female mammals and includes, but is not restricted to, domestic and farm female animals, primates and humans. Preferably, the patient is a female human of any age or race.

20 The *in vitro* method of diagnosis, including screening, of the first aspect of the invention can be performed with a sample of: (a) an asymptomatic subject, (b) a subject which has already been identified as being suspicious of suffering from endometrial cancer, (c) a subject already diagnosed of endometrial cancer, as complementary confirmation diagnostic assay or (d) a subject with high risk of suffering the disease.

25 The term "reference value", as used herein, relates to a predetermined criterion used as a reference for evaluating the values or data obtained from the samples collected from a subject. The reference value or reference level can be an absolute value (i.e., a cut-off value or cut-off discriminating value); a relative value; a value that has an upper or a lower
30 limit; a range of values (i.e., a range of possible cut-off values); an average value; a median value, a mean value, or a value as compared to a particular control or baseline value. A reference value or reference range can be based on an individual sample value, such as for example, a value obtained from a sample from the subject being tested, but at an earlier point in time. The reference value or range can be based on a large number of
35 samples, such as from population of subjects of the chronological age matched group, or based on a pool of samples including or excluding the sample to be tested. Reference values have been determined for the biomarkers of the invention. The reference value for a protein (i.e., MDK), may be from a lower and an upper value as will be disclosed in view of examples below. Range of values of each biomarker (protein levels) and particular

combinations of the values of the different biomarkers provide for correct classification of subjects with high sensitivity and specificity.

If the level of expression is determined at the protein level, then the "reference expression level" is a predefined value of protein quantity, whereas if the level of expression is determined at the mRNA level, then the "reference expression level" is a predefined value of mRNA quantity. The samples are taken from a subject or group of subjects wherein the presence, absence, stage, histological subtype or grade, or course of the disease has been properly performed previously. This value is used as a threshold to discriminate subjects wherein the condition to be analyzed is present from those wherein such condition is absent (i.e., subject having endometrial cancer from subjects free of endometrial cancer), to determine the histological subtype of the disease, the risk of developing or of being suffering from endometrial carcinoma, among others. This reference control level is also useful for determining whether the subject has to initiate a medical regimen and how effective the regimen is. The subject or subjects from whom the "reference control level" is derived may include subject/s wherein the condition is absent, subject/s wherein the condition is present, or both. The skilled person in the art, making use of the general knowledge, is able to choose the subject or group of subjects more adequate for obtaining the reference control level for each of the methods of the present invention. Methods for obtaining the reference value from the group of subjects selected are well-known in the state of the art (Burtis C. A. et al., 2008, Chapter 14, section "Statistical Treatment of Reference Values"). In a particular case "reference control level" is a cut-off value defined by means of a conventional ROC analysis (Receiver Operating Characteristic analysis). As the skilled person will appreciate, optimal cut-off value will be defined according to the particular applications of the diagnostic or prognostic method: purpose, target population for the diagnosis or prognosis, balance between specificity and sensibility, etc.

"Prognosis" as used herein refers to the prediction of the probable progression and outcome of a disease. It includes: neoplasm grading (attempt to express in replicable terms the level of cell differentiation in neoplasms as increasing anaplasia correlates with the aggressiveness of the neoplasm), neoplasm staging (attempt to express in replicable terms the extent of the neoplasm in the patient), neoplasm histological subtype, and neoplasm molecular subtype. As used herein prognosis means, in particular embodiments, differentiation between endometrioid endometrial cancer and non-endometrioid endometrial cancers, or differentiation between low and high histological grades of endometrial cancers, or differentiation between molecular subtypes of endometrial cancers, or differentiation between patients at high or low risk of recurrence.

As previously indicated, the first aspect of the invention is a method of diagnosis and/or for the prognosis of endometrial cancer, the method comprising determining the presence and/or the level of expression of MDK in an isolated sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.

The sample from the female genital tract from the vulvae to the cervix is a sample that results from the common and routine inspection performed usually once a year by gynecologists.

In a particular embodiment of the first aspect, the sample is a pap-smear like sample, and specifically the fluid contained in a pap-smear like and/or cervical fluid. In other words, the sample is a cervical sample and specifically, the fluid contained in a cervical sample. This cervix fluid is easily obtainable using cytobrushes, in the same way they are used for the cytology analysis of the routine gynecological inspection.

In another particular embodiment, the sample is a uterine aspirate (also called pipelle biopsy or uterine fluid). This is commonly known as the sample that results from the common and routine gynecological inspection used for determining the existence of carcinomas in the gynecological tract and which is less damaging and discomforting than a biopsy by hysteroscopy or dilatation&curettage. It is of relevance that MDK allows distinguishing between EC and non-EC also in this kind of sample.

This is the first time in this kind of routine sample MDK is detected as informative. Thus, the invention relates to the use of MDK as diagnostic marker in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear like sample, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof. In particular it relates to the use of MDK as diagnostic and/or prognostic marker of EC.

As will be illustrated in the examples section, when MDK is determined and at certain levels of expression in any sample of this part of the female genital tract, high sensitivities and specificities are achieved (e.g., AUC=0.910, SE=82.8, SP=87.8). When the level of expression of additional markers were also determined in the sample isolated from these structures of the genital tract (i.e., in particular a fluid from the cervix), the specificity was maintained while the sensitivity increased, which allowed the classification of most of the

true positive patients.

Thus, in another particular embodiment of the method of the first aspect, it further comprises determining the presence and/or the level of expression of one or more of the other proteins of Table 1. In another more particular embodiment, determining the presence and/or the level of expression of one or more of the following proteins:

Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF),
 10 Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein
 15 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide
 20 (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Periplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy
 25 Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin
 30 Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and Exportin-2 (XPO2).

In a more particular embodiment, the method comprises determining the presence and/or
 35 the level of expression of two, three, four, five, six, seven, eight, nine, and ten of the proteins always including MDK in the panel of proteins. In yet another particular embodiment of the in vitro method, it comprises determining the presence and/or the level of expression of two, three, or four of the proteins, including MDK in the panel of proteins.

In a more particular embodiment of the method, it comprises determining in the isolated sample the presence and/or the level of expression of the proteins in at least one binary set of the group listed in any one of Tables 4 and 6.

- 5 In a more particular embodiment of the method, it comprises determining in the isolated sample the presence and/or the level of expression of the proteins in at least one binary set of the group consisting of: MDK, LCN2; MDK, FN1; MDK, PLBD1; MDK, APOB; MDK, CD44; MDK, FCGBP; MDK, EPS8L1; MDK, ANXA3; MDK, C4BPA; MDK, MMP8; MDK, NEDD-8; MDK, CAMP; MDK, TIMP2; MDK, HSPE1; MDK, CALU; MDK, C1QA; MDK, LDHA; MDK, TIMP2; MDK, PIGR; MDK, KRT8; MDK, PPL; MDK, SPRR1A; MDK, STMN1; MDK, CAPS; MDK, CA1; MDK, CCT6A; MDK, VIM; MDK, TCP1; and MDK, AGRN.
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- In a more particular embodiment of the method, it comprises determining in the isolated sample the presence and/or the level of expression of the proteins in at least one ternary set of the group listed in any one of Tables 5 and 7.
- 15

- In another particular embodiment, the method comprises determining in the isolated sample the presence and/or the level of expression of the proteins in at least one ternary set of the group consisting of: MDK, ANXA7, CSE1L; MDK, LCN2, FGFBP1; MDK, LCN2, ANXA7; MDK, FGFBP1, LCN2; MDK, APOF, FCGBP; MDK, IMPA1, FCGBP; MDK, LCN2, CD44; MDK, STX7, FCGBP; MDK, ITIH2, FCGBP; MDK, LCN2, EPS8L1; MDK, FGFBP, EPS8L1; MDK, ANXA7, FCGBP; MDK, LCN2, PLBD1; MDK, FCGBP, CD44; MDK, PLBD1, FCGBP; MDK, ANXA3, FCGBP; MDK, PLBD1, LGALS1; MDK, LCN2, IMPA1; MDK, FCGBP, VIM; MDK, FCGBP, LMNB1; MDK, PLBD1, ATP6V1G1; MDK, APOB, PKM; MDK, ITIH2, LCN2; MDK, PLBD1, CALU; MDK, LCN2, APOF; MDK, PLBD1, EPS8L1; MDK, APOC1, ANXA7; MDK, FCGBP, GYG1; MDK, PLBD1, PKM; MDK, FCGBP, LSP1; MDK, TIMP2, CD44; MDK, ANXA3, PLBD1; MDK, PLBD1, HSPE1; MDK, PLBD1, CA1; MDK, FCGBP, HCLS1; MDK, STX7, IMPA1; MDK, ANXA7, STX7; MDK, STX7, EPS8L1; MDK, PLBD1, LSP1; MDK, C1R, ANXA7; MDK, STX7, PEA15; MDK, PLBD1, HCLS1; MDK, LCAT, FCGBP; MDK, PLBD1, LDHA; MDK, APOB, S100A9; MDK, ANXA7, SCEL; MDK, SERPINA3, FCGBP; MDK, FCGBP, ITGB2; MDK, SCEL, ANXA3; MDK, S100A9, PLBD1; MDK, S100A9, LSP1; MDK, C1R, FCGBP; MDK, FGFBP1, FCGBP; MDK, APOC1, S100A9; MDK, PPL, PLBD1; MDK, TCP1, PEA15; MDK, ANXA3, LSP1; MDK, PPL, IMPA1; MDK, S100A9, MMP8; MDK, FGFBP1, PPL; and MDK, S100A9, PPL.
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In a more particular embodiment, the method comprises determining in the isolated sample the presence and/or the level of expression of the set defined by MDK, ANXA7 and CSE1L.

- 5 In yet another particular embodiment, the method comprises determining in the isolated sample the presence and/or the level of expression of the proteins in at least one quaternary set of the group consisting of: MDK, LCN2, PEA15, LDHA; MDK, LCN2, PEA15, PKM; MDK, FGFBP1, FCGBP, EPS8L1; MDK, LCN2, HSPE1, CD44; MDK, FGFBP1, EPS8L1, CTNNB1; MDK, LCN2, IMPA1, LDHA; MDK, IMPA1, FCGBP, PEA15;
- 10 MDK, IMPA1, FCGBP, PKM; MDK, FCGBP, IMPA1, PEA15; MDK, CCT6A, FGFBP1, EPS8L1; MDK, STX7, FCGBP, PKM; MDK, IMPA1, FCGBP, GYG1; MDK, FGFBP1, SCEL, EPS8L1; MDK, STX7, FCGBP, CA1; MDK, STX7, FCGBP, NAMPT; MDK, FGFBP1, EPS8L1, CAPS; MDK, STX7, FCGBP, FCGBP; MDK, SCEL, STX7, FCGBP; MDK, FGFBP1, ANXA7, CAPS; MDK, FGFBP1, EPS8L1, RAB21; MDK, SCEL, STX7,
- 15 FCGBP; MDK, STX7, FCGBP, NEDD8-MDP1; MDK, ITIH2, FCGBP, CAPS; MDK, FGFBP1, EPS8L1, PKM; MDK, IMPA1, FCGBP, HCLS1; MDK, APOF, ANXA3, FCGBP; MDK, FGFBP1, EPS8L1, STMN1; MDK, STX7, FCGBP, LGALS1; MDK, FCGBP, LSP1, VIM; MDK, FGFBP1, EPS8L1, LGALS1; MDK, ANXA3, FCGBP, LMNB1; MDK, FCGBP, IMPA1, MMP8; MDK, FGFBP1, EPS8L1, STMN1; MDK, SCEL, IMPA1, PEA15; MDK,
- 20 FGFBP1, ANXA7, LGALS1; MDK, TCP1, PEA15, LMNB1; MDK, FCGBP, GYG1, CA1; MDK, SCEL, IMPA1, PKM; MDK, FGFBP1, EPS8L1, LDHA; MDK, FCGBP, KRT8, GYG1; MDK, FCGBP, GYG1, LMNB1; MDK, FCGBP, GYG1, LSP1; MDK, FCGBP, KRT8, GYG1; MDK, FCGBP, GYG1, LGALS1; MDK, FCGBP, CAPS, GYG1; MDK, FGFBP1, EPS8L1, LDHA; MDK, FCGBP, CAPS, GYG1; MDK, C1R, FCGBP, LGALS1; MDK,
- 25 FGFBP1, SCEL, PEA15; MDK, FGFBP1, TIMP2, CHMP4B; MDK, ANXA3, STMN1, PKM; MDK, ANXA7, SCEL, CHMP4B; MDK, FCGBP, GYG1, LDHA; MDK, S100A9, MMP8, LGALS1; MDK, C1R, FCGBP, GYG1; MDK, C4BPA, FGFBP1, PPL; MDK, ITIH2, FGFBP1, PPL; and MDK, APOC1, FGFBP1, PPL.
- 30 In another embodiment of the first aspect, optionally in combination with any of the embodiments above or below, the method comprises the following steps:
 - a) determining, *in vitro*, the level of expression of MDK, optionally in combination with one or more of the proteins listed in any one of previous embodiments, in the isolated sample from the genital tract of the female;
 - 35 b) comparing the level of MDK and, if determined, of the one or more other the proteins of step (a) with a corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer, and/or comparing with a cut-off value discriminating among endometrial cancer and other endometriod disorders or conditions of the

endometrium; and

- c) wherein the subject is diagnosed of endometrial cancer if at least the level of MDK is within the value or interval of values from a subject suffering from this cancer, and/or if at least the level of MDK in relation to the cut-off value is classified to the endometrial cancer group.

The expression other gynecological disorders or conditions include, without limitation, a healthy endometrium, endometriosis, endometrial polyps, endometrial myomas, atrophic endometrium, physiological damage of the uterus, or any other condition that can cause abnormal vaginal bleeding.

In another particular embodiment of the methods disclosed in the invention, it further comprises the step of determining one or more clinical or featuring parameters of the subject, in particular selected from the group consisting of blood pressure, glycemia, age, scores for grading or staging (i.e., stages of the Federation of Gynecology and Obstetrics (FIGO)), thickness of the endometrium, CA125 and HE4 molecular markers, AVB, and combinations thereof.

Another particular embodiment of the method according to the first aspect or any of the embodiments thereof, the level of expression of MDK and of other proteins is determined at the protein level.

In a more particular embodiment, the protein level is determined by an assay or technology selected from the group consisting of an immunoassay, a bioluminescence assay, a fluorescence assay, a chemiluminescence assay, electrochemistry assay, a lateral flow assay, mass spectrometry, and combinations thereof.

In another particular embodiment, the level of expression of protein is determined using an antibody or a fragment thereof able to specifically bind to the protein.

More in particular, said antibody or fragment thereof forms part of a kit.

Alternatively, the level of expression is determined at the mRNA level. Below is further explained in more detail this alternative method.

In a second aspect, the invention relates to the use of MDK, as *in vitro* marker for the diagnosis and/or for the prognosis of endometrial cancer in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a

cytology, a pap-smear sample, a pap-smear like sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.

- In a particular embodiment of the second aspect, the sample is a pap-smear, and
5 specifically the fluid contained in a pap-smear and/or cervical fluid.

In also another particular embodiment of the second aspect, MDK is used as in vitro marker in a panel of multiple biomarkers comprising one or more of Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin
10 Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor
15 Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8
20 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Periplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1),
25 Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL),
30 Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and Exportin-2 (XPO2).

In also another particular embodiment of the second aspect, MDK is used as in vitro
35 marker in a panel of multiple biomarkers comprising one or more of the other proteins from the group listed in Table 1.

These pannels of biomarkers including MDK comprise, in a particular embodiment from two to all the listed markers. In particular, the pannels comprise two, three, four, five, six,

seven, eight, nine, and ten biomarkers, being one of them MDK.

Disclosed are also pannels of biomarkers comprising one or more of the proteins of Table 1. In a particular example, pannels including from two to all the listed markers. In particular, the pannels comprise two, three, four, five, six, seven, eight, nine, and ten biomarkers.

Diagnosis of EC with MDK and/or the one or more proteins listed above can be carried out, as indicated, by the use of kits comprising means for determining the level of expression of these proteins. As previously indicated, a third aspect of the invention is a kit comprising a solid support and means for detecting the presence and/or for determining the level of expression of MDK and optionally means for detecting the presence and/or for determining the level of expression of one or more proteins listed in Table 1, in particular one or more selected from the group consisting of Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Periplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and Exportin-2 (XPO2).

- A “device” or “kit” in the sense of the invention is an assay or method to determine a (combination of) biomarker (levels of proteins of interest in a sample) or panel of biomarkers according to the invention that can be used to perform an assay or method for the diagnosis and/or prognosis of EC or for the selection of a patient for a medical regimen once diagnosed. Examples are carrier plates, test stripes, biochip arrays, electrochemical sensors, or the like known in the art including the reagent means to detecting the presence and level of the proteins of interest. The kits, as used herein, refer to a product containing the different reagents (or reagent means) necessary for carrying out the methods of the invention packed so as to allow their transport and storage.
- Materials suitable for packing the components of the kit include crystal, plastic (e.g. polyethylene, polypropylene, polycarbonate), bottles, vials, paper, or envelopes. Instructions in different formats for carrying out the method are, in some embodiments also included in the said kits. Particular formats of the instructions are selected from leaflets, electronic supports capable of storing instructions susceptible of being read or understood, such as, for example, electronic storage media (e.g. magnetic disks, tapes), or optical media (e.g. CD-ROM, DVD), or audio materials.

- In a particular embodiment of the kit of the invention, it comprises means (i.e., reagent means) for detecting and/or quantifying the level of expression of at least one binary set of the group consisting of: MDK, LCN2; MDK, FN1; MDK, PLBD1; MDK, APOB; MDK, CD44; MDK, FCGBP; MDK, EPS8L1; MDK, ANXA3; MDK, C4BPA; MDK, MMP8; MDK, NEDD-8; MDK, CAMP; MDK, TIMP2; MDK, HSPE1; MDK, CALU; MDK, C1QA; MDK, LDHA; MDK, TIMP2; MDK, PIGR; MDK, KRT8; MDK, PPL; MDK, SPRR1A; MDK, STMN1; MDK, CAPS; MDK, CA1; MDK, CCT6A; MDK, VIM; MDK, TCP1; and MDK, AGRN.

In another particular embodiment, the binary set is selected from at least one of Tables 4 and/or 6 below (see in examples).

- In another particular embodiment of the kit of the invention, it comprises means (i.e., reagent means) for detecting and/or quantifying the level of expression of at least one ternary set of the group consisting of: MDK, ANXA7, CSE1L; MDK, LCN2, FGFBP1; MDK, LCN2, ANXA7; MDK, FGFBP1, LCN2; MDK, APOF, FCGBP; MDK, IMPA1, FCGBP; MDK, LCN2, CD44; MDK, STX7, FCGBP; MDK, ITIH2, FCGBP; MDK, LCN2, EPS8L1; MDK, FGFBP, EPS8L1; MDK, ANXA7, FCGBP; MDK, LCN2, PLBD1; MDK, FCGBP, CD44; MDK, PLBD1, FCGBP; MDK, ANXA3, FCGBP; MDK, PLBD1, LGALS1; MDK, LCN2, IMPA1; MDK, FCGBP, VIM; MDK, FCGBP, LMNB1; MDK, PLBD1, ATP6V1G1; MDK, APOB, PKM; MDK, ITIH2, LCN2; MDK, PLBD1, CALU; MDK, LCN2, APOF; MDK, PLBD1, EPS8L1; MDK, APOC1, ANXA7; MDK, FCGBP, GYG1; MDK, PLBD1, PKM;

MDK, FCGBP, LSP1; MDK, TIMP2, CD44; MDK, ANXA3, PLBD1; MDK, PLBD1, HSPE1; MDK, PLBD1, CA1; MDK, FCGBP, HCLS1; MDK, STX7, IMPA1; MDK, ANXA7, STX7; MDK, STX7, EPS8L1; MDK, PLBD1, LSP1; MDK, C1R, ANXA7; MDK, STX7, PEA15; MDK, PLBD1, HCLS1; MDK, LCAT, FCGBP; MDK, PLBD1, LDHA; MDK, APOB, S100A9; 5 MDK, ANXA7, SCEL; MDK, SERPINA3, FCGBP; MDK, FCGBP, ITGB2; MDK, SCEL, ANXA3; MDK, S100A9, PLBD1; MDK, S100A9, LSP1; MDK, C1R, FCGBP; MDK, FGFBP1, FCGBP; MDK, APOC1, S100A9; MDK, PPL, PLBD1; MDK, TCP1, PEA15; MDK, ANXA3, LSP1; MDK, PPL, IMPA1; MDK, S100A9, MMP8; MDK, FGFBP1, PPL; and MDK, S100A9, PPL.

10

In another particular embodiment, the ternary set is selected from at least one of Tables 5 and/or 7 below (see in examples).

In another particular embodiment of the kit of the invention, it comprises means (i.e., 15 reagent means) for detecting and/or quantifying the level of expression of at least one quaternary set of the group consisting of: MDK, LCN2, PEA15, LDHA; MDK, LCN2, PEA15, PKM; MDK, FGFBP1, FCGBP, EPS8L1; MDK, LCN2, HSPE1, CD44; MDK, FGFBP1, EPS8L1, CTNNB1; MDK, LCN2, IMPA1, LDHA; MDK, IMPA1, FCGBP, PEA15; MDK, IMPA1, FCGBP, PKM; MDK, FCGBP, IMPA1, PEA15; MDK, CCT6A, FGFBP1, 20 EPS8L1; MDK, STX7, FCGBP, PKM; MDK, IMPA1, FCGBP, GYG1; MDK, FGFBP1, SCEL, EPS8L1; MDK, STX7, FCGBP, CA1; MDK, STX7, FCGBP, NAMPT; MDK, FGFBP1, EPS8L1, CAPS; MDK, STX7, FCGBP, FCGBP; MDK, SCEL, STX7, FCGBP; MDK, FGFBP1, ANXA7, CAPS; MDK, FGFBP1, EPS8L1, RAB21; MDK, SCEL, STX7, FCGBP; MDK, STX7, FCGBP, NEDD8-MDP1; MDK, ITIH2, FCGBP, CAPS; MDK, 25 FGFBP1, EPS8L1, PKM; MDK, IMPA1, FCGBP, HCLS1; MDK, APOF, ANXA3, FCGBP; MDK, FGFBP1, EPS8L1, STMN1; MDK, STX7, FCGBP, LGALS1; MDK, FCGBP, LSP1, VIM; MDK, FGFBP1, EPS8L1, LGALS1; MDK, ANXA3, FCGBP, LMNB1; MDK, FCGBP, IMPA1, MMP8; MDK, FGFBP1, EPS8L1, STMN1; MDK, SCEL, IMPA1, PEA15; MDK, FGFBP1, ANXA7, LGALS1; MDK, TCP1, PEA15, LMNB1; MDK, FCGBP, GYG1, CA1; 30 MDK, SCEL, IMPA1, PKM; MDK, FGFBP1, EPS8L1, LDHA; MDK, FCGBP, KRT8, GYG1; MDK, FCGBP, GYG1, LMNB1; MDK, FCGBP, GYG1, LSP1; MDK, FCGBP, KRT8, GYG1; MDK, FCGBP, GYG1, LGALS1; MDK, FCGBP, CAPS, GYG1; MDK, FGFBP1, EPS8L1, LDHA; MDK, FCGBP, CAPS, GYG1; MDK, C1R, FCGBP, LGALS1; MDK, FGFBP1, SCEL, PEA15; MDK, FGFBP1, TIMP2, CHMP4B; MDK, ANXA3, STMN1, PKM; 35 MDK, ANXA7, SCEL, CHMP4B; MDK, FCGBP, GYG1, LDHA; MDK, S100A9, MMP8, LGALS1; MDK, C1R, FCGBP, GYG1; MDK, C4BPA, FGFBP1, PPL; MDK, ITIH2, FGFBP1, PPL; and MDK, APOC1, FGFBP1, PPL.

In another particular embodiment of the first aspect, the method for the diagnosis and/or prognosis in which MDK levels are determined, in particular in a gynecological sample and/or cervical sample, is a method for the diagnosis of recurrence or risk of recurrence of endometrial cancer, which further comprises determining in the isolated sample of the female the presence and/or the level of expression of one or more of the proteins selected from the group consisting of: MUC1, PRSS8, PNP, APEH, MUC16, C9, SERPINC1, SERPINA1, F2, AMBP, HP, SERPINA3, CFB, ORM2, CAT, GNAI2, A1BG, FN1, C7, ASTRGL1, B4GALT1, CAPS, CBX3, CD163, CDV3, DMBT1, DSG3, EHD1, GOLM1, MUC5AC, NME1, NT5E, PDLIM5, RDX, and VASP.

10

MDK in combination with the one or more of the other listed in the previous paragraph are, in a particular embodiment determined in a cervical sample and/or in an uterine aspirate.

Thus, also disclosed is a method for the diagnosis of recurrence or risk of recurrence of endometrial cancer, in which one or more of the following proteins (i.e., the presence or absence and/or their levels) are determined in an isolated sample of the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, a pap-smear like sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof: MUC1, PRSS8, PNP, APEH, MUC16, C9, SERPINC1, SERPINA1, F2, AMBP, HP, SERPINA3, CFB, ORM2, CAT, GNAI2, A1BG, FN1, C7, ASTRGL1, B4GALT1, CAPS, CBX3, CD163, CDV3, DMBT1, DSG3, EHD1, GOLM1, , MUC5AC, NME1, NT5E, PDLIM5, RDX, and VASP.

As will be illustrated in the examples below, these markers gave a noteworthy accuracy (AUC > 0.75) to differentiate between recurrent and non-recurrent EC patients. In particular, the one or more markers selected from ASTRGL1, B4GALT1, CAPS, CBX3, CD163, CDV3, DMBT1, DSG3, EHD1, GOLM1, MUC16, MUC5AC, NME1, NT5E, PDLIM5, PRSS8, RDX, and VASP, allowed such a differentiation with only the presence-absence statistical analysis, in the isolated sample.

All the particular embodiments regarding, for example, the type of sample, or number of markers that are determined indicated for the method of the first aspect do also apply to either the method that allows the diagnosis of recurrence or risk of recurrence of endometrial cancer.

For "recurrence", also known as relapse or recidivism is a recurrence of a past (typically medical) condition. The "risk of recurrence" relates to the probability of such a recurrence.

Thus, in a particular embodiment of method for the diagnosis of recurrence or risk of recurrence of endometrial cancer, the method comprises the following steps:

- a) determining, *in vitro*, the level of expression of one or more of the listed proteins, in the isolated sample from the genital tract of the female;
- 5 b) comparing the level of these one or more other proteins of step (a) with a corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer resulting from a recidivism; and
- c) wherein the subject is diagnosed of recurrence of endometrial cancer if at least the
- 10 level of one of the proteins is within the value or interval of values from a subject suffering from a recurrence of this cancer.

In another particular embodiment of the first aspect, the method for the diagnosis and/or prognosis in which MDK levels are determined, in particular in a gynecological sample and/or cervical sample, is a method for the diagnosis of endometrial cancer subtype and/or endometrial cancer molecular classification, and which further comprises

15 determining in the isolated sample of the female the presence and/or the level of expression of one or more of the proteins selected from the group consisting of: LBP, VWF, GPLD1, SAA4, APOF, C4BPA, SPRR1A, SERPIND1, APOB, SCEL, LCAT,

20 SERPINA3, LMO7, C1R, MUC4, FN1, SPRR1B, C1QA, ITIH2, TIMP2, APOC1, GRN, ANXA3, S100A9, PLBD1, PIGR, SERPINH1, HSPE1.

MDK in combination with the one or more of the other listed in the previous paragraph are, in a particular embodiment determined in a cervical sample and/or in an uterine aspirate.

25

Thus, also disclosed herewith is a method for the diagnosis of endometrial endometrial cancer subtype and/or endometrial cancer molecular classification, in which one or more of the following proteins (i.e., the presence or absence and/or their levels) are determined in an isolated sample of the female genital tract part including one or more of the vulvae,

30 vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof: LBP, VWF, GPLD1, SAA4, APOF, C4BPA, SPRR1A, SERPIND1, APOB, SCEL, LCAT, SERPINA3, LMO7, C1R, MUC4, FN1, SPRR1B, C1QA, ITIH2, TIMP2, APOC1, GRN, ANXA3, S100A9,

35 PLBD1, PIGR, SERPINH1, HSPE1.

All the particular embodiments regarding, for example, the type of sample, or number of markers that are determined indicated for the method of the first aspect do also apply to

the method that allows the diagnosis of type of endometrial cancer in terms of molecular distinction between them.

5 In a particular example of the method for the diagnosis of endometrial cancer molecular subtype, the subtypes are selected from the group consisting of POLE ultramutated or POLE mutated (POLEmut), MSI: microsatellite instable (MSI) or MMR deficient, based on loss of mismatch repair protein expression (MMRd); Copy Number Low (CN-Low) or no specific molecular profile (NSMP); Copy Number High (CN-High) or p53 abnormal, based on mutant-like immunostaining (p53abn)

10

Data in examples illustrate the statistical significance between EC subtypes obtained with each of these markers analysed in a pap-smear like sample of patients (also called cervical sample, cervical fluid).

15 In a particular embodiment of method for the diagnosis of for the diagnosis of endometrial cancer subtype, the method comprises the following steps:

a) determining, *in vitro*, the level of expression of one or more of the listed proteins, in the isolated sample from the genital tract of the female;

b) comparing the level of these one or more other proteins of step (a) with a

20 corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from a particular endometrial cancer subtype; and

c) wherein the subject is diagnosed of said endometrial cancer subtype if at least the level of one of the proteins is within the value or interval of values from a subject suffering from

25 that endometrial cancer subtype.

In another particular embodiment of the kits of the invention, the means for detecting the level of expression of the proteins are means for carrying out an assay or technology selected from the group consisting of an immunoassay, a bioluminescence assay, a

30 fluorescence assay, a chemiluminescence assay, an electrochemistry assay, a lateral flow assay, mass spectrometry, and combinations thereof.

In even a more particular embodiment, the means for detecting the level of expression of the proteins in the kits are antibodies or fragments thereof.

35

In yet another more particular embodiment, the kits are for carrying out an enzyme-linked immunosorbent assay (ELISA).

In another particular embodiment of the kits of the invention, they further comprise a panel diagram, to categorize an individual sample.

5 In a preferred embodiment, the reagent means for assaying the levels of the different biomarkers (proteins) comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100% of the total amount of reagents for assaying biomarkers forming the kit. Thus, in the particular case of kits comprising reagents for assaying the levels of MDK (and optionally of one or more of the previous listed proteins), the reagents specific for said biomarkers (i.e.
10 antibodies which bind specifically to the proteins) comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100% of the antibodies present in the kit. These kits are, thus, simplified kits including mainly the reagent means for detecting the levels of the MDK (and optionally of one or more of the previous listed proteins).

15 In another particular embodiment, the kits of the invention are conceived as point of care tests. More in particular they are in form of lateral flow tests or an electrochemical sensor.

In another particular embodiment the kit according to the invention comprises a support
20 (in particular a solid support) and one or more sample inlet ports for deposition of a biofluid sample, in particular whole fluid of the cervix; a reaction area comprising the means /reagents that bind specifically to the marker proteins, in particular antibodies; and wherein the sample inlet port is connected with the reaction area. In another more particular embodiment, the kit comprises as many sample inlet ports as markers (one, two
25 or three or four) to be detected and corresponding reaction areas connected thereto. In another embodiment the kit comprises one single inlet import and as capillary tracks connecting to as many reactive areas, said capillary tracks conducting part of the sample to each corresponding connected reaction area. The kits comprising more than one reaction areas are multiplex kits.

30 In a more particular embodiment, the kits comprise means for detecting the level of expression of a combination from 2 to 80 sets.

35 In the particular case when the kits of the invention are ELISA kits, they comprise a solid support and antibodies or fragments thereof which specifically bind to the target proteins to be detected, these antibodies being conjugated with a reporter molecule capable of producing a signal.

The "solid support" includes a nitrocellulose membrane, glass or a polymer. The most

commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of strips, tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay.

5 The "reporter molecule" as used in the present specification is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e., radioisotopes). In the case of an
10 enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to those skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphatase, among others. The substrates to be
15 used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. For example, 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine, 5-aminosalicylic acid, 3,3',5,5'-tetra methyl benzidine or toluidine are commonly used. It is also
20 possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. Examples of fluorogenic substrates are fluorescein and rhodamine. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody absorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour
25 visually detectable with a light microscope. Immunofluorescence and EIA techniques are both well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent, and bioluminescent molecules and/or dyes and other chromogenic substances, may also be employed.

30

The choice of a particular reporter molecule conjugated antibody will be, for the most part, determined by the intended use and user of the test kit of the present invention.

Binding assays for measuring biomarker levels may use solid phase or homogenous formats. Suitable assay methods include sandwich or competitive binding assays.

35 Examples of sandwich immunoassays are described in U.S. Pat. No. 4,168,146 and U.S. Pat. No. 4,366,241, both of which are incorporated herein by reference in their entireties. Examples of competitive immunoassays include those disclosed in U.S. Pat. No.

4,235,601, U.S. Pat. No. 4,442,204 and U.S. Pat. No. 5,208,535, each of which are incorporated herein by reference in their entireties.

Multiple biomarkers may be measured using a multiplexed assay format, e.g., multiplexing through the use of binding reagent arrays, multiplexing using spectral discrimination of
5 labels, multiplexing of flow cytometric analysis of binding assays carried out on particles, e.g., using the Luminex® system.

The assays (methods and kits) of the present invention may be conducted by any suitable method. In one embodiment, biomarker levels are measured in a single sample, and those measurement may be conducted in a single assay chamber or assay device,
10 including but not limited to a single well of an assay plate, a single assay cartridge, a single lateral flow device, a single assay tube, etc. Biomarker levels may be measured using any of a number of techniques available to the person of ordinary skill in the art, e.g., direct physical measurements (e.g., mass spectrometry) or binding assays (e.g., immunoassays, agglutination assays and immunochromatographic assays). The method
15 may also comprise measuring a signal that results from a chemical reactions, e.g., a change in optical absorbance, a change in fluorescence, the generation of chemiluminescence or electrochemiluminescence, a change in reflectivity, refractive index or light scattering, the accumulation or release of detectable labels from the surface, the oxidation or reduction or redox species, an electrical current or potential, changes in
20 magnetic fields, etc. Suitable detection techniques may detect binding events by measuring the participation of labeled binding reagents through the measurement of the labels via their photoluminescence (e.g., via measurement of fluorescence, time-resolved fluorescence, evanescent wave fluorescence, up-converting phosphors, multi-photon fluorescence, etc.), chemiluminescence, electrochemiluminescence, light scattering,
25 optical absorbance, radioactivity, magnetic fields, enzymatic activity (e.g., by measuring enzyme activity through enzymatic reactions that cause changes in optical absorbance or fluorescence or cause the emission of chemiluminescence). Alternatively, detection techniques may be used that do not require the use of labels, e.g., techniques based on measuring mass (e.g., surface acoustic wave measurements), refractive index (e.g.,
30 surface plasmon resonance measurements), or the inherent luminescence of an analyte.

In another aspect, the invention relates to the use of the kit of the invention according to the third aspect and/or any one of its embodiments, for the diagnosis and/or prognosis of EC.

35

Thus, in a particular embodiment, the invention relates to the use of the kit of the invention in any of the methods of the invention.

In any of the embodiments provided above or below, for any of the aspects and embodiments of the invention, the level of expression of MDK and optionally of any other protein is determined at the protein level. In this embodiment, the protein marker(s)
5 include, but do not limit to, native-sequence peptides, isoforms, chimeric polypeptides, all homologs, fragments, and precursors of the markers, including modified forms of the polypeptides and derivatives thereof.

In particular embodiments provided above or below, the level of expression is determined
10 by immunochemistry, as indicated. Next paragraphs illustrate in more detail this option.

The term "immunochemistry" as used herein refers to a variety of techniques for detecting antigens (usually proteins and peptides, and in the present case any of the proteins listed above alone or in combination) in a sample by exploiting the principle of antibodies
15 binding specifically to said antigens. Visualizing an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, such as fluorescein or rhodamine. The immunochemistry technique can be direct or indirect. The
20 direct method is a one-step staining method and involves a labeled antibody (e.g. FITC-conjugated antiserum) reacting directly with the antigen. While this technique utilizes only one antibody and therefore is simple and rapid, the sensitivity is lower due to little signal amplification, such as with indirect methods, and is less commonly used than indirect methods. The indirect method involves an unlabeled primary antibody (first layer) that
25 binds to the target antigen in the sample and a labeled secondary antibody (second layer) that reacts with the primary antibody. This method is more sensitive than direct detection strategies because of signal amplification due to the binding of several secondary antibodies to each primary antibody if the secondary antibody is conjugated to the fluorescent or enzyme reporter.

30 Further amplification can be achieved if the secondary antibody is conjugated to several biotin molecules, which can recruit complexes of avidin-, streptavidin or Neutravidin-enzyme. The indirect method, aside from its greater sensitivity, also has the advantage that only a relatively small number of standard conjugated (labeled) secondary antibodies
35 needs to be generated. With the direct method, it would be necessary to label each primary antibody for every antigen of interest. It must be borne in mind that immunochemistry techniques can also be used to detect certain nucleic acid sequences if a tagged nucleic acid probe (designed to specifically bind to a certain target nucleic acid sequence) can later on be detected with a labelled antibody. Thus, the detection of the

protein could be performed by using a tagged nucleic acid designed to bind a specific sequence of the target protein RNA, and then detecting said tagged nucleic acid with a labelled antibody which selectively binds to the tag.

- 5 Immunoassay procedures suitable include enzyme-linked immunosorbent assays (ELISA, such as multiplex ELISA), enzyme immunodot assay, agglutination assay, antibody-antigen-antibody sandwich assay, antigen-antibody-antigen sandwich assay, immunocromatography, or other immunoassay formats well-known to the ordinarily skilled artisan, such as radioimmunoassay, chemoluminiscent assays, lateral flow assays or
10 electrochemical assay, as well as protein microarray formats.

Thus, in some embodiments of any of the aspects above or below, the level of expression of protein is determined by an immunoassay. In another embodiment, in combination with any of the embodiments provided above or below, the level of expression of protein is
15 determined by ELISA; more in particular a multiplex ELISA.

Alternatively, the level of expression of a protein can be determined by bioluminescence, fluorescence, chemiluminescence, electrochemistry, or mass spectrometry.

- 20 Alternatively, the level of expression of protein can be determined by measuring the levels of proteotypic peptides of the protein (peptides with an amino acid sequence uniquely associated with the studied protein in a given proteome) by mass spectrometry.

As previously indicated, in some embodiment of any of the aspects of the invention, in
25 combination with any of the embodiments provided above or below, the level of expression of protein is determined using an antibody or a fragment thereof able to bind to the target protein(s). These antibodies can be used as "means" for determining the expression of the target proteins in the kits of the invention. Next paragraphs illustrate in more detail this option.

30 The term "antibody or a fragment thereof able to bind to the target protein(s)" is to be understood as any immunoglobulin or fragment thereof able to selectively bind the target protein. It includes monoclonal and polyclonal antibodies. The term "fragment thereof encompasses any part of an antibody having the size and conformation suitable to bind
35 an epitope of the target protein. Suitable fragments include F(ab), F(ab') and Fv. An "epitope" is the part of the antigen being recognized by the immune system (B-cells, T-cells or antibodies).

The antibodies used for specific detection can be polyclonal or monoclonal. There are well

known means in the state of the art for preparing and characterizing antibodies. Methods for generating polyclonal antibodies are well known in the prior art. Briefly, one prepares polyclonal antibodies by immunizing an animal with the protein; then, serum from the immunized animal is collected and the antibodies isolated. A wide range of animal species
5 can be used for the production of the antiserum. Typically, the animal used for production of antisera can be a rabbit, mouse, rat, hamster, guinea pig or goat.

Moreover, monoclonal antibodies (MAbs) can be prepared using well-known techniques. Typically, the procedure involves immunizing a suitable animal with the protein associated
10 with the disease. The immunizing composition can be administered in an amount effective to stimulate antibody producing cells. Methods for preparing monoclonal antibodies are initiated generally following the same lines as the polyclonal antibody preparation. The immunogen is injected into animals as antigen. The antigen may be mixed with adjuvants such as complete or incomplete Freund's adjuvant. At intervals of two weeks,
15 approximately, the immunization is repeated with the same antigen.

The antibody or fragment thereof for detecting the target protein(s) (i.e., MDK and optionally one or more of the proteins listed) can be included in a kit. The kit may additionally comprise other means (additives, solvents) to visualize the antibody-protein
20 interactions.

Alternatively, the level of expression of MDK and if determined of one or more of the above-listed proteins, is determined at the mRNA level. Next paragraphs illustrate in more detail this option.

25 Thus, in another embodiment of the kits or their use, the kit is a microarray.

In another embodiment, the kit is a microarray including a defined set of genes encoding protein endometrial cancer markers. All the embodiments provided above for particular
30 proteins to be analyzed (from two to eleven of the list), whose expression is significantly altered by endometrial disease, are also particular embodiments of microarrays.

Additionally, the kits of the invention comprise reagents for detecting a protein encoded by a constitutive gene. The availability of said additional reagents allows normalizing the
35 measurements performed in different samples (for example, the sample to be analysed and the control sample) to rule out that the differences in the expression of the biomarkers are due to a different quantity of total protein amount in the sample more than the real differences in the relative levels of expression. The constitutive genes in the present invention are genes that are always active or being transcribed constantly and which

encode for proteins that are expressed constitutively and carry out essential cellular functions. Proteins that are expressed constitutively and can be used in the present invention include, without limitation, β -2-microglobulin (B2M), ubiquitin, 18-S ribosomal protein, cyclophilin, GAPDH, PSMB4, tubulin and actin.

5

In one embodiment, the amount of mRNA of each one of the markers are detected via polymerase chain reaction using, for example, oligonucleotide primers that hybridize to one or more polynucleotide endometrial cancer markers or complements of such polynucleotides. Within other embodiments, the amount of mRNA is detected using a
10 hybridization technique, employing oligonucleotide probes that hybridize to one or more polynucleotide endometrial cancer markers or complements of such polynucleotides.

When using mRNA detection, the methods of the invention may be carried out by combining isolated mRNA with reagents to convert to cDNA according to standard
15 methods well known in the art, treating the converted cDNA with amplification reaction reagents (such as cDNA PCR reaction reagents) in a container along with an appropriate mixture of nucleic acid primers; reacting the contents of the container to produce amplification products; and analyzing the amplification products to detect the presence of one or more of the polynucleotide endometrial cancer markers in the sample. For mRNA,
20 the analyzing step may be accomplished using Northern Blot analysis to detect the presence of polynucleotide endometrial cancer markers in the sample. The analysis step may be further accomplished by quantitatively detecting the presence of polynucleotide endometrial cancer markers in the amplification product, and comparing the quantity of marker detected against a panel of expected values for the known presence or absence of
25 such markers in normal and malignant tissue derived using similar primers.

In another embodiment, the invention provides a method wherein mRNA is detected by:
(a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction reagents and nucleic
30 acid primers that hybridize to one or more of the polynucleotide endometrial cancer markers endometrial cancer marker to produce amplification products; (c) analyzing the amplification products for determining the amount of mRNA present encoding the protein endometrial cancer marker; and (d) comparing the determined amount of mRNA to an amount detected against a panel of expected values for normal and diseased tissue (e.g. ,
35 malignant tissue) derived using similar methods.

In particular embodiments of the invention, RT-PCR can be used to amplify the mRNA for protein endometrial cancer markers for detection and analysis. Other embodiments of the invention use quantitative RT-PCR to quantitatively determine amount of mRNA for

protein endometrial cancer markers. Further embodiments of the invention use real time RT-PCR for quantification and analysis.

5 UniprotKB (<https://www.uniprot.org/help/uniprotkb>) database accession numbers of all the herewith listed proteins or in the corresponding Tables of this description correspond to versions (database release) accessible on July 23, 2021.

10 AGRIN has the Uniprot database accession number O00468. This protein is a heparin sulfate basal lamina glycoprotein involved in the formation and the maintenance of the neuromuscular junction.

PIGR, also known as polymeric immunoglobulin receptor, has the Uniprot database accession number P01833, June 26, 2007 - v4. This receptor binds polymeric IgA and IgM at the basolateral surface of epithelial cells.

15 CD44, also known as CD44 antigen, has the Uniprot database accession number P16070, October 5, 2010 - v3. Mediates cell-cell and cell-matrix interactions through its affinity for HA, and possibly also through its affinity for other ligands such as osteopontin, collagens, and matrix metalloproteinases (MMPs).

20 LDHA, also known as L-lactate dehydrogenase A chain, has the Uniprot database accession number P00338, January 23, 2007 - v2. This protein is involved in step 1 of the subpathway that synthesizes (S)-lactate from pyruvate.

25 Some of these proteins have been related with EC grading/diagnosis in tissue samples. However, no meaningful data are correlated with values in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings
30 and combinations thereof. As above exposed, detection of markers in these kinds of samples imply the advantage of collecting data from routine sampling in clinical practice of the gynecological diseases, avoiding costs and importantly, invasive tissue biopsies.

A further aspect of the present invention was a method of deciding or recommending
35 whether to initiate a medical regimen of a subject suffering endometrial cancer, in particular in function of the prognosis. In a particular embodiment of this method, it comprises

a) determining, *in vitro*, the presence and/or the level of expression of midkine (MDK) in a

- sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof; and
- 5 b) comparing the level of MDK of step (a) with a corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer, and/or comparing with a cut-off value discriminating among endometrial cancer and other gynecological disorders or conditions, and
- 10 c) wherein the subject is diagnosed of endometrial cancer if the level of MDK is within the value or interval of values from a subject suffering from this cancer, and/or if the level of MDK in relation to the cut-off value is classified to the endometrial cancer group, in such a way that:
- 15 i) if the subject is diagnosed of suffering from endometrial carcinoma, or of being suspicious of suffering from endometrial carcinoma, then the initiation of the medical regimen is recommended, which consists of total hysterectomy and bilateral salpingo-oophorectomy, optionally complemented with pelvic and para-aortic lymphadenectomy, and omentectomy;
- 20 ii) if the patient is diagnosed of not suffering from endometrial carcinoma, the follow-up is performed optionally in consideration of the result of an examination of the patient by a physician.
- 25 In another particular embodiment method of deciding or recommending whether to initiate a medical regimen of a subject suffering endometrial cancer, usually in function of the established prognosis, an adjuvant treatment is recommended selected from radiotherapy, brachytherapy, hormone therapy, chemotherapy, targeted therapies and combinations thereof.
- 30 This method of deciding or recommending whether to initiate a medical regimen of a subject suffering endometrial cancer is also applicable to the previously disclosed methods for the diagnosis of recurrence or risk of recurrence of endometrial cancer; and/or for the diagnosis of endometrial cancer subtype and/or endometrial cancer
- 35 molecular classification.

Thus, herewith disclosed is also a method for the treatment of EC in a subject suffering from this disease, the method comprising carrying out the method of the diagnosis and/or prognosis of the first aspect or of any one of its embodiments; and further comprising the

step of treating the subject by means of total hysterectomy and/or bilateral salpingo-oophorectomy, optionally complemented with pelvic and para-aortic lymphadenectomy, and/or omentectomy. In a particular embodiment of the proposed method of treatment, the subject also receives an adjuvant treatment is recommended selected from radiotherapy, brachytherapy, hormone therapy, chemotherapy, targeted therapies and combinations thereof.

Also disclosed is, moreover, a method of detecting, in an isolated sample of a subject, the level of MDK, and/or optionally one or more of the protein listed in Table 1, the method comprising:

- (a) obtaining a sample from the subject, said sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, an uterine fluid, uterine washings and combinations thereof; and
- (b) detecting the presence and/or the level of expression of midkine (MDK) in the isolated sample by: (i) contacting said sample with means capable of binding the corresponding protein and detecting said binding; or (ii) contacting said sample with means capable of binding corresponding RNA going to be translated to the said protein and detecting said binding.

Another aspect of present invention is to provide an algorithm for carrying out any of the methods of diagnosis and/or of prognosis as defined in the above aspects and embodiments, or for carrying out any of the methods disclosed above for the diagnosis of recurrence or risk of recurrence of endometrial cancer and/or endometrial cancer subtype and/or endometrial cancer molecular classification.

As indicated, this fourth aspect relates to computer-implemented methods for carrying out the method as defined in the first aspect, in which after the determination of the level of expression of MDK, and optionally of the one or more of the proteins for the diagnosis and/or for the prognosis of endometrial cancer, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among EC presenting different prognosis, including different histological subtypes and grades and different molecular features.

In a particular embodiment the algorithm is a computer-implemented method for diagnosing EC and/or for prognosing EC, in particular for the prognosis of the disease by

determining EC subtype, in particular EEC or NEEC. This algorithm allows taking the decision of a sample being from a subject suffering from EC or not; and also if a sample being from a subject suffering from EC is suffering from EEC or from NEEC. In a more particular embodiment, the algorithm provides with recommended treatment. Therefore, there is also provided a computer-implemented method for carrying out the method as defined above, in which after the determination of the level of expression of one or more of the proteins for the diagnosis and/or for the prognosis of EC, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC subtypes.

In other words, in another particular embodiment of the algorithm of the invention, after the determination of the level of expression of one or more of the proteins for the diagnosis and/or for the prognosis of EC, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC subtypes.

In a particular embodiment the algorithm is a computer-implemented method for diagnosing EC and/or for prognosing EC, in particular for the prognosis of the disease by determining EC grading, in particular low or high grade EC, according to known gradation or staging systems, such as the one of FIGO. In another particular embodiment the algorithm is a computer-implemented method for diagnosing EC and/or for prognosing EC, in particular for the prognosis of the disease by determining the EC histological subtype, such as endometrioid vs non-endometrioid endometrial cancer. This algorithm allows taking the decision of a sample being from a subject suffering from EC or not; and also if a sample being from a subject suffering from EC is suffering from low or from high grade EC. In a more particular embodiment, the algorithm provides with recommended treatment. Therefore, there is also provided a computer-implemented method for carrying out the method as defined above, in which after the determination of the level of expression of one or more of the proteins for the diagnosis and/or for the prognosis of EC, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC grades.

In other words, in another particular embodiment of the algorithm of the invention, after the determination of the level of expression of one or more of the proteins for the diagnosis and/or for the prognosis of EC, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value;
5 wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC grades.

The *in vitro* methods of the invention provide diagnostic and/or prognostic information. In
10 one embodiment, the methods of the invention further comprise the steps of (i) collecting the diagnostic and/or prognostic information, and (ii) saving the information in a data carrier.

In the sense of the invention a "data carrier" is to be understood as any means that
15 contain meaningful information data for the diagnosis and/or prognosis of endometrial carcinoma, such as paper. The carrier may also be any entity or device capable of carrying the prognosis data. For example, the carrier may comprise a storage medium, such as a ROM, for example a CD ROM or a semiconductor ROM, or a magnetic recording medium, for example a floppy disc or hard disk. Further, the carrier may be a
20 transmissible carrier such as an electrical or optical signal, which may be conveyed via electrical or optical cable or by radio or other means. When the diagnosis/prognosis data are embodied in a signal that may be conveyed directly by a cable or other device or means, the carrier may be constituted by such cable or other device or means. Other carriers relate to USB devices and computer archives. Examples of suitable data carrier
25 are paper, CDs, USB, computer archives in PCs, or sound registration with the same information.

Throughout the description and claims the word "comprise" and variations of the word, are not intended to exclude other technical features, additives, components, or steps.
30 Furthermore, the word "comprise" encompasses the case of "consisting of". Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible
35 combinations of particular and preferred embodiments described herein.

Examples

Example 1. Discovery cohort. Validation cohort and analysis of the information of the markers. Informative data from analysis of MDK levels in cervical fluid samples (routine non-invasive gynecological sampling)

5 **Materials and Methods:** The discovery phase to identify potential biomarkers using the fluid contained in pap-smears, i.e. cervical fluid samples was performed using a shotgun label-free proteomic approach. The study included 60 patients (20 EC patients, 20 controls suffering AVB without endometrial cancer or cervical pathology, and 20 controls without endometrial pathology but cervical pathology). The data was analyzed using
10 MaxQuant and R software. The levels of 110 peptides corresponding to 75 proteins identified in the discovery phase, were further measured in a verification phase of 242 (106 non-EC, 129 EC, 7 premalignancies of EC) patients by mass spectrometry (LC-PRM). Analysis was performed using Skyline software, SPSS and R software. The clinicopathological features of the patients included in the verification study are shown in
15 Table 2.

Results: The discovery study permitted to determine a total number of 2,888 proteins identified with more than a single peptide in our samples. Statistical analysis permitted to identify 75 potential proteins differently expressed between EC and non-EC patients to be
20 further assessed and verified. Verification phase revealed the great potential of 60 of those proteins measured in the cervical samples to reach a non-invasive diagnosis of EC comparing EC vs non-EC patients (adj.p.value < 0.05, Fold-Change >2, AUC > 0.7) (Table 3). Specifically, 19 proteins achieved an AUC > 0.75, and 7 proteins an AUC > 0.8. Additionally, an ELISA assay of the best performing protein was tested in all the samples
25 reproducing the results obtained by mass-spectrometry and reaching an AUC=0.91 in this dataset to diagnose EC (comparing 127 EC vs 106 non-EC patients) (Figure 1). The verification study allowed to identify a set of significant proteins as biomarkers of prognosis to differentiate between histological subtypes and grades of EC patients. Specifically, a set 34 protein biomarkers had a significant adjusted p-value (Table 3, in
30 bold) and 39 proteins a fold-change higher than 1.3 (Table 3, shadowed) when comparing the 35 low grade (G1) vs. the 37 high grade (G3) EC patients. A comparison between low and intermediate grades, G1 and G2, against high grade (G3) was also performed. Significant and highly-expressed proteins are illustrated in Table 3. Regarding histological subtype, a set of of 33 proteins were differentially expressed (fold-change higher than 1.3)
35 and 31 proteins were identified as significant (adj. p-value <0.05) when comparing 102 endometrioid endometrial cancer (EEC) against 25 non-endometrioid endometrial cancer patients. These results are all shown in Table 3. Tables 8 and 9 illustrate also the 2 or 3 protein panels that in combination allowed to differentiate between histological subtypes and grades of EC patients.

Next Table 3 shows AUC, SE and SP of MDK and other markers. Note that MDK provided the best values.

- 5 Table 2. Clinicopathological features of the patients included in the verification phase.

	EC (n=129)	non-EC (n=106)	Hyperplasia (n=7)
Age (years)			
Mean	64	64	50
Minimum	32	31	44
Maximum	92	95	55
Uterine condition			
Premenopausal	29	13	5
Postmenopausal	100	90	2
Histological type			
Endometrioid	103		
Serous	9		
Others (carcinosarcoma)	16		
Histological grade			
Grade 1	36		
Grade 2	56		
Grade 3	37		
FIGO stage			
IA	62		
IB	19		
II	26		
IIIA	4		
IIIB	2		
IIIC1	2		
IIIC2	7		
IVA	2		
IVB	5		
Miometrial invasion			
<50%	80		
>50%	49		
Lymphovascular invasion			
Yes	35		
No	84		

Table 3. 1-protein biomarker detection for EC diagnosis and prognosis, including the differentiation between histological grades and subtypes of ECs. Significant proteins with adjusted p-value <0.05 are in bold, and significant proteins with fold-change (FC) higher than 1.3 are shadowed in grey color.

	Diagnostic biomarkers (EC vs non-EC)			Prognostic biomarkers Histological grade (Low G1 vs High G3 grade)			Prognostic biomarkers (G1G2 vs High G3 grade)			Prognostic biomarkers Histological subtype (Endometrioid vs Non-Endometrioid ECs)		
	FC	adj.p	AUC	FC	adj.p	AUC	FC	adj.p	AUC	FC	adj.p	AUC
A2ML1	0.42	0.00	0.63	0.69	0.06	0.59	0.89	0.84	0.51	0.84	0.74	0.52
AGRN	2.32	0.00	0.72	2.90	0.01	0.65	1.67	0.11	0.59	1.75	0.06	0.61
ANXA3	0.55	0.00	0.69	0.61	0.03	0.67	0.69	0.06	0.64	0.73	0.35	0.58
ANXA7	0.52	0.00	0.72	1.68	0.12	0.62	1.49	0.23	0.59	1.73	0.15	0.61
APOB	3.02	0.00	0.81	2.25	0.00	0.76	1.49	0.03	0.66	1.69	0.03	0.68
APOC1	1.96	0.00	0.77	1.49	0.03	0.67	1.22	0.25	0.59	1.21	0.26	0.59
APOF	2.20	0.00	0.77	2.08	0.00	0.74	1.40	0.02	0.67	1.76	0.02	0.70
APP	2.85	0.00	0.79	1.68	0.08	0.64	0.54	0.34	0.56	0.99	0.40	0.57
ATP6V1G1	0.94	0.33	0.54	1.32	0.41	0.57	1.30	0.45	0.55	1.51	0.15	0.61
BTD	1.45	0.00	0.71	1.06	0.89	0.51	0.98	0.88	0.51	1.08	0.73	0.53
C1QA	2.15	0.00	0.81	1.57	0.00	0.75	1.18	0.09	0.62	1.33	0.10	0.62
C1R	1.89	0.00	0.75	1.62	0.00	0.76	1.20	0.08	0.63	1.41	0.05	0.65
C4BPA	2.14	0.00	0.75	2.36	0.00	0.79	1.52	0.03	0.66	1.78	0.02	0.69
CA1	0.89	0.00	0.61	1.39	0.35	0.58	1.02	0.51	0.55	1.63	0.38	0.57
CALU	1.10	0.01	0.60	1.50	0.01	0.70	1.16	0.25	0.58	1.31	0.04	0.66
CAMP	0.85	0.62	0.52	0.58	0.08	0.64	0.76	0.16	0.60	0.95	0.82	0.52
CAPS	1.49	0.00	0.62	0.53	0.73	0.53	0.57	0.19	0.60	0.69	0.22	0.60
CCT6A	1.94	0.00	0.76	1.55	0.06	0.65	1.23	0.33	0.58	1.67	0.05	0.65
CD44	0.99	0.30	0.54	1.30	0.36	0.58	1.19	0.84	0.52	1.02	0.75	0.53
CHMP4B	0.69	0.01	0.61	1.67	0.12	0.62	1.57	0.16	0.60	1.65	0.22	0.60
COL12A1	3.93	0.00	0.75	1.02	0.40	0.57	0.52	0.72	0.53	0.52	0.60	0.54
COL1A1	4.30	0.00	0.83	1.37	0.41	0.56	1.31	0.36	0.57	1.35	0.41	0.57
COL3A1	2.23	0.00	0.69	1.25	0.36	0.57	1.46	0.14	0.60	1.63	0.24	0.59
CSE1L	1.71	0.00	0.65	2.16	0.01	0.70	1.75	0.06	0.63	2.25	0.00	0.74
CTNNB1	1.11	0.01	0.60	1.60	0.08	0.64	1.13	0.65	0.53	1.28	0.22	0.59
EPS8L1	0.58	0.00	0.65	1.12	0.95	0.51	1.09	0.82	0.52	1.09	0.88	0.51
FAM107B	0.59	0.00	0.68	0.79	0.36	0.57	0.84	0.41	0.56	0.86	0.53	0.55
FCGBP	0.34	0.00	0.69	0.36	0.00	0.78	0.47	0.01	0.70	0.44	0.02	0.68
FGFBP1	2.48	0.00	0.78	1.11	0.75	0.53	0.85	0.41	0.56	1.01	0.60	0.54
FN1	2.34	0.00	0.81	1.86	0.00	0.75	1.32	0.05	0.65	1.24	0.07	0.64
GPLD1	1.63	0.00	0.71	1.58	0.00	0.73	1.27	0.05	0.64	1.45	0.04	0.66
GRN	0.51	0.00	0.67	0.69	0.03	0.67	0.85	0.13	0.61	0.91	0.31	0.58
GYG1	0.68	0.02	0.59	0.84	0.89	0.51	0.94	0.93	0.51	1.06	0.53	0.55
HCLS1	0.74	0.09	0.57	0.73	0.44	0.56	0.80	0.40	0.56	0.93	0.93	0.51
HSPE1	0.95	0.30	0.54	2.15	0.00	0.83	1.44	0.01	0.70	1.33	0.02	0.69
IGFALS	1.68	0.00	0.71	1.30	0.08	0.64	1.10	0.41	0.56	1.20	0.35	0.58
IMPA1	0.70	0.00	0.66	0.99	0.90	0.51	0.99	0.89	0.51	1.10	0.42	0.56

	Diagnostic biomarkers (EC vs non-EC)			Prognostic biomarkers Histological grade (Low G1 vs High G3 grade)			Prognostic biomarkers (G1G2 vs High G3 grade)			Prognostic biomarkers Histological subtype (Endometrioid vs Non-Endometrioid ECs)		
	FC	adj.p	AUC	FC	adj.p	AUC	FC	adj.p	AUC	FC	adj.p	AUC
ITGB2	0.82	0.48	0.53	0.79	0.39	0.57	0.89	0.51	0.55	1.08	0.74	0.53
ITIH2	1.88	0.00	0.76	1.43	0.02	0.69	1.17	0.14	0.61	1.28	0.11	0.62
KRT8	1.07	0.00	0.61	2.15	0.40	0.57	1.33	0.81	0.52	0.88	0.98	0.50
LBP	2.58	0.00	0.76	2.28	0.02	0.68	1.80	0.03	0.66	2.69	0.02	0.71
LCAT	1.89	0.00	0.75	1.71	0.00	0.74	1.26	0.05	0.65	1.48	0.03	0.67
LCN2	0.48	0.00	0.75	0.50	0.00	0.77	0.57	0.01	0.70	0.53	0.02	0.68
LDHA	0.96	0.58	0.52	1.32	0.18	0.61	1.17	0.45	0.56	1.43	0.02	0.69
LGALS1	1.14	0.03	0.59	1.65	0.05	0.66	1.40	0.25	0.59	1.74	0.02	0.69
LMNB1	0.86	0.62	0.52	1.18	0.08	0.64	1.08	0.34	0.57	1.22	0.09	0.63
LMO7	0.34	0.00	0.73	0.70	0.17	0.61	0.77	0.34	0.57	0.53	0.05	0.65
LSP1	0.79	0.21	0.55	0.97	0.70	0.53	0.97	0.99	0.50	1.11	0.56	0.55
MDK	5.54	0.00	0.91	1.81	0.14	0.61	1.45	0.41	0.56	0.69	0.53	0.55
MMP8	0.74	0.30	0.54	0.88	0.18	0.61	1.02	0.32	0.58	1.21	0.82	0.52
MUC4	0.39	0.00	0.75	0.41	0.01	0.70	0.47	0.01	0.70	0.55	0.03	0.67
NAMPT	0.92	0.90	0.51	1.39	0.07	0.64	1.22	0.36	0.57	1.57	0.03	0.67
NEDD8-MDP1	0.71	0.00	0.66	0.99	0.56	0.55	1.03	0.84	0.52	1.21	0.49	0.56
PEA15	0.66	0.01	0.61	1.36	0.34	0.58	1.19	0.59	0.54	1.37	0.26	0.59
PIGR	1.11	0.30	0.54	0.17	0.00	0.91	0.23	0.00	0.83	0.20	0.00	0.85
PKM	0.85	0.07	0.57	3.89	0.00	0.78	2.45	0.01	0.71	1.79	0.00	0.75
PLBD1	0.49	0.00	0.68	0.72	0.09	0.63	0.80	0.18	0.60	0.85	0.53	0.55
PPL	0.39	0.00	0.72	0.79	0.21	0.60	0.84	0.40	0.56	0.71	0.10	0.62
RAB21	0.82	0.10	0.57	1.27	0.18	0.61	1.26	0.26	0.58	1.50	0.03	0.67
S100A9	0.32	0.00	0.73	0.71	0.03	0.67	0.93	0.30	0.58	0.53	0.37	0.57
SAA2-SAA4;SAA4	2.15	0.00	0.76	1.98	0.00	0.74	1.55	0.02	0.67	1.94	0.02	0.70
SCEL	0.34	0.00	0.71	0.56	0.01	0.71	0.65	0.16	0.60	0.37	0.03	0.68
SERPINA3	1.87	0.00	0.69	1.61	0.03	0.67	1.29	0.07	0.63	1.75	0.04	0.66
SERPIND1	2.51	0.00	0.80	1.82	0.01	0.72	1.30	0.06	0.64	1.60	0.02	0.68
SERPINH1	2.55	0.00	0.72	2.63	0.00	0.76	2.14	0.01	0.71	2.62	0.00	0.80
SPP1	3.49	0.00	0.79	1.34	0.39	0.57	1.28	0.41	0.56	1.39	0.42	0.56
SPRR1A	0.32	0.00	0.73	0.34	0.01	0.71	0.46	0.03	0.66	0.25	0.02	0.68
SPRR1B	0.30	0.00	0.74	0.41	0.01	0.71	0.55	0.11	0.62	0.27	0.07	0.64
STMN1	1.04	0.72	0.51	1.91	0.06	0.65	1.69	0.13	0.61	2.06	0.02	0.68
STX7	0.52	0.00	0.68	0.78	0.40	0.57	0.86	0.48	0.55	0.89	0.86	0.51
TCP1	2.26	0.00	0.78	1.46	0.12	0.62	1.21	0.41	0.56	1.51	0.05	0.65
TIMP2	0.59	0.00	0.72	0.53	0.00	0.74	0.70	0.11	0.62	0.67	0.10	0.62
TMPRSS11E	0.52	0.00	0.70	0.29	0.00	0.75	0.34	0.06	0.63	0.12	0.02	0.69

	Diagnostic biomarkers (EC vs non-EC)			Prognostic biomarkers Histological grade (Low G1 vs High G3 grade)			Prognostic biomarkers (G1G2 vs High G3 grade)			Prognostic biomarkers Histological subtype (Endometrioid vs Non-Endometrioid ECs)		
	FC	adj.p	AUC	FC	adj.p	AUC	FC	adj.p	AUC	FC	adj.p	AUC
VIM	1.69	0.00	0.64	1.08	0.08	0.64	1.01	0.57	0.54	1.28	0.11	0.62
VWF	3.38	0.00	0.82	2.41	0.00	0.81	1.52	0.01	0.70	1.78	0.02	0.71

AUC: Area Under the Curve; FC: Fold Change; adj.p.: adjusted p-value; EC: endometrial cancer; G: Grade

When MDK was combined in a two-pannel and three-pannel of protein biomarkers, the AUC value of the combinations was increased in comparison to MDK alone, mainly by increasing the sensitivity to diagnose EC. Data are illustrated in next Tables 4 and 5. As well, proteins

5 providing significant information on prognosis were combined in Tables 6 and 7. As indicated before, the sensitivity is of importance considering the symptomatology of the disease, common with other disorders of the female genital tract.

Table 4. 2-protein biomarker detection for EC diagnosis

Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC
MDK	LCN2	0.94	MDK	TMPRSS11E	0.92	MDK	TCP1	0.91
MDK	MUC4	0.94	MDK	LMNB1	0.92	MDK	CTNNB1	0.91
MDK	MUC4	0.94	MDK	LMNB1	0.92	MDK	CAPS	0.91
MDK	LCN2	0.94	MDK	SPRR1A	0.92	COL1A1	TCP1	0.91
MDK	APOB	0.94	MDK	PEA15	0.92	COL1A1	CCT6A	0.90
MDK	APOB	0.94	MDK	VIM	0.92	TCP1	ANXA7	0.90
MDK	FN1	0.94	MDK	GYG1	0.92	CCT6A	ANXA7	0.90
MDK	VWF	0.94	MDK	IGFALS	0.92	COL1A1	TCP1	0.90
MDK	APOF	0.93	MDK	MMP8	0.92	COL1A1	CCT6A	0.89
MDK	PPL	0.93	MDK	ITGB2	0.92	CCT6A	ANXA7	0.89
MDK	SAA4	0.93	MDK	RAB21	0.92	APP	FGFBP1	0.89
MDK	VWF	0.93	MDK	CAMP	0.92	VWF	FGFBP1	0.89
MDK	FCGBP	0.93	MDK	FGFBP1	0.91	TCP1	ANXA7	0.89
MDK	LMO7	0.93	MDK	LSP1	0.91	COL1A1	FGFBP1	0.89
MDK	SERPIND1	0.93	MDK	PKM	0.91	APP	LCN2	0.89
MDK	STX7	0.93	MDK	COL12A1	0.91	APOB	APP	0.89
MDK	FCGBP	0.93	MDK	APP	0.91	C1QA	SPP1	0.89
MDK	ANXA7	0.93	MDK	CHMP4B	0.91	COL1A1	FGFBP1	0.89
MDK	ITIH2	0.93	MDK	HSPE1	0.91	APOB	SPP1	0.89

Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC
MDK	C1QA	0.93	MDK	BTD	0.91	TCP1	PEA15	0.88
MDK	ITIH2	0.93	MDK	A2ML1	0.91	APOB	SPP1	0.88
MDK	S100A9	0.93	MDK	SERPINA3	0.91	CCT6A	PEA15	0.88
MDK	LBP	0.93	MDK	CA1	0.91	VWF	FGFBP1	0.88
MDK	APOF	0.93	MDK	TMPRSS11E	0.91	VWF	APP	0.88
MDK	ANXA3	0.93	MDK	NAMPT	0.91	SERPIND1	APP	0.88
MDK	IMPA1	0.93	MDK	CALU	0.91	APP	LCN2	0.88
MDK	TIMP2	0.93	MDK	FGFBP1	0.91	C1QA	SPP1	0.88
MDK	C4BPA	0.93	MDK	SERPINH1	0.91	COL1A1	CSE1L	0.88
MDK	APOC1	0.93	MDK	MDK	0.91	FN1	FGFBP1	0.88
MDK	SERPIND1	0.93	MDK	COL3A1	0.91	APOB	APP	0.88
MDK	LCAT	0.93	MDK	LDHA	0.91	COL1A1	C1QA	0.88
MDK	SCEL	0.93	MDK	STMN1	0.91	SERPIND1	APP	0.88
MDK	C4BPA	0.92	MDK	HSPE1	0.91	C1QA	APP	0.88
MDK	EPS8L1	0.92	MDK	LGALS1	0.91	APP	TCP1	0.88
MDK	CD44	0.92	MDK	CCT6A	0.91	COL1A1	APOB	0.88
MDK	PLBD1	0.92	MDK	AGRN	0.91	APOB	FGFBP1	0.88
MDK	LCAT	0.92	MDK	SERPINH1	0.91	APP	MUC4	0.88
MDK	APP	0.92	MDK	LDHA	0.91	FN1	FGFBP1	0.88
MDK	FAM107B	0.92	MDK	CCT6A	0.91	COL1A1	SERPINH1	0.88
MDK	TIMP2	0.92	MDK	TCP1	0.91	APOB	SPP1	0.88
MDK	CD44	0.92	MDK	CALU	0.91	APP	FGFBP1	0.88
MDK	SPRR1B	0.92	MDK	AGRN	0.91	COL1A1	APOB	0.88
MDK	GRN	0.92	MDK	KRT8	0.91	VWF	TCP1	0.87
MDK	COL1A1	0.92	MDK	STMN1	0.91	FN1	FGFBP1	0.87
MDK	PIGR	0.92	MDK	CSE1L	0.91	APP	APOF	0.87
MDK	NEDD8-MDP1	0.92	MDK	PKM	0.91	COL1A1	TIMP2	0.87
MDK	GPLD1	0.92	MDK	VIM	0.91	APOB	SPP1	0.87
MDK	SPRR1B	0.92	MDK	LGALS1	0.91	VWF	CCT6A	0.87
MDK	SPP1	0.92	MDK	ATP6V1G1	0.91	FN1	TCP1	0.87
MDK	PIGR	0.92	MDK	CAPS	0.91	MUC4	AGRN	0.87
MDK	HCLS1	0.92	MDK	KRT8	0.91	VWF	SPP1	0.87
MDK	SPP1	0.92	MDK	CSE1L	0.91	FN1	APP	0.87
MDK	C1R	0.92	MDK	TCP1	0.91	FN1	SPP1	0.87

Prot.: Protein, AUC: Area Under the Curve

Table 5. 3-protein biomarker detection for EC diagnosis

Prot 1	Prot 2	Prot 3	AUC	Prot 1	Prot 2	Prot 3	AUC
MDK	FN1	FCGBP	0.953	MDK	FN1	LMNB1	0.945
MDK	FN1	LCN2	0.952	MDK	FN1	LMO7	0.945
MDK	APOF	MUC4	0.952	MDK	APOC1	MUC4	0.945

Prot 1	Prot 2	Prot 3	AUC	Prot 1	Prot 2	Prot 3	AUC
MDK	FN1	FCGBP	0.952	MDK	SAA4	MUC4	0.945
MDK	FN1	MUC4	0.951	MDK	ITIH2	MUC4	0.945
MDK	ANXA7	CSE1L	0.951	MDK	ANXA7	SERPINH1	0.945
MDK	FN1	LCN2	0.951	MDK	ANXA7	FCGBP	0.945
MDK	FN1	MUC4	0.950	MDK	ITIH2	MUC4	0.945
MDK	APOB	MUC4	0.950	MDK	MUC4	CTNNB1	0.945
MDK	APOB	MUC4	0.950	MDK	COL1A1	FCGBP	0.944
MDK	FN1	LCN2	0.950	MDK	FN1	GRN	0.944
MDK	APOB	MUC4	0.950	MDK	APOF	LCN2	0.944
MDK	FN1	FCGBP	0.949	MDK	FN1	ANXA7	0.944
MDK	FN1	MUC4	0.949	MDK	FN1	CD44	0.944
MDK	FN1	FCGBP	0.949	MDK	LMO7	FCGBP	0.944
MDK	VWF	FCGBP	0.949	MDK	MUC4	TMPRSS11E	0.944
MDK	FN1	LCN2	0.949	MDK	APOB	LMO7	0.944
MDK	FN1	MUC4	0.949	MDK	FN1	ANXA3	0.944
MDK	APOF	MUC4	0.949	MDK	LCN2	PPL	0.944
MDK	LCN2	MUC4	0.949	MDK	LCN2	ANXA7	0.944
MDK	LCN2	MDK	0.949	MDK	APOB	LMO7	0.944
MDK	SERPIND1	MUC4	0.949	MDK	APOB	IMPA1	0.944
MDK	COL1A1	LCN2	0.949	MDK	MUC4	SPRR1B	0.944
MDK	APOB	MUC4	0.948	MDK	COL1A1	APOB	0.944
MDK	APOB	LCN2	0.948	MDK	APP	LCN2	0.944
MDK	LCN2	MUC4	0.948	MDK	MUC4	TIMP2	0.944
MDK	VWF	FCGBP	0.948	MDK	MUC4	CSE1L	0.944
MDK	APOB	LCN2	0.948	MDK	LMO7	FCGBP	0.944
MDK	APOF	FCGBP	0.948	MDK	APOB	FN1	0.944
MDK	MUC4	APP	0.948	MDK	FN1	CD44	0.944
MDK	APOB	LCN2	0.948	MDK	SERPIND1	FCGBP	0.944
MDK	MUC4	FGFBP1	0.948	MDK	FN1	FGFBP1	0.944
MDK	COL1A1	LCN2	0.948	MDK	APOB	SPRR1B	0.944
MDK	MUC4	CCT6A	0.948	MDK	APOB	EPS8L1	0.944
MDK	MUC4	APP	0.948	MDK	ANXA7	MUC4	0.944
MDK	PPL	MUC4	0.947	MDK	SPP1	LCN2	0.944
MDK	LCN2	APP	0.947	MDK	MUC4	TIMP2	0.944
MDK	SPP1	MUC4	0.947	MDK	MUC4	KRT8	0.944
MDK	APOB	ANXA7	0.947	MDK	MUC4	PLBD1	0.944
MDK	APOB	ANXA7	0.947	MDK	MUC4	CSE1L	0.944
MDK	VWF	LCN2	0.947	MDK	STX7	FCGBP	0.944
MDK	VWF	MUC4	0.947	MDK	LBP	MUC4	0.944
MDK	VWF	MUC4	0.947	MDK	LCN2	PPL	0.944
MDK	FN1	SPRR1B	0.947	MDK	APOB	EPS8L1	0.944
MDK	APOB	PPL	0.947	MDK	FN1	EPS8L1	0.944
MDK	CCT6A	ANXA7	0.947	MDK	C4BPA	FCGBP	0.944
MDK	LCN2	MDK	0.947	MDK	MUC4	SERPINH1	0.944

Prot 1	Prot 2	Prot 3	AUC	Prot 1	Prot 2	Prot 3	AUC
MDK	APOB	FCGBP	0.947	MDK	FN1	VIM	0.944
MDK	APOB	FCGBP	0.947	MDK	FN1	S100A9	0.944
MDK	LCN2	MUC4	0.947	MDK	FN1	STX7	0.943
MDK	C1QA	MUC4	0.947	MDK	TCP1	MUC4	0.943
MDK	SPP1	MUC4	0.947	MDK	MUC4	HSPE1	0.943
MDK	TCP1	MUC4	0.947	MDK	SERPIND1	MUC4	0.943
MDK	VWF	LCN2	0.946	MDK	FN1	CAPS	0.943
MDK	APOB	FCGBP	0.946	MDK	FN1	SPP1	0.943
MDK	APOB	SPP1	0.946	MDK	FN1	SCEL	0.943
MDK	APOB	SPP1	0.946	MDK	FN1	IMPA1	0.943
MDK	APOB	PPL	0.946	MDK	APOC1	MUC4	0.943
MDK	FGFBP1	PPL	0.946	MDK	LCN2	LMO7	0.943
MDK	VWF	MUC4	0.946	MDK	MUC4	CSE1L	0.943
MDK	FGFBP1	MUC4	0.946	MDK	LCN2	MMP8	0.943
MDK	APOF	FCGBP	0.946	MDK	PPL	APP	0.943
MDK	SERPIND1	MUC4	0.946	MDK	MUC4	A2ML1	0.943
MDK	SAA4	MUC4	0.946	MDK	SPP1	APOF	0.943
MDK	FN1	PPL	0.946	MDK	FGFBP1	PPL	0.943
MDK	ANXA7	CSE1L	0.946	MDK	FN1	SPRR1B	0.943
MDK	FN1	SCEL	0.946	MDK	LBP	LCN2	0.943
MDK	CCT6A	MUC4	0.946	MDK	MUC4	C4BPA	0.943
MDK	ITIH2	MUC4	0.946	MDK	SPP1	LCN2	0.943
MDK	C1QA	MUC4	0.946	MDK	C4BPA	MUC4	0.943
MDK	APOB	LCN2	0.946	MDK	FN1	CSE1L	0.943
MDK	APOB	IMPA1	0.946	MDK	FN1	CSE1L	0.943
MDK	VWF	MUC4	0.946	MDK	MUC4	AGRN	0.943
MDK	ITIH2	MUC4	0.946	MDK	VWF	PPL	0.943
MDK	MUC4	TCP1	0.946	MDK	APOB	APP	0.943
MDK	COL1A1	APOB	0.946	MDK	FN1	SPP1	0.943
MDK	APOB	SPP1	0.946	MDK	LBP	LCN2	0.943
MDK	LCN2	APP	0.946	MDK	LCN2	SPRR1B	0.943
MDK	LCN2	CAMP	0.946	MDK	C4BPA	FCGBP	0.943
MDK	SCEL	MUC4	0.946	MDK	FN1	CD44	0.943
MDK	VWF	FCGBP	0.946	MDK	MUC4	A2ML1	0.943
MDK	APOB	FCGBP	0.945	MDK	MUC4	CALU	0.943
MDK	SPP1	MUC4	0.945	MDK	VWF	STX7	0.943
MDK	LCN2	MMP8	0.945	MDK	APOB	APP	0.943
MDK	MUC4	FCGBP	0.945	MDK	FN1	ANXA7	0.943
MDK	VWF	FCGBP	0.945	MDK	FN1	LMNB1	0.943
MDK	FN1	S100A9	0.945	MDK	LCAT	MUC4	0.943
MDK	MUC4	LCN2	0.945	MDK	COL1A1	FCGBP	0.943
MDK	ANXA7	FCGBP	0.945	MDK	FN1	APOF	0.943
MDK	FN1	STX7	0.945	MDK	APOB	CD44	0.943
MDK	SPRR1A	MUC4	0.945	MDK	MUC4	HSPE1	0.943

Prot 1	Prot 2	Prot 3	AUC	Prot 1	Prot 2	Prot 3	AUC
MDK	APOF	MUC4	0.945	MDK	APOB	SPRR1B	0.943
MDK	APOB	STX7	0.945	MDK	APOB	CD44	0.943
MDK	SERPIND1	MUC4	0.945	MDK	VWF	LCN2	0.943
MDK	FN1	LMNB1	0.945	MDK	SERPIND1	PPL	0.943
MDK	SERPIND1	FCGBP	0.945	MDK	FN1	PLBD1	0.943
MDK	SPP1	LCN2	0.945	MDK	SAA4	FCGBP	0.943
MDK	APOF	LCN2	0.945	MDK	C4BPA	MUC4	0.943
MDK	APOB	SPP1	0.945	MDK	MUC4	APOF	0.943
MDK	FGFBP1	MUC4	0.945	MDK	MUC4	SERPINH1	0.943
MDK	PPL	FCGBP	0.945	MDK	FGFBP1	LCN2	0.942
MDK	MUC4	STX7	0.945	MDK	APOB	CD44	0.942
MDK	FN1	IMPA1	0.945	MDK	FN1	LMO7	0.942
MDK	MUC4	FCGBP	0.945	MDK	APP	LCN2	0.942
MDK	MUC4	CA1	0.945	MDK	MUC4	SPRR1B	0.942
MDK	SPRR1B	MUC4	0.945	MDK	MUC4	KRT8	0.942
MDK	APOB	STX7	0.945	MDK	MUC4	CSE1L	0.942
MDK	FN1	SPP1	0.945	MDK	APP	MUC4	0.942
MDK	FN1	PLBD1	0.945	MDK	APOB	S100A9	0.942
MDK	SPP1	MUC4	0.945	MDK	FN1	MMP8	0.942
MDK	FGFBP1	MUC4	0.945	MDK	FN1	CAMP	0.942
MDK	LCN2	ANXA7	0.945	MDK	SERPIND1	FCGBP	0.942
MDK	MUC4	PPL	0.945	MDK	FN1	CD44	0.942
MDK	C4BPA	MUC4	0.945	MDK	MUC4	SPRR1A	0.942
MDK	PPL	FCGBP	0.945	MDK	ANXA7	APP	0.942
MDK	MUC4	SPRR1B	0.945	MDK	STX7	FCGBP	0.942
MDK	FN1	SPP1	0.945	MDK	FN1	CCT6A	0.942
MDK	FN1	FGFBP1	0.945	MDK	FN1	CAPS	0.942
MDK	SPP1	LCN2	0.945	MDK	FN1	ANXA3	0.942
MDK	LBP	MUC4	0.945	MDK	SPP1	APOF	0.942

Table 6. 2-protein biomarker detection for EC prognosis: low vs high histological grade

Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC
PIGR	HSPE1	0.97	PIGR	CTNNB1	0.93	PIGR	PEA15	0.92
PIGR	HSPE1	0.96	PIGR	MDK	0.93	PIGR	SPP1	0.92
PIGR	HSPE1	0.96	PIGR	LDHA	0.92	PIGR	SPP1	0.92
PIGR	HSPE1	0.95	PIGR	VWF	0.92	PIGR	C1R	0.91
PIGR	AGRN	0.95	PIGR	ATP6V1G1	0.92	PIGR	S100A9	0.91
PIGR	CD44	0.95	PIGR	PKM	0.92	PIGR	CAPS	0.91
PIGR	CD44	0.95	PIGR	NAMPT	0.92	PIGR	LMNB1	0.91
PIGR	KRT8	0.94	PIGR	PEA15	0.92	PIGR	PKM	0.91
PIGR	AGRN	0.94	PIGR	CSE1L	0.92	HSPE1	FN1	0.91
PIGR	MDK	0.94	PIGR	LMNB1	0.92	PIGR	C4BPA	0.91
PIGR	KRT8	0.94	PIGR	LMO7	0.92	PIGR	CAMP	0.91

Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC
PIGR	CD44	0.94	PIGR	CALU	0.92	PIGR	A2ML1	0.91
PIGR	CD44	0.94	PIGR	STMN1	0.92	PIGR	TCP1	0.91
PIGR	AGRN	0.94	PIGR	TCP1	0.92	PIGR	C1R	0.91
PIGR	STMN1	0.94	PIGR	LDHA	0.92	PIGR	LBP	0.91
PIGR	APP	0.93	PIGR	SPP1	0.92	PIGR	ITIH2	0.91
PIGR	AGRN	0.93	PIGR	VIM	0.92	PIGR	SPRR1B	0.91
PIGR	SERPINH1	0.93	PIGR	CSE1L	0.92	PIGR	VIM	0.91
PIGR	CALU	0.93	PIGR	CCT6A	0.92	PIGR	NAMPT	0.91
PIGR	LGALS1	0.93	PIGR	MDK	0.92	PIGR	CCT6A	0.91
PIGR	ATP6V1G1	0.93	PIGR	SPP1	0.92	VWF	HSPE1	0.91
PIGR	CHMP4B	0.93	PIGR	IMPA1	0.92	PIGR	APOB	0.91
PIGR	KRT8	0.93	PIGR	APP	0.92	PIGR	ITIH2	0.91
FN1	HSPE1	0.93	PIGR	CCT6A	0.92	PIGR	APP	0.91
PIGR	SERPINH1	0.93	PIGR	STX7	0.92	PIGR	CSE1L	0.91
PIGR	CHMP4B	0.93	PIGR	CALU	0.92	PIGR	LMO7	0.91
PIGR	CALU	0.93	PIGR	LDHA	0.92	PIGR	C1QA	0.91
PIGR	STMN1	0.93	PIGR	NEDD8-MDP1	0.92	PIGR	APOF	0.91
PIGR	SERPINH1	0.93	PIGR	EPS8L1	0.92	PIGR	APOC1	0.91
PIGR	CTNNB1	0.93	PIGR	VIM	0.92	PIGR	PPL	0.91
PIGR	LGALS1	0.93	PIGR	STMN1	0.92	PIGR	NEDD8-MDP1	0.91
PIGR	KRT8	0.93	PIGR	LDHA	0.92	PIGR	C4BPA	0.91
PIGR	PKM	0.93	PIGR	VWF	0.92	PIGR	FN1	0.91
PIGR	ANXA7	0.93	PIGR	CAPS	0.92	PIGR	TIMP2	0.91
PIGR	PKM	0.93	PIGR	VWF	0.92	PIGR	TMPRSS11E	0.91
PIGR	VWF	0.93	PIGR	LGALS1	0.92	PIGR	GRN	0.91
PIGR	LGALS1	0.93	PIGR	GYG1	0.92	PIGR	PLBD1	0.91
FN1	HSPE1	0.93	HSPE1	FN1	0.92	PIGR	COL3A1	0.91
PIGR	LMNB1	0.93	PIGR	SPRR1A	0.92	PIGR	COL1A1	0.91
PIGR	RAB21	0.93	PIGR	PPL	0.92	PIGR	C4BPA	0.91
PIGR	SERPINH1	0.93	PIGR	RAB21	0.92	PIGR	FN1	0.91

Prot.: Protein, AUC: Area Under the Curve

Table 7. 3-protein biomarker detection for EC prognosis: low vs high histological grade

Prot 1	Prot 2	Prot 3	AUC	Prot 1	Prot 2	Prot 3	AUC
PIGR	HSPE1	EPS8L1	0.975	PIGR	HSPE1	CA1	0.965
PIGR	VWF	HSPE1	0.975	PIGR	HSPE1	STMN1	0.965
PIGR	C1QA	HSPE1	0.974	PIGR	HSPE1	STMN1	0.965
PIGR	HSPE1	COL3A1	0.974	PIGR	HSPE1	ANXA7	0.965
PIGR	HSPE1	ANXA7	0.973	PIGR	HSPE1	C1QA	0.964
PIGR	HSPE1	COL3A1	0.971	PIGR	HSPE1	C1R	0.964
PIGR	HSPE1	SCEL	0.971	PIGR	HSPE1	NEDD8-MDP1	0.964
PIGR	HSPE1	LMO7	0.971	PIGR	HSPE1	COL1A1	0.964

Prot 1	Prot 2	Prot 3	AUC	Prot 1	Prot 2	Prot 3	AUC
PIGR	HSPE1	PKM	0.971	PIGR	VWF	CD44	0.964
PIGR	VWF	HSPE1	0.971	PIGR	HSPE1	C1R	0.964
PIGR	HSPE1	CSE1L	0.971	PIGR	HSPE1	MUC4	0.964
PIGR	HSPE1	TCP1	0.970	PIGR	HSPE1	ANXA3	0.964
PIGR	HSPE1	SERPIND1	0.970	PIGR	HSPE1	LMO7	0.964
PIGR	HSPE1	CSE1L	0.970	PIGR	HSPE1	TCP1	0.964
PIGR	HSPE1	CALU	0.970	PIGR	HSPE1	HCLS1	0.964
PIGR	HSPE1	PEA15	0.970	PIGR	HSPE1	SERPINH1	0.963
PIGR	HSPE1	GYG1	0.970	PIGR	HSPE1	TCP1	0.963
PIGR	HSPE1	SPP1	0.970	PIGR	HSPE1	CA1	0.963
PIGR	HSPE1	CTNNB1	0.969	PIGR	HSPE1	COL1A1	0.963
PIGR	C1QA	HSPE1	0.969	PIGR	HSPE1	GYG1	0.963
PIGR	HSPE1	COL3A1	0.968	PIGR	HSPE1	CALU	0.962
PIGR	HSPE1	ANXA3	0.968	PIGR	HSPE1	NAMPT	0.962
PIGR	HSPE1	IGFALS	0.968	PIGR	HSPE1	CCT6A	0.962
PIGR	HSPE1	COL1A1	0.968	PIGR	HSPE1	VIM	0.962
PIGR	HSPE1	HCLS1	0.968	PIGR	HSPE1	MUC4	0.961
PIGR	HSPE1	TCP1	0.968	PIGR	HSPE1	FGFBP1	0.961
PIGR	C4BPA	HSPE1	0.968	PIGR	HSPE1	EPS8L1	0.961
PIGR	HSPE1	LDHA	0.968	PIGR	HSPE1	PEA15	0.961
PIGR	HSPE1	CA1	0.968	PIGR	HSPE1	PKM	0.961
PIGR	HSPE1	RAB21	0.967	PIGR	HSPE1	SERPINH1	0.961
PIGR	HSPE1	TCP1	0.966	PIGR	HSPE1	SERPIND1	0.961
PIGR	HSPE1	LDHA	0.966	PIGR	HSPE1	PEA15	0.961
PIGR	HSPE1	MMP8	0.966	PIGR	HSPE1	CD44	0.961
PIGR	HSPE1	ATP6V1G1	0.966	PIGR	HSPE1	APP	0.961
PIGR	HSPE1	PKM	0.966	PIGR	CD44	AGRN	0.961
PIGR	HSPE1	LSP1	0.966	PIGR	CD44	SPP1	0.961
PIGR	HSPE1	EPS8L1	0.966	PIGR	HSPE1	CSE1L	0.961
PIGR	HSPE1	HSPE1	0.965	PIGR	HSPE1	GRN	0.961

Prot.: Protein, AUC: Area Under the Curve

Table 8. 2-protein biomarker detection for EC diagnosis and prognosis, including the differentiation between histological grades; and subtypes of ECs (i.e., Endometrioid, Serous, Others (carcinosarcoma)).

5

Prot 1	Prot 2	AUC	Prot 1	Prot 2	AUC
PIGR_ILLN ***	RAB21_GIEE **	0,89	PIGR_ILLN ***	ANXA7_SEID *	0,86
PIGR_ILLN ***	PKM_NTGI **	0,89	PIGR_ILLN ***	PEA15_ISEE .	0,86
PIGR_ILLN ***	LDHA_VTLT **	0,89	PIGR_ILLN ***	TCP1_SSLG .	0,86
PIGR_ILLN ***	LDHA_LVII **	0,89	PIGR_ILLN ***	HSPE1_VLLP .	0,86
PIGR_VYTV ***	LDHA_VTLT **	0,89	PIGR_ILLN ***	CHMP4B_GGPT .	0,86

Prot 1	Prot 2	AU C	Prot 1	Prot 2	AU C
PIGR_VYTV ***	LDHA_LVII **	0,89	PIGR_ILLN ***	CCT6A_GIDP .	0,86
PIGR_VYTV ***	RAB21_GIEE **	0,89	PIGR_ILLN ***	S100A9_LGHP	0,86
PIGR_ILLN ***	GYG1_GALV *	0,88	PIGR_VYTV ***	CCT6A_VATA .	0,86
PIGR_VYTV ***	PKM_NTGI **	0,88	SERPINH1_GVVE ***	CAPS_EAVI **	0,86
PIGR_ILLN ***	SERPINH1_DTQS **	0,88	PIGR_ILLN ***	SPRR1A_VPEP	0,86
PIGR_ILLN ***	NAMPT_YLLE **	0,88	PIGR_ILLN ***	STX7_ITQC *	0,86
SERPINH1_DTQS ***	CAPS_SGDG ***	0,88	PIGR_ILLN ***	STMN1_AIEE *	0,86
PIGR_ILLN ***	CSE1L_SANV **	0,88	PIGR_ILLN ***	STMN1_SHEA *	0,86
PIGR_VYTV ***	SERPINH1_DTQS **	0,88	PIGR_ILLN ***	CALU_HLVY *	0,86
PIGR_ILLN ***	SERPINH1_GVVE **	0,88	PIGR_VYTV ***	CCT6A_GIDP .	0,86
PIGR_ILLN ***	CSE1L_LLQT **	0,88	PIGR_ILLN ***	LMNB1_AEHD *	0,86
PIGR_VYTV ***	PKM_APIII *	0,88	PIGR_ILLN ***	CTNNB1_LVQL *	0,86
PIGR_ILLN ***	PKM_APIII .	0,88	PIGR_ILLN ***	APP_VESL	0,86
PIGR_ILLN ***	IMPA1_YPSH **	0,88	PIGR_VYTV ***	TCP1_SSLG .	0,86
PIGR_VYTV ***	NAMPT_YLLE **	0,88	PIGR_VYTV ***	ITGB2_ALNE **	0,86
PIGR_ILLN ***	LBP_LAEG *	0,87	PIGR_VYTV ***	HSPE1_VLLP	0,86
SERPINH1_GVVE ***	CAPS_SGDG ***	0,87	PIGR_VYTV ***	ANXA7_SEID *	0,86
PIGR_ILLN ***	CD44_ALSI *	0,87	PIGR_VYTV ***	CALU_HLVY *	0,86
PIGR_VYTV ***	SERPINH1_GVVE **	0,87	PIGR_VYTV ***	LGALS1_SFVL *	0,86
PIGR_VYTV ***	CSE1L_SANV **	0,87	PIGR_VYTV ***	LMNB1_IGDT *	0,86
PIGR_VYTV ***	GYG1_GALV *	0,87	PIGR_ILLN ***	MUC4_SLEP	0,86
PIGR_VYTV ***	IMPA1_YPSH *	0,87	PIGR_ILLN ***	COL12A1_LNWN	0,86
PIGR_VYTV ***	CSE1L_LLQT **	0,87	PIGR_ILLN ***	CALU_TFDQ .	0,86
PIGR_ILLN ***	LGALS1_LPDG **	0,87	PIGR_VYTV ***	CTNNB1_LVQL *	0,86
PIGR_VYTV ***	LBP_LAEG *	0,87	PIGR_VYTV ***	CHMP4B_GGPT .	0,86
PIGR_ILLN ***	AGRN_VLGA **	0,87	PIGR_VYTV ***	MMP8_YYAF **	0,86
PIGR_VYTV ***	CD44_ALSI *	0,87	PIGR_ILLN ***	HCLS1_SAVG *	0,86
PIGR_ILLN ***	ITGB2_ALNE **	0,87	PIGR_VYTV ***	S100A9_LGHP	0,86
PIGR_ILLN ***	CD44_YGFI *	0,87	PIGR_ILLN ***	CAPS_SGDG	0,86
PIGR_ILLN ***	MMP8_YYAF **	0,87	PIGR_ILLN ***	ITIH2_FYNQ	0,86
PIGR_VYTV ***	ATP6V1G1_EEAQ *	0,87	PIGR_VYTV ***	PEA15_ISEE	0,86
PIGR_ILLN ***	HSPE1_FLPL *	0,87	PIGR_ILLN ***	TMPRSS11E_MLC A	0,86
PIGR_VYTV ***	LGALS1_LPDG **	0,87	PIGR_ILLN ***	TCP1_GAND .	0,86
SERPINH1_DTQS ***	CAPS_EAVI **	0,87	PIGR_VYTV ***	NEDD8- MDP1_EIEI *	0,86
PIGR_ILLN ***	LGALS1_SFVL **	0,87	PIGR_VYTV ***	STMN1_AIEE *	0,86
PIGR_ILLN ***	LSP1_EDSD *	0,87	PIGR_ILLN ***	SPRR1B_VPEP	0,86
PIGR_VYTV ***	CD44_YGFI *	0,87	PIGR_VYTV ***	VIM_ISLP *	0,86
PIGR_ILLN ***	ATP6V1G1_EEAQ *	0,87	PIGR_ILLN ***	VIM_ISLP *	0,86

Prot 1	Prot 2	AU C	Prot 1	Prot 2	AU C
PIGR_VYTV ***	AGRN_VLGA **	0,87	PIGR_ILLN ***	ANXA3_DYPD *	0,86
PIGR_ILLN ***	LMNB1_IGDT *	0,87	PIGR_VYTV ***	STMN1_SHEA *	0,86
PIGR_ILLN ***	NEDD8- MDP1_EIEI *	0,87	PIGR_VYTV ***	CALU_TFDQ .	0,86
PIGR_ILLN ***	CCT6A_VATA .	0,86	PIGR_VYTV ***	APP_VESL	0,86
PIGR_VYTV ***	HSPE1_FLPL *	0,86	PIGR_ILLN ***	TMPRSS11E_TDA C	0,86
PIGR_VYTV ***	LSP1_EDSD .	0,86	PIGR_ILLN ***	VIM_ILLA *	0,86

Prot.: Protein, AUC: Area Under the Curve

Continuation of the Table 8

Prot 1	Prot 2	AUC
PIGR_ILLN ***	AGRN_EAAC .	0,855534
PIGR_ILLN ***	EPS8L1_APEP	0,8551456
PIGR_VYTV ***	LMNB1_AEHD *	0,8551456
PIGR_ILLN ***	VWF_VIVI	0,8547573
PIGR_ILLN ***	ITIH2_IQPS	0,8547573
PIGR_ILLN ***	VWF_YAGS	0,8543689
PIGR_ILLN ***	C4BPA_LSCS	0,8543689
PIGR_VYTV ***	TMPRSS11E_MLCA	0,8543689
PIGR_ILLN ***	GRN_QHCC	0,8539806
PIGR_ILLN ***	SPP1_DSYE *	0,8539806
PIGR_VYTV ***	AGRN_EAAC *	0,8539806
PIGR_ILLN ***	PLBD1_LGLD	0,8535922
PIGR_ILLN ***	KRT8_WSLI	0,8535922
PIGR_VYTV ***	SPRR1A_VPEP	0,8535922
PIGR_VYTV ***	HCLS1_SAVG .	0,8535922
PIGR_VYTV ***	TCP1_GAND .	0,8532039
PIGR_VYTV ***	STX7_ITQC .	0,8532039
PIGR_ILLN ***	LCN2_VPLQ .	0,8528155
PIGR_ILLN ***	C1QA_SLGF	0,8528155
PIGR_ILLN ***	PPL_VVLQ	0,8528155
PIGR_ILLN ***	SPRR1B_QPCT	0,8528155
PIGR_ILLN ***	CAPS_EAVI	0,8528155
PIGR_ILLN ***	FAM107B_LEQL	0,8528155
PIGR_VYTV ***	MUC4_SLEP	0,8528155
PIGR_ILLN ***	FCGBP_ISVA .	0,8524272
PIGR_ILLN ***	C1R_ESEQ	0,8524272
PIGR_VYTV ***	COL12A1_LNWN	0,8524272
PIGR_ILLN ***	MUC4_LECL	0,8520388
PIGR_ILLN ***	SERPINA3_NLAV	0,8520388
PIGR_ILLN ***	APOB_FVTQ	0,8516505
PIGR_VYTV ***	ITIH2_FYNQ	0,8516505
PIGR_VYTV ***	CAPS_SGDG	0,8516505

Prot 1	Prot 2	AUC
PIGR_ILLN ***	APOF_SGVQ	0,8512621
PIGR_ILLN ***	LCN2_ELTS .	0,8512621
PIGR_ILLN ***	C4BPA_FSAI	0,8512621
PIGR_ILLN ***	FGFBP1_LVSS	0,8512621
PIGR_VYTV ***	VWF_VIVI	0,8512621
PIGR_ILLN	PIGR_VYTV	0,8508738
PIGR_ILLN ***	APOF_SLPT	0,8508738
PIGR_ILLN ***	SERPIND1_NYNL	0,8508738
PIGR_VYTV ***	SPRR1B_VPEP	0,8508738
PIGR_VYTV ***	KRT8_WSLI	0,8508738
PIGR_ILLN ***	FCGBP_AGCV .	0,8504854
PIGR_ILLN ***	FN1_WCGT	0,8504854
PIGR_ILLN ***	FGFBP1_DTLG	0,8504854
PIGR_VYTV ***	KRT8_LSEL	0,8504854
PIGR_ILLN ***	SERPIND1_SVND	0,8500971
PIGR_ILLN ***	APOB_LLSG	0,8500971
PIGR_ILLN ***	IGFALS_LWLE	0,8500971
PIGR_ILLN ***	KRT8_LSEL	0,8500971
PIGR_VYTV ***	FCGBP_ISVA .	0,8500971

Prot.: Protein, AUC: Area Under the Curve

Table 9. 3- protein biomarker detection for EC diagnosis and prognosis, including the differentiation between histological grades; and subtypes of ECs (i.e., Endometrioid, Serous, Others (carcinosarcoma)).

Prot 1	Prot 2	Prot 3	AUC
PIGR_ILLN ***	PKM_APIII **	MMP8_YYAF ***	0,933
PIGR_VYTV ***	PKM_APIII **	MMP8_YYAF ***	0,929
PIGR_ILLN **	SERPINH1_DTQS ***	CAPS_SGDG **	0,918
PIGR_VYTV **	SERPINH1_DTQS ***	CAPS_SGDG **	0,913
PIGR_ILLN ***	LMO7_EQVP *	PKM_NTGI **	0,912
PIGR_ILLN ***	LBP_LAEG .	PKM_NTGI **	0,911
PIGR_VYTV ***	LBP_LAEG *	PKM_NTGI **	0,911
PIGR_ILLN ***	RAB21_GIEE ***	CAPS_SGDG .	0,910
PIGR_ILLN ***	PKM_NTGI ***	EPS8L1_APEP *	0,910
PIGR_ILLN ***	CHMP4B_GGPT **	MMP8_YYAF ***	0,910
PIGR_ILLN ***	ATP6V1G1_EEAQ **	MMP8_YYAF ***	0,910
PIGR_ILLN **	SERPINH1_GVVE ***	CAPS_SGDG *	0,909
PIGR_VYTV ***	LMO7_EQVP **	PKM_NTGI **	0,909
PIGR_ILLN ***	PKM_APIII **	CAMP_AIDG ***	0,908
PIGR_ILLN ***	PKM_APIII ***	LCN2_VPLQ ***	0,908
PIGR_ILLN ***	PKM_NTGI **	CAPS_SGDG .	0,908

Prot 1	Prot 2	Prot 3	AUC
PIGR_ILLN ***	PKM_APIII **	ITGB2_ALNE ***	0,907
PIGR_ILLN ***	PKM_NTGI *	MMP8_YYAF *	0,907
PIGR_ILLN ***	LBP_LAEG .	NAMPT_YLLE **	0,906
PIGR_VYTV ***	PKM_APIII ***	CAMP_AIDG ***	0,906
PIGR_VYTV ***	PKM_NTGI ***	EPS8L1_APEP *	0,906
PIGR_ILLN ***	PKM_APIII ***	LCN2_ELTS ***	0,906
PIGR_ILLN ***	CSE1L_SANV **	CAPS_SGDG *	0,906
PIGR_ILLN ***	LBP_LAEG *	CD44_ALSI *	0,906
PIGR_ILLN ***	LDHA_LVII **	CAPS_SGDG .	0,906
PIGR_VYTV **	SERPINH1_GVVE ***	CAPS_SGDG *	0,905
PIGR_ILLN **	SERPINH1_DTQS ***	CAPS_EAVI *	0,905
SERPINH1_DTQS ***	PKM_APIII *	CAPS_SGDG ***	0,905
PIGR_ILLN ***	LDHA_LVII **	ITIH2_FYNQ .	0,904
PIGR_ILLN ***	LDHA_LVII ***	APP_VESL *	0,904
SERPINH1_DTQS ***	LBP_LAEG *	CAPS_SGDG **	0,904
PIGR_ILLN *	PIGR_VYTV .	RAB21_GIEE ***	0,904
PIGR_ILLN ***	LDHA_VTLT **	ITIH2_FYNQ .	0,904
PIGR_ILLN ***	NAMPT_YLLE ***	LMO7_EQVP *	0,904
PIGR_ILLN ***	VWF_YAGS	PKM_NTGI **	0,904
PIGR_ILLN ***	LDHA_VTLT **	APP_VESL *	0,904
PIGR_VYTV **	SERPINH1_DTQS ***	CAPS_EAVI *	0,904
PIGR_ILLN ***	LBP_LAEG	LDHA_VTLT *	0,903
PIGR_ILLN ***	LDHA_VTLT	RAB21_GIEE .	0,903
PIGR_ILLN ***	LDHA_VTLT **	COL12A1_LNWN	0,903
PIGR_VYTV ***	PKM_APIII ***	LCN2_ELTS ***	0,903

Continuation of the Table 9

Prot 1	Prot 2	Prot 3	
PIGR_ILLN ***	LDHA_LVII	RAB21_GIEE	0,903
PIGR_VYTV ***	LBP_LAEG	LDHA_LVII *	0,903
PIGR_VYTV ***	NAMPT_YLLE **	LMO7_EQVP *	0,903
PIGR_ILLN ***	LBP_LAEG	LDHA_LVII **	0,903
PIGR_ILLN ***	VWF_VIVI	PKM_NTGI **	0,903
PIGR_ILLN ***	LDHA_VTLT **	S100A9_LGHP	0,903
PIGR_ILLN ***	LDHA_LVII .	NAMPT_YLLE	0,903
PIGR_ILLN ***	LDHA_LVII **	ITIH2_IQPS .	0,903
PIGR_ILLN ***	PKM_NTGI **	COL3A1_FTYT .	0,903
PIGR_ILLN ***	LBP_LAEG	RAB21_GIEE **	0,902
PIGR_ILLN ***	LBP_LAEG *	ATP6V1G1_EEAQ *	0,902
PIGR_ILLN ***	LDHA_VTLT **	ITIH2_IQPS .	0,902
PIGR_ILLN ***	LDHA_VTLT **	CAPS_SGDG	0,902
PIGR_ILLN ***	LDHA_LVII **	ANXA7_SEID	0,902
PIGR_VYTV ***	PKM_APIII **	ITGB2_ALNE ***	0,902
PIGR_VYTV ***	CSE1L_SANV **	CAPS_SGDG *	0,902

Prot 1	Prot 2	Prot 3	
PIGR_VYTV ***	LBP_LAEG	LDHA_VTLT *	0,902
PIGR_ILLN ***	TMPRSS11E_MLCA	LDHA_LVII **	0,901
PIGR_ILLN ***	LDHA_VTLT *	IMPA1_YPSH	0,901
PIGR_VYTV ***	LDHA_LVII **	CAPS_SGDG	0,901
PIGR_ILLN ***	LDHA_VTLT .	NAMPT_YLLE	0,901
PIGR_ILLN ***	LDHA_VTLT *	ANXA7_SEID	0,901
PIGR_ILLN ***	LDHA_LVII **	CAPS_EAVI	0,901
PIGR_ILLN ***	RAB21_GIEE ***	CAPS_EAVI	0,901
PIGR_ILLN ***	PKM_NTGI **	PEA15_ISEE	0,901
PIGR_ILLN ***	PKM_APIII .	RAB21_GIEE **	0,901
PIGR_ILLN ***	CSE1L_LLQT **	CAPS_SGDG *	0,901
PIGR_ILLN ***	LBP_LAEG *	CD44_YGFI *	0,901
PIGR_ILLN ***	LDHA_LVII	PKM_NTGI	0,901
PIGR_ILLN ***	LDHA_LVII **	SPP1_ANDE	0,901
PIGR_ILLN ***	PKM_NTGI **	CAPS_EAVI	0,901
PIGR_VYTV ***	PKM_NTGI **	COL3A1_FTYT *	0,901
PIGR_ILLN **	SERPINH1_GVVE ***	CAPS_EAVI *	0,900
PIGR_ILLN ***	LDHA_LVII **	S100A9_LGHP	0,900
PIGR_ILLN ***	LDHA_LVII **	SPP1_DSYE	0,900
PIGR_ILLN ***	LDHA_LVII *	IMPA1_YPSH	0,900
PIGR_ILLN ***	LDHA_LVII **	COL12A1_LNWN	0,900
PIGR_ILLN ***	PKM_NTGI **	PPL_VVLQ	0,900
PIGR_VYTV ***	PKM_APIII ***	LCN2_VPLQ **	0,900
PIGR_VYTV ***	LDHA_LVII **	S100A9_LGHP	0,900

Prot.: Protein, AUC: Area Under the Curve

Example 2. Markers indicating recurrence of disease.

The discovery study included 20 patients, 9 from which turned to suffer recurrence after 5-
 5 years follow-up. These recurrent women, recur between 10 and 119 months after surgery. Specifically, there were 4 endometrioid EC (2 G2 and 2 G3) and 5 non-endometrioid EC recurring, while 10 EEC (2 G1, 7 G2, 1 G3) and 1 non-EEC did not recur. Hence, it was compared the 9 recurrent vs 11 non-recurrent patients.

Statistical analysis showed 19 proteins with AUC > 0.75 to differentiate between recurrent
 10 and non-recurrent EC patients (MUC1, PRSS8, PNP, APEH, MUC16, C9, SERPINC1, SERPINA1, F2, AMBP, HP, SERPINA3, CFB, ORM2, CAT, GNAI2, A1BG, FN1 and C7). Additionally, by presence-absence statistical analysis, there were identified 18 differential expressed proteins between recurrent and non-recurrent EC patients (ASTRGL1,
 15 MUC5AC, NME1, NT5E, PDLIM5, PRSS8, RDX, and VASP).

Next Table 10 (A) and (B) illustrate the data:

(A) Statistical analysis		
Gene names	Fold change	AUC
MUC1	1.696	0.889
PRSS8	1.613	0.889
PNP	0.975	0.843
APEH	0.704	0.778
MUC16	1.618	0.778
C9	-1.398	0.815
SERPINC1	-0.980	0.88
SERPINA1	1.446	0.815
F2	-1.022	0.806
AMBP	-0.895	0.787
HP	-1.051	0.792
SERPINA3	-1.883	0.815
CFB	-0.563	0.778
ORM2	-0.713	0.759
CAT	0.687	0.769
GNAI2	0.603	0.769
A1BG	-0.626	0.787
FN1	-0.854	0.778
C7	-0.564	0.778

(B) Statistical analysis			
Gene names	No recurrent identifications	Recurrent identifications	significance
ADRGL1	7	2	0.07
B4GALT1	8	2	0.03
CAPS	9	3	0.04

(B)Statistical analysis			
CBX3	11	6	0.07
CD163	4	9	0.00
CDV3	9	3	0.04
DMBT1	9	3	0.04
DSG3	9	4	0.09
EHD1	8	3	0.08
GOLM1	9	3	0.04
MUC16	10	5	0.09
MUC5AC	11	5	0.03
NME1	11	5	0.03
NT5E	9	3	0.04
PDLIM5	10	5	0.09
PRSS8	11	4	0.01
RDX	8	2	0.03
VASP	11	6	0.07

Next Table 11 lists the names of the used biomarkers.

Gene Names	Entry	Entry Name	Protein names
MUC1 PUM	P15941	MUC1_HUMAN	Mucin-1, MUC-1 (Breast carcinoma-associated antigen DF3) (Cancer antigen 15-3, CA 15-3) (Carcinoma-associated mucin) (Episialin) (H23AG) (Krebs von den Lungen-6, KL-6) (PEMT) (Peanut-reactive urinary mucin, PUM) (Polymorphic epithelial mucin, PEM) (Tumor-associated epithelial membrane antigen, EMA) (Tumor-associated mucin) (CD antigen CD227) [Cleaved into: Mucin-1 subunit alpha, MUC1-NT, MUC1-alpha; Mucin-1 subunit beta, MUC1-beta (MUC1-CT)]
PRSS8	Q16651	PRSS8_HUMAN	Prostasin, EC 3.4.21.- (Channel-activating protease 1, CAP1) (Serine protease 8) [Cleaved into: Prostasin light chain; Prostasin heavy chain]

PNP NP	P00491	PNPH_HUMAN	Purine nucleoside phosphorylase, PNP, EC 2.4.2.1 (Inosine phosphorylase) (Inosine-guanosine phosphorylase)
APEH D3F15S2 D3S48E DNF15S2	P13798	ACPH_HUMAN	Acylamino-acid-releasing enzyme, AARE, EC 3.4.19.1 (Acyl-peptide hydrolase, APH) (Acylaminoacyl-peptidase) (Oxidized protein hydrolase, OPH)
MUC16 CA125	Q8WXI7	MUC16_HUMAN	Mucin-16, MUC-16 (Ovarian cancer-related tumor marker CA125, CA-125) (Ovarian carcinoma antigen CA125)
C9	P02748	CO9_HUMAN	Complement component C9 [Cleaved into: Complement component C9a; Complement component C9b]
SERPINC1 AT3 PRO0309	P01008	ANT3_HUMAN	Antithrombin-III, ATIII (Serpine C1)
SERPINA1 AAT PI PRO0684 PRO2209	P01009	A1AT_HUMAN	Alpha-1-antitrypsin (Alpha-1 protease inhibitor) (Alpha-1-antiproteinase) (Serpine A1) [Cleaved into: Short peptide from AAT, SPAAT]
F2	P00734	THRB_HUMAN	Prothrombin, EC 3.4.21.5 (Coagulation factor II) [Cleaved into: Activation peptide fragment 1; Activation peptide fragment 2; Thrombin light chain; Thrombin heavy chain]
AMBP HCP ITIL	P02760	AMBP_HUMAN	Protein AMBP (Protein HC) [Cleaved into: Alpha-1-microglobulin, EC 1.6.2.- (Alpha-1 microglycoprotein) (Complex-forming glycoprotein heterogeneous in charge); Inter-alpha-trypsin inhibitor light chain, ITI-LC (Bikunin) (EDC1) (HI-30) (Uronic-acid-rich protein); Trypstatin]
HP	P00738	HPT_HUMAN	Haptoglobin (Zonulin) [Cleaved into: Haptoglobin alpha chain; Haptoglobin beta chain]
SERPINA3 AACT GIG24 GIG25	P01011	AACT_HUMAN	Alpha-1-antichymotrypsin, ACT (Cell growth-inhibiting gene 24/25 protein) (Serpine A3) [Cleaved into: Alpha-1-antichymotrypsin His-Pro-less]
CFB BF BFD	P00751	CFAB_HUMAN	Complement factor B, EC 3.4.21.47 (C3/C5 convertase) (Glycine-rich beta glycoprotein, GBG) (PBF2) (Properdin factor B) [Cleaved into: Complement factor B Ba fragment; Complement factor B Bb fragment]
ORM2 AGP2	P19652	A1AG2_HUMAN	Alpha-1-acid glycoprotein 2, AGP 2 (Orosomucoid-2, OMD 2)
CAT	P04040	CATA_HUMAN	Catalase, EC 1.11.1.6
GNAI2 GNAI2B	P04899	GNAI2_HUMAN	Guanine nucleotide-binding protein G(i) subunit alpha-2 (Adenylate cyclase-inhibiting G alpha protein)

A1BG	P04217	A1BG_HUMAN	Alpha-1B-glycoprotein (Alpha-1-B glycoprotein)
FN1 FN	P02751	FN1_HUMAN	Fibronectin, FN (Cold-insoluble globulin, CIG) [Cleaved into: Anastellin; Ugl-Y1; Ugl-Y2; Ugl-Y3]
C7	P10643	CO7_HUMAN	Complement component C7
ASRGL1 ALP CRASH	Q7L266	ASGL1_HUMAN	Isoaspartyl peptidase/L-asparaginase, EC 3.4.19.5, EC 3.5.1.1 (Asparaginase-like protein 1) (Beta-aspartyl-peptidase) (Isoaspartyl dipeptidase) (L-asparagine amidohydrolase) [Cleaved into: Isoaspartyl peptidase/L-asparaginase alpha chain; Isoaspartyl peptidase/L-asparaginase beta chain]
B4GALT1 GGTB2	P15291	B4GT1_HUMAN	Beta-1,4-galactosyltransferase 1, Beta-1,4-GalTase 1, Beta4Gal-T1, b4Gal-T1, EC 2.4.1.- (Beta-N-acetylglucosaminyl-glycolipid beta-1,4-galactosyltransferase) (Beta-N-acetylglucosaminylglycopeptide beta-1,4-galactosyltransferase, EC 2.4.1.38) (Lactose synthase A protein, EC 2.4.1.22) (N-acetyllactosamine synthase, EC 2.4.1.90) (Nal synthase) (Neolactotriaosylceramide beta-1,4-galactosyltransferase, EC 2.4.1.275) (UDP-Gal:beta-GlcNAc beta-1,4-galactosyltransferase 1) (UDP-galactose:beta-N-acetylglucosamine beta-1,4-galactosyltransferase 1) [Cleaved into: Processed beta-1,4-galactosyltransferase 1]
CAPS	Q13938	CAYP1_HUMAN	Calcyphosin (Calcyphosine)
CBX3	Q13185	CBX3_HUMAN	Chromobox protein homolog 3 (HECH) (Heterochromatin protein 1 homolog gamma, HP1 gamma) (Modifier 2 protein)
CD163 M130	Q86VB7	C163A_HUMAN	Scavenger receptor cysteine-rich type 1 protein M130 (Hemoglobin scavenger receptor) (CD antigen CD163) [Cleaved into: Soluble CD163, sCD163]
CDV3 H41	Q9UKY7	CDV3_HUMAN	Protein CDV3 homolog
DMBT1 GP340	Q9UGM3	DMBT1_HUMAN	Deleted in malignant brain tumors 1 protein (Glycoprotein 340, Gp-340) (Hensin) (Salivary agglutinin, SAG) (Surfactant pulmonary-associated D-binding protein)
DSG3 CDHF6	P32926	DSG3_HUMAN	Desmoglein-3 (130 kDa pemphigus vulgaris antigen, PVA) (Cadherin family member 6)

EHD1 PAST PAST1 CDABP0131	Q9H4M9	EHD1_HUMAN	EH domain-containing protein 1 (PAST homolog 1, hPAST1) (Testilin)
GOLM1 C9orf155 GOLPH2 PSEC0242 UNQ686/PRO1326	Q8NB4	GOLM1_HUMAN	Golgi membrane protein 1 (Golgi membrane protein GP73) (Golgi phosphoprotein 2)
MUC5AC MUC5	P98088	MUC5A_HUMAN	Mucin-5AC, MUC-5AC (Gastric mucin) (Major airway glycoprotein) (Mucin-5 subtype AC, tracheobronchial) (Tracheobronchial mucin, TBM)
NME1 NDPKA NM23	P15531	NDKA_HUMAN	Nucleoside diphosphate kinase A, NDK A, NDP kinase A, EC 2.7.4.6 (Granzyme A-activated DNase, GAAD) (Metastasis inhibition factor nm23) (NM23-H1) (Tumor metastatic process-associated protein)
NT5E NT5 NTE	P21589	5NTD_HUMAN	5'-nucleotidase, 5'-NT, EC 3.1.3.5 (Ecto-5'-nucleotidase) (CD antigen CD73)
PDLIM5 ENH L9	Q96HC4	PDLI5_HUMAN	PDZ and LIM domain protein 5 (Enigma homolog) (Enigma-like PDZ and LIM domains protein)
RDX	P35241	RADI_HUMAN	Radixin
VASP	P50552	VASP_HUMAN	Vasodilator-stimulated phosphoprotein, VASP

Example 3. ELISA – EC diagnostic (MDK)

- MDK is a good EC diagnostic biomarker when measured in both: mass spectrometry (MS) and antibody-based assay (ELISA technique). The levels of MDK in the cervical fluid of 241 patients (n=128 EC, 113 non-EC) shown statistical differences to distinguish between EC and non-EC patients, achieving AUC of 0.91 when measured by MS and 0.92 when measured by ELISA assay. Showing a correlation of 0.91 between the measurements of both approaches.
- Data are depicted in Figure 2, where in (A) mass spectrometry (LC-MS/MS PRM) data or in (B) an ELISA assay derived data for determining MDK, are illustrated. tested in samples of a cohort of subjects and comparing EC vs non-EC patients. In (C), the correlation between the measurements of both approaches, allowing to see the high correlation level, which makes the marker a one one for a point of care implementation.

15

Example 4.- MDK in uterine fluids

The levels of MDK also demonstrated to be a potential diagnostic marker for EC in the uterine fluid obtained from endometrial biopsies, specifically, from pipelle biopsies. Specifically, MDK levels showed a p-value < 0.0001 and AUC = 0.69 between EC (n=118) and non-EC patients (n=129). Thus, besides the information from MDK that can be obtained from a sample type during routine gynecological inspection, this marker gives also information from other more invasive samplings, such as endometrial biopses. These biopses are sometimes done when suspicion of certain diseases, so that also the analysis of the possibility of an incipient or not EC could be simultaneously detected if this marker is analysed.

10

Data are depicted in Figure 3, where data from an ELISA assay for determining MDK in uterine fluids, tested in samples of a cohort of subjects and comparing EC vs non-EC patients is illustrated. In (A) Dotplot of the distribution of protein concentration between EC and non-EC patients. In (B) the a curve of an ROC analysis (Receiver Operating Characteristic analysis) of MDK assessed by ELISA.

15

Example 5. Molecular classification

A total number of 28 proteins (LBP, VWF, GPLD1, SAA4, APOF, C4BPA, SPRR1A, SERPIND1, APOB, SCEL, LCAT, SERPINA3, LMO7, C1R, MUC4, FN1, SPRR1B, C1QA, ITIH2, TIMP2, APOC1, GRN, ANXA3, S100A9, PLBD1, PIGR, SERPINH1, HSPE1) were able to significantly differentiate between molecular subgroups of EC: presence of POLE exonuclease domain hotspot mutation (POLE) ultramutated or POLE mutated (POLEmut), MSI: microsatellite instable (MSI) or MMR deficient, based on loss of mismatch repair protein expression (MMRd); Copy Number Low (CN-Low) or no specific molecular profile (NSMP); Copy Number High (CN-High) or p53 abnormal, based on mutant-like immunostaining (p53abn)- FC: Fold Change.

25

The data are depicted in Table 12:

Verification phase (n=92)							
Molecular classification							
Entry	Gene name	FC (MMRd vs NSMP)	Adjusted p value (MMRd vs NSMP)	FC (MMRd vs p53 mut)	Adjusted p value (MMRd vs p53 mut)	FC (NSMP vs p53mut)	Adjusted p value (NSMP vs p53mut)
P18428	LBP	-1.64	0.04	1.33	0.85	2.19	0.02
P04275	VWF	-1.77	0.00	1.03	0.94	1.82	0.01
P80108	GPLD1	-1.71	0.00	-1.05	0.72	1.63	0.05
P35542	SAA4	-1.64	0.01	1.14	0.98	1.86	0.07
Q13790	APOF	-1.87	0.00	-1.16	0.88	1.61	0.07
P04003	C4BPA	-1.72	0.01	-1.08	0.80	1.58	0.12
P35321	SPRR1A	3.04	0.02	-1.32	0.88	-4.00	0.05

P05546	SERPIND1	-1.78	0.00	-1.14	0.88	1.56	0.07
P04114	APOB1	-1.51	0.04	1.10	0.80	1.66	0.06
O95171	SCEL	3.47	0.00	1.45	0.97	-2.40	0.07
P04180	LCAT	-1.72	0.00	-1.25	0.69	1.37	0.10
P01011	SERPINA3	-1.96	0.00	-1.32	0.61	1.48	0.13
Q8WWI1	LMO7	2.84	0.01	1.40	0.90	-2.03	0.09
P00736	C1R	-1.52	0.02	-1.19	0.70	1.29	0.13
Q99102	MUC4	1.74	0.04	1.03	0.94	-1.70	0.14
P02751	FN1	-1.36	0.01	-1.07	0.72	1.28	0.10
P22528	SPRR1B	2.10	0.01	-1.65	0.84	-3.47	0.10
P02745	C1QA	-1.33	0.02	-1.16	0.61	1.15	0.19
P19823	ITIH2	-1.61	0.00	-1.24	0.59	1.30	0.13
P16035	TIMP2	1.54	0.04	1.02	0.94	-1.51	0.12
P02654	APOC1	-1.57	0.01	-1.11	0.84	1.41	0.12
P28799	GRN	2.20	0.00	1.44	0.35	-1.53	0.12
P12429	ANXA3	2.15	0.00	1.44	0.41	-1.49	0.18
P06702	S100A9	1.64	0.04	1.00	0.99	-1.64	0.18
Q6P4A8	PLBD1	1.84	0.04	1.60	0.72	-1.15	0.33
P01833	PIGR	1.11	0.41	-3.16	0.18	-3.51	0.01
P50454	SERPINH1	-1.08	0.32	1.99	0.14	2.14	0.00
P61604	HSPE1	-1.32	0.05	1.04	0.89	1.37	0.05

Further aspects/embodiments are defined in the following clauses:

- 5 Clause 1. A method of diagnosis and/or for the prognosis of endometrial cancer, the method comprising determining the presence and/or the level of expression of midkine (MDK) in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries, and selected from a gynecologic sampling, including or selected from a cervical fluid, a cytology, a pap-smear sample, an
- 10 endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.

Clause 2. The method according to clause 1, wherein the sample is a pap-smear, and specifically the fluid contained in a pap-smear and/or cervical fluid.

- 15 Clause 3. The method according to any of clauses 1-2, further comprising determining the presence and/or the level of expression of one or more of the proteins listed in Table 1, in particular, one or more of following proteins:
Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF),
- 20 Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol

acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Perioplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1),

15 Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and

20 Exportin-2 (XPO2).

Clause 4. The method according to any of clauses 1-3, comprising determining the presence and/or the level of expression of two, three, or four of the proteins.

25 Clause 5. The method according to clause 4, comprising determining in the isolated sample the presence and/or the level of expression of the proteins in at least one binary set of the group consisting of: MDK, LCN2; MDK, FN1; MDK, PLBD1; MDK, APOB; MDK, CD44; MDK, FCGBP; MDK, EPS8L1; MDK, ANXA3; MDK, C4BPA; MDK, MMP8; MDK, NEDD-8; MDK, CAMP; MDK, TIMP2; MDK, HSPE1; MDK, CALU; MDK, C1QA; MDK,

30 LDHA; MDK, TIMP2; MDK, PIGR; MDK, KRT8; MDK, PPL; MDK, SPRR1A; MDK, STMN1; MDK, CAPS; MDK, CA1; MDK, CCT6A; MDK, VIM; MDK, TCP1; and MDK, AGRN; and/or at least one binary set listed on any of Tables 4 and 6.

Clause 6. The method according to clause 4, comprising determining in the isolated

35 sample the presence and/or the level of expression of the proteins in at least one ternary set of the group consisting of: MDK, ANXA7, CSE1L; MDK, LCN2, FGFBP1; MDK, LCN2, ANXA7; MDK, FGFBP1, LCN2; MDK, APOF, FCGBP; MDK, IMPA1, FCGBP; MDK, LCN2, CD44; MDK, STX7, FCGBP; MDK, ITIH2, FCGBP; MDK, LCN2, EPS8L1; MDK, FGFBP, EPS8L1; MDK, ANXA7, FCGBP; MDK, LCN2, PLBD1; MDK, FCGBP, CD44;

- MDK, PLBD1, FCGBP; MDK, ANXA3, FCGBP; MDK, PLBD1, LGALS1; MDK, LCN2, IMPA1; MDK, FCGBP, VIM; MDK, FCGBP, LMNB1; MDK, PLBD1, ATP6V1G1; MDK, APOB, PKM; MDK, ITIH2, LCN2; MDK, PLBD1, CALU; MDK, LCN2, APOF; MDK, PLBD1, EPS8L1; MDK, APOC1, ANXA7; MDK, FCGBP, GYG1; MDK, PLBD1, PKM;
- 5 MDK, FCGBP, LSP1; MDK, TIMP2, CD44; MDK, ANXA3, PLBD1; MDK, PLBD1, HSPE1; MDK, PLBD1, CA1; MDK, FCGBP, HCLS1; MDK, STX7, IMPA1; MDK, ANXA7, STX7; MDK, STX7, EPS8L1; MDK, PLBD1, LSP1; MDK, C1R, ANXA7; MDK, STX7, PEA15; MDK, PLBD1, HCLS1; MDK, LCAT, FCGBP; MDK, PLBD1, LDHA; MDK, APOB, S100A9; MDK, ANXA7, SCEL; MDK, SERPINA3, FCGBP; MDK, FCGBP, ITGB2; MDK, SCEL,
- 10 ANXA3; MDK, S100A9, PLBD1; MDK, S100A9, LSP1; MDK, C1R, FCGBP; MDK, FGFBP1, FCGBP; MDK, APOC1, S100A9; MDK, PPL, PLBD1; MDK, TCP1, PEA15; MDK, ANXA3, LSP1; MDK, PPL, IMPA1; MDK, S100A9, MMP8; MDK, FGFBP1, PPL; and MDK, S100A9, PPL; and or at least one ternary set listed on any of Tables 5 and 7.
- 15 Clause 7. The method according to clause 6, comprising determining in the isolated sample the presence and/or the level of expression of the ternary set MDK, ANXA7 and CSE1L
- Clause 8. The method according to any of the clauses 1-7, comprising:
- 20 a) determining, *in vitro*, the level of expression of MDK, optionally in combination with one or more of the proteins listed in any one of claims 3-7, in the isolated sample from the genital tract of the female;
- b) comparing the level of MDK and if determined of the one or more other the proteins of step (a) with a corresponding reference value or reference interval for each protein, said
- 25 reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer, and/or comparing with a cut-off value discriminating among endometrial cancer and other endometriod disorders or conditions of (i.e., a healthy) endometrium, and wherein the subject is diagnosed of endometrial cancer if at least the level of MDK is within the value or interval of values from a subject suffering from
- 30 this cancer, and/or if at least the level of MDK in relation to the cut-off value is classified to the endometrial cancer group.
- Clause 9. The method according to any of clauses 1-8, wherein the level of expression is determined at the protein level.
- 35 Clause 10. The method according to any of clauses 1-9, wherein the protein level is determined by an assay or technology selected from the group consisting of an immunoassay, a bioluminescence assay, a fluorescence assay, a chemiluminescence assay, electrochemistry assay, mass spectrometry, and combinations thereof.

Clause 11. The method according to any of clauses 9-10, wherein the level of expression of protein is determined using an antibody or a fragment thereof able to bind to the protein.

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Clause 12. The method according to clause 11, wherein said antibody or fragment thereof forms part of a kit.

Clause 13. Use of MDK, as *in vitro* marker for the diagnosis and/or for the prognosis of endometrial cancer in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including one or more of a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings.

Clause 14. A kit comprising a solid support and means for detecting the presence and/or for determining the level of expression of MDK and optionally means for detecting the presence and/or for determining the level of expression of one or more proteins listed in Table 1, in particular one or more proteins selected from the group consisting of Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Periplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding

protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B) and Exportin-2 (XPO2).

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Clause 15. A computer-implemented method for carrying out the method as defined in any of clauses 1-3, in which after the determination of the level of expression of MDK, and optionally of the one or more of the proteins for the diagnosis and/or for the prognosis of endometrial cancer, said level(s) are given a value and/or a score, and optionally are

10 computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC presenting different prognosis, including different histological subtypes and grades and different molecular features.

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Citation List

- DeSouza LV, et al, "Endometrial cancer biomarker discovery and verification using differentially tagged clinical samples with multidimensional liquid chromatography and tandem mass spectrometry", Mol Cell Proteomics MCP- 2007, vol. no.6, pp.:1170–8.
- EP3452829B1
- EP3655778A1
- 25 - Tanable et al., "Midkine and its clinical significance in endometrial carcinoma", cancer Sci-2008, vol. no. 99(6), pp.: 1125-1130
- Kemik P, et al. "Diagnostic and prognostic values of preoperative serum levels of YKL-40, HE-4 and DKK-3 in endometrial cancer", Gynecol Oncol- 2016; vol. no.140, pp.:64–9.
- 30 - Burtis C. A. et al., 2008, Chapter 14, section "Statistical Treatment of Reference Values"

Claims

1. A method of diagnosis and/or for the prognosis of endometrial cancer, the method comprising determining the presence and/or the level of expression of midkine (MDK) in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries, and selected from a gynecologic sampling, including or selected from a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.
2. The method according to claim 1, wherein the sample is a pap-smear, and specifically the fluid contained in a pap-smear and/or cervical fluid.
3. The method according to any of claims 1-2, further comprising determining the presence and/or the level of expression of one or more of the proteins listed in Table 1, in particular, one or more of following proteins:
 Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 (KRT8), Periplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1

protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B), and Exportin-2 (XPO2).

4. The method according to any of claims 1-3, comprising determining the presence
5 and/or the level of expression of two, three, or four of the proteins.

5. The method according to claim 4, comprising determining in the isolated sample the presence and/or the level of expression of the proteins in at least one binary set of the group consisting of: MDK, LCN2; MDK, FN1; MDK, PLBD1; MDK, APOB; MDK, CD44;
10 MDK, FCGBP; MDK, EPS8L1; MDK, ANXA3; MDK, C4BPA; MDK, MMP8; MDK, NEDD-8; MDK, CAMP; MDK, TIMP2; MDK, HSPE1; MDK, CALU; MDK, C1QA; MDK, LDHA; MDK, TIMP2; MDK, PIGR; MDK, KRT8; MDK, PPL; MDK, SPRR1A; MDK, STMN1; MDK, CAPS; MDK, CA1; MDK, CCT6A; MDK, VIM; MDK, TCP1; and MDK, AGRN; and/or at least one binary set listed on any of Tables 4 and 6.

15 6. The method according to claim 4, comprising determining in the isolated sample the presence and/or the level of expression of the proteins in at least one ternary set of the group consisting of: MDK, ANXA7, CSE1L; MDK, LCN2, FGFBP1; MDK, LCN2, ANXA7; MDK, FGFBP1, LCN2; MDK, APOF, FCGBP; MDK, IMPA1, FCGBP; MDK, LCN2, CD44;
20 MDK, STX7, FCGBP; MDK, ITIH2, FCGBP; MDK, LCN2, EPS8L1; MDK, FGFBP, EPS8L1; MDK, ANXA7, FCGBP; MDK, LCN2, PLBD1; MDK, FCGBP, CD44; MDK, PLBD1, FCGBP; MDK, ANXA3, FCGBP; MDK, PLBD1, LGALS1; MDK, LCN2, IMPA1; MDK, FCGBP, VIM; MDK, FCGBP, LMNB1; MDK, PLBD1, ATP6V1G1; MDK, APOB, PKM; MDK, ITIH2, LCN2; MDK, PLBD1, CALU; MDK, LCN2, APOF; MDK, PLBD1,
25 EPS8L1; MDK, APOC1, ANXA7; MDK, FCGBP, GYG1; MDK, PLBD1, PKM; MDK, FCGBP, LSP1; MDK, TIMP2, CD44; MDK, ANXA3, PLBD1; MDK, PLBD1, HSPE1; MDK, PLBD1, CA1; MDK, FCGBP, HCLS1; MDK, STX7, IMPA1; MDK, ANXA7, STX7; MDK, STX7, EPS8L1; MDK, PLBD1, LSP1; MDK, C1R, ANXA7; MDK, STX7, PEA15; MDK, PLBD1, HCLS1; MDK, LCAT, FCGBP; MDK, PLBD1, LDHA; MDK, APOB, S100A9; MDK,
30 ANXA7, SCEL; MDK, SERPINA3, FCGBP; MDK, FCGBP, ITGB2; MDK, SCEL, ANXA3; MDK, S100A9, PLBD1; MDK, S100A9, LSP1; MDK, C1R, FCGBP; MDK, FGFBP1, FCGBP; MDK, APOC1, S100A9; MDK, PPL, PLBD1; MDK, TCP1, PEA15; MDK, ANXA3, LSP1; MDK, PPL, IMPA1; MDK, S100A9, MMP8; MDK, FGFBP1, PPL; and MDK, S100A9, PPL; and or at least one ternary set listed on any of Tables 5 and 7.

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7. The method according to claim 6, comprising determining in the isolated sample the presence and/or the level of expression of the ternary set MDK, ANXA7 and CSE1L

8. The method according to any of the claims 1-7, comprising:

- a) determining, *in vitro*, the level of expression of MDK, optionally in combination with one or more of the proteins listed in any one of claims 3-7, in the isolated sample from the genital tract of the female;
- b) comparing the level of MDK and if determined of the one or more other the proteins of
- 5 step (a) with a corresponding reference value or reference interval for each protein, said reference value or interval selected from a value or interval of values from a subject suffering from endometrial cancer, and/or comparing with a cut-off value discriminating among endometrial cancer and other endometriod disorders or conditions of (i.e., a healthy) endometrium, and wherein the subject is diagnosed of endometrial cancer if at
- 10 least the level of MDK is within the value or interval of values from a subject suffering from this cancer, and/or if at least the level of MDK in relation to the cut-off value is classified to the endometrial cancer group.

9.- The method according to any of claims 1-8, which is a method for the diagnosis of

15 recurrence or risk of recurrence of endometrial cancer, which further comprises determining in the isolated sample of the female the presence and/or the level of expression of one or more of the proteins selected from the group consisting of: MUC1, PRSS8, PNP, APEH, MUC16, C9, SERPINC1, SERPINA1, F2, AMBP, HP, SERPINA3, CFB, ORM2, CAT, GNAI2, A1BG, FN1, C7, ASTRGL1, B4GALT1, CAPS, CBX3, CD163,

20 CDV3, DMBT1, DSG3, EHD1, GOLM1, MUC5AC, NME1, NT5E, PDLIM5, RDX, and VASP.

10.- The method according to any of claims 1-9, which is a method for the diagnosis of endometrial cancer subtype, and which further comprises determining in the isolated

25 sample of the female the presence and/or the level of expression of one or more of the proteins selected from the group consisting of: LBP, VWF, GPLD1, SAA4, APOF, C4BPA, SPRR1A, SERPIND1, APOB, SCEL, LCAT, SERPINA3, LMO7, C1R, MUC4, FN1, SPRR1B, C1QA, ITIH2, TIMP2, APOC1, GRN, ANXA3, S100A9, PLBD1, PIGR, SERPINH1, HSPE1

30 11. The method according to any of claims 1-10, wherein the level of expression is determined at the protein level.

12. The method according to any of claims 1-11, wherein the protein level is determined

35 by an assay or technology selected from the group consisting of an immunoassay, a bioluminescence assay, a fluorescence assay, a chemiluminescence assay, electrochemistry assay, mass spectrometry, and combinations thereof.

13. The method according to any of claims 11-12, wherein the level of expression of

protein is determined using an antibody or a fragment thereof able to bind to the protein.

14. The method according to claim 13, wherein said antibody or fragment o thereof forms part of a kit.

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15. Use of MDK, as *in vitro* marker for the diagnosis and/or for the prognosis of endometrial cancer in a sample in a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries and selected from a gynecologic sampling, including one or more of a cervical fluid, a cytology, 10 a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings.

16. A kit comprising a solid support and means for detecting the presence and/or for determining the level of expression of MDK and optionally means for detecting the presence and/or for determining the level of expression of one or more proteins listed in

15 Table 1, in particular one or more proteins selected from the group consisting of Apolipoprotein B (APOB), Complement C1q subcomponent subunit A (C1QA), Fibronectin 1 (FN1), Serpin Family D Member 1 (SERPIND1), apolipoprotein F precursor (APOF), Apolipoprotein C1 (APOC1), Chaperonin Containing TCP1 Subunit 6A (CCT6A), lipopolysaccharide-binding protein precursor (LBP), Serum Amyloid A4 (SAA4), Inter- 20 Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Lipocalin 2 (LCN2), Lecithin:cholesterol acyltransferase (LCAT), C4b-binding protein alpha chain (C4BPA), Complement C1r (C1R), Fibroblast Growth Factor Binding Protein 1 (FGFBP1), Small Proline Rich Protein 1B (SPRR1B), Small Proline Rich Protein 1A (SPRR1A) and Tissue inhibitor of metalloproteinases 2 (TIMP2), Liopocalin-2 (LCN2), Phospholipase B Domain Containing 25 1 (PLBD1), CD44 antigen, Fc Fragment Of IgG Binding Protein (FCGBP), Epidermal growth factor receptor kinase substrate 8-like protein 1 (EPS8L1), Annexin A3 (ANXA3), matrix metalloproteinase-8 (MMP8), NEDD-8 protein, Cathelicidin Antimicrobial Peptide (CAMP), Heat Shock Protein Family E (Hsp10) Member 1 (HSPE1), Calumenin (CALU), Lactate Dehydrogenase A (LDHA), Polymeric Immunoglobulin Receptor (PIGR), Keratin 8 30 (KRT8), Periplakin (PPL), Stathmin 1 (STMN1), Calcyphosin (CAPS), Carbonic anhydrase 1 (CA1), Vimentin (VIM), T complex 1 (TCP1), Agrin (AGR), Annexin A7 (ANXA7), Inositol Monophosphatase 1 (IMPA1), Syntaxin 7 (STX7), Inter-Alpha-Trypsin Inhibitor Heavy Chain 2 (ITIH2), Galectin 1 (LGALS1), ATPase H⁺ Transporting V1 Subunit G1 (ATP6V1G1), Pyruvate kinase isozymes M1/M2 (PKM), Glycogenin 1 (GYG1), 35 Lymphocyte-specific protein 1 (LSP1), Hematopoietic Cell-Specific Lyn Substrate 1 (HCLS1), Proliferation And Apoptosis Adaptor Protein 15 (PEA15), S100 calcium-binding protein A9 (S100A9), Sciellin (SCEL), Serpin Family A Member 3 (SERPINA3), Integrin Subunit Beta 2 (ITGB2), Fc Fragment Of IgG Binding Protein (FCGBP), NEDD8-MDP1 protein (NEDD8-MDP1), Charged Multivesicular Body Protein 4B (CHMP4B) and

Exportin-2 (XPO2).

17. A computer-implemented method for carrying out the method as defined in any of claims 1-14, in which after the determination of the level of expression of MDK, and optionally of the one or more of the proteins for the diagnosis and/or for the prognosis of endometrial cancer, said level(s) are given a value and/or a score, and optionally are computed in a mathematical formula to obtain a computed value; wherein in function of the said level(s), score(s) and or computed value(s), a decision is taken between the options of suffering or not from EC and/or between the options of suffering among different EC presenting different prognosis, including different histological subtypes and grades and different molecular features.
18. A method for the diagnosis of recurrence or risk of recurrence of endometrial cancer, which comprises determining in an isolated sample of a female the presence and/or the level of expression of one or more of the proteins selected from the group consisting of: MUC1, PRSS8, PNP, APEH, MUC16, C9, SERPINC1, SERPINA1, F2, AMBP, HP, SERPINA3, CFB, ORM2, CAT, GNAI2, A1BG, FN1, C7, ASTRGL1, B4GALT1, CAPS, CBX3, CD163, CDV3, DMBT1, DSG3, EHD1, GOLM1, MUC5AC, NME1, NT5E, PDLIM5, RDX, and VASP, wherein the sample is a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries, and selected from a gynecologic sampling, including or selected from a cervical fluid, a cytology, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.
- 19.- A method for the diagnosis of endometrial cancer subtype, and/or endometrial cancer molecular classification, which comprises determining in the isolated sample of a female the presence and/or the level of expression of one or more of the proteins selected from the group consisting of: LBP, VWF, GPLD1, SAA4, APOF, C4BPA, SPRR1A, SERPIND1, APOB, SCEL, LCAT, SERPINA3, LMO7, C1R, MUC4, FN1, SPRR1B, C1QA, ITIH2, TIMP2, APOC1, GRN, ANXA3, S100A9, PLBD1, PIGR, SERPINH1, HSPE1, wherein the sample is a sample from the female genital tract part including one or more of the vulvae, vagina, cervix, uterus, fallopian tubes, and ovaries, and selected from a gynecologic sampling, including or selected from a cervical fluid, a cytology, a pap-smear like sample, a pap-smear sample, an endometrial biopsy, a uterine fluid, uterine washings and combinations thereof.