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(54) **METHOD AND DIAGNOSTIC KIT FOR DIAGNOSIS OF ENDOMETRIOSIS**

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(52) **U.S. Cl.** **435/7.24**; 435/7.1; 435/7.2; 435/7.21; 435/7.23; 435/7.4; 435/7.5; 435/7.92; 435/7.94; 435/287.2; 435/960; 435/975; 436/503; 436/519; 436/547; 436/548; 436/63; 436/64; 436/172

(58) **Field of Search** 435/7.2, 7.21, 435/7.23, 7.1, 7.24, 6, 7.92, 7.4, 7.94, 7.5, 287.2, 960, 975; 436/503, 519, 547, 548, 63, 64, 172

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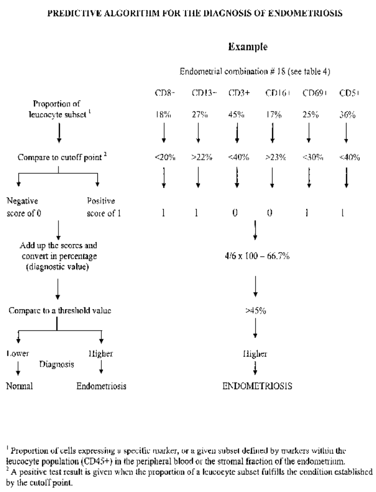
(57) **ABSTRACT**

The invention relates to a method and a kit for the diagnosis of endometriosis using blood and endometrial leukocyte markers or a combination thereof. The marker is a surface antigen from endometrial or blood leukocytes.

3 Claims, 1 Drawing Sheet



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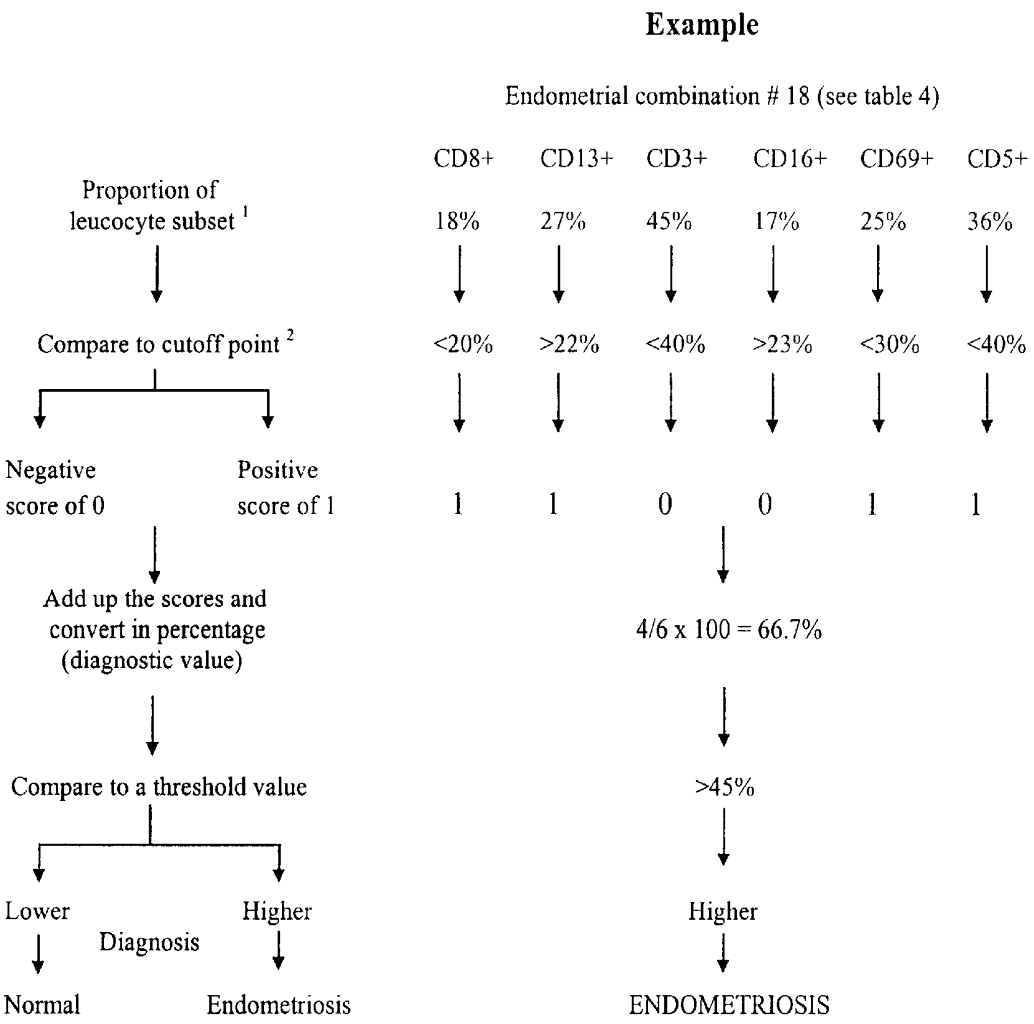
PREDICTIVE ALGORITHM FOR THE DIAGNOSIS OF ENDOMETRIOSIS



[1] Proportion of cells expressing a specific marker, or a given subset defined by markers within the leucocyte population (CD45+) in the peripheral blood or the stromal fraction of the endometrium.
[2] A positive test result is given when the proportion of a leucocyte subset fulfills the condition established by the cutoff point.

Fig. 1

METHOD AND DIAGNOSTIC KIT FOR DIAGNOSIS OF ENDOMETRIOSIS

This application claims the benefit of provisional application No. 60/117,031 filed Jan. 25, 1999.

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a method and a kit for the diagnosis of endometriosis using blood and endometrial leukocyte markers.

(b) Description of Prior Art

Endometriosis is one of the most common gynecological disorders, affecting up to 15% of women within reproductive age. It is closely associated with severe pelvic pain, dysmenorrhea, dyspareunia, infertility and several other symptoms such as intraperitoneal bleeding, back pain, constipation and/or diarrhea. It is a major threat to physical, psychological and social integrity of the patients.

Endometriosis is characterized by the implantation and growth of endometrial cells (which normally constitute the lining of the uterus) in extra-uterine sites such as the peritoneal cavity. Although the etiology and pathogenesis of endometriosis remain mainly unclear, the theory of retrograde menstruation is the most widely accepted to explain the presence of ectopic endometrial cells in the peritoneal cavity. However, this phenomenon occurs in most women and, thus, several other factors must be invoked to explain the implantation of endometrial cells and the subsequent development of endometriotic lesions. It is generally believed that initiation of endometriosis implies a complex cascade of events requiring several essential features. Retrogradely seeded endometrial cells must remain viable, be capable of adhering to the mesothelium and of proliferating. Local degradation of the extracellular matrix, as well as extensive vascularization, are also believed to play an essential role in promoting the invasion of the peritoneal cavity by endometrial cells. Furthermore, once implanted, ectopic endometrial cells must have the capacity to counteract the cytolytic action of the immune system. Indeed, this is supported by the observation of several immunological abnormalities in patients with endometriosis.

At present, direct visualization of the endometriotic lesions under surgical procedures (laparascopy or laparotomy) is the golden standard and the only reliable method available to diagnose endometriosis. However, this method is highly invasive (i.e. surgery under general anesthesia), costly (i.e. direct cost and indirect cost due to convalescence) and requires a well-trained surgeon who has the ability to identify endometriotic lesions with a variety of appearances. The type of lesions, their size and their localization will determine the stage of the disease (stage I minimal, stage II mild, stage III moderate, stage IV severe). However, there is still no clear consensus on how these parameters correlate with the stage of the disease and the prognostic of endometriosis. In addition, early or minimal endometriosis (which can involve microlesions) can be hardly diagnosed by surgical methods, as they are unlikely to be detected by direct visualization. Indeed, several studies have reported microscopic endometriotic lesions that were not detected laparoscopically. Because the diagnosis of endometriosis by surgical procedures is difficult, costly and invasive, in some cases, several physicians and patients tend to avoid it or at least seriously delay it. Hence, the length of time between the onset of symptoms and the diagnosis can be as long as 8 to 12 years. The possibility to diagnose endometriosis at an early stage would certainly improve the efficacy of the treatments, and reduce dramatically the number of years during which patients endure acute or chronic pain.

Imaging methods such as transvaginal ultrasound and magnetic resonance imaging have been designed for the diagnosis of endometriosis. However, these techniques can only be reliable for the detection of large (>1 cm diameter) endometriomas lesions detected among a very small proportion of patients with endometriosis. Moreover, the high cost of these techniques has limited their use for the diagnosis of endometriosis.

Serum proteins such as CA-125 and placental protein-14 have been proposed as diagnostic markers for endometriosis. Elevated levels of CA-125 have been observed in serum, menstrual effluent and peritoneal fluid of patients with endometriosis. However, these markers, when used alone, are of very limited value for a diagnosis test. Indeed, these markers are not suitable for screening or diagnostic purposes because they provide poor sensitivity. Furthermore, levels of CA-125 and placental protein-14 vary according to several factors such as the assay, the stage of the disease and the menstrual cycle. Finally these markers are known to be modulated by conditions other than endometriosis.

High concentrations of antibodies to endometrial antigens were found in the serum of patients with endometriosis, and thus were proposed as markers for a diagnostic test (International patent application publications WO 94/28021 and WO 92/18535). However, the levels of specificity and sensibility with these tests remain very low. In most cases, the antigens recognized by these antibodies are still poorly characterized or yet totally unknown.

In U.S. Pat. No. 5,478,725, low levels of $\alpha v\beta 3$ integrin expression in endometrial samples during the secretory phase of the menstrual cycle is described as a predictor of endometriosis in infertile but not in fertile patients with endometriosis. This observation was associated with milder form of endometriosis (stages I and II) only and, thus, is not useful to detect advanced stages of the disease. Moreover, this method yielded a specificity of 91% but a very low sensitivity (38%).

Taking into account that a number immunological abnormalities have been reported in patients with endometriosis, it is conceivable that the proportion of leukocyte populations and/or their activation status may be modulated during the course of the disease and, thus, may provide some diagnostic value. Previous flow cytometric studies have shown that some T lymphocyte subpopulations (CD8+, CD45+/HLADR+, CD45+/CD3+/HLADR+or CD3+/CD25+) can be slightly modulated in the peritoneal fluid of subjects with endometriosis relative to normal controls (Oosterlynck D. J., et al., *Am J reprod. Immunol.*, 31: 25–31, 1994; Becker J. L., et al., *Am J Reprod. Immunol.*, 34: 179–187, 1995; Wu M. Y., et al., *Am. j. Reprod. Immunol.* 35: 510–516, 1996). However, these observations have limited value for the diagnosis of endometriosis because peritoneal fluid collection is an invasive, non-conventional procedure. Proportions of leukocyte populations have also been studied in peripheral blood and endometrium of patients with endometriosis. Wu et al., (supra) have reported a modest but significant decrease in the proportion of CD3+ T lymphocytes expressing either CD69 or CD25 activation marker in the blood of patients with advanced endometriosis but not in patients with mild stage of endometriosis or normal controls. This difference was observed in advanced cases of endometriosis only and it was too modest to be used as a diagnostic marker. In contrast, Oosterlynck et al., (Oosterlynck D. J., et al., *Am J reprod. Immunol.*, 31: 25–31, 1994) and Ho et al. (Ho H. N., et al., *Hum Reprod.*, 97: 2528–2533, 1997) reported no significant difference in term of T lymphocyte subpopulations when comparing endometriosis subjects with normal controls. These inconsistent results may be explained by the very low number of samples tested in these studies.

Several studies have investigated whether leukocytes are also modulated in eutopic endometrium from patients with endometriosis. Results arising from these studies are contradictory, probably due to the fact that in most cases the methods used were only semi-quantitative and the number of samples tested were very low. For instance, by means of immunohistochemistry, Ota et al. (Ota H., et al., *Am J Reprod. Immunol.*, 35: 477–482, 1996) have reported that the number of CD3+, CD4+, or CD8+ T lymphocytes, cells bearing adhesion molecules (i.e. ICAM-1, LFA-1, CD2) or CD68+ cells were upregulated in the endometrium of patients with endometriosis compared with infertile controls. In contrast, several other studies using similar techniques have reported no difference in the proportion of T lymphocyte subsets (Klentzeris L. D., et al., *Eur. J Obstet gynecol Reprod Biol.*, 63:41–47, 1995; Jones R. K., et al., *Fertil Steril*, 66:81–89, 1996). In addition, a decrease in CD3 positive T cells has been shown by flow cytometry analysis but no difference in the proportion of CD4+, CD8+ stromal leukocytes in the endometrium of patients with endometriosis compared with fertile controls. When these observations are tentatively used in a diagnostic test, they give only low levels of sensibility and specificity because of a significant overlap between the groups.

Therefore, the diagnostic methods presented in the literature so far do not solve the problems encountered with the diagnosis of endometriosis by surgical procedures. It thus remains imperative to be provided with a less invasive, cheaper and reliable method that could allow detection of females suffering from endometriosis as early as possible.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a less invasive, cheaper and reliable method that could allow detection of females suffering from endometriosis as early as possible.

In accordance with the present invention there is provided a specific blood and/or endometrial leukocyte marker for endometriosis selected from the group consisting of CD3+, CD4+, CD5+, CD8+, CD13+, CD14+, CD20+, CD36+, CD44+, CD56+, CD57+, CD69+, CD122+, HLADR+, CD16+, CD45RA+, CD45RO+, CD56–CD122+, CD3+CD4–CD69+, CD3–CD8+HLADR–, CD3+CD4+, CD3+CD4–, CD3–CD4–, CD3+CD5+, CD3–CD5+, CD3–CD5–, CD3+CD8+, CD3+CD8–, CD3–CD8–, CD3+CD16+, CD3–CD16+, CD3+CD16–, CD3+CD20–, CD3–CD20–, CD3+CD44–, CD3–CD44+, CD3–CD44–, CD3+CD45RA–, CD3–CD45RA–, CD3+CD45RA+, CD3+CD45RO+, CD3–CD45RO+, CD3+CD45RO–, CD3+CD56–, CD3–CD56–, CD3+CD57–, CD3–CD57+, CD3–CD57–, CD3+CD69–, CD3+CD69+, CD3–CD69+, CD3+CD122–, CD3+HLADR+, CD3+HLADR–, CD3–HLADR+, CD3–HLADR–, CD4+CD13+, CD4+CD13–, CD4–CD13+, CD4+CD14–, CD4–CD14–, CD4–CD16–, CD4–CD36+, CD4+CD45RA–, CD4–CD45RA–, CD4+CD45RO+, CD4+CD45RO–, CD4–CD45RO+, CD4+CD69–, CD4–CD69+, CD4–CD69–, CD4+HLADR–, CD4-HLADR+, CD8–CD44+, CD8–CD44–, CD8+CD69–, CD8+HLADR–, CD8–HLADR–, CD13+CD16–, CD13–CD16+, CD13+CD44+, CD13–CD44–, CD13+CD45RO–, CD13–CD45RO+, CD13–CD69+, CD13–CD122+, CD13–CD122–, CD13+HLADR+, CD13–HLADR+, CD14+CD13+, CD14+CD13–, CD14+CD16–, CD14+CD44+, CD14–CD44–, CD14+CD45RO+, CD14–CD69–, CD14+CD122–, CD14+HLADR+, CD14–HLADR+, CD20–CD5+, CD20–CD5–, CD20+CD44–, CD20–CD44+, CD20–CD44–, CD20–CD69+, CD20–CD69–, CD20+HLADR+, CD20–HLADR+, CD20–HIADR–, CD36–HLADR+, CD56–CD16+, CD56–CD16–, CD56–CD44–, CD56+CD69–, CD56–CD69+, CD56–CD69–, CD56+CD122+, CD56+CD122–, CD56–CD122–, CD56+HLADR+, CD57–CD44+, CD57–CD44–, CD3–CD4–CD44+, CD3–CD4+CD45RA–, CD3–CD4–CD45RA+, CD3–CD4–CD45RA–, CD3–CD4–CD45RO+, CD3–CD8–CD44+, CD3+CD8+CD69–, CD3+CD8+HLADR–, CD3+CD8–HLADR+, CD3+CD8–HLADR–, CD3–CD8–HLADR–, CD3+CD20–CD5+, CD3+CD20–CD5–, CD3–CD20–CD5–, CD3+CD56+CD16+, CD3+CD56–CD16+, CD3+CD56–CD16–, CD3–CD56+CD16+, CD3–CD56+CD16–, CD3+CD56–CD44+, CD3+CD56–CD44–, CD3+CD56–CD122+, CD3+CD56–CD122–, CD3–CD56+CD122+, CD3–CD56–CD122–, CD3–CD56–HLADR–, CD3–CD57+CD44–, CD3–CD57–CD44+, CD3–CD57–CD44–, CD3+CD57–HLADR+, CD4–CD13+CD16+, CD4–CD13–CD16+, CD4–CD13–CD16–, CD14+CD13+CD16b+, CD14+CD13+CD16b–, CD14+CD13–CD16b–, CD14–CD13–HLADR+, CD14–CD13–HLADR–, CD14+CD20+CD44+, CD14+CD20+CD44–, CD14+CD20–CD44+, CD14+CD20–CD44–, CD14–CD20+CD44–, ratio CD3/CD45RO, Ratio CD13/CD3, Ratio CD13/CD8, Ratio CD14/CD3, and Ratio CD14/CD8.

Also in accordance with the present invention, there is provided a diagnostic method for the detection of endometriosis in a patient sample. The method comprises the step of detecting at least one specific marker as described above, whereby detection of this specific marker is indicative of endometriosis.

Further in accordance with the present invention, there is provided a diagnostic method for the detection of endometriosis in a patient sample. The method comprises the step of detecting at least two different surface antigens from blood or endometrial leukocytes, whereby detection of at least two different surface antigens is indicative of endometriosis.

In accordance with a further embodiment of the invention, there is provided a diagnostic method for the detection of endometriosis in a patient sample. The method comprises the step of detecting a specific marker combination for endometriosis as defined above, whereby detection of this combination is indicative of endometriosis.

Further in accordance with the present invention, there is provided a diagnostic kit for the detection of endometriosis. The kit comprises an antibody specific for the specific maker as described above. Preferably, the kit comprises at least two different antibodies, each specific for different surface antigens as defined in the specific marker combination defined above. Most preferably, the specific marker combination of the diagnostic kit is selected from the combination described below in Tables 1 and 2.

For the purpose of the present invention, the following symbol "/" is intended to mean a ratio between an expression in front of the symbol and another expression after the symbol.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 illustrates a predictive algorithm for the diagnosis of endometriosis.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided reliable diagnostic test for endometriosis that is less invasive and less costly than the actual surgical procedure accepted as the golden standard. An extensive study was undertaken by means of flow cytometric analysis, in which the proportion of several blood and endometrial leukocyte subsets was compared in patients with endometriosis and normal controls.

The present invention identifies a series of leukocyte subsets that can be used as markers in a diagnostic test for endometriosis. These leukocyte subsets are defined according to the expression of cell surface antigens. Several cell surface antigens may define the same population of cells, and thus they are included in the present invention.

Any other antibodies or molecules recognizing the same antigen or a different epitope, isoform, subunit, chain, glycosylation or phosphorylation form or an allelic variant of the same antigen, a member of the same complex, or an antigen with the same cell distribution is also included in the present invention.

Further in accordance with the present invention, there is provided examples showing how at least two different surface antigens from blood and/or endometrial leukocytes can be used in combinations in a diagnostic test for endometriosis (Tables 1 and 2).

TABLE 1

Levels of sensibility and specificity provided by several examples of endometrial and/or blood marker combinations used as a diagnostic method for endometriosis

Marker combination	Threshold value[1]	Specificity	Sensibility
Endometrial leukocyte markers			
1.			
CD4+ (<17%)[2]	>67%	90%	67%
CD8+CD69− (<21%)			
CD13+CD45RO− (<17.5%)			
2.			
CD4+ (<15.5%)	>60%	89%	65%
CD8+CD69− (<21%)			
CD56+CD122− (>19%)			
CD3+CD45RA− (<35%)			
CD13+CD45RO− (<17.5%)			
3.			
CD4+ (<17%)	>67%	88%	65%
CD8+CD69− (<21%)			
CD13−CD122+ (>28%)			
CD13+CD45RO− (<17.5%)			
4.			
CD4+ (<17%)	>67%	89%	63%
CD8+CD69− (<21%)			
CD14+CD13−CD16b− (>14.5%)			
5.			
CD3+CD16− (<40%)	>55%	84%	62%
CD13+CD45RO− (<13.5%)			
CD3+ (<40%)			
CD8+ (<20%)			
CD3+CD69+ (<15%)			
6.			
CD3+ (<40%)	>65%	84%	63%
CD3+CD8+ (<16%)			
CD13+CD45RO− (<17.5%)			
CD3−CD20− (>56%)			
7.			
CD3+CD8+ (<16%)	>65%	81%	65%
CD13+CD45RO− (<17.5%)			
CD3+CD5+ (<37%)			
CD3+CD122− (<42.5%)			
CD3−C020− (>56%)			
CD3+CD45RO− (<30%)			
8.			
CD3+CD8+ (<16%)	>60%	82%	64%
CD13+CD45RO− (<17.5%)			
CD3+CD5+ (<37%)			
CD3+CD122− (<42.5%)			
9.			
CD3+CD20-CD5− (>7.7%)	>60%	81%	66%
CD4+CD13− (<20.5%)			
CD56−CD122− (<47%)			
10.			
CD3+CD8+ (<16%)	>60%	80%	65%
CD13+CD45RO− (<17.5%)			
CD4+CD45RA− (<16%)			
CD3+CD45RO− (<30%)			
11.			
CD3+ (<40%)	>35%	79%	67%
CD8+CD69− (<18%)			
CD3−CD4−CD45RO+ (>56%)			
Ratio CD13+/CD3+ (>0.675%)			
CD13+CD45RO− (<21%)			
12.			
CD3+CD8+ (<16%)	>70%	81%	61%
CD13+CD45RO− (<17.5%)			
CD3−CD5− (>54%)			
CD20−CD5+ (<44%)			
13.			
CD8+ (<20%)[2]	>51%	81%	60%
CD5+ (<37%)			
CD3−CD20− (>58%)			
CD3−HLADR− (>54.5%)			
14.			
CD3+CD8+ (<16%)	>60%	81%	60%

TABLE 1-continued

Levels of sensibility and specificity provided by several examples of endometrial and/or blood marker combinations used as a diagnostic method for endometriosis

Marker combination	Threshold value[1]	Specificity	Sensibility
CD13+CD45RO− (<17.5%)			
CD5+ (<40%)			
15.			
CD4+ (<17%)	>50%	76%	71%
CD13−CD122+ (>28%)			
CD8+CD69− (<19.5%)			
CD3+CD45RA− (<37%)			
16.			
CD4+ (<15.5%)	>35%	71%	78%
CD8+CD69− (<21%)			
CD13−CD122+ (>28%)			
CD3+CD45RA− (<35%)			
CD13+CD45RO− (<17.5%)			
17.			
CD4+ (<15.5%)	>40%	70%	78%
CD8+CD69− (<21%)			
CD56−CD122− (<47%)			
CD3+CD45RA− (<35%)			
CD13+CD45RO− (<17.5%)			
18.			
CD3+ (<40%)	>35%	72%	76%
CD4+ (<17%)			
CD3+CD8+ (<16%)			
CD13+CD45RO− (<21%)			
CD3+CD5+ (<37%)			
19.			
CD3+ (<40%)	>40%	74%	74%
CD4+ (<17%)			
CD3+CD8+ (<16%)			
CD13+CD45RO− (<21%)			
20.			
CD3+ (<40%)	>40%	75%	73%
CD3+CD8+ (<16%)			
CD13+CD45RO− (<21%)			
21.			
CD3+ (<40%)	>25%	71%	69%
CD5+ (<40%)			
CD3+CD5+ (<37%)			
CD69+ (<33%)			
CD4−CD69+ (<35%)			
22.			
CD3+ (<40%)	>30%	68%	83%
CD3+CD8+ (<13.5%)			
CD13+CD45RO− (<17.5%)			
23.			
CD3+ (<40%)	>30%	61%	86%
CD3+CD8+ (<16%)			
CD13+CD45RO− (<17.5%)			
24.			
CD3+ (<40%)	>22%	62%	80%
CD3+CD8+ (<16%)			
CD13+CD45RO− (<17.5%)			
CD3−CD20− (>58%)			
CD56−CD16− (<46%)			
25.			
CD3+CD16− (<47.5%)	>45%	66%	79%
CD3−CD4−CD45RO+ (>31.5%)			
CD3+ (<40%)			
CD8+ (<20%)			
CD3+CD69+ (<15%)			
CD13+CD45RO− (<17.5%)			
26.			
CD3+ (<40%)	>40%	66%	75%
CD3−CD45RO+ (>15%)			
Ratio CD13+/CD3+ (>0.675%)			
CD3+CD8+ (<16%)			
CD8+CD69− (<21%)			
27.			
CD3+CD20−CD5− (>7.7%)	>45%	61%	80%
CD4+CD13− (<20.5%)			
CD56−CD122− (<47%)			
CD4+CD45RO− (<16%)			
28.			
CD3+ (<40%)	>20%	61%	86%
CD3+CD8+ (<16%)			
CD13+CD45RO− (<17.5%)			
CD3−CD20− (>58%)			
29.			
CD13+CD45RO− (<17.5%)	>70%	90%	54%
CD4+CD45RA− (<16%)			
CD3+CD122− (42.5%)			
CD8+CD69− (<21%)			
30.			
CD3+CD8+ (<16%)	>70%	84%	60%
CD13+CD45RO− (<17.5%)			
CD4+CD45RA− (<16%)			
CD3+CD45RO− (<30%)			
CD3−CD5− (>54%)			
Blood leukocyte markers			
31.			
CD3−CD5+ (>14.5%)	>55%	66%	60%
CD3−CD45RA− (>14.5%)			
CD3−CD44+ (>13%)			
CD13+ (>17.5%)			
CD3−CD57−CD44 (<41.3%)			
32.			
CD3−CD45RA (>17%)	>22%	61%	64%
CD20−CD44+ (>17%)			
CD20−HLADR+ (>20%)			
CD3−CD4−CD44+ (>40.5%)			
CD36−HLADR+ (<5.6%)			
33.			
CD3−CD45RA− (>14.5%)	>40%	62%	64%
CD3−CD45RO+ (>19%)			
CD20-HLADR+ (>14.5%)			
Blood (in italics) and endometrial leukocyte markers			
34.			
CD57+ (>10%)	>50%	76%	72%
CD14+ (>10%)			
CD3−CD69+ (>17.5%)			
CD3+ (<40%)			
CD4+ (<15.5%)			
CD3+CD8+HLADR− (<35%)			
35.			
CD3−CD69+ (>17.5%)	>33%	70%	79%
CD3+ (<40%)			
CD4+ (<15.5%)			
CD3+CD8+HLADR− (<35%)			
36.			
CD4−CD36+ (>14.4%)	>43%	70%	74%
CD3−CD69+ (>17.5%)			
CD8+ (<20%)			
CD13+ (>29%)			
CD3+ (<40%)			
CD16+ (>27%)			
CD69+ (<33%)			
CD5+ (<40%)			
37.			
CD3−CD45RA− (>14.5%)	>50%	73%	71%
CD3−CD45RO+ (>19%)			
CD20−HLADR+ (>14.5%)			
CD14+CD44+ (>15%)			
CD8+ (<20%)			
CD5+ (<37%)			
CD3−CD20− (>58%)			
CD3−HLADR− (>54.5%)			

[1]Value above which a diagnosis of endometriosis is given.
[2]Cutoff point established for each individual marker.

TABLE 2

Examples of logistic regression models provided by endometrial or blood leukocyte markers for the identification of patients with endometriosis

Marker combination	B value	Threshold value[1]	Specificity	Sensibility	Number of sample tested
Endometrial leukocyte markers					
Combination no. 1					
1. CD3+ (<40%)[2]	−7.9747	>.55	83%	79%	41
2. CD3−CD5− (>60%)	7.2921				
3. CD13+CD45RO−	−0.1410				
(<17.5%)	−1.6259				
4. CD3−CD20− (>58%)	9.5142				
Interaction of 1 to 4					
Constant = 2.0516					
Combination no. 2					
1. CD3+ (<40%)	−6.7753	>.55	74%	73%	67
2. CD3−CD5− (>60%)	5.8240				
3. CD13+CD45RO−	−1.9298				
(<17.5%)	−0.0262				
Interaction 1 to 4	2.8385				
Constant = 2.7910					
Combination no. 3					
1. CD3+CD8+ (<16%)	−0.1308	>.50	84%	72%	51
2. CD13+CD45RO−	−2.6688				
(<17.5%)	−1.1778				
3. CD3+CD5+ (<37%)					
Constant = 3.1417					
Combination no. 4					
1. CD3+ (<40%)	−1.6965	>.50	78%	75%	81
2. Length of menstruation	−1.8160				
(>7 days)	−1.9656				
3. CD13+CD20− (<21%)	10.3064				
4. Pelvic pain[3]					
Constant = 3.1984					
Blood leukocyte markers					
Combination no. 1					
1. CD14+CD44+ (>15%)	0.9298	>0.55	80%	70%	140
2. CD57+ (>10%)	0.7423				
3. CD3−CD45RA− (>12%)	−0.8147				
4. CD14+ (>10%)	0.8629				
Combination no. 2					
1. CD14+ (>10%)	10.5891	>.50	65%	71%	125
2. CD57+ (>10%)	0.7326				
3. CD3+CD69+ (>17.5%)	0.6899				
4. CD3+HLADR+ (<4%)	1.2004				
5. CD3−CD45RA− (>12%)	−0.1137				
Constant = −1.2062					
Combination no. 3					
1. CD14+ (>10%)	1.1994	>.55	76%	75%	142
2. CD57+ (>10%)	0.8080				
3. CD3+HLADR− (<4%)	1.3593				
4. CD3−CD45RA− (>12%)	−0.63				
5. Pelvic pain	2.1506				
6. Length of menstruation	.7489				
(>7 d)					
Constant = −1.771					
Combination no. 4					
1. CD14+ (>10%)	.9727	>.50	71%	78%	141
2. CD57+ (>10%)	.4489				

TABLE 2-continued

Examples of logistic regression models provided by endometrial or blood leukocyte markers for the identification of patients with endometriosis

Marker combination	B value	Threshold value[1]	Specificity	Sensibility	Number of sample tested
3. CD3+CD69+	.8129				
(>17.5%)	1.3368				
4. CD3+HLADR− (<4%)	−0.8805				
5. CD3−CD45RA− (>12%)	2.1574				
6. Pelvic pain	1.5164				
7. Age (>40)					
Constant = −1.7686					

[1]Value above which a diagnosis of endometriosis is given.
[2]Cutoff point established for each individual marker.
[3]presence of pain at any time other than menstruation and intercourse

The predictive models for endometriosis were established according to the following equation:

$$P(r) = \frac{e^{c+B1*(marker1)+B2*(marker2)+\ldots\ Bn*(marker\ n)}}{1+e^{c+B1*(marker1)+B2\pm(marker2)+\ldots\ Bn\pm(marker\ n)}}$$

Where: P(r)=probability of having endometriosis;

c=constant established for a particular combination;

B=coefficient of regression; and

n=total number of markers in the combination.

In the present invention, a series of endometrial and peripheral blood leukocyte subpopulations for which proportions were modulated in patients with endometriosis (stage I–IV;I–II or III–IV) compared with those of normal controls, have been identified. The novelty of the present invention is to use these leukocyte subpopulations, either alone or in combination, as markers for the diagnosis of endometriosis. Moreover, risk factors for endometriosis identified amongst personal information and menstrual characteristics were shown to be of significant value when used in combination with blood or endometrial leukocyte subsets in a predictive test for endometriosis.

Two methods were used for the combination of markers.

Method 1

A cutoff point is established for the proportion of each leukocyte markers in order to obtain the best discrimination between patients with endometriosis and controls. The proportion obtained for each marker is compared to the cutoff point. A positive test result gives a score of 1, whereas a negative test result gives a score of 0. The diagnostic value is obtained by adding the scores of all the markers of a particular combination and converting it in percentage. The final diagnostic value is then compared to a threshold value that was established to provide the best levels of sensibility and specificity. A positive diagnosis of endometriosis is given when the final diagnostic value exceeds the threshold value established for a particular combination of markers. On the opposite, a negative diagnosis of endometriosis is given when the final diagnostic value is lower than the threshold value (see FIG. **1**).

Method 2

A predictive model for endometriosis is established by including each marker of a particular combination in the following logistic regression equation:

$$P(r) = \frac{e^{c+B1*(marker1)+B2*(marker2)+\ldots\ Bn*(marker\ n)}}{1+e^{c+B1*(marker1)+B2\pm(marker2)+\ldots\ Bn\pm(marker\ n)}}$$

Where:

P(r)=probability of having endometriosis;

c=constant established for a particular combination;

B=coefficient of regression; and

n=total number of markers in the combination.

The probability of having endometriosis (P(r)) is then compared to a threshold value that provides the best discriminative value. A positive diagnosis of endometriosis is given when the P(r) value exceeds the threshold value established for a particular combination of markers. Alternatively, a negative diagnosis of endometriosis is given when the P(r) value is lower than the threshold value.

In the present invention, there is reported a series of 102 endometrial CD45+ leukocyte populations and 93 blood mononuclear CD45+ leukocyte populations which were shown by flow cytometric analysis to be modulated in patients with endometriosis compared with normal controls and, thus are candidate markers for the diagnosis of endometriosis (Tables 3, 4, 5, and 6). An innovative feature of the present invention is to use these markers in combination to increase their level of sensibility and specificity in the diagnostic test.

TABLE 3

Endometrial leukocyte populations proposed as good predictive markers for the identification of patients with endometriosis

	Mean proportion (% ± s.d.) of leukocyte subsets			Number of samples tested		area under				Sen-		
Leukocyte Subsets	Controls	Endo stage I–IV	P[1]	Control	Endo	ROC curve[2]	P[3]	Cutoff point	Specificity	sitivity	Odds ratio	(CI)[4]
CD3+	47.7 ± 12.3	38.7 ± 12.6	3.37 × 10^{-5}	58	88	0.703	3.3 × 10^{-5}	<40	84	55	6.5	(2.9–14.9)
CD4+	18.3 ± 5.6	15.7 ± 5.9	0.008	55	88	0.632	0.008	<17	63	63	3.0	(1.5–6.2)
								<15.5	72	52	2.9	(1.4–6.0)
CD5+	45.1 ± 11.6	36.3 ± 12.3	1.6 × 10^{-4}	46	74	0.702	0.0002	<37	80	53	4.2	(1.8–9.7)
								<40	73	58	3.9	(1.7–8.8)
CD8+	24.3 ± 8.5	18.5 ± 8.6	1.4 × 10^{-4}	54	87	0.688	0.00019	<20	74	62	4.8	(2.3–10.2)
CD3+CD4+	17.2 ± 5.5	14.4 ± 5.7	0.004	55	88	0.641	0.004	<15	67	57	2.7	(1.3–5.5)
CD3+CD4−	29.9 ± 9.6	23.6 ± 9.1	1.2 × 10^{-4}	55	88	0.687	0.00017	<24	80	52	4.4	(2.0–9.6)
CD3−CD4−	51.1 ± 14.2	60.8 ± 12.6	4.1 × 10^{-5}	55	88	0.698	6.9 × 10^{-5}	>61	83	50	5.1	(2.2–11.7)
CD3+CD8+	18.9 ± 7.5	13.7 ± 7.7	1.1 × 10^{-4}	54	84	0.714	2.3 × 10^{-5}	<16	70	70	5.6	(2.6–11.8)
								<13.5	81	54	5.3	(2.3–11.9)
CD3+CD8−	26.1 ± 7.8	23.1 ± 7.1	0.022	54	84	0.609		<23.5	68	51	2.2	(1.1–4.7)
CD3−CD8−	49.6 ± 12.0	58.2 ± 13.0	1.3 × 10^{-4}	54	84	0.688	1.9 × 10^{-4}	>53.5	70	63	4.0	(1.9–8.5)
CD3+CD69+	20.4 ± 9.6	15.5 ± 8.0	0.003	44	76	0.642	0.010	<15	67	53	2.4	(1.1–5.2)
CD3+CD122−	41.4 ± 10.0	34.4 ± 12.4	0.011	29	53	0.669	0.012	<42.5	64	76	2.9	(1.1–7.5)
CD3+HLADR−	38.1 ± 10.3	30.6 ± 12.3	2.8 × 10^{-4}	51	80	0.681	0.0005	<35	72	63	4.0	(1.8–8.5)
CD3−HLADR−	46.4 ± 13.0	55.6 ± 13.5	1.9 × 10^{-4}	51	80	0.693	0.0002	>54.5	80	51	4.1	(1.8–9.3)
CD3+CD45RA+	7.4 ± 4.7	5.7 ± 3.0	0.018	56	85	0.608	0.030	<4.9	77	40	2.2	(1.0–4.7)
CD3+CD45RA−	40.3 ± 11.2	32.7 ± 12.0	2.5 × 10^{-4}	56	85	0.684	0.0002	<37	69	66	4.7	(2.2–9.7)
								<35	73	60	4.1	(1.9–8.5)
CD3−CD45RA−	31.4 ± 12.7	39.6 ± 13.4	4.2 × 10^{-4}	56	85	0.667	8.2 × 10^{-4}	>32	51	69	2.3	(1.1–4.6)
CD3+CD45RO−	31.0 ± 11.1	25.0 ± 9.8	0.002	50	73	0.661	0.002	<28	65	62	3.1	(1.4–6.6)
								<30	55	70	2.7	(1.3–5.7)
CD3+CD16−	45.5 ± 12.1	37.2 ± 13.1	2.1 × 10^{-4}	57	83	0.680	0.0003	<38	80	49	4.5	(2.0–9.9)
								<40	75	58	4.2	(2.0–8.9)
								<47.5	40	80	2.6	(1.2–5.6)
CD3+CD56−	46.5 ± 12.2	38.5 ± 12.8	3.2 × 10^{-4}	56	83	0.674	0.00053	<40	78	55	4.6	(2.1–9.9)
CD3+CD5+	41.9 ± 11.4	33.3 ± 12.3	2.1 × 10^{-4}	45	74	0.695	0.00036	<37	77	61	4.7	(2.1–10.9)
CD3−CD5−	50.6 ± 12.0	59.0 ± 12.9	0.001	45	74	0.690	0.00052	>60	82	51	4.6	(1.9–11.2)
								>54	66	66	4.3	(1.9–9.6)
CD4+CD69−	16.4 ± 4.8	13.8 ± 5.1	0.012	37	72	0.648	0.012	<14	78	53	4.1	(1.6–10.1)
CD4+CD45RA−	16.7 ± 5.3	14.2 ± 5.7	0.010	54	85	0.632	0.009	<16	62	66	3.3	(1.6–6.7)
CD8+CD69−	24.0 ± 7.9	18.9 ± 8.3	0.007	30	59	0.687	0.004	<18	83	53	5.9	(1.9–17.6)
								<19.5	76	59	5.1	(1.9–13.9)
								<21	65	68	4.2	(1.6–10.7)
CD8+HLADR−	23.3 ± 7.7	18.1 ± 8.5	0.001	49	79	0.673	0.001	<18	77	54	4.1	(1.8–9.2)
CD8−HLADR−	61.6 ± 9.5	68.1 ± 9.5	2.2 × 10^{-4}	49	79	0.675	0.0009	>61.5	52	77	3.8	(1.7–8.3)
CD13−CD122+	27.0 ± 9.8	33.6 ± 18.7	0.031	32	58	0.605		>28	64	59	2.5	(1.0–6.2)
CD13−CD122−	47.1 ± 14.4	40.4 ± 15.0	0.043	32	58	0.635	0.035	<46	58	64	2.6	(1.1–6.2)
CD20−CD5+	44.6 ± 12.0	36.4 ± 12.6	0.001	41	66	0.681	0.002	<41	60	62	2.6	(1.2–5.7)
								<44	57	71	3.5	(1.5–7.9)
CD20−CD5−	52.0 ± 12.8	60.9 ± 12.9	0.001	41	66	0.692	0.0009	>60	77	50	3.6	(1.5–8.6)
CD56−CD16+	22.0 ± 12.2	27.0 ± 16.6	0.044	56	84	0.571						
CD56−CD16−	51.6 ± 12.2	42.8 ± 13.5	1.2 × 10^{-4}	56	84	0.687	1.8 × 10^{-4}	<46	71	57	3.3	(1.6–6.7)
ratio CD3+/CD45RO+	1.5 ± 1.0	1.2 ± 0.7	0.020	51	80	0.626	0.015					
CD14+CD13−	1.4 ± 0.9	2.3 ± 1.8	0.041	21	36	0.639						
CD3+CD20−	44.4 ± 11.2	36.9 ± 13.5	0.024	24	45	0.667	0.023	<40	78	58	4.1	(1.4–12.3)
CD3−CD20−	52.3 ± 11.5	61.0 ± 14.9	0.016	24	45	0.669	0.022	>58	83	53	6.2	(1.8–21.2)
								>56	70	60	4.5	(1.4–13.5)
CD3−CD4−CD45RA+	40.1 ± 13.7	34.9 ± 15.1	0.046	51	79	0.618	0.023					
CD3−CD4−CD45RA−	57.9 ± 14.1	63.3 ± 15.2	0.042	51	79	0.620	0.021					
CD3+CD8+CD69−	40.4 ± 10.2	35.1 ± 11.7	0.039	29	56	0.635	0.042	<34.5	75	52	3.4	(1.2–9.2)
CD3+CD8+HLADR−	39.5 ± 9.2	33.6 ± 11.5	0.003	48	74	0.665	0.002	<35	72	55	3.5	(1.6–7.7)
CD3+CD8−HLADR−	43.1 ± 7.5	46.3 ± 10.9		48	74	0.603		>47	69	50	2.2	(1.0–4.7)
CD3−CD8−HLADR−	76.7 ± 8.9	80.3 ± 7.9	0.021	48	74	0.597						
CD14+CD13−CD16b−	19.8 ± 16.4	33.4 ± 22.9	0.026	19	36	0.703	0.014	>23	68	69	4.9	(1.4–16.3)
								>14.5	53	83	4.6	(1.4–15.6)
CD4+CD14−	20.7 ± 7.8	14.8 ± 5.8	0.014	14	23	0.738	0.017	<16.6	79	65	6.9	(1.5–32.0)
CD4−CD14−	75.7 ± 8.1	81.0 ± 5.7	0.025	14	23	0.711	0.033	>76	57	83	6.3	(1.4–28.7)
CD4+HLADR−	16.0 ± 5.7	12.3 ± 4.1	0.018	14	30	0.715	0.023	<17	57	87	8.7	(1.9–38.6)
CD13−CD69+	54.2 ± 14.3	42.3 ± 18.2	0.039	14	30	0.705	0.030	<51	71	70	5.8	(1.4–23.6)
CD13+CD45RO−	22.1 ± 8.8	15.5 ± 10.7	0.009	25	54	0.746	4.6 × 10^{-4}	<17.5	76	70	7.5	(2.5–22.3)
								<21	52	80	4.2	(1.5–11.8)
CD56−CD122−	49.0 ± 12.2	42.8 ± 15.8		29	51	0.631		<47	65	69	3.6	(1.4–9.3)
CD3+CD69−	26.4 ± 7.0	23.3 ± 8.4	0.036	44	76	0.612	0.041	<27	57	67	2.2	(1.0–4.8)
CD4+CD45RO−	14.9 ± 5.3	13.0 ± 6.2		47	74	0.615	0.034	<16	51	72	2.6	(1.2–5.6)
CD56+CD122+	3.5 ± 2.2	2.6 ± 1.7		29	51	0.651	0.025	<3.0	55	73	3.3	(1.3–8.5)
CD3−CD56+CD16+	8.2 ± 3.8	6.8 ± 4.0	0.049	53	78	0.615	0.026	<6.5	72	55	3.0	(1.4–6.2)
CD3−CD56+CD122+	3.6 ± 2.0	2.6 ± 1.8	0.033	27	49	0.638	0.048	<2.7	63	65	3.2	(1.2–8.5)

TABLE 3-continued

Endometrial leukocyte populations proposed as good predictive markers for the identification of patients with endometriosis

Leukocyte Subsets	Mean proportion (% ± s.d.) of leukocyte subsets: Controls	Endo stage I–IV	P[1]	Number of samples tested: Control	Endo	area under ROC curve[2]	P[3]	Cutoff point	Specificity	Sensitivity	Odds ratio	(CI)[4]
CD14+CD13+	3.8 ± 1.9	3.0 ± 2.6		21	36	0.672	0.032	<2.3	81	53	4.8	(1.3–16.9)
CD3+CD20−CD5+	93.0 ± 2.7	88.0 ± 13.8		24	40	0.659	0.034	<91.5	79	55	4.6	(1.4–14.9)
CD4−CD13+CD16+	31.0 ± 14.0	22.4 ± 18.9		16	36	0.699	0.023					
CD69+	41.8 ± 12.9	38.8 ± 17.8		43	78	0.557		<33	81	41	3.0	(1.2–7.4)
ratio CD13+/CD3+	0.56 ± 0.54	0.78 ± 0.71		46	78	0.596		>0.68	80	40	2.6	(1.1–6.1)
CD3−CD20−CD5−	88.0 ± 6.9	90.7 ± 5.2		24	40	0.598		>84	37	90	5.4	(1.4–20.3)
CD3+CD20−CD5−	5.1 ± 2.3	9.5 ± 13.6		24	40	0.683	0.015	>7.7	87	50	9.9	(2.1–48.1)
CD4+CD13−	17.5 ± 6.9	15.8 ± 5.4		36	63	0.594		<20.5	42	86	4.3	(1.6–11.3)
CD3+CD44−	41.7 ± 12.0	38.3 ± 13.6		31	56	0.596		<37.8	74	50	2.9	(1.1–7.5)
CD56+	26.2 ± 12.5	30.2 ± 17.2		57	87	0.562		>32	81	41	2.9	(1.3–6.5)
CD13−CD45RO+	21.4 ± 8.7	26.3 ± 11.3		25	54	0.625		>28	80	45	3.2	(1.0–9.8)
CD56+CD69−	19.6 ± 12.9	24.3 ± 14.3		33	53	0.610		>26	85	40	3.4	(1.1–10.2)
CD13−CD16+	8.0 ± 7.5	6.7 ± 3.3		39	71	0.562		<6	72	51	2.6	(1.1–6.1)
CD56+CD122−	21.8 ± 11.8	28.7 ± 17.3		29	51	0.621		>19	59	71	3.1	(1.2–7.9)
								>18	55	72	3.3	(1.3–8.5)
CD3+CD4−CD69+	37.2 ± 10.0	34.0 ± 13.8		34	66	0.572		<33.5	73	49	2.6	(1.1–6.4)
CD4−CD13−CD16+	8.5 ± 3.6	8.7 ± 10.8		16	36	0.642		<7.1	75	58	4.2	(1.1–15.6)
CD4−CD13−CD16−	54.5 ± 13.0	62.2 ± 21.4		16	36	0.655		>65	81	56	5.4	(1.3–22.3)
CD14+CD13+CD16b+	11.0 ± 12.5	6.5 ± 6.2		19	36	0.616		<16	32	94	7.8	(1.4–43.9)
CD4−CD69−	40.7 ± 12.9	46.4 ± 18.6		37	72	0.593		>47	76	47	2.8	(1.2–6.7)
CD3−CD45RO+	23.0 ± 12.5	27.1 ± 16.3		50	73	0.556		>15	34	80		
CD4−CD69+	39.3 ± 11.8	37.7 ± 18.0		37	72	0.462		<35	68	46		
CD3−CD4−CD45RO+	41.3 ± 18.1	43.4 ± 20.9		43	72	0.530		>31.5	33	71		
								>56	81	28		

[1]P value (when ≦0.05) obtained in a student "t" test when mean proportion found in patients with endometriosis stage I–IV was compared to normal controls.
[2]Discriminative value of each marker established by area under ROC curve.
[3]P value (when ≦0.05), significance of area under ROC curve.
[4]Confidence interval for odds ratio.

TABLE 4

Peripheral blood leukocyte populations proposed as good predictive markers for the identification of patients with endometriosis

Leukocyte Subsets	Mean proportion (% ± s.d.) of leukocyte subsets: Control	Endo. Stage I–IV	P[1]	Number of samples tested: Control	Endo. Stage I–IV	area under curve[2]	P[3]	Cutoff point	Specificity	Sensitivity	Odds ratio	(CI)[4]
CD3+	66.6 ± 8.5	64.5 ± 8.7	0.032	132	172	0.570	0.037					
CD8+	17.3 ± 5.2	16.4 ± 4.8		129	172	0.549		<18.9	33	77	1.7	(1.0–2.8)
CD13+	16.0 ± 6.0	17.6 ± 6.5	0.039	122	155	0.575	0.032	>17.5	63	51	1.8	(1.1–2.9)
CD14+	11.8 ± 4.9	13.4 ± 6.0	0.020	124	167	0.575	0.029	>10	45	71	2.0	(1.3–3.3)
CD20+	5.7 ± 3.1	4.8 ± 2.3	0.006	124	162	0.582	0.017	<6	39	74	1.8	(1.1–3.0)
CD36+	15.7 ± 6.8	17.2 ± 7.3		112	140	0.560		>19	77	37	2.1	(1.2–3.6)
CD44+	17.1 ± 5.6	19.1 ± 6.5	0.009	113	148	0.585	0.018	>18.5	61	51	1.7	(1.0–2.7)
CD57+	8.0 ± 3.9	9.2 ± 4.9	0.023	114	148	0.569		>10	75	39	1.8	(1.1–3.1)
CD69+	19.4 ± 8.2	21.0 ± 7.1		109	144	0.590	0.014	>21.5	71	45	2.0	(1.2–3.4)
CD122+	29.2 ± 8.4	31.2 ± 11.7		122	166	0.567		>34	74	42	2.1	(1.2–3.4)
CD3+CD5+	66.6 ± 8.5	63.7 ± 10.4	0.017	115	146	0.586	0.017	<69	44	70	1.8	(1.1–2.9)
CD3+CD45RA−	39.3 ± 9.5	37.2 ± 8.3	0.044	124	168	0.583	0.015	<42	40	72	1.7	(1.1–2.8)
CD3+CD56−	65.3 ± 8.8	63.2 ± 8.6	0.035	126	169	0.571	0.037	<68	42	71	1.8	(1.1–2.9)
CD3+CD57−	63.7 ± 8.3	60.7 ± 9.7	0.009	113	146	0.592	0.011	<67	40	77	2.3	(1.3–3.9)
CD3+CD69−	60.1 ± 9.6	57.9 ± 9.2		107	141	0.584	0.023					
CD3+CD122−	62.2 ± 8.4	59.6 ± 9.8	0.021	121	164	0.578	0.024	<58	69	42	1.6	(1.0–2.7)
CD3+HLADR+	3.9 ± 1.4	3.5 ± 1.2	0.006	121	154	0.601	0.004	<4	40	77	2.2	(1.3–3.7)
CD3−CD5+	15.5 ± 5.3	18.3 ± 8.4	0.003	115	146	0.619	0.001	>14.5	50	72	2.5	(1.5–4.2)
CD3−CD16+	23.3 ± 7.9	25.4 ± 8.7	0.036	123	166	0.572	0.037					
CD3−CD44+	13.1 ± 5.3	15.6 ± 6.3	0.001	111	143	0.615	0.002	>11.5	44	73	2.2	(1.4–3.7)

TABLE 4-continued

Peripheral blood leukocyte populations proposed as good predictive markers for the identification of patients with endometriosis

	Mean proportion (% ± s.d.) of leukocyte subsets			Number of samples tested		area				Sen-		
Leukocyte Subsets	Control	Endo. Stage I–IV	P[1]	Con-trol	Endo. Stage I–IV	under curve[2]	P[3]	Cutoff point	Specif-icity	si-tivity	Odds ratio	(CI)[4]
CD3−CD57+	4.2 ± 2.5	5.0 ± 3.1	0.023	113	146	0.573	0.044					
CD3−CD69+	14.2 ± 5.6	16.4 ± 6.5	0.006	107	141	0.602	0.006	>17.5	75	41	2.1	(1.2–3.6)
CD3−CD45RO+	16.0 ± 5.6	18.1 ± 6.8	0.006	117	148	0.595	0.008	>19	77	44	2.6	(1.5–4.5)
CD3−CD4−	31.1 ± 9.5	33.3 ± 9.0	0.043	132	171	0.566	0.050					
CD3−CD8−	33.4 ± 9.2	35.5 ± 10.0		122	166	0.568	0.050					
CD3−CD45RA−	14.2 ± 5.2	16.2 ± 6.2	0.004	124	168	0.595	0.006	>14.5	61	60	2.3	(1.4–3.7)
CD3−CD56−	21.6 ± 6.3	23.1 ± 7.0		126	169	0.562		>25	73	39	1.7	(1.0–2.8)
CD4−CD13+	14.5 ± 6.0	16.3 ± 6.8	0.030	108	131	0.586	0.023	>16.5	69	51	2.4	(1.4–4.1)
CD4−CD36+	13.8 ± 7.4	19.0 ± 6.2	0.037	13	21	0.771	0.009	>19	92	62	19.5	(2.1–179.9)
CD4−CD69+	16.0 ± 6.1	18.1 ± 6.5	0.021	94	120	0.603	0.009	>19	75	45	2.4	(1.3–4.3)
CD4−CD45RO+	23.4 ± 6.7	27.0 ± 7.7	0.043	27	50	0.620						
CD4−CD45RA−	22.7 ± 6.5	24.4 ± 8.3		125	168	0.562						
CD8−CD44+	16.8 ± 5.5	18.9 ± 6.7	0.017	88	119	0.588	0.030					
CD8−CD44−	66.4 ± 7.0	64.4 ± 6.6	0.039	88	119	0.584	0.040	<68	48	70	2.1	(1.2–3.7)
CD13+CD44+	13.3 ± 5.3	15.4 ± 5.9	0.006	96	121	0.605	0.008	>11	38	76	1.9	(1.1–3.4)
CD13+HLADR+	13.0 ± 5.3	14.7 ± 6.1	0.024	108	135	0.581	0.031	>15.5	71	42	1.8	(1.1–3.1)
CD13+CD16−	1.4 ± 0.9	3.7 ± 4.6	0.005	21	37	0.727	0.004	>2	76	57	4.2	(1.3–13.9)
CD13−HLADR+	8.2 ± 3.2	7.0 ± 3.0	0.005	108	135	0.619	0.001	<8	50	70	2.4	(1.4–4.0)
CD13−CD44−	79.9 ± 6.2	76.9 ± 8.6	0.004	96	121	0.606	0.007	<82	39	76	2.0	(1.1–3.6)
CD14+HLADR+	11.0 ± 4.6	12.7 ± 5.9	0.009	109	147	0.587	0.017	>9.5	48	70	2.2	(1.3–3.6)
CD14+CD44+	10.7 ± 4.9	12.9 ± 5.7	0.003	85	123	0.612	0.006	>15	85	33	2.7	(1.3–5.4)
CD14+CD45RO+	12.2 ± 4.4	14.3 ± 6.1	0.004	102	118	0.586	0.028					
CD14+CD16−	0.7 ± 0.8	2.7 ± 5.4	0.023	23	41	0.603						
CD14+CD122−	0.4 ± 0.3	2.4 ± 5.2	0.029	14	34	0.648		>0.7	86	47	5.3	(1.0–27.5)
CD14−HLADR+	10.3 ± 3.3	9.1 ± 2.7	0.001	109	147	0.611	0.002	<8.5	69	47	2.0	(1.2–3.3)
CD14−CD44−	83.5 ± 5.3	81.6 ± 6.3	0.023	85	123	0.588	0.032	<80	79	37	2.2	(1.2–4.2)
CD20+HLADR+	5.2 ± 2.9	4.3 ± 2.1	0.005	106	141	0.596	0.010	<5.5	39	75	1.9	(1.1–3.3)
CD20+CD44−	5.0 ± 2.8	4.2 ± 2.2	0.033	95	126	0.571		<4	62	55	2.0	(1.2–3.4)
CD20−CD44+	14.4 ± 5.1	17.3 ± 6.2	0.0004	95	126	0.636	0.001	>17	74	47	2.5	(1.4–4.4)
CD20−CD69+	16.4 ± 4.3	21.0 ± 6.2	0.016	14	29	0.719	0.021	>16	57	76	4.2	(1.1–16.3)
CD20−HLADR+	15.4 ± 4.9	17.6 ± 6.9	0.004	106	141	0.591	0.014	>14.5	49	70	2.2	(1.3–3.7)
CD20−CD44−	79.5 ± 5.1	77.5 ± 6.1	0.008	95	126	0.590	0.022					
CD20−CD69−	79.4 ± 6.7	75.3 ± 6.5	0.065	14	29	0.711	0.027	<75.6	71	52	11.6	(2.3–57.0)
CD36−HLADR+	7.8 ± 3.2	6.4 ± 2.0	0.0005	95	121	0.629	0.001	<5.6	77	40	2.2	(1.2–4.0)
CD56−CD69+	18.1 ± 4.2	21.8 ± 6.7	0.083	13	23	0.667		>20.5	85	52	6.0	(1.1–33.3)
CD56−CD122+	19.5 ± 5.7	21.4 ± 8.6	0.028	113	154	0.578	0.030	>23	74	40	2.0	(1.2–3.3)
CD56−CD69−	67.9 ± 5.3	62.7 ± 9.4	0.075	13	23	0.671	0.093					
CD56−CD122−	67.7 ± 8.3	64.7 ± 11.5	0.014	113	154	0.586	0.017	<64.5	67	51	2.1	(1.3–3.5)
CD57−CD44−	74.3 ± 6.9	71.7 ± 7.7	0.015	84	117	0.602	0.014	<76	44	71	1.9	(1.1–3.5)
CD3+CD57−HLADR+	4.3 ± 1.5	4.0 ± 1.4		96	118	0.584	0.034	<3.7	72	43	1.9	(1.1–3.5)
CD3−CD4−CD44+	34.4 ± 11.0	38.1 ± 12.6	0.032	88	112	0.577		>40.5	69	45	1.8	(1.0–3.3)
CD3−CD56+CD16−	1.2 ± 0.6	1.4 ± 1.0	0.023	121	163	0.553						
CD3−CD56−CD122−	23.4 ± 11.1	21.2 ± 11.4		113	148	0.581	0.024					
CD3−CD57−CD44−	48.7 ± 10.1	44.5 ± 10.3	0.004	84	114	0.610	0.008	<41.3	80	41	2.8	(1.4–5.3)
CD14+CD20+CD44−	0.2 ± 0.2	0.1 ± 0.1	0.036	65	75	0.613	0.037					
CD14+CD20−CD44+	91.8 ± 4.6	93.5 ± 3.5	0.016	65	75	0.614	0.021	>95	75	43	2.3	(1.1–4.7)
CD14+CD20−CD44−	5.9 ± 4.5	4.6 ± 3.3	0.037	65	75	0.604	0.035	<3	75	43	2.3	(1.1–4.7)
CD14−CD13−HLADR+	8.7 ± 3.5	7.6 ± 2.6	0.021	77	82	0.600	0.029	<7	71	45	2.1	(1.1–4.0)
Ratio CD13+/CD3+	0.25 ± 0.12	0.29 ± 0.14	0.025	121	153	0.583	0.018	>0.30	71	43	1.8	(1.1–3.0)
Ratio CD13+CD8+	1.04 ± 0.55	1.20 ± 0.69	0.039	114	153	0.574	0.039					
Ratio CD14+/CD3+	0.19 ± 0.10	0.22 ± 0.12	0.011	123	165	0.575	0.028	>0.14	40	74	1.9	(1.1–3.1)
Ratio CD14+/CD8+	0.78 ± 0.47	0.91 ± 0.55	0.040	116	164	0.574	0.036					

[1]P value (when ≦0.05) obtained in a student "t" test when mean proportion of leukocyte subsets was compared between patients with endometriosis (stage I–IV) and normal controls.
[2]Discriminative value of each marker established by area under ROC curve.
[3]P value (when ≦0.05), significance of area under ROC curve.
[4]Confidence interval for odds ratio.

TABLE 5

Endometrial leukocyte populations used as markers to discriminate between patients with endometriosis stage I–II or stage III–IV and normal subjects

	Mean proportion (% ± s.d.) of leukocyte subsets					Number of samples tested		
Leukocyte Subsets	Controls	Endo stage I–II	P[1]	Endo stage III–IV	P[2]	Control	Endo I–II	Endo III–IV
CD3−CD44−	50.0 ± 12.9	50.5 ± 11.9		61.8 ± 16.6	0.015	31	43	13
CD3+HLADR+	8.3 ± 5.7	6.1 ± 2.6	0.013	7.6 ± 3.3		51	57	23
CD3+CD45RO+	14.9 ± 8.4	12.0 ± 5.6	0.043	13.2 ± 9.5		50	50	23
CD4+CD13+	2.3 ± 1.9	2.2 ± 1.7		1.4 ± 1.1	0.025	36	44	19
CD13+HLADR+	5.7 ± 2.7	5.3 ± 2.3		3.6 ± 1.3	0.017	27	37	12
CD14−HLADR+	14.7 ± 7.5	10.2 ± 3.9	0.049	10.8 ± 5.1		14	24	4
CD56+HLADR+	2.8 ± 1.7	1.5 ± 0.7	0.011	4.6 ± 3.3		16	17	5
CD56−CD44−	63.5 ± 13.0	63.5 ± 13.4		51.1 ± 20.4	0.023	30	41	12
CD3−CD4+CD45RA−	1.5 ± 0.9	1.5 ± 0.9		1.0 ± 0.7	0.022	51	54	25
CD3+CD8−HLADR+	15.9 ± 8.1	15.8 ± 8.6		23.6 ± 12.1	0.011	48	52	22
CD3−CD8+HLADR−	9.5 ± 6.5	8.6 ± 6.1		6.1 ± 5.2	0.036	48	52	22
CD3+CD56+CD16+	3.0 ± 3.4	2.9 ± 3.2		6.0 ± 5.7	0.027	53	56	22
CD3+CD56−CD16+	6.6 ± 7.5	7.0 ± 6.2		11.0 ± 9.1	0.350	53	56	22
CD3+CD56−CD16−	87.0 ± 8.1	87.2 ± 7.1		79.5 ± 12.9	0.016	53	56	22
CD3+CD56−CD44+	7.0 ± 6.1	6.1 ± 4.8		13.2 ± 10.5	0.025	28	38	12
CD3+CD56−CD44−	87.1 ± 6.1	88.5 ± 6.2		78.0 ± 13.9	0.049	28	38	12
CD3−CD56−HLADR−	29.7 ± 15.6	42.7 ± 19.6	0.050	29.3 ± 21.3		15	17	6
CD3+CD56−CD122+	12.1 ± 7.5	11.9 ± 6.3		21.7 ± 14.0	0.005	27	33	16
CD3+CD56−CD122−	81.9 ± 8.2	83.0 ± 6.8		68.0 ± 21.7	0.024	27	33	16
CD4−CD16−	51.5 ± 12.9	59.1 ± 12.7	0.033	49.3 ± 21.4		24	31	11
CD14+CD13+CD16b−	62.7 ± 20.7	56.2 ± 25.8		38.2 ± 26.4	0.011	19	26	10
CD16+	27.4 ± 12.4	30.0 ± 16.2		35.4 ± 19.5		58	62	23
CD45RA+	28.3 ± 9.1	27.6 ± 9.0		27.9 ± 16.0		56	60	25
CD45RO+	38.4 ± 13.6	38.0 ± 15.5		44.6 ± 18.1		50	54	24
CD13+	24.8 ± 12.7	25.5 ± 14.7		31.3 ± 20.3		47	57	22

Leukocyte Subsets	area under ROC curve	P	Cutoff point	Specificity	Sensitivity	Odds ratio	(CI)[4]
CD3−CD44−	0.766	0.007	>48	52	92	12.9	(1.4–113.8)
CD3+HLADR+	0.587		<5.5	67	48.3		
CD3+CD45RO+	0.565		<15	37.3	71		
CD4+CD13+	0.658		<1.7	50	79	3.8	(1.0–13.8)
CD13+HLADR+	0.740	0.021	<5	54	92	13	(1.4–117.2)
CD14−HLADR+	0.679		<13	57	75		
CD56+HLADR+	0.741	0.018	<1.7	75	71	7.2	(1.5–33.6)
CD56−CD44−	0.708	0.041	<65	58	83	6.8	(1.2–37.5)
CD3−CD4+CD45RA−	0.668	0.021	<0.7	84	40	3.4	(1.1–10.7)
CD3+CD8−HLADR+	0.701	0.009	>14.8	55	73	3.3	(1.1–10.1)
CD3−CD8+HLADR−	0.691	0.013	<5	78	59	5.0	(1.6–15.4)
CD3+CD56+CD16+	0.670	0.024	>1.8	49	77	3.5	(1.0–10.3)
CD3+CD56−CD16+	0.698	0.009	>9	84	50	5.4	(1.7–17.3)
CD3+CD56−CD16−	0.695	0.010	<83	81	50	4.1	(1.4–12.5)
CD3+CD56−CD44+	0.721	0.034	>5	42	92		
CD3+CD56−CD44−	0.668		<81	83	58	7.0	(1.5–33.7)
CD3−CD56−HLADR−	0.706	0.047	>36	73	59		
CD3+CD56−CD122+	0.715	0.024	>20	87	50	6.7	(1.4–31.7)
CD3+CD56−CD122−	0.715	0.024	<76.5	71	63	4.0	(1.1–15.5)
CD4−CD16−	0.659	0.045	>58	76	47		
CD14+CD13+CD16b−	0.753	0.035	<61	69	80	8.8	(1.3–57.4)
CD16+	0.603		>39	85	39	3.5	(1.2–10.5)
			>27	50	51		
CD45RA+	0.562		<23.5	75	48	2.8	(1.0–7.5)
CD45RO+	0.620		>52	88	43	4.4	(1.3–14.4)
CD13+			>29	68	34		

[1]P value obtained in a student "t" test when mean % of leukocyte subsets found in patients with endometriosis stage I–II was compared to normal controls.
[2]P value obtained in a student "t" test when mean % of leukocyte subsets found in patients with endometriosis stage III–IV was compared to normal controls.

TABLE 6

Peripheral blood leukocyte populations used as markers to discriminate between patients with endometriosis stage I–II or stage III–IV and normal subjects

	Mean proportion (% ± s.d.) of leukocyte subsets					Number of samples tested		
Leukocyte Subsets	Controls	Endo stage I–II	P[1]	Endo stage III–IV	P[2]	Control	Endo I–II	Endo III–IV
CD3−CD57+CD44−	12.5 ± 6.2	14.9 ± 7.5	0.030	12.2 ± 6.9		84	81	33
CD14−CD13−HLADR−	85.6 ± 5.0	87.6 ± 3.9	0.016	85.9 ± 3.3		77	54	28
CD14−CD20+CD44−	5.7 ± 2.9	4.7 ± 2.6	0.043	5.6 ± 2.7		65	50	23
HLADR+	21.6 ± 6.0	21.7 ± 6.6		23.8 ± 6.7	0.037	120	102	52
CD3+CD8−	49.9 ± 8.5	49.0 ± 9.2		46.3 ± 8.8	0.012	122	113	53
CD3+CD44−	60.2 ± 8.6	59.2 ± 8.8		57.2 ± 8.0	0.047	111	98	45
CD3+HLADR−	63.3 ± 8.7	63.0 ± 9.1		59.6 ± 7.9	0.009	121	102	52
CD3−HLADR+	18.1 ± 5.8	18.7 ± 6.5		20.8 ± 6.9	0.010	121	102	52
CD3+CD16+	9.3 ± 13.3	8.4 ± 12.1		6.0 ± 4.3	0.016	123	113	53
CD3−CD57−	28.3 ± 6.9	29.0 ± 7.2		32.3 ± 9.5	0.004	113	101	45
CD4−HLADR+	19.8 ± 6.0	19.8 ± 6.2		22.0 ± 6.6	0.036	112	96	49
CD4+CD45RA−	31.4 ± 8.1	29.7 ± 7.2		27.7 ± 8.5	0.006	125	114	54
CD4+CD45RO+	19.5 ± 5.6	20.5 ± 6.4		16.2 ± 4.3	0.029	27	31	19
CD13−CD16+	18.9 ± 8.2	16.8 ± 8.7		14.3 ± 3.1	0.035	21	28	9
CD14−CD69−	81.0 ± 6.1	77.4 ± 9.1		76.0 ± 6.7	0.031	20	32	14
CD20−HLADR−	79.3 ± 5.2	78.5 ± 7.0		77.1 ± 6.6	0.025	106	97	44
CD57−CD44+	17.2 ± 5.5	18.3 ± 6.4		19.7 ± 7.7	0.046	84	82	35
CD3−CD4+CD45RA−	6.9 ± 4.4	6.4 ± 4.0		5.4 ± 3.5	0.043	122	109	50
CD3−CD8−CD44+	37.2 ± 11.9	37.2 ± 12.7		43.2 ± 12.6	0.019	87	80	32
CD3−CD57−CD44+	38.5 ± 12.1	40.3 ± 12.5		44.0 ± 12.7	0.029	84	81	33
CD14+CD20+CD44+	2.1 ± 1.3	2.1 ± 1.5		1.4 ± 0.7	0.001	65	52	23

Leukocyte Subsets	area under ROC curve	P	Cutoff point	Specificity	Sensitivity	Odds ratio	(CI)[4]
CD3−CD57+CD44−	0.586		>15.6	74	43	2.1	(1.1–4.1)
CD14−CD13−HLADR−	0.637	0.008	>88.3	74	47	2.6	(1.1–5.3)
CD14−CD20+CD44−	0.607	0.049	<4.5	66	53	2.2	(1.0–4.7)
HLADR+	0.586		>24.5	73	45	2.2	(1.1–4.5)
CD3+CD8−	0.654	0.002	<51.5	48	85	5.2	(2.2–12.1)
CD3+CD44−	0.605	0.047	<61.2	51	73	2.8	(1.3–6.1)
CD3+HLADR−	0.628	0.010	<63.5	55	73	3.2	(1.5–6.6)
CD3−HLADR+	0.607	0.030	>21.5	73	45	2.3	(1.1–4.6)
CD3+CD16+	0.528		>3.7	31	81		
CD3−CD57−	0.644	0.007	>28	53	71	2.7	(1.2–5.7)
CD4−HLADR+	0.588		>19.3	48	67		
CD4+CD45RA−	0.625	0.010	<29.5	60	60	2.3	(1.2–4.5)
CD4+CD45RO+	0.645		<19.8	50	90	8.5	(1.6–44.5)
CD13−CD16+	0.698		<13	86	56	7.5	(1.2–45.1)
CD14−CD69−	0.729	0.025	<82	50	86	6.0	(1.1–34.0)
CD20−HLADR−	0.573		<80.3	41	72		
CD57−CD44+	0.573		>21.6	80	40	2.7	(1.1–6.7)
CD3−CD4+CD45RA−	0.587		<5.5	59	61	2.3	(1.1–4.5)
CD3−CD8−CD44+	0.588		>40.5	54	63		
CD3−CD57−CD44+	0.575		>44	63	58		
CD14+CD20+CD44+	0.647		<2.1	39	91	6.4	(1.3–30.9)

[1]P value obtained in a student "t" test when mean proportion found in patients with endometriosis stage I–II was compared to normal controls.
[2]P value obtained in a student t test when the % leukocyte subsets found in patients with endometriosis stage III–IV was compared to normal controls.

Cutoff points established for each individual marker are presented in Table 3, 4, 5, 6 and threshold value established for a particular marker combination are presented in Table 1. Any other cutoff points or threshold values providing a valuable diagnostic test for endometriosis are meant to be included in the present invention

In accordance with a preferred embodiment of the present invention, there is provided a series of 34 different combinations of endometrial leukocyte markers (Tables 1 and 2), 7 combinations of blood leukocyte markers (Tables 1 and 2) and 4 combinations of endometrial and blood leukocyte markers providing a diagnostic test with levels of sensibility and specificity up to 89 and 90%, respectively. The different marker combinations of the present invention may serve several important clinical needs. Hence in the general population, these markers could be used to evaluate the risk factor to develop endometriosis or to identify women with high likelihood of suffering from the disease. Furthermore in patients with endometriosis, these markers could serve to monitor the disease or to give a prognosis.

Study Subjects and Samples

Uterine endometrial tissues were obtained from 146 subjects undergoing laparoscopy or laparotomy. The experimental group was formed of up to 88 subjects with endometriosis stage I–IV confirmed by laparoscopy or laparotomy and the control group consists of up to 58 healthy subjects who underwent surgery for tubal ligation (or reanastomosis) and had no clinical evidence, nor family history of endometriosis. Table 7 gives details concerning the age, menstrual cycle and indication of laparoscopy or laparotomy for the subjects included in experimental and control groups.

Stromal Cell Preparation From Endometrial Samples

Endometrial tissue samples were mechanically disrupted with a Pyrex™ glass Broeck tissue grinder (Fisher) to obtain a single cell suspension. Stromal cell fraction was isolated by filtration through a 250 μm stainless steel sieve (Millipore) to retain the glandular fraction and was washed twice with 10 ml phosphate buffered saline (PBS) (Sigma) containing 1% BSA (Boehringer Mannheim), 0.1% sodium azide (Fisher) (thereafter called PBS washing buffer).

TABLE 7

Description of the experimental groups used in the analysis of endometrial leukocyte populations

Experimental groups	Number of subjects	Mean age ± s.d.	Menstrual cycle		Percentage of patients* Indication of laparoscopy			
			ES[1*]	LS[2*]	ligation or reanastomosis	Hysterectomy and/or ovariectomy	Diagnostic laparoscopy	Other**
Controls	58	34.2 ± 5.3	54.5%	45.5%	100%			
Endometriosis								
Stage I–IV	88	34.4 ± 6.8	47.0%	53.0%	21.6%	22.7%	52.3%	3.4%
Stage I–II	63	34.4 ± 7.3	50.9%	49.1%	28.6%	22.2%	47.7%	1.5%
Stage III–IV	25	34.4 ± 5.4	36.4%	63.6%	4.0%	24.0%	64.0%	8.0%

[1]Early secretory (days 14–21)
[2]Late secretory (days 22–28)
*% patients among control or endometriosis groups

Endometrial biopsies were taken with a Pipet Curette (Milex) (approximately 0.5 g of tissue). All samples were harvested in the secretory phase (day 14–28) of the menstrual cycle as confirmed by histological evaluation. The samples were collected into sterile RPMI-1640 medium (Gibco) supplemented with 2% heat-inactivated fetal calf serum (Bio-Media) and 1% penicillin-streptomycin and kept at 4° C. until cell isolation.

Blood samples were obtained from up to 172 subjects with endometriosis (stage I–IV) confirmed by laparascopy or laparotomy and from up to 132 healthy subjects with no evidence of endometriosis at surgery, and no family history of endometriosis. Blood samples (30 ml) were collected in heparin-tubes (Vacutainer™, Becton Dickinson) and kept at 20° C. until mononuclear cell separation. The age, menstrual dating and indication for laparoscopy of the subjects included in the study are given in Table 8.

Isolation of Mononuclear Cells From Peripheral Blood

Blood samples were diluted 1:1 with Hank's Balanced Salt Solution (HBSS) (Gibco), layered on an equal volume of Ficoll-Paque™ (Pharmacia Biotech) and centrifuged at 1500 rpm for 40 minutes at room temperature. Leukocytes were isolated at the interface of Ficoll and HBSS and they were washed in 50 ml of HBSS. Contaminating red blood cells were lysed with 6 ml of ammonium chloride solution (0.15M) (6 minutes at room temperature). The peripheral blood mononuclear cells were then washed twice in 10 ml PBS and resuspended in PBS washing buffer.

Endometrial and Peripheral Blood Leukocyte Surface Antigen Staining

Endometrial stromal cells or peripheral blood mononuclear cells were distributed in 5 ml tubes (1 to 1.5×10^6

TABLE 8

Description of the subjects included in the study of peripheral blood mononuclear leukocytes

Experimental groups	Number of subjects	Mean age ± s.d.	Menstrual cycle		Percentage of patients* Indication of laparoscopy			
			Proliferative*	Secretory*	ligation or reanastomosis	Hysterectomy and/or ovariectomy	Diagnostic laparoscopy	Other
Control	132	34.30 ± 5.5	43.8%	56.2%	100%			
Endometriosis								
Stage I–IV	172	36.40*	42.8%	57.2%	22.1%	33.7%	38.9%	5.3%
Stage I–II	116	35.96 ± 6.39	41.0%	59.0%	31.1%	30.2%	37.1%	1.6%
Stage III–IV	56	34.30 ± 5.5	46.2%	53.8%	3.6%	41.1%	42.9%	12.4%

*% of patients amongst control or endometriosis groups

cells/tube) or in 96 well plates (5×10^5 cells/well), respectively and incubated in the presence of 0.1 μg of human γ-globulin for 5 minutes at room temperature. The cells were then incubated 30 minutes in the dark (at room temperature for endometrial cells and at 4° C. for peripheral blood mononuclear cells) with a panel of 4 different mouse monoclonal antibodies (MAbs) in a total volume of 100 μl. The cell samples were stained with mouse anti-human CD45 MAbs conjugated to peridinin chlorophyl protein (PerCP) and with several sets of three different mouse MAbs labeled with distinct fluorochromes (fluorescein isothiocyanate —FITC—, phycoerythrin —PE or with phycoerythrin-texas red —ECD—) directed toward cell surface markers for specific cell populations such as T lymphocytes, B lymphocytes, NK cells, macrophages and/or activation markers (Table 9).

TABLE 9

Description of mouse monoclonal antibodies used for immunophenotyping

Specificity	Clone	Isotype	Supplier	Fluoro-chrome
CD3	HIT3A	mouse IgG2a	Beckman/Coulter	ECD
CD4	SK3	mouse IgG1	Becton Dickinson	PE
CD5	BL1A	mouse IgG2a	Beckman/Coulter	FITC
CD8	SK1	mouse IgG1	Becton Dickinson	PE
CD13	SJ1D1	mouse IgG1	Beckman/Coulter	RPE
CD14	RM052	mouse IgG2a	Beckman/Coulter	PE
CD16	NKP15	mouse IgG1	Becton Dickinson	FITC
CD16B	1D3	mouse IgM	Beckman/Coulter	FITC
CD20	H299	mouse IgG2a	Beckman/Coulter	RDI
CD36	SMf	mouse IgM	Sigma	RPE
CD44	L178	mouse IgG1	Becton Dickinson	FITC
CD45	2D1	mouse IgG1	Becton Dickinson	PerCP
CD45-RA	ALB11	mouse IgG1	Beckman/Coulter	FITC
CD45-RO	UCH1	mouse IgG2a	Beckman/Coulter	FITC
CD56	N901(NKH-1)	mouse IgG1	Beckman/Coulter	PE
CD57	VC1.1	mouse IgM	Sigma	RPE
CD69	L78	mouse IgG1	Becton Dickinson	FITC
CD122	2RB	mouse IgG1	Beckman/Coulter	FITC
HLA-DR	L243	mouse IgG2a	Becton Dickinson	FITC

Table 10 below lists the distribution of the antigens listed in Table 9.

TABLE 10

Main distribution of antigens

Antigen	Main Cell Distribution
CD3	Expressed on all mature T cells associated with TCR complex (α/b, γ/δ)
CD4	Expressed on T helper lymphocytes. It can be also expressed on cells of the monocyte/macrophage lineage
CD5	Found on all mature T lymphocytes and a subset of B lymphocytes
CD8	Found on a subset of T lymphocytes called suppressor/cytotoxic T cells.
CD13	Detected on most cells of myeloid origin polymorphonuclear cells or cells of the monocyte/macrophage lineage. Member of metalloproteinase family
CD14	Expressed strongly on the surface of monocytes Found on most tissue macrophages Weakly expressed on the surface of granulocytes and B lymphocytes Receptor for lipopolysacharride (LPS) and LPS binding protein
CD16	Expressed mainly on NK cells, monocytes macrophages and polymorphonuclear leukocytes Low affinity receptor for IgG
CD16b	Found on granulocytes including polymorphonuclear cells (PMN)
CD20	Present on all B lymphocytes
CD36	Expressed on platelets, monocytes or macrophages, microvascular endothelial cells, mammary endothelial cells, during stages of erythroid cell development
CD44	Widely expressed on the surface of most cell types. Including most leukocytes and epithelial cells. Family of core/link peptidoglycan
CD45	Present on the surface of all leukocytes
CD45RA	Isoforms of CD45 Found on naive/resting T cells Also expressed on B lymphocytes and monocytes
CD45RO	Isoforms of CD45 expressed on memory/activated T cells also expressed on monocytes
CD56	Marker for NK cells Can also be found on a population of T lymphocytes
CD57	Found on a subset of cells with natural killer activity
CD69	Expressed on activated leukocytes including T cells, B cells, NK cells, neutrophils, eosinophils and cells of the monocyte/macrophage lineage. Activation marker detected early after cell activation
CD122	Expressed on NK cells B, T lymphocytes or monocytes/macrophages Component of the IL-15 receptor
HLADR+	HLA class II molecule Found on antigen presenting cells or on other cells upon activation such as T cells.

Blood cells were washed twice with 0.15 ml of PBS washing buffer. Endometrial cell samples were incubated with a red blood cell lysing solution, (FACS™ Lysing Solution, Becton Dickinson) for 10 minutes at room temperature in the dark and washed with 3 ml of PBS washing buffer. Endometrial and blood cells were fixed in 1% paraformaldehyde (diluted in PBS) at a concentration of 1×10^6 cells/ml and kept at 4° C. in the dark until the immunofluorescence reactivity was determined by flow cytometry.

Flow Cytometry Analysis

The immunofluorescence reactivity was carried out on a Coulter EPICS XL™ flow cytometer (Coulter Corporation, Hialeah, Fla.) equipped with an argon laser operating at 488 nm, 15 mW and detectors at 525, 575, 610, and 675 nm. Calibration of the flow cytometer parameters for forward scatter, side scatter and fluorescence were the same for all the samples. Cells expressing CD45 pan leukocyte antigen were gated using the Coulter system II software. The percentage of cells bearing markers for T, B lymphocytes, macrophages or NK cells and/or activation markers was evaluated within the CD45 positive populations only. A minimum of 6000 CD45+ cells were analyzed for each sample.

Use of Leukocyte Markers in a Diagnostic Test for Endometriosis

A cutoff point was established for the proportion of the endometrial or blood leukocyte subpopulations identified as diagnostic markers. The value obtained for each marker is compared to the cutoff point (FIG. 1). A positive result was given when the proportion of a particular leukocyte subset fulfills the condition established by the cutoff point (for example<40% for CD3+ cells). When these markers are used in combination, a positive result for each marker gives a score of 1, whereas a negative result gives a score of 0. A diagnosis of endometriosis is given, when the final diagnostic score obtained from adding the results of all the markers of a particular combination is higher than a predetermined threshold value. The levels of sensibility and/or specificity measured for the marker combination represents the number of positive test results obtained among the patients already confirmed with endometriosis and the number of negative test results among the subjects within the control group, respectively.

Endometrial and blood leukocyte markers can be used in combinations in logistic regression model:

$$P(r)=\frac{e^{c+B1*(marker1)+B2*(marker2)+\dots\ Bn*(marker\ n)}}{1+e^{c+B1*(marker1)+B2\underline{*}(marker2)+\dots\ Bn\underline{*}(marker\ n)}}$$

Where:

P(r)=probability of having endometriosis

c=constant established for a particular combination

B=coefficient of regression

n total number of markers in the combination

The probability of having endometriosis (P(r)) is then compared to a threshold value that provides the best discriminative value. A positive diagnosis of endometriosis is given when the P(r) value exceeds the threshold value established for a particular combination of markers. Alternatively, a negative diagnosis of endometriosis is given when the P(r) value is lower than the threshold value.

Results

Endometrial and blood leukocyte subsets defined as good potential markers for the diagnosis of endometriosis are presented in Tables 3 and 4 respectively. Selection of these markers was done on the basis of 1) a significant difference in the mean proportion of leukocyte subsets between patients with endometriosis (stage I–IV) and control groups; 2) several endometrial and blood leukocyte markers were also selected according to the area under the ROC curve (>0.500), an indication of the discriminative value of the markers. The ROC curve allowed the determination of one or more cutoffs (proportion % of leukocyte subpopulations) that best discriminate between patients with endometriosis (stage I–IV) and normal controls. In an attempt to use these differences for identifying patients with endometriosis, a positive test result was given when the proportion measured for a particular leukocyte subset fulfills the condition established by the cutoff point (for example<40% for CD3+ cells). The levels of specificity and sensibility were calculated for each marker used alone to diagnose endometriosis and are presented in Table 3 (for endometrial leukocyte markers) and Table 4 (for blood leukocyee markers). Moreover, 3) a significant odds ratio calculated with a particular cutoff point gave an additional indication that the leukocyte markers selected in Tables 3 and 4 are associated with an increased risk to develop endometriosis and can, thus, be used for identifying women with high likelihood of suffering from endometriosis.

The mean proportion of some endometrial (Table 5) and blood (Table 6) leukocyte subsets was found to be significantly modulated only in patients with stage I–II endometriosis or with stage III–IV endometriosis when compared to normal controls. These markers remain good candidates for a diagnostic test for endometriosis, but their use may be limited to a specific stage of the disease.

Several of endometrial and blood leukocyte markers were found to be even more reliable as diagnostic markers when they are analyzed in combination with other markers. Table 1 gives a series of 33 combinations in which endometrial or blood leukocyte markers are used in a diagnostic test for endometriosis. For each marker, a positive test result (as described above; see also FIG. **1**) gives a score of 1, whereas a negative test result gives a score of 0. The final diagnostic value obtained from adding the scores of all the markers of a particular combination is then compared to a threshold value, which is indicated in Table 1. A diagnosis of endometriosis is given, when the diagnostic value exceeds the threshold value established for each set of combination markers. The use of leukocyte marker subsets in combination in this new method clearly improves the levels of sensibility and/or specificity for diagnosing endometriosis. Table 1 also provides 4 examples showing that blood leukocyte markers, when used in combination with endometrial markers, can also increase the predictive value of the diagnostic test.

The present invention also demonstrate that logistic regression models can also be used to combine endometrial as well as blood leukocyte markers for the development of a predictive model of endometriosis (Table 2). In some cases, these models need to be adjusted with risk factors associated with endometriosis such as the length of the menstrual cycle, the duration of menstruation, pain (during intercourse, menstruation or in other circumstances) and age. In some instances, these factors were shown to increase the predictive value of the model.

The present invention identifies several examples of marker combinations, which give rise to diagnostic methods yielding improved levels of sensibility and specificity. Indeed, the different marker combinations of the present invention may serve different clinical applications including screening, diagnosis, monitoring and prognosis of endometriosis.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims. For example, other blood or endometrial markers, alone or in combination, might also be suitable to practice the method of the present invention, and are thus intended to be included in the present invention.

What is claimed is:

1. A method for determining likelihood of endometriosis in a female subject, comprising:

a) obtaining from said female subject a sample of blood;

b) measuring in said sample a proportion of at least one specific population of blood leukocytes expressing or not expressing at least one specific surface molecule among total blood leukocytes, said specific population of blood leukocytes being selected from the populations of blood leukocytes defined in the following Table B; and

TABLE B

Population of blood leukocytes	Proportion of the population of leukocytes in the female subject as compared to the cutoff value that is indicative of an increased likelihood of endometriosis
CD3 − CD44 +	Higher
CD3 − CD69 +	Higher
CD3 − CD45RO +	Higher
CD3 − CD45RA −	Higher
CD4 − CD69 +	Higher
CD14 + CD44 +	Higher
CD14 + HLADR +	Lower
CD20 − CD44 +	Higher
CD36 − HLADR +	Lower
CD3 − CD57 − CD44 −	Lower

c) comparing the quantitative level measured at step (b) to a predetermined cutoff value for evaluating whether said measured proportion is higher or lower than the cutoff value; wherein a correlation as defined in Table B between the measured proportion and the cutoff value is indicative of an increased likelihood of endometriosis in said female subject as compared to an endometriosis-free female subject.

2. A method for determining likelihood of endometriosis in a female subject, comprising the steps of:

a) obtaining uterine endometrial tissues from said female subject;

b) measuring said tissues for a quantitative level of a population $CD3^-CD45RA^-$ endometrial leukocytes; and

c) comparing the quantitative level measured in step (b) to a predetermined cut-off value,

wherein a higher quantitative level of said population of leucocytes as compared to the cut-off value is indicative of an increased likelihood of endometriosis in said female subject as compared to an endometriosis-free female subject.

3. The method of claim **2**, further comprising the step of measuring the quantitative level of at least one further population of endometrial leukocytes selected from the group consisting of: CD3−HLADR−, CD3+, CD56−CD16+, CD3+CD16−, CD3+CD56−, and CD16+.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

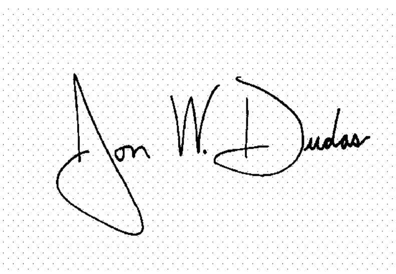
PATENT NO. : 6,743,595 B1
DATED : June 1, 2004
INVENTOR(S) : Diane Gosselin et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

<u>Column 29,</u>
Line 13, "CD14+HLADR+ Lower" should read -- CD14-HLADR+ Lower --.
Before line 14, add -- CD20+HLADR+ Lower --.

Signed and Sealed this

Seventh Day of February, 2006



JON W. DUDAS
Director of the United States Patent and Trademark Office

专利名称(译)	用于诊断子宫内膜异位症的方法和诊断试剂盒		
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申请(专利权)人(译)	METRIOGENE生物科学公司.		
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摘要(译)

本发明涉及使用血液和子宫内膜白细胞标记物或其组合诊断子宫内膜异位症的方法和试剂盒。标记物是来自子宫内膜或血液白细胞的表面抗原。