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Identification of anti-syntaxin 5 autoantibody as a novel serum marker of endometriosis

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ABSTRACT

The sensitivity and specificity of CA125, as a sole serum marker of endometriosis, are not high enough for routine clinical assessment. To explore new markers for the diagnosis of endometriosis, serum autoantibodies in endometriotic patients were investigated employing a fibroblast cell line, two-dimensional (2D) gel electrophoresis and Western blotting. Proteins reacting with serum autoantibodies by Western blotting were identified using MASCOT analysis. ELISAs were then prepared using recombinant proteins and titers of serum autoantibodies were determined in the endometriotic patients, disease controls, and healthy subjects. Among the autoantibodies identified, anti-syntaxin 5 (STX5) autoantibody levels were significantly elevated in endometriotic patients. Sensitivity (53.6%) and accuracy (72.2%) of the serum anti-STX5 autoantibody assay were better than those of serum CA125 levels (36.2% and 62.9%, respectively) for diagnosis. The sensitivity of anti-STX5 autoantibody was remarkably high in Stage II (80.0%) compared with that of CA125 (40.0%). A combination assay of anti-STX5 autoantibody with CA125 improved the overall sensitivity to 69.6%. We conclude that serum anti-STX5 autoantibody, which was discovered by a proteomic approach, is a potential new serum marker for the diagnosis of endometriosis. This initial study now requires validation by further clinical evaluation.

1. Introduction

Endometriosis arises in no less than 10% of fertile women (Simoens et al., 2007; Ozkan et al., 2008; Bulun, 2009). This disorder causes various aggravating symptoms such as dysmenorrhea and infertility (Simoens et al., 2007; Ozkan et al., 2008), and these disturb the quality of life of premenopausal women profoundly. Because of recent changes in the lifestyle of fertile women, particularly in industrialized countries, the chances of becoming pregnant have decreased and the number of lifetime menstruations has increased. Therefore, the risk of endometriosis is continuing to grow (Cramer and Missmer, 2002).

Theoretically, diagnosis of endometriosis should depend on the direct inspection of the abdominal cavity by laparoscopy or laparotomy (Simoens et al., 2007; Bulun, 2009). Histological confirmation of ectopic endometrial tissues is also desirable (Marchino et al., 2005; Ozkan et al., 2008). However, the majority of endometriotic patients are diagnosed clinically without these invasive maneuvers. Indeed, it is not an easy task to diagnose endometriosis using laparoscopy or laparotomy in all patients because of its benign nature. Therefore, patients who only have dysmenorrhea without endometriosis could be given hormone therapy (Practice Committee of American Society for Reproductive Medicine, 2008).

The significance of serum CA125 in the diagnosis of endometriosis has been studied (Bedaiwy and Falcone, 2004; Rosa E. Silva et al., 2007). This is currently the only clinical marker for endometriosis. However, the

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sensitivity of CA125 in the diagnosis of this disease is not high, especially in the early stages. Therefore, this assay is inadequate for the routine clinical assessment of endometriosis (Somigliana et al., 2004). The specificity of CA125 is also limited by its marked elevation in ovarian cancers (Kashyap, 1999; Check et al., 2001; Ghaemmaghami et al., 2007). A new serum endometriotic marker that possesses high sensitivity and reliable specificity has long been desired.

Previous studies have suggested that some autoantibody-reactions are provoked in patients with endometriosis (Mathur et al., 1982; Gorai et al., 1993; Inagaki et al., 2003; Matarese et al., 2003). Accordingly, we have investigated autoantibodies as serum biomarkers for the diagnosis of endometriosis. In the course of this work, we have found anti-α-enolase and anti-PDIK1L autoantibodies to be useful (Nabeta et al., 2009, 2010). In this study, we attempted to a find more specific autoantibody than those described above.

2. Materials and methods

2.1. Patients

Serum samples were collected from 69 laparotomically or laparoscopically confirmed endometriotic patients (aged 20–51, 35.2 ± 8.0, mean ± SD). Sera were also collected from 44 healthy female donors (22–51, 34.2 ± 9.3) and from 38 control subjects with laparotomically or laparoscopically confirmed disease but no endometriosis (18–48, 34.4 ± 8.2; Table 1). Ethical approval for this project was granted by the Institutional Review Board (IRB) and the Ethics Committee of Ehime University Graduate School of Medicine and Matsuyama Red Cross Hospital. Written informed consent was obtained from all subjects before collection of both blood and tissue samples. Subjects with autoimmune diseases were excluded from this study. Stages of the endometriotic patients were determined according to the Revised American Society for Reproductive Medicine classification (1997). Blood was collected in a tube containing coagulation accelerator and placed for 1–2 h at room temperature. Serum was centrifuged at 3000 rpm for 20 min, separated in a tube and stored at −20 °C until assayed.

2.2. Cells

MRC-5 human fetal fibroblast cells were obtained from ATCC and cultivated in DMEM supplemented with 10% fetal bovine serum (10% DMEM) at 37 °C, 5% CO_2.

2.3. Two-dimensional gel electrophoresis and Western blotting

Two-dimensional (2D) gel electrophoresis and Western blotting were carried out as described previously (Murase et al., 2008; Nabeta et al., 2009, 2010). Cells were collected and solubilized with 8 M urea, 2% NP-40, 2% 2-ME, 10 mM PMSF. Ten to 50 μg of protein was mixed with the sample loading buffer with 0.5% IPG buffer, pI 3–10 (GE Healthcare, Tokyo, Japan) and loaded for isoelectric focusing using the Immobiline DryStrip, pH 3–10, 7 cm (GE Healthcare) and Ettan IPGphor II (GE Healthcare) followed by SDS-PAGE using a 10% acrylamide gel (BIO CRAFT, Tokyo, Japan). Proteins in the gel were transferred to a PVDF membrane (Millipore, Tokyo, Japan). The PVDF membrane was blocked with 5% nonfat skim milk, 0.1% Tween 20 in PBS for 1 h at room temperature, then incubated with the 1:2000 diluted serum samples in 5% BSA, 0.1% Tween 20 in PBS (5% BSA–PBS) for 1 h at room temperature. After washing, the membrane was incubated with anti-human IgG HRP conjugate (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The membrane was developed using ECL solution (GE Healthcare) and exposed to Hyper-Film ECL (GE Healthcare). The 2D gels were also stained with Coomassie brilliant blue (CBB).

2.4. MALDI TOF-MS analysis

For protein identification, peptide mass fingerprinting (PMF) was used employing Voyager DE-PRO (Applied Biosystems, Tokyo, Japan) (Murase et al., 2008; Nabeta et al., 2009, 2010). Identified spots on CBB-stained 2D gels were excised using a scalpel. They were incubated with 50% acetonitrile in 12.5 mM NH_4HCO_3, pH 8.0, for 15 min at room temperature. Gels were then incubated in 100% acetonitrile for 5 min at room temperature. After drying, they were mixed with trypsin, 15 μg/ml in 25 mM NH_4HCO_3, pH 8.0 (Promega, Tokyo, Japan) and incubated at 37 °C overnight. The gels were mixed with 50% acetonitrile, 5% trifluoroacetic acid for 60 min. After drying, samples were dissolved in 0.1% trifluoroacetic acid and purified with a ZipTip C18 (Millipore). After mixing with matrix solution, a-cyano-4-hydroxycinnamic acid (Sigma, Tokyo, Japan), TOF-MS analysis was carried out using the Voyager DE-PRO. Data were analyzed on the Protein Inspector page at http://trypsin.nichd.nih.gov/ucsfhtml3.2/msfit.htm.

2.5. Recombinant protein

Recombinant protein was elaborated as previously described (Abe et al., 2000; Murase et al., 2008; Nabeta et al., 2009). STX5-cDNA was cloned using the specific primers: STX5F-*Bam*HI: 5′-CGGGATCCATGATCCCGCGGAAACGC-3′, STX5B-*Xho*I: 5′-CCCTCGAGTCAAGCAAGGAAGACC-3′ into a pGEX-6P-2 vector (Amersham). The BL21 *Escherichia coli* cells were transformed and GST fusion protein was induced by the addition of IPTG. The supernatant of *E. coli* cell lysate was purified using Glutathione-Sepharose 4FF gel (GE Healthcare).

2.6. ELISA

ELISA was developed for estimation of the serum autoantibody using recombinant proteins for the solid phase (Murase et al., 2008; Nabeta et al., 2009, 2010). The recombinant GST-STX5 full length protein was diluted with carbonate buffer (0.1 M, pH 9.5) and 100 μl was added to the wells of an ELISA plate (Nunc Immunoplate, Thermo Scientific, Kanagawa, Japan) at a final concentration of 1 μg/ml. Following incubation at 4 °C overnight, plates

Table 1
Demographics of the study participants.

Group	*n*	Mean age (range)	Details	*n*
Endometriosis patients	69	35.2 (20–51)	Stage I	11
		35.2 ± 8.0	Stage II	10
			Stage III	20
			Stage IV	28
Disease controls	38	34.4 (18–48)[a]		
		34.4 ± 8.2	Leiomyoma uteri	8
			Ovarian carcinoma	4
			Endometrial carcinoma	4
			Infertility	
			Adenomyosis	3
			Tubal occlusion	2
			Cervical carcinoma	2
			Septate uterus	1
			Primary amenorrhea (Turner Syndrome)	1
			Hydatidiform mole	1
Healthy controls	44	34.2 (22–51)		
		34.2 ± 9.3[a]		

[a] Statistically insignificant difference vs. endometriosis (Welch *t*-test).

were washed with washing buffer (0.05% Tween 20 in PBS). For the assay, 100 μl of 1:2000 diluted serum samples in 1% BSA–PBS were added to wells and incubated for 2 h at room temperature. After washing, 100 μl anti-human IgG-HRP conjugate solution (Santa Cruz Biotech.) was added and incubated for 1 h at room temperature. After washing, 100 μl of tetramethylbenzidine solution, 10 mg/ml in 0.05 M sodium acetate buffer, pH 5.5 with 0.01% H_2O_2, was added and incubated for 30 min at room temperature. Reactions were stopped by adding 50 μl 10% H_2SO_4 solution. OD at 450/620 nm was estimated using the ELISA reader (Labsystems, Helsinki, Finland). A standard serum of an endometriotic patient was selected and 1000 units/ml (U/ml) were given after a 1:2000 dilution with 1% BSA–PBS. The inter- and intra-assay variances of this ELISA were less than 10%. The serum CA125 antigen levels were estimated by a CLEIA assay performed at the Ehime Medical Laboratory (Ehime, Japan). The statistical analysis employed in this study was Welch's *t*-test. The receiver-operating characteristic (ROC) plot was calculated and depicted.

3. Results

3.1. Identification of autoantibodies in endometriotic patients using fibroblast cells as the target

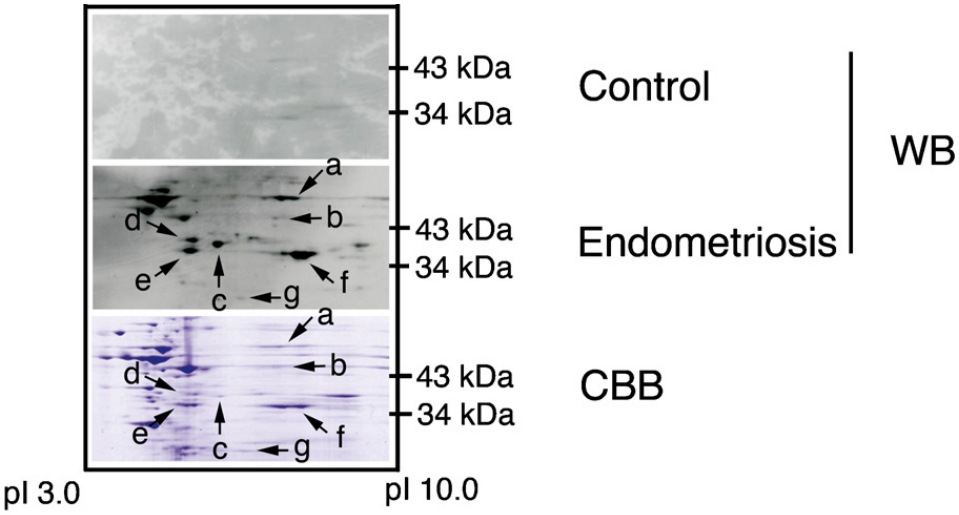
Using 2D gel electrophoresis and Western blotting, autoantibodies in sera of endometriotic patients were investigated. Western blot analysis revealed seven major spots that were produced by specific reactions in sera of endometriotic patients; however, no such spots were produced from the sera of healthy controls in preliminary Western blotting experiments (Fig. 1A). We checked the existence of these autoantibody-spots using the sera of seven endometriotic patients and three healthy controls (Table 2). By MASCOT analysis, these spots were: (a) PHD finger protein 6, (b) β-actin, (c) cyclin D2, (d) PDIK1L (PDLIM1-interacting kinase 1 like), (e) galactosyl transferase 3, (f) syntaxin 5, and (g) Rab32 (Table 2). We finally obtained recombinant proteins of β-actin, PDIK1L, and syntaxin 5. Employing these recombinants, ELISAs for estimating serum autoantibodies were established. By the screening ELISA analyses using a panel of sera from patients with endometriosis, disease controls, and healthy subjects, anti-STX5 autoantibody was shown to be useful as an endometriotic marker.

3.2. Serum anti-STX5 autoantibody in endometriotic patients

In order to evaluate the serum anti-STX5 autoantibody levels, the ELISA system was developed. One patient's serum with a high anti-STX5 autoantibody titer was selected and assigned the value of 1000 units/ml (U/ml) after 1:2000 dilution. A standard curve was determined using this serum thereafter. Serum anti-STX5 autoantibody levels were estimated in the 1:2000 diluted sera. Using this ELISA system, serum levels of anti-STX5 autoantibody were estimated for clinical samples.

Results were analyzed according to diseases (Fig. 2A, left panel). The mean ± SD (U/ml) levels of serum anti-STX5 autoantibody were 505.4 ± 381.0 in the endometriotic patients, 287.9 ± 161.8 in the disease controls and 201.5 ± 107.2 in the healthy volunteers. There is a statistically significant difference between endometriotic patients and disease controls ($p = 9.0 \times 10^{-6}$; Welch's *t*-test) and between endometriotic patients and healthy controls ($p = 2.2 \times 10^{-8}$). Considering the ROC curve and a low false-positive rate, the cut-off level was set at 400 U/ml. As a result, the serum anti-STX5 autoantibody for diagnosis of endometriosis shows 53.6% sensitivity and 87.8% specificity against controls, including both disease controls and healthy subjects (Fig. 2B, left table). Data of CA125 in the same serum samples are also presented (Fig. 2A, right panel). There is no statistically significant difference between endometriosis patients and disease controls ($p = 0.36$) or between endometriosis patients and healthy controls ($p = 0.063$) in CA125 levels. When using the regular cut-off level of 35 U/ml, the serum CA125 in the diagnosis of endometriosis shows 36.2% sensi-

A



B

PMF Analysis of Spot f

	MOSE Score	#/78(%) Masses Matched	%Cov	%TIC	Mean Err ppm	Data Tol ppm	MS-Digest Index #	Protein MW (Da)/pI	Accession #	Protein Name
1	3.396e+004	4(5)	17.0	5.6	-25.1	165	182672	34086/9.0	Q13190	Syntaxin-5
2	1453	4(5)	17.0	5.6	69.1	87.2	196594	33540/4.7	Q9UFF9	CCR4-NOT transcription complex subunit 8 (CAF2)
3	550	4(5)	22.0	5.6	-95.2	189	119506	35710/9.1	P09758	Tumor-associated calcium signal transducer 2 precursor (Cell surface glycoprotein Trop-2)
4	452	4(5)	17.0	5.6	-4.02	42.0	108461	36889/9.1	Q8TDV5	Probable G-protein coupled receptor 119
5	427	4(5)	9.0	5.6	62.3	233	202438	39595/5.2	Q9NZR1	Tropomudulin-2

Fig. 1. Identification of STX5 as an autoantigen in patients with endometriosis. (A) Western blotting using the serum of a healthy control (Control) and an endometriotic patient (Endometriosis). Seven major endometriosis-specific spots (a–g) were found. These spots were identified in the CBB-stained 2D PAGE gel (a–g). (B) Typical data on MASCOT analysis of spot f. Spot f was thus identified as STX5.

tivity and 85.4% specificity against controls, including both disease controls and healthy subjects (Fig. 2B, right table). Thus, the sensitivity of anti-STX5 autoantibody is shown to be better than CA125 in the serum samples we tested.

Anti-STX5 autoantibody levels were compared according to clinical stages (Fig. 2C, left panel). The mean ± SD (U/ml) levels were 428.6 ± 444.2, 634.2 ± 330.9, 529.6 ± 383.2, and 472.4 ± 355.6 for Stages I, II, III and IV, respectively. The positive rates were 27.3% (3/11), 80.0%

Table 2
Protein Identification of the reacted spots.

Spot	Protein name	Accession	Theoretical pI[a]	Theoretical MW[a]	Cover rate (%)	MOWSE score	Mass tolerance	WB (+)[b] in ctrl	WB (+)[b] in endometriosis
a	PHD finger protein 6	Q8IWS0	9.0	41291	15.0	7.8E+02	100	1/3	4/7
b	β-Actin	P60709	5.3	41737	14.0	5095	150	0/3	3/7
c	G1/S-specific cyclin-D2	P30279	5.1	33068	25.0	1030	150	0/3	5/7
d	Serine/threonine-protein kinase PDIK1L	Q8N165	6.4	38546	10.0	3324	100	0/3	6/7
e	β-1, 3-Galactosyl-transferase 3	O75752	7.7	39512	22.0	1155	50	0/3	5/7
f	Syntaxin 5	Q13190	9.0	34086	17.0	3.396E+04	200	3/3	5/7
g	Ras-related protein Rab-32	Q13637	6.1	24997	28.0	103	80	0/3	1/7

[a] Theoretical molecular pI and weight (kDa) from the Swiss-Prot database.
[b] WB (+): positive in Western blotting.

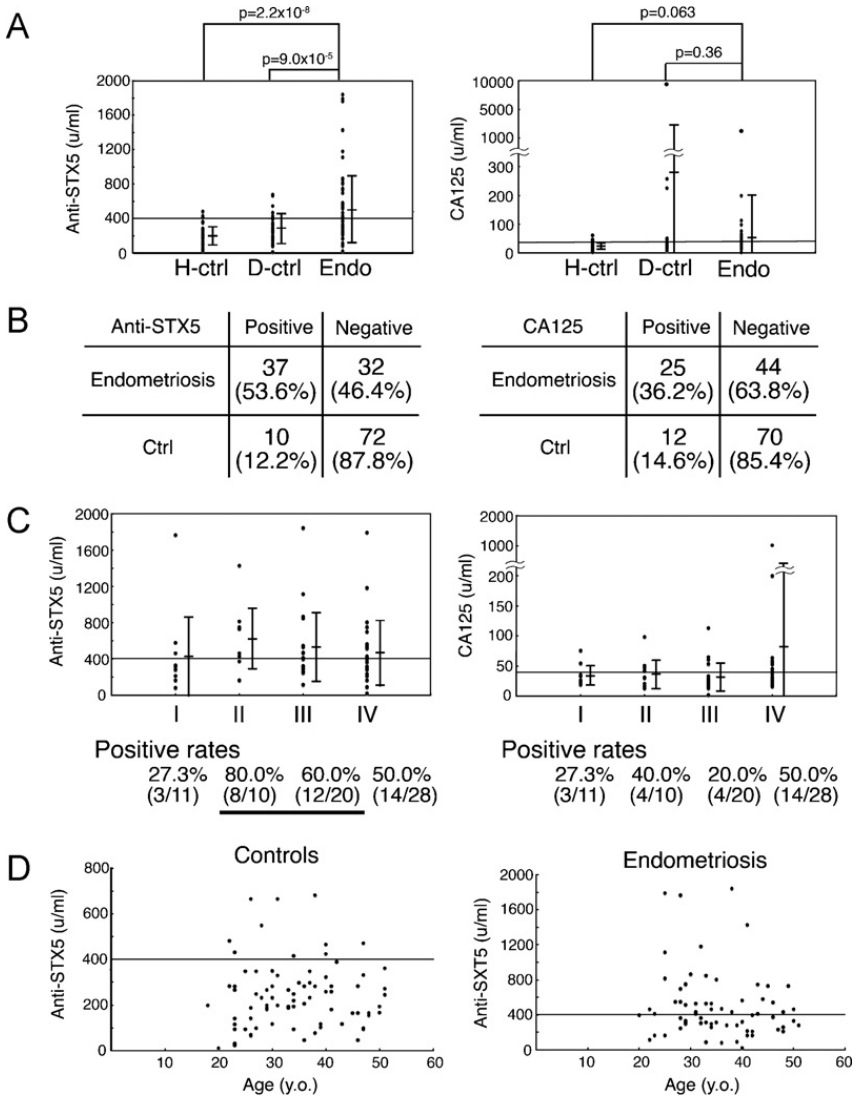


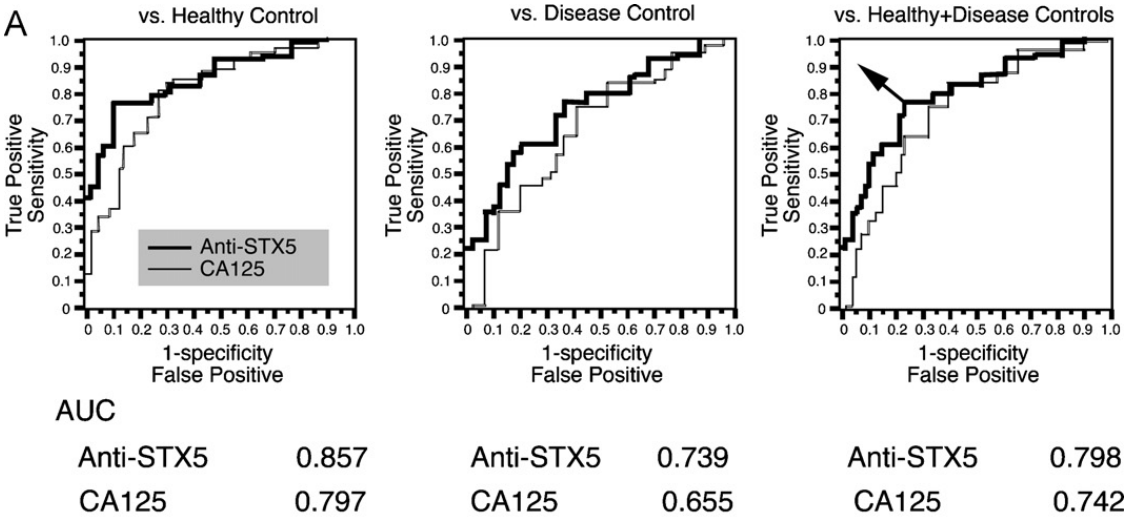
Fig. 2. Establishment of ELISA for serum anti-STX5 autoantibody estimation in clinical serum samples. (A) Dilution linearity of three sample sera (left) and a typical standard curve (right). (B) Scatter plots of the serum anti-STX5 autoantibody (left) and CA125 (right) levels in healthy controls (H-ctrl), disease controls (D-ctrl), and endometriotic patients (Endo). Levels in the endometriotic patients were significantly elevated against healthy controls ($p = 2.2 \times 10^{-8}$; Welch's *t*-test) and disease controls ($p = 9.0 \times 10^{-6}$). The cut-off levels are indicated in the figures at 400 U/ml and 35 U/ml for anti-STX5 autoantibody and CA125, respectively. (C) The positive and negative rates of anti-STX5 autoantibody (left) and CA125 (right) assays in the diagnosis of endometriosis. (D) Scatter plots of the serum anti-STX5 autoantibody (left) and CA125 levels (right) according to the stages of endometriosis. (E) Scatter plots of the serum anti-STX5 autoantibody levels in the sera of controls (left) and endometriotic patients (right) against age.

(8/10), 60.0% (12/20), and 50.0% (14/28) for Stages I, II, III and IV, respectively. The serum anti-STX5 autoantibody levels were thus evidently elevated at clinical Stage II. The levels of Stages III and IV are somewhat lower than that of Stage II. Serum CA125 levels are compared in the same series of sera according to stages (Fig. 2C, right panel). CA125 appeared to be elevated at the late stage.

The serum anti-STX5 autoantibody levels were plotted according to age (Fig. 2D). No age-related changes were observed in either controls or endometriotic patients.

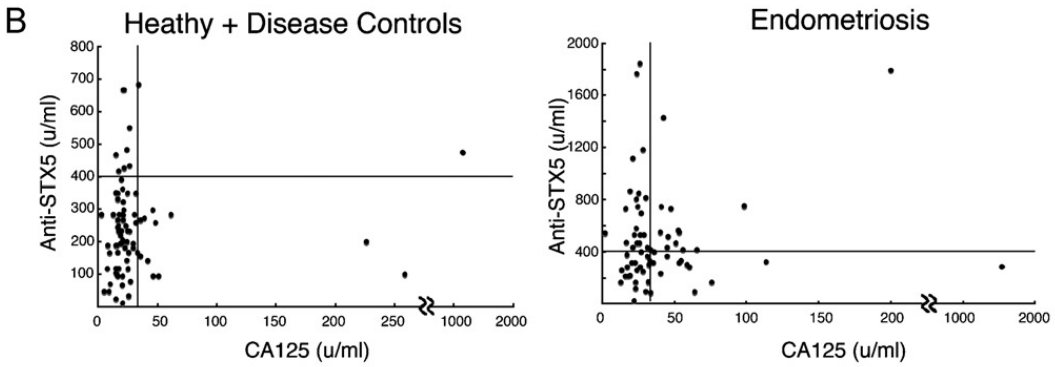
3.3. Direct comparisons of anti-STX5 autoantibody with CA125

The efficiency of the serum anti-STX5 autoantibody as an endometriotic marker was compared with that of serum CA125 antigen directly. ROC curves in healthy controls were calculated for these two markers and compared (Fig. 3A, left panel). The AUC (area under the curve) was calculated as 0.857 for anti-STX5 autoantibody and 0.797 for CA125. Because the AUC is larger in anti-STX5 autoantibody than in CA125 and the plotted line of anti-STX5 autoantibody shifts almost evenly to the upper left side from that of CA125, the competence of anti-STX5 autoantibody is better than that of CA125. Next, ROC curves against disease controls were calculated (Fig. 3A, center panel). The AUC area was calculated as 0.739 for anti-STX5 autoantibody and 0.655 for CA125. Lastly, ROC curves in controls, including both healthy volunteers and disease controls, were calculated (Fig. 3A, right panel). The AUC was 0.798 for anti-STX5 autoantibody and 0.742 for CA125. Thus, the diagnostic efficacy of the serum anti-STX5 autoantibody is better than that of CA125.



AUC

Anti-STX5	0.857	Anti-STX5	0.739	Anti-STX5	0.798
CA125	0.797	CA125	0.655	CA125	0.742



Dot counts

9	1
61	11

23	14
21	11

C

	Sensitivity	Specificity	Accuracy
Anti-STX5	53.6%	87.8%	72.2%
CA125	36.2%	85.4%	62.9%
Anti-STX5 and/or CA125	69.6%	74.4%	72.2%

Fig. 3. Direct comparison of anti-STX5 autoantibody and CA125 levels. (A) ROC curves of serum anti-STX5 autoantibody and CA125 in endometriotic patients against healthy controls (left), disease controls (center), and healthy + disease controls (right). Thick lines indicate anti-STX5 autoantibody and thin lines show CA125. AUC (area under the curve) values are presented underneath. (B) Scatter plots of serum anti-STX5 autoantibody against CA125 levels in both healthy and disease controls (left) and in endometriotic patients (right). The dot counts in each of the four areas are shown below. (C) Comparison of the sensitivity, specificity and accuracy (true positive + true negative cases/total cases) of serum anti-STX5 autoantibody and CA125 levels in the diagnosis of endometriosis. When combining both assays, sensitivity improved.

The anti-STX5 autoantibody and CA125 levels in the control subjects and endometriotic patients were directly plotted and the correlation between these two markers was investigated (Fig. 3B). No correlation was thus observed between the anti-STX5 autoantibody and CA125 levels in both control subjects and endometriotic patients. In 69 endometriotic patients, 14 were positive and 21 were negative in both markers (Fig. 3B, right panels). Twenty-three patients were positive only in the anti-STX5 autoantibody levels. The anti-STX5 autoantibody level was an exclusive

marker in these 23 endometriotic patients. On the other hand, 11 subjects were positive only in their serum CA125 levels.

The competence of the serum anti-STX5 autoantibody was compared with that of CA125 and the combination effect by both assays was addressed (Fig. 3C). As shown, the sensitivity and accuracy of anti-STX5 autoantibody are 53.6% and 72.2%, respectively, while those of CA125 are 36.2% and 62.9%, respectively. Thus, the parameters of anti-STX5 autoantibody are evidently better than CA125. When both markers were combined (determined as positive when one or both markers were elevated), the sensitivity improved to 69.6%.

4. Discussion

In this study, we discovered anti-STX5 autoantibody to be a novel serum endometriotic marker by the 2D mapping method using patients' sera and fibroblast cells. The result supports the concept that endometriosis possesses an autoimmune nature (Mathur et al., 1982; Gorai et al., 1993; Gitlits et al., 2001; Matarese et al., 2003; Nabeta et al., 2009, 2010). The concept has been claimed mainly due to the existence of autoantibodies in the sera of patients. Our previous study and this study showed that some specific autoantibody reactions occur in patients with endometriosis, which can be applied to the clinical diagnosis of this disorder (Nabeta et al., 2009, 2010). The main advantage of the anti-STX5 autoantibody assay for the diagnosis of endometriosis will be its high sensitivity in the early stages. We estimated both CA125 and anti-STX5 autoantibody in the same serum samples and directly compared these results. In this study, the sensitivity of CA125 was 36.2%, which is compatible with previous reports (Somigliana et al., 2004), while that of anti-STX5 autoantibody was 53.6% with a specificity of 87.8%. Thus, the sensitivity of anti-STX5 autoantibody appears to be higher than that of CA125. ROC curves support this. Additionally, no correlation was seen in the serum levels of anti-STX5 autoantibody and serum CA125. Therefore, the combination assay is useful in the diagnosis of this disorder. The sensitivity of combined assays improved to 69.6% while keeping the specificity above 70%.

It is noteworthy that anti-STX5 autoantibody shows a high sensitivity (80%) at Stage II. This sensitivity was better than those of our previous markers (Nabeta et al., 2009, 2010). This is significant for the clinical diagnosis of endometriosis. The diagnosis of endometriosis after Stage III is not necessarily difficult because of the common existence of chocolate cysts. The endometriotic chocolate cyst can be assessed at the bedside; however, when chocolate cysts were identified in patients, the clinical stages had commonly advanced far beyond Stage III. The diagnosis of endometriosis in the early stages is important for effective hormone therapy, but it is not an easy task. Here, anti-STX5 autoantibody shows a competent diagnostic ability at Stage II, which could provide benefit for the diagnosis of this disorder.

The level of serum anti-STX5 autoantibody does not appear to elevate further in the advanced stages of endometriosis. The mean levels of anti-STX5 autoantibody at Stages III and IV were rather lower than that at Stage II. This is compatible with the evidence that the existence of chocolate cysts in endometriotic patients does not affect the mean serum level of anti-STX5 autoantibody. On the other hand, the serum CA125 level appears to increase according to the clinical stages seen between Stages III and IV. Although there was no statistically significant difference, its mean level increased when a chocolate cyst existed. Thus, the serum anti-STX5 autoantibody levels might not strictly reflect the "volume" of an endometriotic cell burden. This resembles the result in our previous study on the anti-sideroflexin 3 autoantibody in the diagnosis of oral cancers. Anti-sideroflexin 3 autoantibody was elevated in the early stages of oral cancer, but it did not elevate further even in the advanced stages (Murase et al., 2008).

The mean serum anti-STX5 autoantibody levels were not affected by the existence of adenomyosis, and the serum CA125 levels were also not affected (Supplementary figure). This is compatible with the current concept that adenomyosis is not considered to be endometriosis.

The elevation of a certain autoantibody may predict the occurrence of diseases pre-clinically. It is curious that some control subjects possessing relatively high anti-STX5 autoantibody levels could be diagnosed with this disorder in the future. A cohort study in healthy subjects should be carried out to address this.

In summary, serum anti-STX5 autoantibody can be a novel clinical diagnostic marker for endometriosis. The estimation could contribute to the non-invasive diagnosis of this disorder. Further clinical and basic analyses are needed.

Conflict of interest

The authors declare that the result of this study is currently being applied to a patient.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jri.2011.04.012.

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