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Alternative antibody for the detection of CA125 antigen: a European multicenter study for the evaluation of the analytical and clinical performance of the Access® OV Monitor assay on the UniCel® Dxl 800 Immunoassay System

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Abstract

Background: Cancer antigen CA125 is known as a valuable marker for the management of ovarian cancer.
Methods: The analytical and clinical performance of the Access OV Monitor Immunoassay System (Beckman Coulter) was evaluated at five different European sites and compared with a reference system, defined as CA125 on the Elecsys System (Roche Diagnostics).
Results: Total imprecision (%CV) of the OV Monitor ranged between 3.1% and 8.8%, and inter-laboratory reproducibility between 4.7% and 5.0%. Linearity upon dilution showed a mean recovery of 100% (SD+8.1%). Endogenous interferents had no influence on OV Monitor levels (mean recoveries: hemoglobin 107%, bilirubin 103%, triglycerides 103%). There was no high-dose hook effect up to 27,193 kU/L. Clinical performance investigated in sera from 1811 individuals showed a good correlation between the Access OV Monitor and Elecsys CA125 (R=0.982, slope=0.921, intercept=+1.951). OV Monitor serum levels were low in healthy individuals (n=267, median=9.7 kU/L, 95th percentile=30.8 kU/L), higher in individuals with various benign diseases (n=549, medians=10.9–16.4 kU/L, 95th percentiles=44.2–355 kU/L) and even higher in individuals suffering from various cancers (n=995, medians=12.4–445 kU/L; 95th percentiles=53.4–4664 kU/L). Optimal diagnostic accuracy for cancer detection against the relevant benign control group by the OV Monitor was found for ovarian cancer [area under the curve (AUC) 0.898]. Results for the reference CA125 assay were comparable (AUC 0.899).
Conclusions: The Access OV Monitor provides very good methodological characteristics and demonstrates an excellent analytical and clinical correlation with Elecsys CA125. The best diagnostic accuracy for the OV Monitor was found in ovarian cancer. Our results also suggest a clinical value of the OV Monitor in other cancers.
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Keywords: CA125; diagnosis; method comparison; ovarian cancer.

Introduction

Ovarian cancer is the fifth most common cause of tumor-related death in women in the developed world and the most lethal gynecological malignancy (1). Unlike other tumor entities with few histological subtypes, ovarian cancer subsummarizes a variety of distinct tumor types, including epithelial, germ cell, sex-cord stromal and metastatic tumors (2). Because prognosis is best in early stages but clinical symptoms often appear only late, there is a great need for parameters which improve early diagnosis of ovarian cancer (3).

Besides progress in radiological diagnostics, serum related markers have shown to provide valuable differential diagnostic and prognostic information and to be useful for the management of the disease in the further follow-up care after the primary therapy was applied (4–6). Among ovarian cancer serum markers, the tumor-associated antigen CA125 has proven a high sensitivity for cancer detection, particularly if used in serial measurements (2, 3, 6, 7).

CA125 is an antigenic determinant found on a high-molecular weight glycoprotein of 200–2000 kDa originally detected by the OC125 monoclonal antibody on a human serous cystadenocarinoma cell line AVCA433 (8). It is physiologically present in a number of normal adult tissues derived from the coelomic epithelium, as well as in cells of mesothelial origin, such as pleural, pericardial and peritoneal cells (2, 3, 9–11).

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Recent studies indicate that CA125 has a widespread distribution in human tissues, such as epithelia of kidney, lung, stomach, gall bladder, pancreas, and colon, as well as in malignancies of these organs (2, 9).

Despite this lack of organ specificity, CA125 has a very high sensitivity for ovarian cancer, already in early stages, and was supposed to be a valuable marker for differential diagnosis of ovarian cancer (2, 9, 12–17). Preoperative sensitivity and specificity could be improved by combination with other cancer antigens, such as CA15-3 and CA72-4 (14, 16). Further, prognostic value of CA125 values was found (18, 19). In addition, CA125 mirrors the recurrence of disease in blood accurately and frequently with a lead time of several months prior to radiological detectable tumor manifestations (2, 9, 20–22), Finally, the therapy efficacy can be monitored effectively by the course of CA125, if interpreted by experts (2, 9, 20–22).

In the present study, a new assay for detection of CA125 antigen was evaluated for its analytical and clinical performance, and compared with an established reference method. This Access OV Monitor assay is applied on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter Eurocenter S.A., Nyon, Switzerland) and uses OVK 95 as monoclonal tracer antibody, which recognizes practically the same epitope as the Centocor OC125 antibody (Centocor Inc., Horsham, PA, USA) (23), and OV185 as monoclonal capture antibody, which detects a similar epitope as the Centocor M11 antibody (23). As most of the currently available assays are based on the original Centocor antibody, it is challenging to compare the clinical relevance of the new assay using an alternative antibody, which in consequence has led to the new name ''Access OV Monitor assay''.

The present evaluation was performed as a European multicenter trial including five sites in various countries.

Materials and methods

Assay procedure

Access OV Monitor (CA125 antigen) assay on the UniCel® Dxl 800 Immunoassay System (Beckman Coulter) The Access OV Monitor assay is a paramagnetic particle, two-site immunoenzymatic (''sandwich''), chemiluminescent immunoassay for the quantitative determination of CA125 antigen levels in human serum and plasma using the Access Immunoassay Systems. A sample is added to a reaction vessel along with paramagnetic particles coated with polyclonal goat anti-biotin antibody, mouse monoclonal-biotin conjugate and a buffered protein solution. After incubation in a reaction vessel, separation in a magnetic field and washing remove materials not bound to the solid phase. A monoclonal-alkaline phosphatase conjugate is then added. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of CA125 antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.5–5000 kU/L).

For calibration, Access OV Monitor Calibrators (Cat. No. 386358: S0–S5, 2.5 mL/vial) were used. The Access OV Monitor Calibrators are provided at six levels – zero and approximately 25, 100, 500, 2000 and 5000 kU/L. Controls, Bio-Rad Lyphochek Tumor Marker Controls (Cat. No. 580 Bilevel, 6×2 mL; Bio-Rad Laboratories, Munich, Germany), were run in duplicates every day of the study.

CA125 assay on Elecsys 2010 Immunology System (Roche Diagnostics GmbH) The CA125 assay is an electrochemoluminescence immunoassay for the quantitative determination of CA125 antigen levels in human serum and plasma using the Elecsys 2010/1010 and Modular Analytics E170 Immunology Systems (Roche Diagnostics, Penzberg, Germany). The assay is based on a sandwich principle with the monoclonal antibodies OC125 and M11: 20 μL of sample, a biotinylated monoclonal CA125-specific antibody, and the monoclonal antibody labeled with a ruthenium complex form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell buffer. Application of a voltage to the electrode then induces chemiluminescent emission that is measured by a photomultiplier tube. Results are determined via a calibration curve which is instrument-specifically generated by two-point calibration and a master curve provided by the manufacturer via the reagent pack barcode.

Samples can be accurately measured within the analytic range of 0.5–5000 kU/L.

For calibration, Elecsys CA125 CalSet (Cat. No. 11776240, for 4×1 mL) was used. Controls, Elecsys PreciControl Tumor Markers 1 and 2 (Cat. No. 11776452, 2×3 mL), were run in duplicates every day of the study.

Analytical evaluation

The analytical performance of the Access OV Monitor assay was evaluated by all five centers in parallel, in particular imprecision, inter-laboratory reproducibility, minimum detectable concentration and linearity upon dilution. The influence of endogenous interferents and high-dose hook effect was tested in the laboratories of Munich, Barcelona and Aachen; interferences of sample type and sample storage were only tested in Munich.

Imprecision Two controls (Bio-Rad) and three human serum pools prepared by each center, including a low, medium and high concentration pool, were tested in triplicate, with two runs per day for at least 10 days according to the guidelines of the CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS; document NCCLS-EP5-A). Data analysis included calculation of within-run and total imprecision and was performed by Acomed Statistics, Leipzig, Germany.

Inter-laboratory reproducibility Inter-laboratory reproducibility was evaluated using the two controls across the five evaluation sites.

Minimum detectable concentration The minimum detectable concentration was defined as the OV Monitor concentration corresponding to a signal two standard deviations

above the main value of 10 replicates of the S0 calibrator tested on each of 3 days.

Linearity upon dilution A total of 27 samples, with five of them above the assay dynamic range (>5000 kU/L) and 16 of them between 1000 and 5000 kU/L, were diluted with the appropriate Access OV Monitor diluent to obtain a minimum of four dilutions within the assay dynamic range. Dilutions were prepared separately in one to two steps using calibrated pipettes and were carried out in four replicates. Recoveries were calculated with respect to the highest concentration in the dynamic range.

Sample type interference Samples were obtained from 10 patients with unsuspicious laboratory findings, one serum in tubes with kaolin, one lithium-heparinate plasma, one EDTA plasma and one citrate plasma. Sample type interference was tested in duplicates. Recoveries were calculated with respect to the concentration in the serum sample.

Sample storage interference Samples from seven of these patients were measured natively and after storage at 4°C and –20°C for 1 day. Both storage modalities were compared with the native measurements.

Further, serum and lithium-heparinate plasma samples of the 10 patients were stored at –20°C for 6 months and measurements were compared with the original –20°C data to test the long-term stability.

Endogenous interferents The influence of bilirubin, hemoglobin and triglycerides was tested on a human serum pool with high CA125 concentrations. The serum pool was diluted with a serum containing high bilirubin concentrations (>0.062 μmol/L), with a serum with high hemoglobin (ca. 0.855 mmol/L; normal serum spiked with hemolyzed blood sample) and with a serum with high triglyceride concentration (>0.006 mmol/L). These test solutions were tested with the Access OV Monitor assay and compared to the control solutions obtained from the same pool diluted in the same way with the Sample Diluent A (Catalog Number 81908) instead of the interfering substance. Each test solution and each control solution were assayed 10 times in constantly decreasing proportions. Recoveries were calculated with respect to the concentration of the undiluted serum pool. Additionally, 10 samples with high known rheumatoid factor concentration were tested in duplicate.

High-dose hook effect Serial 10-fold dilutions of two different samples with very high CA125 concentrations above 20,000 kU/L were tested. Recoveries were calculated with respect to the highest concentration in the dynamic range.

Clinical performance

The clinical performance of the Access OV Monitor assay was evaluated by two sites (Munich and Barcelona). All clinical samples were sent to the Institute of Clinical Chemistry of the University Hospital Munich, to be tested using the Access OV Monitor assay on the UniCel® DxI 800 Immunoassay System (Beckman Coulter) and compared to the reference CA125 assay on the Elecsys 2010 Immunology System (Roche Diagnostics).

Healthy individuals The normal reference interval for the OV Monitor was established from 267 samples, including 113 sera from men and 154 sera from non-pregnant women. The subject inclusion/exclusion criteria were as follows:

- normal, apparently healthy subjects (evaluated clinically and by clinical chemistry parameters),
- adults older than 18 years were tested,
- no personal history of cancer disease, renal failure or liver disease.

Age and sex were mandatory for all samples enrolled. Samples with hemolysis, bilirubin or lipemia were excluded.

Individuals with benign diseases OV Monitor results were determined in a total of 549 individuals diagnosed with benign diseases, among them 109 benign gynecological diseases (ovarian cysts, endometriosis, uterine leiomyoma, etc.), 148 benign breast diseases, 155 benign gastrointestinal diseases (ulcerous colitis, Crohn's disease, liver cirrhosis, hepatitis, pancreatitis, cholelithiasis, etc.), 44 benign lung diseases (tuberculosis, sarcoidosis, pneumonia, etc.), 66 benign urological diseases (nephrolithiasis, renal failure, etc.) and 27 other benign diseases, and compared with the reference system.

Individuals with malignant diseases OV Monitor results were determined in a total of 995 individuals diagnosed with cancer diseases and compared with the reference system. The cancer diseases included 81 ovarian cancers, 416 breast cancers, 57 other gynecological cancers, 62 pancreatic cancers, 26 gastric cancers, 58 hepatocellular cancers, 113 colorectal cancers, 82 lung cancers, 57 urological cancers (bladder and kidneys) and 43 prostate cancers.

All samples were obtained from patients with active disease, typically before surgery as first treatment modality, or in some cases at time of recurrent disease.

Statistical analysis

The OV Monitor assay and reference method were compared using regression equations according to Passing and Bablok. Normalized differences from mean values were calculated according to Bland and Altman.

In healthy individuals, the frequency distribution for the OV Monitor and reference method was defined including 25th percentile, median, mean and upper reference limit (URL) of a normal population at 95th, 97.5th and 99th percentiles.

In all studied groups, distribution of the OV Monitor and reference method concentrations were presented graphically, as well as statistically (median, range, 95th percentile).

The analysis of the sensitivity/specificity for ovarian cancer included receiver operator characteristics (ROC) curves, using benign gynecological diseases as the control group. Similarly, ROC curves were established for breast cancer vs. benign breast diseases, lung cancer vs. benign lung diseases, and colorectal cancer vs. benign gastrointestinal diseases. Further, at 95% specificity against the respective benign control group, the sensitivity for each cancer type was calculated, and also the area under the curve (AUC) of the corresponding ROC curves with the corresponding 95% confidence interval.

Results

Analytical evaluation

Imprecision Within-run imprecision of the low control (26.4–30.2 kU/L) ranged in the various centers between 2.1% and 3.4%, and of the high control

(118.2–127.2 kU/L) between 1.9% and 2.8%. Within-run imprecision of the low serum pools (ranging from 6.3 to 46.7 kU/L) was between 2.4% and 3.2%, of the medium serum pools (ranging from 46.1 to 345 kU/L) between 2.0% and 2.7%, and of the high serum pools (ranging from 390 to 1939 kU/L) between 2.2% and 2.8%.

Total imprecision of the low control (26.4–30.2 kU/L) ranged in the various centers between 3.7% and 5.9%, and of the high control (118.2–127.2 kU/L) between 3.7% and 5.8%. Total imprecision of the low serum pools (6.3–46.7 kU/L) was between 3.6% and 8.8%, of the medium serum pools (46.1–345 kU/L) between 3.0% and 7.9%, and of the serum pools (390–1939 kU/L) between 4.2% and 6.0%.

Inter-laboratory reproducibility Inter-laboratory imprecision of the low control (26.4–30.2 kU/L) was found to be 5.0%, and of the high control (118.2–127.2 kU/L) 4.7%.

Minimum detectable concentration The minimum detectable concentration was found to be <2.0 kU/L in all centers. All these results are in the very low range and have no clinical relevance.

Linearity upon dilution In total, 27 samples were investigated on linearity upon dilution. Dilutions were performed with the appropriate Access OV Monitor diluent in one to two steps, down to the low value range (<30 kU/L), with a minimum of four dilutions within the assay dynamic range. Mean recovery of all dilutions in all centers was 100%, with a standard deviation of 8.1% (minimum 84.0%, maximum 138%) (Figure 1).

Sample type interference Samples from 10 patients with unsuspicious laboratory findings were tested on sample type interference. OV Monitor measurements in kaolin serum and lithium-heparinate plasma were very comparable. Mean recovery in heparinate plasma was 101%, with a standard deviation of 3.7% (minimum 95.6%, maximum 108%). OV Monitor values in EDTA plasma and citrate plasma were very comparable and lower than OV Monitor values in serum. For EDTA plasma, mean recovery was 91.8%, with a standard deviation of 7.0% (minimum 83.3%, maximum 106%). For citrate plasma, mean recovery was 81.0%, with a standard deviation of 6.4% (minimum 72.1%, maximum 90.9%).

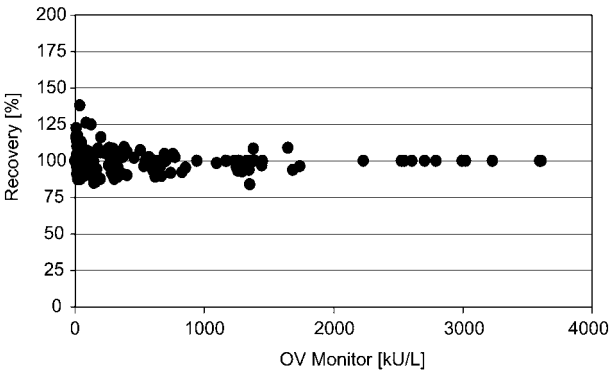

Figure 1 Linearity upon dilution.
Samples with high OV Monitor levels were diluted by one to two steps and recoveries were calculated for various dilution steps.

Sample storage interference Samples from seven of these patients were measured natively, after storage at 4°C and at –20°C for 1 day. Both storage conditions tested did not affect the OV Monitor values. After storage at 4°C, mean recovery was 99.3%, with a standard deviation of 6.3% (minimum 92.4%, maximum 107%). After storage at –20°C, mean recovery was 98.4%, with a standard deviation of 4.5% (minimum 94.0%, maximum 105%).

In addition, serum and lithium-heparinate plasma samples of the 10 patients were stored at –20°C for 6 months and measurements were compared with the original –20°C data to test the long-term stability. Again, storage had no influence on marker levels. When serum was stored at –20°C for 6 months, mean recovery was 93.6%, with a standard deviation of 6.3% (minimum 86.1%, maximum 104%). When lithium-heparinate plasma was stored at –20°C for 6 months, mean recovery was 93.2%, with a standard deviation of 10.4% (minimum 71.5%, maximum 108%).

Endogenous interferents The potentially confounding impact of endogenous interferents, such as hemoglobin, bilirubin and triglycerides, was tested at two centers. Stepwise dilution of a serum pool having high CA125 levels with a serum sample with high concentrations of the relevant interferent and, alternatively, with sample diluent which was free of any contamination showed that neither interferent had any influence on OV Marker levels.

Dilution with hemoglobin-spiked serum resulted in a mean recovery of 107%, with a standard deviation of 11.4% (minimum 95.9%, maximum 144%). There was no trend of continuously changing OV Monitor values when increasing amounts of hemoglobin were added (Figure 2).

Dilution with bilirubin-rich serum showed a mean recovery of 103%, with a standard deviation of 13.0% (minimum 88.1%, maximum 152%). There was no trend of continuously changing OV Monitor values when increasing amounts of bilirubin were added (Figure 2).

In the dilution series with triglyceride-rich serum, mean recovery was 103%, with a standard deviation of 9.2% (minimum 93.2%, maximum 133%). There was no trend of continuously changing OV Monitor values when increasing amounts of triglycerides were added (Figure 2).

Rheumatoid factor In total, 14 serum samples with high rheumatoid factor concentrations (mean 209.4 kU/L, standard deviation 298.0 kU/L, minimum 26.7 kU/L, maximum 1131 kU/L) were tested on a potential confounding effect on OV Monitor values.

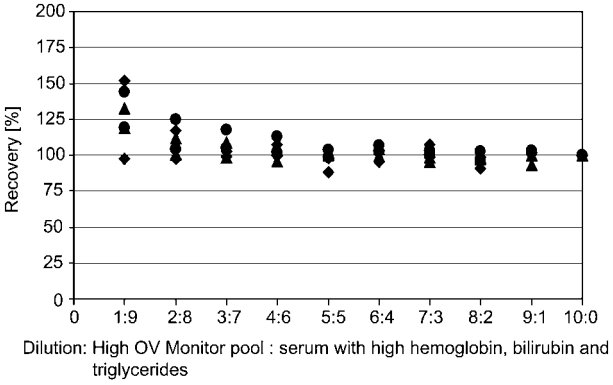

Figure 2 Influence of endogenous interferences. Samples were spiked with various concentrations of hemoglobin (♦), bilirubin (▲) and triglycerides (●) and recoveries of OV Monitor levels were calculated for various dilution steps.

However, all OV Monitor levels were very low in the range of healthy individuals. Mean value was 11.8 kU/L, with a standard deviation of 4.6 kU/L (minimum 5.7 kU/L, maximum 19.1 kU/L).

High-dose hook effect In total, two serum samples with extremely high CA125 levels (24,950 and 27,193 kU/L) were tested in dilution series on a potential high-dose hook effect. In all samples a linear dilution response was observed with a mean recovery of 103% and a standard deviation of 8.6% (minimum 95.1%, maximum 122%).

Clinical performance

Method comparison Comparison of the Access OV Monitor (CA125 antigen) assay on the UniCel® DxI 800 Immunoassay System and the CA125 assay on the Elecsys 2010 Immunology System, calculated on all serum samples (n = 1811), yielded a correlation coefficient of R = 0.982, with a slope of 0.921 and an intercept of +1.951.

A large number of samples (1751 out of 1811) were found to have values up to 500 kU/L. For this group, an excellent correlation was still found. The coefficient of correlation was R = 0.937, with a slope of 0.946 and an intercept of +1.660 kU/L. Values up to 100 kU/L were found in 1600 out of 1811 samples. For this clinically relevant group, a similarly good correlation was found. The coefficient of correlation was R = 0.939, with a slope of 0.992 and an intercept of +1.208 kU/L (Figure 3).

Healthy individuals For the Access OV Monitor, the 95th percentile URL of a healthy population (n = 267) was found at 30.8 kU/L. The value distribution ranged from 1.3 to 175 kU/L. Mean was at 13.2 kU/L, median at 9.7 kU/L. The 25th percentile was calculated at 6.8 kU/L, 97.5th percentile at 36.6 kU/L and 99th percentile at 55.8 kU/L. Females (median at 9.4 kU/L, 95th percentile at 27.2 kU/L) and males (median at 11.1 kU/L, 95th percentile at 32.4 kU/L) had a similar value distribution. Both methods showed a very comparable distribution and a good correlation (R = 0.949, slope 1.026, intercept of +0.415) (Figure 4, Table 1).

Individuals with benign diseases Of 549 individuals diagnosed with benign diseases, patients with benign gastrointestinal diseases showed the highest levels for the Access OV Monitor (median at 15.4 kU/L, 95th percentile at 355 kU/L). The lowest levels were found in benign breast diseases (median at 12.0 kU/L, 95th percentile at 44.2 kU/L), which were in the range of healthy individuals. For all benign diseases, both methods showed comparable results and a good correlation (R = 0.903, slope 0.959, intercept of +1.602). Details of value distribution are listed in Table 1 and Figure 4.

Individuals with malignant diseases Of 995 individuals diagnosed with malignant diseases, patients with ovarian cancer showed the highest levels for the Access OV Monitor (median at 445 kU/L, 95th percentile at 4664 kU/L), with maximum levels of more than 15,000 kU/L. Some strongly elevated values were found in single individuals with gynecological, breast, hepatocellular, pancreatic, colorectal and lung cancers too, but median and 95th percentiles were considerably lower than in ovarian cancer. Lower values with maximum levels <200 kU/L were observed in gastric, urological and prostate cancer. In general, results of both methods were very comparable and a good correlation was found (R = 0.943, slope 0.908, intercept of +2.384). Details of value distribution are listed in Table 1 and Figure 5.

The frequency distribution of OV Monitor values showed that most of the samples of healthy individuals, and individuals with various benign diseases and individuals with malignant diseases without ovarian cancer had very low OV Monitor levels not only in the reference range <35 kU/L but even below 15 kU/L (Figure 6). In contrast, only a few individuals with ovarian cancer with or without distant metastases had such low OV Monitor concentrations. This non-release of OV Monitor is, thus, particularly important to estimate the probability of not having ovarian cancer if there are suspicious pelvic masses. Concerning high OV Monitor levels, they could be found in some cases of benign diseases up to 2000 kU/L and in some cases of cancer other than of ovarian origin up to 5000 kU/L. However, if there are suspicious pelvic masses and OV Monitor concentrations are >300 kU/L the presence of ovarian cancer seems to be very probable (Figure 6).

Sensitivity for cancer disease In addition to the comparison of the absolute concentrations of both methods in various patient groups with benign and malignant diseases, the diagnostic capacity of the Access OV Monitor (CA125 antigen) assay on the UniCel® DxI 800 Immunoassay System was tested by ROC curves showing the profile of sensitivity and specificity over the whole range of values and was compared to that of CA125 assay on the Elecsys 2010 Immunology System. According to the guidelines of

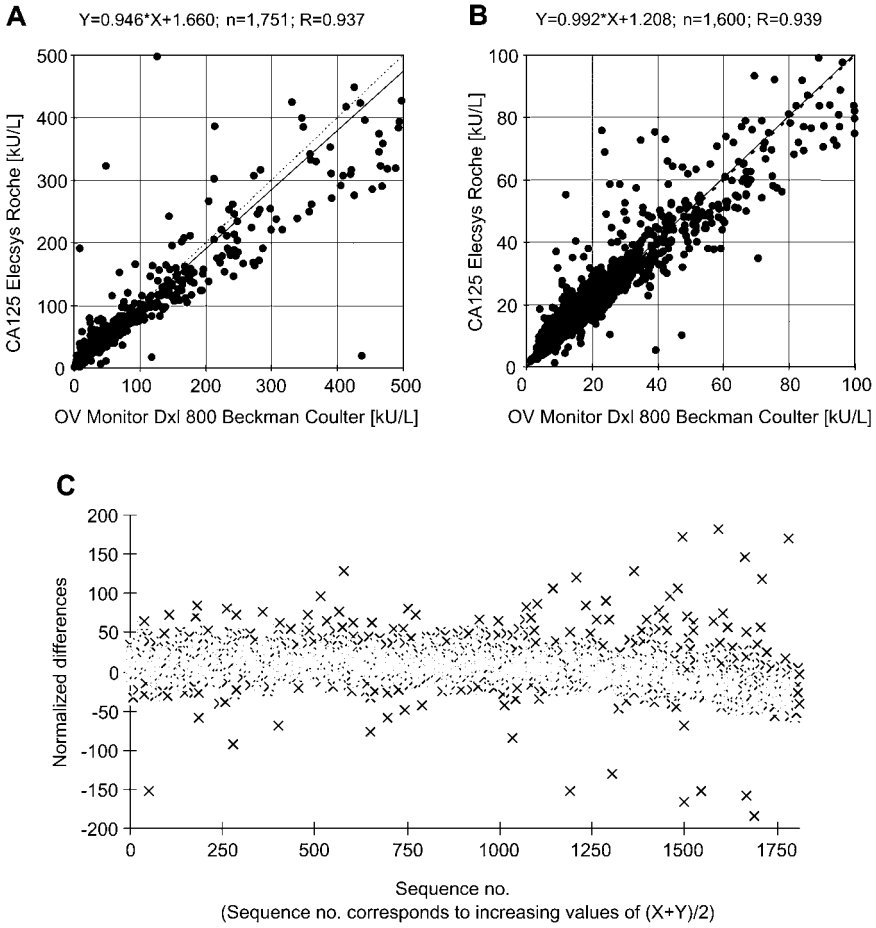

Figure 3 Method comparison of the OV Monitor with the reference method.
Correlation of Access OV Monitor (Beckman Coulter) and Elecsys CA125 (Roche Diagnostics) concentrations were calculated (A) for the range <500 kU/L and (B) for the range <100 kU/L. (C) Normalized differences from mean values were calculated according to Bland and Altman.

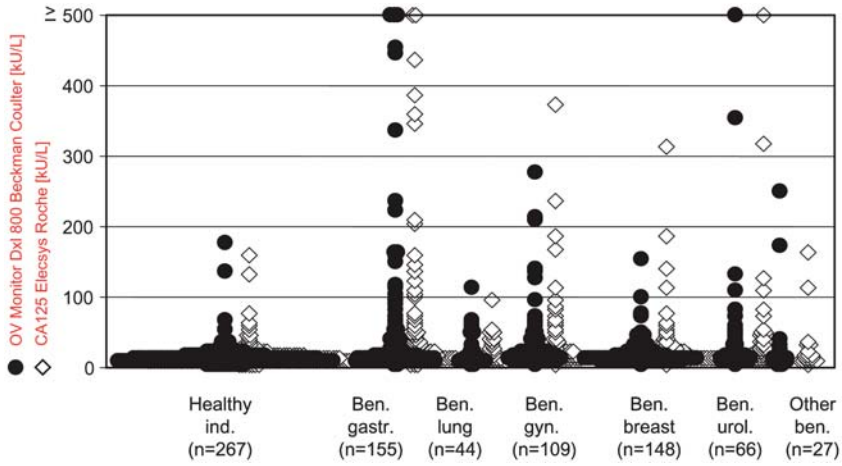

Figure 4 Value distribution of the OV Monitor and reference method in controls.
(●) Dot plot of Access OV Monitor (Beckman Coulter) and (◇) Elecsys CA125 (Roche Diagnostics) concentrations in serum samples of healthy individuals and individuals with various benign diseases.

the European Group on Tumor Markers (EGTM), all cancer types were compared with the respective benign disorders as the relevant control group.

Concerning their diagnostic capacity, both methods showed very comparable results for all cancer types investigated. This good diagnostic correlation was expressed by the similar values for the AUC with overlapping confidence intervals, as well as by the sensitivity for cancer detection at the 95% specificity of benign diseases (Table 2). Among all cancers, ovarian cancer showed the highest AUC value for both methods when compared with benign gynecological

Table 1 OV Monitor concentrations in sera of cancer patients and controls.

Diagnosis	n	Method	Median, kU/L	Range, kU/L	95th percentile, kU/L
Healthy individuals	267	Dxl 800	9.7	1.3–175.0	30.8
		Elecsys	10.7	1.6–158.0	32.7
Benign gynecological diseases	109	Dxl 800	16.4	3.7–276.0	130.0
		Elecsys	16.1	1.2–371.0	101.6
Benign breast diseases	148	Dxl 800	12.0	3.5–150.0	44.2
		Elecsys	13.7	2.5–310.0	55.9
Benign gastrointestinal diseases	155	Dxl 800	15.4	2.9–1126.0	355.0
		Elecsys	17.1	3.4–897.0	232.8
Benign lung diseases	44	Dxl 800	11.7	3.2–111.2	65.1
		Elecsys	13.7	4.9–93.2	52.5
Benign urological diseases	66	Dxl 800	15.7	4.0–763.5	121.8
		Elecsys	18.2	5.9–590.0	117.1
Other benign diseases	27	Dxl 800	10.9	3.6–247.1	217.1
		Elecsys	12.0	4.3–160.0	140.8
Ovarian cancer	81	Dxl 800	445.0	5.2–15,019	4664
		Elecsys	305.5	5.0–9908	4266
Gynecological cancer	57	Dxl 800	26.4	4.5–2625	1698
		Elecsys	27.0	5.0–1784	979.2
Breast cancer	416	Dxl 800	14.2	2.4–2110	242.7
		Elecsys	17.5	2.6–1843	225.6
Gastric cancer	26	Dxl 800	14.2	3.2–83.0	70.9
		Elecsys	15.4	4.0–72.0	62.9
Hepatocellular cancer	58	Dxl 800	31.3	4.5–2806	1582
		Elecsys	29.9	4.6–2203	1187
Pancreatic cancer	62	Dxl 800	40.3	6.2–1064	623.8
		Elecsys	37.9	6.0–669.0	622.2
Colorectal cancer	113	Dxl 800	15.2	3.4–2814	275.9
		Elecsys	16.9	4.1–2653	221.3
Lung cancer	82	Dxl 800	30.4	3.4–3496	538.8
		Elecsys	29.1	4.0–1904	460.3
Bladder and renal cancer	57	Dxl 800	12.4	3.4–66.9	53.4
		Elecsys	13.8	3.3–64.0	53.0
Prostate cancer	43	Dxl 800	12.8	4.0–189.6	152.9
		Elecsys	15.7	4.3–151.0	129.7

Median, range and 95th percentile limit of the Access OV Monitor (Beckman Coulter) for healthy individuals, patients with benign and malignant diseases. Comparison with Elecsys CA125 (Roche Diagnostics) concentrations.

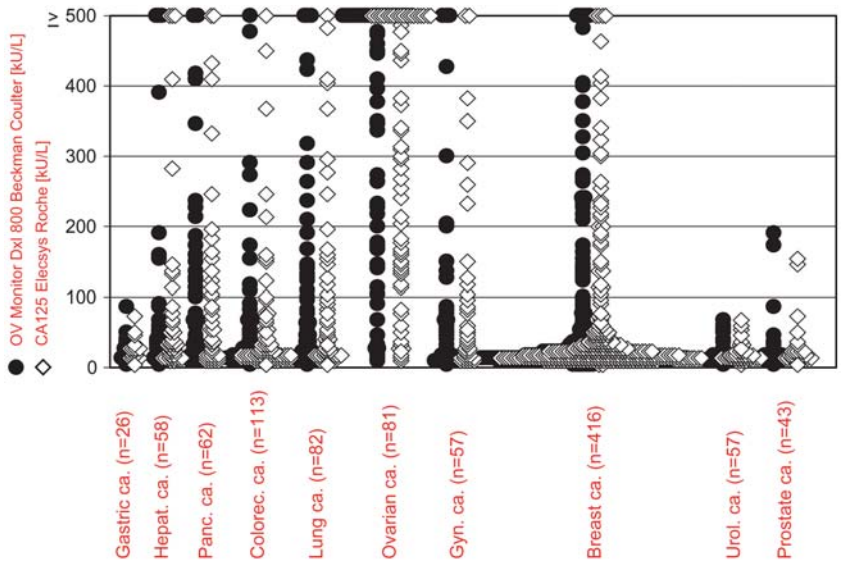

Figure 5 Value distribution of the OV Monitor and reference method in cancer patients.
(●) Dot plot of Access OV Monitor (Beckman Coulter) and (◇) Elecsys CA125 (Roche Diagnostics) concentrations in serum samples of individuals with various malignant diseases.

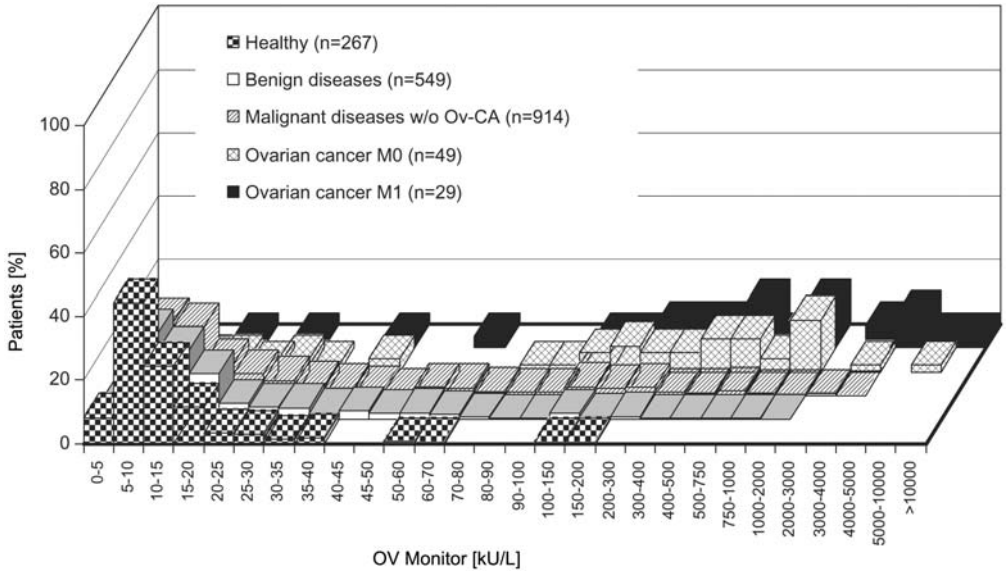

Figure 6 Frequency distribution of OV Monitor concentrations in various patient groups.
Frequency of Access OV Monitor values in serum samples of healthy individuals, individuals with various benign diseases, individuals with various malignant diseases without ovarian cancer, individuals with ovarian cancer without distant metastases (M0) and individuals with ovarian cancer with distant metastases (M1).

diseases (Access OV Monitor: AUC 0.898, Elecsys CA125: AUC 0.899) and the highest sensitivity at 95% specificity of benign gynecological diseases (Access OV Monitor: sensitivity 74.1%, Elecsys CA125: sensitivity 75.3%) (Figure 7, Table 2). For ovarian cancer with distant metastases, the discrimination was even better with an AUC of 0.931 for the Access OV Monitor and an AUC of 0.930 for the Elecsys CA125. However, the Access OV Monitor and Elecsys CA125, respectively, showed diagnostic power for other cancers, such as lung and gynecological cancers, too. It is noteworthy to mention that the diagnostic sensitivity was highest in the adenocellular histological subtype, whereas it was considerably lower in the squamous cellular subtype for both gynecological and lung cancers. Because in lung cancer, CA125 may aid in finding the histological differential diagnosis, the differences are shown in Figure 8. In adeno cell lung cancer, the AUC reached 0.781 for the Access OV Monitor and 0.773 for the Elecsys CA125, whereas in

Table 2 Diagnostic capacity of OV Monitor for various cancer diseases.

Diagnosis	n	Method	Sensitivity at 95% specificity vs. respective benign diseases	AUC	Confidence Interval
Ovarian cancer	81	Dxl 800	74.1	0.898	0.848–0.949
		Elecsys	75.3	0.899	0.850–0.949
Gynecological cancer	57	Dxl 800	21.1	0.587	0.487–0.688
		Elecsys	24.6	0.622	0.526–0.718
Breast cancer	416	Dxl 800	16.8	0.573	0.523–0.624
		Elecsys	14.7	0.575	0.524–0.626
Gastric cancer	26	Dxl 800	0.0	0.464	0.357–0.571
		Elecsys	0.0	0.476	0.367–0.585
Hepatocellular cancer	58	Dxl 800	12.1	0.659	0.579–0.738
		Elecsys	12.1	0.652	0.570–0.734
Pancreatic cancer	62	Dxl 800	11.3	0.681	0.602–0.760
		Elecsys	12.9	0.680	0.599–0.761
Colorectal cancer	113	Dxl 800	4.4	0.520	0.450–0.589
		Elecsys	6.2	0.531	0.462–0.601
Lung cancer	82	Dxl 800	35.4	0.733	0.645–0.821
		Elecsys	35.4	0.693	0.601–0.786
Bladder and renal cancer	57	Dxl 800	0.0	0.399	0.299–0.499
		Elecsys	0.0	0.387	0.287–0.487

Survey on the diagnostic capacity of the Access OV Monitor (Beckman Coulter) for various cancer diseases when compared with their respective benign diseases as control groups and comparison with the Elecsys CA125 (Roche Diagnostics). Area under the curve (AUC) of the receiver operating characteristic curves and sensitivity at 95% specificity vs. the respective benign diseases indicate the discriminating power.

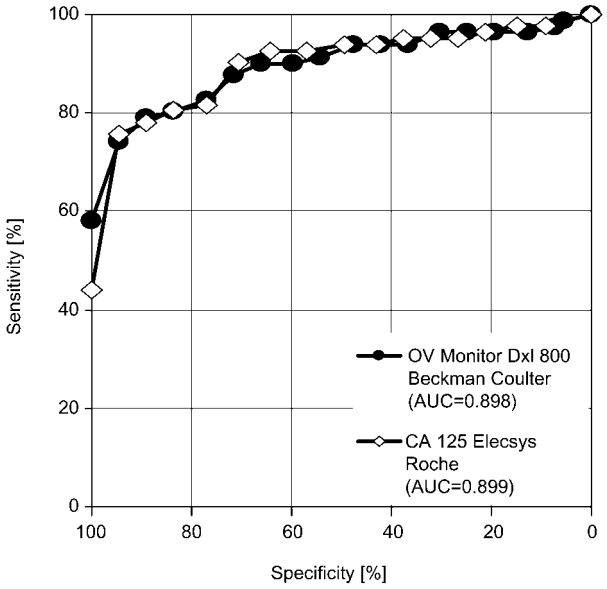

Figure 7 Diagnostic capacity of the OV Monitor and reference method for detection of ovarian cancer.
Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for ovarian cancer (n = 81) vs. benign gynecological diseases (n = 109). (●) Access OV Monitor (Beckman Coulter) and (◇) Elecsys CA125 (Roche Diagnostics).

squamous cell lung cancer it was only 0.587 for the Access OV Monitor and 0.546 for the Elecsys CA125 (Figure 8).

Discussion

Several studies have shown that CA125 is the marker of first choice for diagnosis of ovarian cancer (2, 9, 12–17). However, specificity of CA125 is limited by various benign diseases, particularly those affecting the visceral epithelia, e.g., when pleural, pericardial or peritoneal effusions are present (10, 11). Besides diagnosis, CA125 levels have shown to be relevant for the prognosis of ovarian cancer patients undergoing surgery and/or receiving systemic chemotherapy (17–19). Similarly, the usefulness of CA125 for therapy monitoring, as well as early detection of disease progression in ovarian cancer patients is widely recognized and accepted (2, 9, 20–22). In addition to ovarian cancer, CA125 is detected in endometrial, cervical, lung, renal and gastrointestinal cancer tissues, as well as in the serum of these patients (2, 9, 24–26).

In the present study, the new Access OV Monitor assay, which uses the monoclonal antibodies OVK95 and OV185 for detection of the CA125 antigen (23), was tested on its analytical and clinical performance. The guidelines of the EGTM (27) require a new diagnostic method to be investigated for potential influence of organ-specific and non-specific influences which might alter the metabolism of the antigen. Further, the new method has to be compared with a current accepted method to demonstrate its superiority, or at least equivalence, for the intended indication.

First, we therefore performed a thorough analytical evaluation at five European centers, to test the basic preconditions for routine application. Then, a large panel of sera from 1811 individuals was investigated. These individuals included healthy individuals, patients with gastrointestinal and other benign diseases and many patients with various cancer diseases that might be relevant for differential diagnosis by CA125. The entire clinical evaluation of the Access OV Monitor was carried out in parallel with the Elecsys CA125, a current standard method, using the same sera from the same patients, to enable a fair comparison of both methods.

The analytical performance for the OV Monitor assay was very good with a low within-run, total and inter-laboratory imprecision. Additionally, we observed high recoveries during linearity upon dilution testing, and no high-dose hook effect up to 27,193 kU/L. Sample type interference studies demonstrated that serum and lithium-heparinate plasma can be used interchangeably. However, OV Monitor levels in EDTA plasma and citrate plasma were approximately 10%–20% lower than in serum. Concerning sample storage, it is important to note that freezing did not affect the marker values, and long-term storage for 6 months at –20°C still yielded stable results. Of clinical relevance is the finding that endogenous interferents, such as hemoglobin, bilirubin, triglycerides and rheumatoid factor, do not influence OV Monitor concentrations.

Comparison of the Access OV Monitor with Elecsys CA125 showed an excellent correlation for all patients and for the various subgroups investigated. This observation is all the more valuable as the slope and intercept were only minimal, meaning that the absolute values of both methods were very comparable. Nevertheless, it has to be pointed out that in single patients considerable differences in the concentrations were observed showing the necessity to plan carefully the potential change of the CA125 antigen methods and to measure CA125 antigen in parallel with both methods if kinetic interpretations are carried out.

In our study, healthy individuals had very low concentrations, as measured with both methods. Cut-offs for the URLs were very comparable and were in the range of the values indicated by both methods (Beckman Coulter: 35 kU/L, Roche Diagnostics: 35 kU/L).

Concentrations in sera of individuals diagnosed with benign gastrointestinal, lung, breast, gynecological diseases and other disorders were higher than in healthy individuals. The differences were only slight in benign lung and breast diseases, whereas single individuals with benign gastrointestinal, gynecological and urological diseases reached higher values. As expected, the highest CA125 levels were observed in benign gastrointestinal diseases, which were affected with peritoneal effusion. In this group, the median level was in the range of other benign diseases, but single individuals reached extremely high levels (> 1000 kU/L).

CA125 concentrations in patients suffering from various cancers were also elevated. However, in

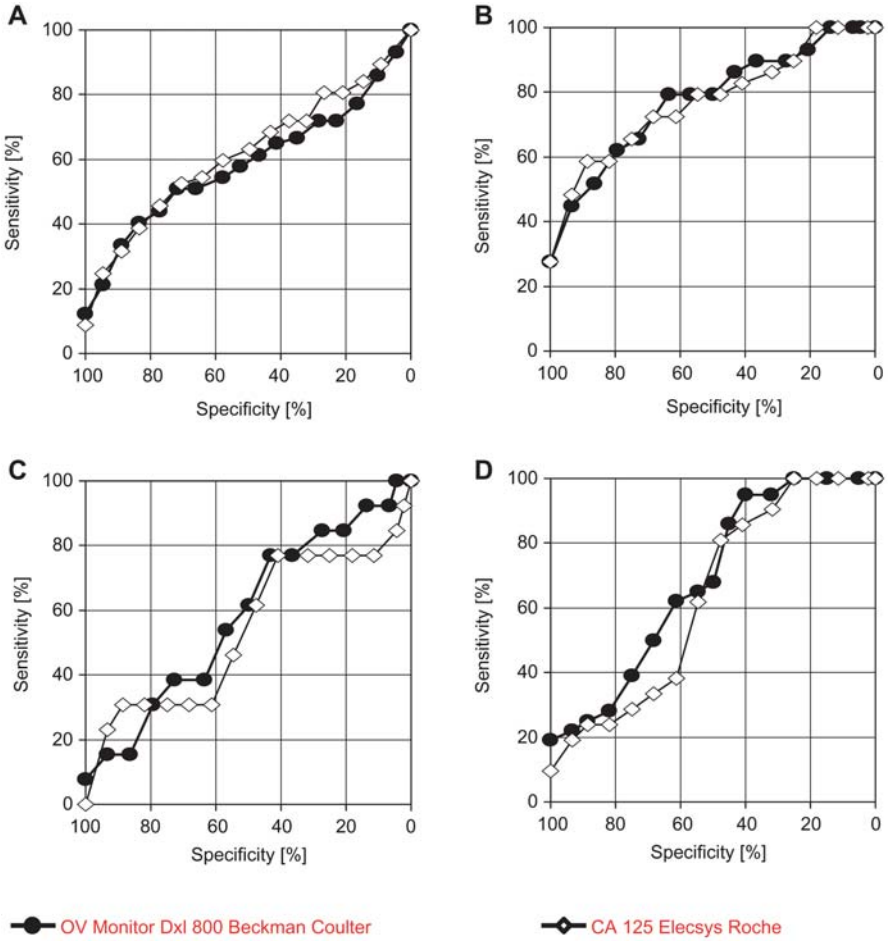

Figure 8 Diagnostic capacity of the OV Monitor and reference method for detection of other gynecological cancers and lung cancer.
Profiles of sensitivity and specificity over the whole range of cut-off values are shown by receiver operating characteristic (ROC) curves for (A) gynecological cancers (n = 57) vs. benign gynecological diseases (n = 109), (B) adeno cell lung cancer (n = 29) vs. benign lung diseases (n = 44), (C) squamous cell lung cancer (n = 13) vs. benign lung diseases (n = 44), and (D) small cell lung cancer (n = 21) vs. benign lung diseases (n = 44). (●) Access OV Monitor (Beckman Coulter) and (◇) Elecsys CA125 (Roche Diagnostics).

some cancer types, the medians and 95th percentiles were comparable with those of benign diseases, e.g., for bladder, renal and prostate cancer. In contrast, gastrointestinal, gynecological and lung cancers demonstrated greater CA125 elevations, which reached > 1000 kU/L in some patients.

However, it has to be pointed out that most of the samples of healthy individuals, and individuals with various benign diseases and individuals with malignant diseases without ovarian cancer had very low OV Monitor levels not only in the reference range < 35 kU/L but even below 15 kU/L. In contrast, only a few individuals with ovarian cancer with or without distant metastases had such low OV Monitor concentrations suggesting that the non-release of CA125 antigen is essential to estimate the probability of not having ovarian cancer if there are suspicious pelvic masses. On the contrary, even if high CA125 antigen levels were found in some individuals with benign diseases or cancer other than of ovarian origin, CA125 antigen levels > 300 kU/L and suspicious pelvic masses are very suggestive for ovarian cancer with or without distant metastases.

As expected, the best diagnostic accuracy of the OV Monitor for cancer detection against the relevant benign control group was found for ovarian cancer (AUC: 0.898, sensitivity at 95% specificity vs. benign gynecological controls 74.1%). This excellent differential diagnostic result cannot be achieved by other established tumor-associated antigens and underlines the high relevance of CA125 antigen in ovarian cancer. Importantly, OV Monitor results also correspond very well with the diagnostic accuracy of the CA125 reference method for cancer detection, demonstrated by the similar AUC values and the broadly overlapping confidence intervals. Though the diagnostic power of the OV Monitor is lower for other tumor types, it has to be emphasized that for lung and gynecological cancer the AUC was still higher than or near to 0.6 and the sensitivity at 95% specificity vs. the relevant benign control group was higher than 20% – once again, both methods were very comparable. As

already mentioned by some previous reports (26), the diagnostic sensitivity of CA125 antigen was highest in the adenocellular histological subtype, whereas it was considerably lower in the squamous cellular subtype for both gynecological and lung cancers. This diagnostic performance in cancer types for which CA125 was not considered as a relevant marker suggests there may be value in including CA125 with other diagnostically relevant markers in future multiparametric analyses. In combination with well-known markers, e.g., CYFRA 21-1, carcino-embryonic antigen, neuron-specific enolase and squamous cancer cell antigen in lung cancer, CA125 might be helpful to further improve the diagnostic accuracy and may aid in finding the histological differential diagnosis (26, 28).

Conclusions

The Access OV Monitor is a new assay based on an alternative antibody for CA125 antigen detection. The Access OV Monitor provides very good methodological characteristics for use in routine laboratory and demonstrates an excellent analytical and clinical correlation with the Elecsys CA125. The OV Monitor shows a high diagnostic accuracy in ovarian cancer and it is a valuable marker in the management of this disease. Our results also suggest a clinical value of the OV Monitor in lung and gynecological cancers. If the CA125 antigen method is changed, parallel measurements of CA125 with both methods for an appropriate time span are strongly recommended.

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