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Analytical and clinical evaluation of an electrochemiluminescence immunoassay for the determination of CA 125

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The CA 125 II assay on the Elecsys® 2010 analyzer was evaluated in an international multicenter trial. Imprecision studies yielded within-run CVs of 0.8–3.3% and between-day CVs of 2.4–10.9%; CVs for total imprecision in the manufacturer's laboratory were 2.4–7.8%. The linear range of the assay extended to at least 4500 kilounits/L (three decades). Interference from triglycerides (10.3 mmol/L), bilirubin (850 μmol/L), hemoglobin (1.1 mmol/L), anticoagulants (plasma), and several widely used drugs was undetectable. Method comparisons with five other CA 125 II assays showed good correlation but differences in standardization. A 95th percentile cutoff value of 35 kilounits/L was calculated from values measured in 593 apparently healthy (pre- and postmenopausal) women. In 95% of patients with benign gynecological diseases CA 125 was ≤190 kilounits/L; 63% of patients with newly diagnosed ovarian carcinoma had values >190 kilounits/L. A comparison of CA 125 values obtained with the Elecsys test and with other common CA 125 tests in monitored patients being treated for ovarian cancer showed identical patterns. In conclusion, the Elecsys CA 125 II assay is linear over a broad range, yields precise and accurate results, is free from interferences, and compares well with other assays.

CA 125 is a glycoprotein that occurs in blood as high molecular weight (M_r >200 000) aggregates. High concentrations are associated with ovarian cancer and with a range of benign and malignant diseases. Although the specificity and sensitivity of CA 125 assays are somewhat limited, especially in the early diagnosis of ovarian cancer, the assay has found widespread use in the differential diagnosis of adnexal masses, in monitoring disease progression and response to therapy in ovarian cancer, and in the early detection of recurrence after surgery or chemotherapy for ovarian cancer. The use of CA 125 assays has been reviewed excellently *(1, 2)*. Most assays for the determination of CA 125 use the M11 antibody for capture and the OC125 antibody as tracer. Such assays are named CA 125 II. Recently, Boehringer Mannheim GmbH introduced a fully automated CA 125 II assay on their Elecsys® 2010 analyzer. This study describes the results of an extensive multicenter, international evaluation of the analytical and clinical performance of this assay.

Materials and Methods

TEST PRINCIPLE

The Elecsys CA 125 II test is a sandwich immunoassay that is provided for Elecsys immunoassay systems (Boehringer Mannheim GmbH). As described recently *(3)*, these instruments use electrochemiluminescence as the detection technology, thereby offering the advantage of short reaction times and high sensitivity. A 40-μL sample is incubated with biotinylated M11 capture antibody and ruthenylated OC125 tracer antibody. After 9 min, streptavidin-coated paramagnetic beads are added, followed by an additional 9-min incubation period. The reaction mixture is then drawn into the measuring cell where the

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following steps take place: capture of the magnetic beads, addition of buffer containing tripropylamine, and voltage application and measurement of the resulting electrochemiluminescence by a photomultiplier. Results are available after 18 min.

The CA 125 test is calibrated against the Enzymun-Test® CA 125 II (Boehringer Mannheim GmbH). Reagent-specific application data and a master calibration curve are stored on two-dimensional bar codes supplied with the reagents. Two-point calibration in the user's laboratory adapts the master calibration curve to the analyzer. Reagents and calibrators are ready to use.

ANALYTICAL EVALUATION OF ELECSYS CA 125 II

The test was evaluated according to a standardized protocol based on the concepts of a European Committee for Clinical Laboratory Standards document *(4)* in eight clinical laboratories on fully automated Elecsys 2010 systems.

Imprecision. Imprecision studies were carried out using control materials and human pool sera with defined concentration ranges of CA 125. Within-run imprecision (CV; 21 replicates per analysis) was determined in seven laboratories, and between-day imprecision (measurement once daily for 21 days) was determined in five laboratories. Total imprecision was measured in the laboratory of the manufacturer following the NCCLS EP5-T protocol.

Accuracy. Five laboratories participated in a small interlaboratory survey by measuring CA 125 (twice daily for 5 days) in two human pool sera that were shipped to the participants in frozen aliquots. The results obtained in the individual laboratories were compared with the overall median.

Method comparison studies with the Elecsys assay were carried out in the participating laboratories with the Enzymun-Test CA 125 II on ES 300, 600, and 607 enzyme immunoassays, two microparticle enzyme immunoassays (MEIA 1 on AxSYM® and MEIA 2 on IMx® immunoanalyzers, Abbott Laboratories) and two IRMAs (IRMA 1, ELSA-CA 125 II; CIS bio international; IRMA 2, Centocor® CA 125 II; Centocor Inc.). The tests were performed according to the manufacturers' protocols. Regression equations were calculated according to Passing and Bablok *(5)*.

Analytical range. The lower limit of detection was determined by two different methods. In the one, the calibrator matrix without antigen was measured in nine independent analyses (21 replicates per analysis) in three laboratories. The lower limit of detection was defined as the mean +2 SD of these measurements. In the other, a human serum sample with a very low CA 125 concentration was used for the same kind of measurements in one laboratory. The linearity of the test was analyzed by the dilution of human pool sera with CA 125 concentrations up to 4500 kilounits/L with either low-concentration human pool sera or Elecsys Diluent Universal according to a recently described protocol *(6)*. The method was considered linear if the measured concentration deviated <10% from the expected concentration. The possible occurrence of a high-dose hook effect was studied by measuring serial dilutions (with the Elecsys diluent) of samples with very high concentrations of CA 125.

Interferences. Possible interference by hemolysis, icteria, or lipemia was analyzed by addition experiments performed according to a Société française biologie clinique protocol *(7)* as well as by dilution experiments. The effects of rheumatoid factors, biotin, and dysproteinemia were checked in the laboratory of the manufacturer. Results were rated acceptable if the diluted sample or the sample containing a potentially interfering substance showed a recovery range of 90–110% of the undiluted sample or the sample without the interfering substance. Eighteen widely used drugs and 11 cytotoxic drugs were tested as described previously *(8)*. The comparability of results obtained in serum and various types of plasma (heparin, EDTA, or citrate plasma) derived from the same donors was studied in four laboratories.

HEALTHY INDIVIDUALS/REFERENCE RANGE

Five laboratories participated in the determination of the reference range, using sera from 593 nonpregnant women (195 pre- and 293 postmenopausal) and 289 men. Individuals included were >18 years of age and judged apparently healthy by clinical and clinical chemistry parameters. In one laboratory, CA 125 was also measured in sera derived from 32 women in the first trimester of pregnancy (weeks 2–9). The results were analyzed by nonparametric fractile limits, and the 95th percentile was taken as the upper limit.

BENIGN DISEASES

Samples from 342 patients with benign diseases were analyzed. These diseases could be summarized as follows: 80 gynecological diseases (ovarian cysts, ovarian metaplasia, endometriosis, uterine leiomyoma, cervicitis, and squamous metaplasia), 87 liver diseases (cirrhosis, hepatitis, necrosis, cysts, cholecystitis, and hemochromatosis), 46 gastrointestinal diseases (acute and chronic pancreatitis, colitis ulcerosa, Crohn disease, diverticulosis, and colon polyps), 33 renal insufficiency patients, and 96 other benign diseases.

MALIGNANCIES OTHER THAN OVARIAN CARCINOMA

Sera from 505 patients were included: 56 patients with endometrial carcinoma, 169 patients with gastrointestinal carcinoma (colonic, pancreatic, gastric, rectal, or esophageal carcinoma), 58 patients with bronchial carcinoma, 128 patients with breast carcinoma, and 94 patients with other malignancies (genito-urinary, liver, prostate, hematological, and thyroid).

Table 1. Summary of imprecision studies in control serum (CS) and human serum (HS) by ECCLS[a] protocol.

Imprecision	No. of analyses/ laboratories	Material	Concentration, kilounits/L	CV, %		
				Min	Max	Median
Within-run	19/7	CS 1	41.0	0.7	3.3	1.7
	19/7	CS 2	126	0.8	2.8	1.5
	17/6	HS 1	10.6–22.0	1.0	3.0	2.1
	17/5	HS 2	41.1–110	0.8	3.3	1.6
	17/5	HS 3	406–1026	1.0	4.8	1.5
Between-day	99/5	CS 1	41.0	2.9	9.0	5.7
	98/5	CS 2	126	2.9	7.5	6.2
	99/5	HS 1	10.7–21.7	3.0	10.9	10.1
	99/4	HS 2	42.0–104	2.4	6.0	4.3
	99/4	HS 3	400–1015	5.5	8.8	6.2

[a] ECCLS, European Committee for Clinical Laboratory Standards.

OVARIAN CARCINOMA PATIENTS

Samples drawn at the time of primary diagnosis from 100 patients with ovarian carcinoma were measured [35 stage I, 17 stage II, 25 stage III, and 11 stage IV according to the International Federation of Gynecology and Obstetrics (FIGO) criteria *(9)*]. The majority of patients had nonmucinous ovarian carcinoma. Additional sera (n = 171) were derived from patients during treatment and follow-up.

MONITORING OF OVARIAN CARCINOMA

Measurements with the Elecsys test and IRMA 2 (Centocor CA 125 II; Centocor Inc.) were carried out on samples obtained serially from 50 clinically well-characterized patients during treatment and follow-up. Measurements with Elecsys and Enzymun-Test reagents were performed in sera collected serially from 10 patients. At least four points per patient at different monitoring intervals were tested.

ETHICS CONSIDERATIONS

All procedures followed in this study were in accordance with the guidelines of the various local ethics committees.

Results and Discussion

ANALYTICAL PERFORMANCE OF THE ELECSYS CA 125 II TEST

Imprecision. In the diagnostically most important range from 10 to ~100 kilounits/L, within-run CVs of 0.8–3.3% and between-day CVs of 2.4–10.9% were obtained in human pool sera (Table 1). Total imprecision CVs ranged from 2.4% to 7.8%. CVs >10% were found in low-concentration samples (<13 kilounits/L). There were no major differences at comparable concentrations in the CVs for control sera and human sera.

Accuracy. Measurement of CA 125 in two human pool sera revealed median recoveries of 94–110% for the low-concentration serum pool (32 kilounits/L) and 86–112% for the high-concentration serum pool (332 kilounits/L) by referring the results of the individual laboratories to the median of all laboratories (data not shown). These results indicate a good interlaboratory transferability of the Elecsys CA 125 II test.

The results of the method comparison studies are summarized in Table 2. Representative examples of

Table 2. Summary of method comparison studies between Elecsys CA 125 (*y*) and routine immunochemical methods (*x*).

Laboratory no.	Method (*x*)	Range, kilounits/L		n	Passing/Bablok regression		Correlation coefficient	$s_{y\|x}$, kilounits/L
		Min	Max		Slope	Intercept, kilounits/L		
3	EIA[a]	1	488	286	0.94	2.27	0.987	9.64
4	EIA	1	489	341	0.98	−2.84	0.987	12.45
7	EIA	3	457	133	1.07	2.82	0.989	9.14
8	EIA	1	209	136	0.96	1.57	0.932	11.18
4	MEIA 1	3	480	329	1.03	0.81	0.986	13.26
4	MEIA 2	1	405	330	1.23	2.41	0.987	11.67
7	IRMA 1	7	337	96	1.42	−6.52	0.977	16.95
1	IRMA 2	1	464	139	0.93	5.57	0.981	9.93
8	IRMA 2	2	240	137	0.89	−0.38	0.979	5.06

[a] EIA, enzyme immunoassay.

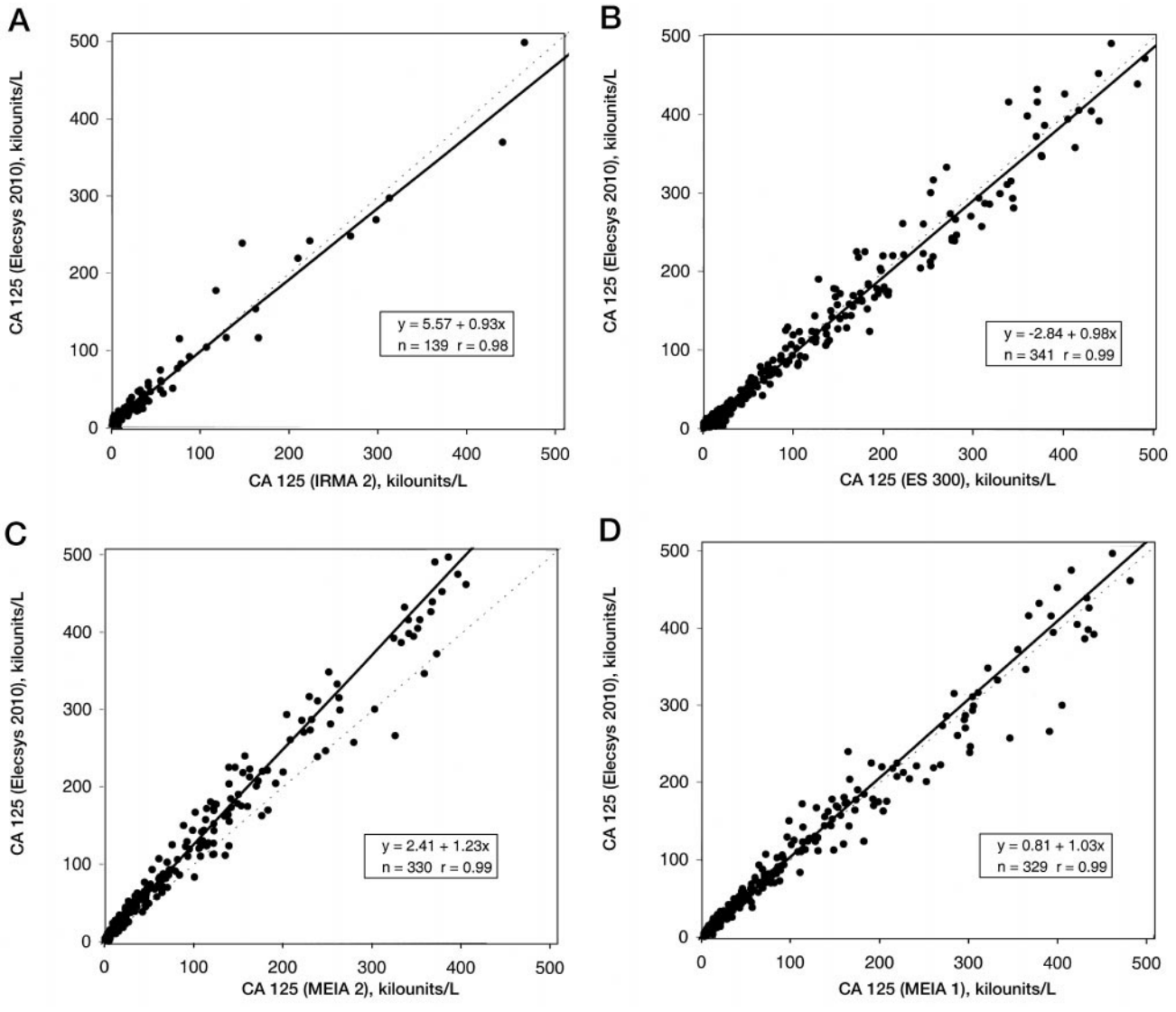

Fig. 1. Examples of method comparison studies between the Elecsys CA 125 II and routine immunochemical methods. (· · · · ·), identity lines; (———), Passing-Bablok regressions.

method comparison plots are shown in Fig. 1. The data presented include only values up to 500 kilounits/L (measuring range of the comparison methods) to exclude dilution effects. Similar results were obtained if higher values were included in the calculation. Although some outliers were detected, good comparability of the Elecsys CA 125 II test with Enzymun-Test CA 125 II, MEIA 1, and IRMA 2 was obtained. Systematic differences were found in the comparison with MEIA 2 and IRMA 1, which can be explained by differences in the standardization of these methods. Comparable systematic deviations between MEIA 2 and IRMA 2 as well as another CA 125 test were described in a recent paper *(10)*.

Analytical range. The limit of detection calculated from repeated measurements of the zero calibrator was found to be <0.6 kilounits/L in all analyses. This is equivalent to the readable limit of the analyzer. One laboratory performed measurements in a serum sample with a CA 125 value of 0.8 kilounits/L. The limit of detection obtained for serum was 1.1 kilounits/L, which is highly sufficient for diagnostic purposes.

According to the manufacturer, the assay is linear up to 5000 kilounits/L. This was checked in four laboratories in a total of 24 dilution series covering three decades of CA 125 concentrations, using either human sera or diluent for dilution. On the basis of an acceptance range of 90–110% recovery, the linearity of the assay when serum was used as diluent was confirmed throughout the range studied (highest serum concentration ~4500 kilounits/L) with the exception of the highest dilution, which exceeded the acceptance criteria in 5 of 12 experiments. The acceptance criteria were not met with the low-concentration human pool sera (5–10 kilounits/L) used for dilution. Analytical

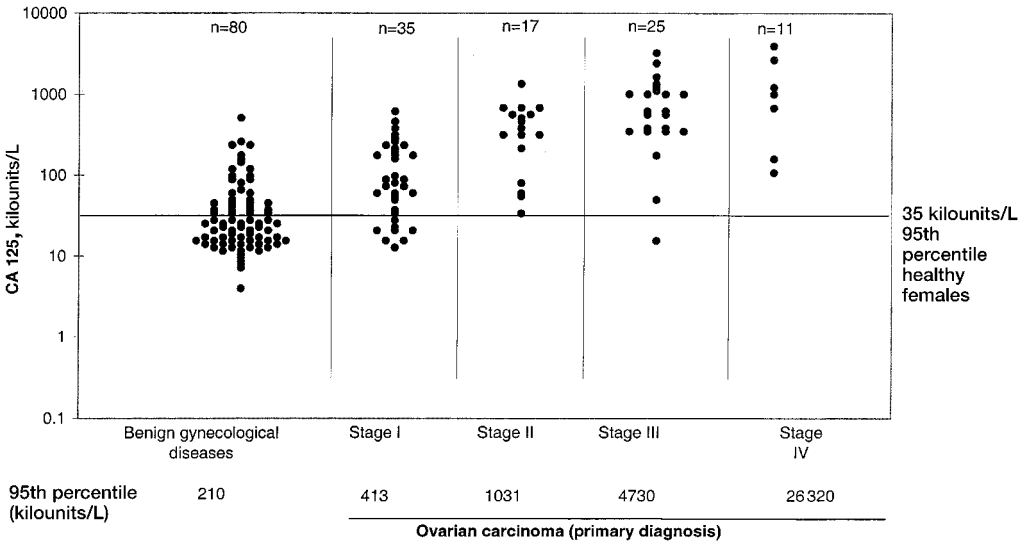

Fig. 2. CA 125 values measured with the Elecsys CA 125 II in patients with benign gynecological diseases and ovarian carcinoma patients classified according to the FIGO stage.

imprecision at these very low concentrations was a probable cause for this phenomenon. The Elecsys diluent was found to be suitable provided that the concentration of the diluted sample was not below ~500 kilounits/L.

No high-dose hook effect was observed up to 26 000 kilounits/L, the highest concentration available. Higher concentrations can occasionally be encountered in fluids obtained from ovarian cysts; however, in such cases the laboratory will already be alerted to the possible presence of extremely high concentrations by the nature of the sample. It should be noted that a high-dose hook effect can occur when samples with extremely high concentrations are measured. Such samples are encountered very rarely.

Interferences. Addition experiments revealed no effect by bilirubin up to 850 μmol/L (recovery, 97.2–100.1%), hemoglobin up to 1.1 mmol/L (recovery, 92.3–101.2%), or triglycerides up to 10.3 mmol/L (recovery, 100.7–102.7%; data not shown). The concentrations given are the highest tested and are representative of interferences encountered in practice. Comparable results were obtained in dilution experiments. No influence on the results of the Elecsys CA 125 II test was observed by rheumatoid factor activity

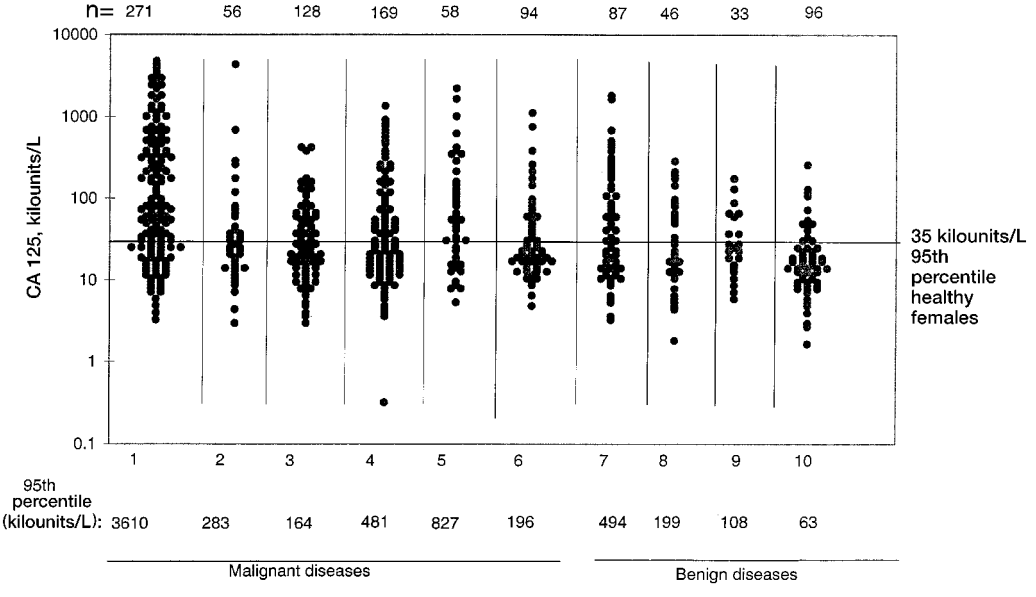

Fig. 3. CA 125 values measured with the Elecsys CA 125 II in benign and malignant diseases.

1, ovarian cancer; *2*, endometrial cancer; *3*, mammary cancer; *4*, gastrointestinal cancer; *5*, bronchial cancer; *6*, other cancers; *7*, benign liver disease; *8*, benign gastrointestinal disease; *9*, renal insufficiency; *10*, other benign diseases.

(1500 kIU/L), biotin (50 μg/L), or in dysproteinemic sera. The addition of 18 widely used drugs (fivefold therapeutic concentration) and 11 cytotoxic drugs (one- or fivefold therapeutic concentration) did not affect the results.

Comparison studies between serum and heparin, EDTA, and citrate plasma yielded slopes from 0.91 to 1.03, intercepts <2.0 kilounits/L, and correlation coefficients of 1.00, thereby indicating that these types of plasma are suitable as sample material (data not shown).

CLINICAL EVALUATION

Reference range. CA 125 was measured in 593 apparently healthy women (pre- and postmenopausal, ages >18 years) in five laboratories. A cutoff value of 35 kilounits/L (95th percentile) was obtained for the Elecsys CA 125 II test. This value is identical to the generally accepted cutoff for other CA 125 assays, which are described in several publications *(11–13)*. Slightly higher 95th percentile cutoff CA 125 concentrations were found in premenopausal women (38 kilounits/L) compared with postmenopausal women (31 kilounits/L). CA 125 concentrations in women in the first trimester of pregnancy were frequently >35 kilounits/L (median, 38 kilounits/L), as reported in the literature *(1, 11)*. A 95th percentile cutoff of 28 kilounits/L was found in 289 men.

Distribution of CA 125 in Different Groups of Patients. The distribution of CA 125 values in ovarian carcinoma patients at the time of primary diagnosis related to the FIGO stage was compared with that of women suffering from benign gynecological diseases (Fig. 2). A large overlap existed in the two groups studied. As shown previously *(11)*, the magnitude of CA 125 increases was clearly related to the tumor stage.

CA 125 concentrations in malignancies other than ovarian carcinoma and various benign diseases are presented in Fig. 3. As is known, CA 125 is an unspecific tumor marker [see Ref. *(1)*]. Although the highest concentrations measured with Elecsys CA 125 II were in ovarian carcinoma patients, very high values frequently occurred in other malignancies studied. Benign diseases were also frequently associated with CA 125 values >35 kilounits/L. Especially high CA 125 concentrations were found in the group of benign liver disease patients (95th percentile, 494 kilounits/L).

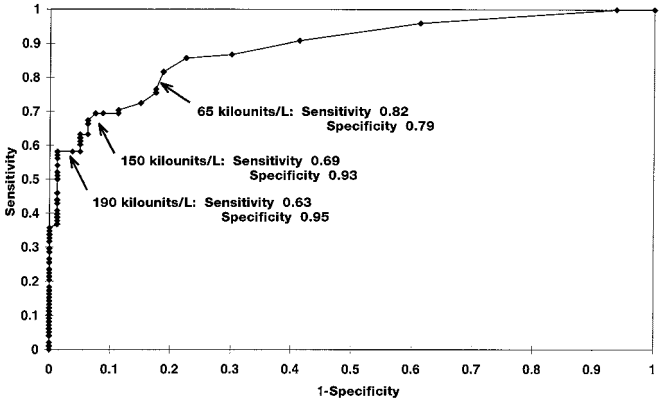

Fig. 4. ROC plot for patients with ovarian carcinoma (primary diagnosis, n = 100) vs benign gynecological diseases (n = 80).

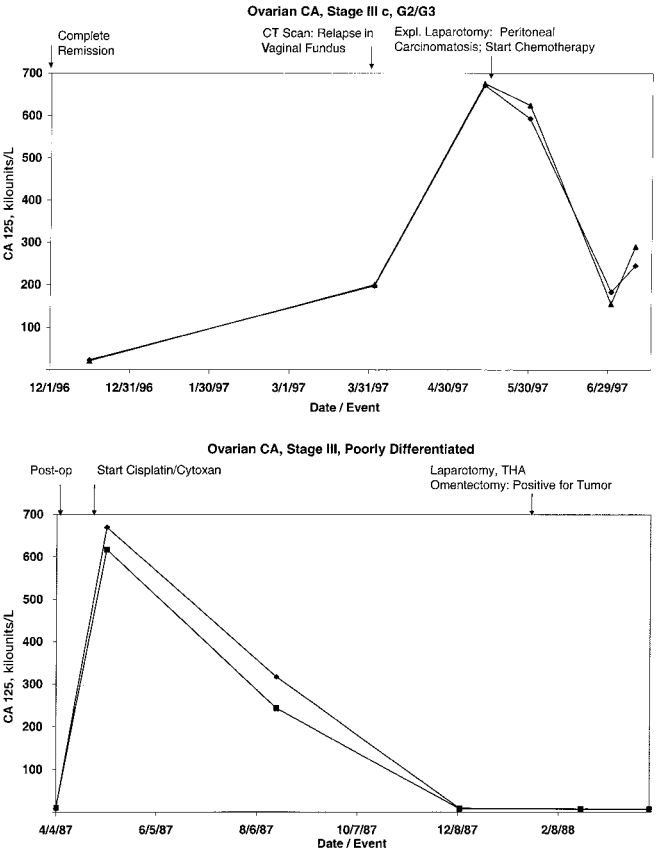

Fig. 5. Follow-up of ovarian carcinoma patients, using the Elecsys CA 125 and Enzymun-Test CA 125 (*top*) or IRMA 2 (*bottom*).
♦, Elecsys CA 125 II; ▲, Enzymun-Test CA 125 II; ■, IRMA 2.

Diagnostic Sensitivity and Specificity. For the calculation of diagnostic sensitivity and specificity, CA 125 values in 100 ovarian carcinoma patients at the time of primary diagnosis were compared with 80 patients with benign gynecological diseases (clinically relevant reference group). The maximum diagnostic efficiency was reached at 150 kilounits/L (specificity, 93%; sensitivity, 69%; Fig. 4). When specificity was fixed at 95%, following the recommendation of the Hamburg Group for Standardization of Tumor Markers *(14)*, the sensitivity was 63% (cutoff, 190 kilounits/L). This sensitivity is in agreement with previously published results for other CA 125 methods, which described sensitivities from 50% to 60% *(10, 15, 16)*. At the historical cutoff of 65 kilounits/L *(13)*, the specificity decreased to 82%.

Apparently healthy women were also used as the reference group for ovarian carcinoma patients to simulate the screening situation. At 35 kilounits/L (95% spec-

ificity), a sensitivity of 87% was obtained, which confirms other reports *(1, 17)* that indicated that measurement of CA 125 alone has insufficient diagnostic efficiency to be used as a screening test for the general population [prevalence of ovarian cancer, 0.05% *(17)*] for ovarian cancer. In this situation the positive predictive value would be only 0.86%.

Follow-up of Ovarian Carcinoma Patients. Representative examples of the 60 patients studied with the Elecsys CA 125 II and either the Enzymun-Test CA 125 II or IRMA 2 are shown in Fig. 5. When the results obtained by the different methods were compared, a clear agreement was found. The CA 125 values were correctly reflecting the status of the disease and the effect of various therapeutic measures.

Conclusion

The Elecsys CA 125 II assay has proven to be precise and accurate. The assay has an extended range (from 0.6 to at least 4500 kilounits/L) and is free from the interferences usually encountered. Clinical evaluation showed that the assay performed very comparably to other established CA 125 II assays. In our view, the Elecsys CA 125 II assay is suitable for routine use in the diagnosis and follow-up of ovarian cancer patients.

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