PT program criteria, one can assess the impact of proposed regulations. In six cases the CDC criteria permit overly large intralaboratory CVs, in seven cases the CDC criteria are approximately correct, and in two cases (AST and thyroxin) the CDC criteria may be tighter than necessary. Clearly, for calcium, chloride, and cholesterol the proposed limits would allow grossly larger intralaboratory errors than are compatible with the needs of physicians. Intuitively, there appears to be a subtle compounding effect in the approach. By setting the performance criteria based on a group of samples, i.e., 67% (four of six) or 70% (seven of 10), but recognizing that the laboratory performs only one analysis per patient, the proposed PT regulations may be promoting the exact opposite of the desired effect.

The present study is not designed merely to criticize the proposed criteria, but it suggests that some revision is needed. Rather, we wish to propose, based on our model, a positive, quantitative approach to selecting PT criteria. PT limits, both the range and frequency of acceptable intralaboratory performance, could be based on medical need and selected from Figure 1. In setting PT criteria, the regulators have several variables to work with. The size of the fixed limit can be altered; this has the most pronounced effect on the level of intralaboratory error that is tolerated. Also, the minimum frequency of acceptable results could be modified; i.e., a change from 70% to 80% translates to about a 25% decrease in the allowable intralaboratory CV. Other approaches to PT program grading are also possible (13).

PT programs always suffer from trying to draw very precise conclusions about intralaboratory performance based on very small numbers of PT data. As clinicians better define intralaboratory performance needs, increasing the number of PT challenges and using more effective PT program grading criteria will increase the ability of PT programs to identify substandard intralaboratory performance so that passing PT will actually denote that medically useful data are being provided.

References

Improved Specificity of the CA 125 Enzyme Immunoassay for Ovarian Carcinomas by Use of the Ratio of CA 125 to Carcinoembryonic Antigen
James T. Wu, Terry Mllyi, Joseph A. Knight, and David P. Knight

We found that ovarian cyst fluids contained carcinoembryonic antigen (CEA) and CA 19-9 and CA 125 tumor markers. However, only the ratio of CA 125 to CEA concentrations provided sufficient specificity to differentiate serous from mucinous cysts. For CEA measurement, our results suggested the use of a monoclonal CEA kit. When CEA was determined with a Hybritech monoclonal CEA kit, all ratios in mucinous ovarian cysts were <10 and most of the ratios were >1000 in serous ovarian cysts. We also found that the ratio of CA 125 to CEA in serum could be used to differentiate ovarian from nonovarian malignant diseases when both sera contain increased CA 125 concentrations. The nonovarian malignancies consisted of colorectal, breast, lung, and pancreatic carcinomas. The mean ratio for serum from patients with nonovarian cancers was 0.94 (n = 19); for ovarian-cancer patients (n = 45), 916. Therefore, determining this ratio will greatly improve the specificity of the CA 125 test for ovarian cancer.

Ovarian cancer is the most lethal form of gynecological malignancy in the United States (1). Its association with a mortality rate is due in part to the lack of a specific and sensitive test for the early detection of ovarian cancer or for

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monitoring patients' response during chemotherapy. Although ovarian tumors are histologically heterogeneous, about 90% are epithelial (1). Serous tumors, the most common form of ovarian malignancies, account for 35% to 50% of all ovarian tumors (1, 2). The less common—and usually benign—mucinous tumors account for 6% to 10% (1).

Although several tumor-associated markers have been identified with ovarian epithelial tumors (3, 4), none has sufficient sensitivity and specificity for clinical use. Recently, a radiolmmunossay (RIA) kit, based on the use of a monoclonal antibody against an ovarian tumor cell line, has been developed (5) to measure the antigenic determinant CA 125 in serum.2 As measured with this kit, 82% to 92% of patients with ovarian carcinoma had increased concentration of CA 125 in their serum (6, 7); as many as 96% of the patients with serous cyst adenocarcinoma had increased CA 125 (7). However, increased concentrations were also found in 43% of the patients with various nonovarian malignancies, particularly colonic and mammary adenocarcinomas at advanced stages (7). In addition, CA 125 values for serum were increased during pregnancy (8), thereby further decreasing the usefulness of CA 125 RIA as a screening or diagnostic test.

Here we report our study of fluid from histologically proven serous and mucinous cysts, used to reassess the specificity of several newly developed monoclonal antibody-based immunoassay kits. Besides evaluating the specificity of individual antigenic determinants, we wanted to improve overall test specificity by measuring patterns of antigenic determinants through the use of various combinations of monoclonal defined markers. We postulated that any test design capable of differentiating between two morphologically distinct, but closely related, ovarian cysts might also be sufficiently specific for the diagnosis of ovarian epithelial carcinomas.

We found that by applying the ratio of CA 125 to carcinoembryonic antigen (CEA) to serum measurements, we could identify abnormal CA 125 values in patients with nonovarian tumors, thereby greatly improving the specificity of the CA 125 test.

Materials and Methods

Three kits for measuring tumor-associated antigens—CEA by EIA, CA 19-9 by RIA, and CA-125 by EIA—were purchased from Abbott Laboratories, North Chicago, IL 60064. CEA-Roche was obtained from Roche Diagnostics, Nutley, NJ 07110. The Tandem-CEA kit was a gift from Hybritech Inc., San Diego, CA 92121.

We stored 27 specimens of serous and mucinous ovarian cyst fluids, removed at surgery, at 4 °C until analyzed for CA 125, CA 19-9, and CEA with the kits named above. The anatomical diagnoses were established by standard light-microscopic criteria for tissue sections, processed in the usual manner and stained with hematoxylin and eosin.

We collected 64 serum specimens from the clinical laboratory: 19 from cases of nonovarian cancers with increased values for both CA 125 and CEA, which included colorectal, breast, pancreatic, and lung carcinomas; and 45 with increased CA 125 values from cases of ovarian cancer.

2 Nonstandard abbreviations: CEA, carcinoembrionyc antigen; CA 125, the antigenic determinant(s) to which the monoclonal antibody OC 125 binds; CA 19-9, carbohydrate antigen; EIA, enzyme immunoassay.

Results

Specificity of Individual Kits

In Figure 1 the values of CA 19-9 and CEA in cystic fluids are grouped according to whether they were from serous or mucinous ovarian cysts. The CEA concentrations were determined by use of both Roche and Abbott polyclonal-antibody kits. Neither CEA measurement nor the CA 19-9 test was useful in differentiating mucinous from serous ovarian cysts. Although both CEA kits showed that most mucinous ovarian cysts contained greater concentrations of CEA than did the serous cysts, there was considerable overlap. However, the Abbott CEA kit performed better than the Roche kit, generally yielding lower CEA values for serous fluids (and hence higher CA 125/CEA ratios), thereby providing better differentiation from mucinous fluids. Both cystic fluids contained relatively high concentrations of CA 19-9.

Replacing the polyclonal anti-CEA kits with the Hybritech monoclonal antibody kit resulted in lower CEA measurements for serous fluids without appreciably changing the CEA content measured in mucinous cyst fluids (Figure 2). This improvement, however, still did not provide a complete distinction between these two types of ovarian cysts, nor did the use of the more specific CA 125 EIA test, which gave much higher values in the serous cyst fluids.

CA 125/CEA Ratio

The finding that CA 125 and CEA had opposite relative concentrations in serous and mucinous cyst fluids suggested that the use of the ratio of CA 125 to CEA might improve the specificity of the CA 125 test in cyst differentiation. As

![Fig. 1. Differentiation between serous (S, ●) and mucinous (M, ○) ovarian cysts with Roche (R) and Abbott (A) CEA kits [based on polyclonal (P) anti-CEA] and with CA 19-9 RIA.](image-url)
shown in Figure 2, the use of this ratio allowed a clear distinction of serous from mucinous ovarian cysts, especially when the CEA concentrations were determined with the Hybritech monoclonal-antibody-based CEA kit. Most of the ratios were >1000 for serous cyst fluids, whereas all mucinous fluids had a ratio of <10. Therefore, most serous fluids had ratios almost 100 times higher than that of most mucinous cysts. Interestingly, the only serous fluid with a ratio of <100 was retrospectively found to be a morphologically mixed serous–mucinous cyst.

We also tested several different commercial CEA kits to determine how they might affect the CA 125 to CEA ratio (Figure 3). Although use of the Abbott polyclonal kit also allowed complete separation of cyst types, the ratios calculated with CEA concentrations provided by the Hybritech monoclonal antibody kit gave the best differentiation. We did not test the monoclonal anti-CEA kits from Abbott and Roche, and their numerical values must be assessed before calculated ratios can be reliably used clinically. However, it seems reasonable to expect that, if these kits react with the same CEA determinant, there should be only minor differences among them. Using other ratios, such as CA 19-9/CEA or CA 19-9/CA 125, failed to distinguish as clearly between these two types of ovarian cysts (Figure 3).

These results suggest that the difference between serous and mucinous cysts lies in the pattern of specific antigenic determinants. Thus, selection of the specific determinants for monitoring and use of kits based on more specific monoclonal antibodies are important for obtaining the desired specificity.

Increased Concentrations of CA 125 in Serum

CEA and CA 125 concentrations in sera from ovarian and nonovarian cancer patients were determined by using monoclonal-antibody-based kits. Figure 4 (left), where only CA 125 concentrations are plotted, shows that increased CA 125 concentrations are common in patients with nonovarian carcinomas; moreover, there are some overlap between the concentrations in ovarian- and nonovarian-cancer patients. However, comparing the ratios of CA 125 to CEA (Figure 4, right) gives a clear differentiation. There is an average ratio difference of almost 100 between the two groups. These results strongly suggest that the above-normal CA 125 values for serum reported by investigators in nonovarian patients (7,8)—that is, high concentrations of CA 125 in the absence of ovarian tumors—could be clearly ruled out by using the CA 125/CEA ratio.

Discussion

In the past, no laboratory test of sufficient specificity or sensitivity was available to either diagnose or manage patients with ovarian epithelial cancers. Although CEA concentrations in plasma have been measured with polyclonal anti-CEA kits (9), only a small percentage of ovarian cancer patients had CEA values above normal (6, 7, 10). As a result, the recent addition of CA 125 EIA has been welcome. Despite a lack of specificity, its relatively high sensitivity allows one to successfully manage most cases of epithelial ovarian cancer (11, 12). In addition, two other monoclonal-antibody-based immunoassay kits, the CA 19-9 RIA and "ECEA" from Hybritech, are now available. Therefore, we decided to reassess their usefulness, as individual tests and in combination. CA 19-9 RIA was included because its determinant has been demonstrated in ovarian cysts (7) and because the monoclonal antibody used in CA 19-9 RIA was prepared against a colon tumor cell line and it reacts with mucin in serum (13-15).
Although the morphological difference between serous and mucinous cells is usually distinct, there are several advantages in examining ovarian cyst fluids also.

- Kabawat et al. (16), using an indirect immunofluorescence technique, reported that monoclonal anti-CA 125 reacted with most serous tumors but not with mucinous cyst. On the other hand, CEA was demonstrated in mucinous but not in serous tumors (17).
- Cyst fluids contain the highest concentration of tumor-associated molecules, which are released directly into the cyst by the tumor cells (9).
- Cyst fluids are less contaminated from other sources, because most of their contents are products of tumor cells.
- Because cells of mucinous cysts are morphologically similar to those of well-differentiated colon carcinomas (17), our success in differentiating between serous and mucinous cysts prompted us to attempt to differentiate non-mucinous ovarian malignancies from colon and other nonovarian cancers and to identify above-normal CA 125 in patients with nonovarian cancers (Figure 4).

Apparently, only tumor-associated antigens, not tumor-specific antigens, exist. Advances in the hybridoma technique have led to the development of monoclonal antibody kits with improved specificity, making it possible to examine the specificity of individual determinants on tumor-associated molecules. We learned from the work on CEA that CEA contains multiple antigenic determinants (18–22). Many of these antigenic determinants are shared by other glycoproteins that are present in a wide variety of tumors, various normal tissues, and physiological fluids. This explains why so many CEA-reactive or CEA-like molecules have been described, and why it is practically impossible to render anti-CEA antiserum specific by absorbing unwanted antibodies with extracts from normal tissues. Similarly, all the newly identified antigenic determinants, such as CA 125, CA 19-9, and CA 15-3 are also expressed by more than one type of cancer cell (23). The use of monoclonal antibodies will improve the specificity for tumor diagnosis, but will probably never attain 100% specificity. We believe that the only difference among these CEA-reactive molecules is the pattern of various antigenic determinants.

As with studies of other CEA-active molecules, none of the immunoassay kits we used to measure the individual determinants of tumor-associated antigens could differentiate between serous and mucinous cysts. However, these two morphologically distinct cysts could be differentiated by using the ratio of CA 125 to CEA or the pattern of antigenic determinants. The critical consideration is to select the proper antigenic determinants for ratio determinations. Our selection of CA 125 to CEA appears to be an excellent one, at least for ovarian epithelial tumors. Calculating ratios with different epitopes may be necessary for diagnosing different neoplasms.

Perhaps more important than the information obtained on cyst fluids are the results with serum specimens. Even though the data are preliminary, our results strongly suggest that the serum CA 125/CEA ratio can be used to distinguish increased CA 125 values of nonovarian malignancies from those of ovarian cancers. For example, even with a logarithmic scale, there is a clear difference between the ratios found in the sera of patients with ovarian and nonovarian tumors (Figure 4). Given the high sensitivity of the CA 125 EIA kit (6, 7), the improved specificity of using the CA 125 to CEA ratio suggests a possible screening method for ovarian carcinoma.

References

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Improved Agarose Electrophoretic Method for Separating Alkaline Phosphatase Isoenzymes in Serum

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A modified agarose electrophoretic system for the separation of alkaline phosphatase (ALP, EC 3.1.3.1) isoenzymes is described. Bone, liver, high-molecular-mass, and intestinal ALP are separated with high reproducibility. The sensitivity of the agarose system is superior to cellulose acetate in detecting high-M, ALP. Correlation is good between bone ALP fractions scanned before and after treatment with neuraminidase. Immunoglobulin-bound ALPs, the ALP–lipoprotein-X complex, and the additional ALP fraction observed in transient hyperphosphatasemia in children are detected by their peculiar electrophoretic mobility in the proposed system. Approximately 25% of the samples contained an additional fraction of intestinal-type ALP, as evidenced by neuraminidase treatment and use of polyclonal and monoclonal antibodies. Because the electrophoretic mobilities of this "intestinal variant" and of some immunoglobulin-bound ALP fractions are identical to those of bone and intestinal ALP, respectively, treatment of the samples with a polyclonal antibody that reacts with intestinal ALP is advised.

Additional Keyphrases: agarose and cellulose acetate electrophoresis compared • neuraminidase treatment • polyclonal vs monoclonal antibodies

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Human alkaline phosphatase (ALP, orthophosphoric-monoester phosphohydrolase (alkaline optimum); EC 3.1.3.1) consists of a group of isoenzymes encoded for by at least three different gene loci. They are membrane-bound sequenced isoenzymes and glycosylated dimeric metalloenzymes for which the exact biological function is still unknown (1–3). Their clinical interest stems from the existence of at least six more or less tissue-specific isoenzymes, whose cellular expression and liberation in biological fluids is influenced by physio-pathological factors (4).

The biochemical determination of ALP activity is technically easy and reliable. For electrophoretic separation of ALP isoenzymes, various supporting media have been proposed: agar (5), agarose (6), starch (7), polyacrylamide (8), and cellulose acetate (9). Some methods are too cumbersome for routine use. In others, a poor resolution of the liver- and bone-derived isoenzymes, an absent or poor visualization of high-molecular-mass ALP, and a lack of sensitivity and reproducibility make interpretation of the isoenzyme patterns difficult.

Here we report our evaluation of the separation of serum ALP isoenzymes with a commercially available agarose electrophoretic system ("Tsopal"; Beckman Europe, Analis S.A., Namur, Belgium).

Materials and Methods

Instruments

For absorbance measurements we used an Hitachi 705 apparatus (Boehringer, Mannheim, F.R.G.). Gels were scanned in a computerized densitometer ("Appraise"; Beckman Instruments Inc., Brea, CA).