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Efficiency of Canonical Discriminant Function versus Mahalanobis Distance in Differentiating Groups: Screening Ovarian Cancer in a Multivariate System Analysis Using Enzyme Markers

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Due to its low prevalence, high mortality and uniquely hidden intrapelvic position, ovarian cancer remains a subject of intense interest to researchers. Statistical calculation and new technology both have major roles to play in the effort to screen this cancer at an early stage. Advanced statistics, such as multivariate analysis, remain at the root of screening endeavors. Multivariate analysis has the power to combine many tests and to produce better results in terms high specificity and positive predictive value. Multivariate analysis techniques include Mahalanobis distance (D^2), canonical stepwise discriminant function (Z) and Posterior Probability. These may have varied efficacy, but to date comparisons have not been conducted to determine which is best in the context of ovarian cancer screening.

Key words: Multivariate analysis, Mahalanobis distance (D^2), canonical stepwise discriminant function (Z), posterior probability, ovarian cancer screening, tumor marker.

Introduction

With an overall survival rate of 30%, ovarian cancer remains the fifth leading cause of cancer death. This disease, which is neither common nor rare (Bast, 2004), has remained enigmatic amongst gynecological cancers with agonizing prospects. Ovarian cancer is the second most common gynecologic malignancy, and little is known about the progression of its early changes (dysplasia).

Ovarian cancer has the highest mortality rate among gynecologic malignancies (70%) and its mortality rate has not lowered in the last 50 years. Only 25% of cases are diagnosed in an early stage and late case diagnosis survival is very poor. Though tests such as tumor markers and ultrasounds are available, no cost-effective screening method with adequate sensitivity and specificity is available to detect early ovarian cancer.

Combining markers and tests results in higher sensitivity and specificity, thus, many scientists have used multivariate analysis in their experiments. In an ovarian cancer screening system, multivariate stepwise discriminant function analysis is described using different tumor markers, for example, CA125, TPA, IAP, CEA, and ferritin (Yabushita, et al., 1985; LaHousen, et al., 1987). Kobayashi and Terao (1992) combined CA 125, TPA, Ferritin, CEA, AFP and Sialyl Lewis Xi using Mahalanobis distance and were able to decrease both false positive and false negative cases. Bose and Mukherjea (1994) statistically combined several enzymatic tumor markers to increase specificity, positive predictive value (PPV) and to decrease false positive tests.

Other groups described combining multiple markers, but they either combined them in a statistically unacceptable way (Inoue, Fujita, Nakazawa, Ogawa & Tanizawa, 1992), in a simple Euclidian relationship, such as the risk of malignancy Index (RMI, Oram, et al.,1990; Jacobs, et al., 1990) or otherwise (Jacobs, et al.,

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1990). Jacobs, Oram & Bast (1992) used a multivariate system while also using apolipoprotein A1 (down-regulated in cancer); a truncated form of transthyretin (down-regulated) and a cleavage fragment of inter-α-trypsin inhibitor heavy chain H4 (up-regulated).

Zhang, Bast, et al. (2004) described the risk of ovarian cancer (ROC) algorithm. They combined the parameters Serial CA125 assay value, changes in CA125 levels over time and woman's age, and assay variability by a multivariate based software program, which they called the ROC algorithm. However, they did not describe the actual procedure they followed. They speculated sensitivity 86%, specificity 99.7%, and PPV up to 19% which is encouraging (Menon, et al., 2005). They are now conducting a massive trial on population screening in the UK, which will take another two years to complete.

Timmerman, et al. (2005) combined 12 useful independent prognostic variables in a logistic regression model and found a probability cut off value of 0.10 that gave a sensitivity of 93% and a specificity of 76%. Curling, et al. (1998) conducted a multivariate analysis of DNA ploidy, steroid hormone receptors and CA 125 as prognostic factors in ovarian carcinoma, and Kozak, et al. (2005) used multivariate analysis to greatly improve the detection of early stage ovarian tumors compared to cancer antigen CA125 alone with the help of differential expression of transthyretin (TTR), beta-hemoglobin (Hb), apolipoprotein AI (ApoAI) and transferrin (TF).

Multivariate procedures include Mahalanobis distance (D^2), canonical stepwise discriminant function (Z) and Posterior Probability, but no research has been conducted to determine whether they are equally effective in detection systems for screening ovarian cancer using multiple parameters.

Methodology

Serum levels of four enzyme makers (placental alkaline phosphatase, lactate dehydrogenase, 5' nucleotidase and Amylase) were measured using a commercially available kit, in 50 ovarian cancer patients and 31 patients with benign gynecological disease before initiation of any treatment. These were compared with the levels in a control group of 30 healthy women using different multivariate parameters Mahalanobis distance (D^2), canonical stepwise discriminant function (Z) and Posterior Probability. The goal was to determine if any difference exists in the power of detection of disease state by these methods and if one is more or most efficient in detecting disease state.

Data for all enzyme levels in different groups were fed into a DIGITAL-VAX 8650 computer using a VMS operating system. BMDP 1990 version software program packages 3D and 7M were used to analyze the data. In BMDP 3D, mean, standard deviation, standard error of mean and pooled T test were used to show significant group differences separately for each enzyme. Sensitivity and specificity for each enzyme were determined at different cut off scores and a Receiver Operator Characteristic Curve (ROC) was prepared to compare the efficacy of individual enzyme.

In the same program, Hotelling's T^2 test, F, p for four enzymes taken together at a time (multivariate analysis) were obtained and were analyzed to observe significant differences between different groups. The F value was observed and, if it significantly exceeded unity, the two groups were assumed to be statistically significantly different.

If a random sample of size n yields the sample value $x_1, x_2, x_3, \ldots, x_n$

$$\overline{X} = \sum_{i=1}^{n} \frac{x_i}{n},$$

and the sample estimate of variance is

$$s^2 = \sum_{i=1}^{n} \frac{(x_i - x)^2}{(n-1)},$$

then these are estimates of corresponding population parameters – the population mean μ and the population variance σ^2.

In a similar way, multivariate population can be summarized by mean vectors and covariance matrices. These are defined as follows. If there are p variables $x_1, x_2, x_3, \ldots, x_p$ and the values of these for the i^th individual in a sample are $x_{i1}, x_{i2}, x_{i3}, ..., x_{ip}$ respectively, then the sample mean of variable *j* is

$$\overline{X}_j = \sum_{i=1}^{n} \frac{X_{ij}}{n}$$

and the sample variance is

$$s_j^2 = \sum_{i=1}^{n} \frac{(x_{ij} - x_j)^2}{(n-1)}.$$

In addition the sample covariance between variable *j* and *k* is defined as

$$c_{jk} = \sum_{i=1}^{n} \frac{(x_{ij} - x_j)(x_{jk} - x_k)}{(n-1)}.$$

The pooled estimate of variance from the two sample n_1 and n_2 is,

$$s^2 = \frac{[(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2]}{(n_1 + n_2 - 2)},$$

the matrix of covariances (C_1 and C_2)

$$C = \begin{pmatrix} c_{11} & c_{12} & \dots c_{1p} \\ c_{21} & c_{22} & \dots c_{23} \\ c_{p1} & c_{p2} & \dots c_{pp} \end{pmatrix},$$

the pooled estimate of covariance matrix is

$$C = \frac{[(n_1 - 1)C_1 + (n_2 - 1)C_2}{(n_1 + n_2 - 2)},$$

and Hotelling's T^2 statistics is defined as

$$T^2 = \frac{n_1 n_2 (\overline{X}_1 - \overline{X}_2) C^{-1} (\overline{X}_1 - \overline{X}_2)}{(n_1 + n_2)}.$$

A significantly large value for these statistics is evidence that the mean vectors are different for the two sample populations. The significance or the lack of significance of T^2 is most simply determined by using the null hypothesis case of equal population means for the transformed statistics.

The analysis of variance (also known as Snedecor's F or the Fisher-Snedecor F) test is based on the continuous F-distribution, which is a random variate arising as the ratio of two Chi-squared variates:

$$\frac{U_1 / d_1}{U_2 / d_2},$$

where U_1 and U_2 have Chi-square distributions with d_1 and d_2 degrees of freedom respectively, and U_1 and U_2 are independent. Thus,

$$F = \frac{(n_1 + n_2 - p - 1)T^2}{[(n_1 + n_2 - 2)p]}$$

Because T^2 is a quadratic form it is scalar, and can be written in as

$$T^2 = \frac{n_1 n_2}{n_1 + n_2} \sum_{i=1}^{p} (\overline{X}_{1i} - \overline{X}_{2i}) c_{ik} (\overline{X}_{1k} - \overline{X}_{2k}),$$

which is simpler to compute. Here x_{ji} is the mean of the variable x_i in the j^{th} sample and c_{ik} is the element in the i^{th} row and the k^{th} column of the inverse matrix C^{-1}.

BMDP 7M was used for multivariate stepwise canonical discriminant function analysis. To separate the different groups of patients, following simple linear combination was used

$$Z = K + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_4 X_4.$$

Z is the canonical discriminant function of variable enzymes namely X_1 = PLAP, X_2 = LDH, X_3 = 5'N, X_4 = Amylase, whereas a_1, a_2, a_3, and a_4 are the coefficients of the above variable respectively and K is the constant. Coefficient and constant were determined by using BMDP.

Mahalanobis distance of individuals to group centers can be calculated by the following formula

$$D_i^2 = (x - \overline{X}_i)' C^{-1} (x - \overline{X}_i)$$
$$= \sum_{r=1}^{p} \sum_{s=1}^{p} (x_r - x_{ri})' c_{rs} (x_s - x_{si}),$$

while the posterior probability is

$$P(A:PT) = \frac{P(PT:A)P(A)}{P(PT:A)P(A) - P(PT:NotA)P(NotA)}$$

where p is probability, A is the abnormality, and PT is the positive test result. Thus, the expression on the left, P (A: PT) is equivalent to the probability P of abnormality A given the positive test result PT. This is described as posterior probability and is in Bayes' theorem, which relates the conditional and marginal probabilities of stochastic events A and B:

$$\Pr(A|B) = \frac{\Pr(A|B)\Pr(B|A)}{\Pr(B)} \propto L(A|B)\Pr(A)$$

where L(A|B) is the likelihood of A given fixed B. Each term in Bayes' theorem has a conventional name. Pr(A) is the prior probability or marginal probability of A. It is prior in the sense that it does not take into account any information about B. Pr(A|B) is the conditional probability of A given B; it is also called the posterior probability because it is derived from or depends upon the specified value of B. Pr(B|A) is the conditional probability of B given A. Pr(B) is the prior or marginal probability of B, and acts as a normalizing constant. The posterior probability is proportional to the prior probability times the likelihood.

Both D^2 and PP were determined through the same package. The ROC curve of individual marker enzymes showed LDH to be most sensitive and specific. Thus, LDH was compared with these three multivariate systems in Receiver Operator Characteristic (ROC) chart.

In healthy women discriminant function (Z), Mahalanobis distance (D^2) and posterior probability (PP) were determined for each case. Their mean and standard deviation were compared with the values of corresponding multivariate parameters for each individual in both the benign gynecological disease (BGD) and ovarian cancer group. When plotted with chosen cut-off scores with their corresponding (1−specificity) in *x* axis and sensitivity in *y* axis the ROC curve will show comparative efficacy of Z over D^2, PP and LDH in terms of largest area under the graph.

Results

Table 1 shows the serum concentration of four enzymes markers, placental alkaline phosphatase, lactate dehydrogenase, 5' nucleotidase and Amylase, with their mean ± standard error of mean. Results of enzyme estimations of three groups, Healthy control, Benign gynecological disease (BGD) and Ovarian cancer are shown in three columns. Significant differences are also shown as p values. Although activities of all enzymes have been found to be significantly higher in ovarian cancer cases than in healthy women, positivity rates were not very high. The positivity rate measured for each enzyme in ovarian cancer, showed LDH to be the most sensitive (positivity 48%), whereas amylase showed least sensitivity at a positivity rate of 30%. However, these are much lower compared to the positivity rate (75%) of cancer antigen CA 125, the most sensitive tumor marker in ovarian cancer (Heinonen, Kallinoiemi & Koivula, 1987).

Test results in the ovarian cancer group were significantly different from the healthy control group, but showed no statistically significant difference with the benign gynecological disease (BGD) group.

Table 2 shows sensitivity and specificity of serum enzymes markers at different cut-off concentrations. Table 3 summarizes the cut-off scores of the markers that had the highest sensitivity and specificity. They were compared with CA125 at a suitable cut off level of 35 IU/L. LDH had the highest sensitivity and specificity at a cut off score of 157.88 IU/L but it still fell behind CA125.

With different sensitivity and specificity at cut-off values of those enzyme markers, a Receiver Operator Characteristic Curve (ROC) was prepared to compare the power and efficacy of individual enzymes to differentiate between groups in a univariate system (Figure 1).

Four serum enzymes are used at a time in multivariate analysis by the BMDP package (program 3D), where a significant group difference was observed between healthy versus ovarian cancer and BGD versus ovarian cancer patients; in healthy versus BGD there was no statistically significant difference (Table 4.). Z, D^2 and PP were obtained through program 7M for healthy versus ovarian cancer patients. Sensitivity and specificity of LDH, Z, D^2 and PP at different cut-off scores or action lines with their confidence interval (derived from the binomial distribution chart) for healthy women and ovarian cancer cases are shown in Table 5. Table 6 compares sensitivity and specificity of different multivariate parameters such as, LDH, Z, D^2 and PP.

The performance of the canonical discriminant function (Z) at various upper limits is illustrated in the ROC chart (Figure 2) and is observed to combine higher levels of sensitivity and specificity than those achieved by Mahalanobis distance (D^2), PP and LDH. Table 7 shows the positive and negative predictive values for malignancy of ovary for different levels of Z (cutoff scores 1.377, 2.907. 3.437 and 5.967). A cut-off score of 3.437 produced the best results. No statistically significant group difference was predicted between healthy and BGD, which was corroborated by the determination of Z value.

Conclusion

The population screening of ovarian cancer has remained elusive due to low disease prevalence and low positive predictive value of the tests. Some groups are trying to combine different test results in different software packages using algorithms based on multivariate systems of data processing, but many alternatives in this multivariate system exist which are not based on some type of mathematical calculation. As a result, finding more efficacious methods in terms of higher specificity and higher positive predictive value is a priority. This system is applicable in many areas in biology and medicine. This article presented an example of the use of multivariate analysis in ovarian cancer screening to illustrate the comparative efficacy of stepwise discriminant function (Z) Mahalanobis distance (D') and posterior probability (PP).

It is expected that this example will be replicated in other experimental circumstances, but will need further verification and establishment of mathematical proof as to why it occurs. In the experiment presented, it was observed that the Multivariate stepwise discriminant function (Z) analysis of enzyme variables establishes an easy quantitative assessment method of the risk of malignancy in the ovary. A Z value with a cut-off score of 3.437 has a higher predictive value and relative risk than LDH, Mahalanobis distance (D^2) or posterior probability (PP). This system of combining four enzymes for improvement of ovary screening must be established in clinical practice through further research.

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Table 1: Serum Concentration of Enzymes Markers Mean ± SEM

Enzymes	Healthy Control	Benign Gynecological Disease (BGD)	Ovarian Cancer
Placental alkaline phosphatase (IU/L)	0.81± 0.09	1.76 ± 0.47 (P< .0615)	4.47 ± 0.89 (P< .0011)
Lactate dehydrogenase (IU/L)	157.88 ± 8.61	155.65 ± 7.88 (P< .8497)	255.44 ± 16.19 (P< .0001)
5' nucleotidase (IU/L)	5.94 ± 0.75	5.22 ± 0.42 (P< .4098)	9.13 ± 0.93 (P< .0191)
Amylase (IU/L)	79.1 ± 3.83	77.8 ± 3.38 (P< .6828)	121.54 ± 8.23 (P< .0001)

Table 2: Sensitivity and Specificity of Serum Enzymes Markers at Different Cut-off Concentrations

Test and Action Line (cutoff score in IU/L)	Sensitivity	Specificity
LDH		
110.73	94	20
157.88	88	63
205.03	50	90
252.18	34	93.3
Amylase		
75.25	76	43.3
79.1	74	53.3
83.93	68	60
86.76	64	60
PLAP		
0.72	64	50
0.81	60	60
0.90	58	66.6
5’Nucleotidase		
5.19	56	56.6
5.94	54	63.6
6.69	42	66.6
7.44	39	66.6

Table 3: Cut-off Score Offering Highest Sensitivity and Specificity

Cutoff Scores (IU/L)	Enzyme Markers	Sensitivity	Specificity
0.90	Placental alkaline phosphatase (IU/L)	58	66.6
157.88	Lact dehydrogenase (IU/L)	88	63
83.93	Amylase (IU/L)	68	60
5.95	5' nucleotidase(IU/L)	54.8	64.3
35	CA125	72	75

Figure 1: Receiver Operator Characteristic Curve (ROC) to Compare the Power of Individual Enzymes

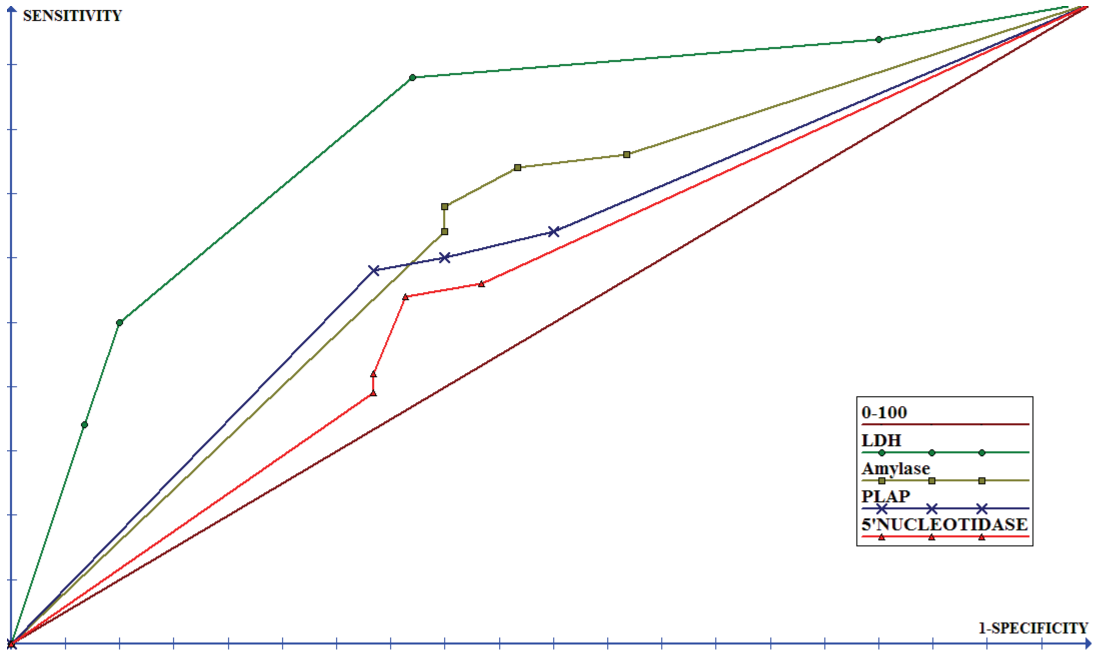


Table 4: Mahalanobis Distance (D^2) Hotelling's T^2 F & P Value to Predict Multivariate Based Statistical Significance of Different Between Groups

Group Difference	Mahalanobis Distance (D^2)	Hotelling's T^2	F	P
Healthy women vs. ovarian cancer	2.7061	50.7390	12.1969	0.0001
Healthy women vs. Benign gyn. disease	0.2992	4.5611	1.0823	0.3741
Ovarian cancer vs. Benign gyn. disease	2.4808	54.3602	13.0740	0.0001
Ovarian cancer vs. non-responder	0.0530	1.0721	0.2582	0.9028
Ovarian cancer vs. responder	1.3802	12.444	2.9528	0.0277

Table 5: Sensitivity and Specificity of Multivariate Based Statistical Parameters Compared With LDH at Different Cut-off Concentrations

Test and action line (cutoff score in IU/L)	Sensitivity		Specificity	
	%	(95% CI)	%	(95% CI)
LDH				
110.73	94	(82-99)	20	(82-99)
157.88	88	(82-99)	63	(82-99)
205.03	50	(82-99)	90	(82-99)
252.18	34	(82-99)	93.3	(82-99)
Z				
1.337	98	(88-100)	13	(82-99)
2.907	96	(86-99)	70	(82-99)
3.437	96	(86-99)	83	(82-99)
5.967	76	(61-87)	93.3	(82-99)
D^2				
0.09	100	(92-100)	0	(0-12)
0.93	86	(72-94)	73	(54-87)
1.77	82	(68-91)	86.6	(66-96)
2.61	74	(59-86)	93	(77-99)
PP				
0.525	80	(67-90)	90	(72-97)
0.726	92	(80-92)	76.6	(56-89)
0.887	96	(86-99)	20	(10-58)
1.048	100	(92-100)	0	(0-12)

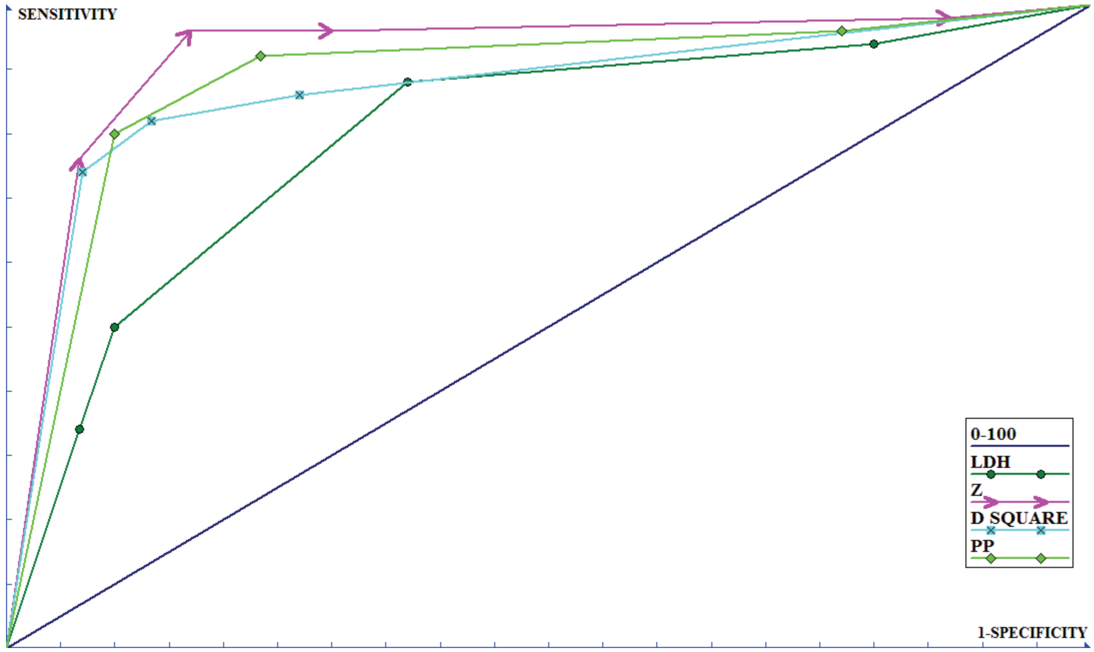
Table 6: Sensitivity and Specificity of Different Multivariate Parameters Such as, LDH, Z, D^2 and PP

Statistics	Sensitivity	Specificity
Mahalanobis Distance $D^2 = 1.77$	82	86.3
Posterior Probability Pp = 0.726	92	76.6
Discriminant Function Z = 3.437	96	83

Table 7: Positive and Negative Predictive Value for Different Levels of Z

Z Score	Predictive Value (%)	
	Positive	Negative
1.337	91.3	3.7
2.907	91.3	9.6
3.437	84	92
5.967	70	95

Figure 2: Receiver Operator Characteristic Curve (ROC) to Compare the Power of Multivariate Based Statistical Parameters Compared with LDH



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