Tumour M2-PK as a predictor of surgical outcome in ovarian cancer, a prospective cohort study

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Summary

Background: Optimal cytoreduction is a major prognostic factor in ovarian cancer; several clinical, radiological and biochemical predictors have been studied. Tumour M2-PK (TU M2-PK) is over-expressed in tumour cells and can be detected in plasma samples but its role in ovarian cancer has not yet been evaluated. *Objectives:* To assess the potential clinical applications of TU M2-PK in ovarian cancer particularly in relation to surgical cytoreduction. *Settings:* The Gynaecological Cancer Centre at both King's College and St Thomas' Hospitals; London; UK. *Methods:* Patients with suspected ovarian cancer were recruited prospectively during the years 2004-2005. Blood samples were collected before surgery for plasma TU M2-PK assays. Data were analysed in relation to cancer diagnosis and outcome. Statistical analysis was performed using Analyse-It® and SPSS® V13. *Results:* 100 patients were recruited; 52 diagnosed with invasive ovarian cancer, 13 with borderline tumours and 35 patients had benign conditions. The mean M2-PK concentration in cancer patients was 52 U/ml vs 31 U/ml in patients with borderline tumours and 22 U/ml in those with benign conditions ($p < 0.001$); it was significantly raised in association with late stage disease and higher grade ($p < 0.05$). Taking 35 U/ml as a reference point, TU M2-PK predicted sub-optimal cytoreduction in advanced stage disease with a sensitivity of 69%, specificity of 60% and overall efficacy of 61% (95% CI: 44-75%). *Conclusion*: TU M2-PK was significantly raised in ovarian cancer patients, particularly those with higher stage disease. The potential clinical application as a predictor of surgical outcome in ovarian cancer needs further evaluation.

Key words: Ovarian cancer; Pyruvate Kinase; Diagnosis; Tumour Marker; Cytoreduction.

Introduction

Ovarian cancer is the leading cause of death from gynaecological malignancies in the Western world with a lifetime risk of one in 75 [1, 2]. The majority of patients are diagnosed with FIGO Stage III or above due to the lack of specific early symptoms or a reliable screening tool. The five-year survival rate is 90% for patients with Stage I disease compared with 30% for patients with Stage III and IV [3, 4]. The aim of surgery in ovarian cancer is optimum cytoreduction of tumour load in addition to accurate staging [5]. Patients in whom optimum tumour cytoreduction was achieved demonstrate a survival advantage compared with those in whom residual disease deposits after initial surgery was greater than 2 cm across [5, 6]. Therefore, the importance of identifying a reliable predictive tool for optimum primary cytoreduction in patients with advanced ovarian cancer has attracted significant attention. Serum tumour markers, mainly CA125, in addition to other clinical and/or radiological predictors have previously been assessed for this purpose, albeit with varying results [7-11].

Pyruvate kinase is a key enzyme for ATP synthesis and energy production in the glycolytic pathway and has been shown to be over-expressed in a variety of cancers. It is present in four identifiable tissue-specific isoenzymes (L, R, M1 and M2). The unstable nutritional environment allows tumour cells to employ an adaptation mechanism whereby the energy production is slowed down in exchange for substrate availability required for synthetic processes and cellular proliferation. During tumour formation, the pyruvate kinase isoenzyme M2 (M2-PK) predominates over other isoforms regardless of the cancer cell line [12]. Two forms exist for M2-PK isoenzyme, an active tetrameric form and inactive dimeric form. The active form is part of the glycolytic pathway responsible for energy production, whereas the inactive form is responsible for channeling phosphometabolite substrates towards protein and nucleic acid synthesis necessary for cell proliferation. Therefore, it is the dimeric form that is abundant in tumour cells and often referred to as Tumour M2-PK (TU M2-PK); it can be detected in plasma samples from cancer patients using radioimmunoassay techniques [12-14]. This study explores the potential role of Tumour M2-PK as a marker in ovarian cancer particularly with respect to surgical outcome.

Patients and Methods

The study was set out with the purpose to: a) investigate the difference in TU M2-PK plasma concentrations between benign, borderline and malignant ovarian neoplasm, and b) determine the correlation between TU M2-PK plasma concentrations and different parameters relating to tumour characteristics and surgical outcome. This is a prospective observational cohort study conducted at the gynaecological oncology centre and the gynaecology unit at two University Teaching Hospitals,

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London, UK. Seventy-two patients with suspected ovarian malignancy and 28 with benign gynaecological conditions were recruited. All patients with suspected ovarian cancer had their serum CA125 tested and were recruited when the risk of malignancy index (RMI) exceeded 250 [15]. Exclusion criteria were concomitant treatment with chemotherapy, moderate or severe renal failure and patients participating in other studies. Ethical approval was granted and patients consented after receiving the appropriate information leaflets detailing the study. This prospective study was conducted under single-blind conditions to minimise bias on the part of subjects, investigators and analysts.

Patient characteristics and clinical details were tabulated and stored in a secure database. Preoperative blood samples for M2-PK were obtained using venepuncture with minimal stasis. Samples were withdrawn into 5 ml EDTA tubes and centrifuged for 10 min at 3000 rpm. Plasma aliquots were separated and stored in –70°C freezers until analysed. Preoperative blood samples for CA125 concentrations were collected into 5 ml tubes without anticoagulant. Serum aliquots were separated by centrifugation as above. CA125 concentrations were measured using an immunometric sandwich assay on the ADVIA Centaur analyser (Bayer Diagnostics, Newbury, Berks) with a cut-off value for normal of ≤ 35 kU/l.

Patients underwent staging laparotomy as described in the FIGO surgical staging for ovarian cancer [16]. The result of cytoreduction was documented and the amount of any residual disease was noted together with its distribution. Optimum cytoreduction was defined as residual disease deposits of 2 cm or less [6]. Removed tissues were histologically examined using conventional and immunohistochemistry methods. Histological diagnosis of ovarian cancer and grading were established according to the WHO and Gynecologic Oncology Group (GOG) criteria [17, 18].

The TU M2-PK isoenzyme was quantitatively measured in EDTA plasma via an enzyme-linked immunosorbent assay (ELISA) developed by ScheBo-Tec®, Giessen, Germany. The test is based on two monoclonal antibodies which specifically react with TU M2-PK and do not cross-react with the other isoenzymes. The first step comprises binding of the plasma TU M2-PK to the first monoclonal antibody whereby it is immobilised on the antibody coated ELISA plate. During the next incubation period, a second monoclonal antibody which is biotinylated binds to the immobilised TU M2-PK. Then a conjugate of peroxidase and streptavidin binds to the biotin moiety. Subsequently the peroxidase oxidizes 3,3'5,5'-tetra-methyl benzidine (TMB) which turns yellow. Finally, the concentration of the oxidized TMB is determined photometrically. The test kit allows the quantification of TU M2-PK within the range of 5 to 100 units/ml (U/ml) in EDTA plasma; however, values above 100 U/ml were accurately recorded for the purpose of this study. The manufacturer average intra-assay coefficient of variance (CV) is 3.5% (2.4-7.0%).

Statistical analysis was carried out using Analyse-It® and SPSS® V13 software. Descriptive statistics of patient characteristics were calculated using simple methods for the mean, median, standard deviation (SD), minimum and maximum values. Comparison of group means was carried out using non-parametric tests such as Mann Whitney and Kruskal-Wallis tests. The chi-square test was used to compare categorical data. The receiver-operator characteristic (ROC) curve was used for analysis of the presurgical results of TU M2-PK to determine cut-off values for the diagnosis of ovarian cancer and assess the potential role as a predictor of surgical outcome. Confidence intervals for positive and negative likelihood ratios were calculated with the method described by Simel and colleagues [19]. A correlation matrix and coefficient of correlation (r) for relationship of different variables were calculated using the Spearman rank non-parametric correlation statistics.

Results

There were 100 patients recruited for the study; 52 patients with histological diagnoses of cancer, 35 with benign conditions and 13 patients had histology results confirming borderline ovarian tumours (BOT). The overall age range was 14-88 years with a mean of 55.5 and a median of 57 years. Patients with cancer had a mean age of 61 years (95% CI: 57-65 years), with a minimum of 19 and a maximum of 88 years. Table 1 demonstrates important patient characteristics. Thirty-eight patients had surgico-pathological FIGO Stage III and above. The majority of tumours were of epithelial origin and demonstrated high-grade features (Table 2). Thirty-eight patients out of the 65 (58%) diagnosed with invasive cancer or borderline ovarian tumour (BOT) were left with no macroscopic residual disease; ten (15%) patients had residual disease of 2 cm or less, and 17 (26%) had residual disease deposits larger than 2 cm. Overall, optimum cytoreduction was therefore achieved in 48 patients (74%; 95% CI: 62-83%; Table 2). Out of the 38 patients presenting in FIGO Stage III and above, 22 (58%) patients underwent optimal cytoreductive surgery.

Table 1. — *Selected patient characteristics.*

Patient characteristcs		Cancer diagnosis Yes (n = 52)	No (n = 48)	% Total
Patient age (years)	Mean	61.2	44	
	Median	65	49	
	(Min-Max)	19-88	14-83	
Menopausal status (%)	Postmenopausal	41%	19%	60%
	Premenopausal	11%	29%	40%
Patient parity (%)	P0	11%	16%	27%
	P1	12%	6%	18%
	> P1	29%	26%	55%
Presence of ascites (%)	Yes	31%	10%	41%
	No	21%	38%	59%

Table 2. — *Important features for patients with invasive cancer and BOT.*

Characteristcs		Total number = 65 N	%
FIGO stage	I & II	27	42.5
	III &IV	38	58.5
Epithelial origin	Yes	54	83
	No	11	17
Grade	I	15	23
	II	19	18.5
	III	38	58.5
Residual disease	None	38	58.5
	< 2 cm	10	15.4
	> 2 cm	17	26.2
Optimum cytoreduction	Yes	48	73.8
	No	17	26.2

BOT: borderline ovarian tumour.

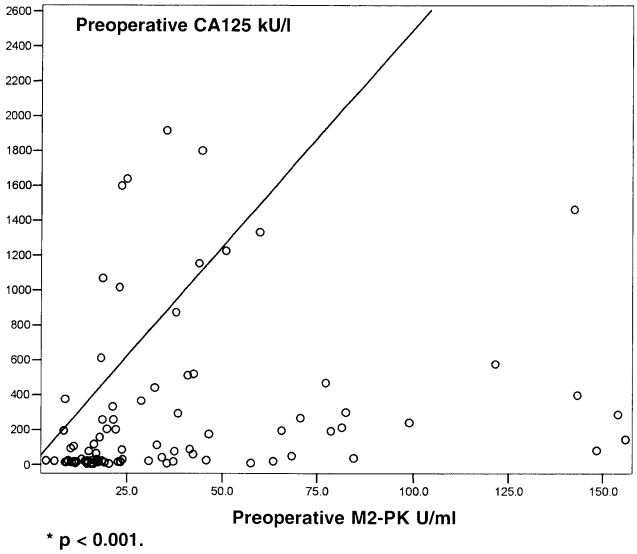

Figure 1. — Correlation between TU M2-PK and CA125*.

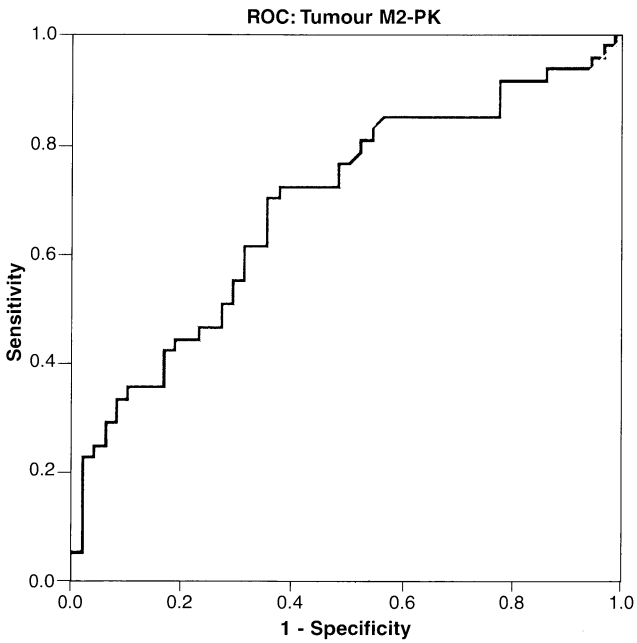

Figure 2. — ROC curve for TU M2-PK for cancer diagnosis.

Plasma preoperative TU M2-PK results were available for all the 100 patients; however five cases were excluded from the analysis as they had received neoadjuvant chemotherapy prior to their staging laparotomy. Only patients with histological confirmation of invasive cancer (n = 52) are defined as cancer positive. The median preoperative plasma TU M2-PK value for cancer patients was 35 U/ml, with a mean of 52 U/ml (95% CI: 38-65 U/ml); for those with BOT, concentrations were 24 U/ml and 31 U/ml (95% CI: 18-45 U/ml) for the median and mean, respectively, compared with 17 U/ml and 22 U/ml (95% CI: 17-27 U/ml) for the median and mean in patients with benign conditions. The difference between

Table 3. — *Plasma Tumour M2-PK by patient category.*

Plasma Tumour M2-PK (U/ml)	Diagnosis			p
	Invasive cancer (n = 52)	BOT (n = 13)	Benign (n = 35)	
Median	35	23	17	
Mean	52	31	22	< 0.001
95% CI of the mean	38-65	18-45	17-27	
Range	186	69	59	
Interquartile range	52	27	12	

BOT: borderline ovarian tumour.

Table 4. — *Diagnostic parameters for Tumour M2-PK and CA 125 blood tests.*

Test	Tumour M2-PK	CA125
Sensitivity %	70	82
Specificity %	65	60
Positive predictive value %	66	68
Negative predictive value %	69	75
Test efficacy % (95% CI)*	67 (57-76)	71 (61-79)

* p > 0.05.

Table 5. — *Plasma Tumour M2-PK by Stage and Grade category.*

Plasma Tumour M2-PK (U/ml)	FIGO Stage		p
	I-II	III-IV	
Median	18.1	37.8	
Mean	40.7	52.6	< 0.05
95% CI of the mean	23.8-57.7	37.8-67.4	
Range	186.1	147.4	
Interquartile range	55.5	39.2	
	FIGO Grade		p
	I-II	III-IV	
	I-II	III	
Median	22.8	36.5	
Mean	36.8	55.2	< 0.05
95% CI of the mean	23.4-50.2	38.7-71.7	
Range	137.5	183.4	
Interquartile range	46.5	50	

the three groups was highly significant on the Kruskal-Wallis non-parametric test (p = 0.001) (Table 3).

Plasma TU M2-PK concentrations strongly correlated with serum CA125 concentrations (p < 0.001) (Figure 1) but not with either patient age (p = 0.37) or serum creatinine (p = 0.27). At a cut-off value of 22 U/ml, the sensitivity of TU M2-PK in detecting cancer was 70% with a specificity of 65%. The positive and negative predictive values (PPV, NPV) at this cut-off point were 66% and 69%, respectively, with an overall test efficacy of 67% (Figure 2).

The equivalent results for the preoperative serum CA125 test in the study cohort taking the 35 kU/l concentration as the cut-off point are listed in Table 4. The overall diagnostic efficacy is not statistically significant (71% vs 67%; p > 0.05). With respect to patients with either invasive cancer or BOT, plasma TU M2-PK concentrations were significantly elevated in late stage (FIGO III & IV) disease and higher histological grade (Grade 3) compared with early stage (FIGO I & II) disease or lower grade (Grade 1 & 2) histology (p < 0.05) (Table 5).

Table 6. — *Tumour M2-PK by cytoreduction outcome.*

Tumour M2-PK (U/ml)	Optimum cytoreduction		p
	Yes	No	
Median	26.6	42.6	
Mean	43.3	57.1	< 0.05
95% CI of the mean	30.6-56.1	34.4-79.9	
Range	186	143	
Interquartile range	48.5	59.5	

Table 7. — *Prediction of suboptimal cytoreduction for both Tumour M2-PK and CA125 blood tests.*

Test	Tumour M2-PK	CA125
Sensitivity %	69	75
Specificity %	60	33
PLR (95% CI)	1.46 (0.8-2.7)	1.12 (0.71-1.77)
NLR (95% CI)	0.59 (0.2-1.3)	0.75 (0.24-2.27)
Test efficacy % (95% CI)*	61 (44-75)	55 (38-71)

*p > 0.05.

Patients who underwent suboptimal cytoreduction at the initial staging laparotomy had significantly higher concentrations of TU M2-PK (Table 6, Figure 3; p < 0.05, one-tailed). The ROC curve was performed for plasma TU M2-PK and serum CA125 concentrations with respect to the outcome of cytoreductive surgery in both cancer and BOT patients with FIGO Stage III and IV. The best cut-off value on the TU M2-PK ROC for predicting patients in whom optimal cytoreduction could not be achieved was 35 U/ml (Figure 4). Plasma TU M2-PK concentrations of 35 U/ml or above had sensitivity for predicting suboptimal cytoreduction of 69% and specificity of 60%. The positive likelihood ratio (PLR) was 1.46 (95% CI: 0.8-2.7) and the negative likelihood ratio (NLR) was 0.59 (95% CI: 0.2-1.3). The overall efficacy of TU M2-PK in predicting suboptimal cytoreduction was 61% (95% CI: 44-75%) which was not significant. This was also demonstrated by the wide confidence interval of the diagnostic odds ratio (DOR) if we employed the 35 U/ml cut-off point (DOR = 2; 95% CI: 1-7). The corresponding reference point for serum CA125 for predicting suboptimal cytoreduction in our cohort was 250 kU/l. Table 7 lists the relevant performance parameters of plasma TU M2-PK and serum CA125 in the study population.

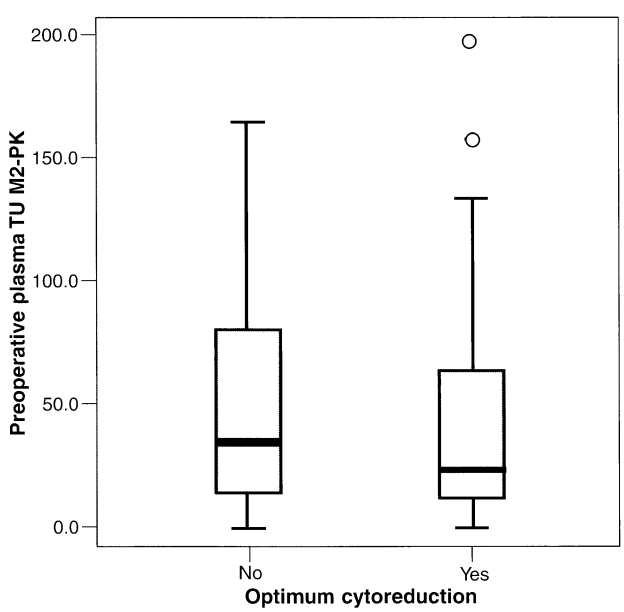

Figure 3. — Plasma Tumour M2-PK per cytoreduction (p < 0.05).

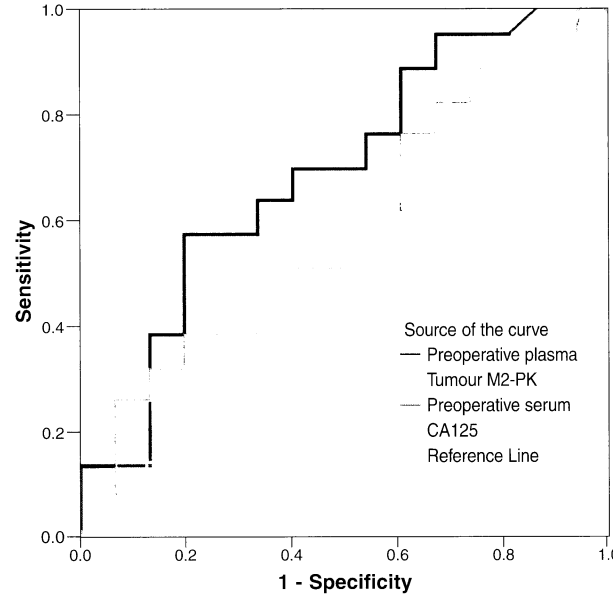

Figure 4. — ROC curve for plasma Tumour M2-PK and serum CA125 per cytoreduction (diagonal segments are produced by ties).

Discussion

The potential role of TU M2-PK as a cancer marker has been explored in a variety of cancers including lung, breast, prostate, renal and gastrointestinal cancers [20-26]. However, there has been no previous attempt to investigate its possible clinical application in ovarian cancer. Previously, we have investigated the value of TU M2-PK as a diagnostic tool in ovarian cancer in comparison with the standard test of serum CA125. The reference cut-off points were determined using the ROC curve and a best value of 22 U/ml was chosen as a diagnostic level (in press). This yielded a sensitivity of 70% and a specificity of 65% with overall accuracy reaching 67% which was not significantly different from the 71% test accuracy of serum CA125 (p > 0.05). The present study, however, mainly addresses the use of TU M2-PK as a predictor of surgical outcome with respect to tumour characteristics and level of cytoreduction.

There is little doubt that surgery remains the most important modality in the management of ovarian cancer; although on its own, may only be curative in a small minority of patients. For decades, the concept of optimal cytoreductive surgery has established itself as a strong predictor of ovarian cancer outcome [27]. Surgical procedures that would leave patients with more than 2 cm of

residual disease have failed to show any significant survival advantage [5, 6]. Moreover, further attempts at secondary cytoreduction in an effort to further eradicate or reduce residual disease to the acceptable minimum have not demonstrated significant improvements in outcome [28, 29]. Subsequently, the concept of deferred primary surgery until after initial treatment with neoadjuvant chemotherapy in patients with large volume disease was investigated. Nevertheless, those who underwent primary optimal cytoreductive surgery responded either better than or similar to those who received neoadjuvant chemotherapy [30-33]. Hitherto, the most pertinent question to this clinical dilemma is how to predict patients in whom primary optimum cytoreduction is not feasible; thus saving them the burden of a futile major surgery.

Several predictors of surgical outcome and degree of cytoreduction have been tested [7-11]; notably the CA125 tumour marker featured in most of these studies. In a retrospective multicentre study, Gemer and coworkers [10] reviewed 424 patients with FIGO Stage III and IV ovarian cancer who underwent primary cytoreductive surgery. Optimum cytoreduction was achieved in 242 patients. The validity of pre-operative CA125 as a single predictor of suboptimal cytoreduction was evaluated. The median CA125 concentration in optimally cytoreduced patients was lower than those who underwent suboptimal debulking (304 vs 863 kU/l, $p < 0.001$). The CA125 threshold derived from the ROC curve was 400 kU/l; the accuracy of the test at this concentration was 62% [10]. Another study by Saygili *et al.* [34] employed CA125 concentration of 500 kU/L, as shown on the ROC curve coordinate points, to assess surgical outcome in 92 patients with Stage IIIC epithelial ovarian cancer. At a 500 kU/l cut-off point, the sensitivity, specificity, true and false positive rates for predicting optimal cytoreduction were 73%, 77%, 73%, and 23%, respectively [34]. In essence, the sensitivity of CA125 in predicting surgical outcome ranged from 62-73% with specificity between 62-83%, using cut-off points between 400-600 KU/l [8-11, 34, 35].

To our knowledge, this is the first study to evaluate the potential role of TU M2-PK in predicting surgical outcome in ovarian cancer patients. TU M2-PK was significantly elevated in ovarian cancer patients and demonstrated high association with the serum CA125 blood test ($p < 0.001$). Furthermore, significantly higher concentrations of plasma TU M2-PK were associated with high stage as well as high-grade disease. This could reflect the degree of tumour burden and the carcinogenesis process. However, according to the ROC analysis, the test performed inadequately as a predictor of suboptimal cytoreduction, with overall efficacy of 61%.

The presented results mean that approximately one third of patients out of those deemed not to be suitable for primary cytoreduction, would actually have benefited from the primary surgery in that optimum cytoreduction would have been achieved. Similarly, around 40% of those expected to undergo optimal cytoreduction would be left with residual disease greater than 2 cm. Interestingly, the CA125 test performed even worse in the study cohort, with an overall test efficacy of 55%.

The small number of patients in this study with advanced stage disease means that these results should be viewed with caution and highlights the need for further larger research into this important aspect of the management of ovarian cancer patients. The employment of more than one predictor may improve the overall efficacy and help overcome potential problems of lack of specificity.

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