Abstract
Ovarian cancer ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system. Since diagnosis at an early stage is associated with improved rates of survival, an effective screening strategy that detects early-stage ovarian cancer could have a significant impact on mortality from this disease. Cancer researchers are under way to develop more accurate tumor markers that can be used to identify disease in its early stages, to predict the effectiveness of treatment, or to predict the chance of cancer recurrence after treatment has ended. Carbohydrate antigen 125 (CA125) is an established biomarker for ovarian cancer detection. As CA125 effectiveness in the identification of the malignancy is threatened by its low diagnostic specificity, measurements of two other tumor markers, carcinoembryonic antigen (CEA) and Human epididymis protein 4 (He4) in serum have been proposed for improving the specificity of laboratory identification of the disease.

The aim of our study was to evaluate the diagnostic performance of CA125, CEA and He4 in discriminating ovarian cancer from other benign gynecologic diseases. Our study group consisted of 100 women outpatients aged between 15 and 78 years old (mean age 48 years), which were diagnosed with an ovarian cyst during a visit to a gynecologist or during investigation of unknown abdominal pain by computer tomography. Fasting blood samples were collected and centrifugated using standardized procedure. All analyses were performed in plasma. CA125 and He4 plasma concentrations were determined using automated analyzerCobas®6000 (Rosche Diagnostics). The data were statistically treated by using Descriptive Statistics. A good correlation was found between values of CA125, CEA and He4. Measuring together CA125, CEA and He4 biomarkers can be used for a better classification and diagnosis of women with an ovarian cystic pelvic mass.

Key words: He4, CA125, tumor marker, ovarian, cancer

Introduction
Ovarian cancer (OC), uterine cervical cancer, endometriial cancer, and trophoblastic neoplasms are gynecologic malignancies for which tumor markers are in clinical use. Ovarian cancer is the fourth leading cause of cancer death worldwide and is responsible for 5% of all cancer deaths in women. The main challenge for laboratory biomarkers of OC diagnosis is to allow the accurate detection of malignancy as early as possible to improve clinical outcome and survival of patients (Moore et al. 2007. However, fewer than 30% of all ovarian cancers are diagnosed in stages I/II and worldwide mortality from ovarian cancer has decreased only by 12% since 1973 (Ferlay J. et al. 2008). The 5-year survival rate is less than 20% for women diagnosed at the late stage, whereas it is 90% if detected in the early stage (Scholler et al.
Many new tumor markers have been discovered since the development of monoclonal antibodies, and most tumor markers are now detected with them. No marker is completely specific. Therefore, diagnostic laboratory immunohistochemistry, and immunohistochemistry tests must be used in conjunction with morphologic and clinical findings in order to improve the early diagnostics of ovarian cancer (Kaspar et al. 2015). CA125 (carcinoma antigen 125, or carbohydrate antigen 125); CEA (Carcinoembryonic antigen) and HE4(human epididymis protein 4), are important gynecologic established tumor markers used nowadays for detecting OC recurrence and monitoring therapeutic response. CA125 is a protein that in humans is encoded by the MUC16 gene which is a member of the mucin family glycoproteins. It is commonly referred to as a “biomarker” or “tumor marker”, because it provides information about the biological state of a ovarian tumor (Köbel et al. 2008). This glycoprotein is widely distributed on the surface of cells of mesothelial origin in various benign and malignant conditions other than OC (Miralles et al.). Therefore, early detection may result in better outcomes. CA125 is the most widely used serum marker in the detection and management of the disease. It was first identified in the early ‘80s and can be helpful in determining whether an ovarian mass is malignant or not. As CA125 effectiveness in the identification of the malignancy is threatened by its low diagnostic specificity, two other biomarkers (CEA and HE4) measurements have been proposed for improving the specificity of laboratory identification of OC (Rosen et al. 2005). As mentioned above CEA is one of the most extensively used clinical tumor markers. The main reasons why CEA is useful as a serum tumor marker for colorectal, breast, lung, pancreas, stomach, and ovary cancers are probably because, CEA is a stable molecule, has a fairly restricted expression in normal adult tissue and is expressed at high levels in positive tumors; CEA could be defined as a glycoprotein containing approximately 50% carbohydrate with a molecular weight of approximately 200 kDa (Westwood JH et al. 1974). Another major step in the CEA field was the discovery of CEA-cross-reactive antigens in normal human tissue including blood (Krupey J. et al. 1966). The CEA is often positive in malignancies and can be used to monitor the progress of disease or response to treatment. CEA in combination with other tumor markers, (eg. mucin tumour markers CA19-9, CA242, CA 125) can be used in pre-operative staging and thereby assist in the planning of the type of surgery required and future management options. (Levy M et al 2008). The WFDC2 (He4) gene is a member of a family of stable 4-disulfide core proteins that are secreted at high concentrations (Hellström et al. 2003). It is amplified in ovarian carcinomas, whereas its expression in normal tissues, including ovary, is low (Havrilesky et al. 2008). Serum concentrations of He4 are less affected by menstruation, ovulation and other benign ovarian conditions (e.g. endometriosis) compared with CA125 (Ono et al. 2000). Also the He4 assay may have an advantage over the CA125 assay as it is less frequently positive in patients with nonmalignant disease (Hellström et al. 2003). The combination of CA125 and He4 is a more accurate predictor of malignancy than either marker alone (Moore et al. 2008). The women with a gynecological disease and increased concentrations of He4, CA125 and CEA are at higher risk for malignant pathologies, as expected from immunohistochemically data (Rosen et al. 2005). He4 is found in high concentrations in the serum of women with serous epithelial ovarian tumor confirmed that tumor markers can be very helpful in following response to treatment and recurrence, but they cannot replace physical examination, evaluation of symptoms, and radiologic studies (CT scan, MRI, PET, etc.). (Carolyn Vachani RN. 2016).

Material and method

Study population

Our study group consisted of 100 women outpatients presented in Gynecological Department of Kavaja General Hospital, who were diagnosed with an ovarian cyst during a visit to a gynecologist or during investigation of unknown abdominal pain by computer tomography. The inclusion criteria were the
availability of complete clinical records and the consent to have additional testing for new markers for ovarian tumor. The exclusion criteria were pregnancy and significant concomitant disease. The mean age was 48 years old (range 15-78).

Sample collection
Venous blood samples were collected following an overnight fasting (serum and EDTA samples) using standardized procedure for collection and storing. All analyses were performed on plasma. Fasting blood samples were collected, centrifugated and stored using standardized procedure.

CA125, CEA and He4 Analysis
Complete blood picture was performed on Mythich-18 fully automated cell counter. Serum tumor markers’ concentrations were determined using respectively Cobas e601 automated analyzer for Ca125 and Elecsys 2010 automated analyzer for He4 (Rosche Diagnostics), which are based on electrochemilumineshence immunoassay ECLIA. Results are measured via a calibration curve which is instrument-specifically generated by 2-point calibrations and a master curve provided via the reagent barcode. The resulting chemiluminescent reaction was measured as relative light units. A direct relationship exists between the concentration of CA125, CEA and He4 and respectively antigen in the sample and relative light units.

Statistical Analysis
Descriptive Statistics were applied to each data set in order to analyze and interpret the results. The mode of the data distribution was discussed according to kurtosis and skewness test. Minimum and maximum values obtained for each parameter were estimated, as well. MINITAB 17 software package was used for data analysis.

Results and discussion
Tumor marker concentrations in all patients (N=100)
The analytical values of CA125 and CEA in 100 patients' serum samples are presented in Figure 1 and Figure 2. We have considered the concentrations 35U/ml and 5.0 ng/ml as the upper limits (cut-off values) of normality for CA125 and CEA, respectively. In 33% of serum samples CA125 concentrations exceeded the cut-off value. CEA concentrations exceeded the cut-off value in only 11% of serum samples. In these last samples, CA125 values were above the limit, while for CEA five of positive results were been found closely near the cut off value.

![Figure 1. Distribution profile of CA125 concentrations in all serum samples compared with cut-off values (35U/ml for CA125)]
Simultaneous measurements of ...

Figure 1. Distribution profile of CEA concentrations in all serum samples compared with cut-off values (5.0 ng/ml for CEA)

In all 11 patients' serum samples, no value of HE 4 was found higher than 150 pmol/l. This is because, in different benign gynecologic conditions in premenopausal women, the elevated values of CA125 are more frequent than He4 ones. This is the reason why He4 can be a good complement to distinguish CA125 false positives from true positives (Figure 3).

Figure 3. Distribution profile of HE 4 concentrations in all serum samples compared with cut-off values (150 pmol/ml for HE 4)

Percentages of positive results, obtained for CA 125, CEA and HE 4 tumor markers in our study are presented in Figure 4.

Percentages of positive results, obtained for CA 125, CEA and HE 4 tumor markers in our study are presented in Figure 4.
Statistical analyses
In order to obtain new information for the correlation between tumor markers, all the analytical data were treated using Descriptive Statistics. MINITAB 17 software package was used for data analysis. Statistical results are presented in Table 1. The analytical results for CA125 and CEA represented different variability. CA125 values had a high variability (CV% = 368.537), also CEA values had a high variability (CV = 280.561%). Minimum values were 6.010 and 0.430 for CA 125 and CEA and maximum values measured were 5426.00 and 104.800 for both CA 125 and CEA respectively.

Table 1. Descriptive Statistics for CA125 and CEA values in all serum samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Count</th>
<th>Mean</th>
<th>Median</th>
<th>StD</th>
<th>Variance</th>
<th>CV %</th>
<th>Min</th>
<th>Max</th>
<th>Skewness</th>
<th>Kurtosis</th>
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<tr>
<td>CA125</td>
<td>100</td>
<td>169.850</td>
<td>19.460</td>
<td>625.960</td>
<td>391825</td>
<td>368.537</td>
<td>6.010</td>
<td>5426.00</td>
<td>6.735</td>
<td>52.162</td>
</tr>
<tr>
<td>CEA</td>
<td>100</td>
<td>4.522</td>
<td>1.865</td>
<td>12.687</td>
<td>160.950</td>
<td>280.561</td>
<td>0.430</td>
<td>104.800</td>
<td>6.459</td>
<td>45.505</td>
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</table>

High disparity is evident for the analytical data of both parameters which are characterized by high values of skewness (6.735 for CA 125 and 6.459 for CEA) and kurtosis value (52.162 for CA 125 and 45.505 for CEA) which indicate the asymmetrical distribution of CA125 and CEA, as well as the influence of complicated factors. These high positive values indicate that the data are positively skewed and are affected by complicated factors (skewness value > 0 and kurtosis value > 3). The high variance of CA125, He4 and Ca125/He4 values is shown in Figure 5.
Simultaneous measurements of …

The data correlation analysis between parameters (Figure 6) indicates that CA125 and CEA serum concentration values do not obey the normal distribution (p-value <0.005).

![Figure 5. Boxplots of CA125 and CEA values for all samples](image)

![Figure 6. Probability plot of CA125 and CEA for all samples](image)

It explains that both tumor markers represent positive values in cases with suspect of OC and also it is an indicia for further examinations. A statistically significant positive correlation has been found between CEA and CA125. The correlation coefficient is good (r =0.730), while between CEA and HE 4 a moderate correlation was found(r =0.528). The data confirms a moderate correlation between CA 125 and HE 4 tumor markers (r =0.501), indicating that experimental results of CA 125, HE 4 and CEA antigen in the blood serum have a very good correlation with each other. For this reason, by combining CA125, CEA and He4 measurements, we can improve the diagnostics performance for OC (Table 2).

**Table 2. Statistical correlation between CA 125, CEA and HE4**

<table>
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<tr>
<th></th>
<th>CEA</th>
<th>Ca125</th>
<th>He-4</th>
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<td>CEA</td>
<td>1.000</td>
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<td></td>
</tr>
<tr>
<td>Ca125</td>
<td>0.730</td>
<td>1.000</td>
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<tr>
<td>He-4</td>
<td>0.528</td>
<td>0.501</td>
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To compare the variance of both tumor markers, statistical Anova single factor treatment was done and the obtained results are shown in Table 3.
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<td>Average</td>
<td>Variance</td>
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<tr>
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<td>160.9504</td>
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<tr>
<td>Column 2</td>
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<td>16985</td>
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<td>MS</td>
<td>F</td>
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<td>1</td>
<td>1366669</td>
<td>6.97</td>
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<tr>
<td>Within Groups</td>
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<td>195993</td>
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From the Table 3, and by comparison of the results obtained for both tumor marker, we conclude that there are significant changes between the variances of the CA 125 and CEA serum levels, and the difference between the averages of the results obtained was significant (F = 6.97 > Fcrit = 3.889, and P value = 0.0089 < 0.05). Also, it is obvious that the variability is higher within groups (SS = 388806642) than between groups (SS = 1.3667).

**Conclusions**

The analytical results for CA125, CEA and He4 represented different variability. The mean and the median values for CEA parameter were positioned close to the cut off values, for HE 4 the mean and median values were positioned close to the minimum value, while CA 125 mean and median values were higher than cut off value, showing high variability. The data correlation analysis between parameters indicates that CA125 and CEA serum concentrations values do not obey the normal distribution (p-value < 0.005). Both tumor markers represent positive values in cases of suspected OC and also give an indicia for further examinations. In our study group according to different benign gynecologic conditions, CA125 is often more elevated than He4. This is also true for our set of data, suggesting that He4 is a good complement to distinguish CA125 false positive, from true positive cases, especially in fertile women. We took different values for CA125 and He4 tumor markers. The results confirmed that by combining CA125, CEA and He4 measurements, we can improve the diagnostics performance for OC. The use of this combination can improve the detection of ovarian tumor, as compared with use of either marker alone for the discrimination of benign from malignant ovarian lesions. Due to the high prevalence of OC in the post-menopausal women and the need for data related to early tumor stages, more tailored studies on this specific subgroup are needed. In conclusion, tumor markers can be very helpful in following response to treatment and recurrence, but they cannot replace physical examination, evaluation of symptoms, and radiologic studies (CT scan, MRI, PET, etc.).

**References**