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CA125 reference values change in male and postmenopausal female subjects

Abstract

Background: In cancer patients, including women with a diagnosis of ovarian cancer, cancer antigen 125 (CA125) is used to evaluate the presence of peritoneal involvement. The aims of the present study were to assess CA125 reference intervals and reference change values (RCV) in postmenopausal reference women, postmenopausal women breast cancer free, reference men and cancer free men.
Methods: The series consisted of 433 subjects: 105 postmenopausal breast cancer free women and 56 cancer free men in addition to a total of 272 reference subjects (145 postmenopausal women and 127 men). Repeated CA125 measurements were made in a subset of 149 women and 54 men to calculate RCV and index of individuality. Serum CA125 levels were evaluated by a chemiluminescent assay.
Results: In postmenopausal reference women, the mean CA125 value and 2.5th–97.5th percentiles were 6.70, 2.60–11.00 kU/L, respectively, with a unidirectional RCV of 38.4%. In postmenopausal breast cancer free women, the mean CA125 value and 2.5th–97.5th percentile were 7.45, 4.09–10.92 kU/L, respectively, with a RCV of 34.5%. The difference between the means was statistically significant (t=–3.02, p=0.003). In the two male subgroups, the difference between the means for CA125 was not statistically significant (t=0.43, p=0.665). On considering the entire male population, the mean CA125 value and 2.5th–97.5th percentiles were 7.50 and 2.40–13.2 kU/L, respectively, while the unidirectional RCV was 34.3%. In all the studied groups, the indices of individuality were equal to or below 0.6.
Conclusions: The extremely low index of individuality found underlines the importance of using the RCV instead of absolute values as a parameter when interpreting the CA125 data in the monitoring and follow-up of patients with ovarian cancer.

Keywords: CA125 antigen; index of individuality; ovarian cancer; postmenopausal women; reference change values; reference values.

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Introduction

Cancer antigen 125 (CA125) is commonly used for the detection and management of patients with ovarian cancer (OC) but, in spite of its clinical usefulness, CA125 measurement has several limitations. CA125 may be elevated in women with benign gynaecological conditions and, in the presence of peritoneal inflammation (hyperstimulation, salpingitis, ruptured ectopic pregnancy, laparotomy), peritoneally derived CA125 markedly contributes to circulating CA125 concentrations, leading to increased CA125 values [1]. Moreover, influenced by hormones, the normal endometrium produces CA125, and so its levels can fluctuate during the menstrual cycle. CA125 cut-off in serum samples has been set at 35 kU/L [2], but this cut-off may be unreliable at the time of menstruation and leads to a suspicion of cancer in pre- and postmenopausal women [3–5].

Whey-acidic human epididymis protein 4 (HE4), a new promising biomarker for ovarian carcinoma, has recently been introduced into clinical practice and several publications have demonstrated superiority of HE4 over CA125 as biomarker for OC in patients with pelvic mass [6]. In particular, HE4 was more frequently expressed in early stage disease when compared to CA125 [7, 8]. Interestingly, the Risk of Ovarian Malignacy Algorithm (ROMA) is a predictive index which basically takes into account the

serum concentration of both biomarkers (CA125 and HE4) together with patients pre- or postmenopausal status [9, 10]. Despite that, HE4 and ROMA sensitivity and specificity in OC detection have been shown to vary between pre- and postmenopausal women [8, 11]. Furthermore, the data reported in some very large studies, such as the recently published Asian study [12] which evaluated 2182 women, are based on information given by the patient rather than an accurate physical examination and extensive diagnostic tests, so that the distribution of CA125 and HE4 values in these apparently healthy women should be considered with caution.

OC is rare in women under the age of 40 whereas its incidence increases steeply thereafter, peaking in the 65 to 75 year age category [13]. It is therefore of utmost importance to improve our understanding of OC tumour markers patterns in older women, especially in view of the increase in life expectancy, which may incur an increase in the incidence of OC in the future.

In several studies, the presence of the CA125 tumour marker has also been found in blood samples from men with testicular carcinoma, regardless of its histotype [14, 15], and seminal vesicular adenocarcinoma [16]. Moreover, it has been shown that CA125 is measurable not only in blood samples, but also in amniotic [17] and peritoneal fluid, and in the seminal plasma of men with and without infertility [18].

Since female hormones influence CA125, males should have CA125 values lower than those of fertile women. Due to the recent applications of CA125 measurement for other diseases [14, 15], evidence is needed to adapt the reference values not only for postmenopausal women, but also for men, in order to improve the potential application of this marker to clinical practice.

We evaluated CA125 serum level in a series of age matched males and postmenopausal females. In both groups, reference subjects and cancer free subjects (clear for ≥5 years) were studied to evaluate the variability of CA125 and to estimate: 1) reference values; 2) reference change values and 3) indices of individuality.

Materials and methods

Subjects

At the Department of Laboratory Medicine, blood samples were taken from 433 subjects who had been recruited from the Department of Oncology of Padua University Hospital. The series included 105 postmenopausal women with a previous history of breast cancer and 56 cancer free men. The types of cancer in men were of the lung, colon, stomach, pancreas or kidney and/or melanoma and lymphoma. In addition, a total of 272 reference subjects (127 men and 145 postmenopausal women) were included in the study. Men and women with a previous history of cancer were recruited from the list of follow-up controls for cancer patients. The group of reference subjects, who were volunteers, included relatives of the cancer subjects; all agreed to undergo CA125 free of charge, and also gave their clinical history during an interview conducted before the study. Subjects with a history of cancer were admitted if they stated they had been in complete remission for ≥5 years; this was confirmed by objective, instrumental and biochemical negative findings. The criteria used to define menopausal status were: amenorrhoea ≥2 years, no hysteroadnexectomy, follicle stimulating hormone (FSH), luteinising hormone (LH) and 17 β-oestradiol levels consistent with menopausal status. Subjects with diabetes type I or II and/or arterial hypertension were admitted. The exclusion criteria were: conditions with pleural irritation like history of spring allergic reaction, smoking habit of more than five cigarettes/day, chronic asthma, chronic chest disease with recurrent lung/bronchial infection; pathologies which altered the hepatic metabolism, such as HBV or HCV positive serology, liver cirrhosis, chronic hepatitis alcoholism, congenital hyperbilirubinaemia, and biliary cholestasis. Pharmacological treatment, whether for acute or chronic disease, was an exclusion criterion unless related to drugs administration for diabetes or chronic hypertension.

Subjects who had travelled to Asia, Africa and Central-South America at least 6 months before the study were also excluded. Nor were subjects who had undergone the following events within the 6 months prior to the study: bone, muscle and/or soft tissue trauma, deep vein thrombosis, major dental surgery, major surgery of another nature during the previous 2 months, 2 months recent vaccinations, infectious disease in the last 6-month period, because they could have a potential and uncertain effect on mesenchymal cells and in CA125 production [19, 20]. Moreover, rheumatoid arthritis [21], collagen disease [22] and autoimmune gastritis requiring therapy were exclusion criteria because rheumatologic and autoimmune pathologies seem to increase the CA125 concentration [23]. Also either acquired or inherited blood disease, thalassaemia, haemoglobinopathy and haemolytic anaemia were excluded because alteration of blood components could modify the serum concentration of CA125 [24]. Finally, cancer treatment, hormone therapy, contraception or menopause-related disturbances and confirmed endometriosis were also exclusion criteria.

For a subset of 149 women and 54 men, in a period of 2 years following admission to the study, a series of repeat CA125 measurements were collected, the number of specimens for each individual ranging from two to 10.

The study was performed in accordance with the Declaration of Helsinki; all subjects gave their fully informed consent in writing to take part in the study, to undergo blood collection; they also consented to the evaluation of their serum and clinical data before enrolment.

Sample collection and CA125 assay

All blood samples, collected from seated subjects by an experienced phlebotomist using conventional procedures with minimal stasis, were processed by centrifuge at 4000 *g* for 5 min, and serum was

aliquoted and stored at −80°C until analysis. Serum concentrations of CA125 were determined by a chemiluminescence method (ADVIA Centaur® CP Immunoassay System, Siemens Healthcare Diagnostic, Deerfield, IL, USA), which is a fully automated, single-step sandwich immunoassay using direct, chemiluminescent technology. The coefficient of analytical variation (CV_a) was obtained by analysing the internal quality control data related to the study timeframe. The value reported is the mean CV_a at the quality control level closer to the mean CA125 level observed in the study population.

Statistical methods

Data were collected and analysed. First, the means and standard deviations of the CA125 repeated measures were calculated. Second, Cochran and Read tests were used to assess the outliers, discarding them whenever present, as suggested by Fraser and Harris [25]. Then, the distribution of the total CA125 values was assessed using the Shapiro-Wilk test for normality. The Student's t-test was used to assess differences in group's mean distributions. For each group, the CA125 means and the corresponding reference values were calculated. As reference values the P_{95} was taken as the 2.5th and 97.5th percentile of the evaluated distribution. We then applied the nested analysis of variance to estimate the within and between subject CV (CV_i and CV_g, respectively). The index of individuality was calculated by dividing CV_i by CV_g. The reference change value (RCV), also known as the critical difference, was calculated from the data generated with the following formula: $RCV=2^{1/2}\,Z\cdot(CV_A^2+CV_I^2)^{1/2}$ where Z is the Z-statistic. The value of Z is 1.65 if the expected change is unidirectional (either an increment or decrement) and 95% probability is conventionally regarded as significant, while it is 1.96 if the expected change is bidirectional.

All statistical analyses were made using STATA® (StataCorp LP, TX, USA) version 10.1.

Results

CA125 reference values

After the outliers had been removed following Fraser and Harris [25], the CA125 data had a normal distribution (p=0.442). The difference between the mean values of CA125 in postmenopausal reference women and those in postmenopausal breast cancer free women was statistically significant (t=−3.02, p=0.003); they were therefore analysed separately. In the subgroup of postmenopausal reference women (mean age: 61.7; range: 33–92), the mean value of CA125 was 6.70 kU/L with a SD of 2.06 and the 2.5th and 97.5th percentile were 2.60–11.00 kU/L, respectively. In postmenopausal breast cancer free women (mean age: 59 years; range: 26–91 years), the mean CA125 value was 7.45 kU/L with a SD of 1.74 and the 2.5th and 97.5th percentiles were 4.09 and 10.92 kU/L, respectively.

In reference male subjects subgroup (mean age: 61.4; range: 49–80) the mean value of CA125 was 7.57 kU/L with SD of 2.77 (95% CI 6.86–7.94) while in the subgroup of cancer free men (mean age: 61.5 years; range: 38–77 years), the mean value of CA125 was 7.49 kU/L with SD of 2.66 (95% CI 7.28–7.86). As the mean CA125 values of the latter two groups were quite similar, no statistically significant difference being found (t=0.43, p=0.665), the two were considered together in further analyses. On considering the entire male population, the mean CA125 value was 7.50 with an SD of 2.79, while the 2.5th and 97.5th percentiles were 2.40 and 13.2 kU/L, respectively. The latter mean value was statistically different from the one of postmenopausal reference women (t=−2.65, p=0.008), but not from the mean value of postmenopausal breast cancer free women (t=−0.16, p=0.863).

RCV and index of individuality

The analytical variation coefficient (CV_a) for the determination of CA125 with the automated chemiluminescence method, previously calculated as described above, was 4.30%.

Forty-six of the 145 postmenopausal reference women and 103 of the 105 breast cancer free women underwent repeated CA125 evaluations. In women from whom specimens were repeatedly collected, the resulting variability for both reference subjects and free from breast cancer patients is displayed in Figure 1 (panels A and B).

In the subgroup of postmenopausal reference women, after Nested ANOVA, the within-subject CV_i was 15.93%, while the between-subject CV_g was 26.6%. With these CVs, the bidirectional RCV with a confidence level of $p<0.05$ was 45.6% and the unidirectional RCV, 38.4%, while the index of individuality was 0.6. In the subgroup of postmenopausal breast cancer free women, the within-subject CV_i was 14.2%, and the between-subject CV_g, 23.71%. The bidirectional RCV with a confidence level of $p<0.05$ was 41.0% and the unidirectional RCV, 34.5%, while the index of individuality was again 0.6.

Regarding male subjects, it was only possible to collect and measure serial samples in a subset of 54 of 183 subjects. Figure 1 (panel C) reports the CA125 variability for the male subjects who repeatedly underwent specimen collection. In this group, the within-subject CV_i was 14.1%, while the between-subject CV_g was 35.59%, calculated using nested ANOVA. The bidirectional RCV with a confidence level of $p<0.05$ was thus 40.7% and the unidirectional RCV, 34.3% while the index of individuality was 0.39.

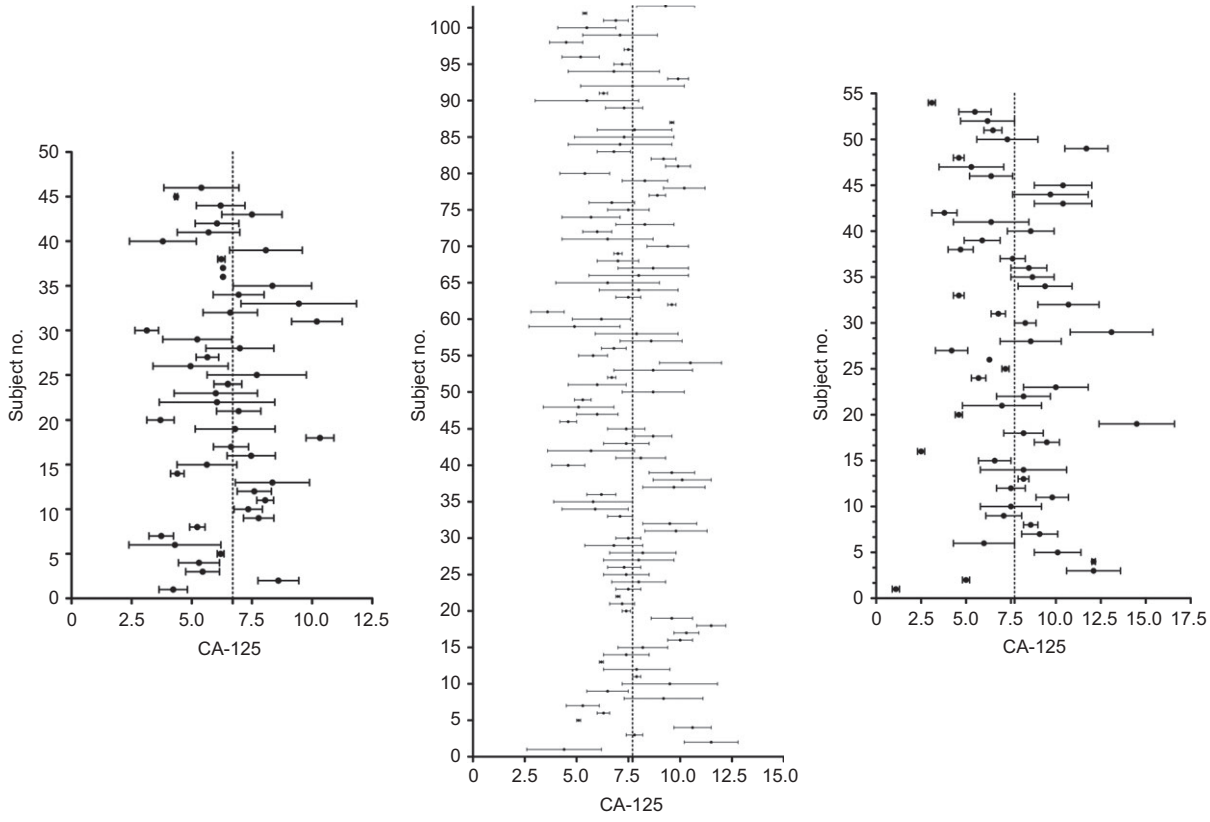

Figure 1 CA125 variations in repeated evaluation. Each point represents the subject's mean of the CA125 determinations and the corresponding standard deviation (expressed as kU/L).
The dotted lines show the mean of the CA125 in the corresponding group. (A) postmenopausal reference women; (B) postmenopausal breast cancer free women; (C) male subjects.

Discussion and conclusions

In 1983, the cut-off level for CA125 was first determined as 35 kU/L by Bast et al. [2], who demonstrated that 82% of patients with OC had a level exceeding 35 kU/L. Since then, the CA125 cut-off of 35 kU/L has been consolidated, and is widely used in routine clinical practice. However, it is well-known that the CA125 distribution is strongly affected by gynaecological conditions and menopausal status, and findings reported in several studies [26, 27] have demonstrated that in postmenopausal women the mean CA125 level is lower than that in fertile women. Therefore, the CA125 cut-off should be reappraised in order to enhance the clinical utility of preoperative evaluation and postoperative surveillance [27].

Recently, the novel serological marker HE4 has been introduced in clinical practice for aiding diagnosis of OC and most investigations have revealed that a combination of CA125 and HE4 (ROMA) results in a higher accuracy of OC diagnosis then if either marker is used alone [9, 10]. However, a newly published study by Lenhard et al. concluded that the diagnostic performance of ROMA is better than CA125 or HE4 alone in postmenopausal women, but not in premenopausal women [8] while Montagnana et al. found that ROMA is not superior to that of HE4 alone [11]. Moreover, different ROMA performances are found by using different analytical methods [8]. Therefore, results of CA125, HE4 and ROMA seem to be still partly controversial [28].

However, it goes beyond the scope of the present study to propose a new CA125 cut-off or to compare the diagnostic performance of CA125 with that of ROMA or HE4. Our aim was to evaluate CA125 variability and suggest changes to reference values, in an attempt to provide clinicians with information conducive to a reliable patient follow-up. On evaluating the serum levels of CA125 of 145 postmenopausal reference women and 105 postmenopausal breast cancer free women (with complete remission) we found that the mean value of this tumour marker was lower in postmenopausal control women than in postmenopausal

breast cancer free women. Although this slight difference was statistically significant, the upper reference values in these two groups were the same (11 kU/L), this finding being compatible to that made by Takami et al. (13.7 kU/L), who studied 291 women with a mean age of 59 (range 49–90) years [26]. Yet Bjerner et al., on studying the CA125 serum levels in 250 women (aged 30–39) and in 250 men (aged 18–29) with a multiple linear regression model, found an upper reference value close to 35 kU/L, for both women and men [29]. These findings underlined the similarity between these two groups, despite the different sites of CA125 production in men and women [29]. The CA125 reference interval specified by Pauler et al., wider than that reported by us, ranges from 4.6 to 52.7 kU/L for non-young Caucasian healthy women [30]. Moreover, Pauler et al., found that for women with a history of cancer, reference intervals shifted slightly upward, at 5.2–59.4 kU/L. These observations, however, do not contradict those made by us in postmenopausal breast cancer free women, who had a mean CA125 value higher than that of postmenopausal reference women. The differences between reference values found by Pauler et al., Bjerner et al., and ourselves may have depended, at least in part, on the different analytical methodologies used to measure the CA125 serum levels. Indeed, Bjerner et al. used an in-house immunofluorometric assay while Pauler et al., used a radioimmunometric assay, both of which are different from the ADVIA Centaur chemiluminescent methods that we used.

There are few studies investigating the reference values in male subjects in literature. In our first analysis we made separate evaluations of the CA125 level in men with and those without a history of cancer, and found no statistically significant difference; we therefore considered them together, as a single group. The mean values obtained by us (7.50 kU/L) confirm the results reported by Barcelo et al. [31], who found a value of 8 kU/L on using the same immunochemiluminiscent assays as us.

Interestingly, the indices of individuality of all the studied groups (Table 1) are equal to or <0.6. Our findings support those of Tuxen et al. [32], who studied postmenopausal women and found not only an index of individuality below 0.6, but also a unidirectional critical difference in the region of 50%, which is slightly higher than the value found by us in a comparable group.

The false-positives for tumour markers are related to the patient's condition as well as to pre-analytical or iatrogenic factors, interference due to the assay method used and, finally, various non-cancer diseases. So, identifying false-positives by observing the evolution of the tumour marker concentration over a period of 3–4 weeks between two consecutive readings can really contribute to the diagnosis and follow-up of patients [33]. In this context, it is widely known that an index of individuality below 0.6 indicates that the serial measurements from a subject in a steady state condition provide a better basis for the early detection of recurrence than conventional reference intervals. Therefore, findings made in an individual subject may be highly unusual for that particular person, but may still lie below the upper reference limit, and a significant change in concentrations may occur when both measurements are within the normal range. Moreover, when a subject has a homeostatic setting point close to a cut-off value, serial results should be expected to fluctuate across that cut-off value.

Based on the above results, we suggest that in patients with a follow-up including the measurement of CA125, the reference change values rather than the reference values, should be considered and, especially for tumour markers, such as CA125, the unidirectional RCV should be used in monitoring cancer recurrence. Indeed, for two serial measurements of CA125 to be considered significantly different each other, the difference in numerical results must be greater than the reference change value.

The reference values and the reference change values may vary widely depending on the measurement methods used. The performance of chemiluminescent methods,

	Postmenopausal females		Males
	RS	BCF	
n	145	105	183
Age			
Mean	61.7	59.1	61.4
SD	8.66	10.8	10.2
CA 125			
Mean	6.70	7.45	7.50
SD	2.60	1.74	2.79
Reference values	2.6–11.0	4.1–10.9	2.4–13.2
n	45	103	54
RCV			
Bidirectional	45.6%	41.0%	40.7%
Unidirectional	38.4%	34.5%	34.3%
II	0.6	0.6	0.4

Table 1 Summary table reporting, for each considered groups, the number of subjects included, the mean and SD of age and CA125 and also the CA125 2.5th and 97.5th percentiles (reference values). The reported reference change values (RCV) and the individuality index were calculated in the subjects from whom specimens were repeatedly collected (46 postmenopausal reference women, 103 breast cancer free women and 54 men subjects). For RCV calculation, the 95% probability ($p<0.05$) of the considered Z statistic was used. BCF, breast cancer free women; II, individuality index; RS, reference subjects.

such as the ADVIA Centaur® CP Immunoassay System, is good, with a low analytical variation; RCV therefore is enhanced by this analytical precision.

The major limitation of the present study lies in the relatively small series, which is not representative of the general population, and excludes young male and female subjects. Moreover, in some recruited subjects, particularly in reference subjects, we were unable to collect multiple samples for CA125 assay. However, the strength of the study lies in its use of well-defined selection criteria and its extended follow-up of patients with a previous history of cancer after our study's time (all the of subjects underwent objective/instrumental re-examination for at least 12 months following the study period to search for some insidious potential disease).

Finally, while efforts to provide a better standardisation of current assays for CA125 measurement are welcomed, the current recommendation is still to use the same clinical laboratory and related quality specifications in order to obtain laboratory-specific RCV information for interpreting the data provided by this marker in monitoring patients with ovarian cancer.

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Conflict of interest statement

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