and could contribute to the high incidence of infections at mucosal surfaces.

Some authors have pointed out that nutritional deficiency may influence the biological gradient and the natural history of AIDS infection [1]. The results of this preliminary study emphasize the need to design an age-appropriate nutritional support for children with AIDS, which might improve their longevity and quality of life.

We thank Patricia Ronaye de Ferrer, Department of Food Science, School of Pharmacy and Biochemistry, for the revision of manuscript. This study was supported by grants of Raffo SA and Rotary Club (Barracas), Buenos Aires, Argentina.

References


Immunoluminometric Assay for Determining CA 72-4 in Patients with Gynecological Tumors, Thomas Henze*1 and Edith Greinert-Mai2 [1 Dept. of Gynaecol. (Hormone Lab.), Virchow-Klinikum, Faculty of Med., Humboldt Univ. Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany; 2 Byk-Sangtec Diagnostica KG, Product Management, Marketing and Sales Dept., von Hevesy-Str. 3, D-63128 Dietzenbach, Germany; *author for correspondence: fax + 49-30-450-64932]

TAG72 (CA 72–4; tumor-associated mucinous glycoprotein) was discovered by Colcher et al. in 1986 [1] and is frequently used to monitor patients with gastrointestinal and mucinous ovarian carcinomas [2–4]. The aim of our study was the technical evaluation of a new immunoluminometric CA 72–4 assay and a comparison with established IRMA methods. Furthermore, we established reference data for women without tumors and obtained comparative data on patients with gynecological tumors.

The LIA-mat® CA 72–4 immunoluminometric assay (Byk-Sangtec Diagnostica, Dietzenbach, Germany) is based on the "sandwich" principle. Monoclonal antibodies cC9 and B72.3 [5, 6] are used for the solid-phase (coated tubes) and the detection (luminescence-labeled tracer) antibodies, respectively. Both antibodies react simultaneously with the CA 72–4 antigen to form the sandwich, which is detected by light emission. The antigen used in the calibrator is protein purified from tumors by Centocor.

The immunoradiometric determinations were carried out with the CA 72–4 ELSA® (CIS Diagnostica, Dreieich, Germany) and the IRMA-mat® CA 72–4 (Byk-Sangtec Diagnostica). All assays were performed according to the instruction manuals.

Serum samples were taken from 315 apparently healthy premenopausal and 82 postmenopausal women, 60 women in all terms of pregnancy, 9–11 samples from each of 5 young women during a menstrual cycle, 53 women with benign gynecological disorders (endometriosis 41, uterine myomatosis 6, ovarian cysts 3, benign tumors 2, myoma 1), 61 female patients with thyroid dysfunction, 73 patients undergoing sterility treatment, and 168 patients with malignant gynecological tumors of various sites.

The immunoluminometric determination of CA 72–4 was shown to correlate well with the established immunoradiometric determinations (r = 0.946). The values for LIA-mat CA 72–4 were slightly higher, especially in the pathological range (Fig. 1). The within-run precision data for LIA-mat CA 72–4 from duplicate analyses (n = 447) show a CV of 5.8% (total mean). The useful working range is 1.7–100 kU/L. The total imprecision (CV, n = 12) over 2 months was 7.0% (mean = 5.8 kU/L), 8.8% (mean = 9.1 kU/L), 7.3% (mean = 18.6 kU/L), and 9.4% (mean = 58.7 kU/L).

Four samples with high CA 72–4 concentrations were diluted serially with the kit diluent. Strict linearity over the whole calibration range was found in all cases (r = 0.997–0.999). One-step assays bear the risk of a high-dose hook effect. Therefore, high-titered serum samples were used for detection.

Fig. 1. Correlation between LIA-mat CA 72–4 and CA 72–4 ELSA, by standard principal component calculation.
The LIA-mat CA 72-4 showed a high-dose hook risk at concentrations >8500 kU/L.

To establish reference data for women, we analyzed CA 72-4 in seven different reference groups. For all groups, the values did not follow a gaussian distribution.

In 315 healthy young women who were included in a screening program and did not show any benign abnormalities, median = 1.9 and 95th percentile = 4.8 kU/L. Only 1.9% of subjects showed concentrations >6 kU/L.

Because ovarian cancer prevails in postmenopausal women, this group was of special interest. Values ranged from 0.8 to 11.7 kU/L, median = 2.0, 95th percentile = 6.2 kU/L. Rarely (6% of subjects) were concentrations >6 kU/L.

A group of patients evaluated for infertility also had higher values, ranging from 0.1 to 12.0 kU/L, median = 2.4, 95th percentile = 8.5 kU/L. More than 10% of the subjects exceeded the 6 kU/L cutoff.

CA 72-4 values analyzed in 5 young women on different days of the menstrual cycle showed only small statistical variations with the following ranges: 1.9-3.5 kU/L (subject 1), 1.6-2.7 kU/L (subject 2), 1.5-2.9 kU/L (subject 3), 1.5-2.6 kU/L (subject 4), and 2.2-3.2 kU/L (subject 5). However, CA 72-4 concentrations demonstrated no relevant dependence on hormonal changes during the menstrual cycle.

Slight increases in CA 72-4 concentrations were seen in the group of 60 pregnant women, in whom median = 1.3 and 95th percentile = 8.3 kU/L. No rise in CA 72-4 concentrations occurred in relation to pregnancy term. It remains to be clarified whether or not there is a relation between increased CA 72-4 concentrations and high-risk pregnancies.

In the 61 patients being screened for thyroid dysfunction, CA 72-4 concentrations were quite frequently increased (median = 3.4 kU/L). This group exhibited the highest 95th percentile at 10.3 kU/L, and 11.4% of patients' values exceeded 6 kU/L.

The majority of the 53 patients with benign gynecological disorders (mainly endometriosis) exhibited concentrations <5 kU/L; however, increases up to 19.4 kU/L were also observed. Median value = 1.9 and 95th percentile at 9.5 kU/L were well below the thyroid patient group.

In 100 patients with ovarian carcinomas of different histological types, we found median = 132 kU/L for serous type, 750 kU/L for mucinous, and 15 kU/L for endometrial carcinoma. Ovarian tumors of both mucinous and serous type showed values >1000 kU/L. Patients with other gynecological carcinomas were also tested. In most of these cases CA 72-4 values were significantly higher than in reference groups, 68% were positive at a 4 kU/L cutoff, 60% positive at a 4 kU/L cutoff.

For receiver-operating characteristic (ROC) curve analyses, CA 72-4 values obtained for the benign patient group and the cancer group were used. With a cutoff of 6 kU/L (95% specificity), the resulting sensitivity is 44% for LIA-mat CA 72-4 and 35% for CA 72-4 ELSA. A cutoff of 4 kU/L decreases the specificity to 90% and increases the sensitivity to 48.4% for both assays.

The first objective of our study was the technical evaluation of the LIA-mat CA 72-4. The data obtained for precision, linearity upon dilution, and high-dose hook revealed a reliable performance of the assay for routine use.

The comparison with IRMA methods by means of correlation and ROC analyses showed the equivalence of the LIA assay. However, the subjects included in this study may not be representative of the patient spectrum normally seen in clinical practice. This should be taken into consideration when looking at absolute ranges of the clinical sensitivity of CA 72-4.

Reference ranges for CA 72-4 have so far been determined for a mixed cohort of men and women, which is reasonable for gastric cancer. In earlier results from our laboratory, where ~200 male and female blood donors were measured, a cutoff was set at 10 kU/L, which is also widely used [1, 7]. In the present study, however, in which only female subjects were included as reference, higher values were obtained. Therefore, a slightly higher cutoff at 6 kU/L should be emphasized when CA 72-4 is used for female patients, especially since clinical sensitivity is only marginally affected. Moreover, moderate increases up to 10 or 20 kU/L seem to occur in nonmalignant disorders and during pregnancy. This should be taken into consideration when using CA 72-4 as a tumor marker in gynecology. An increased cutoff is also proposed in the literature [8-11]. The cancer patients included in this trial showed in most cases median values well above 6 kU/L, especially in ovarian and endometrial cancer, indicating a good discrimination between normal and malignant cases.

The results in cancer patients obtained in this study show that CA 72-4 is a useful marker in the follow-up of ovarian cancer and other gynecological neoplasms such as carcinoma of endometrium, cervix, collum, and fallopian tubes. Thus the clinical findings of other studies could be confirmed [4, 10, 12-15]. Outcome studies are needed to define the clinical value of immunoluminometric determination of CA 72-4 as a supplement to the established tumor markers in gynecological malignancies.

References

1. Klug TL, Sattler MA, Colcher D, Schjom J. Monoclonal antibody immunoradiometric assay for an antigenic determinant (CA 72) on a novel pancarcino-


Cardiac Troponin T in Patients with High Creatinine Concentration but Normal Creatine Kinase Activity in Serum, Hannsjorg Baum,* Margit Obst, Ursula Huber, and Dieter Neumeier (Inst. für Klin. Chem. und Pathobiochem., Klinikum rechts der Isar, TU München, Ismaningerstr. 22, D-81675 München, Germany; *author for correspondence: fax +49 89 4140 4875, e-mail tbb01ac@sunmail.lrz-muenchen.de)

Troponin T is part of the regulatory system of the contractile complex of skeletal and heart muscle. It is expressed in two different isoforms, namely, skeletal muscle troponin T and cardiac troponin T. After loss of the integrity of the cell membrane, it is released into the circulation similarly to myoglobin or creatine kinase [1]. Because cardiac troponin T is ordinarily undetectable in healthy individuals [2], its measurement is a powerful tool in the diagnosis of acute myocardial infarction. Moreover, about one-third of patients with unstable angina pectoris show increased cardiac troponin T in serum [3]. These patients may be a subgroup with higher risk for complications, i.e., acute myocardial infarction [4].

In patients with chronic renal failure, creatine kinase, the MB isoenzyme of creatine kinase, or myoglobin is often increased without evidence of ischemic myocardial injury [5]. Using the first-generation unspecific troponin T assay, which had 1–2% cross-reactivity with skeletal muscle [6], Hafer et al. [7] and Bhayana et al. [8] showed increased “cardiac” troponin T in patients with chronic renal failure.

We determined “cardiac” troponin T with this first-generation assay in patients with high creatinine concentration but normal creatine kinase activity in serum to look for the clinical relevance of falsely increased “cardiac” troponin T in routine patients (i.e., other than those selected for possible cardiac complications).

For this purpose, over a period of 4 days, we selected from those samples routinely submitted to our laboratory for conventional analyte testing 174 samples from 92 patients with high creatinine concentrations but normal creatine kinase activity in serum and no clinical signs of ischemic myocardial injury. The serum samples for “cardiac” troponin T measurement were stored at −20 °C until analysis.

The patients could be divided into 10 diagnostic groups: chronic renal failure (49 serum samples from 29 patients), compensated renal failure (7 serum samples from 3 patients), acute renal failure (20 serum samples from 6 patients), infectious disease (25 serum samples from 7 patients), kidney transplantation (15 serum samples from 10 patients), urinary tract obstruction (7 serum samples from 4 patients), cancer (19 serum samples from 10 patients), liver transplantation (9 serum samples from 2 patients), heart diseases (12 serum samples from 10 patients), and other diseases (12 serum samples from 12 patients). The serum samples from the patients with heart disease were excluded from further analysis. Furthermore, in all patients with increased concentrations of “cardiac” troponin T, possible ischemic heart disease was retrospectively ruled out by using anamnestic and clinical data.

Creatinine concentration and creatine kinase activity in serum were measured by dry-film technology on a fully automated analyzer (Ektachem 700C XR; Johnson & Johnson, Rochester, NY) at 25 °C. The upper reference limits in this laboratory for creatinine in serum are 114.92 μmol/L for men and 97.24 μmol/L for women; for creatine kinase activity in serum, 80 U/L for men and 70 U/L for women.

“Cardiac” troponin T was measured with the first-generation troponin T assay, a single-step sandwich assay on an ESR300 automated analyzer (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s recommendations. The upper reference limit for “cardiac” troponin T in this assay is 0.1 μg/L.

We found that, of 162 serum samples from patients without clinical signs of ischemic myocardial injury or other heart diseases and normal values for creatine kinase activity, 64 serum samples (40%) showed “cardiac” troponin T values >0.2 μg/L. The range for these samples was 0.21–5.37 μg/L, with a median value of 0.88 μg/L.

The samples of the individual subgroups exhibited great differences. In patients with cancer, compensated renal failure, urinary tract obstruction, kidney transplantation, or “other” diseases, predominantly no increase of “cardiac” troponin T was measurable—although some single serum samples in these subgroups gave falsely positive results, mostly slightly increased values. These included 16% of the cancer samples (range 0–0.94, median 0.02 μg/L), 7% of the kidney transplantation samples (range 0–1.15, median 0.02 μg/L), and 25% of the “other” diseases samples (range 0–1.6, median 0.03 μg/L). On the other hand, none of the samples from patients with compensated renal failure (range 0–0.02, median 0 μg/L) or urinary tract obstruction (all values 0 μg/L) were falsely positive. The serum sample with the most-increased “cardiac” troponin T, 1.6 μg/L, was from a patient with an infected knee prosthesis.

In the other subgroups, either the median for “cardiac” troponin T was distinctly higher than the upper reference limit or values were often detectable, which is an “indicator” of possible acute ischemic myocardial injury. In the 49 serum samples from the patients with chronic renal failure, 40% showed increased “cardiac” troponin T values (range 0–4.95, median 0.03 μg/L). In serum samples from patients with acute renal failure, 75% showed increased values (range 0–3.06, median 0.37 μg/L); in the samples from patients with septic infections, 67% showed increased values (range 0.04–1.68, median 0.5 μg/L); and in samples from patients after liver transplantation, 78% showed increased values (range 0.5–5.37, median 2.39 μg/L).

In Fig. 1, serum creatinine is plotted vs “cardiac” troponin T for each serum sample. Note the lack of correlation between serum creatinine and “cardiac” troponin T, both in patients with renal or postrenal diseases and in the other groups.

In concordance with previous findings [7,8] of increased “cardiac” troponin T values in patients with chronic renal failure and increased creatine kinase activity but without evidence of ischemic myocardial injury, we demonstrate increased “cardiac” troponin T also in patients without increased creatine kinase activity. These increased values were predominantly found in patients with chronic or acute renal failure, septic infection, or liver transplantation.