

Development of time-resolved immunofluorometric assays for CA 72-4 and application in sera of patients with gastric tumors

Shi Le Sheng¹, Qing Wang¹, Gang Huang*

Department of Nuclear Medicine, Renji Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 200001, China
Institute of Clinical Nuclear Medicine, Shanghai JiaoTong University School of Medicine, Shanghai 200001, China

Received 21 September 2006; received in revised form 12 January 2007; accepted 12 January 2007

Available online 30 January 2007

Abstract

Background: Immunoradioassay (IRMA) with ¹²⁵I-labeled monoclonal antibodies is widely used to measure levels of cancer antigen (CA) 72-4. We investigated a time-resolved immunofluorometric assay (TR-IFMA) to determine its sensitivity and specificity compared with those of IRMA. **Methods:** We used 2 CA 72-4-specific monoclonal antibodies with different specificities. One was immobilized on a solid phase, and one was labeled with Eu³⁺. Serum from 44 adults with newly diagnosed gastric cancer and 208 control subjects was assayed by TR-IFMA and a conventional IRMA.

Results: The detection limit of TR-IFMA was 0.55 U/ml. The calibration curve was linear to 1000 U/ml, with no hook effect up to 1000 U/ml. Intraassay CVs were 4.2–10.26% over the calibration range. Compared with IRMA, TR-IFMA had a broader analytical range and a shorter analysis time. At a cutoff of 99% specificity, TR-IFMA and IRMA results were positive in 37 and 26 patients, respectively.

Conclusions: TR-IFMA is a rapid and sensitive nonradioactive method applicable to large-scale screening for CA 72-4. In addition to its broad, linear analytical range, TR-IFMA was more sensitive and specific than conventional IRMA. It was easy to perform and automate.

© 2007 Elsevier B.V. All rights reserved.

Keywords: CA 72-4; Gastric tumors; Time-resolved immunofluorometric assay; Immunoradioassay

1. Introduction

Over the past 5 decades or longer, the mortality rate associated with gastric cancer has markedly decreased in most areas of the world [1–6]. However, the likelihood that an individual patient will survive 5 y after receiving a diagnosis of gastric cancer is still low [7,8]. Gastric cancer remains the second leading cause of cancer-related deaths worldwide [2,3,9]. Among recently proposed tumor markers, tumor-associated glycoprotein (TAG)-72, also called cancer antigen (CA) 72-4, is of great interest. TAG-72 is a mucin with high molecular weight (220–400 kDa). It is identified by using monoclonal antibody B72-3. TAG-72 has been widely used to

diagnose cancer and to monitor immunotherapy. Its reported specificity (92%) and positive predictive value (86%) are high [10–12]. Preoperative levels of this tumoral marker may aid in predicting the invasiveness of gastric cancer and in providing prognostic information for patients [13–15].

After europium chelates were developed as labels in the 1980s [16,17], the time-resolved immunofluorometric assay (TR-IFMA) has been reported as an ideal immunoassay technique. Sensitivity is higher with this technique than with methods based on tritiated tracers or enzyme-conjugated tracers. In addition, TR-IFMA offers an alternative challenging immunoassays based on ¹²⁵I labels with its advantage of a stable, nonradioactive tracer [18]. Fluorescence of the Eu³⁺ tracer is long lived and allows for the differentiation of the short-lived background fluorescences of biological material, plastics, and optics [19]. The highly specific activity of the label increases the sensitivity of immunoassays while minimizing nonspecific binding of the labeled bioaffinity molecule [20]. Other favorable features of Eu³⁺ complexes have been reviewed [21–23].

* Corresponding author. Department of Nuclear Medicine, Renji Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 200001, China. Fax: +86 21 63111384.

E-mail address: shengsl2002@163.com (G. Huang).

¹ These authors contributed equally to this work.

2. Materials and methods

2.1. Instrumentation

We used chromatographic separation system (EP-1 Econo Pump and EM-1 Econo ultraviolet [UV] monitor; Bio-Rad Co., Hercules, CA). Time-resolved fluorescence measurements of liquids were performed with a fluorometer (Arcus 1235; Wallace, Turku, Finland). Radioactivity was counted with a gamma counter (1260 Multigamma II; Labsystems Oy, Helsinki, Finland). Manual pipetting was done with disposable plastic tips and pipettes (Finnpipette; Labsystems Oy).

2.2. Chemicals

Anti-CA 72-4 monoclonal antibodies McAb-C003 and McAb-C004 were from Wolobiotec (Shanghai, China). These antibodies were generated by immunizing a mouse with TAG-72 previously purified by means of affinity chromatography. BALB/c mice were immunized with specific TAG-72 (whole human protein of TAG-72 obtained from human ascites fluids) by means of multisite hypodermic injections. The splenic cells of the mice were fused with SP2/0 mouse myeloma cells. Immunohistochemical analysis demonstrated that the antibody could bind with TAG-72 specifically. McAb-C003 recognized an epitope different from the one McAb-C004 recognized. These antibodies demonstrated no cross-reactions. The McAb-C003 isotype was IgG1, and the McAb-C004 isotype was IgG1-K. The antibodies were strictly produced by using an ISO9001 quality-certified system.

Other materials were microtitration strips (Nunc, Roskilde, Denmark), N1-[*p*-isothiocyanatobenzyl]-diethylene-triamine-N1,N2,N3,N4-tetraacetate chelated with Eu^{3+} (DTTA- Eu^{3+} ; Tianjin Radio-Medical Institute, Tianjin, China), and bovine serum albumin (Sigma-Aldrich Co., St. Louis, MO). An IRMA CA 72-4 kit (ELSA; CIS Bio International, Gif sur Yvette France) was used as comparative method. Other chemicals used were of analytical grade.

2.3. Buffers

The coating buffer was 100 mmol/l sodium carbonate buffer (pH 9.3) containing 0.9% NaCl and 0.04% NaN_3 . The washing buffer was 10 mmol/l Tris-HCl (pH 8.0) containing 0.04% Tween 20 and 0.9% NaCl. The blocking buffer was 50 mmol/l Tris-HCl (pH 8.0) that contained 0.9% NaCl, 0.04% NaN_3 and 1% bovine serum albumin. The assay buffer was 100 mmol/l Tris-HCl (pH 8.4) containing 0.1% bovine serum albumin, 0.04% NaN_3 , 0.9% NaCl, 0.08% Tween 20, and 0.5% bovine globulin. The buffer for eluting the Eu^{3+} -labeled reagents was 50 mmol/l Tris-HCl (pH 7.8) that contained 0.9% NaCl and 0.05% NaN_3 . The enhancement solution was used as supplied (Wallace). A volume of 1 l contained 1 g of Triton X-100, 6.8 mmol of potassium hydrogen phthalate, 100 mmol of acetic acid, 50 μmol of tri-*n*-octylphosphine oxide, and 15 μmol of 2-naphthoylethylfluoroacetone.

2.4. CA 72-4 standards

CA 72-4 antigen was a product of Fitzgerald Industries International, Inc. (Concord, MA). Purified CA 72-4 was obtained from human ascites fluids and calibrated by radioimmunoassay (Centocor B.V., Leiden, the Netherlands). The preparation was reconstituted according to the manufacturer's recommendations and diluted in 10 mmol/l phosphate-buffered saline (pH 7.4) containing 2.0% bovine serum albumin and 0.04% NaN_3 . This step resulted in the desired standard concentrations of 3, 20, 50, 100, and 1000 U/ml.

2.5. Patients and specimens

We examined 44 adults with newly diagnosed and previously untreated gastric cancer (17 women and 27 men aged 38–78 y) and 40 adults (18 women and 22 men aged 21–88 y) with benign stomach disease (stomach ulcers in 18 patients and gastritis in 22). The patients had been admitted to the Departments of Gastroenterology and General Surgery, Renji Hospital, Shanghai JiaoTong

University School of Medicine. All clinical diagnoses of gastric cancer were confirmed with microscopic examination of material obtained during gastroscopy and/or surgery. All tumors were histologically verified to be adenocarcinomas. The tumors were classified in accordance with the stages of the Fifth International Union Against Cancer [24].

We also enrolled 168 healthy subjects (78 women and 90 men, aged 18–60 y) without a history of neoplastic or recent inflammatory disease as control subjects. Blood samples were collected before the gastric cancers and ulcers were treated. None of the patients had received chemotherapy or radiation therapy before this collection. Venous blood samples were collected into sterile tubes and centrifuged to obtain serum samples, which were stored at -20°C until they were assayed. All the samples were concurrently tested with TR-IFMA and IRMA.

2.6. Purification and labeling of McAb-C004

Before McAb-C004 was labeled with Eu^{3+} , columns prepacked with gel-filtration medium (Sephadex G-25M) were used for desalting and buffer exchange. We dissolved 1 mg of purified McAb-C004 in 50 mmol/l sodium carbonate buffer (pH 9.8) containing 0.9% NaCl. This solution was added to a glass vial containing 0.8 mg of DTTA- Eu^{3+} and then incubated overnight at 4°C .

Labeled McAb-C004 was separated from excess free label by gel filtration by using $1.5 \times 50\text{-cm}$ beaded column (Sephadex CL-6B) and the elution buffer. The elution was monitored at 280 nm with the UV monitor, and 8.8 ml of labeled McAb-C004 was collected. The concentration of McAb-C004 in the purified conjugate solution was determined with the Beer–Lambert method in which the UV absorbed by the DTTA- Eu^{3+} was subtracted. McAb-C004 at 1 mg/ml gave an absorbance of 19 at 280 nm. The concentration of Eu^{3+} was measured against Eu^{3+} calibrators in the fluorometer after fluorescence enhancement. The mean number of Eu^{3+} ions coupled to one McAb-C004 molecule was 8.9, as calculated from the molar concentrations of Eu^{3+} and McAb-C004. Labeled McAb-C004 could be stored at 37°C for at least 4 weeks without any loss of immunoreactivity.

2.7. Coating of polystyrene microtiter strips

Concentrated McAb-C003 (1 mg/ml) was diluted to 5 $\mu\text{g}/\text{ml}$ in coating buffer. We added 200 μl of the diluted antibodies per well and incubated them overnight at 4°C . The strips were aspirated and washed twice with washing buffer. Then, 200 μl of blocking buffer was dispensed into each well, and the strips were incubated for 4 h at room temperature. After we removed the solution from the wells, the strips were stored at $4\text{--}8^\circ\text{C}$ in a dry sealed bag. The coated strips were stable. No loss of immunoreactivity was observed during storage for 4 weeks at 37°C .

2.8. TR-IFMA

One-step assay procedures were used. In brief, 50 μl of the standards or samples was incubated at room temperature for 2 h in antibody-coated wells with 200 μl of assay buffer containing diluted Eu^{3+} -labeled McAb-C004. The assay wells were subsequently aspirated and washed 6 times with the washing buffer. The bound Eu^{3+} -label was then dissociated from the surface with 200 μl of enhancement solution. The resulting fluorescent chelate solution was subjected to single photon counting with the time-resolved fluorometer.

2.9. IRMA

The assay was performed according to the instructions enclosed in the kits. In brief, 200 μl of buffer was dispensed into all tubes. We added 100 μl of the standards, controls, or samples to the corresponding groups of tubes and then gently mixed them with a vortex-type mixer. After a 4-h incubation at room temperature with continuous shaking at 400 rpm, the tubes were washed twice. We added 300 μl of anti-CA 72-4 ^{125}I -labeled monoclonal antibody to all tubes and gently mixed them again with a vortex-type mixer. The tubes were incubated overnight at $2\text{--}6^\circ\text{C}$ and washed as previously described. The

Download English Version:

<https://daneshyari.com/en/article/1967459>

Download Persian Version:

<https://daneshyari.com/article/1967459>

[Daneshyari.com](https://daneshyari.com)