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Original article

Detection of M2 antimitochondrial antibodies by dot blot assay is more specific than by enzyme linked immunosorbent assay

La technique dot blot est plus spécifique que la technique Elisa pour la détection des anticorps antimitochondries

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Abstract

Aims. – The objective of our study was, in one hand, to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ELISA and dot blot assay to investigate IgG M2 antimitochondrial antibodies (M2 AMA) and, on the other hand, to compare these results with those of indirect immunofluorescence technique (IIF).

Methods. – Sera from patients suffering from primary biliary cirrhosis (PBC) ($n = 55$), systemic lupus erythematosus ($n = 21$), celiac disease ($n = 30$) and blood donors ($n = 75$) were analyzed. M2 AMA were detected by ELISA and dot blot using pyruvate dehydrogenase purified from porcine heart and by IIF on cryostat sections of rat liver-kidney-stomach.

Results. – IIF was more sensitive (98%) than ELISA (93%) and dot blot (91%). The specificity of AMA for PBC using IIF, ELISA and dot blot reached 100%, 92% and 100%, respectively. The PPV of IIF, ELISA and dot blot was 100%, 93% and 100%, respectively. The NPV was 98% for IIF, 92% for ELISA and 91% for dot blot.

Conclusion. – Dot blot, using purified pyruvate dehydrogenase, had a higher specificity than ELISA and may be useful in confirming the specificity of AMA in cases of doubt with IIF.

Résumé

L'objectif. – De notre travail consiste d'une part à déterminer la sensibilité, la spécificité et les valeurs prédictives positive (VPP) et négative (VPN) de la technique Elisa et de la technique dot blot pour la recherche des anticorps antimitochondries de type M2 et d'autre part, de comparer ces résultats à ceux de la technique d'immunofluorescence indirecte (IFI).

Matériel et méthodes. – Notre étude a porté sur 55 sérums de patients atteints de cirrhose biliaire primitive (CBP), 21 sérums de patients atteints de lupus érythémateux systémique, 30 sérums de patients atteints de maladie coeliaque et 75 sérums prélevés chez des donneurs de sang. La recherche des anticorps antimitochondries de type M2 a été effectuée par les techniques Elisa et dot blot utilisant la pyruvate déshydrogénase extraite a partir du cœur de porc et par IFI sur coupes de tissus de rein/foie/estomac de rat.

Résultats. – La sensibilité de la technique IFI (98 %) est supérieure à celle de la technique Elisa (93 %) et à celle de la technique dot blot (91 %). La spécificité des techniques IFI, Elisa et dot blot est de 100, 92 et 100 % respectivement. La VPP des techniques IFI, Elisa et dot blot est 100, 93 et 100 % respectivement. La VPN est de 98 % pour la technique IFI, 92 % pour L'Elisa et 91 % pour la technique dot blot.

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Conclusion. – La technique dot blot utilisant la pyruvate déshydrogénase purifiée possède une spécificité meilleure que la technique Elisa et pourrait être utile pour confirmer la spécificité de l'IFI dans les cas douteux.

Keywords: Antimitochondrial antibody; ELISA; Dot blot; Indirect immunofluorescence; Primary biliary cirrhosis

Mots clés : Anticorps anti-mitochondries ; Elisa ; Dot blot ; Immunofluorescence indirecte ; Cirrhose biliaire primitive

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver that primary affects women and is associated with a high titre of M2 antimitochondrial antibody (M2 AMA) [1]. PBC is marked by progressive inflammatory destruction and obliteration of intrahepatic bile ducts, followed by development of cirrhosis and liver failure. AMA are detected in the serum of the vast majority of patients, may be detected several years before the manifestation of any clinical, biochemical cholestasis or histological signs of the disease, yet are not associated with its severity or rate of progression [2].

The major mitochondrial autoantigens were identified as components of the 2 oxo-acid dehydrogenase complex (2-OADC) such as the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2), the E2 subunit of branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2), the E2 subunit of oxoglutarate dehydrogenase complex (OGDC-E2), the E1α subunit and the E3 binding protein of PDC (PDC-E1α and E3BP, respectively) [3].

Sequestration of antigen is one mechanism by which peripheral tolerance can be maintained, and it would appear logical that mitochondrial antigens might not be easily seen by the immune system. However, several studies have suggested that in patients with PBC, these specific mitochondrial autoantigens may be present on the surface of the hepatocytes or biliary epithelium [4,5].

In the clinical environment, the routine method for serum M2 AMA detection is indirect immunofluorescence (IIF), on frozen sections of rodent liver-kidney-stomach, that produces a typical pattern. More recently, recombinant mitochondrial antigens (in the case of pML-MIT3, the three main autoepitopes are conjugated in one molecule) have been available. The use of these antigens, either by immunoblotting or ELISA, has significantly increased the sensitivity and specificity of the test [6–8].

The objective of our study was, in one hand, to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of both ELISA and dot blot assay to investigate IgG M2 AMA using pyruvate dehydrogenase purified from porcine heart and, on the other hand, to compare these results with those of IIF.

2. Patients and methods

2.1. Study population

Serum samples from 55 patients (54 women, one man) with biopsy proven PBC were studied. The mean age of these patients was 58.9 ± 13.4 years. Serum samples were stored at -80 °C until use. The study was approved by Local Ethics Committee and all patients gave their informed consent.

2.2. Control population

As a control population, we studied 51 patients with other auto-immune diseases (21 patients with systemic lupus erythematosus (SLE) and 30 patients with untreated celiac disease (CD)). Seventy-five sera from blood donors served as healthy controls.

2.3. M2 AMA by indirect immunofluorescence

Cryostat sections of rat liver-kidney-stomach were incubated with sera diluted 1:100 in phosphate buffered saline (PBS) for 30 min in a humidified chamber. After three washing in PBS, the sections were incubated for 30 min with fluorescein-isothiocyanate- conjugated anti-human IgG antibodies (Bio-Rad®, Marnes–La Coquette, France) used as secondary antibody diluted 1:10. The immunofluorescence patterns were assessed under fluorescence microscope. The typical ''granular'' positivity within the cytoplasm of cells in kidney, stomach and hepatocytes is considered as M2 AMA reactivity.

2.4. M2 AMA by ELISA

Wells were coated either with pyruvate dehydrogenase purified from porcine heart (cat. no. P-7032; Sigma®) at 30 μg/ml in PBS, pH 7.2 or coated with PBS containing 1% skimmed milk. Microplates were incubated overnight at 4 °C. After three washing with PBS containing 0.1% Tween 20, sera were diluted at 1:1000 in PBS Tween 0.1% containing 1% skimmed milk and incubated for 30 min at room temperature. After three washing with PBS Tween 0.1%, peroxydase conjugated anti-human IgG antibodies (Bio-Rad®, Marnes–La Coquette, France) diluted at 1:400 in PBS Tween 0.1%, were incubated for 30 min at room temperature. The reaction was revealed using ortho-phenylenediamine as substrate. The colorimetric reaction was measured at 492 nm by spectrophotometry (optical density of sera = optical density in wells coated with pyruvate dehydrogenase – optical density in wells coated with skimmed milk). The serum was considered positive for a value strictly greater than 0.2. This value corresponds to the mean absorbance plus five standard deviation obtained with 75 sera from healthy blood donors.