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CASE REPORT

A case of acute febrile illness with multiple IgM positives: risk of dependence on IgM-based serological tests

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Abstract

A rapid onset of fever and symptoms such as headache, chills or muscle and joint pains is common in the tropics and sub-tropics and can be caused by very diverse pathogens. Differential diagnosis of these etiologies based on clinical criteria alone is not possible as clinical signs and symptoms of most of these infections are very similar and the correct diagnosis is only possible by using pathogen specific diagnostic tests. For patient treatment and management, differential diagnosis of causative agent is required. Commercial IgM-ELISA kits are used for the diagnosis of many acute infections. This study report describes a case of acute febrile illness with IgM positivity for Brucella species, Rickettsia species, Leptospira species and scrub typhus. Real time PCR for the above pathogens and Epstein Barr virus (EBV) were negative. From our search of the literature, this report documents, for the first time, an EBV and HIV negative individual with multiple IgM-positivity. The study reports a rare case of multiple -IgM positivity in a febrile illness patient. We therefore infer that it is important to interpret IgM results with caution and establish provisional diagnosis which is in consonance with clinical findings.

Keywords: ELISA, IgM detection, acute febrile illness

Introduction

IgM-based serodiagnostic tests have found a place in rapid and early diagnosis of infectious diseases. Several assay modes are available for IgM detection including Enzyme Immunoassays (EIAs) and lateral flow technologies. Many assays for diagnosis of infectious diseases are available commercially for which the manufacturers claim good sensitivity and specificity; however, the performance in a clinical setting may vary [1]. These tests are widely used in health care settings for ease of use and interpretation. Multiple reports exist on the evaluation of IgM based serological tests for different pathogens.

The development of nucleic acid detection and genome sequencing technology has considerably improved early and specific diagnosis of infectious diseases. Many different molecular assays have been developed for the detection of infectious pathogens with higher sensitivity and specificity as compared to traditional methods [2].

IgM detection is useful usually between 7 and 21 days post-clinical manifestation [3,4]. The most widely used serodiagnosticassays are lateral flow immunoassays and enzyme-linked immunosorbent assays (ELISA) [5,6]. This article focuses on the uses and limitations in using IgM detection kits with reference to false positive IgM results in a case of acute febrile illness.

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Case Report

A 61 year old male presented with acute febrile illness. He had pre-existing type 2 diabetes mellitus and systemic hypertension and was evaluated at a multi-specialty hospital in Vellore in June 2018. His chief complaints included chest discomfort at the right infrascapular and inframammary regions of two days duration. He gave a history of fever, cough and headache for one week. The patient also had been treated for extra pulmonary tuberculosis several years ago.

The markers of renal function except estimated-GFR were normal. The eGFR value was 99ml/min/1.73m^2. The patient had plasma protein elevation (5.6 gm/dL); other liver function markers were within normal limits. He had high glycemiclevels with elevated HbA1C (9.4%), glucose fasting (174 mg/dL) and post prandial glucose (219 mg/dL). Acute phase reactant values [ESR (37 mm/hr), CRP (166mg/dL),procalcitonin (0.96 ng/ml)] were elevated. The cell counts were as follows: total leucocyte count was 17270 cells/mm^3, polymorphs 82.4%, lymphocytes 11.5% and

eosinophils 1.4%. The biochemistry markers were tested using AU480 Beckman Coulter (Atlanta, Georgia, USA and hematology markers were tested using XN-1000 Sysmex (Kobe, Japan).

A CT scan and Ultrasound Sonography Test of the chest and the abdomen respectively showed contracted left lobe of the liver with right lobe showing surface nodularity and liver parenchymal disease with a final impression of active infection. The pleural fluid for cytology indicated inflammatory cells and were negative for malignancy in smears and cell block section.

The blood-borne virus serology by ELISA showed that the sample was negative for antibodies to HIV and HCV, and HBsAg. Routine microscopic pleural fluid analyses for physical characteristics, cell count and protein and albumin levels were all within normal limits and negative for acid fast bacilli by smear microscopy. Adenosine deaminase (63U/L) and lactate dehydrogenase (2829 U/L) levels were elevated in the pleural fluid. The sample was negative for *Mycobacterium tuberculosis* by GenXpert Dx (Sunnyvale, CA, USA) system. Culture reports from bronchial and pleural fluid were negative. The sputum smear revealed no acid fast bacilli but showed many pus cells and few epithelial cells in the smears while showed moderate growth of *Staphylococcus aureus*. Blood culture was negative.

The provisional diagnosis was extra pulmonary tuberculosis. The patient was treated conservatively with adequate IV and oral antibiotic (augmentin), nebulization, antiemetics, antihypertensive, insulin and other supportive measures. The patient was discharged in stable condition with advised medication for anti-tuberculosis treatment.

As part of an on-going research project, additional tests were carried out later on the archived sample. The blood sample was tested for seven bacterial pathogens *viz. Salmonella Typhi*, *Salmonella Paratyphi* A, *Burkholderia pseudomallei*, *Orientia tsutsugamushi*, *Leptospira* species, *Rickettsia* species and *Brucella* species using an in-house multiplex realtime PCR assay. Appropriate negative and positive controls were included in every run. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal amplification control. The results were negative for all the seven pathogens.

Commercial IgM ELISA testing was performed for *O. tsutsugamushi* (InBios International Inc, Seattle, Washington, USA), *Leptospira* species (Panbio diagnostics, Gyeonggi-do, Republic of Korea), *Brucella* species (Novatec Immundiagnostica, GmbH, Germany), typhus group of rickettsia and spotted fever group of rickettsia (Fuller laboratories, Fullerton, California, USA). IgM ELISA assay kits for Brucella, Rickettsia SFG and Rickettsia TG anti-IgG (RF-adsorbent) in the sample dilution buffer for the specific detection of IgM-class antibody. IgM ELISA kits for *Leptospira* species and *O. tsutsugamushi* did not contain RF-adsorbent. To rule out malaria and dengue fever, rapid immunochromatography tests (J Mitra & Co Pvt Ltd, New Delhi, India) were carried out on the samples. The assays were carried out according to manufacturers' instructions and all the kits are CE certified.

Results

The patient was found to be IgM positive for Leptospira, typhus group of rickettsia, spotted fever group of rickettsia, Brucella and scrub typhus. The immunochromatographic test was positive for malarial antigen and negative for Dengue NS1, IgM and IgG. The plasma sample was negative for anti-nuclear antibody by ELISA. The absorbance values of ELISA positives and sensitivities and specificities of ELISA tests and rapid card tests are shown in table 1. The patient sample was positive for rickettsia SFG with high titre and for other pathogens with slightly high titre.

DNA extracted from the buffy coat was tested for EBV DNA by an in-house PCR and while the plasma sample was tested for EBV IgM by ELISA (Epstein-Barr virus capsid antigen IgM with anti-IgG as RF-adsorbent, EuroImmun, Luebeck, Germany) and both were negative. These two assays were carried out in the Department of Virology, tertiary care teaching hospital in Vellore.

Discussion

The patient with acute febrile illness was IgM positive by ELISA for several pathogens (*Leptospira* species, *Rickettsia* species (typhus group and spotted fever group), *Brucella* species and scrub typhus). ELISA for rickettsia uses native Outer Membrane Proteins and recombinant outer membrane proteins A and B heteroduplex from Rickettsia rickettsii with no traces of lipopolysaccharides (LPS) to decrease cross-reactivity with other endobacterial LPS. The assays used in the study had good sensitivity and specificity as established by the manufacturers. It is known that EBV infection could be associated with pleural effusion [7]. Multiple IgM elevations have been shown to occur in certain viral infections possibly due to polyspecific IgM antibodies [8,9]. Our patient did not show evidence of EBV infection.

The patient had the history of three days of fever and the sample was collected during the acute phase of febrile illness. IgM ELISA for rickettsia and scrub typhus have been shown to last for 2 weeks [10]; though IgM persistence for two to six months such as that for Dengue fever and sometimes for several years in Leptospiral infection has been reported.

We present for the first time an EBV and HIV negative individual showing multiple IgM-positivity. It is our inference that the finding of multiple IgM response could

be biological or technical issues leading to false positives. It is hence important to interpret IgM results with caution and establish provisional diagnosis which is in consonance with clinical findings. Additionally, these finding need to be correlated with PCR findings.

Conclusions

The study reports an EBV and HIV negative individual showing IgM-positivity for multiple infectious agents. The study reports on only one patient and lacks statistical sampling, but signifies the need for attention while utilizing IgM based detection for infectious diagnostics. While there could be multiple interferences in immunological assays leading to false positives, it is imperative to interpret results with caution when used in isolation and correlate with molecular findings.

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