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MINIREVIEW

Immunoglobulin M for Acute Infection: True or False?

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Immunoglobulin M (IgM) tests have clear clinical utility but also suffer disproportionately from false-positive results, which in turn can lead to misdiagnoses, inappropriate therapy, and premature closure of a diagnostic workup. Despite numerous reports in the literature, many clinicians and laboratorians remain unaware of this issue. In this brief review, a series of virology case examples is presented. However, a false-positive IgM can occur with any pathogen. Thus, when an accurate diagnosis is essential for therapy, prognosis, infection control, or public health, when the patient is sick enough to be hospitalized, or when the clinical or epidemiologic findings do not fit, IgM detection should not be accepted as a stand-alone test. Rather, whenever possible, the diagnosis should be confirmed by other means, including testing of serial samples and the application of additional test methods.

The diagnosis of an acute infectious disease most commonly involves the detection of the pathogen by culture, immunoassay, or molecular methods. However, these tests may be costly, may require collection of swab samples or body fluids, may be sent out to reference laboratories, or may have a long time to a result or may not be available at all. For many infections, a more convenient and less expensive alternative is the detection of IgM antibody (Table 1).

When a patient presents early in illness, IgM antibodies may not yet be detectable in peripheral blood. But for immune-mediated diseases in particular, IgM serology can be the test of choice since the patient presents when IgM levels are rising and virus titers have declined. Examples include Epstein-Barr virus (EBV)-associated infectious mononucleosis, parvovirus B19-associated Fifth disease, and acute hepatitis B. The rashes of measles and rubella are also immune mediated, and PCR and culture tests are not readily available; thus, IgM detection remains a mainstay of diagnosis. In infections by pathogens such as cytomegalovirus (CMV), with a long incubation period before symptoms develop, IgM antibodies are usually detectable at presentation. For arbovirus central nervous system (CNS) infection, cerebrospinal fluid (CSF) IgM has a higher yield than CSF PCR and remains the preferred test. Thus, IgM tests have proven useful and are commonly performed (1).

However, despite having clear clinical utility, IgM tests also suffer disproportionately from false-positive results, which can lead to misdiagnoses, inappropriate therapy, and premature closure of a diagnostic workup. In our Clinical Virology Laboratory, the vagaries of IgM tests are readily apparent, but our vantage point may be unique. In addition to IgM and IgG serology, our laboratory performs culture, antigen detection, and nucleic acid amplification tests (NAAT). Consequently, we have the opportunity to correlate multiple test methods, and we do this routinely in order to monitor and better understand the performance of various tests. Furthermore, since our laboratory is located within a large medical center, daily communication with clinicians and access to patient medical records is standard practice. In contrast, in many facilities, serology may be done in Immunology, culture in Microbiology, and NAAT in a Molecular Diagnostics Laboratory, or some or all of these tests may be sent out to a reference laboratory.

TABLE 1 Diagnosis of acute viral infections[a]

IgM use	Infection(s)
IgM commonly used for diagnosis	Arbovirus neurologic disease (e.g., WNV, EEE virus, and SLE virus infections)
	Arbovirus rash illness (e.g., dengue virus, CHIK virus, and Zika virus infections)
	CMV and EBV infectious mononucleosis
	Hantavirus pulmonary syndrome
	Acute hepatitis A, B, and E virus infections
	Acute HIV-1 and HIV-2 infections (3rd- and 4th-generation tests)
	Acute measles, rubella, mumps
	Parvovirus B19 Fifth disease
IgM use should be discouraged	HHV-6[b]
	HSV and VZV[b]
	Enterovirus infections[c]

[a] WNV, West Nile virus; EEE virus, Eastern equine encephalitis virus; SLE virus, St. Louis encephalitis virus; CHIK virus, Chikungunya virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HHV-6, human herpesvirus type 6; HSV, herpes simplex virus; VZV, varicella-zoster virus.
[b] Detect virus for diagnosis of active infection. For documentation of primary infection, determine seroconversion of IgG.
[c] Detect virus for diagnosis of active infection. Serology not useful.

Having witnessed numerous instances of misleading IgM test results, the impact on clinical care, and the lack of awareness of many clinicians and laboratorians that IgM test results can be falsely positive, we thought it useful to focus attention on this issue through a brief case series and review.

In the case descriptions and comments presented below and summarized in Table 2, a few examples from our laboratory are presented. Although the cases focus on virology due to the nature

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TABLE 2 Summary of selected case examples of false-positive IgM results[a]

Case no.	IgM falsely positive for:	True etiology	Clinical presentation	Diagnosis confirmed by:	Clue(s) to erroneous IgM results	Potential impact
1	Hantavirus IgM and Sin Nombre virus IgM	Adenovirus	26 year old with pneumonia, rapid onset of ARDS, and renal failure	Positive adenovirus PCR of NP swab and blood, confirmed at CDC; negative hantavirus IgM and PCR at CDC	Hantavirus IgG negative; two concurrent rare diseases unlikely	Public health and treatment options
2	Mycoplasma IgM	WNV	45 year old with meningoencephalitis in August	Positive WNV CSF PCR; conversion of WNV IgM and IgM in CSF and serum	No respiratory symptoms; mycoplasma IgG strongly positive and unchanged in 3 weeks	Unnecessary tests, consultations, and treatment
3	EBV VCA IgM	CMV	39 year old with headache, fever, myalgias, and elevated liver enzymes	High positive CMV IgM and low positive CMV IgG; positive CMV PCR	Strongly positive EBNA antibody; low positive VCA IgM	Unnecessary hospitalization, tests, and consultations
4	HAV IgM	CHF	78 year old with cardiovascular disease, vol overload, and CHF	Resolution of mildly elevated liver enzymes with diuresis	Patient did not have acute hepatitis; low positive HAV IgM	Public health investigation, exclusion from adult day care center
5	HEV IgM	HAV	14-year-old recent immigrant with acute hepatitis	High positive HAV IgM; HEV IgM and PCR negative at CDC	Concurrent acute infection unusual	Inappropriate management of contacts
6	Measles virus IgM	Sulfa drug allergy	28 year old with rash illness 1 week after starting sulfa drug therapy	Negative measles virus IgM and NP PCR at CDC	Measles virus IgG higher than IgM; measles virus IgM borderline; recent sulfa drug and 7% eosinophilia	Callback of >100 patients and HCW for testing and furlough of HCW
7	HSV-2 IgM	HSV-1	23 year old with primary HSV infection and hepatitis	Positive HSV-1 PCR plasma and liver tissue; HSV-1 isolated from lip lesion and liver tissue	HSV-1 PCR and culture results	Etiology attributed to an STD with different future implications
8	WNV IgM	HSV-2	20 year old with sacral vesicles and aseptic meningitis	Positive HSV-2 PCR of CSF; HSV in sacral vesicles; negative WNV PRNT	Dual infection unlikely; WNV IgM low positive; case occurred outside WNV season	Failure to receive antiviral therapy or counseling for genital infection and recurrent meningitis

[a] HCW, health care workers; STD, sexually transmitted disease.

of our work, false-positive IgM tests have been reported for any pathogen for which IgM tests are used. These include *Salmonella enterica* serovar Typhi (2), *Bordetella pertussis* and *Legionella pneumophila* (3), *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* (4, 5), *Toxoplasma gondii* (6–8), the *Coccidioides* spp. causing coccidiomycosis (9), and *Borrelia burgdorferi* (10).

CASE EXAMPLES

(i) Case 1: adenovirus pneumonia (false-positive hantavirus IGM). A 26-year-old male presented to an emergency department (ED) in July with a 3-day history of the worst headache of his life, associated with photophobia, nausea, vomiting, and chills. He had been treated with azithromycin by his doctor without improvement. Lumbar puncture test results were normal, but a chest X-ray showed a left upper lobe infiltrate. The patient had had asthma as a child but did not smoke or use illicit drugs. He was admitted and treated with ceftriaxone and azithromycin. The following day, he developed severe respiratory distress and was intubated and transferred to intensive care. The patient progressed to acute respiratory distress syndrome (ARDS) and then to renal failure requiring dialysis. Nasopharyngeal (NP) swabs collected on the first and third hospital days were positive by both a direct immunofluorescent antibody (DFA) test and PCR for adenovirus (estimated at >8 $\log_{10}$ copies/ml). Plasma PCR was positive for adenovirus at 4.10 $\log_{10}$ copies/ml. Adenovirus was confirmed at CDC and sequenced as adenovirus type 4. The clinicians, however, did not accept the diagnosis of adenovirus because the patient was not immunocompromised. Instead, they suspected hantavirus pulmonary syndrome (HPS), though it is very rare in the Northeast. The patient had not traveled but had cleaned a very dirty apartment and could have had rodent exposure. Serum was sent to a reference laboratory and was reported positive by a hantavirus IgM screen at an index of 4.97; IgG was negative. Sin Nombre virus IgM was also positive. However, this sample and a second serum sample collected 7 days later were tested at CDC and were negative for hantavirus by IgM and IgG serology and by PCR of the NP swab.

Comments. The commercial hantavirus testing laboratory had previously published that, with their assay, 7 of 16 hantavirus IgM-positive but IgG-negative patients were shown to have had conditions other than HPS, including EBV and dengue virus infections and Rocky Mountain spotted fever (11). A comment to that effect was included in their test interpretation but was not read by the clinicians. Although a corrected report with the negative CDC results was issued and the infectious disease team notified, the patient was discharged with the diagnosis of hantavirus pulmonary syndrome. Of note, several years later, the commercial laboratory reported that increasing the cutoff value for a hantavirus positive improved test specificity (12).

(ii) Case 2: WNV meningoencephalitis (false-positive mycoplasma IGM). A 45-year-old woman was admitted in August with fever to 102.6°F after completing her 3rd cycle of chemotherapy for breast cancer. She denied bug bites but was a gardener. Despite antibiotic therapy, she continued to spike fevers and complained of severe headache. She developed confusion and delusions, and brain magnetic resonance imaging (MRI) showed abnormalities in the thalami bilaterally. CSF analysis revealed 102 nucleated cells/μl (normal range [NR], <6) and 78% mononuclear cells, with a normal glucose level and an elevated protein level of 81 mg/dl (NR, <50). CSF PCR was negative for herpes simplex virus (HSV), varicella-zoster virus (VZV), CMV, EBV, human herpesvirus type 6 (HHV-6), enterovirus, and adenovirus. Arbovirus and Lyme disease serology results were negative, as well as cryptococcal antigen results and bacterial and acid-fast bacillus (AFB) cultures. Mycoplasma IgM (qualitative result) and IgG (index, 4.12) were both positive. Thus, the patient was treated with two courses of doxycycline, with no improvement. On day 23 of illness, the lumbar puncture was repeated; the West Nile virus (WNV) IgM test result was now positive at an index of 4.48 (NR, <1.10) and IgG at 1.55 (<1.50). Serum WNV antibodies also seroconverted, and a retrospective CSF PCR test result for WNV was positive. Mycoplasma serology results were unchanged.

Comments. In normal hosts, CSF WNV IgM tests detect more positives than CSF PCR (13, 14). However, in immunocompromised patients, the appearance of antibody may be delayed or the antibody may be absent (15). Thus, WNV PCR of CSF should be ordered instead, or antibody studies of CSF or serum should be repeated within a few days to a week. In this case, antibody studies were not repeated for 23 days.

(iii) Case 3: CMV hepatitis (false-positive monospot and EBV IGM). A 39-year-old woman reported myalgias, low-grade fevers, chills, headache, and polyarthralgia to her physician. She was noted to have mildly elevated liver enzyme levels, leukopenia, and thrombocytopenia. She was treated empirically with doxycycline for Lyme disease and anaplasma without improvement. Symptoms continued for 3 weeks. Liver enzyme levels increased, hepatitis A, B, and C tests were negative, and she was admitted for evaluation. Laboratory results on admission included a total bilirubin level of 2.07 mg/dl (NR, <1.2), an aspartate transaminase (AST) level of 947 U/liter (<35), an alanine aminotransferase (ALT) level of 936 U/liter (<35), an alkaline phosphatase level of 174 U/liter (<130), and a white blood cell (WBC) count of 4,700 with 28% atypical lymphocytes. The heterophile antibody result was weakly positive, and EBV viral capsid antigen (VCA) IgM and IgG and Epstein-Barr nuclear antigen 1 (EBNA-1) test results were all positive, as were the CMV IgM and IgG results. CMV PCR of plasma revealed 17,204 copies/ml, whereas EBV PCR results were negative.

Comments. Positive IgM antibodies to both CMV and EBV, as well as false-positive monospot test results, commonly occur in mononucleosis (16–19), which obscures the true etiology. To confirm primary EBV infection, testing all three antibodies is key. While test results for IgM and IgG to EBV viral capsid antigen (VCA) are positive during acute primary infections, test results for IgG to EBNA-1 are negative and levels increase in convalescence (19, 20). A strong positive EBNA-1 result excludes diagnosis of an acute primary infection. Rather, positive results for all three EBV antibodies occur not infrequently due to subclinical reactivation of EBV or false-positive IgM test results or to a heterologous rise in IgM levels. Performing PCR to determine viral loads in blood also helps to identify the true pathogen.

(iv) Case 4: congestive heart failure (false-positive HAV IGM). A 78-year-old woman with a history of hypertension and myocardial infarction was admitted with congestive heart failure (CHF). She had mildly abnormal liver enzyme levels, which resolved as her CHF was treated. An acute hepatitis panel was ordered, and hepatitis A virus (HAV) IgM test results were positive, with a low index of twice the cutoff. After discharge, a public health investigation ensued and the patient was not allowed to

return to her adult senior center due to her "acute hepatitis A infection."

Comments. For the 15 years since this case occurred, we have monitored all positive HAV IgM test results in our laboratory. Of approximately 2,000 samples tested annually, only 5 or 6 have been IgM positive, and of these, only 40% have represented true cases of acute hepatitis A. The poor positive predictive value of HAV IgM reflects inappropriate testing of patients who do not have acute hepatitis and the low prevalence of HAV disease (21, 22). The true positives represent values that are usually 9 to 10 times the cutoff in acute HAV. We report all low positive values that are less than 4 times the cutoff as likely false positives. Unfortunately, there are no HAV NAAT or antigen or culture tests to confirm positive IgM test results.

(v) Case 5: hepatitis A (false-positive HEV IGM). A 14-year-old recent immigrant from India presented to the emergency room with 1 week of epigastric abdominal pain, increased stool frequency, 2 days of vomiting, and jaundice. His ALT level was 1,830 U/liter, his AST level was 398 U/liter, his alkaline phosphatase level was 218 U/liter, and his direct bilirubin level was 7.10 mg/dl. His HAV IgM level was 9.4 times the cutoff. His hepatitis E virus (HEV) IgM test results were reported to be positive by the reference laboratory. However, retesting of his serum at CDC revealed negative HEV IgM and PCR results.

Comments. Having two simultaneous acute hepatitis infections is highly unlikely, and the HEV IgM level represented a low positive (23, 24). Samples were sent to the CDC and were confirmed to be HEV negative.

(vi) Case 6: sulfa drug allergy (false-positive measles virus IGM). A 28-year-old nanny presented in the ED with a 5-day history of fever and a new-onset erythematous rash starting on her face and spreading to her trunk and extremities. She had moved to the United States from Puerto Rico at age 4 and remembered receiving some vaccinations. The child in her care was becoming sick with fever and respiratory symptoms. The attending physician identified white spots on her left buccal mucosa as Koplik's spots. Subsequently, the State Laboratory reported her measles virus IgM as positive at index 1.85 (NR, <1.0) and her measles virus IgG as positive at index 2.089 (<1.0). Infection Control identified over 100 exposed staff members and patients for immune status testing, and a number of the staff members were furloughed. Repeat testing of serum at CDC revealed a negative measles virus IgM test result and a positive IgG value of 2.49 (<1.10). The result of a measles virus PCR of her NP swab at CDC was negative. The patient had started sulfamethoxazole-trimethoprim (Bactrim) therapy 1 week prior and had 7% eosinophils in a complete blood count (CBC). The final diagnosis was sulfa drug allergy.

Comments. The clinical diagnosis of Koplik's spots and the positive measles virus IgM test result imposed a substantial burden on Infection Control, hospital staff members, and "exposed" patients, followed by the diagnosis of a flurry of other "measles" cases. The stronger positive measles virus IgG result, as well as the history of sulfa drug ingestion and 7% eosinophils, should have raised flags of doubt and the possibility of a false-positive IgM test result. Instead, the diagnosis of measles was considered confirmed until the Virology Laboratory insisted that the State Laboratory forward the samples to the CDC for PCR as well as repeat IgM testing. False-positive IgM test results for measles virus, rubella virus, parvovirus B19, and HHV-6 have all been reported in outbreaks and cases of rash illness (25–27).

(vii) Case 7: primary HSV-1 hepatitis (false-positive HSV-2 IGM). A 23-year-old teacher developed fever, myalgias, watery diarrhea, and abdominal pain with cramps 3 days prior to admission. The fever did not subside despite antibiotic treatment. He developed nausea and vomiting, and his fever rose to 104°F. Physical examination results were unremarkable, but anemia, leukopenia, thrombocytopenia, and elevated liver enzyme levels were noted. Liver enzyme levels continued to rise to an ALT level of 1,767 U/liter and an AST level of 2,272 U/liter, with a direct bilirubin level of 0.4 mg/dl, and his WBC count fell to 1,200/μl and his platelet count to 63,000/μl. A diagnosis of herpes simplex virus (HSV) hepatitis was made on hospital day 4 by detection of HSV type 1 in plasma by PCR (>8 $\log_{10}$ copies/ml), by PCR and immunostain of liver tissue, and by isolation of HSV-1 in cell culture from a newly recognized lip lesion and from liver biopsy tissue. Both HSV-1 IgG and HSV-2 IgG results were negative. However, the HSV type 2 IgM test result was reported to be positive by the reference laboratory and not the HSV-1 IgM test result.

Comments. This case of primary HSV-1 hepatitis was clearly documented by HSV-1 detection in multiple samples and by multiple methods. The HSV-1 IgM test result was negative despite the presence of a life-threatening acute infection, whereas the HSV-2 IgM test result was positive. False-positive results have been reported for both HSV and VZV (28, 29). Of note, IgM may not develop during active reactivation. With the ready availability of antigen, culture, and PCR test options, HSV IgM tests should not be used to diagnose acute infections.

(viii) Case 8: HSV-2 meningitis (false-positive WNV IGM). A 20-year-old female presented in May in Connecticut with 2 days of fever, headache, stiff neck, nausea, and vomiting. She was noted to have a few new sacral herpetic vesicles. The patient was sexually active, and her partner did not use condoms. Her CSF PCR was positive for HSV-2, and skin lesions were HSV positive by DFA testing. The test result for WNV IgM in CSF was also positive at less than twice the cutoff. The sample was retested at CDC, and while the WNV IgM test result was positive, the plaque reduction neutralization (PRNT) test result for WNV was negative. Of note, WNV has never been detected in mosquitoes in Connecticut in May; rather, positives are detected from July to October.

Comments. Although critical to the diagnosis of WNV (14, 30), false-positive IgM test results have been well documented (31, 32) due to cross-reactivity with other arboviruses, faulty kits (31), and failure to remove nonspecific reactants as required in the manufacturer's instructions (30). Background subtraction was performed for this sample as recommended, and yet the WNV IgM test result remained positive. In this case, however, clinical, laboratory, and epidemiologic data all pointed to HSV-2.

DISCUSSION

False-positive IgM test results tend to come to light in three situations. In the first situation, multiple tests are performed for the same clinical syndrome and multiple positive results are generated, e.g., mononucleosis and CMV, EBV, and HIV infections (16–19); acute hepatitis and HAV, HEV, CMV, and EBV infections (16, 23, 33); rash illness and measles virus, parvovirus, rubella virus, and HHV-6 infections (26, 28, 34, 35); arbovirus CNS disease and WNV, St. Louis encephalitis (SLE) virus, and Jamestown Canyon virus infections (32, 36); and arbovirus rash illness and Zika, dengue, and chikungunya virus infections (37, 38). In the second situation, another etiology is confirmed by another

TABLE 3 Mechanisms for false-positive IgM results

Mechanism	Reference(s)
Polyclonal B cell activation	27, 33
Vigorous immune response	27, 33
Influenza vaccination	41
Cross-reactive antibodies	16, 32, 36
Autoimmune disease	42
Heterologous reactions to similar viruses	17, 19
Subclinical reactivation of latent viruses	18
Interfering substances such as rheumatoid factor	40, 43
Naturally occurring biotin IgM antibodies	44
Cutoff values set too low	5, 12
Faulty reagents	31
Technical errors, such as overreading weakly reactive bands on immunoblots	10
Low pretest probability	21
Inappropriate test ordering	21, 22

method. In the third situation, the IgM test result clearly does not match the clinical situation. If an IgM test is done for a single pathogen with no confirmatory testing and the clinical syndrome is compatible, a misdiagnosis may go undetected.

The risks of accepting a false-positive IgM as a true result include delays in appropriate therapy, unnecessary tests and therapies, premature closure of an investigation of etiology, erroneous counseling or a lack of counseling of the patient, and inappropriate public health and infection control interventions.

A variety of mechanisms have been proposed to account for false-positive IgM test results, as shown in Table 3. For certain pathogens, false-positive IgM test results either occur more often or have been more readily detected because of common parallel IgM testing for multiple pathogens.

Arboviruses are notorious for their association with cross-reactive antibodies (32, 36). The current Zika virus outbreak has highlighted this issue (37, 38). The screening test for recent exposure is Zika virus IgM detection, but if the result is positive, a more specific plaque reduction neutralization test (PRNT) is performed. Ultimately, the positive Zika virus IgM test result may be found to have been due to the presence of cross-reactive dengue virus antibodies and not to the presence of Zika virus. In other cases, the test results may be inconclusive and may be reported as representative of a recent flavivirus infection (http://www.cdc.gov/zika/hc-providers/diagnostic.html). Herpesviruses are commonly associated with increases in heterologous IgM levels, cross-reactivity, or subclinical reactivations leading to multiple positive IgM test results (16, 17). Lyme disease IgM immunoblotting is prone to false positives because of inappropriate ordering, weak criteria for positivity, and inaccurate reading (10).

The key message is that false-positive IgM test results can occur with any pathogen, and if the stakes are high, the accuracy of the result should be verified. Diagnostic accuracy can be improved by assessing the relative strengths of the IgM and IgG reactivities (in general, the reactivity of IgM should be higher than that of IgG), obtaining serial samples to determine if the IgM and IgG levels are rising, using the class capture IgM format, raising the cutoff value for a positive result, using a second and more specific serologic test such as PRNT or immunoblotting, testing for the pathogen itself rather than IgM, documenting seroconversion of IgG, and examining the clinical findings, other laboratory values, and epidemiologic risk factors.

IgG avidity testing can help determine whether an infection was recent. This is commonly used for pregnant women who are found to have a positive CMV IgM test result (39). For some viruses such as EBV, tests for late-appearing antibodies such as IgG to EBNA-1 can distinguish an acute primary infection from a subclinical reactivation of a past infection (19). In addition, samples can be treated to remove interfering substances such as rheumatoid factor (RF) (40), and serum can be preabsorbed to remove nonspecific reactants.

CONCLUSIONS

Although false-positive IgM test results have been described in many case reports and case series, many clinicians and laboratorians remain unaware of this issue. IgM tests have proven valuable in many situations, but it is important to recognize that false positives may be more common than with other diagnostic methods for a variety of reasons and for some pathogens in particular.

While this small case series presents a limited number of examples, a false-positive IgM test result can occur with any pathogen. Thus, when the diagnosis is important for therapy, prognosis, or public health, when the patient is sick enough to be hospitalized, or when the clinical or epidemiologic findings do not fit, IgM detection should not be accepted as a standalone test. Rather, the diagnosis should be confirmed by other means, including testing of serial samples and the application of additional test methods.

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