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Are IgG antibody avidity assays useful in the diagnosis of infectious diseases? A review

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Abstract

The reliability of tests for the identification of low avidity specific IgG in infectious diseases was investigated. The IgG antibody avidity assays were proposed in order to examine the probable aetiology, the accurate moment of primary infection and to distinguish between reactivation, reinfection or primary infection, in cases with false positive IgM or without specific IgM response. Such assays improve the usual serological techniques.

Introduction

The detection of IgM antibodies for serological diagnosis of viral, bacterial, or parasitic acute infections has several drawbacks. Frequently, it is not possible unequivocally to differentiate between primary infection, reinfection or reactivation. Moreover, the presence of antigens common to various viruses can lead to errors in diagnosis. The B-lymphocyte clonal stimulation produced in certain infections can result in specific protracted IgM synthesis to different pathogens.

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Recently, the IgG antibody avidity assays have been proposed in order to investigate the probable aetiology, the accurate moment of primary infection and to distinguish between reactivation, reinfection or primary infection. Thus, they improve the usual serological methods.

Theoretical justification

The overall strength of antigen-antibody bonds is known as antibody avidity or functional affinity. The B-cell selection process brings about maturation of IgG antibody affinity which is initially low and increases during subsequent weeks and months (1 to 7 months). This selection produces an increase in the complementarity of the antigen-antibody binding site which results from somatic mutations in variable regions of the IgG antibodies. A few days after antigenic contact, the antibodies produced still originate from unmutated plasma cells. At the end of the first month, there are mutations in the antibody variable region and an increase in IgG antibody affinity (Steele, 1990; Thomas and Morgan-Capner, 1991).

Avidity maturation seems to be proportional to antigen dose. Indeed, low doses of antigen give rise to a more rapid maturation and higher doses to a slower maturation. Therefore, low avidity antibodies are produced during the first stage of infection when a high level of antigens is usually present (Thomas and Morgan-Capner, 1991).

The measurement of the avidity of specific IgG antibodies for serodiagnosis of various infections can be carried out using methods such as agglutination, radio-immunoassay (RIA), complement fixation, enzyme-linked immunosorbent assay (ELISA), immuno-fluorescence assay (IFA), electroblotting and electrophoresis. It is necessary to add partially denatured substances to these avidity assays, and these are included in the dilution of serum (dilution principle methods) or applied after the formation of the antigen-antibody complex (elution principle methods). Denaturing substances such as diethylamine, that is inactive to physiological pH, potassium thiocyanate, guanidine or urea may be used. There have been various reports that treatment with 8 M urea may be the simplest method for IgG antibody avidity measurement (Kangro *et al.*, 1991; Meurman *et al.*, 1992; Schoub *et al.*, 1992), but is probably less sensitive than diethylamine because it cannot detect low avidity antibodies after the first 3 months following primary infection (Thomas *et al.*, 1992; Thomas *et al.*, 1993a,b).

In our laboratory we investigated the avidity of specific IgG antibodies using commercial reagents and automated systems. The procedure consisted of two parallel determinations of specific IgG: one was processed according to the standard protocol; the other included a washing stage after the antigen-antibody reaction, using washing buffer supplemented with 8 M urea. Determinations were read in a spectrophotometric assay and the results expressed in titres. If a reduction in titres higher than, or equal to 50% was seen after treatment with urea, the sample was considered positive for low avidity IgG.

Clinical justification

The application of avidity assays may be of interest in some or all of the following infections of humans.

Tuberculosis

A serological test for the detection of circulating IgG antibodies against the mycobacterial antigen A60 was used to analyse the avidity of antibodies raised in the serum of tuberculous patients. The avidity of the antibodies detected appeared variable and was quite low in patients with primary infection (Maes, 1991). However, its real value was

relative because the adult tuberculosis may have occurred by reactivation and there may have been no decrease in IgG antibody affinity. Indeed, this affinity was only observed to decrease in primary infections of adults and children.

Periodontitis by *Porphyromonas gingivalis*

Patients with periodontitis have chronic symptoms, reactivations and persistent infections due to multiple micro-organisms. The detection of low avidity IgG antibodies has been suggested for diagnosis of primary infection by *P. gingivalis* which may be associated with the pathology of periodontal illness (Lopatin *et al.*, 1991; Chen *et al.*, 1991).

Q Fever

Specific IgM titres persist for more than 6 months in the majority of cases of Q fever. Consequently there are no criteria for primary infection diagnosis. However, relative avidity changed significantly, and was related to the time of clinical evolution (Guigno *et al.*, 1992).

Chickenpox

Although chickenpox is an infantile illness it can also develop in elderly people. It is difficult to differentiate from other cutaneous illnesses and from the disseminated herpes zoster (although symptoms of the latter are stronger) (Schoub *et al.*, 1992). Reinfection or reactivation (mainly in risk groups) are both possible, sometimes without cutaneous manifestations (Kangro *et al.*, 1991; Schoub *et al.*, 1992). Diagnosis by conventional techniques is difficult in all cases because of unclear serological dates of primary infection or the detection of specific antibodies to another herpes virus.

Infections by herpes simplex virus, cytomegalovirus, Epstein-Barr virus and human herpes virus 6

During infections by herpes simplex virus, cytomegalovirus, Epstein-Barr virus and human herpes virus-6, the IgM antibodies do not act in a sufficiently consistent way for use in the diagnosis of primary infection, reinfection or reactivation, whether or not there are symptoms. Indeed, such antibodies can give false negative results, they are not synthetized, or since the quantity synthetized is difficult to detect, or there is an early appearance of anti-Epstein-Barr nucleotide antigen (anti-EBNA) antibodies (De Ory, 1991). Moreover, they can yield false positive results because of (1) their persistence and their presence in processes unrelated to the infection (Grazia *et al.*, 1991); (2) the fact that they may be detected in reactivations or reinfections (De Ory *et al.*, 1993; De Ory *et al.*, 1995, 1995; Ward *et al.*, 1993; Ward *et al.*, 1995) as in subjects infected with the human immunodeficiency virus; (3) epitopes common to different viruses, like herpes simplex

Infections by respiratory syncytial virus

The respiratory syncytial virus is the most important pathogen causing sever lower respiratory tract infections in infants and neonates. Its special features include the incomplete protection of infants by maternal antibodies, frequent reinfections and the possible role of antibodies in disease pathogenesis. Avidity studies permitted us to consider the time course of specific antibodies. The newborn child has IgG mother antibodies which display high avidity and act as protectors, but in the following months the decrease of these high avidity antibodies accompanied by an increase in low avidity IgG make the patient susceptible to infection by this virus. When reinfections occurred, IgG avidity was also high (Meurman *et al.*, 1992).

Epidemic nephropathia

In patients with acute epidemic nephropathy, antibody titres are high and the IgM antibodies remain stable for a considerable time thus making diagnosis difficult. Partly because of the straightforward nature of these tests (Hedman *et al.*, 1991) avidity assays are thus suitable for use in patients with acute epidemic nephropathia.

Mumps and infections by parainfluenza virus

In patients with mumps the specific IgM may react with parainfluenza virus antigens (PiV) in indirect ELISA. Moreover, in patients with PiV infection the sera cross-reacted with the mumps virus antigens. Estimation of antibody avidity even by the arbitrary method used, can distinguish between homotypic and cross-reactive heterotypic antibodies after mumps or PiV infections (Frankova *et al.*, 1988; Lethonen and Meurman, 1986).

Hepatitis C virus diagnosis

Since techniques of IgM antibody detection are relatively new and are still not well understood (Wreghitt *et al.*, 1990), the serological investigation of patients infected with the hepatitis C virus can be carried out by research into low avidity IgG antibodies.

Toxoplasmosis

The only reliable serological markers until now for recent active toxoplasmosis have been an IgM-positive finding and/or a rise in the IgG antibodies titre. The main drawback of IgM antibodies are their persistence in the blood, which makes it difficult to determine the limits of the disease, and they are not synthetized in certain categories of patients such as children. For these reasons, it seems more useful to resort to serial determination of IgG antibodies in two or three serum samples, with the consequent delay in diagnosis (Ades, 1991; Remington and Desmonts, 1990; Gutiérrez *et al.*, 1993). The measurement of IgG avidity is a highly specific and sensitive method

suitable for verification of acute primary *Toxoplasma gondii* infections during pregnancy. An assay measuring the antigen-binding avidity (functional affinity) of IgG antibodies against *T. gondii* was recently developed to separate the low affinity antibodies produced at an early stage of infection from those with a higher binding affinity which reflect past immunity. By means of this technique, primary infection could be identified using a single serum specimen (Joyson *et al.*, 1990; Lappalainen *et al.*, 1993).

Clinical significance

The study of IgG and IgM antibodies does not satisfactorily resolve the diagnosis of infections, and so new serological markers are being evaluated, such as IgG antibodies avidity.

Several workers have shown that the avidity of IgG antibodies changes with the time since the initial infection, whether viral (Meurman *et al.*, 1992; Schoub *et al.*, 1992; Thomas *et al.*, 1992; De Ory *et al.*, 1993; Hedman *et al.*, 1991; Blackburn *et al.*, 1991), or bacterial (Maes, 1991; Lopatin *et al.*, 1991; Chen *et al.*, 1991; Guigno *et al.*, 1992; Lahesmaa-Rantala *et al.*, 1987). This suggests that when the percentage of IgG antibodies with a low avidity for the antigen is equal to or higher than 50% we are dealing with a recent primary infection.

We found that, using break-points of 50%, the study of IgG class antibody avidity may help to distinguish a short-term from a long-term acute infection, since the yield of this assay is close to 100% for all parameters (sensitivity, specificity, positive and negative predictive value) and micro-organisms. In some cases, it was not 100%, and this may have been due to various causes, such as persistence of immature (or not very protective) antibodies in patients with a past infection or reactivation; rapid maturation of antibody avidity in patients with a primary infection; problems in the commercial antigen used, which would mean it did not adequately and sensitively detect antibodies to a particular virus subtype; or the need to use different denaturing agents which would make it possible to distinguish the presence of low-avidity antibodies with greater sensitivity. It is evident that 8 M urea may be less sensitive than diethylamine because urea may not be capable of detecting low-avidity antibodies more than 3 months after infection, as in the case of rubella (Hedman *et al.*, 1993). However, the use of one or other denaturing agent is affected by the type of organism inducing the antibody response and the strength of the denaturing effect caused, and must be investigated for each case.

The break-points for considering that there are low-avidity antibodies vary from 70–75% (Grazzia *et al.*, 1991; Joyson *et al.*, 1990; Lappalainen *et al.*, 1993; Thomas *et al.*, 1993) to 50% (Kangro *et al.*, 1991; Schoub *et al.*, 1992), depending on the denaturing agent employed, the type of organism and the requirements of the technique used. Grazzia *et al.* (1991), Joyson *et al.* (1990) and Lappalainen *et al.* (1993) argued in favour of higher break-points, and they used 6 M urea or diethylamine as a denaturing agent. Initially, we used a 50% break-point and later 55%, discarding samples with values between 50 and 55%, and we found that the yield improved slightly, except for HHV-6, where the break-point was not set in the same way. In this manner, we obtained a diagnostic yield of 100% except with cytomegalovirus, for which it was close. If we take into account that the diagnostic yield of IgM antibodies investigated in acute infections was not 100%, the combination of an IgG avidity result with an IgM study could resolve conflicting cases. Thus, there are IgM-negative primary infections with low-avidity IgG antibodies, and IgM-positive primary infections with IgG antibodies with normal avidity, which would indicate a non-recent infection.

In patients for whom we were able to carry out a serological follow-up, under the terms of reference of our study, we found a normalization of avidity varying from 75 to 90 days. These results confirmed the importance for differentiation, with a high degree of significance, of a short-term or a long-term infection. Taking into account the avidity behaviour of IgG antibodies in recent primary infections, the study of this parameter may be useful in ascertaining the aetiology of an infection when there are various IgM antibodies with cross-reactivity in antigen populations (between herpes virus and varicella zoster virus) or because of heterotypical reactions between herpes virus.

The drawback of this type of technique is its low sensitivity hence it can only be used on samples which have high IgG antibody titres. When antibody titres are <1/1,000, from ELISA, this method was not sensitive enough to detect low avidity antibodies. The antibody avidity studies are unfortunately not applicable in the investigation of cerebrospinal fluid samples. In serum they can only be used during the advanced and not the early stages of the illness.

In conclusion, in recent primary infections most IgG antibodies have low avidity for the antigen. In past infections or reactivation most IgG antibodies have a high avidity for the antigen. Recent primary infections have higher levels than 55% of IgG with low avidity for the antigen. If it is lower than 55% and the infection is suspected the sequential study of the normalization of avidity is diagnostic. Indeed

the study of avidity for IgG is relevant for the diagnosis of primary infection with IgM negative or positive limits and infections with multiple IgM antibodies; for diagnosis between primary infection, reactivation or reinfection; and for diagnosis of past infection with persistent IgM positive limits.

The avidity test is simple and automated in the laboratory and thus differs from commercial disposable tests. Techniques quantifying the avidity of specific IgG antibodies, together with traditional serological methods (tests for IgG and IgM), are very useful for ascertaining, from a single sample, the time which has elapsed since the initial infection.

References

ADES A. E. 1991. Evaluating the sensitivity and predictive value of test of recent infection: toxoplasmosis in pregnancy. *Epidemiol. Infect.* **107** 527–35.

BLACKBURN N. K., Besselaar T. G., Schoub B. D. and O'Connell K. F. 1991. Differentiation of primary cytomegalovirus infection from reactivation using the urea denturation test for measuring antibody avidity. *J. med. Virol.* **33** 6–9.

CHEN H. A., Johnson B. D., Sims T. J., Darveau R. P., Moncla B. J., Whitney C. W., Engel D. and Page R. C. 1991. Humoral immune responses to *Porphyromonas gingivalis* before and following therapy in rapidly progressive periodontitis patients. *J. Periodontol.* **62** 781–91.

CRADOCK-WATSON J. M., Ridehalgh M. K. S., Anderson M. J. and Pattison J. R. 1985. Rubella reinfection and the foetus. *Lancet* 1039.

CRADOCK-WATSON J. M., Ridehalgh M. K., Anderson M. J. and Pattison J. R. 1981. Outcome of asymptomatic infection with rubella virus during pregnancy. *J. Hyg. Camb.* **87** 147–54.

DE ORY F. 1991. Viral infections: interpretation of specific IgM detection assays. *Rev. Esp. Microbiol. Clin.* **25** 110–2.

DE ORY F., Antonaya J., Fernández V. and Echevarria J. M. 1993. Application of low-avidity inmunoglobulin G studies to diagnosis of Epstein-Barr virus infections mononucleosis. *J. clin. Microbiol.* **31** 1669–71.

DE ORY F., Casa I., Domingo C. J. and Echevarria J. M. 1995. Application of fluoro-immunoassay to identification of low avidity specific IgG against pathogenic human viruses and *Toxoplasma gondii*. *Clin. Diag. Virol.* **3** 323–5.

FIKAR C. R. and McKee M. D. 1994. False positivity of IgM antibody to Epstein-Barr viral capsid antigen during acute hepatitis A infection. *Pediat. Infect. Dis. J.* **13** 413–4.

FRANKOVA V., Holubova J., Grubhoffer L. and Kasova V. 1988. Contribution to laboratory diagnosis of mumps and parainfluenza. *Acta Virol. Praha.* **32** 503–14.

GRAY J. J., Caldwell J. and Sillis M. 1992. The rapid serological diagnosis of infectious mononucleosis. *J. Infect.* **25** 39–46.

GRAZZIA M., Percivalle E., Zannini M., Rossi V. and Gerna G. 1991. Development and evaluation of a capture ELISA for IgM antibody to the human cytomegalovirus major DNA binding protein. *J. virol. Meth.* **35** 315–29.

GUIGNO D., Coupland B., Smith E. G., Farrell I. D., Desselberger U. and Caul E. O. 1992. Primary humoral antibody response to *Coxiella burnetii*, the causative agent of Q fever. *J. clin. Microbiol.* **30** 1958–67.

GUTIÉRREZ J., De la Higuera A. and Maroto M. C. 1993. A comparative study between the determination of immunoglobulin A and immunoglobulin M in acute toxoplasmosis. *Rev. Invest. Clin.* **45** 585–8.

GUTIÉRREZ J., Rodríguez M., Maroto M. C. and Piédrola G. 1996. Reliability of four methods for the diagnosis of acute infection by Epstein-Barr virus. *J. clin. Lab. Anal.* In press.

GUTIÉRREZ J., Rodríguez M., Maroto M. C., Piédrola G. and Peiron J. 1997. Behaviour of IgG antibody avidity for the antigen of IgA in the active cytomegalovirus, Epstein-Barr virus, herpes simplex virus and human herpes virus-6 infections. Adaptation of a commercial test. *J. Infect.* In press.

HEDMAN K., Vaheri A. and Brumemmer-Korvenkontio M. 1991. Rapid diagnosis of Hantanvirus disease with an IgG-avidity assay. *Lancet* **338** 1353–6.

HEDMAN K., Lappalainen M., Söderlund M. and Hedman L. 1993. Avidity of IgG in serodiagnosis of infectious diseases. *Rev. Med. Microbiol.* **4** 123–9.

JOYSON D. H. M., Payne R. A. and Rawal B. K. 1990. Potential role of IgG avidity for diagnosing toxoplasmosis. *J. clin. Path.* **43** 1032–3.

KANGRO H. O., Manzoor S. and Harper D. R. 1991. Antibody avidity following varicella-zoster virus infections. *J. med. Virol.* **33** 100–5.

KURTZ J. B. and Anderson M. J. 1985. Cross-reactions in rubella and parvovirus specific IgM test. *Lancet* 1356.

LAHESMAA-RANTALA R., Lehtonen O., Granfors K. and Toivaven A. 1987. Avidity of anti-yersinia antibodies in yersiniosis patients with and without *Yersinia*-triggered reactive arthritis. *Arthr. Rheum.* **30** 1176–80.

LAPPALAINEN M., Koskela P., Koskiniemi M., Ammala P., Hiilesmaa V., Teramo K., Raivio K. O., Remington J. S. and Hedman K. 1993. Toxoplasmosis acquired during pregnancy: improvement in serodiagnosis based on avidity of IgG. *J. infect. Dis.* **167** 691–7.

LETHONEN O. P. and Meurman O. H. 1986. Avidity of IgG antibodies against mumps, para-influenza 2 and Newcastle disease viruses after mumps infection. *J. virol. Meth.* **14** 1–7.

LOPATIN D. E., LaBelle D. and Lee S. W. 1991. Measurement of relative avidity of antibodies reactive with *Porphyromonas (Bacteroides) gingivalis* in the sera of subjects having adult periodontitis. *J. Periodont. Res.* **26** 167–75.

MAES R. F. 1991. Evaluation of the avidity of IgG anti-mycobacterial antibodies in tuberculous patients serum by an A-60 immunoassay. *Eur. J. Epidemiol.* **7** 188–90.

MEURMAN O., Waris M. and Hedman K. 1992. Immunoglobulin G antibody avidity in patients with respiratory syncytial virus infection. *J. clin. Microbiol.* **30** 1479–84.

MORGAN-CAPNER P. 1986. Does rubella reinfection matter? pp 50–62. Edited by P. P. Mortimer. Public Health Virology, 12 reports.

MORGAN-CAPNER P., Tedder R. S. and Mace J. E. 1983. Rubella specific IgM reactivity in serum from cases of infectious mononucleosis. *J. Hyg. Camb.* **90** 407–13.

REMINGTON J. S. and Desmonts G. 1990. Toxoplasmosis. *In* Infectious Diseases of Foetus and Newborn Infant. pp 98–195. Edited by J. S. Remington and J. O. Klein.

SCHOUB B. D., Blackburn N. K., Johnson S., McAnerney J. M. and Miller B. 1992. Low antibody avidity in elderly chickenpox patients. *J. med. Virol.* **37** 113–5.

STEELE E. J. 1990. Somatic hypermutation in V-regions. pp 1–185. CRC Press.

THOMAS H. I. J., Morgan-Capner P., Enders G., O'Shea S., Calicott D. and Best J. M. 1992. Persistence of specific IgM and low avidity specific IgG_1 following primary rubella. *J. virol. Meth.* **39** 149–55.

THOMAS H. I. J. and Morgan-Capner P. 1991. The use of antibody avidity measurements for the diagnosis of rubella. *Rev. Med. Virol.* **1** 41–50.

THOMAS H. I. J., Morgan-Capner P., Cradeck-Watson J. E., Enders G., Best J. M. and O'Shea S. 1993. Slow maturation of IgG_1 avidity and persistence of specific IgM in congenital rubella: implications for diagnosis and immunopathology. *J. med. Virol.* **41** 196–200.

THOMAS H. I. J., Morgan-Capner P. and Connor N. S. 1993. Adaptation of a commercial rubella-specific IgG kit to assess specific IgG avidity. *Serodiagn. Immunother. Infect. Dis.* **1** 13–6.

WARD K. N., Gray J. J., Fotheringham M. W. and Sheldon M. J. 1993. IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection. *J. med. Virol.* **39** 131–8.

WARD K. N., Gray J. J., Joslin E. and Sheldon M. J. 1995. Avidity of IgG antibodies to human herpes virus-6 distinguishes primary from recurrent infection in organ transplant recipients and excludes cross-reactivity with other herpes virus. *J. med. Virol.* **39** 44–9.

WREGHITT T. G., Gray J. J., Aloyisus S., Contreras M. and Barbara J. A. 1990. Antibody avidity test for recent infection with hepatitis C virus. *Lancet* **31** 335–9.

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