

Rapid diagnosis of hantavirus infections

A. VAHERI, K. HEDMAN, O. VAPALAHTI, H. KALLIO-KOKKO, H. PIIPARINEN, M. TEMONEN
and M. BRUMMER-KORVENKONTIO

Department of Virology, University of Helsinki, Haartmaninkatu 3, SF-00290, Helsinki,
Finland

Received in September 1991

Approved in October 1991

INTRODUCTION

Hantaviruses are globally important pathogens causing hemorrhagic fevers with renal syndrome (HFRS) with acute renal insufficiency as the cardinal manifestation (Lee and Van der Groen, 1989; Editorial, 1990). A severe form of HFRS, caused by Hantaan virus, occurs in Asia and eastern parts of Europe, a moderate form caused by Seoul virus, occurs in Asia, and a milder form, nephropathia epidemica (NE), is found widely in Europe (Lee *et al.*, 1990; Van Ypersele de Strihou and Méry, 1989).

HFRS is difficult to diagnose on clinical grounds alone and specific diagnostic procedures are thus often necessary. Moreover, it seems that many of the clinical manifestations of hantavirus infections have not yet been identified and that the full epidemiology of hantavirus infections world-wide in humans and carrier animals is not known. An additional reason for the need of rapid diagnosis of hantavirus infections is that ribavirin, and possibly also recombinant Interferon- α , are clinically useful in HFRS (see Monath, 1990).

IgG ANTIBODY TESTS

The first methods for serological diagnosis of HFRS were based on demonstration of a ≥ 4 -fold rise in serum titer of antibodies detected the indirect immunofluorescence (IF) test and *Apodemus agrarius* (for Korean hemorrhagic fever, KHF) or *Clethrionomys glareolus* (for NE) lung sections as antigens (Lee *et al.*, 1978; Brummer-Korvenkontio *et al.*, 1980) adaptation of the different hantaviruses to grow in cell culture (Yanagihara *et al.*, 1984; Schmaljohn *et al.*, 1985), virus-infected Vero E6 and A549 cells have been widely used as antigen in the indirect IF test.

After Schmaljohn and co-workers (1985) provided the evidence that hantaviruses belong to the *Bunyaviridae* family, their hemagglutination (HA) activity was detected and hemagglutination-inhibition (HI) tests were developed. Tsai and co-workers (1984) reported on HA activity of a sucrose-acetone extract of Hantaan virus-infected suckling mouse brain. Puumala virus-infected cell culture material

provided a more convenient source of antigen for an HI test (Brummer-Korvenkontio *et al.*, 1986).

The HI test has two disadvantages. Firstly, some false-positive results may be obtained unless special precautions are used, and secondly, the HI antibodies develop somewhat slower than those detected using the IF test. On the other hand, the broad cross-reactivity (Puumala virus antigen reacts with both NE and KHF sera) makes the HI test a valuable screening method for detecting hantavirus-positive human and animal sera.

More recently, reports have appeared on the development of enzyme immunoassays (EIA) or solid-phase radioimmunoassays (SPRIA) for the detection of hantavirus antibodies. These tests have been based on the use of hantavirus-infected Vero E6 cell homogenates (Groen *et al.*, 1989; Groen *et al.*, 1991; Ivanov *et al.*, 1988; Meegan *et al.*, 1989), of purified virus (Niklasson *et al.*, 1990) or tissue extracts (Tkachenko *et al.*, 1989) as antigen. In our experience purified detergent (β -octylglucoside) -disrupted Puumala virus in a solid-phase EIA procedure has not provided a test reliable enough to compete with the IF and HI tests we developed earlier. The high density particle agglutination test, developed by Tomiyama and Lee (1990), has the special advantage that the results are obtained within 40 minutes and that no expensive equipment is needed.

IgM ANTIBODY ASSAYS

Detection of hantavirus-specific serum IgM antibodies may provide a way for rapid diagnosis of recent infection. We have used earlier in selected cases the ultracentrifugation

method to separate serum IgM and IgG antibodies for the HI test (Brummer-Korvenkontio *et al.*, 1980) but this is clearly a technique too tedious for routine diagnostic purposes.

Other laboratories have reported on successful use of IF and EIA procedures for detection of hantavirus-specific IgM antibodies using cell culture material or purified virus as antigen (Lee *et al.*, 1989; Ivanov *et al.*, 1988; Meegan *et al.*, 1989; Niklasson *et al.*, 1990; Niklasson and Kjellson, 1988) but in our experience purified Puumala virus in solid-phase IgM EIA or in IgM immunoblotting has not provided optimal rapid tests we have developed (see below). The same is true for all modifications of IF IgM tests we have applied. In addition to the problems derived from the very common presence of rheumatoid factor (that may yield false-positive results), the unpredictable persistence of IgM antibodies, may be another major reason for the poor "field performance" of these IgM tests.

RAPID IF TEST TO EXCLUDE NE

In Finland according to our serological surveys about 5% of the adult population have serum antibodies to Puumala virus; in Eastern Finland in certain areas the prevalence of seropositivity is up to 20% (Brummer-Korvenkontio *et al.*, 1982). Since the antibodies develop in the early phase of illness, the absence of serum antibodies in practice excludes NE according to the following. In our experience 83% are seropositive within three days after onset of symptoms and 100% within 6 days.

GRANULAR IF PATTERN AS AN INDICATION OF ACUTE PUUMALA VIRUS INFECTION

We have made the observation that the IF pattern in the Puumala virus-infected Vero E6 cells is closely dependent on the phase of infection. Sera collected during the first 10 days after onset of illness give a coarsely granular pattern while late convalescent sera give a diffuse IF staining pattern. Thus we can quite reliably estimate the phase of infection from the IF pattern which changes during the course of the infection from a coarsely granular pattern to finely granular, then to granular-diffuse, and finally to a diffuse IF pattern.

RAPID DIAGNOSIS OF NE BY AVIDITY ASSAY OF IgG ANTIBODIES

We have recently developed for serodiagnosis of NE a novel type of assay which measures the avidity (functional affinity) of IgG antibodies against Puumala virus (Hedman *et al.*, submitted for publication). The principle is to elute the antigen-bound IgG antibodies with a protein denaturant (6-8 M urea), and to quantitate the proportion of the residual antibodies which have a high binding avidity (Hedman *et al.*, 1989). This new test was shown to be highly specific and sensitive, with the diagnosis (low avidity) or exclusion (high avidity) of NE achieved rapidly from single samples of serum.

The diagnostic sensitivity of this new method is almost 4-fold compared to conventional IgG serology utilizing paired samples, and has multiplied the annual numbers of confirmed diagnoses of NE in Finland (Hedman *et al.*, submitted for publication). Measuring IgG avidity we

have, during 22 months in 1989-1991, verified NE for > 1 300 patients. This result represents an unexperienced nationwide incidence of serologically confirmed hantavirus disease. This IgG-avidity method is technically straight-forward and utilizes only simple reagents plus a fluorescence microscope, and should therefore be suitable also for technically and economically less advanced conditions.

DIAGNOSTIC POTENTIAL OF RECOMBINANT HANTAVIRUS PROTEINS, SYNTHETIC PEPTIDES AND OF THE POLYMERASE CHAIN REACTION

The hantavirus genome consist of three genome segments coding for the nucleocapsid protein (S segment), two glycoproteins G1 and G2 (M segment) and a polymerase (L segment). Several of the genome segments of the pathogenic hantaviruses have been cloned and sequenced: Hantaan S, M and L segments (Schmaljohn *et al.*, 1986; Schmaljohn *et al.*, 1987; Yoo and Kang, 1987; Schmaljohn, 1990), Seoul (strain SR-11) S and M segments (Arikawa *et al.*, 1990), Puumala (Hällnäs strain) M and S segments (Giebel *et al.*, 1989; Stohwasser *et al.*, 1990), and Puumala (Sotkamo strain) M and S segments (Vapalahti *et al.*, submitted for publication). These sequences show that in its M and S segments Hantaan virus differs at the amino acid level from Puumala virus by about 40% and from Seoul virus by about 20%. Furthermore, the two strains of Puumala virus, Hällnäs and Sotkamo isolated in Sweden and Finland, respectively, differ from each other by about 5% (Vapalahti *et al.*, submitted for publication).

The cloning and sequencing of these hantavirus genome segments has made several new diagnostic approaches possible. Recombinant nucleocapsid proteins have been expressed both in *E. coli* and in eukaryotic cells using baculovirus and vaccinia virus vectors (Vapalahti *et al.*, submitted for publication; Schmaljohn *et al.*, 1990; Rossi *et al.*, 1990; Gött *et al.*, 1990). Similarly, using DNA technology the hantavirus envelope proteins can be expressed for diagnostic purposes (Schmaljohn *et al.*, 1990), thus eliminating the health hazards involved in the conventional preparation of hantavirus antigens from virus-infected cultured cells or tissues. These recombinant hantavirus antigens are currently being tested for suitability in routine diagnostic procedures such as EIA.

Chemical synthesis of antigenically active peptides provides an attractive alternative as antigen for diagnostic virology. Synthetic peptides have the additional advantage that by appropriate selection of the peptide sequences either widely cross-reactive or type- and even strain-specific antigens can be obtained. The successful use of synthetic peptides as solid-phase antigen in antibody EIA by the immunologically cross-reactive human immunodeficiency viruses HIV-1 and HIV-2 (e.g. Närvänen *et al.*, in press) show that this approach is feasible in practice. The genetic variability within hantaviruses suggests that, like in the case of human retrovirus infections, synthetic peptides can be useful for serological screening and typing. We are actively exploring these possibilities.

The polymerase chain reaction (PCR) is a potentially powerful tool for rapid viral diagnosis. Our recent experience, while still quite preliminary, suggests that PCR may be useful also in the diagnosis of hantavirus infections.

REFERENCES

- ARIKAWA, J.; H.F. LAPENOTIERE; L. IACONO-CONNORS; M. WANG and C.S. SCHMALJOHN (1990). Coding properties of the S and M segments of Sapporo rat: comparison to other causative agents of hemorrhagic fever with renal syndrome. *Virology* **176**: 114-125.
- BRUMMER-KORVENKONTIO, M.; A. VAHERI; T. HOVI; C-H. VON BONSDORFF; J. VUORIMIES; T. MANNI; K. PENTTINEN; N. OKER-BLOM and J. LÄHDEVIRTA (1980). Nephropathia epidemica: Detection of antigen in bank voles and serological diagnosis of human infection. *J. Infect. Dis.* **141**: 131-134.
- BRUMMER-KORVENKONTIO, M.; H. HENTTONEN and A. VAHERI (1982). Hemorrhagic fever with renal syndrome in Finland: ecology and virology of Nephropathia epidemica. *Scand. J. Infect. Dis. (Suppl.)* **36**: 88-91.
- BRUMMER-KORVENKONTIO, M.; T. MANNI; S. UKKONEN and A. VAHERI (1986). Detection of hemagglutination-inhibiting antibodies in patients with nephropathia epidemica and Korean hemorrhagic fever by using Puumala virus cell culture antigen. *J. Infect. Dis.* **153**: 997-998.
- Editorial (1990). *Lancet* **336**: 407-408.
- GIEBEL, L.B.; R. STOHWASSER; L. ZÖLLER; E.K.F. BAUTZ and G. DARAI (1989). Determination of the coding capacity of the M genome segment of Nephropathia epidemica virus strain Hällnäs B1 by molecular cloning and nucleotide sequence analysis. *Virology* **172**: 498-505.
- GÖTT, P.; L. ZÖLLER; S. YANG; R. STOHWASSER; E.K.F. BAUTZ and G. DARAI (1990). Antigenicity of hantavirus nucleocapsid proteins expressed in *E. coli*. *Virus Research* **19**: 1-16.
- GROEN, J.; G. VAN DER GROEN; G. HOOFD and A. OSTERHAUS (1989). Comparison of immunofluorescence and enzyme-linked immunosorbent assays for the serology of Hantaan virus infections. *J. Virol. Meth.* **23**: 195-205.
- GROEN, J.; H.G.M. JORDANS; J.P.G. CLEMENT; E.J.M. ROOJAKKERS; F.G.C.M. UYTDEHAAG; J. DALRYMPLE; G. VAN DER GROEN and A.D.M.E. OSTERHAUS (1991). Identification of hantavirus serotypes by testing of post-infection sera in immunofluorescence and enzyme-linked immunosorbent assays. *J. Med. Virol.* **33**: 26-32.

- HEDMAN, K.; M. LAPPALAINEN; I. SEPPÄLÄ and O. MÄKELÄ (1989). Recent primary toxoplasma infection indicated by a low avidity of specific IgG. *J. Infect. Dis.* 159: 736-740.
- HEDMAN, K.; A. VAHERI and M. BRUMMER-KORVENKONTIO. Rapid diagnosis of hantavirus disease by an avidity assay for IgG antibody. (Submitted for publication).
- IVANOV, A.P.; E.A. TKACHENKO; V.A. PETROV; A.J. PASHKOV; T.K. DZAGUROVA; T.P. VLADIMIROVA; G.M. VORONKOVA and G. VAN DER GROEN (1988). Enzyme immunoassay for the detection of virus specific IgG and IgM antibody in patients with haemorrhagic fever with renal syndrome. *Arch. Virol.* 100: 1-7.
- LEE, H.W.; P.W. LEE and K.M. JOHNSON (1978). Isolation of the etiologic agent of Korean hemorrhagic fever. *J. Infect. Dis.* 137: 298-308.
- LEE, H.W. and G. VAN DER GROEN (1989). "Hemorrhagic fever with renal syndrome". In: *Prog. Med. Virol.* Ed. J.L. Melnick, 36: 62-102.
- LEE, H.W.; P.W. LEE; L.J. BAEK and J.K. CHU (1990). Geographical distribution of hemorrhagic fever with renal syndrome and hantavirus. *Arch. Virol. Suppl.* 1: 5-18.
- MEEGAN, J.M. and J.W. LEDUC (1989). "Enzyme immunoassays". In: *Manual of Hemorrhagic Fever with Renal Syndrome*. Eds. H.W. Lee and J.M. Dalrymple. WHO Collaborating Center for virus Reference and Research (Hemorrhagic fever with renal syndrome). Institute for Viral Diseases, Korea University: 83-87.
- MONATH, T.P. (1990). Ribavirin, interferon, and antibody approaches to prophylaxis and therapy for viral hemorrhagic fevers. *Curr. Opinion Infect Dis.* 3: 824-833.
- NÄRVÄEN, A.; M. KORKOLEINEN; F. LILLO; O. VARNIER; R. RESCALDANI; A. VISCONTI; E. DE GOURVILLE; A. VAHERI and M-L. HUHTALA. "Synthetic peptides derived from HIV-1, HIV-2 and HTLV-1 envelope proteins in human retroviral serology". In: *Rapid Methods and Automation in Microbiology and Immunology*. Eds. A.Vaheri, R.C. Tilton and E. Balows. Springer-Verlag, Heidelberg (in press).
- NIKlasson, B. and T. KJELLSON (1988). Detection of Nephropathia epidemica (Puumala virus)-specific immunoglobulin M by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 26: 1519-1523.
- NIKlasson, B.; E. TKACHENKO; A.P. IVANOV; G. VAN DER GROEN; D. WIGER; H.K. ANDERSEN; J. LEDUC; T. KJELSON and K. NYSTROM (1990). Haemorrhagic fever with renal syndrome: evaluation of ELISA for detection of Puumala-virus-specific IgG and IgM. *Res. Virol.* 141: 637-648.
- ROSSI, C.A.; C.S. SCHMALJOHN; J.M. MEEGAN and J.W. LEDUC (1990). Diagnostic potential of a baculovirus-expressed nucleocapsid protein of hantaviruses. *Arch. Virol.* 1: 19-28.
- SCHMALJOHN, C.S.; S.E. HASTY; J.M. DALRYMPLE; J.W. LEDUC; H.W. LEE; C-H. VON BONSDORFF; M. BRUMMER-KORVENKONTIO; A. VAHERI; T.F. TSAI; H.L. REGNER; D. GOLDVAGER and P.W. LEE (1985). Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science* 227: 1041-1044.
- SCHMALJOHN, C.S.; G.B. JENNINGS; J. HAY and J.M. DALRYMPLE (1986). Coding strategy of the S genome segment of Hantaan virus. *Virology* 155: 633-643.
- SCHMALJOHN, C.S.; A.L. SCHMALJOHN and J.M. DALRYMPLE (1987). Hantaan virus M RNA: coding strategy, nucleotide sequence and gen order. *Virology* 157: 31-39.
- SCHMALJOHN, C.S. (1990). Nucleotide sequence of the L genome segment of Hantaan virus. *Nucleic Acids Res.* 18: 6738-6740.
- SCHMALJOHN, C.S.; Y-K. CHU; A.L. SCHMALJOHN and J.M. DALRYMPLE (1990). Antigenic subunits of Hantaan virus expressed by baculovirus and vaccinia virus recombinants. *J. Virol.* 64: 3162-3170.
- STOHWASSER, R.; L.B. GIEBEL; L. ZÖLLER; E.K.F. BAUTZ and G. DARAI (1990). Molecular characterization of the RNA S segment of Nephropathia epidemica virus strain Hällnäs B1. *Virology* 174: 79-86.
- TKACHENKO, E.A.; A.P. TVANOV; G.V. REZAPKIN and S.G. DROZDOV (1989). "Immunosorbent methods for the detection of HFRS antigens and antibodies". In: *Manual of Hemorrhagic Fever with Renal Syndrome*. Eds. H.W. Lee and J.M. Dalrymple. WHO Collaborating Center for virus Reference and Research (Hemorrhagic fever with renal syndrome). Institute for Viral Diseases. Korea University, pp. 88-93.
- TOMIYAMA, T. and H.W. LEE (1990). Rapid serodiagnosis of hantavirus infections using high density particle agglutination. *Arch. Virol. Suppl.* 1: 29-33.

- TSAI, T.F.; Y.W. TANG; S.L. HU; K.L. YE; G.L. CHEN and Z.Y. XU (1984). Hemagglutination-inhibiting antibody in hemorrhagic fever with renal syndrome. *J. Infect. Dis.* **150**: 895-898.
- YANAGIHARA, R.; D. GOLDGABER; P.W. LEE; H.L. AMYX; D.C. GAJDUSEK; C.J. GIBBS and A. SVEDMYR (1984). Propagation of nephropathia epidemica virus in cell culture. *Lancet* **1**: 1013.
- YOO, D. and C.Y. KANG (1987). Nucleotide sequence of the M segment of the genomic RNA of Hantaan virus 76-118. *Nucleic Acids Res.* **15**: 6299-6300.
- VAN YPERSELE DE STRIHOU, C. and J.P. MÉRY (1989). Hantavirus-related acute interstitial nephritis in Western Europe. Expansion of a world-wide zoonosis. *Quart. J. Med. (New Series 73)* **270**: 941-950.
- VAPALAHTI, O.; H. KALLIO-KOKKO; E-M. SALONEN; M. BRUMMER-KORVENKONTIO and A. VAHERI. Cloning and sequence of Puumala virus Sotkamo strain S and M segments: evidence for strain variation in hantaviruses and expression of the nucleocapsid protein. (Submitted for publication).