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P1437 Simultaneous Detection of Mononucleosis Syndrome Serology Markers Using an EIA Dipstick System

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Objective and Methods: Dipsticks detecting IgG to Epstein-Barr virus viral capsid antigen (VCA), Epstein-Barr virus recombinant nuclear antigen type-1 (EBNA-1), cytomegalovirus (CMV) and toxoplasma and IgM to heterophile, EBV-VCA, and CMV are evaluated and compared to latex (heterophile), indirect immunofluorescence (IFA) and enzyme immunoassay (EIA) results.

Results: Six (1.8%) out of four hundred twenty-five samples submitted from patients with suspect mononucleosis syndrome were heterophile latex positive and the dipstick product classified all as positives. The corresponding EBV profiles supported EBV mononucleosis for all eleven samples. One (0.3%) additional sample is identified as a current or recent EBV infection based on VCA-IgG reactivity and EBNA-IgG nonreactivity. An additional 25 (7.6%) samples are CMV IgM reactive. Twenty samples (6.0%) that are neither EBV nor CMV reactive and toxoplasma IgG were detected.

Conclusions: Agreement between individual assay methods are all greater than 90%. Simultaneous detection of all EBV mononucleosis syndrome markers is feasible. Additionally, detection of a significant number of primary and/or recurrent CMV infections that presumably may cause the mononucleosis episode is illustrated.

P1438 Comparison of Immunofluorescence with Enzyme Immunoassay for Detection of Serum Immunoglobulin G Response to Human Herpesvirus 6

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Objectives: To evaluate indirect immunofluorescence assay (IFA) and enzyme immunoassay (EIA) for the detection of immunoglobulin G antibodies to human herpesvirus 6 (HHV-6).

Methods: We tested 250 serum samples by IFA (HHV-6 IgG IFI Kit, Bios GmbH, München, Germany) and EIA (HHV-6 IgG EIA, Biotrin International, Dublin, Ireland).

Results: The frequency of distribution of the samples according to IFA test titers and results obtained by EIA were:

IFA test titer	No. of serum samples	No. positive by EIA (%)	No. negative by EIA (%)
Negative	65	16 (24.6)	49 (75.4)
1/20	62	29 (46.8)	33 (53.2)
1/40	22	12 (54.5)	10 (45.5)
1/80	48	42 (87.5)	6 (12.5)
1/160	14	13 (93)	1 (7.1)
1/360	13	13 (100)	0
1/640	9	9 (100)	0
1/1280	5	5 (100)	0
Inspecific	12	12 (100)	0

Conclusions: We have found discrepancies in the results obtained by IFA and EIA, mainly when low titers resulted by IFA analysis.

Viral hemorrhagic fevers

P1439 Coagulation Disorders in Children with Viral Hemorrhagic Fever

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Objectives: Viral hemorrhagic fever e.g. Dengue hemorrhagic fever (DHF), is an acute febrile disease complicated by bleeding and/or shock. Activation of the coagulation cascade and fibrinolysis is studied in a group of children with severe hemorrhagic fever.

Methods: During the period July/October 1996 all children (28) admitted to the intensive care unit with the clinical diagnosis of Dengue hemorrhagic fever enrolled the study. Patients were clinically classified as DHF III and IV, according to WHO criteria. Markers of coagulation and fibrinolysis were sampled on three successive days, starting at the day of admittance and one sample on the day of discharge.

Results: Eight patients died. Mean age of all patients 6.5 years. Early in the disease there was an evident increase in markers of thrombin generation. (values at day of admittance, survivors vs. deaths). F1 + 2 fragments (median 2.4 vs. 5.9; $p < 0.05$; normal < 1.1 nmol/l), TAT complexes (median 22 vs. 81 mg/l; $p < 0.05$; normal < 4.1 mg/l), fibrinogen was decreased (mean \pm sd 1.6 \pm 0.8 vs. 1.4 \pm 0.3 g/l; ns; normal 1.7–4.0 g/l). Fibrinolysis increased during hospital stay, most obvious in the non survivors, measured as D dimers (third day after admittance, median 457 vs. 649 ng/ml; $p < 0.05$; normal < 39 ng/ml).

Conclusions: Hemorrhagic fever in children is characterized by disseminated intravascular coagulation (DIC), probably one of the contributive factors to the often dramatic outcome.

P1440 Disorders of Hemostasis and Fibrinolysis in the Hemorrhagic Fever

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In 57 patients suffering from hemorrhagic fever /Hantaa = Puumala virosis/hemostasis and fibrinolysis were followed. The analysis showed the frequent acceleration of trombocitopenia and fibrinolysis. The most frequent was trombocitopenia-at 32 patients, primary trombocitopenia 19, and secondary 13. Fibrinolysis was acceleration in 25 out of 57 observed patients, DIC was detected at two patients.

Primary fibrinolysis is more frequently/15/, secondary at 10 patients. It was more frequently verified at the onset of the disease, when the greatest acceleration was noticed 98/min with the approximate one of 116. In the later course of the disease, fibrinolysis was present together which the developed azotemia gave the grave course of disease. It was noticed that early fibrinolysis had more benign course. Also, trombocitopenia and fibrinolysis in this stage were shorter in duration and ended without the therapy, while the secondary with azotemia can provoke malignant bleeding, because of which it was necessary to apply antiplasmin in fibrinolysis, or heparin in DIC.

P1441 Viral Hemorrhagic Fever in Pregnancy

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Evolution of pregnancy and development of embryo during existence of infectious diseases has been the subject of many investigations due to pathological changes in pregnancy with numerous complication for a mother.

Within 1986–1995 we have treated 300 patients suffering from Hemorrhagic Fever, type Hantaan (with kidney syndrome) and Congo-Crimean Fever (CCHF). The latter had severe course with evident consumable coagulopathy manifested by hemorrhagic shock and abundant skin and mucous membrane bleeding and also bleeding from gastro-intestinal organs.

Only four pregnant women suffering from CCHF (table 1) were registered.

Age	Ordinal Pregnancy	Grav. (months)	Duration of illness (days)	Result
29	6	VIII	8	Ex Ex
30	8	VI	10	Ex Ex
39	7	IV	8	Ex Ab
24	4	III	3	Ab

In the eight month of pregnancy we have premature baby dies immediately after being born: mother dies 8 days later. In the sixth month a baby is born dead: mother dies 10 days later. In the fourth month embryo is dead: mother dies 8 days later. The fourth pregnant woman was in third month of pregnancy, had abortion at home followed by rich bleeding at the beginning of illness and after being treated at our clinic she survived and has been cured without negative results.

We conclude that CCHF is very severe acute infectious disease for mother and her foetus, too. There is also danger for medical staff and family members due to high risk of transmitting virus by blood.

P1442 Serological Evidence of the Presence of Hantaviral Serotypes in Montenegro

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The aim of this study was to assess the circulation of different Hantaviral serotypes in Montenegro in 1995.

Hantavirus infection has been explored for almost 30 years. These viruses are maintained in nature as chronic infection and cause diseases in humans every five to ten years.

In 1995, we treated 128 patients (Clinical Hospital Centre, Ward of Infectious Disease). Clinical picture varied from mild to severe. Sera from 128 patients were examined by IFA using different hantaviral antigens (Institute of Virology, Belgrade, S. Tomanović).

The serological investigation showed the following results: diagnosis was serologically confirmed in 82% cases. The seropositivity was found in a high percentage: both serotypes Hantaan (HTN) and Puumala (PUU) in 14%, HTN in 58%, and PUU in 20% cases.

In conclusion we can say that HTN serotype infection ranked first. The findings for both serotypes (HTN, PUU) corresponded to the severity of illness. PUU infection caused mild form of disease.

P1443 Cellular Immune Defence in the Pathogenesis of Hemorrhagic Fever with Renal Syndrome (HFRS)

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Objectives: The aim of our study was to measure proinflammatory cytokines that may have a role in the pathogenesis of HFRS.

Methods: We use EIA-test (Quantikine, R&D Systems Inc., UK) for the detection of IL-2, IL-2sR α , IL-6 and IL-6sR in sera of 41 HFRS patients. Samples were collected among the Croatian soldiers during the greatest HFRS outbreak in Croatia in 1995. During the outbreak, we were looking for immunophenotypic changes in the main lymphocyte populations and activation markers in 22 HFRS patients.

Results: We found elevated levels of IL-2sR α , IL-6 and IL-6sR in patients with HFRS in comparison to healthy controls. IL-2sR α and IL-6 showed negative correlation with the day after the onset of HFRS. In 14 patients 2sR α positively correlated with the CD25, an early activation marker (detected during the study in 1995). In the same patients we also found positive correlation among the IL-6 and CD20⁺CD21⁺ double-positive B-lymphocytes, CD23, B-cell activation marker and CD4⁺ and CD8⁺-lymphocytes simultaneously expressing both CD45RA and CD45RO markers.

Conclusions: We found an increase of tested proinflammatory cytokines in the early phase of HFRS. Also we could consider that measured cytokines acts as an autocrine and paracrine factors, driving the expansion of antigen-specific lymphocytes and influencing the activity of the other cells within the immune system. Our next aim would be to correlate clinical and biochemical data with the immune response, and to look for their role in the prognosis of HFRS.

P1444 Evaluation of Two Commercial Dengue Fever Assays verses an IgM Specific Neutralization

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Objective: To assess the specificity and sensitivity of two commercial DFV assays in comparison to IgM specific viral neutralization.

Method: A panel of 30 selected low IgM positive samples provided by the CDC, San Juan, P.R. and 5 normal sera from a non-endemic region were tested. Prior to performing the neutralization assay, IgG was removed by treating the samples with goat anti-human IgG. The commercial assays compared included the MRL Diagnostics μ capture ELISA and Genelabs Diagnostics IgM Blot. Samples were tested per kit manufacturers' instructions.

Results: The thirty IgM low positive samples all had specific IgM neutralizing antibodies. Two samples had only type 1 DFV neutralizing antibodies, 11 had only type 2, 1 had only type 4. There were no samples with type 3 DFV neutralizing antibodies. The remaining 16 samples had antibodies specific to more than one DFV. All five normal samples were negative. Viral neutralization was detected using IFA. The MRL Diagnostics μ capture ELISA detected IgM in 29 of the 30 samples for sensitivity of 96.7%. The Genelabs Diagnostics Blot detected IgM in 20 of 30 for a sensitivity of 66.7%; seven samples were indeterminate. Both the MRL and the Genelabs assays had 100% specificity.

Conclusion: The MRL μ capture ELISA showed significantly better correlation with IgM viral neutralization than the Genelabs Blot.

P1445 Dengue Shock Syndrome (DSS): Liver Involvement in Fatal versus Non Fatal Cases

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Objectives: To get clinical pictures and laboratory findings concerning liver involvement in DSS.

Methods: Cross sectional study. Fifty children DSS patients admitted to Dr. Kariadi Hospital from June 24, 1996 to December 10, 1996, were evaluated for physical examination, laboratory tests: SGOT, SGPT, Total protein, Albumine, Alkaline phosphatase, Gamma-GT and Prothrombin time. We also measured TNF level in 13 out of 50 DSS cases.

Results: Hepatomegaly was found in 88% of cases. Laboratory findings fatal vs non fatal cases were: the median of SGOT was (143 vs 76) u/l, SGPT (41.50 vs 26) u/l, Alk. Phosphatase (115 vs 138) u/l, Gamma-GT (31 vs 17) u/l, Total protein (4.50 vs 4.65) g/dl, Albumine (3.30 vs 3.50) g/dl, Prothrombin time (18 vs 14.6) seconds. The TNF data from 13 cases showed, that there seems to be very high level in fatal cases.

Conclusions: 1. Hepatomegaly was found in 88% cases of DSS. 2. High level of SGOT, SGPT, low level of total protein and albumine, and prolonged of prothrombin time were found in DSS, but more remarkable in fatal cases. 3. The level of alkaline phosphatase and Gamma-GT were relatively normal. 4. There seems to be very high level TNF in fatal cases. 5. The correlation between the prolonged of prothrombin time and high level of TNF needs further investigation.

P1446 C1Q Induces Conformational Changes into the NP of Lassa Virus Antigen

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It was shown that a guinea pig complement, being added to antigen in Lassa virus ELISA-test, intensified sensitivity and specificity of the assay. The analysis of mice antibodies spectrum, involved in this phenomenon, demonstrated that the intensification of method's sensitivity as much as 8-16 times took place in polisterol plate sensibilization with antibodies of IgG2a and IgG2b subclasses and when the complement was added to antigen.

On the basis of footprinting data it was proved, that after binding to antigen-antibody immune complex, the complement induced conformational changes in antigen.

Footprinting and ELISA data permitted us to assume, that the C1q component of the complement and MAbs of various isotypes, specific to NP protein of Lassa virus, induced changes in antigen in different ways, affecting various antigen-active sites.

Miscellaneous viruses

P1447 False Positive Results in the Serologic Diagnostic of the Human Parvovirus B19 Infection

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Parvovirus B19 (PVB) is a DNA virus recently implicated in a wide spectrum of human diseases. IgM antibody detection constitutes the easiest and most available diagnostic procedure for recent infections.

From January 1993 to December 1995, our laboratory received the request to determine IgM-PVB (Parvo-B19 EIA IgM test, MarDx Diagnostics) in 584 clinical samples. Of these, 95 samples from 65 patients were positive. We were able to find adequate clinical information in 46 cases. Twelve cases (26%) were considered "false positive": Ten adults with liver transplantation with acute CMV infection (positive viral culture), a child with measles infection (clinical presentation and seroconversion) and one case with EBV infectious mononucleosis (IgM). The remaining 34 cases had clinical manifestations well described in relation to PVB: Seven were pediatric patients, the most of them with erythema infectiosum, 7 healthy young women presented arthralgias or erythematous-rash illness, 16 were HIV-positive patients with persistent anemia and 4 other patients had hematological disorders. Most frequent signs were: anemia (58%), skin lesions (47%), fever (38%) and arthralgias (20%).

The high rate of "false positive" in our study warns against over-interpretation of the results.

P1448 Persistence of Human Parvovirus B19 DNA in Bone Marrow of Healthy Donors

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Objectives: To investigate the presence of human parvovirus B19 (B19V) in bone marrow of healthy bone marrow donors.

Methods: Presence of B19V DNA was tested in blood and/or bone marrow samples obtained from a total of 45 healthy bone marrow donors using a nested polymerase chain reaction assay (nPCR). The serological status of the donors was determined by enzyme immunoassay (EIA).

Results: B19V DNA was detected in the bone marrow of 4 out of 45 donors (9%). Among serum samples available from 39 donors none tested positive for B19V DNA, 28 (72%) contained anti-B19 IgG antibody only as a sign of past infection, and none contained anti-B19 IgM antibody. Anti-B19 IgG antibody was detected in each serum sample available for 3 of the 4 individuals with B19-DNA in bone marrow.

Conclusions: These results indicate that B19V may persist in the bone marrow of 11% (3/28) of donors with serologically documented past B19V infection.

P1449 The Polyomyelitis in Kosovo and Metohia in 1996 Year

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In places where antiepidemic measures were not applied properly and hygienic conditions of life are bad, epidemic form of disease are possible. The aim of this work is to point on differency of clinical picture and epidemiological characteristics epidemy of polyomyeli-