Acta virol. **36**: 304-312, 1992

SEROLOGICAL DIAGNOSIS OF PARAINFLUENZA VIRUS INFECTIONS: VERIFICATION OF THE SENSITIVITY AND SPECIFICITY OF THE HAEMAGGLUTINATION-INHIBITION (HI), COMPLEMENT-FIXATION (CF), IMMUNOFLUORESCENCE (IFA) TESTS AND ENZYME IMMUNOASSAY (ELISA)

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Received October 5, 1990; revised September 18, 1991

Summary. - Using four serological tests paired sera were examined of 117 patients with acute respiratory diseases, in whom parainfluenza viruses (PIV) infection was demonstrated by virus isolation, and of 41 patients with typical clinical mumps symptoms. Comparative analysis showed the high sensitivity of IFA and ELISA. A significant rise of antibodies in convalescent sera with homologous antigen of PIV was found in nearly 100 percent of cases. Only the sera of youngest children with high titres of persisting maternal antibodies remained without seroconversion. Cross heterologous antibody responses could be found by means of ELISA in 45 % and by IFA in 10 %, of patients who in the past experienced infection with one or more PIV or mumps virus - apart from homologous antibody reaction. HI and CF test proved to be less sensitive for detection of postinfections antibodies, especially in primoinfections with PIV types 1 and 2.

Key words: parainfluenza; cross-reactions; enzyme immunoassay; immunofluorescence

Introduction

Laboratory diagnosis of parainfluenza virus infections is not easy. Only virus isolation or detection of viral antigens directly in clinical specimens allow to determine the type of parainfluenza virus (PIV) which caused the disease. Antigenic relationship between the individual types of human PIV and known antigenic relationship with mumps virus (PaV) considerably impairs interpretation of the results obtained by serological examination. (Chanock *et al.*, 1963; Lennette *et al.*, 1963; Julkunen, 1984; Lehtonen and Meurman, 1986; Glikmann and Mordhorst, 1986; Vuorinen and Meurman, 1989.)

This paper reports the results of examining the paired sera from patients in whom PIV infection was confirmed by virus isolation. We tried to define the sensitivity and specificity of several serological tests. For comparison, the same procedure was used to examine the sera of mumps patients. In addition, we tried to find out which cross-reacting antibody responses occur in infection with various PIV types, and tentatively recommend the test or tests preferably to be used in the routine diagnostic.

Materials and Methods

Samples and sera. In the period from 1983 to 1988 a systematic virological and serological study was conducted in children and adults with clinical diagnosis of acute respiratory diseases (ARD). The group of 117 patients followed in this study included cases in which PIV ifection was demonstrated by virus isolation. The sera were collected in the acute phase of illness up to day 5 since the appearance of first clinical symptoms and at 10–27 days thereafter. The sera of 41 patients with mumps were collected at the same intervals.

Virus isolation of PIV was performed from nasopharyngeal swabs on secondary monkey kidney cells. PaV were isolated on continuous line of Vero cells (Lennette and Schmidt, 1979).

Viruses. Parainfluenza virus (PIV) type 1, strain C-35, PIV type 2, strain Greer, PIV type 3, strain C-243, PIV type 4, strain M 25 and mumps virus, strain Enders. All strains were supplied by Microbiol. Assoc. MD., Bethesda, U.S.A. PIV type 1 and 4 were passaged in primary or secondary monkey kidney cells, PIV type 2 and 3 in LLC-MK 2 cells, mumps virus in chicken embryos.

Serological tests. Haemagglutination-inhibition (HI) and complement-fixation (CF) tests were performed by means of standard WHO recommended micromethods (Lennette and Schmidt, 1979).

Immunofluorescence test (IFA) was performed using the indirect method. Suspension of secondary monkey kidney cells infected with PIV and a suspension of Vero cells, infected with mumps virus were used as antigens. The conjugates, SwAHu/IgG FITC and SwAHu/IgM FITC (SEVAC, Czechoslovakia) were diluted consistently with the control tests. The specific fluorescence was evaluated according to the intensity of the granular fluorescence in the cytoplasm of infected cells. Antibody titre in human sera was given by serum dilution with fluorescence intensity ++/+++. The human sera were diluted from 1 : 8 upwards. (Brown *et al.*, 1970.)

Enzyme immunoassay (ELISA) was performed by indirect method on flat bottom plates (IMUNOLON B, Dynatech). The mumps virus antigen was prepared as described by Grubhoffer *et al.* (1987). PaV antigen was concentrated by means of PEG 6000 (Serva) precipitation and purified by ultracentrifugation in a linear sucrose gradient. The parainfluenza virus antigens were prepared in a similar way, with certain modification. All antigens were lyophilized and immediately before use in ELISA sonified (2 min.). The antigens were diluted according to box-titration; 1 ml of diluted PaV antigen contained 5 μg of proteins, PIV antigens 7–10 μg. The quality of the antigens was evaluated electrophoretically in SDS PAGE, the specificity of the antigens in cross test with specific guinea-pig antisera. The human sera were serially double-diluted from 1 : 200 upwards.

The antigens, and a day later also the antisera, were adsorbed on microplates in an amount of 0.1 ml per well at 4 °C for overnight. The conjugates, horse-radish peroxidase-labelled swine antibody to human IgG or IgM (SwAHu/IgG Px and SwAHu/IgM Px - SEVAC Czechoslovakia) were diluted according to the previous titration with each antigen. As a substrate, 5-aminosalicylic acid (Fluka) was used. The test was evaluated photometrically using an Automatic ELISA Reader (Dynatech) by absorbance of samples *in situ* at 450 nm.

Results

Serologically examined were altogether 41 patients with mumps and 117 patients with ARD in whom infection with PIV was demonstrated by virus isolation. PIV type 1 infection was diagnosed in 36, PIV type 2 in 32, PIV type 3 in 46 and PIV type 4 in 3 cases. Age distribution of the followed cases is given in Table 1. Paired sera from all patients were examined with four antigens (PIV type 1, 2, 3 and PaV) in four serological tests; HI, CF, IFA and ELISA. In IFA and ELISA specific immunoglobulin G (IgG) and M (IgM) were determined. As the criterion of positivity in all tests the significant i.e. fourfold or higher increase of antibodies in the convalescent sera was considered.

The results of serological examinations are given in Table 2. Generally, infections due to all four PIV types and PaV showed a significant increase of antibodies in convalescent sera not only with the homologous antigen, but frequently also with one or more antigens of other types of PIV or PaV.

In the sera of patients infected with PIV type 1 heterological cross-reactions with PIV type 3 antigen (in HI, CF, IFA, ELISA) and with PaV antigen (ELISA) were found. On the other hand sera of patients with PIV type 3 infection cross-reacted with PIV type 1 antigen (CF, IFA, ELISA) and with PaV antigen (ELISA). Sera of patients with PIV type 2 infections cross-reacted with PIV type 3 and PaV antigens (HI, ELISA). In one of three cases of PIV type 4 isolations there was a positive seroconversion with PaV antigen (ELISA). In patients with mumps frequent cross-reactions with all three types of PIV occurred (Table 3).

Table 1. Age destribution of 117 patients in whom parainfluenza virus infection was demonstrated by virus isolation, and 41 patients with manifest mumps virus infection

Age	Number of patients infected with the virus:				
	Parainfluenza				Mumps
	type 1	type 2	type 3	type 4	
2 - 6 months	1	2	6	-	-
7 - 12 months	5	5	6	1	-
13 - 23 months	6	3	12	-	-
24 - 35 months	5	5	7	-	8
5 - 5 years	13	9	8	-	17
6 - 15 years	4	5	3	2	12
16 - 30 years	2	3	4	-	4
Total:	36	32	46	3	41

Table 2. Summarized results of serological examinations in 114 cases with confirmed parainfluenza virus and in 41 mumps cases

Viral++ agents	Number of cases	Test	Number of seroconversions+ with parainfluenza (PIV) and mumps virus (PaV) antigens												
			PIV type 1	PIV type 2	3	PaV	PIV 1+3	type 2+3	1 +PaV	2 +PaV	3 +PaV	1+2 +PaV	1+3 +PaV	2+3 +PaV	Neg.
PIV type 1	36	HI	8		6		5								17
		CF	10		4		6								16
		IFA	33				3								0
		ELISA	24				7		2				3		0
PIV type 2	32	HI		18	2			2		1				1	8
		CF		21											11
		IFA		31											1
		ELISA		24				2		3				2	1*
PIV type 3	46	HI			44										2*
		CF			40		1								5
		IFA			43		2								1
		ELISA			38		3						3		2*
Mumps virus	41	HI				26			1	7	3			4	0
		CF				33				6	2				0
		IFA				35				5	1				0
		ELISA				26			1	7	4	1		2	0

++ = positive PIV isolation; + = seroconversion – fourfold or higher rise of antibodies in convalescent sera; * = high titres of persisting maternal antibodies in both sera; HI = haemagglutination-inhibition test; CF = complement-fixation test; IFA = immunofluorescence test; ELISA = enzyme immunoassay.

Table 3. Cross-reactions between human parainfluenza virus type 1, 2 and 3, and mumps virus. Evaluated according to the number of significant increases of antibodies in convalescent sera

Viral agents	Number of cases	Number of seroconversions with parainfluenza (PIV) and mumps virus (PaV) antigens in test											
		HI				IFA-IgG				ELISA-IgG			
		PIV 1	PIV 2	PIV 3	PaV	PIV 1	PIV 2	PIV 3	PaV	PIV 1	PIV 2	PIV 3	PaV
PIV type 1	36	13 **36 %**		11 31 %		36 **100 %**		3 8 %		36 **100 %**		10 28 %	5 14 %
PIV type 2	32		22 **69 %**	5 16 %	2 6 %		31 **97 %**				31 **97 %**	4 13 %	5 16 %
PIV type 3	46			44 **96 %**		2 4 %		45 **98 %**		6 15 %		44 **96 %**	3 7 %
Mumps virus	41	1 2 %	11 27 %	7 17 %	41 **100 %**		5 12 %	1 2 %	41 **100 %**	2 5 %	10 24 %	6 15 %	41 **100 %**

Table 4. Homologous and heterologous antibody response in patients infected with parainfluenza virus type 1, 2 and 3. Evaluation of age-dependent sensitivity and specificity of the tests

Age	Viral agents		Number of cases	Antibody responses in patients paired sera in tests											
				HI			CF			IFA-IgG			ELISA-IgG		
				Ho.	He.	Neg.	Ho.	He.	Neg.	Ho.	He.	Neg.	Ho.	He.	Neg.
2–35 months	PIV	type 1	17	2	2	13	3	1	13	17	0	0	16	1	0
		type 2	15	7	0	8	5	0	10	14	0	1	14	0	1*
		type 3	31	29	0	2	26	0	5	30	0	1	28	1	2*
		Total:	63	38	2	23	34	1	28	61	0	2	58	2	3*
				60 %	3 %	37 %	54 %	2 %	44 %	**97 %**		3 %	**92 %**	3 %	5 %
3–30 years	PIV	type 1	19	6	9	4	7	9	3	16	3	0	8	11	0
		type 2	17	11	6	0	16	0	1	17	0	0	10	7	0
		type 3	15	15	0	0	14	1	0	13	2	0	10	5	0
		Total:	51	32	15	4	37	10	4	46	5	0	28	23	0
				63 %	29 %	8 %	72 %	20 %	8 %	90 %	**10 %**		55 %	**45 %**	

Ho. — homologous antibody response-only with antigen of virus isolated from the patient; He. — heterologous antibody response-with both homologous and heterologous antigen, or with heterologous antigen of another PIV type or PaV only; Neg. — negative seroconversion; * — high titres of maternal antibodies in both sera.

Test sensitivity and number of cross-reactions depended on the age of the patients (Table 4). Paired children sera were divided into two age groups. The first group contained sera of younger children (aged 2–35 months), the second group consisted of sera of older children including 7 adults. In the first group of 63 younger children a high percentage of sera with negative seroconversion in HI (37 %) and CF (44 %) tests was recorded. These sera were predominantly obtained from children with PIV type 1 and 2 isolations. The same sera, if tested in IFA or ELISA, were positive in high number of cases. Homologous antibody response was demonstrated 61 times (97 %) by IFA and 58 times (98 %) by ELISA. Without seroconversion remained only infant sera of patients up to 3 months of age with persisting maternal antibodies. Cross-reactions were found by ELISA in only 2 children (3 %). In the second group of 51 older children an adults negative seroconversion was found only 4 times by HI and CF tests (8 %). Nevertheless, in all serological tests high percentage of cross heterologous antibody responses was recorded: in ELISA 45 %, in HI 29 %, in CF 20 % and in IFA test 10 %. In ELISA and IFA a significant increase of antibodies, together with homologous and heterologous antigens was recorded; in HI and CF tests often only with heterologous antigens. Most specific results were obtained by means of immunofluorescence. If the antibody titre was evaluated according to the fluorescence intensity of the granules of replicating virus in the cytoplasm of infected cells, specific fluorescence with homologous antigen only was diagnosted 46 times (in 90 %). Cross-reactions were found only 5 times (10 %), and, in contrary to other tests, the antibody increase with the homologous antigen was many times higher than with the heterologous one.

From the point of differential diagnosis cross-reactions with PaV antigen were important (HI, ELISA-IgG). Yet, in sera of patients with PIV infection a significant increase of IFA-IgG and IgM or ELISA-IgM antibodies with mumps virus antigen could never be demonstrated. Without seroconversion, was also the CF test with soluble ribonucleoprotein PaV antigen (Orion, Finland).

Discussion

The problem of cross-reactions and assumed antigenic relationship between human and animal Paramyxoviruses has been known since the 60'ies. Yet, only approximately in the early 80'ies, when studies on structural proteins purified by various biochemical methods had been started, and monoclonal antibodies introduced, the relatedness between the individual PIV types and PaV could be demonstrated (Goswami and Russel, 1982, 1983; Örvell *et al.*, 1986; Ito *et al.*, 1987). The present, so far incomplete data indicate that a certain antigenic relationship exists among all types of human PIV and that it would be difficult to select some of the structural proteins as an antigen for type-specific serological

diagnosis. Julkunen (1984) used virion envelope glycoproteins and nucleoprotein antigens in ELISA. Yet, the number and the nature of cross-reactions in paired sera from patients with both different antigens could be evaluated only with difficulties, as the actual infective agent was not known.

The disadvantage of the present study was that we had no opportunity to examine the paired sera of confirmed parainfluenza virus infections with antigens prepared from at least some main structural proteins. Nevertheless, our results in ELISA with purified whole-virion antigens yielded some new data. They confirmed that ELISA was highly sensitive, and demonstrated that young children with some PIV primoinfections experience seroconversion only with homologous antigen which was simultaneously isolated from the patient. Seroconversion occured almost at 100 % but was negative in children with maternal antibodies persisting in high titres. In addition to seroconversion with homologous antigen, numerous cross-reactions with heterologous antigens were present in the sera of older children or adults with previous history of infection or reinfection with one or more type of PIV or PaV.

It is interesting that immunofluorescence test proved to be equally sensitive as ELISA and gave more specific results. The disadvantage of IFA test has been in its laboriousness, in the impossibility of automation and the necessary experience of the virologist at reading the result. However, IFA test can be applied as check method in cases of positive seroconversion in ELISA. HI and CF tests were little sensitive especially in PIV type 1 and 2 primoinfections. Both tests are less suitable for routine serological diagnostics.

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