The Czech National External Quality Assessment of Monoclonal Immunoglobulin in the Period of 1996 – 2005

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The aim of the presented study was to evaluate the results of SEKK "Gammopathy" (GP) control cycle (Czech National External Quality Assessment) that assessed the success rate of monoclonal immunoglobulin determination by clinical laboratories for the 1996 – 2005 period.

The study summarizes the results of 20 "Gammopathy" control cycles during the ten-year period. Control cycles were repeated every 6 months.

Patients who provided samples for individual SEKK "Gammopathy" control cycles were selected during routine diagnostic process in the University Hospital Hradec Kralove. Correct paraprotein typing in both A and B control samples (plasma, serum or urine) is required prior to certification. Assessment of paraprotein concentration is optional.

The number of participating laboratories was gradually increasing from 26 in 1996 to 79 in 2005 (including 6 Slovak laboratories). The majority of laboratories used immunofixation electrophoresis as the method of paraprotein typing. In 2005, only one laboratory was still using immunoelectrophoresis. Typing was successful in approximately 70% of cases during the first 3 cycles and the success rate gradually increased to almost 96% by 2005. The only exception was GP 1/02 cycle with a sample of relatively rare IgD-lambda paraprotein and the success rate of 38% only. A sample of plasma without paraprotein was distributed 4 times. Several laboratories falsely identified fibrinogen as paraprotein each time.

Results of "Gammopathy" control cycle for the past 10 years confirmed the value and legitimacy of this control cycle in the system of external quality control of SEKK laboratories.

Key words: monoclonal immunoglobulin, paraprotein, external quality control, gammopathy

Monoclonal gammopathy (MG), also referred to as paraproteinemia or dysproteinemia, is a group of disorders defined by proliferation of one or more B-lymphocyte clones producing immunologically homogenous immunoglobulin called paraprotein or monoclonal immunoglobulin [1]. This M-protein is composed of either complete immunoglobulin molecule or of isolated monoclonal immunoglobulin light chains or (very rarely) of isolated immunoglobulin heavy chains. Monoclonal gammopathies are a very heterogeneous group of disorders [2, 3] which can be basically divided into two major categories – Malignant Monoclonal Gammopathies (MMG) and Monoclonal Gammopathies of Undetermined Significance (MGUS). Multiple myeloma (accounts for 1% of all malignant disorders and 10-15% of hematologic malignancies) is the most common and most serious MG.

Paraprotein (M-protein) is a tumor marker specific for MG and reflects clonal production of immunoglobulin. It is the oldest tumor marker described by Bence Jones as early as in 1847. Because of the importance of paraprotein diagnosis, particularly in patients with MMG, it has been included in the SEKK system of external quality control 10 years ago.

Methods

The first "Gammopathy" control cycle was performed in 1996. Since then, control cycles have been organized twice

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a year by SEKK s.r.o., Czech Republic. Control samples are collected by laboratories of the Institute of Clinical Biochemistry and Diagnostics as well as Institute of Clinical Immunology and Allergic Disorders of Medical Faculty and University Hospital in Hradec Kralove, Czech Republic. Patients suitable for sample collection for the purpose of individual SEKK "Gammopathy" control cycles are selected during the regular diagnostic process performed in the laboratory. Following consultation with a physician, the selected patient is informed about the collection of the blood sample (50 – 100ml) or about the use of the sample collected during therapeutic plasmapheresis. The patient is also offered a reward for the sample collected. If the patient agrees, the sample is collected at an agreed date. The sample is centrifuged and acquired plasma or serum is divided in two portions. The first portion (at a volume of approximately 2ml) is used for preliminary verification analyses, the second (remaining and greater) portion is poured into a suitable airtight container. The control sample is protected from bacterial contamination by the addition of sodium azide (at an amount that ensures sodium azide concentration of 0.01%). The sample is stored at –30°C until the date of the respective control cycle. The control sample is always tested for the following infection markers: HbsAg, Treponema pallidum antibodies, anti-HIV 1,2 as well as p24 Ag and anti-VHC. Samples are distributed among laboratories signed up to the respective control cycle (the control samples A and B were always obtained from different patients) along with the request for determination of paraprotein presence or absence and for its typing. – i.e. determination of paraprotein immunoglobulin class and antigenic type of light chains. Compliance with this requirement is a prerequisite for the issuance of the certificate. Determination of paraprotein concentration from electrophoresis densitometric analysis or from the capillary electrophoresis curve is optional (Cappillarys, SEBIA; Paragon 2000, Beckman). We used ALTM (All Laboratory Trimmed Mean) with tolerance limit ± 33% for evaluation of success in paraprotein quantification.

Results

The results of 20 already performed "Gammopathy" control cycles are summarized in Table 1. The first two cycles were performed in 1996 and were characterized by a low number of participating laboratories and relatively low accuracy (76% resp. 69%). The majority of laboratories performed paraprotein typing by immunofixation. However 6 laboratories in the first cycle and 4 laboratories in the second cycle still used immunoelectrophoresis. Only about 20% of laboratories were successful in the quantification of paraprotein during these two cycles. 7 laboratories falsely identified fibrinogen as paraprotein during the typing of paraprotein-free plasma in cycle 92/97. The success rate of typing and quantification increased to approximately 90% and 54% respectively during both 1998 cycles. Therefore, certificates have been issued since 1999 for successful typing of paraprotein in both samples (A and B). In 1999, typing was successful in both cycles and determination of paraprotein concentration improved significantly to 56% and 74% respectively. The success rate of quantification was very good in both cycles (around 70%). In 2001, 7 laboratories (10%) falsely determined the presence of paraprotein in paraprotein-free sample of plasma in the first control cycle. In 2002, a sample-containing IgD-lambda was distributed to laboratories during the GP 1/02 control cycle. This was successfully determined by the lowest number of laboratories (38%) throughout the whole duration of "Gammopathy" control cycles. Samples with low concentration of paraprotein were used in cycle GP1/03. This was reflected by the low accuracy of quantification, which was successfully performed by only 31% of laboratories. Quantification was very successful (94%) in GP2/03 cycle, during which samples with high paraprotein concentration were assessed. The 2004 and 2005 cycles were also successful in both typing and determination of paraprotein concentration.

Discussion

Immunoelectrophoresis was a method of choice for the typing of monoclonal immunoglobulins for over 30 years. This method was relatively complicated, both in its procedures and even in the interpretation of its results. Immunofixation electrophoresis was introduced into our clinical laboratories some 10-12 years ago. Compared to immunoelectrophoresis, this method is easier to perform, saves time (approximately 2 hours vs. 2-3 days necessary for immunoelectrophoresis) and ensures easier interpretation of results. For the above reasons, immunoelectrophoresis was performed by a relatively low number of Czech laboratories. The majority of laboratories quickly replaced immunoelectrophoresis by immunofixation. Despite the fact that immunofixation is a relatively simple method, it requires considerable experience from both the lab technician as well as from scientist or physician. This method has several pitfalls, which can (particularly in laboratories performing immunofixation of only several samples per month) be the source of erroneous or inaccurate results [4, 5]. For the above reasons and with respect to the need for gradual standardization of laboratories, "Gammopathy" control cycle was included in the SEKK control system in Czech Republic. Interest of laboratories in this control cycle has gradually increased and the number of participating labs has increased to 80 from the original 26. For control, samples of plasma, serum and also urine are distributed, because paraprotein typing in urine samples has its specifics. The concentration of protein in the urine must be frequently adjusted by dilution or concentration of samples in order to achieve the desired 1.5 – 2.0 g/l. Attempts are also made to include all paraproteins in the control cycle. So far, the following paraproteins have been included: IgG-kappa, IgG-lambda, IgA-kappa, IgM-kappa, IgM-lambda, IgD-lambda, kappa free and lambda free. A sample of plasma

Table 1. The Czech National External Quality Assessment of Monoclonal Immunoglobulin in the Period of 1996-2005.

Year	Survey	Number of Laboratories	Sample		Qualitative determination			Quantitative determination		
					Paraprotein	Successful [%]		Concentration [g/l]	Successful [%]	
						One sample	Both samples		One sample	Both samples
1996	82/96	29	A	Plasma	IgG-lambda	93	76	21,5	77	23
			B	Urine	kappa	76		0,4	27	
	83/96	26	A	Plasma	IgM-kappa	88	69	37.9	64	21
			B	Urine	free kappa	77		2.66	36	
1997	92/97	40	A	Plasma	Negative	80	70	–	73	33
			B	Urine	free kappa	80		0.22	40	
	93/97	39	A	Plasma	IgG-lambda	100	90	48.5	84	40
			B	Urine	free kappa	90		7.67	48	
1998	096/98	57	A	Plasma	free kappa	93	88	8.05	53	40
			B	Urine	free kappa	95		0.978	65	
	097/98	49	A	Plasma	IgM-lambda	96	86	33.8	89	54
			B	Urine	free kappa	90		1.46	63	
1999	GP1/99	59	A	Plasma	Negative	97	95	–	56	56
			B	Plasma	IgA-kappa	97		53.7	98	
	GP2/99	54	A	Plasma	IgG-lambda	98	91	16.30	100	74
			B	Urine	IgG-lambda	91		1.28	74	
2000	GP1/00	72	A	Plasma	IgG-lambda	99	90	49.2	98	72
			B	Urine	free kappa	90		1,04	72	
	GP2/00	53	A	Plasma	IgM-kappa	100	96	35.6	95	68
			B	Urine	free kappa	96		2.14	70	
2001	GP1/01	70	A	Plasma	Negative	89	86	–	87	45
			B	Urine	free lambda	97		0,828	49	
	GP2/01	60	A	Plasma	IgG-lambda	100	95	45.2	98	54
			B	Urine	IgG-lambda	95		0,844	57	
2002	GP1/02	73	A	Plasma	IgD-lambda	38	38	3.30	90	75
			B	Urine	free lambda	95		0,689	69	
	GP2/02	61	A	Plasma	IgM-kappa	98	93	58,5	98	55
			B	Urine	free lambda	93		6,06	55	
2003	GP1/03	78	A	Plasma	IgG-lambda	92	89	1.46	49	31
			B	Urine	free lambda	95		0.113	54	
	GP2/03	64	A	Plasma	IgM-kappa	89	89	57.80	94	94
			B	Plasma	IgG-lambda	100		42.20	98	
2004	GP1/04	79	A	Plasma	IgG-kappa	92	92	30,7	99	96
			B	Plasma	IgG-lambda	97		42.3	97	
	GP2/04	66	A	Plasma	Negative	95	91	–	–	60
			B	Plasma	IgM-lamda IgG-kappa free lambda	94		62,3 2.08 1,15	91 64 60	
2005	GP1/05	79	A	Plasma	IgG-lambda	99	91	1.98	75	n.a.*)
			B	Plasma	IgM-kappa	92		14.20	87	
	GP2/05	66	A	Plasma	IgG-kappa	97	96	23,2	100	n.a.*)
			B	Urine	free kappa	98		1.84	69	

*) Starting 2005 the success is calculated strictly separately for each sample in quantitative determination.

without paraprotein has been included several times. Despite this fact, several laboratories determined incorrect typing of fibrinogen gradient and classified is as paraprotein each time. The concentration of paraprotein varied between 62.3 g/l (GP2/04, B) and 1.46g/l (GP1/03, A). Sample GP1/02 A was very problematic. It was collected from a patient with multiple myeloma and included paraprotein IgD-lambda + free lambda. Correct typing was performed by only 19 of 73 participating laboratories. Another 9 laboratories stated that such samples (with isolated immunoglobulin light chains) are forwarded to other laboratories that can perform immunofixation with IgD and IgE antiserum. Results were regarded as correct in all 28 laboratories (38%). This sample was also intended to point out at the difficulties associated with diagnosis of IgD myeloma, which is only rarely considered. Samples A and B from the GP1/03 cycle reflected the requirements of American Association of Clinical Pathologists that suggest occasional inclusion of samples with marginal concentration

of paraproteins in the control cycle [4]. This concentration is 1g/l of paraprotein in plasma and 0.15 g/l in urine (this equals to the level of physiologic proteinuria). This control was very successful as it was passed by almost 90% of participating laboratories. Optional step of "Gammopathy" control is the determination of paraprotein concentration. On average, these results are submitted by 2/3 of laboratories (approximately 68%). This field is very difficult to standardize, however, the results and comparability of laboratories are constantly improving. Determination of paraprotein plasma concentration is influenced by many factors, which can have significant effect on the results (subjective evaluation of M-gradient, type of densitometer used, use of capillary electrophoresis, overlapping with other plasma proteins, etc). Quantification of paraprotein in urine is even more difficult. Results are influenced particularly by the applied method of proteinuria quantification. Despite the above facts, this optional part of "Gammopathy" control cycle is beneficial, because the success increased to 70-90% from the original 21% in participating laboratories.

To a certain extent, our "Gammopathy" control system can be compared to French "National External Quality Assessment of Monoclonal Immunoglobulin". This cycle has been running in France since 2001 and is based on once yearly assessment of one serum sample [6]. Since 2004, it has been organized in association with the Scientific Institute of Public Health (Belgium). 6 French university laboratories have been selected as references for this control cycle. Samples are collected during therapeutic plasmapheresis. The number of French laboratories participating in the first cycle during 2001 reached 1118 and increased to 1423 in 2004. The number of Belgian laboratories reached 152 in 2004. The review of paraprotein typing methods during 2004 is interesting and worth mentioning. 95.8% of French laboratories used immunofixation, 2.6% used immunoelectrophoresis and 1.5% used capillary electrophoresis. As for the Belgian laboratories, 80.6% used immunofixation, 3.9% used immunoelectrophoresis and 15.5% used capillary electrophoresis. The control cycle was initiated in France in 2001 with a very difficult sample of paraprotein IgD-lambda and corresponding success rate of 34.9%, which was very similar to our GP1/02 cycle – 38%. In the following years, the following paraprotein samples were assessed: 2002 IgA-kappa, 2003 IgM-lambda and 2004 again IgM-lambda. Success rate was 96.6%, 90.9% and 96.3%, which is very similar to the accuracy of paraprotein typing in our laboratories during the past two years. In the Czech Republic, only one laboratory used immunoelectrophoresis in 2005. All other laboratories performed immunofixation electrophoresis (SEBIA, DAKO, Beckman Coulter, The Binding Site).

The results of "Gammopathy" control cycle presented for the past 10-year period confirmed the value and legitimacy of this control cycle and substantiated its introduction. This control cycle substantially contributed to the improved determination and to certain extent also quantification of monoclonal immunoglobulins in Czech and Slovak (6 labs) clinical laboratories.

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