Lessons from diagnostic investigations of poliomyelitis patients and their direct contacts for the present surveillance of acute flaccid paralysis

Tineke Herremans, Marion Koopmans, Harrie van der Avoort, Anton van Loon

Submitted for publication
Abstract

One of the key strategies for the global eradication of poliomyelitis is the virological investigation of stool samples from all cases of acute flaccid paralysis (AFP) to exclude poliovirus as a possible cause. However, adequate virological investigation of the stool specimens from these patients is carried out in fewer than the required number of cases, especially in industrialised countries. Poliomyelitis cannot be excluded in all of these cases. Other diagnostic assays using both immunological and molecular biological methods have been developed within the last decade for the detection of poliovirus infection. Clinical specimens from the 1992/93 poliovirus serotype 3 outbreak in The Netherlands provided an opportunity to examine the potential of the various methods for the diagnosis of poliomyelitis. The virus isolation rate in poliomyelitis patients was maximal (89.6%) during the first two weeks after the onset of paralysis and then dropped sharply to 18.6%. In contrast, a high percentage of the patients tested positive for serotype 3-specific IgM (93.3%) and IgA (87.1%) in the early phase of the infection and remained positive up to eight weeks after the onset of paralysis. While the serotype 3-specific IgM rate dropped to 20% at 8 weeks after infection, the poliovirus-specific IgA persisted in many patients (56.7%) for more than 8 weeks after infection and is therefore less suitable for the diagnosis of recent infections. The use of virus isolation would have correctly identified only 54.9% of AFP cases in the theoretical event that all of these cases were due to poliovirus infection. The identification rate would have been increased to 92% through the use of the poliovirus-specific IgM ELISA. Virus isolation from stool specimens will remain the standard diagnostic method. The speed and ease of performance of the IgM ELISA, however, could allow it to serve as an important additional tool for the rapid diagnosis of poliomyelitis. Moreover, the IgM ELISA may facilitate the resolution of AFP cases when stool investigation has been less than adequate or in cases analysed late after the onset of disease.

Introduction

In 1988, the World Health Organisation (WHO) launched an initiative to eradicate poliomyelitis by the year 2000 [10]. One of the key strategies for the global eradication of poliomyelitis is the virological investigation of stool samples from all cases of acute flaccid paralysis (AFP) to identify poliovirus as a possible cause [29]. Acute flaccid paralysis (AFP) is characterised by the rapid onset of muscle weakness in the extremities, or in some cases the muscles involved in respiration and swallowing. The maximum severity of symptoms is apparent within one to ten days post-onset [30]. AFP can have numerous other causes apart from poliovirus infection, including infections with other enteroviruses, acute myelopathy, peripheral neuropathy (such as the Guillain-Barré Syndrome) and neuromuscular transmission disorders [30].

The incidence of AFP in the absence of poliomyelitis is estimated to be at least one case per 100,000 children younger than 15 years of age [3]. The timely collection of adequate stool samples from AFP cases is considered crucial for viral detection, and the collection of two samples 48 hours apart within 14 days after onset of paralysis is used by the WHO as performance criteria for the quality of the AFP surveillance [30]. However, compliance with these criteria is limited. This is especially true in industrialised countries where, because of the absence of poliomyelitis cases, clinicians do not always recognise the need for intensive AFP surveillance [10].
Both our laboratory [15,20] and other groups [11,14,21,22,25,32] have developed poliovirus serotype-specific IgM- and IgA-enzyme-linked immunosorbent assays (ELISAs) and methods for the detection of poliovirus based on RT-PCR. While most of these assays have been used in seroprevalence or epidemiological studies, they may also be useful for the rapid diagnosis of poliovirus-related illness as well as for a resolution of cases reported late after the onset of disease in the AFP surveillance system. Therefore, this study re-examines specimens that have been collected from patients and their contacts during a poliovirus serotype 3 outbreak in The Netherlands in 1992/1993 [7,23,27].

Stool samples, sera, cerebrospinal fluids (CSF) and/or throat swabs were examined from most of the 71 patients from this outbreak. In addition, stool specimens and sera from 86 family members or close contacts of the poliomyelitis patients were examined for poliovirus excretion and poliovirus-specific antibodies. This article describes the application of this broader panel of diagnostic assays in order to determine whether these tests may provide an added value for the surveillance of AFP as a crucial step in the poliomyelitis eradication program.

Material and methods

Patients and clinical samples

Seventy-one patients were notified during the outbreak of type 3 poliomyelitis in The Netherlands in 1992/1993 [23]. Clinical samples from 69 poliomyelitis patients were available for analysis in the laboratory. One or more serum samples were collected from 69 cases (97.2%), one or more stool samples were available from 58 patients (81.7%), a CSF sample was obtained from 44 poliomyelitis cases (62.0%) and throat-swabs were collected from 25 patients (35.2%).

Contacts of poliomyelitis patients

Stool and serum samples were obtained from 86 contacts of 25 poliomyelitis patients. These specimens were examined for the presence of poliovirus in the stool samples as well as for the presence of poliovirus-specific IgM and IgA antibodies in the serum samples. Poliovirus-Binding Inhibition assays were performed to determine levels of poliovirus type 3-specific binding antibodies [16].

Acute Flaccid Paralysis (AFP) surveillance

Recent AFP surveillance data from The Netherlands were used to calculate the added value of the new diagnostic methods in the present AFP surveillance. Surveillance of AFP among children younger than 15 years of age in The Netherlands started in 1992. The measured incidence of non-polio AFP was between 0.39 to 0.87 per 100,000 children [8,24]. This was lower than the required non-polio AFP incidence of 1:100,000, indicating inadequate surveillance [8,24]. The median reporting time of AFP cases in The Netherlands was 91 days [8]. None of the non-polio AFP cases met the required stool sampling criterion of two samples taken 24-48 hours apart within the first 14 days after onset of paralysis. Virus isolation was carried out in only a low percentage (31%) of the AFP cases [8].
Isolation of poliovirus from stool and throat swabs

Virus isolation and typing was carried out on HEp-2C and RD cells as recommended by the WHO [29]. Poliovirus strains were differentiated as wild- or vaccine-derived strains in an enzyme immuno assay using intra-typic cross-absorbed rabbit antibodies [28]. The wild-type character of all of these isolates was confirmed by RNA sequencing [19].

Poliovirus serotype-specific IgM and IgA ELISAs

The ELISAs for poliovirus serotype-specific IgM or IgA antibodies in serum from patients and contacts were performed as described [15,20]. Poliovirus-specific IgM was also determined in patients’ CSF [20]. Inactivated poliovirus was used as the antigen in both assays. A positive and a negative control serum sample were included in each assay.

Reverse Transcriptase (RT)-PCR for the detection of poliovirus in CSF

A generic enterovirus-specific RT-PCR was used to examine CSF for the presence of polioviruses as described [26]. Viral RNA was purified from the CSF samples by binding to silica particles in the presence of GuSCN [5].

Poliovirus-Binding Inhibition assay (PoBI)

A Poliovirus-Binding Inhibition assay was used to determine the poliovirus-specific antibody titers as an indicator of neutralising antibodies. The PoBI assay was performed as described [16]. The reciprocal of the first serum dilution that was positive in the inhibition test was taken as the titer of the test sample.

Results

Virus isolation from poliomyelitis patients

Stool samples from 58 poliomyelitis patients were examined for the presence of serotype 3 poliovirus at different times after the onset of paralysis. Stool samples provided during the first two weeks after the onset of paralysis were most frequently positive (89.6%) [Figure 1, Table 1]. The virus isolation rate dropped sharply to approximately 20% in samples provided between three to ten weeks after the onset of paralysis. No virus was isolated from any of the stool samples collected more than ten weeks after the onset of disease [Figure 1, Table 1]. The first stool sample examined was poliovirus serotype 3 positive in 50 of 54 polio patients (84.7%); the accumulating positivity rate increased to 93.2% and 94.8% after examination of a second and third specimen respectively. Throat swabs were collected during the first two weeks after the onset of paralysis only (n=25). Virus was isolated from the throat of 32% of the patients during this period [Figure 1, Table 1].
Diagnostic investigations of poliomyelitis patients

Figure 1. Positivity rates of virus isolation from stool, and of serum IgM and IgA antibodies to poliovirus type 3 in poliomyelitis patients. Results are from consecutive serum samples collected during the 1992/1993 outbreak in The Netherlands.

Table 1. Sensitivity of assays per time period after onset of paralysis

<table>
<thead>
<tr>
<th>Assays</th>
<th>0-2 Weeks (%)</th>
<th>2-8 Weeks (%)</th>
<th>&gt; 8 Weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stool</td>
<td>89.6</td>
<td>18.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Throat</td>
<td>31.0</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Serology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>89.5</td>
<td>93.9</td>
<td>33.3</td>
</tr>
<tr>
<td>IgA</td>
<td>60.7</td>
<td>87.1</td>
<td>56.7</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>63.6</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>0.0</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>
Detection of poliovirus-specific IgM in poliomyelitis patients

Serotype 3-specific IgM was detectable in all serum samples from patients obtained between three to six weeks after the onset of paralysis (n=24); a high percentage was also positive before this period (89.5%) [Figure 1, Table 1]. Twenty percent of the patients had low but detectable levels of virus-specific IgM three months after the onset of paralysis.

Detection of poliovirus-specific IgA in the serum of poliomyelitis patients

Sera from 49 patients were available for the determination of poliovirus-specific IgA. The kinetics of the IgA response in patients have been reported elsewhere [15] and show that the IgA peak is maximal between three to eight weeks after the onset of paralysis. Five months after the onset of paralysis, 33 (60%) of the patients still had detectable type 3 specific IgA in their circulation [Figure 1, Table 1].

Examination of the CSF of poliomyelitis patients

A CSF sample collected during the first two weeks after the onset of paralysis was available from 44 patients. Poliovirus type 3-specific IgM was present in 28 (63.6%) of these CSF samples [Figure 1, Table 1]. All matching serum samples were also positive for serotype 3-specific IgM. Sera from fourteen patients (31.8%) without IgM in their CSF was positive for serotype 3-specific IgM.

Twenty-one CSF samples from poliomyelitis patients were examined by RT-PCR for the presence of poliovirus RNA [Figure 1, Table 1]. Viral RNA was not detected in any of these samples, whereas the same extracts yielded positive RT-PCR signals after spiking with poliovirus.

Comparison of IgM, IgA and virus culture in contacts

A comparison of assays was possible for 86 contacts of poliomyelitis patients with a complete diagnostic data set (virus isolation, IgM, IgA and PoBI). A total of 68 stool samples were positive for the epidemic wild-type serotype 3 strain in culture (79%). Fifty-nine (68.6%) and 46 (53.5%) contacts were positive for serotype 3-specific IgM and IgA respectively. Eighty-five (67.5%) contacts were positive in the IgM-ELISA and secreted poliovirus. Only ten (11.6%) contacts were excreting poliovirus in the absence of an IgM response. Seventy-seven (89.5%) contacts had PoBI titers to serotype 3.

The prevalence of serotype 3-specific IgM antibodies was high (86.1%) in the young age groups (three to ten years) and declined with age [Figure 2]. The prevalence of serotype 1- and 2-specific IgM was below 10% in all age groups. Virus excretion in the stool was high (96.7%) within the age group ranging from three to 15 years, and followed the same decreasing pattern with age as did the IgM seroprevalence for serotype 3 [Figure 2C]. In contrast, poliovirus-specific IgA followed the same patterns as the poliovirus-specific antibodies for all three serotypes [Figures 2A and 2C]. The presence of serotype 1-specific IgA, serotype 2-specific IgA and poliovirus-binding antibodies (PoBI) increased with age and was highest in the oldest (31-80 years) age group (46.2-53.8%) [Figures 2A and 2B].
Figure 2. Prevalence of IgM, IgA and neutralizing antibodies to poliovirus type 1 [Fig 2A], type 2 [Fig 2B] and type 3 [Fig 2C] in contacts of poliomyelitis patients of different ages.
Calculation of the theoretical benefit of the application of IgM and IgA ELISAs for AFP surveillance in The Netherlands

Virus isolation was attempted during the first to the seventh week after the onset of paralysis in 31% of all AFP cases reported in The Netherlands in 1995 and 1996. This virus isolation was carried out within the recommended time period of two weeks after the onset of disease in only 51% of these cases. The sensitivity of virus isolation is 89.6% during this time period [Figure 1], which theoretically leads to 45.8% correctly diagnosed AFP cases in the virologically investigated group [Figure 3A]. Stool samples from 49% of these cases were examined between three to seven weeks after the onset of paralysis. The chance of successful isolation during this period is 18.6%, leading to a calculated percentage of correct poliovirus diagnoses in the virologically-examined AFP patients of only 9.1% [Figure 3A]. Theoretically then, the overall virological investigation of these AFP cases could only yield a correct diagnosis in 54.9% of the virologically examined cases, or 17% of all reported AFP cases.

A similar calculation was performed for the IgM ELISA [Figure 3B]. Only 15% of the reported AFP cases were serologically examined by IgM ELISA. In the first two weeks after the onset of paralysis, IgM-expected positivity rates were almost identical to the rates of virus isolation from the stool (89.5%). Expected rates remained high (93.9%) three to seven weeks later, leading to an additional 46.0% of theoretically correctly diagnosed cases. The overall percentage of theoretically correctly diagnosed AFP cases with the IgM ELISA was 92.0%.

Discussion

Historically, the diagnosis of poliomyelitis has relied on poliovirus isolation. Virus isolation is still the diagnostic method of choice and was, until recently, the only method that could reliably distinguish between wild or vaccine-virus infection. However, several new diagnostic methods have been developed in recent years that have not been previously evaluated under field conditions due to the lack of poliomyelitis cases. An epidemic of serotype 3 poliovirus in The Netherlands in 1992/1993 provided the opportunity to examine the potential of a number of these new methods [7,23,27].

Serotype 3 poliovirus from the outbreak was isolated most frequently from the stool of poliomyelitis patients at one to two weeks after the onset of paralysis (89.6%) but positivity rates dropped sharply thereafter to a level of less than 20%. As has been previously described, 63% to 93% of serotype 1 stool samples test positive during the first two weeks after the onset of disease [2]. During the third and fourth weeks after onset, isolation frequencies for serotype 1 generally decline to a range of 35% to 75%. Specimen positivity is usually below 50% after four weeks [2]. There is limited data on the duration of excretion of wild strains of poliovirus types 2 and 3 [2]. However, our study shows that poliovirus type 3 excretion seems to be about two weeks shorter than that of serotype 1 [2].

In this study, poliovirus was isolated from the throat in only 32% of patients during the first two weeks after the onset of disease. Because poliovirus is excreted from the intestine for several weeks after infection, it is most frequently isolated from the stool samples. These stool samples are, therefore, the most suitable materials for isolation of poliovirus, confirming WHO criteria [29].
Diagnostic investigations of poliomyelitis patients

Figure 3. Calculations of the number of correct exclusions of poliomyelitis cases by A] virus isolation and B] IgM-ELISA.

The poliovirus-specific IgM ELISA does not discriminate between vaccine and wild-type poliovirus-induced antibodies. However, our data show that in addition to virus isolation from the stool, the detection of poliovirus serotype-specific IgM in AFP patients may facilitate the laboratory diagnosis of poliomyelitis and may help to exclude poliovirus as the causative agent. The detection of poliovirus-specific IgM has the additional value of indicating proof of a recent infection with poliovirus even in the absence of virus isolation. Seven cases (10%) of poliomyelitis were diagnosed solely on the basis of IgM serology during the 1992/1993 outbreak in The Netherlands. Virus-specific IgM in poliomyelitis patients could be detected for at least six weeks longer than virus could be isolated from the stool [Figure 1]. An additional advantage of the IgM-ELISA lies in the fact that it provides a result within 24 hours compared to the five to seven days required for routine virus isolation and typing. Poliovirus serotype 1- and 2-specific IgM antibodies were detected in a small number (<10%) of patient contacts. The presence of these IgM antibodies is probably due to circulating OPV vaccine strains that were offered to control the epidemic.
The percentage of samples with serotype 3-specific IgM positive results was remarkably lower in CSF (63.0%) than in serum samples (89.5%). In addition, all patients with serotype 3-specific IgM-positive CSF had virus-specific IgM in their serum. Similar results were described by Roivainen et al [25] who reported that 58% of the patients in their study had detectable poliovirus-specific IgM in their CSF, while 94% tested positive for poliovirus-IgM in serum. These results indicate that there is no additional diagnostic benefit of poliovirus-specific IgM detection in CSF.

Poliovirus RNA could not be detected with an enterovirus-specific RT-PCR in the CSF samples of poliomyelitis patients. This is in contrast to results obtained in the diagnosis of infections with other enteroviruses, where infection can easily be confirmed in CSF by PCR and/or virus culture [1,6,9,18]. In conclusion, laboratory examination of CSF either by PCR or by IgM assay is not recommended for the laboratory confirmation of poliomyelitis, although it may be useful for the determination of other causes of paralytic disease.

Not much is known about how polioviruses are able to cross the blood brain barrier. Data from transgenic mouse experiments shows that polioviruses permeate through the blood brain barrier at a high rate, independently of the poliovirus receptor [31]. It has been proposed that poliovirus-infected monocytes that cross the blood brain barrier into the CNS are a possible route [12,17].

As for IgM, the detection of serotype 3-specific IgA antibodies was much more sensitive than was virus isolation from the stool for diagnosis of poliovirus infection between two to eight weeks after the onset of illness. However, the presence of IgA to serotype 3 might also reflect past rather than recent infection because of the persistence of the IgA isotype in the bloodstream after infection. For this reason, the detection of poliovirus-specific IgA is less suitable for the detection of recent poliovirus infection.

Previously, we described that the seroprevalence of poliovirus-specific IgA was significantly lower in young IPV-vaccinated children compared to older IPV-vaccinated individuals and to a group of naturally exposed persons. These results could not be explained by the IPV vaccination schedule [15] and we therefore hypothesised that the presence of circulating IgA in the population of The Netherlands reflects past contact with live poliovirus (wild poliovirus or OPV vaccine strains) rather than vaccination with IPV [15]. In the present study, we found a similar age-related increase of poliovirus-specific antibodies to serotype 1 and 2 in these contacts (determined with the PoBI) and poliovirus-specific IgA for serotype 1 and 2 in up to 53.8% of the contacts of poliomyelitis patients. Because these contacts belong to the same community that rejects vaccination, these IgA levels probably indicate natural exposure at a level similar to that observed in the general Dutch population. The serotype 1 outbreak that occurred within the risk group in 1978 [4,13] can explain the presence of antibodies to serotype 1 poliovirus. During this outbreak, serotype 1 circulated extensively, while at the same time, monovalent OPV type 1 was offered to the community at risk [4].

One of the strategies used for the eradication of poliomyelitis is the surveillance of patients with AFP. Performance criteria have been established to provide compelling evidence for high quality AFP surveillance. Adequate AFP case investigation includes the collection and laboratory investigation of two stool samples within 14 days after the onset of paralysis [30]. There is a long delay in reporting AFP cases in The Netherlands, which makes AFP surveillance in its present form insufficient as a tool
Diagnostic investigations of poliomyelitis patients

for the documentation of the absence of poliovirus. Only 31% of the reported AFP cases were virologically examined in The Netherlands [8], implying that poliovirus infection could not be excluded with certainty in 69% of these cases [24]. In addition, a further 12.4% of the virologically examined group could not be successfully diagnosed due to late sampling. When serum samples are available, the IgM ELISA may be helpful in resolving those cases of AFP that cannot be definitely classified as poliomyelitis retrospectively.

Reliable diagnostic virology depends upon the timely and correct collection of clinical specimens. Although stool samples should be collected as soon as possible, preferably within the first two weeks after the onset of paralysis, this does not always occur. Therefore, other methods such as the poliovirus serotype-specific IgM ELISA could serve as an important complementary tool for the diagnosis or exclusion of poliomyelitis in AFP patients, especially in the post-eradication era. Although the poliovirus-specific IgM assay cannot replace virus isolation from a clinical case, the method has proved to be very helpful for the rapid diagnosis of poliomyelitis and for the exclusion of poliovirus infection as the cause of AFP.

Acknowledgements

We greatly acknowledge the technical assistance of Johan Reimerink, Albert Ras, Jan Vinjé, Piet Poelstra, Herman Kooy, Ton Wermenbol, Ankje de Vries, Paul Oostvogel and Cecile Holweg. We would also like to thank the participating Municipal Public Health Services for their contribution of public surveys during the epidemic.
References


