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Review

# Post-poliomyelitis syndrome as a possible viral disease

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SUMMARY

This review summarizes current concepts on post-polio syndrome (PPS), a condition that may arise in polio survivors after partial or complete functional recovery followed by a prolonged interval of stable neurological function. PPS affects 15–20 million people worldwide. Epidemiological data are reported, together with the pathogenic pathways that possibly lead to the progressive degeneration and loss of neuromuscular motor units. As a consequence of PPS, polio survivors experience new weakness, generalized fatigue, atrophy of previously unaffected muscles, and a physical decline that may culminate in the loss of independent life. Emphasis is given to the possible pathogenic role of persistent poliovirus infection and chronic inflammation. These factors could contribute to the neurological and physical decline in polio survivors. A perspective is then given on novel anti-poliovirus compounds and monoclonal antibodies that have been developed to contribute to the final phases of polio eradication. These agents could also be useful for the treatment or prevention of PPS. Some of these compounds/antibodies are in early clinical development. Finally, current clinical trials for PPS are reported. In this area, the intravenous infusion of normal human immunoglobulins appears both feasible and promising.


## 1. Introduction

Paralytic poliomyelitis is caused by infection with the poliovirus and dates back to the eighteenth Egyptian dynasty (c. 1543–1292 BC), or earlier. Large epidemics occurred in the early nineteenth century, but with the introduction of vaccination, the number of new cases dropped dramatically in the 1960s. Today, paralytic poliomyelitis has been essentially forgotten, both by people and the medical community. Nevertheless, large numbers of individuals who have survived the illness are alive today in the world. Resisting vaccination efforts, scattered poliomyelitis cases continue to surface each year in a few countries. These cases are holding back or hindering the expected goal of polio eradication.[1,2]

Abrupt asymmetrical flaccid paralysis is the clinical manifestation of anterior poliomyelitis, as acute polio may also present with other manifestations. After the acute attack, survivors experience a period of neurological and functional recovery followed by a phase of almost complete stability. During the stability phase, many patients become over-achievers, working hard both physically and emotionally to overcome their disabilities.[3] In 1875, new weakness, muscle atrophy, and fatigue occurring years after poliomyelitis were recognized by the French neurologist Raymond and his famous peer Jean-Martin Charcot.[4] Since the 1970s, a multitude of cases have been published worldwide, and in the 1980s, post-polio syndrome (PPS) was accepted as a new medical condition.[5,6] Whereas the late consequences of polio (i.e., biomechanical decline such as scoliosis, kyphosis, arthrosis, etc.) can manifest for any survivor of polio,[7] PPS (i.e., neurological decline) may develop in 20–75% of polio survivors, 15 to >60 years after acute paralytic or non-paralytic disease.[8–16]

Common manifestations of PPS include central and peripheral fatigue, muscle atrophy and weakness, musculoskeletal pain, and new disabilities that may also affect many other body functions such as respiration, the digestive tract, voiding, and sleep. It is estimated that there are 15–20 million polio survivors worldwide. Medicine has been slow to address the morbidity and cost of chronic disease and the growing number of elderly persons.[17] However, today PPS is recognized as the most prevalent disease of

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anterior horn motor neurons (http://www.post-polio.org). The US Social Security Disability Insurance (SSDI) program first acknowledged the late effects of poliomyelitis in 1987 (http://www.postpolioinfo.com). Subsequently, PPS was recognized by the European Parliament,[18] and a specific code for PPS (G14) was adopted by the International Classification of Diseases 2010.[19] In 2012, the problems of polio survivors were presented at the Commonwealth Parliament of Australia[20] and in the Italian Parliament.

In spite of the numbers of affected patients, the aetiology and pathogenesis of PPS are still unclear and no effective therapy is available.[21] Current treatments are based on a conservative approach consisting of exercise, avoidance of muscular overuse, orthoses, and assistive devices (http://www.post-polio.org; http://www.polioplace.org). Diagnosis is based on the medical history and clinical–instrumental examination, as well as on the exclusion of medical conditions other than polio that could explain the symptoms.[22]

## 2. Structure and genome of polioviruses

The three poliovirus (PV) types (PV1, 2, 3) belong to Enterovirus group C of the *Picornaviridae* family. Virions are non-enveloped icosahedral particles, about 28 nm in diameter. As shown in Figure 1, the genome consists of a single-stranded, positive-sense RNA of about 7.4 kb, with a 22-aa virus-encoded protein (viral protein genome-linked, VPg) covalently linked to the 5′ end.[23] The 5′ non-translated region (approximately 740 nt) has a complex secondary structure consisting of region 1 (regulatory) and region 2 that represents the internal ribosome entry site (IRES). The single open reading frame encodes a polyprotein of about 2200 amino acids that is processed to yield four different capsid proteins (viral proteins VP1, VP2, VP3, and VP4) and seven non-structural proteins (2A (protease), 2B (endoplasmic reticulum localization, viroporin), 2C (ATPase, helicase), 3A (Golgi localization), 3B (VPg), 3C (major viral protease), and 3D (RNA-dependent RNA polymerase). The 3′ non-translated region contains a variable poly-A tail of approximately 70 nt.

Recently, human enteroviruses have been re-classified, based largely on genome structure (Table 1). Excluding rhinoviruses, the Enterovirus genus contains four species of human pathogen (A, B, C, and D). PVs belong to the C species. Humans are the only natural host of PVs. Many different human cell types express the CD155 poliovirus receptor[24] that is essential for infection, possibly together with a co-receptor. All enteroviruses are quite resistant in the environment. Transmission occurs through the faecal–oral route and the respiratory route.

## 3. Acute poliovirus infection: poliomyelitis

Poliomyelitis is an acute disease caused by infection with any one of the three PV serotypes. The virus multiplies in the pharynx and intestine for 1 to 3 weeks. In the majority of cases, virus spread is contained by the local immune response. Thus, over 95% of infections are either asymptomatic or characterized by flu-like symptoms. In 5% of cases, a viraemia phase occurs and virus can cross the blood–brain barrier by ways that are possibly independent from the expression of poliovirus receptor (PVR).[24] Upon arrival in the central nervous system (CNS), patients may develop a meningitis-like illness characterized by fever with pharyngitis, myalgia, anorexia, nausea, vomiting, headache, and neck stiffness. The onset of spinal poliomyelitis is associated with myalgia and severe muscle spasms, with the subsequent development of an asymmetrical (predominantly lower limb) flaccid weakness that becomes paretic within a few days.[23] A purely bulbar form with minimal limb involvement may also occur. This form of polio has a particularly high mortality because of vasomotor disturbances and other complications (hypertension, hypotension and circulatory collapse, autonomic dysfunction, dysphagia, dysphonia, and respiratory failure).

In the epidemics of the last century, most paralytic cases were attributed to PV1. Epidemics of polio occurred throughout the USA and Europe, including one severe outbreak from 1943 to 1956 in which 400 000 people were infected, resulting in 22 000 deaths. The introduction of Jonas Salk's inactivated polio vaccine (1955) and Albert Sabin's live oral vaccine (1961) dramatically reduced the number of cases. In 1965, only 61 infections were reported in the USA and by 1991 the disease had been virtually wiped out in the Western Hemisphere. The massive MECACAR immunization program, launched in 1995, started to rapidly clear virus from the 18 countries with residual poliomyelitis, spanning two continents. In most of the world where the four core eradication strategies were introduced, the numbers of both cases of polio-paralyzed children and polio-infected countries began to fall rapidly. The sense that eradication might soon be inevitable was reinforced in 1999 by the global eradication of type 2 wild poliovirus. This suggested that the eradication of the other serotypes would follow quickly in all countries.[25]

New cases of poliomyelitis due to PV1 and PV3 have now been reduced to a few hundred per year. In 2014, cases were found in scattered countries such as Pakistan, Afghanistan, Nigeria, Somalia,

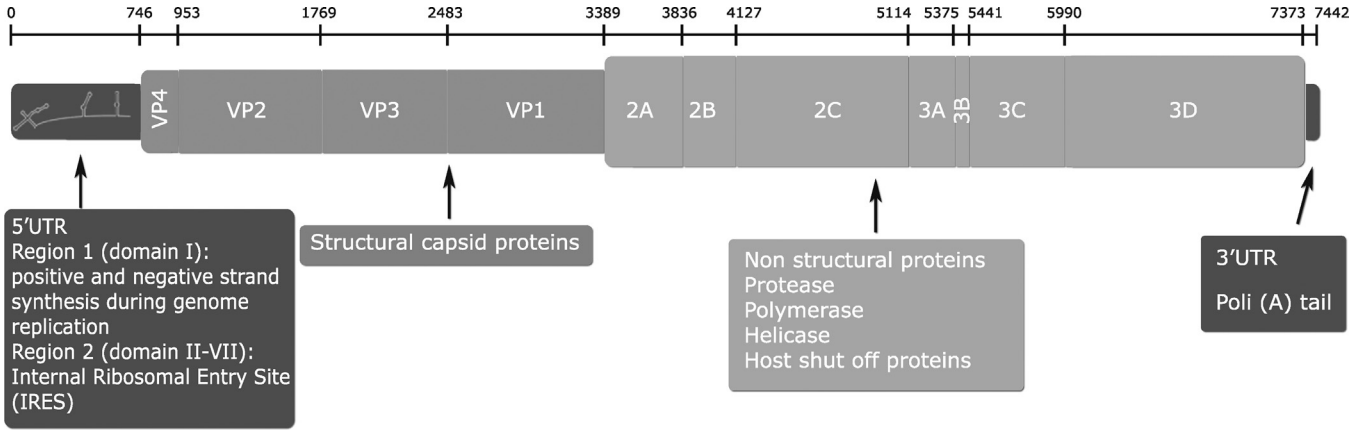


**Figure 1.** Schematic diagram of the poliovirus genome. Structural and non-structural virus-coded proteins are indicated. The 5′ non-translated genome terminus (5′-UTR) regulates virus replication and plays a fundamental role in the synthesis of viral proteins.

**Table 1**
Classification of human enteroviruses within the *Picornaviridae* family (excluding rhinoviruses)

| Species (No. of serotypes) | Serotypes |
|---|---|
| Enterovirus A (20) | CV-A2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16; EV-A71, 76, 89, 90, 91, 114, 119, 120, 121<br>CV-B1, 2, 3, 4, 5, 6; CV-A9 |
| Enterovirus B (56) | E-1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 26, 27, 29, 30, 31, 32, 33; EV-B69, 73, 74, 75, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 97, 98, 100, 101, 106, 107, 111 |
| Enterovirus C (13) | CV-A-1, 11, 13, 17, 19, 20, 21, 22, 24; EV-C95, 96, 99, 102, 104, 105, 109, 113, 116, 117, 118<br>PV-1, 2, 3 |
| Enterovirus D (3) | EV-D68, 70, 94, 111 |

CV-A, coxsackievirus group A; CV-B, coxsackievirus group B; E, echovirus; EV-D, enterovirus group D; PV, poliovirus. Modified from http://www.picornaviridae.com (2015).

Equatorial Guinea, Iraq, Cameroon, the Syrian Arab Republic, and Ethiopia. All of these areas have been affected by war, famine, and forced human migration (http://www.polioeradication.org; date of consultation: 2015-03-15).

## 4. Innate and adaptive response to poliovirus infection

In the early phases of infection, the innate response plays a prominent role.[26] Toll-like receptor-3 (TLR3), retinoic acid-inducible gene 1 (RIG-I), and melanoma differentiation-associated protein 5 (MDA-5) are the major cytoplasmic receptors of single-stranded and double-stranded viral RNA. Upon binding of the viral genome, these sensors activate interferon (IFN) regulatory factor 3, which is translocated into the nucleus and induces the expression of type I IFNs. IFNs secreted by infected cells bind to the receptors of neighbouring cells and activate multiple IFN-stimulated genes (ISG). Protein kinase R (PKR) and oligoadenylate synthetase 1 (OAS1) inhibit protein synthesis and virus replication, thus blocking or limiting virus replication and cell damage. In productively infected cells, PV-coded proteases (2A, 3C) degrade ISG proteins, PKR, and components of the RIG-I/MDA-5 pathway, thus curbing the antiviral effect of IFNs.

What has been described above is a dynamic response, thus the success of limiting local viral replication and virus spread is dependent on both the rapidity and magnitude of the innate response. In non-polio enteroviral infections, genetic polymorphisms of components of the innate response system decisively influence the virus susceptibility of mice.[27] Type I IFNs also stimulate the expression of surface major histocompatibility complex class I (MHC-I) molecules and the activation of natural killer cells, which are early antiviral effectors. Soon after infection, proinflammatory cytokines and chemokines are secreted by infected and bystander cells and play a critical role in both inflammatory processes and initiating the adaptive immune response.

Poliovirus infection and vaccination are followed by the production of neutralizing PV antibodies of the IgM, IgG, and IgA classes,[28] and by the appearance in peripheral blood of CD4 helper T-cells and CD8 cytotoxic/memory T-cells that recognize viral capsid epitopes.[29–31] These cells secrete IFN-γ in response to PV capsid proteins. Neutralizing antisera produced in humans and animals are type-specific (i.e., sera against PV1 do not neutralize PV2 or PV3) and are directed to PV neutralization epitopes that are conformational and have been described in great detail.[32,33] IgM and IgG antibodies appear 3–4 days after infection. Secretory IgA becomes detectable 1 week post-exposure.

It has recently become possible to produce human and chimpanzee neutralizing monoclonal antibodies specific to each PV serotype. Monoclonals capable of neutralizing two different serotypes have also been obtained. These monoclonals bind the capsid recognition site for the cellular receptor.[32,34–36] The recently obtained antibodies may be of particular value for human treatment (chronic PV carriers, possible polio epidemics, accidental exposure to PVs).

Anti-poliovirus immunity is protective and life-long. This is also true for polio victims, but only for the type of PV the victim had. Thus, vaccination is also advisable for polio survivors. A person is considered to be fully immunized if he/she has received a primary series of at least three doses of inactivated poliovirus vaccine (IPV), live oral poliovirus vaccine (OPV), or four doses of any combination of IPV and OPV (http://www.cdc.gov/vaccines/vpd-vac/polio/#vacc).

## 5. Persistent poliovirus infection in vitro and in vivo

To establish persistent infection, a virus must be able to reduce its cytopathic effects (i.e., its ability to kill or damage the infected cell), maintain its genome within host cells over time, and avoid elimination by the host immune system. Although PVs are cytolytic to their host cells, mainly due to the shutdown of the host transcriptional and translational machinery resulting in substantial inhibition of host cell metabolism,[37] some evidence suggests that persistent PV infection may be associated with PPS,[38–41] as well as with myasthenia gravis.[42]

A number of in vitro models of PV persistence have been reported: monkey kidney cells,[43] human cells such as the HEp2 line,[44] cells of neural and non-neural origin,[45] and a neuroblastoma line.[46] Cell cultures persistently infected by PVs show peculiar characteristics: (1) only a small percentage of cells express viral antigens, and (2) viral titres (i.e., the amount of infectious viral particles released by infected cells in the medium) are usually low ($\leq 10^3$ plaque-forming units/ml). In persistently infected cultures, and possibly in vivo, virus spread to uninfected cells is not only achieved by a lytic mechanism, but also by the release of host cell-derived microvesicles and the formation of cell protrusions or intercellular bridges.[47–49] The high genetic variation of these agents (high mutation rate of single-stranded RNA viruses and recombination events) certainly contributes to the selection of attenuated variants. In vitro, the possible role of poliovirus receptor mutations has been documented in neuroblastoma cells persistently infected with PV.[50] Experimental studies in mice have shown that PV may cause persistent infection and paralysis upon immunosuppression,[51] that infection can be traced to motor neurons,[52] and that hindered replication of the PV genome possibly contributes to PV persistence in the CNS.[53]

Genomic changes in persistent PV isolates (especially in the 5′-untranslated region (5′-UTR) and VP1 region) have been reported in immunodeficient individuals who are chronic carriers of the virus. In the majority of cases, the persisting virus has been recovered from the intestine.[54–61]

Persons with a primary humoral immunodeficiency (e.g., hypogamma/agammaglobulinemia), or an immunodeficiency secondary to HIV infection or other reasons, may suffer a chronic PV infection (not only upon infection with wild-type virus, but also following vaccination with attenuated virus). These individuals may release large amounts of virus for months or years.[54,57,62–64] In a study of our group in Italy, persistent PV1 infection of primary

cultures of human skeletal myoblasts was found to be associated with the upregulation of pro-inflammatory cytokines and chemokines (interleukin (IL)-1, IL-6, IL-8, monocyte chemoattractant protein 1 (MCP1; manuscript in preparation)). Thus, chronic infection may be accompanied by the upregulation of cytokines, as seen in PPS patients.[65]

## 6. Diagnostic criteria for PPS

Late functional deterioration after poliomyelitis is common, and unlike a typical syndrome where signs and symptoms are relatively constant, this takes many forms and has many causes. No disabilities are truly static. The causes of late deterioration may include those related to previous polio (orthopaedic, respiratory, peripheral nerve entrapment) and those related to subsequent illness. It is vital to diagnose comorbidities, as many can be resolved with simple treatment.

There are no diagnostic tests or specific biomarkers for PPS.[22,66,67] The early diagnostic criteria of Halstead represent the basis of the criteria presented by Trojan and Cashman[68] have been integrated into current diagnostic guidelines:[22] (1) Prior paralytic poliomyelitis with evidence of motor neuron loss, as confirmed by history of the acute paralytic illness, signs of residual weakness, and atrophy of muscles on neurological examination, and signs of denervation on electromyography. (2) A period of partial or complete functional recovery after acute paralytic poliomyelitis, followed by an interval (usually 15 years of more) of stable neurological function. (3) Gradual or sudden onset of progressive and persistent muscle weakness or abnormal muscle fatigability (decreased endurance), with or without generalized fatigue, muscle atrophy, or muscle and joint pain. Sudden onset may follow a period of inactivity, or trauma, or surgery. Less commonly, symptoms attributed to PPS include new problems with swallowing or breathing. (4) Symptoms persist for at least 1 year. (5) Exclusion of other neurological, medical, and orthopaedic problems as causes of the symptoms.

Symptoms are often mixed with the physiological changes of aging (e.g., decreased number of nerve cells, decline in vital capacity, decline in maximum heart rate and cardiac output, increased mean systolic blood pressure, decline in bone mineral mass, altered carbohydrate metabolism, and decline of immune functions). Health issues for ageing polio survivors and young polio survivors differ. In young survivors, the challenge is to prevent and treat severe deformities, to reduce disability, to improve social participation, and to prevent PPS. In aging polio survivors, treatment focuses on the management of PPS and neuromuscular decline, with the aim of preserving independence and quality of life.

## 7. Epidemiology: polio survivors and PPS

There is a growing need for health professionals with the knowledge to adequately treat the estimated 15–20 million people who have survived polio.[69] Polio survivors can be found in every country around the world, although those in the Western World tend to belong to an ageing group, whereas those in developing countries represent the whole age spectrum. For the diagnosis of PPS, clinicians should not simply require a history of paralytic polio and electromyographic evidence of denervation, but should also be aware that non-paralytic polio (and possibly even poliovirus-induced 'minor illnesses') can be associated with CNS damage and late-onset muscle weakness and fatigue.[14,15,70]

Data on the numbers of polio survivors per country and numbers of those who have developed PPS are based mostly on local estimates and are summarized in Table 2. For many geographic areas these data are totally missing. Data from polio associations and/or publications are available for some countries, including Australia (http://www.polio.org.au/about-polio-australia/),[71,72] Brazil,[73,74] Canada,[16] Denmark (http://www.polioplace.org/resources/ptu-danish-society-polio-and-accident-victims-landsforeningen-af-polio-trafik-og-ulykkessk),[13] France,[75] Germany,[76] Italy,[77,78] Japan,[79] Norway,[10] Poland,[80] Spain,[81] Sweden,[82,83] the UK,[84] and the USA (http://www.post-polio.org/ir-usa.html).[85] In some countries, the absence of data on the numbers of polio survivors may reflect problems of public health organizations as well as difficulties in confirming the diagnosis of PPS due to the symptoms being similar to those associated with natural aging. In fact, case ascertainment is sometimes difficult and may expose statistics to bias. Taken together, the data support the statement that at least 15 million polio survivors are alive worldwide and that the percentage of polio survivors developing PPS may vary widely from 20% to 75% depending on the criteria used for diagnosis and on the age of the groups investigated.

Figure 2 gives the estimated numbers of polio survivors in the USA with age distributions for the years 2006 and 2016. The data were obtained from the morbidity and mortality reports made to public health authorities by the United States Public Health Service (USPHS) taking into account life-expectancy. Numbers of polio survivors are probably underestimated, since many polio cases are underreported (mild or non-paralytic cases, cases treated outside

**Table 2**
Reported numbers of polio survivors in different countries and percentages of polio survivors developing PPS. An estimate is provided for the whole world based on population size

| Country | Population mid-2014 (millions) | No. polio survivors (paralytic and non-paralytic cases) | % Polio survivors | Reported prevalence of PPS among polio survivors (% range) |
|---|---|---|---|---|
| Australia | 23.5 | 400 000 | 1.74 | 20–45 |
| Brazil | 202.8 | 14 0000 | 0.07 | 68–78 |
| Canada | 35.5 | 30 000 | 0.08 | 20–40 |
| Denmark | 5.6 | 12 000 | 0.21 | 14–60 |
| France | 64.1 | 60 000 | 0.09 | 10–45 |
| Germany | 80.9 | 100 000 | 0.12 | 15–55 |
| Italy | 61.3 | 80 000 | 0.13 | 15–75 |
| Japan | 127.1 | 30 000 | 0.02 | 50–85 |
| Norway | 5.1 | 7500 | 0.15 | 14–85 |
| Poland | 38.5 | 30 000 | 0.08 | 25–80 |
| Spain | 46.5 | 45 000 | 0.10 | Not available |
| Sweden | 9.7 | 15 000 | 0.15 | 50–80 |
| USA | 317.7 | 640 000 | 0.20 | 25–40 |
| UK | 64.5 | 30 000 | 0.05 | 30–60 |
| Whole world (estimate) | 7238.0 | 15 000 000 | 0.21 | Not available |

PPS, post-poliomyelitis syndrome. Population data: http://www.prb.org/pdf14/2014-world-population-data-sheet_eng.pdf.

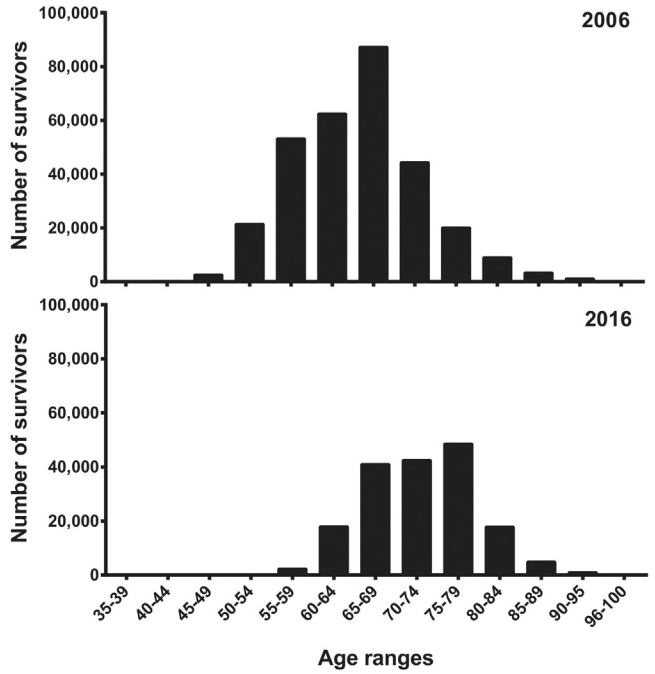


**Figure 2.** Predicted age distribution of polio survivors in the USA in the years 2006 and 2016 (source: Becker LC. Polio survivors in the U.S., 1915–2000. Age distribution data. Saint Louis, MO: Post-Polio Health International; 2006).

the official medical system, cases in subpopulations without easy access to physicians, and rare cases among immigrants). In fact, the National Center for Health Statistics (NCHS, of the Centers for Disease Control and Prevention), on analyzing data collected through the US Census Bureau process (1994–95), estimated the numbers to be higher (774 500 in 2006 and 573 000 in 2016). The trend indicates that in Western countries, future treatments will be directed at the ageing population, predominantly those in the age range of 60–80 years. In contrast, the youngest and largest proportion of these survivors reside in developing countries, and a disproportionate number of these are female.[86]

## 8. Pathogenesis of PPS

Pathological descriptions of PPS have been scarce and have emphasized the presence of persistent or new inflammation in the meninges, spinal cord, and muscles of affected patients.[87–92] At variance with amyotrophic lateral sclerosis, no ubiquitin-reactive inclusion bodies are present in anterior horn cells of PPS cases.[90] Axonal spheroids in anterior horns and moderate Wallerian degeneration in lateral columns have been reported.[91] Postmortem studies of 70 years ago documented frequent and severe poliovirus-induced lesions within the reticular activating system located in the brain stem that controls basic functions, including sleep, waking, and behavioural motivation.[93] In PPS patients, magnetic resonance imaging has revealed small discrete or multiple punctate areas of hyperintense signal in the reticular formation, putamen, medial lemniscus, or white matter tracts in 55% of the subjects reporting high fatigue and in none of those reporting low fatigue.[93] Thus, lesions in these areas might contribute to the generalized fatigue proper of PPS. The inflammatory changes found mainly in the spinal cord and in muscle suggest different pathogenic hypotheses, including persistent poliovirus infection, autoimmune attack on the central and peripheral nervous systems, and increased vulnerability of poliovirus-damaged tissue to further infectious events. In subjects with PPS, motor units are enlarged and symptoms are likely due to degeneration and dysfunction of terminal axons.[94]

### 8.1. Distal degeneration hypothesis

Following the original observation of Wiechers and Hubbell in polio patients,[95] Emeryk et al. reported the progressive disintegration of the motor units in individuals who were developing PPS.[96] A combination of distal degeneration of enlarged motor units due to increased metabolic demands and the normal aging process, as well as the ongoing inflammatory processes, were thought to be involved.[97] The most likely aetiology of new weakness is the distal degeneration of the abnormally enlarged motor units that form after poliomyelitis. This results in patchy denervation of muscle fibres (Figure 3). The denervated muscle fibres may become reinnervated from surviving motor neurons, producing a continuous 'remodelling' of the motor unit. In longitudinal studies with macro electromyography (macro-EMG), a continuous loss of neurons has been demonstrated, with exaggerated speed compared to normal age-dependent degeneration.[98] New weakness appears when reinnervation mechanisms are no longer sufficient and occurs when macro motor unit potential (MUP) exceeds 20-times the normal size. Macro-MUP amplitudes have been shown to be smaller in post-polio muscles with new weakness and atrophy than in stable post-polio muscles.[99]

### 8.2. Autoimmunity and co-factors

Currently, there is no evidence for PPS being an immune-mediated condition. Levels of immune complexes are normal in PPS patients,[100] and no autoantibodies to CNS or muscle tissues

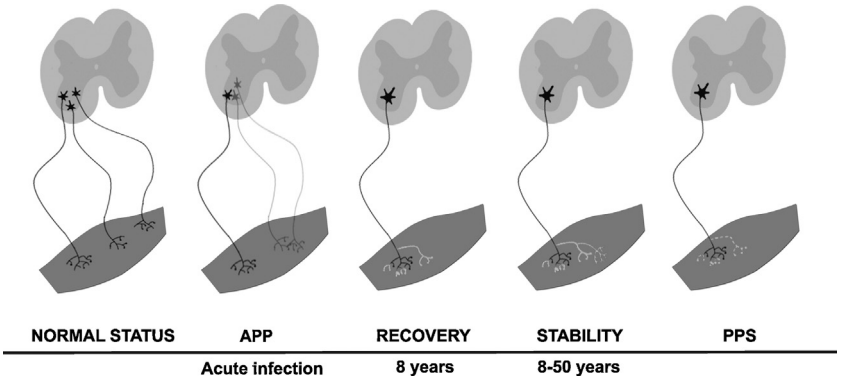


**Figure 3.** Idealized representation of changes in motor units from the time of acute poliovirus attack and the possible development of PPS. Distal degeneration of innervating fibres and loss of motor units accompanies the appearance of new weakness and the atrophy of previously unaffected muscles.

have been reported so far. Autoantibodies to ganglioside GM1 (a biomarker of Guillain–Barré syndrome) have not been detected.[101] However, other CNS autoantibodies have not been adequately investigated in PPS. For instance, antibodies to receptors, ion channels, glutamic acid decarboxylase (GAD),[102] and neuronal surface antigens[103] require appropriate research. Numerical alterations in peripheral blood CD4 T-cells have been reported,[104,105] but T-cell autoreactivity and the immunoregulatory functions of lymphoid cells have not been explored. Human macrophages and dendritic cells are susceptible to PV infection.[106] Some data have suggested that the numbers of regulatory T-cells in PPS are increased as compared to non-polio controls.[104]

### 8.3. Persistent poliovirus infection in polio survivors and PPS

In the 1990s, a series of studies attempted to clarify whether PVs could establish a persistent infection in some polio survivors. The hypothesis was suggested by the finding of oligoclonal IgM bands of PV antibodies in the cerebrospinal fluid (CSF) of PPS patients, but none in controls.[107] The results supported the intrathecal production of anti-polio antibodies, hence a continuous infection within the CNS. Other investigators challenged the data.[108,109] Muir et al. used PCR assays directed at the 5′-UTR region of enteroviruses and investigated a cohort of PPS patients. It was shown that a small percentage of PPS patients had enterovirus RNA in the CSF, together with PV-specific oligoclonal IgM bands.[110] The results strongly pointed to persisting infection, at least in a few PPS patients. Using different PCR assays, positivity for PV genomes was confirmed in CSF and peripheral leukocytes,[38] as well as in CSF by others.[39] In 1999, Julien et al. concluded that 'PV genome fragments' were detectable in a significant percentage of PPS patients.[40] Based on the above findings, an observational study was started in a cohort of polio survivors who were attending hospitals in northern Italy, looking for new weakness and neuromuscular problems. The results of virology studies (detection of poliovirus genome and biological effects of the persisting virus) showed that in polio survivors diagnosed with PPS, a low-level infection is sustained by either PV1, PV2, or PV3 (not by non-polio enteroviruses).[41,111–113] Additional evidence has shown that family members of PPS patients do not carry PV genomes, thus confirming that PPS patients are not infectious. So far, it has not been possible to completely sequence the PV genome and to demonstrate that the persisting virus plays a role in the development of this progressive disorder. Novel technologies will possibly help clarify the nature of PV genomes found in PPS patients.

## 9. Possible treatments

The effectiveness of pharmacological treatment and the management of rehabilitation in PPS is not yet established. A meta-analysis has been conducted of randomized and quasi-randomized trials of any form of pharmacological or non-pharmacological treatment for people with PPS.[21] The primary outcome was self-perceived activity limitations, and secondary outcomes were muscle strength, muscle endurance, fatigue, pain, and adverse events. Nine pharmacological studies (modafinil, intravenous human immunoglobulin (IVIg), pyridostigmine, lamotrigine, amantadine, prednisone) and three non-pharmacological studies (muscle strengthening, rehabilitation in warm or cold climates, static magnetic fields) were examined. Due to issues with the quality of the data and the lack of randomized studies, it was not possible to draw definite conclusions on the effectiveness of these interventions for PPS. However, treatment with IVIg, lamotrigine, muscle strengthening exercises, and static magnetic fields may be beneficial.

### 9.1. Intravenous human immunoglobulin (IVIg)

Over the last decade, several trials have explored the potential of a single IVIg course for PPS patients. Immunoglobulin infusions of 1–2 g/kg have been used. Patients have been evaluated at time 0 and at 2 to 12 months post-treatment for a variety of parameters: health-related quality of life (Short Form 36), Physical Activity Scale for the Elderly, pain intensity (visual analogue scale), Six-Minute Walk Test, and muscle strength. Results have varied somewhat among trials, but patients have reported a reduction of pain,[114] an increased quality of life at 6 months (pain, vitality, social function, and role emotional),[115] and, in the first months of treatment, improvements in the mental component score.[116] Age $<$65 years, paresis in the lower extremities, and lack of concomitant disorders may represent indicators for identifying responders. A further study showed that up to 1 year after treatment, concentrations of IFN-γ and IL-23 were reduced in the CSF of PPS patients, while those of the anti-inflammatory IL-13 cytokine were increased.[65] Taken together, the results support a beneficial effect of a single IVIg course. Anecdotal reports of repeated IVIg courses confirm this conclusion.

What mechanisms of action form the basis of IVIg efficacy? The Fc fragment and the constant domain of the Fab fragment contain binding sites for activated complement fragments, such as C3a, C5a, C3b, and C4b. The interaction may prevent binding of complement fragments to their receptors on target cells, thus attenuating the immune damage.[117] Fc sialylation of a small percentage of human IgG has also been proposed as an anti-inflammatory factor. Although glycosylation may not be so critical for IVIg activity, results for the Guillain–Barré syndrome suggest that sialylation is associated with improved clinical outcomes. Antiviral antibodies may represent a further contributing factor. For instance, in the experimental infection of mice with West Nile virus (WNV), encephalitis and inflammation are prevented, in part, by IVIg infusion (likely via anti-inflammatory activity). Interestingly, human WNV convalescent serum has been shown to be more effective than IVIg in controlling encephalitis.[118]

### 9.2. Anti-poliovirus drugs

In 2005, the National Research Council Panel of the US National Academies was invited to consider the development of antiviral drugs to conclude the polio eradication effort.[119–121] It was thought that the development of at least two drugs active on distinct targets was required. The primary application of anti-polio drugs would be for the resolution of chronic poliovirus excretion in persons with primary immunodeficiency, but the control of possible polio outbreaks post-eradication and the sporadic treatment of accidental exposure would represent additional goals.[121] In Europe, the FP6-supported project VIZIER was set up to investigate enzymes involved in viral replication and to develop specific inhibitors.[122] In Japan, a successful effort was started to control the possible zoonotic spread of foot-and-mouth disease virus. An important compound, favipiravir, effective against multiple RNA viruses, was developed.[123] As shown in Table 3, the numerous anti-polio drugs derived from these studies are directed at a variety of targets.[32,34–36,121–132]

So far, only a few compounds have entered clinical development, but no drugs have yet been approved for the treatment of enterovirus infections. Monoclonal antibodies have recently been added to the anti-polio armamentarium. Human and chimpanzee monoclonals capable of neutralizing wild-type and vaccine PV strains have become available.[32,34,35,133] In transgenic mice expressing the receptor PVR, small doses of select monoclonals have been shown to provide pre- and post-exposure protection from challenge with a lethal dose of poliovirus. The treatment of

**Table 3**
Candidate antivirals for the treatment of poliovirus infections

| Compound | Target | Effective dose in vitro or in animals | Clinical development for PV infection | Reference |
|---|---|---|---|---|
| Pirodavir-related compounds: BTA39, BTA188 | Capsid inhibitor. Interferes with binding to receptor and uncoating | Nanomolar | No | 124 |
| Pocapavir (V-073) | Capsid inhibitor. Binds to VP1. Interferes with binding to receptor and uncoating | Nanomolar | Yes | 121 |
| H1PVAT | Capsid inhibitor. Binds to VP1 in the pocket underneath the floor of the capsid canyon involved in capsid binding | Nanomolar | Yes | 125 |
| Substituted flavanoids: 3(2H)-isoflavene, 6-chloro-3(2H)-isoflavene | Possible capsid inhibitors. Bind to capsid protein site | Micromolar | No | 126 |
| Amiloride | Docks in the VPg-binding site of 3D RNA polymerase and inhibits initiation of RNA synthesis | Micromolar | No | 127 |
| GPC-N114 (2,2′-[(4-chloro-1,2-phenylene)bis(oxy)]bis(5-nitro-benzonitrile)) | Non-nucleoside inhibitor of 3Dpol targeting the RNA template-primer binding site in the core of 3Dpol | Micromolar | No | 128 |
| Favipiravir (T-705) | Is phosphoribosylated by cell enzymes to a triphosphate compound. Inhibits RNA-dependent RNA polymerase of many different viruses | Nano/micromolar | No | 123 |
| Seven potential inhibitors of RNA polymerase | Block 3D polymerase initiation of RNA synthesis. However, do not inhibit NTP binding during elongation | Micromolar | No | 122 |
| Thiazolobenzimidazoles | Interfere with 2C (helicase, ATPase) functions and inhibit RNA replication | Micromolar | No | 129 |
| TTP-8307 | Binds to 3A protein (possible membrane anchor) and inhibits RNA replication | Nanomolar | No | 129 |
| Enviroxime-like compounds (A4, E5, E7, GW5074) | Interfere with the processing of viral polyprotein affecting both 3C- and 2A-dependent cleavage | Micromolar | No | 130 |
| AG-7404 | Inhibits the viral 3C protease. Synergistic with capsid inhibitors | Nanomolar | Yes | 131 |
| OSW-1 (candidate antitumor drug) | Interferes with the human oxysterol-binding protein | Nanomolar | No | 132 |
| Poliovirus-neutralizing chimpanzee/human monoclonal antibodies | Six neutralizing antibodies active against vaccine and virulent strains of polioviruses | 5 μg/mouse | No | 34 |
| Poliovirus-neutralizing human monoclonal antibodies 12F8 and 1E4 | Neutralizing antibodies active against vaccine and virulent strains of polioviruses | 5 μg/ml | Pre-clinical development | 35 |
| Chimpanzee monoclonal antibody capable of neutralizing both PV1 and PV2 | Extended-spectrum neutralizing antibody directed to the capsid site that recognizes the cellular receptor | 5 μg/ml and 25 μg/mouse | Pre-clinical development | 32,36 |
| Single-domain antibody fragments (VHHs) against PV-1 | Interfere with virus attachment to cell, viral uncoating, induce aggregation of virus–VHH complexes | Nanomolar | No | 133 |

PV, poliovirus; VP1, viral protein 1; VPg, viral protein genome-linked; 3Dpol, poliovirus RNA-dependent RNA polymerase; NTP, nucleotide triphosphate; VHHs, variable domains of heavy chain antibodies.

animals with the antibody does not prevent an immune response to the polio vaccine.[36] This suggests that human monoclonals could be used in combination with polio vaccine and/or drugs to improve their efficacy and to prevent the emergence of resistant variants. These experimental data provide a proof of concept for initiating the clinical evaluation of these biological products.

Anti-polio drugs can be grouped as follows: (1) capsid inhibitors (interfere with virus binding to cellular receptor(s) and with the uncoating process), (2) inhibitors of RNA-dependent RNA polymerase, (3) inhibitors of 2C helicase/ATPase, (4) inhibitors of the lipophilic 3A protein, (5) inhibitors of PV proteases, (6) ligands of human oxysterol-binding protein, and (7) PV neutralizing monoclonal antibodies (whole antibody or single-domain antibody fragment).

### 9.3. Ongoing clinical trials

The design of therapeutic studies in patients with PPS raises methodological concerns due to the multiform nature of this progressive disease.[134] Registered clinical trials in PPS patients are currently underway (http://www.who.int/ictrp/search/en/; date of consultation: 2015-03-16). A multicenter interventional, double-blind, randomized, and placebo-controlled trial involving 10 countries aims to evaluate whether monthly IVIg infusions for 1 year is superior to placebo, and to determine the effective IVIg dose. Efficacy is being measured simply as the physical performance of the subjects in a 2-min walking test. A second trial aims to compare the potential of a microprocessor-controlled orthosis vs. a stance orthosis in individuals with impairments of the lower extremities. The physical performance of subjects is being assessed with a 6-min walking test together with measurements of oxygen consumption. Other trials are observational in nature. These aim to define the reproducibility of the Six-Minute Walk Test that is used to measure the physical performance of PPS subjects, the type of mental fatigue in polio survivors, and the swallowing and facial impairments in PPS patients.

Among current trials, the largest one is in the promising area of IVIg treatment. The trial is based on a human IVIg preparation whose IgG molecules have been shown to be functional both in Fc fragment assays and in neutralization assays against polioviruses and other pathogens.[135] Since during production, IgG fractions are exposed to multiple inactivation treatments, including nanofiltration, this IVIg preparation has wide margins of biological safety.

As of today, no experimental work or clinical trials have attempted to assess the efficacy of anti-polio compounds or antibodies in curing PPS or in preventing its development. It is our hope that this review will stimulate translational medicine to find a cure for this prevalent, progressive, and disabling disease.

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*Conflict of interest:* The guarantor of this study is Antonio Toniolo and there are no conflicts of interest to disclose.

*Author contributions:* AT and AB designed the study, analyzed data, and wrote the manuscript. AB and MC executed laboratory experiments. JLH, JRMF, and MAL contributed data, and reviewed and edited the manuscript.

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