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POST-POLIO SYNDROME – ANALYSIS OF INFLAMMATION AND IMMUNE MODULATION

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Post-polio syndrome – analysis of inflammation and immune modulation
THESIS FOR DOCTORAL DEGREE (PhD)

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To my father Lennart Melin, my late mother Elisabet Melin Annell, my late “morfar” Gunnar Annell, my late “mormor” Margareta Annell, and also to all the women pioneers in science and medicine who made it possible for me to combine family with research and clinical work. Thank you!

ABSTRACT

Poliomyelitis is a viral infection, which affects the anterior horn cells of the spinal cord leaving patients with different levels and distribution of muscle weakness and loss of, or decreased, function. This situation has previously been regarded as a stable condition. However, following recovery from poliomyelitis, with or without remaining symptoms and after a stable period, some polio patients may develop Post-polio Syndrome (PPS), a syndrome characterized by new or increasing muscular weakness, pain, fatigue, and sensitivity to cold. The findings of increased cytokine levels in cerebrospinal fluid and peripheral blood has led to the conclusion that there is an inflammatory process underway in PPS.

One of the most common symptoms, in addition to muscle weakness, is pain and some earlier studies have presented evidence of an inflammatory process in muscle. This was confirmed in this research based on the findings of an elevated expression of the enzymes in the Prostaglandin E_2 (PGE_2) pathway, i.e. mPGES-1, COX-2, COX-1 and cPGES. This may explain muscle pain and opens new perspectives for specific treatment.

It has been speculated that the inflammation in PPS may have an autoimmune background. In this research there were no circulating immune complexes in the blood of the PPS patients. This reduces the possibility that the inflammation is driven by a complement mediated autoimmune process.

In some chronic inflammatory conditions there is an increase of blood lipids. In earlier studies of PPS patients, blood lipid levels have been found to be increased. In this study the blood lipids were normal, and thus, the inflammation does not appear to give rise to hyperlipidaemia.

The inflammation in PPS patients is down-regulated by immune-modulatory treatment, by means of IvIg, which is followed by a clinical improvement. However, it has been difficult to pinpoint and characterize responders and non-responders. When re-evaluating the PPS diagnosis 2-8 years after the treatment, patients still considered as suffering from PPS experienced a better outcome of the treatment than patients with a stable condition, i.e. non-PPS. This opens up for speculation that PPS may be divided into two different subgroups, unstable patients with PPS driven by an inflammatory process and stable patients with a slower deterioration possibly due to ageing and concomitant disorders.

LIST OF SCIENTIFIC PAPERS

I. **Elevated Expression of Prostaglandin E_2 Synthetic Pathway in Skeletal Muscle of Prior Polio Patients**

Eva Melin, Eva Lindroos, Ingrid E Lundberg, Kristian Borg, Marina Korotkova

J Rehab Med. 2014; 46 (1): 67-72

II. **Normal serum levels of immune complexes in postpolio patients**

Eva Melin, Azita Sohrabian, Johan Rönnelid, Kristian Borg

Res in Immunol. 2014; 4: 54-57

III. **Dyslipidaemia is uncommon in patients with post-polio syndrome – a case-control study**

Eva Melin, Thomas Kahan, Kristian Borg

Submitted 2014

IV. **Quality of life and pain intensity after immunoglobulin treatment in post-polio patients – re-evaluation after a follow-up**

Eva Melin, Lars Werhagen, Kristian Borg

Submitted 2014

CONTENTS

LIST OF ABBREVIATIONS

PPS	Post-polio syndrome
CNS	central nervous system
PNS	peripheral nervous system
CSF	cerebrospinal fluid
PB	peripheral blood
IvIg	intravenous immunoglobulin
HRQoL	health related quality of life
TNF	tumour necrosis factor
IFN	interferon
IL	interleukin
IgG	immunoglobulin G
IgM	immunoglobulin M
NSAID	non steroid anti-inflammatory drugs
PGE2	prostaglandin E2
PGH2	prostaglandin H2
COX	cyclooxygenase
mPGES	microsomal prostaglandin E synthase
cPGES	cytosolic prostaglandin E synthase
LDL	low density lipoprotein
HDL	high density lipoprotein
SLE	systemic lupus erythematosus
RA	rheumatoid arthritis
HIV	human immunodeficiency virus
IC	immune complex

1 INTRODUCTION

1.1 ACUTE PARALYTIC POLIOMYELITIS

In 2014 three countries, Afghanistan, Nigeria and Pakistan, are still polio endemic, while 25 years ago there were more than 125 such countries. Polio cases have decreased by more than 99% since 1988, from about 350 000 cases (1) to 406 worldwide. This reduction is the result of the global vaccination program (2) as there is no other cure for polio. Despite the decline in acute polio there are still, and will be for decades to come, a substantial number of patients all over the world, including in the developed countries, with sequelae of polio, who are still in need of care and medical attention (3, 4).

Acute poliomyelitis is caused by an enterovirus, of which there are three serotypes (Poliovirus 1, 2 and 3) (5). The Poliovirus is transmitted via the faecal-oral pathway and causes fever, fatigue, headache, vomiting, stiffness in the neck, and pain in the limbs (2). In less than 1% of the cases, mostly associated with infection with Poliovirus 1, paresis or paralysis occur, caused by destruction of the anterior horn cells. The paresis is flaccid, asymmetrically distributed, and most often found in the lower extremities. Among those paralysed, 5-10% die, mainly due to immobilised breathing muscles and respiratory failure (6). After the acute poliomyelitis, there is a recovery of muscle strength, reaching a plateau after a couple of years, leaving the patients with sequelae of differing levels of severity. Recovery from the acute poliomyelitis is mostly due to a reinnervation process, where the remaining nerves take over the innervation of muscles from the damaged nerves by means of collateral sprouting (7, 8).

1.2 LATE EFFECTS OF POLIO AND THE POST-POLIO SYNDROME (PPS)

Acute polio leaves the patients with different levels and distribution of muscle weakness and loss of, or decreased, function. This situation has been regarded as a stable condition. However, most polio patients will experience late effects of polio i.e. a slow deterioration of function over the decades. Following the recovery from poliomyelitis, with or without remaining symptoms and after a stable period, some polio patients may develop the Post-polio Syndrome (PPS), a syndrome characterized by new or increasing muscular weakness, pain and fatigue (9-14). Different diagnostic criteria for PPS have been used, aimed at

evaluating muscular atrophy and muscle dysfunction (14-16). Nowadays the criteria given by March of Dimes are those most often applied (17), and includes five different criteria: 1. A history of paralytic poliomyelitis, as confirmed by history, neurologic examination, and electromyography. 2. A period of partial or complete functional recovery, followed by a stable period, usually of 15 years or more. 3. Gradual or sudden onset of progressive and persistent new muscle weakness or decreased endurance, with or without generalized fatigue, muscle atrophy, or muscle and joint pain. (Sudden onset may follow a period of inactivity, trauma or surgery.) 4. Symptoms should persist for at least a year. 5. Exclusion of other causes (17).

Differential diagnoses of PPS, which should be considered include; lumbar spinal stenosis, disc hernia, stroke, entrapment/peripheral nerve injury, amyotrophic lateral sclerosis, incomplete lumbar spinal cord injury, spina bifida, and adverse effects of medication i.e. Statins (18). The age of the PPS patients at the out-patient clinics in the developed world today is high, and thus cardiovascular and other concomitant disorders that may cause general fatigue and muscle weakness should be excluded.

1.3 PATHOGENESIS OF PPS

Since first described in 1875 by French neurologists JM Charcot and M Raymond (19), different theories for the mechanisms behind PPS have been presented. The reinnervation process no longer being able to compensate for the denervation (9, 12, 20), overuse or degeneration of enlarged motor units (21-24), genetic factors (25), part of a normal ageing process (22), a persistent viral infection (26, 27) and immunological factors have all been suggested (9). Most likely a combination of different factors gives rise to the syndrome. Grimby et al. (28) divided the patients into stable who did not report new weakness, and unstable, showing new or severe muscular weakness. Dalakas (29) also divided the syndrome into; patients with musculoskeletal symptoms, with diminished functional capacity but a relatively stable condition, and patients with late postpolio myelitis muscular atrophy, with new weakness, wasting, fasciculations and myalgia. It must, in fact, be considered that perhaps PPS is not one, but two conditions. One form being stable, or progressing slowly, and another form, referred to as PPS, being more progressive. One possible explanation is that the progressive form, PPS, is driven to a greater degree by immunological/inflammatory processes.

1.4 COMPENSATORY MECHANISMS AFTER POLIOMYELITIS

After recovery from acute poliomyelitis, motor nerves and their corresponding muscle fibres, i.e. the motor unit, adapts in several ways to compensate for the loss of muscle power. In order to be able to re-gain and maintain muscle strength, there is a reinnervation process with collateral sprouting, and muscle fibre hypertrophy (24, 30-33). There is also a transition of muscular fibers from the fast type II to slow type I (24, 30, 31, 34, 35), and a changed motor unit activation pattern (24, 36). In muscles there are high and low threshold motor units. For example, normally only the low threshold motor units are activated in the tibialis anterior muscle during walking, while high threshold motor units are used during running or corrective movements (36, 37). In patients who have recovered from poliomyelitis, there appers to be no such differentiation, leading to all their residual muscle fibres being used at the same time, regardless of activity, and there are muscle fibres containing slow as well as fast myosin (30, 36, 38, 39). This could be of significance for the polio patient's balance and ability to compensate for unexpected, rapid movements.

1.5 INFLAMMATION IN PPS

Inflammation is the immune system's response to infection and injury. In PPS patients, signs of inflammation have been detected in several locations in the body including muscle, blood, cerebrospinal fluid (CSF), central nervous system (CNS), spinal cord and peripheral nerves. In the muscle, lymphocyte infiltrates have been reported (40). Semino-Mora and Dalakas (41) found endomysial inflammation with rimmed vacuoles in PPS similar to those found in inclusion body myositis (IBM). Leon-Monzon and Dalakas (42) found evidence of an ongoing antibody response to a viral antigen, by detecting high titres of IgM anti-poliovirus antibodies in the blood. In peripheral blood alterations of CD4+ T-cells have been found (43), as well as an enhanced activity of pro-inflammatory myeloperoxidase in leukocytes (44).

Cytokines are small proteins acting through cell signalling as immune mediators (45). In the immune system, they regulate the balance between humoral and cell-based response (45). They are also involved in regulating maturation, growth and responsiveness of different cell populations (45, 46). Fordyce et al. (47) and Nollet et al. (48) detected elevated levels of the cytokines TNF-α, IL-6 and Leptin in PPS patient serum. Nollet et al. also found elevated IL-8 and IL-18 in PPS patients and also concluded that the cytokine increase was not correlated to decline of function (48).

1.5.1 The central nervous system (CNS)

In the CNS, immune responses to infection may differ from other parts of the body (49-51). The blood-brain barrier, and the restricted space inside the cranium, both contribute to this (49, 51). However, inflammation in the CNS may contribute to acute, chronic and psychiatric disorders such as stroke, multiple sclerosis, traumatic brain injury, and even depression and schizophrenia (52).

In the spinal cord of diseased patients with previous poliomyelitis, without any new neurological diseases, a mild to moderate perivascular and interparenchymal inflammation, as well as severe reactive gliosis has been reported (53). Ongoing immune activation has been detected in CNS and spinal cord (54). Oligoclonal IgG bands as well as elevated levels of IgM antibodies to poliovirus have also been discovered in the CSF (55-57).

Elevated levels of pro-inflammatory cytokines have been reported in the CSF. Increased levels of the cytokines TNF-α, IFN-γ and IL-10 in the CNS were reported (58-61) in PPS patients, and an intrathecal inflammatory process was suggested. The inflammatory process was further supported by a proteomic study report. Five altered proteins, all involved in neuroinflammation and apoptosis, in the CSF of PPS patients were identified (62).

1.5.2 The peripheral nervous system (PNS)

Inflammation of the PNS is usually caused by damage to the nerve cell or axon. The nerve cells can be damaged due to, for example, a viral infection, such as polio or herpes virus, anoxia, mechanical trauma, cancer or immune mediated nerve inflammation, as seen in Guillian-Barrés syndrome (63, 64). In one study, evidence of inflammation in the peripheral nerves has been presented and neuropathy with inflammation has been shown in nerve biopsies from patients (65).

1.6 LATENT VIRUS AND PPS

Since the immune system in CNS differs from the rest of the body, it makes it possible for some pathogens, such as HIV and herpes virus, to survive, or to remain latent for a long period of time (66). Thus, it is of interest to know if the PPS can be caused by a persistent

poliovirus, hiding in the CNS. The presence of poliovirus-RNA has been detected in the CSF and in lymphocytes, suggesting the presence of a persistent mutated virus or defective poliovirus particles (42).

1.7 AUTOIMMUNITY AND PPS

Autoimmunity occurs when the body mistakes its own parts for hostile invaders, leading to an immunological response to its own tissues or cells (67, 68). Autoimmune disease can be divided into systemic and local autoimmune diseases (67), where the systemic autoimmunity is associated with autoantibodies to antigens, which are not tissue specific e.g. systemic lupus erythematosus (SLE), while local autoimmunity affects a specific organ or tissue, e.g. Type I Diabetes (68).

In order to eliminate foreign substances from the body during infections, immune complexes (IC) containing antigens and their corresponding antibodies are formed (69). In some autoimmune diseases, such as SLE, rheumatoid arthritis (RA), and Type I Diabetes, autoantibodies and their corresponding auto-antigen form circulating IC (70-72). Both patients with IC mediated disease and PPS frequently report similar types of pain (73-75) which may indicate a common background. Gonzalez et al. (76) speculated that there might be autoimmune processes behind the inflammation in PPS, but there has been no previous studies made on PPS and autoimmunity including IC.

1.8 PROSTAGLANDINS AND PPS

Prostaglandins are hormone-like lipid compounds derived from arachidonic acid, present in the cell membranes of the body. The prostaglandins are produced by the enzymes cyclooxygenase (COX) 1 and 2, and microsomal prostaglandin E synthase 1 and 2 (mPGES) and cytosolic prostaglandin E synthase (cPGES) (77). Prostaglandins in the body both contribute to homeostatic functions and to pathogenic actions, such as inflammation and pain (77, 78). Prostaglandins are important in the generation of the acute inflammatory response and their synthesis is up-regulated in tissue affected by inflammation (78-80). The Non-Steroid Anti Inflammatory Drugs (NSAID) mostly works by inhibiting Arachidonic acid transformation into prostaglandin H2 (PGH2), and hence the production of prostaglandin E2 (PGE2) (81), as seen in Figure 1.

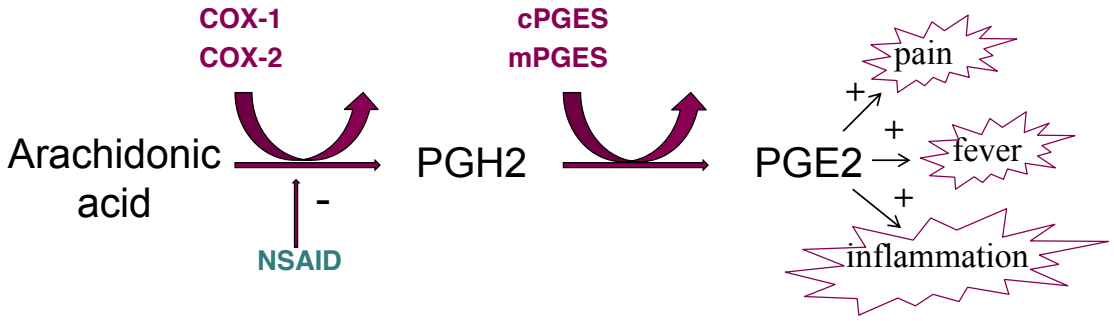


Figure. 1. Synthesis of Prostaglandins, for description see text.

1.9 BLOOD LIPIDS AND PPS

Blood lipids are lipids in the bloodstream which are insoluble in water (82). The most common blood lipids are cholesterol, including low density lipoprotein (LDL) and high density lipoprotein (HDL), and triglycerides (83). Although blood lipids are often associated with heart disease, they carry out important functions in the body, such as forming cell membranes, energy storage and formation of hormones (84). Previous studies found blood lipids in PPS patients to be elevated (85, 86). The study by Agre et al. (85) found that two thirds of the men and one in four of the women with PPS had hyperlipidemia, with the men also having low HDL, and high total cholesterol/HDL ratios. In the study by Gawne et al. (86), the prevalence of dyslipidemia in PPS patients was higher than in the total population in the USA at the time. However, there have been reports that there is no higher risk of cardiovascular disease in PPS patients (87).

Atherosclerosis is often considered to be an inflammatory disease (88), and in some chronic inflammatory conditions such as systemic lupus erythematosus (SLE), and rheumatoid arthritis, the metabolism of lipids is altered and gives rise to elevated lipid levels (89-91). With all of this taken into account, and the hypothesis of an inflammatory process in PPS as discussed above, it might be expected that the increased blood lipids earlier reported are due to inflammatory processes.

1.10 TREATMENT WITH INTRAVENOUS IMMUNOGLOBULIN (IVIG) AND PPS

IvIg consists of IgG antibodies extracted from plasma of blood donors, and is used for suppressing of harmful inflammation (92). Its actions are mainly unknown (93), but are thought to be inhibition of complement deposition, neutralization of cytokines, modulation of Fc-receptor phagocytosis, affecting activation and effector function of T- and B-cells, and suppression of antibody production (92, 94, 95). IvIg is primarily used to treat autoimmune diseases, immune deficiencies, and acute infections. The most common adverse effects of IvIg include headaches, skin reactions, fever, flushing, chills, changes in blood pressure, tachycardia, thromboembolism, aseptic meningitis, and renal failure (96). However, IvIg is usually well tolerated, and the serious adverse effects are rare (94, 97). Over the last decade, IvIg has been used in the treatment of PPS (9, 58, 60, 61, 76, 98, 99). The effect has mainly been on pain and quality of life, especially vitality (98-100). The clinical impression is that in some PPS patients the IvIg treatment is very effective, but not in others. IvIg has side-effects and is an expensive treatment and, thus, trying to pinpoint and characterize responders and non-responders to IvIg is a primary goal for research at the moment.

1.11 ETHICAL ASPECTS

Enrolment to the studies followed the recommendations of the Declaration of Helsinki and the studies were approved by the Ethical Review Board in Stockholm, Sweden.

I. Ethical approval: 2006/1452-31/2
II. Ethical approval: 2007/492-31/1
III. Ethical approval: 2007/492-31/1
IV. Ethical approval: 2010/411-31/1

2 AIMS

2.1 GENERAL AIM

The overall aim of the thesis was to study inflammation, and immune-modulatory therapy with IvIg in PPS patients.

2.2 STUDY I

Signs of inflammation have earlier been reported in the muscle tissue of PPS patients (40). As PPS is clinically characterized by muscle weakness and myalgia, this study was performed with the aim of investigating signs of inflammation in skeletal muscle, by means of staining with an immunohistochemical technique, and analyzing for the enzymes COX-1, COX-2, m-PGES, c-PGES as well as T-cells and monocytes/macrophages in muscle biopsies. The enzymes analyzed are all part of the PGE_2 pathway, a known mechanism of pain.

2.3 STUDY II

As there have been speculations concerning an autoimmune process in PPS, this study was performed in order to evaluate whether the initial infection is followed by a delayed exacerbated IC response. Circulating IC levels were analyzed both by means of a conventional C1q binding technique and by analyzing the cytokine-inducing properties of polyethylene glycol (PEG)-precipitated IC.

2.4 STUDY III

Abnormal blood lipids in PPS have previously been reported (85, 86), but there seems to be no increase of mortality due to cardiovascular disease in PPS patients. As dyslipidemia is found in disorders with inflammation, and an inflammatory process has been suggested in PPS, this study was undertaken in order to evaluate whether dyslipidemia is common in Swedish PPS patients and if there is a correlation between the lipid levels and inflammatory markers i.e. C-reactive protein and sedimentation rate. Furthermore, the risk of coronary heart disease in PPS patients was estimated by means of the Framingham risk score (101).

2.5 STUDY IV

Treatment with Ivig in PPS has led to increase of muscle power, physical activity and increased health related quality of life (HRQoL) (60, 76, 98, 99). However, there have been difficulties in pinpointing responders and non-responders to this treatment. This study was undertaken in order to further characterize responders and non-responders to IvIg. The patients receiving IvIg-treatment earlier were re-evaluated regarding the diagnosis after a follow-up period of 2-8 years and characterized as PPS or non-PPS, and the outcome of the IvIg treatment was compared between the two groups.

3 PATIENTS AND METHODS

Demographic data of the patients included in studies I-IV are seen in Table 1.

Table 1. Demographic data of the patients included in the studies

	Study I	Study II	Study III	Study IV
Number of patients	8	20	89	150
Female	4	17	53	84
Male	4	3	36	66
Mean age(SD)	39(21)	64(12)	65(9)	65(10)

3.1 STUDY I

Eight patients, and six age and gender-matched healthy controls, were included in the study. The patients were recruited consecutively from the out-patient clinic at the Department of Neurology at the Karolinska University Hospital in Stockholm, Sweden, and the healthy controls were volunteers without neurological disease. All patients had previously had poliomyelitis, diagnosed by clinical neurological and neurophysiological examinations and were suffering from new or increasing neurological symptoms in the lower extremities, mainly muscular pain, weakness and fatigue.

In the study, an immunohistochemical technique for detecting proteins in tissue sections was performed by using the principle of specifically binding, labelled antibodies (102). Different methods can be used to visualize the antibody-antigen complex. Often, an antibody conjugates to an enzyme that can catalyze a reaction, which produces colour (102) and is then analyzed in a microscope. The procedure is shown in Figure 2.

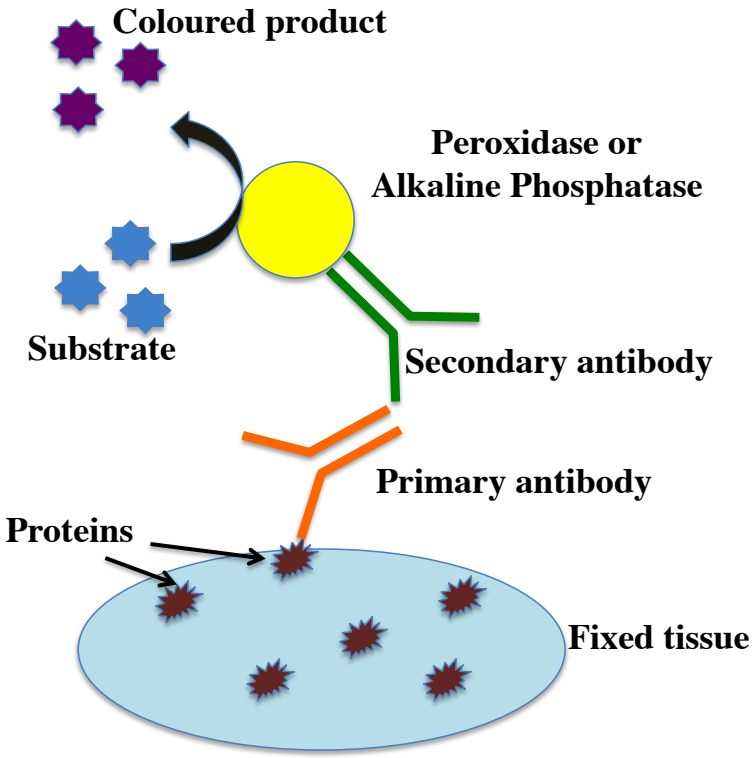


Figure. 2. Principles for staining with immunohistochemistry. For description see text.

In study I, a standard immunohistochemistry protocol, described by Frostegård et al. (103) was used. Staining was performed for CD3 (T-cells), CD68 (marker for monocyte/macrophages), CD163 (marker for resident tissue macrophages), and the enzymes COX-1, COX-2, mPGES-1 and cPGES. Primary antibodies for the stainings are shown in Table 2.

Table 2. Primary antibodies used in the immunohistochemical stainings for T-cells, monocytes and macrophages, and the prostaglandin enzymes

Name	Primary antibody		
	Clone	**Species**	**Isotype**
Anti-CD3	SK7	Mouse	IgG1
Anti-CD68	EBM11	Mouse	IgG1
Anti-CD163	Ber-MAC3	Mouse	IgG1
Anti-mPGES-1	Polyclonal	Rabbit	Ig
Anti-cPGES	Polyclonal	Rabbit	Ig
Anti-COX1	Monoclonal	Mouse	IgG
Anti-COX2	Polyclonal	Rabbit	Ig

3.2 STUDY II

20 PPS patients from the Post-Polio outpatient clinic at Danderyd University Hospital, Stockholm, Sweden, 95 healthy controls, and 162 positive controls were included in the study. PPS was diagnosed with the criteria given by March of Dimes (17). The healthy controls were recruited from blood donors in the Department of Clinical Immunology and Transfusion Medicine at Uppsala University Hospital. As positive controls, data of 162 sera from SLE patients previously studied with the same technique were used. To compensate for differences in age between patients and controls, a sub-analysis was performed using only the 30 oldest controls. Circulating immune complexes were compared between the PPS patients and the controls.

Measurement of circulating C1q-binding IC was performed using ELISA. Data from the patients and control groups were compared using non-parametric Mann-Whitney's U-test.

3.3 STUDY III

89 consecutive PPS patients from the Post-Polio out-patient clinic, Danderyd University Hospital, Stockholm, Sweden were included in the study. Total cholesterol, high density lipoprotein cholesterol (HDL) and triglycerides, were analyzed in serum, and low density lipoprotein cholesterol (LDL) was calculated using Friedewald's formula (104). C-reactive protein, erythrocyte sedimentation rate and leukocyte count were measured as markers of inflammation, in addition to creatinine and HbA1c. All the samples were analyzed using standard techniques at accredited regional laboratories.

Lipid data were compared to reference values provided by two earlier published studies. The Nordic Reference Interval Project (NORIP) (105) is based on blood samples from 3 036 healthy individuals in the Nordic countries and provides reference data for total cholesterol, LDL, HDL and triglycerides. Jungner et al. (106) reported on a Swedish population sample of 147 576 males and females obtained from general health screening, and provides results for total cholesterol and triglycerides. The results of the PPS patients were matched for age and gender.

3.4 STUDY IV

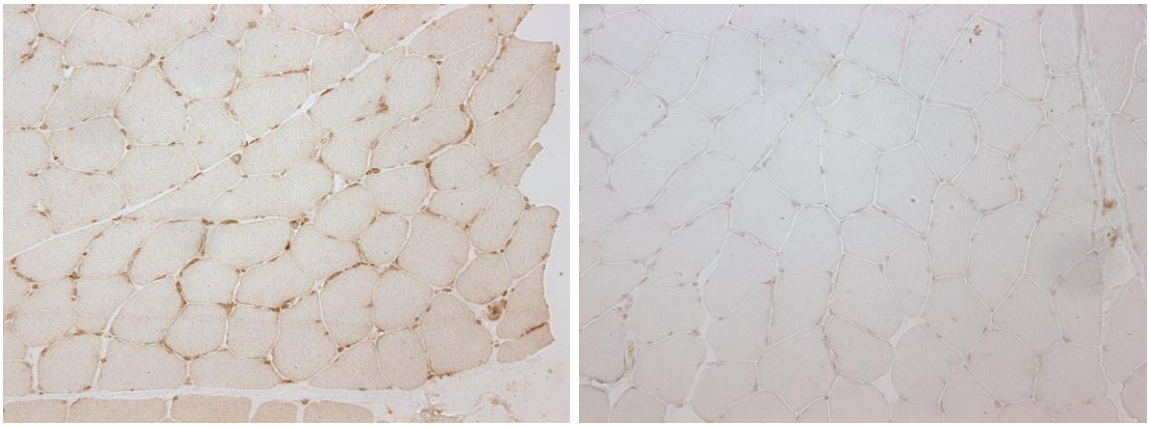
150 PPS patients diagnosed using either the Halstead and Rossi (11) or the March of Dimes (17) criteria were included in the study. All were treated with IvIg at the Post-Polio out-patient clinic at Danderyd University Hospital, Stockholm, Sweden. A medical examination of the patients was performed before IvIg, and at a 6-months follow-up. This included a general examination and a neurological examination, and the patients filled in the health-related quality of life questionnaire SF 36, and Visual analogue scale (VAS) for pain intensity. Two of the authors (LW and EM) re-evaluated the patients independently according to the criteria for PPS given by March of Dimes (17) 2-8 years after the IvIg treatment, and the patients were diagnosed as PPS or non-PPS. Data from PPS and non-PPS patients were compared.

4 RESULTS AND DISCUSSION

4.1 STUDY I

No inflammatory cell infiltrates were found, and only scattered inflammatory cells (T-cells, activated and non-activated macrophages) could be detected in the muscle tissue of the patients. There was no significant difference in the number of inflammatory cells when the patients were compared with the healthy controls. Previously published research has found inflammatory infiltrates (55). However, this finding could not be repeated in the present study, and thus it contradicts earlier findings.

An elevated expression of the enzymes in the PGE_2 pathway, i.e. the inflammatory induced mPGES-1 and COX-2, as well as the constitutively expressed COX-1 and cPGES was found in the muscle biopsies from the patients. An example of staining with COX-1 is shown in Figure 3. The expression of the enzymes in the PGE_2 pathway was mainly localized to scattered mononuclear cells and endothelial cells. This points to that different cellular sources than the inflammatory cells which are most common in infiltrates and that may contribute to inflammation in the skeletal muscle of the patients, and PGE_2 may contribute to muscle pain and weakness since those symptoms were present in the patients who were examined.



PPS Control

Figure 3. COX-1, immunohistochemistry staining of the tibialis anterior muscle in a PPS patient and a healthy control. Positive staining in brown. Note the positive staining inbetween the cells, mostly in the capillaries, in the PPS patient.

Since the enzymes of the PGE_2 pathway were mostly found in blood vessels, it would point to an inflammatory process that might be generalized to a systemic inflammation. This is consistent with the findings in previous studies showing an up-regulation of inflammatory molecules such as TNF-α and IL-6 in peripheral blood (47, 48, 61), as well as the cytokines TNF-α, IFN-γ, IL-4 and IL-10 in cerebrospinal fluid (CSF) (58-60). Gonzalez et al. (61) were not able to correlate the cytokine levels to clinical parameters and in the study by Nollet et al. (107) there was no correlation between cytokine levels in PB and functional decline. However, only a small number of inflammatory molecules have been analyzed and there is reason to believe that there might be a connection between molecular and clinical parameters i.e. a biomarker for PPS.

Pain is a common complaint in PPS patients. The pain in PPS has been characterized as mostly nociceptive, of high intensity, and mostly localized to muscles (74). It was, however, recently shown that although the pain has a high intensity it does not decrease quality of life for PPS patients, as would be expected (107). This could be due to a long-standing pain, forcing PPS patients to develop effective coping strategies. It could be speculated that the effect on pain of IvIg may be due to an altered regulation of the prostaglandin enzymes. The present finding may increase knowledge of the background of pain in PPS, and the effect of IvIg as well as opening up for new treatment strategies.

4.2 STUDY II

When comparing levels of circulating IC in PPS patients to the entire control group, as well as to the 30 oldest controls, there were no statistically significant differences between the groups. No difference was found in TNF levels induced by immune complexes when comparing patients and controls. There was no increase in circulating immune complex or in TNF-inducing effects of circulating immune complex in the PPS patients when compared to healthy controls. This indicates that the background of PPS is not due to a complement mediated autoimmune reaction.

Gonzalez et al. (58) speculated that an autoimmune process might drive the inflammation in PPS. There are several studies showing inflammation in PPS and there is also evidence of increased antibodies directed against the Polio virus (K. Borg 2015, personal communication), indicating an active immune response. It may be concluded that there are several different factors, which may be involved in inflammation in PPS, and an autoimmune

background cannot be excluded, since other autoimmune mechanisms may be involved. Increased knowledge of the factors behind the inflammatory reaction and what drives the inflammation is important for future treatment strategies.

4.3 STUDY III

Compared to reference populations, PPS patients in the present study have low or normal total cholesterol and low density lipoprotein (LDL) levels, whereas high density lipoprotein (HDL) and triglyceride levels are low. The Framingham risk score calculated for the PPS patients gave a 10-year mean risk of 8% for a coronary artery disease event. When the Framingham score is less than 10%, this is considered a low risk. Results from an earlier study (86) showed increased values of blood lipids in up to 60% of the PPS patients. This could not be confirmed in the present study. However, the findings of normal blood lipids and the low risk of coronary heart disease are in accordance with the findings of a normal incidence of heart disease as described by Farbu et al. (87).

Furthermore, due to the results of earlier studies it has been suggested that doctors should be generous with statin treatment in PPS. This is not supported by the present findings. Statin treatment may also give rise to Statin myopathy (18), which may enhance the functional decline.

Increased blood lipids may be due to an inflammatory process, as seen in disorders such as rheumatoid arthritis and SLE (89-91). However, this seems not to be the case in PPS. Hence, the persisting inflammatory process in post-polio does not seem to be associated with increased lipids or an increased risk of coronary heart disease events.

4.4 STUDY IV

Of the 150 patients (84 females, 66 males), 61 patients (41%) were diagnosed with PPS and 89 (59%) with non-PPS.

The PPS and non-PPS groups were analyzed separately and individually, and divided into three different categories - Improvement, decline or no difference. Improvement being an increase of 10 points or more on the SF-36 scale or 20 or more points on VAS, decline was a decrease of 10 points or more on SF-36, and 20 points or more on VAS, and no difference were the patients who had a difference of less than 10 points on SF 36 or 20 points on VAS before and after the IvIg treatment. A higher number of patients who experienced an improvement and a lower number of patients who experienced decline in the SF-36 subdomains were found in the PPS group, compared with the non-PPS group. The difference

was statistically significant only for PF (p=0.013). When analyzing mean values the PPS group alone had statistically significant effects on the SF-36 subdomains PF (p=0.006), RP (p=0.027), BP (p=0.043), VT (p=0.002) and MH (p=0.033). In the non-PPS group there were no statistically significant differences on SF-36 or VAS for pain intensity after the IvIg treatment.

Overall, both the PPS and the non-PPS patients experienced a significant effect of the IvIg treatment on four of the SF-36 subdomains BP (p=0.029), VT (p=0.05), RE (p=0.010) and the sum score MCS (p=0.009).

The PPS patients showed significant effects in five of the SF-36 subdomains i.e. PF (p=0.006), RP (p=0.027), BP (p=0.043), VT (p=0.002) and MH (p=0.033). The non-PPS patients alone showed no statistically significant effect on SF-36 or VAS for pain intensity of the IvIg treatment. Also of interest, however not statistically significant, in all but one of the parameters of SF-36 and VAS the PPS patients showed more improvement than the non-PPS patients. When comparing the groups, the PPS patients experienced a significantly better effect of IvIg treatment on VAS, and three areas on SF-36, than the non-PPS patients.

Hypertonia and cancer were the dominating concomitant disorders in both PPS and non-PPS patients. Concomitant disorders were more common in the non-PPS group when compared with the PPS group with the exception of depression/anxiety and osteoporosis, which were more common in the PPS than in the non-PPS patients.

The general belief has been that all or almost all patients with sequelae after polio would develop PPS. Dalakas (29) and Grimby et al. (28) divided the patients into stable and unstable. On the basis of the present study, it could be speculated that PPS might be at least two different conditions. One which is more stable, with slow deterioration of function and strength, due to factors such as ageing, inactivity, and the increase of concomitant disorders, and one which is progressive, with a more rapid deterioration of function, and to a greater extent accompanied by other symptoms such as fatigue, “polio-pain”, cold-sensitivity, swallowing problems, atrophy and progressive weakness of the muscles.

It seems from the present study that an accurate diagnosis after a relatively long period of observation and the exclusion of concomitant disorders is of great importance for the outcome of IvIg treatment in PPS patients. There are several side-effects and the IvIg treatment is expensive and consequently only patients who will benefit from the treatment should receive it. Further studies are necessery on the subject of taxonomy and diagnostic criteria of late effects of polio and PPS as well as the IvIg treatment of PPS patients.

5 GENERAL DISCUSSION

PPS has, for a couple of decades been regarded as a neurophysiological condition with a background of non-compensated denervation of enlarged re-innervated motor units. With the background of results from further research, there may also be other factors, such as immunological and genetic mechanisms behind PPS. The pathophysiology of PPS is beginning to be revealed and there is increasing evidence of an inflammatory process reported from several groups involved in post-polio (47, 48, 59). However, there is no inflammatory marker that is correlated directly to decrease of clinical function and, could consequently be a biological marker for PPS. From the proteomic study by Gonzalez et al. (62) there are three proteins that are of major interest as possible biomarkers, but further research is needed in order to correlate clinical and molecular parameters. Due to the identification of an inflammatory process in PPS, several studies on treatment by IvIg have been performed. The outcome has been positive but there have been difficulties in pinpointing responders and non-responders. With a longer follow-up and identification of concomitant disorders the outcome improved, as shown in this study. Increased prostaglandin enzymes were found mainly in the capillaries of muscle tissue of PPS patients and further support a systemic inflammation in PPS, as suggested earlier. This may provide an explanation for fatigue and pain in PPS but it must be more firmly established. These findings may be the beginning of a new field of pain treatment in PPS i.e. with specific blockers of prostaglandin enzymes, which are already available today.

There is reason to discuss the effect of IvIg on PPS. Since Gonzalez et al. (76) showed a decrease of cytokines in CSF combined with clinical improvement of PPS patients were treated with IvIg, it is supposed that the effect would be central, in the CNS. However, if prostaglandin up-regulation in the capillaries is down-regulated by IvIg, the effect would be peripheral. The dosage of IvIg has been high in order for IvIg to pass over the blood-brain barrier. If the effect of IvIg is on prostaglandin enzymes outside the CNS, the dosage could be dramatically decreased and administration would not have to be intravenous which would decrease the cost and open up for administration outside hospital. It also opens the door to alternative treatments as discussed above.

There are probably several factors that drive the immune response in PPS. Although the present study did not point to an autoimmune background to PPS, other autoimmune

reactions are possible. The breakdown of nerve and muscle may give rise to antibody production. There are also several other possibilities that have to be considered. A persistent Poliovirus cannot be excluded and there may be genetic factors behind the active immune response.

The persisting inflammation in Swedish PPS patients does not seem to be associated with dyslipidaemia or an increased risk of coronary heart disease, which is the case in other diseases associated with inflammation such as SLE and RA. In these diseases, altered metabolism of lipoproteins gives rise to changes in the lipid profile and hyperlipidaemia may be secondary to the systemic inflammatory process. Further studies categorizing the inflammation and its background are of interest, and in order to be able to treat the inflammation adequately the factors driving the inflammation must be identified. The previously reported increase in blood lipids (86) could not be repeated in this study, and thus blood lipids could not be monitors of the inflammatory response and the treatment of PPS. Furthermore, the recommendation to be generous with Statin treatment for PPS patients is not valid.

6 CONCLUSIONS

This thesis shows evidence of an inflammatory process in muscle based on an elevated expression of the enzymes in the PGE_2 pathway, i.e. mPGES-1, COX-2, COX-1 and cPGES, which opens new perspectives for specific treatment. There is no evidence of immune complexes in PPS based on the measurement of circulating immune complexes in blood in the patients, reducing the possibility that there is an immune complex mediated explanation to the inflammation. The inflammation does not seem to give rise to hyperlipidaemia, since no elevation of blood lipids was seen in the PPS patients. When reevaluating the PPS diagnosis, patients who were treated with IvIg 2-8 years earlier, and are still considered to have PPS in the follow-up experienced a better outcome of the treatment than patients with a stable condition, mostly explained by concomitant diseases i.e. non-PPS. A thorough diagnosis of PPS and making a distinction between the progressive form of PPS and the form that is more stable, and often dependent on concomitant diseases, non-PPS, is important in treatment with immunomodulatory drugs, such as immunoglobuline. It may also indicate that all PPS may be divided into different subgroups; one with a more progressive course and with an inflammatory background, and one showing slow progress, which may be mostly due to ageing and concomitant disorders.

7 FUTURE RESEARCH

For the future, there are several fields of interest to explore:

To evaluate taxonomy and diagnostic criteria for PPS in order to establish if there are two different subgroups and if so, evaluate prognosis and different treatment options for the different groups.

Search for and evaluate specific biomarkers for PPS in order to explore the immunological mechanism behind the up-regulation of prostaglandin enzymes and cytokines in PPS, as well as correlating them to clinical parameters.

A possible correlation between pain in the PPS patients and the prostaglandin enzymes has to be established, and if a correlation is found, evaluation of treatment with low dose of IvIg and specific medication directed against the prostaglandin synthesis, such as like COX-1 and -2 inhibitors.

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