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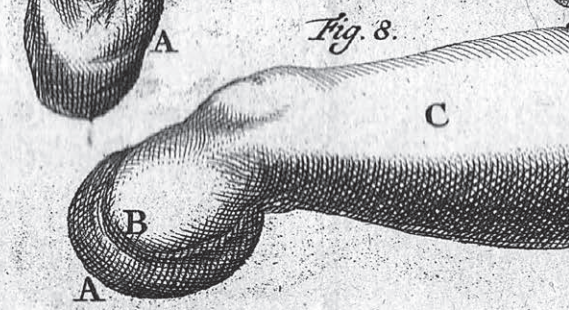
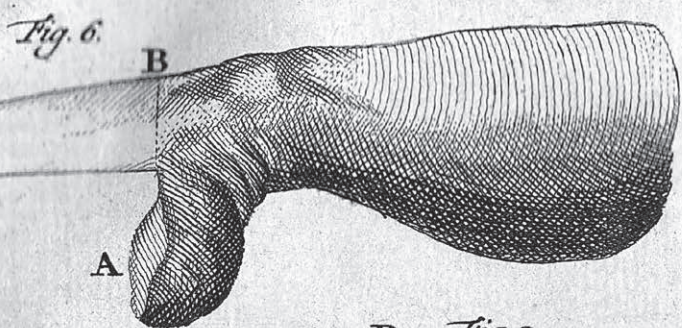
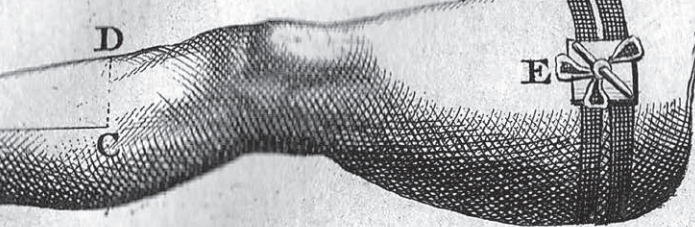
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Liposteroid Therapy for Idiopathic Pulmonary Hemosiderosis: A Scoping Review of the Literature

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Abstract: Idiopathic pulmonary hemosiderosis (IPH) is a rare cause of diffuse alveolar hemorrhage (DAH). Glucocorticosteroids (CS) represent the first line therapy for IPH. Although most patients respond to CS, steroid refractoriness is seen in an appreciable minority of patients. This paper reviews and evaluates the efficacy and safety profile of liposomal dexamethasone 21-palmitate (liposteroid) for the treatment of IPH. Medline, Embase and Web of Science biomedical databases were searched between 1980 and 2020 to identify papers describing patients with IPH, who were treated with liposteroid. A total of five articles were identified. Four in the form of case reports and one as a case series. A total of 12 pediatric patients (5 boys, 7 girls) were identified, with a median age of 2.3 years (range 0.5–8.6). Liposteroid therapy in intravenous doses ranging 0.06–0.1 mg/kg body weight appeared to be effective for both remission induction therapy, and maintenance therapy. There was no mortality among patients treated with liposteroid, either in the acute phase or during follow-up. The majority of patients for whom long-term follow-up data were available, were cured or in disease remission. No acute adverse events were reported, and long-term side effects were minimal and tolerable. Liposteroid represents a potential alternative or supplement to conventional CS therapy, as it appears to be more efficacious and associated with fewer side effects. Larger prospective, controlled trials are necessary to be able to define more precisely the therapeutic role of liposteroid in IPH.

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Introduction

Idiopathic pulmonary hemosiderosis (IPH) is a rare cause of diffuse alveolar hemorrhage (DAH) (Milman and Pedersen, 1998). The classic presentation of IPH includes hemoptysis, radiologic chest infiltrates, and anemia. Although hemoptysis is present in the majority of patients, the classic triad is less common (Chen et al., 2017). In pediatric patients, the identification of hemoptysis may be challenging due to swallowing of the sputum, and patients may present with unexplained anemia. The intensity of DAH and the degree of respiratory impairment in IPH are highly variable (Saha, 2020). The majority of patients with IPH present with recurrent episodes of hemoptysis without respiratory failure. However, a minority of patients suffer from massive pulmonary hemorrhage resulting in acute respiratory failure and occasionally death (Gutierrez et al., 2014; Matsumoto and Nakagawa, 2019). Recurrent episodes of DAH may result in pulmonary fibrosis and progressive respiratory failure (Saha and Chong, 2022).

The etiology of IPH is so far unknown (Ioachimescu et al., 2004; Saha, 2020). Based on recent evidence, IPH appears to be a disease of immunologic origin with a possible genetic background (Taytard et al., 2013; Saha and Milman, 2021a). Therefore, clinicians are focused on using immunosuppressive agents, such as glucocorticosteroids (CS) and antimetabolites, for the treatment of IPH (Ali et al., 1998; Saha and Milman, 2021a). Early administration of these agents has significantly reduced overall mortality in IPH during recent years (Soergel and Sommers, 1962; Ohga et al., 1995; Kiper et al., 1999; Taytard et al., 2013). Currently, there is no consensus on a standardized treatment for IPH, and there is significant variability in the treatment regimens used throughout the world (Chin et al., 2015).

The treatment of IPH can be classified into “remission induction” and “remission maintenance” treatment phases. “Remission induction” treatment refers to the early therapy that is given during an acute episode of DAH. “Remission maintenance” treatment denotes the therapeutics employed to prevent the recurrence of DAH. Generally, systemic CS is the first line of therapy for remission induction. Although most patients improve on CS, steroid refractory cases are not uncommon. In these patients, second line therapy, such as cyclophosphamide or azathioprine, can be used as add-on therapy (Naithani et al., 2006; Flanagan et al., 2013). Liposteroid has shown promising results as a remission inducing agent, both as a single therapy and in combination with other CS. In addition, liposteroid has demonstrated efficacy as a remission maintenance agent.

Dexamethasone sodium phosphate (DSP) is a long-acting CS with a distinctly higher potency than prednisone or prednisolone, and liposteroid is the liposomal preparation of dexamethasone palmitate (Yokoyama et al., 1985; Furst and Saag, 2021). Due to its high lipid solubility and affinity for phagocytic and inflammatory cells, liposteroid achieves high concentrations in inflamed tissues (Yokoyama et al., 1985), and has an anti-inflammatory effect that is significantly higher than DSP

(Mizushima et al., 1982). The properties of liposteroid and the indications for treatment has recently been reviewed (Saha and Milman, 2021b).

The purpose of the present paper is to review and evaluate the efficacy and safety profile of liposteroid treatment in IPH, based on the existing reports. This may be of help to the clinician in the decision-making process of treatment modalities in the individual patient with IPH.

Methods

This manuscript is a scoping review of the literature. The literature was scrutinized to identify appropriate studies. The Medline, Embase and Web of Science databases were searched between 1980 and 2020 to identify all patients with IPH treated with liposteroid. The bibliographies of the identified reports were subsequently scrutinized to find additional reports. Both pediatric and adult patients were included in the search. Papers published in any language were included in this review. The databases were searched with the following terms: “idiopathic pulmonary hemosiderosis”; “idiopathic pulmonary hemosiderosis AND liposteroid”; “idiopathic pulmonary hemosiderosis AND treatment”.

In total, 292 articles were found in the initial search. After examining the abstracts, full texts were reviewed for 13 papers. We finally identified five manuscripts that fulfilled our inclusion criteria. Out of the five papers, four were case reports (Table 1), and one was a small series of patients (Ohga et al., 1994; Doi et al., 2013, 2015; Sakamoto et al., 2018; Tobai et al., 2020). One article in Japanese (Sakurai et al., 1995) was translated into English.

Results

Demographics

A total of 12 patients were identified, 5 patients from case reports, and 7 from the patient series as shown in Tables 1 and 2. Two patients in the patients series (Doi et al., 2013) had previously been published as case reports (Ohga et al., 1994). All patients were Japanese children being started on liposteroid therapy at a median age of 2.3 years (range 0.5–8.6) (Table 2). Among the 12 children, 5 were boys and 7 girls. At birth, three were low birth weight babies being small for gestational age.

Autoimmune associations

All patients were examined for serum antinuclear antibody (ANA). Two patients had a positive ANA, but the titre was low in both, and none demonstrated symptoms or signs of autoimmune disease (Tables 1 and 2). The ANA developed during the follow up period in both patients (Doi et al., 2013). A positive serum antineutrophil cytoplasmic antibody (ANCA), milk protein allergy antibody or serum tissue transglutaminase antibodies were not reported in any patient. Two patients had trisomy 21, one of them developed thyroiditis during follow-up. Both patients with a positive ANA were alive at the end of the follow-up period.

Table 1 – Previous cases of IPH treated with liposteroid therapy

Report	Age	Sex	Country	First IPH diagnosis	Hemoglobin of presentation (g/dl)	Auto-immune workup	Dia-gnosis
	22 months	M	Japan	yes	6.1	negative	Sputum cytology and MRI
Ohga	14 months	F	Japan	yes	4.9	negative	Gastric aspirate cytology and MRI
Sakurai	6 months	M	Japan	yes	3.1	negative	Gastric aspirate cytology
Sakamoto	6 year	F	Japan	10 months of age	NR	NR	Gastric aspirate and sputum cytology
Tobai	2 year	F	Japan		2.2	negative	Gastric aspirate cytology

IPH – idiopathic pulmonary hemosiderosis; M – male; F – female; NR – not reported; MRI – magnetic resonance imaging

Treatment before liposteroid	Liposteroid dose	Outcome	Duration of therapy	Adverse effect	Recurrence
Methylprednisolone bolus Prednisolone 2 mg/kg Cyclosporin	0.05 mg/kg biweekly	Remission	14 months	none	One, upon discontinuation with a bacterial infection
Methylprednisolone	0.05 mg/kg every 10–14 days	Remission		none	Recurrence after 4 months managed with addition of azathioprine and inhaled corticosteroid
Methylprednisolone bolus (30 mg/kg/day × 5 days and prednisolone 2 mg/kg × 5 days failed, then another pulse steroid therapy was tried for 3 days with remission of symptoms	0.1 mg/kg/day every 2 weeks	Remission	7 months afterwards still no recurrence	none	none
Intravenous dexamethasone sodium	0.8 mg/kg/day for 3 days followed by maintenance dose weekly (0.5 mg/kg) with methyl prednisolone	Remission	NS	PRES Palmitate induced inflammation	Patients discharged from hospital 5 months after initiation of liposteroid
Intravenous prednisolone followed by oral prednisolone for maintenance Complicated by recurrent bleed and side effects	0.06 mg/kg/day for 3 days, followed by maintenance therapy every 4 weeks at the time of report		24 months		Recurrent bleed twice responded to bolus dose for 3 days

Table 2 – Demographic data, clinical presentation, and treatment in 12 children with idiopathic pulmonary hemosiderosis (IPH)

Variables	Number of patients /total number of reported patients
Sex	
– Boys	5/12
– Girls	7/12
Age (year)	2.3 (0.5–8.6)
Ethnicities	
– Asian	12/12
Admission hemoglobin (g/l)	4.8 (2.2–7.4)
Blood transfusion on admission	10/12
Antinuclear antibody	2/12
Genetic disorder	2/12
Liposteroid as remission induction therapy	10/12
– Steroid refractory patients	7/10
– Initial therapeutic agent	3/10
– Immunosuppressive agent before liposteroid	3/10
Liposteroid as maintenance therapy	2/12
– With corticosteroids	12/12
– With immunosuppressive agents	7/12
Follow-up period (years)	9.5 (0.6–16.9)
Hospitalizations during liposteroid therapy	7 (2.5–10.5)
Adverse effects	
Short-term adverse events	
– Posterior reversible encephalopathy	0/9
– Systemic inflammatory response	0/9
Long-term adverse events	
– Weight gain, cushingoid face, cataract, cutaneous striae, hypertension, low height	0/9
Reduced bone mineral density	4/9
Outcome	
– Cured	3/12
– Remission	6/12
– Active disease	3/12

Data presented as median (range)

Dosing of liposteroid therapy

Liposteroid given as intravenous infusion was initiated for remission induction treatment in 10/12 patients and for maintenance of remission in 2/12 patients (Table 2). For remission induction treatment, 8/12 patients received liposteroid

in doses ranging from 0.06 to 0.1 mg/kg body weight/day for three consecutive days and 2/12 received a dose of 0.05 mg/kg once every 10–14 days. In addition to liposteroid, 7/10 patients received other systemic CS as part of the induction treatment. Prior to treatment with liposteroid, 3/10 patients were treated with an antimetabolite.

The doses used for remission induction was also used for maintenance therapy. Typically, the maintenance therapy was initiated one week after the last induction dose, and the interval between the subsequent doses was gradually increased based on the patients' response. If the patients remained stable, without any evidence of relapse, the interval between infusions was increased up to every four weeks. If there was a recurrence of bleeding during the maintenance phase, the induction treatment regimen was repeated to control bleeding. During maintenance therapy, 7/12 patients required other immunosuppressive medications in addition to liposteroid.

Duration of liposteroid therapy

The duration liposteroid treatment was variable. Tobai et al. (2020) treated one patient with liposteroid for 2 years. In the study by Doi et al. (2013), all 9 patients received at least four years of monthly liposteroid treatment as maintenance therapy, and 2/9 patients received liposteroid therapy for more than 10 years. These 9 patients received liposteroid therapy for a median of 6.1 years, with a range of 2.3–15.6 years (Doi et al., 2013). In this review of 12 patients, the median duration of liposteroid therapy was 5.8 years, with a range of 0.6–15.6 years.

Efficacy of liposteroid therapy

Improvement during liposteroid treatment was reported in all 12 patients. The occurrence of respiratory failure requiring mechanical ventilation was not specified for all patients. However, two patients with respiratory failure could be weaned from mechanical ventilation after initiation of liposteroid therapy. Likewise, hemoptysis and anemia improved during liposteroid therapy. The long-term outcomes were assessed only in the study by Doi et al. (2013); all 9 patients were alive after a median follow-up period of 11 years (2.4–16.9 years); three patients remained symptom-free without medication for more than 1.4 years, three patients achieved long-term remission lasting more than two years, while the three remaining patients still had active disease.

The majority of patients did not report any limitation in physical activity during rest or exercise. At the end of the follow-up period, pulmonary function tests were available in 6/12 patients, while 6/12 patients could not cooperate. The forced vital capacity (FVC) was normal in three, mildly reduced in two, and moderately reduced in one patient. The only patient reporting exertional dyspnea during the observation period had a mild reduction of FVC, he was in the active phase of the disease and was reportedly non-compliant with the treatment (Doi et al., 2013). The serum

KL-6 level, an indicator for activity in pulmonary fibrosis was normal in all 9 patients (Doi et al., 2013).

Recurrence

After induction treatment, 11/12 patients had recurrence of bleeding, leading to hospitalization for median 7 times (range 2–20) (Table 2). The median blood hemoglobin concentration on first admission was markedly decreased, 48 g/l (normal reference interval 110–130 g/l) but had normalized without blood transfusion in all patients by the end of the observation period.

Adverse events

The long-term adverse effects of liposteroid therapy were assessed in the 9 patients of Doi et al. (2013). There were no cases of obesity, cutaneous striae or abnormal body fat distribution, such as cushingoid appearance, and no cases with hypertension or visual impairment. Low bone mineral density was found in four patients, but none had bone fractures. Although the final height of the liposteroid treated patient series was comparable to the normal population, no patient exceeded the 85th height percentile at the final follow-up.

Sakamoto et al. (2018) reported acute adverse events following the first infusion of liposteroid in a 6-year-old girl consisting of restlessness and irritability possibly due to posterior reversible encephalopathy syndrome. In addition, the patient presented a systemic inflammatory response due to palmitic acid causing elevated inflammatory markers, such as C-reactive protein (Sakamoto et al., 2018; Korbecki and Bajdak-Rusinek, 2019). However, this patient erroneously received an overdose of liposterid (see discussion).

Discussion

This paper discusses the role of liposteroid therapy in the management of DAH caused by IPH. Dexamethasone is a long-acting CS with antiinflammatory properties that is 6 times as potent compared to an equivalent dose of prednisone or prednisolone (Furst and Saag, 2021). Liposteroid is the liposomal preparation of dexamethasone palmitate (Yokoyama et al., 1985). The liposome particle is spherical in shape and composed of a phospholipid bilayer, which can be single or multi-lamellar (Benameur et al., 1993). The specific drug molecule is carried within the center of the liposome. Liposteroid was originally designed to target CS therapy to inflamed tissues, in order to reduce the unwanted systemic side effects of CS (Yokoyama et al., 1985). Liposteroid was developed in 1980 by Dr. Yutaka Mizushima in Japan and is currently approved for clinical use in several inflammatory disorders.

Compared to the conventional parenteral preparation of dexamethasone (DSP), which is hydrophilic, liposteroid is more lipophilic, and the tissue distribution and distribution half-life vary between these preparations. Liposteroid is administered

via the intravenous route as an infusion and taken up by phagocytic cells, e.g. macrophages in the reticuloendothelial system by phagocytosis; the rate of uptake is approximately 8 times faster than of free dexamethasone palmitate and DSP (Yokoyama et al., 1985; Wakiguchi and Ohga, 2016). Therefore, liposteroid achieves a higher concentration in the spleen compared to DSP (Mizushima et al., 1982). In contrast, due to its hydrophilic nature, DSP demonstrates a higher concentration than liposteroid in skeletal muscle. The elimination half-life is longer for DSP than for liposteroid (Yokoyama and Watanabe, 1996).

By virtue of its lipid solubility and predilection for phagocytic cells and other inflammatory cells, liposteroid achieves a two-fold higher concentration in inflamed tissues (Yokoyama et al., 1985), and the anti-inflammatory effect is 5–6 times more potent than DSP (Mizushima et al., 1982). Liposteroid exerts more potent inhibitory effect than DSP on the proinflammatory functions of the macrophages, such as receptor-mediated phagocytosis, production of superoxide, lipid peroxidation, and chemotaxis (Yokoyama and Watanabe, 1996).

As liposteroid is distributed primarily in the reticuloendothelial system, the suppression of the hypothalamic-pituitary axis is lower than by conventional steroid formulations. In animal studies, the level of dexamethasone in the pituitary was significantly lower with liposteroid compared to DSP (Mizushima et al., 1982) and there appears to be fewer metabolic side effects with liposteroid compared to the free dexamethasone palmitate (Schiffelers et al., 2006).

Liposteroid has been utilized for several rheumatologic and non-rheumatologic diseases. The role of liposteroid has been studied more comprehensively in patients with rheumatoid arthritis than in patients with other diseases (Mizushima et al., 1983; Hoshi et al., 1985). In a multicenter, double-blind comparative trial of 138 patients with rheumatoid arthritis, Hoshi et al. (1985) reported a significantly higher rate of improvement and lower adverse effects with biweekly intravenous or intramuscular liposteroid (0.05–0.08 mg/kg/body weight) compared to DSP. Efficacy of liposteroid has also been seen in patients with the macrophage activation syndrome or hemophagocytic lymphohistiocytosis, graft versus host disease, inflammatory myopathy, and immune thrombocytopenic purpura (Sakurai et al., 1999; Funauchi et al., 2003; Kobayashi et al., 2007; Nishiwaki et al., 2009, 2014; Filipovich et al., 2010; Wakiguchi et al., 2015; Wakiguchi and Ohga, 2016).

IPH is a rare disease. Based on the literature, IPH appears to be more common in children compared to adults (Ioachimescu et al., 2004). Among children, the incidence varies between 0.24 and 1.23 cases per million individuals per year (Kjellman et al., 1984; Ohga et al., 1995). Chen et al. (2017) reported only 37 adult cases in the period 2000–2015. We found that all reported patients who had received liposteroid therapy were in the pediatric age group. Based on the French database of rare pediatric diseases, IPH is more prevalent among girls (Taytard et al., 2013). This trend was present in our patient series as well. In contrast, there is a distinct male predominance among the reported adult cases (Chen et al., 2017). In

children, IPH frequently presents before the age of 10 years, as in this patient series. IPH in childhood appears to be more aggressive and difficult to treat compared to adult patients (Chen et al., 2017).

Despite first being reported by Virchow in 1864, the pathogenesis of IPH has remained obscure (Virchow, 1864; Zhang et al., 2019). There are significant suggestions of an immunologic and/or autoimmune pathogenesis for IPH (Saha, 2021). For example, autoantibodies are found in a considerable number of patients with IPH, either at diagnosis or later during the course of the disease (Iijima et al., 1988; Taytard et al., 2013; Freitas et al., 2015; Yanagihara et al., 2018; Stainer et al., 2019). Some patients with biopsy proven IPH have developed ANCA positive vasculitis many years after the initial diagnosis of IPH (Freitas et al., 2015). There may also be a genetic contribution (Milman and Pedersen, 1998; Watanabe et al., 2015; Alimi et al., 2018). IPH is often seen in patients with trisomy 21 and is in these patients associated with pulmonary hypertension and worse outcomes (Taytard et al., 2013). We have proposed renaming IPH to immune or pauci immune-mediated pulmonary hemosiderosis due to the immunologic association (Saha, 2021). In this liposteroid series, two patients had ANA without overt autoimmune disease. One patient with trisomy 21 developed thyroiditis during follow-up. A recent study found the overall prevalence of autoantibodies to be 26.4% in pediatric patients.

The diagnosis of IPH is often delayed (Chen et al., 2017). A definitive diagnosis of IPH requires histopathologic evaluation of lung biopsy specimens (Saha, 2020). DAH from IPH is often referred to as “bland pulmonary haemorrhage”, denoting the absence of vasculitis and inflammatory cellular infiltration of the lung parenchyma. It is crucial to emphasize that other causes of “bland pulmonary haemorrhage” such as cardiac diseases, anticoagulation therapy, and some autoimmune/ rheumatologic diseases need to be ruled out before making a definitive diagnosis of IPH (Ioachimescu et al., 2004; Imtiaz et al., 2019; Saha and Chong, 2021; Saha et al., 2021; Saha et al., 2022). In children, IPH is often diagnosed by a compatible clinical history, radiologic findings, and identification of hemosiderin-laden macrophages from the sputum, bronchoalveolar lavage or gastric aspirate. However, this could lead to an erroneous diagnosis of IPH as patients with small-vessel vasculitis may have a negative serologic workup (Fullmer et al., 2005; Thompson et al., 2016). All patients in this liposteroid series were diagnosed with IPH without having a lung biopsy. Thus, there is a possibility that some of these patients may have suffered from a vasculitic disorder rather than IPH.

The treatment of IPH can be divided into remission induction and maintenance phases. There is no agreed-upon treatment regimen for IPH, and we have recently proposed a treatment algorithm (Saha and Milman, 2021a). Most clinicians would choose CS as the first-line therapy for inducing remission in the acute phase (Kiper et al., 1999; Chin et al., 2015). A questionnaire-based multinational study that surveyed pediatric physicians taking care of IPH patients revealed that CS was the medication

of choice during active bleeding (Chin et al., 2015). Similarly, CS also represented the first-choice treatment for maintenance therapy. The choice of a second-line agent varied among physicians. The majority favoured hydroxychloroquine, azathioprine, cyclophosphamide, and inhaled corticosteroids. Although some authors have questioned the long-term efficacy of the CS in the overall outcome, there appears to be a significant improvement in the outcomes among patients treated with CS. Indeed the survival rate in IPH has improved significantly during recent years, possibly due to the early initiation of immunosuppressive therapy (Soergel and Sommers, 1962; Kiper et al., 1999; Taytard et al., 2013).

Clinicians are often faced with challenges while treating patients with IPH. First, a subgroup of patients appears to be refractory to CS and requires additional immunosuppressive agents, such as cyclophosphamide or azathioprine (Colombo and Stolz, 1992; Rossi et al., 1992; Saeed et al., 1999; Airaghi et al., 2001; Helman et al., 2003; Naithani et al., 2006; Kamienska et al., 2009). However, these agents may be associated with severe adverse events, including the occurrence of malignancy (Radis et al., 1995; Bernatsky et al., 2008). Second, long-term use of CS therapy is associated with significant side effects, such as obesity, cushingoid appearance, cataracts, hyperlipidemia, hypertension, diabetes mellitus and osteoporosis.

The liposteroid was formulated as a targeted drug therapy to increase efficacy and reduce side effects from CS therapy. Liposteroid has been used as both induction therapy and maintenance agent. Intravenous methylprednisolone is typically used during acute IPH with respiratory failure (Li et al., 2017; Milman, 2020). As a remission inducing agent, liposteroid was predominantly used in the steroid-refractory patients in this review. In the majority of cases, liposteroid was used as add-on therapy to another CS. As liposteroid is a more potent anti-inflammatory agent and demonstrates enhanced inhibition of macrophage activation, a beneficial effect is seen even if the patients are refractory to conventional CS therapy. When liposteroid is used as a maintenance agent, the long-term side effects of CS are distinctly minimized. In a minority of patients, other immunosuppressive agents were used in addition to liposteroid therapy.

The dosing regimen used for liposteroid therapy was variable. During the acute phase of the disease with bleeding, the medication is typically infused for three consecutive days. Subsequently, infusion is performed at weekly intervals. When the pulmonary hemorrhage is controlled, the interval between infusions can be progressively increased from one to two weeks with the goal to have maintenance infusion therapy every four weeks. The dose used in maintenance therapy varied between 0.06 and 0.08 mg/kg body weight. Most patients also receive another systemic CS, the dose of which can also be tapered based on the response to liposteroid therapy. Doi et al. (2013) continued the maintenance liposteroid therapy for at least four years. The optimal duration of therapy for IPH is unknown. After a symptom-free period of 12–18 months without bleeding it seems reasonable to taper and discontinue liposteroid (Saha and Milman, 2021a).

The liposteroid was efficacious. Several patients were either cured or maintained long-term remission on liposteroid therapy. More importantly, all patients survived during the follow-up period. Also, the majority reported no functional impairment at exercise, and the pulmonary function tests revealed a relatively well-preserved lung function. There was no evidence of pulmonary fibrosis in any of the patients. End-stage lung disease can occur as a complication of IPH, but was not observed in this patient series. Lung transplantation represents the only available long-term therapy in patients with end-stage lung disease (Wroblewski et al., 1997; Calabrese et al., 2002; Ross et al., 2020; Gocho et al., 2021; Saha and Chong, 2022).

Liposteroid appears to be safe both in the short and long-term treatment regimens. Doi et al. (2013) did not report any acute adverse events. In contrast, Sakamoto et al. (2018) reported the development of posterior reversible encephalopathy syndrome and a systemic inflammatory syndrome following liposteroid infusion. However, the infused overdose of liposteroid was 0.8 mg/kg/day (Sakamoto et al., 2018), similar to the dosing regimen erroneously reported by Doi et al. in 2013, which in 2015 in an *erratum* was changed to the correct dose of 0.08 mg/kg/day (Doi et al., 2015). Therefore, Sakamoto's patient received a 10-fold higher dose than used in a standard dosage regimen (Sakamoto et al., 2018).

Long-term therapy with CS is associated with significant side effects. One of the reasons why liposteroid had been used was to reduce the cumulative steroid exposure and reduce overall side effects. Doi et al. (2013) did not report any patient with cushingoid appearance, metabolic abnormalities, or increased body mass index due to obesity in patients who received liposteroid therapy. The main concern was the overall height of this patient cohort. Although no patient was below two standard deviations in the final height, no patient was above their estimated 85th percentile.

IPH causes significant short and long-term morbidity and mortality, and can affect patients of any age. The mortality in the acute phase of the disease in adult patients, who often demonstrates relative refractoriness to CS, is approximately 14% (Chen et al., 2017). The mortality in pediatric patients can be as high as 100% within the first 2.5–5 years, unless an aggressive immunosuppressive regimen is used (Soergel and Sommers, 1962; Kjellman et al., 1984; Kiper et al., 1999). Liposteroid is a relatively less known formulation of dexamethasone palmitate that is not available globally. Based on the literature, liposteroid is more potent than DSP and causes more effective inhibition of proinflammatory cellular function than traditional CS preparations. It can be used as the stand-alone therapy for remission induction or used in association with other systemic CS in patients who demonstrate steroid refractoriness. The use of liposteroid as a maintenance agent could be associated with fewer adverse effects and possibly better outcomes.

Limitations of this study

Our study has several limitations. We identified a small series of 12 Japanese pediatric patients who received liposteroid therapy for IPH, and long-term follow-up was not

performed in all patients. None of the patients had a lung biopsy. Additionally, there might be a selection bias in the reported cases as all patients were reported from Japan because liposteroid is not available in many countries of the world, including the United States and Denmark.

Conclusion

IPH is a rare disease. Although the majority of patients respond well to CS therapy, a significant minority does not. Immunosuppressive agents have been tried in IPH patients with variable success. Liposteroid is a liposomal preparation of dexamethasone palmitate with an anti-inflammatory effect that is 25 times more potent than prednisone. Liposteroid appears to be an effective treatment in remission induction and remission maintenance regimens. Liposteroid should be considered in patients with refractory IPH, and in maintenance therapy to reduce the side effects of long-term steroid therapy. This review supports the efficacy and relative safety of liposteroid as a therapeutic option for IPH in children, while the efficacy in an adult population remains speculative.

Prospective studies in both pediatric and adult patients are needed to evaluate whether liposteroid has specific advantages compared to other CS, and whether it should be used as single treatment or in combination with other conventional CS and/or alkylating agents or antimetabolites.

References

- Airaghi, L., Ciceri, L., Giannini, S., Ferrero, S., Meroni, P. L., Tedeschi, A. (2001) Idiopathic pulmonary hemosiderosis in an adult. Favourable response to azathioprine. *Monaldi Arch. Chest Dis.* **56(3)**, 211–213.
- Ali, A. M., Milman, N., Clausen, P. P., Reinert, P., Pedersen, F. M., Jacobsen, G. K. (1998) Idiopathic pulmonary haemosiderosis. Favourable effect of corticosteroids in two women aged 16 and 55 years. *Eur. Respir. Topic* **4**, 53–57.
- Alimi, A., Taytard, J., Abou Taam, R., Houdouin, V., Forgeron, A., Lubrano Lavadera, M., Cros, P., Gibertini, I., Derelle, J., Deschildre, A., Thumerelle, C., Epaud, R., Reix, P., Fayon, M., Roullaud, S., Troussier, F., Renoux, M.-C., de Blic, J., Leyronnas, S., Thouvenin, G., Perisson, C., Ravel, A., Clement, A., Corvol, H., Nathan, N.; French RespiRare® Group (2018) Pulmonary hemosiderosis in children with Down syndrome: A national experience. *Orphanet J. Rare Dis.* **13(1)**, 60.
- Benameur, H., De Gand, G., Brasseur, R., Van Vooren, J. P., Legros, F. J. (1993) Liposome-incorporated dexamethasone palmitate: Chemical and physical properties. *Int. J. Pharm.* **89(3)**, 157–167.
- Bernatsky, S., Clarke, A. E., Suissa, S. (2008) Hematologic malignant neoplasms after drug exposure in rheumatoid arthritis. *Arch. Intern. Med.* **168(4)**, 378–381.
- Calabrese, F., Giacometti, C., Rea, F., Loy, M., Sartori, F., Di Vittorio, G., Abudurehman, A., Thiene, G., Valente, M. (2002) Recurrence of idiopathic pulmonary hemosiderosis in a young adult patient after bilateral single-lung transplantation. *Transplantation* **74(11)**, 1643–1645.
- Chen, X.-Y., Sun, J.-M., Huang, X.-J. (2017) Idiopathic pulmonary hemosiderosis in adults: Review of cases reported in the latest 15 years. *Clin. Respir. J.* **11(6)**, 677–681.
- Chin, C. I. C., Kohn, S. L., Keens, T. G., Margetis, M. F., Kato, R. M. (2015) A physician survey reveals differences in management of idiopathic pulmonary hemosiderosis. *Orphanet J. Rare Dis.* **10**, 98.

- Colombo, J. L., Stolz, S. M. (1992) Treatment of life-threatening primary pulmonary hemosiderosis with cyclophosphamide. *Chest* **102(3)**, 959–960.
- Doi, T., Ohga, S., Ishimura, M., Takada, H., Ishii, K., Ihara, K., Nagai, H., Hara, T. (2013) Long-term liposteroid therapy for idiopathic pulmonary hemosiderosis. *Eur. J. Pediatr.* **172(11)**, 1475–1481.
- Doi, T., Ohga, S., Ishimura, M., Takada, H., Ishii, K., Ihara, K., Nagai, H., Hara, T. (2015) Erratum to: Long-term liposteroid therapy for idiopathic pulmonary hemosiderosis. *Eur. J. Pediatr.* **174(12)**, 1701.
- Filipovich, A., McClain, K., Grom, A. (2010) Histiocytic disorders: Recent insights into pathophysiology and practical guidelines. *Biol. Blood Marrow Transplant.* **16**, S82–S89 (Suppl. 1).
- Flanagan, F., Glackin, L., Slattery, D. M. (2013) Successful treatment of idiopathic pulmonary capillaritis with intravenous cyclophosphamide. *Pediatr. Pulmonol.* **48(3)**, 303–305.
- Freitas, A., Senra, V., Marinho, A., Guedes, M. (2015) Chronic alveolar haemorrhage in a paediatric patient: A diagnostic and treatment challenge. *BMJ Case Rep.* **2015**, bcr2014206856.
- Fullmer, J. J., Langston, C., Dishop, M. K., Fan, L. L. (2005) Pulmonary capillaritis in children: A review of eight cases with comparison to other alveolar hemorrhage syndromes. *J. Pediatr.* **146(3)**, 376–381.
- Funauchi, M., Ohno, M., Yamagata, T., Nozaki, Y., Kinoshita, K., Kanamaru, A. (2003) Effects of liposteroid on the hemophagocytic syndrome in systemic lupus erythematosus. *Lupus* **12(6)**, 483–485.
- Furst, D. E., Saag, K. G. (2021) Determinants of glucocorticoid dosing. *UpToDate*. Retrieved January 22, 2021, from: https://www.uptodate.com/contents/determinants-of-glucocorticoid-dosing?search=relative%20potency%20of%20corticosteroids&source=search_result&selectedTitle=1~150&usage_type=default&display_rank=1
- Gocho, K., Sato, K., Imasaka, K., Hamanaka, N., Takahashi, M., Shimizu, K., Takemura, T. (2021) A case of idiopathic pulmonary hemosiderosis associated with emphysematous change in an adult who underwent lung transplantation. *Intern. Med.* **60(1)**, 117–122.
- Gutierrez, S., Shaw, S., Huseni, S., Sachdeva, S., Costello, J. P., Basu, S., Nath, D. S., Klugman, D. (2014) Extracorporeal life support for a 5-week-old infant with idiopathic pulmonary hemosiderosis. *Eur. J. Pediatr.* **173(12)**, 1573–1576.
- Helman, D. L., Sullivan, A., Kariya, S. T., Deering, S. H., Hueppchen, N. A., Shorr, A. F. (2003) Management of idiopathic pulmonary haemosiderosis in pregnancy: Report of two cases. *Respirology* **8(3)**, 398–400.
- Hoshi, K., Mizushima, Y., Shiokawa, Y., Kageyama, T., Honma, M., Kashiwazaki, S., Shichikawa, K., Tsunematsu, T., Kaneko, K. (1985) Double-blind study with liposteroid in rheumatoid arthritis. *Drugs Exp. Clin. Res.* **11(9)**, 621–626.
- Iijima, N., Torii, Y., Ito, S., Hiramatu, K., Suzuki, M., Ito, T., Senda, Y. (1988) A case of idiopathic pulmonary hemosiderosis recurrent after remission of fifteen years and associated with Sjögren's syndrome. *Nihon Kyobu Shikkan Gakkai Zasshi* **26(11)**, 1191–1194. (in Japanese)
- Imtiaz, M., Saha, B., Sana Ullah, U., Saha, A. (2019) A case of acute life-threatening pulmonary hemorrhage from synthetic cannabinoid abuse. *Case Rep. Pulmonol.* **2019**, 8137648.
- Ioachimescu, O. C., Sieber, S., Kotch, A. (2004) Idiopathic pulmonary haemosiderosis revisited. *Eur. Respir. J.* **24(1)**, 162–169.
- Kamienska, E., Urasinski, T., Gawlikowska-Sroka, A., Glura, B., Pogorzelski, A. (2009) Idiopathic pulmonary hemosiderosis in a 9-year-old girl. *Eur. J. Med. Res.* **14**, 112–115 (Suppl. 4).
- Kiper, N., Göçmen, A., Özçelik, U., Dilber, E., Anadol, D. (1999) Long-term clinical course of patients with idiopathic pulmonary hemosiderosis (1979–1994): Prolonged survival with low-dose corticosteroid therapy. *Pediatr. Pulmonol.* **27(3)**, 180–184.
- Kjellman, B., Elinder, G., Garwicz, S., Svan, H. (1984) Idiopathic pulmonary haemosiderosis in Swedish children. *Acta Paediatr.* **73(5)**, 584–588.
- Kobayashi, Y., Salih, H. M., Kajume, T., Nakamura, K., Miyagawa, S., Sato, T., Nishimura, S., Kobayashi, M.

- (2007) Successful treatment with liposteroid followed by reduced intensity stem cell transplantation in an infant with perforin deficiency presenting with hemophagocytic lymphohistiocytosis. *J. Pediatr. Hematol. Oncol.* **29(3)**, 178–182.
- Korbecki, J., Bajdak-Rusinek, K. (2019) The effect of palmitic acid on inflammatory response in macrophages: An overview of molecular mechanisms. *Inflamm. Res.* **68(11)**, 915–932.
- Li, Y.-T., Guo, Y.-X., Cai, L.-M., Pan, L., Duan, M.-Q., Yang, L.-F., Sun, Y.-Y., Tan, W.-P., Chen, Z.-G. (2017) Methylprednisolone pulse therapy rescued life-threatening pulmonary hemorrhage due to idiopathic pulmonary hemosiderosis. *Am. J. Emerg. Med.* **35(11)**, 1786.e3–1786.e7.
- Matsumoto, S., Nakagawa, S. (2019) Extracorporeal membrane oxygenation for diffuse alveolar hemorrhage caused by idiopathic pulmonary hemosiderosis: A case report and a review of the literature. *J. Pediatr. Intensive Care* **8(3)**, 181–186.
- Milman, N. (2020) Idiopathic pulmonary hemosiderosis. *UpToDate*. Retrieved October 11, 2020, from: https://www.uptodate.com/contents/idiopathic-pulmonary-hemosiderosis?search=idiopathic%20pulmonary%20hemosiderosis§ionRank=1&usage_type=default&anchor=H17&source=machineLearning&selectedTitle=1~17&display_rank=1#H17
- Milman, N., Pedersen, F. M. (1998) Idiopathic pulmonary haemosiderosis. Epidemiology, pathogenic aspects and diagnosis. *Respir. Med.* **92(7)**, 902–907.
- Mizushima, Y., Hamano, T., Yokoyama, K. (1982) Tissue distribution and anti-inflammatory activity of corticosteroids incorporated in lipid emulsion. *Ann. Rheum. Dis.* **41(3)**, 263–267.
- Mizushima, Y., Kaneko, K., Hoshi, K. (1983) Targeting steroid therapy in rheumatoid arthritis. *Ann. Rheum. Dis.* **42(4)**, 479–480.
- Naithani, R., Chandra, J., Singh, V., Kumar, V., Dubey, N. K. (2006) Life threatening exacerbation in idiopathic pulmonary hemosiderosis salvaged by cyclophosphamide infusion. *Indian J. Chest Dis. Allied Sci.* **48(4)**, 287–289.
- Nishiwaki, S., Terakura, S., Ito, M., Goto, T., Seto, A., Watanabe, K., Yanagisawa, M., Imahashi, N., Tsukamoto, S., Shimba, M., Ozawa, Y., Miyamura, K. (2009) Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: A clue to refractory graft-versus-host disease. *Blood* **114(14)**, 3113–3116.
- Nishiwaki, S., Nakayama, T., Murata, M., Nishida, T., Terakura, S., Saito, S., Kato, T., Mizuno, H., Imahashi, N., Seto, A., Ozawa, Y., Miyamura, K., Ito, M., Takeshita, K., Kato, H., Toyokuni, S., Nagao, K., Ueda, R., Naoe, T. (2014) Dexamethasone palmitate ameliorates macrophage-rich graft-versus-host disease by inhibiting macrophage functions. *PLoS One* **9(5)**, e96252.
- Ohga, S., Nomura, A., Suga, N., Hikino, S., Kira, R., Matsuzaki, A., Ueda, K., Masuda, K. (1994) Liposteroid against refractory pulmonary haemorrhage in idiopathic pulmonary haemosiderosis. *Eur. J. Pediatr.* **153(9)**, 687–690.
- Ohga, S., Takahashi, K., Miyazaki, S., Kato, H., Ueda, K. (1995) Idiopathic pulmonary haemosiderosis in Japan: 39 possible cases from a survey questionnaire. *Eur. J. Pediatr.* **154(12)**, 994–995.
- Radis, C. D., Kahl, L. E., Baker, G. L., Wasko, M. C., Cash, J. M., Gallatin, A., Stolzer, B. L., Agarwal, A. K., Medsger, T. A., Kwok, C. K. (1995) Effects of cyclophosphamide on the development of malignancy and on long-term survival of patients with rheumatoid arthritis. A 20-year followup study. *Arthritis Rheum.* **38(8)**, 1120–1127.
- Ross, B., Halloran, K., Adam, B., Laing, B., Hirji, A. (2020) Disease recurrence after lung transplantation for idiopathic pulmonary hemosiderosis. *Respir. Med. Case Rep.* **30**, 101128.
- Rossi, G. A., Balzano, E., Battistini, E., Oddera, S., Marchese, P., Acquila, M., Fregonese, B., Mori, P. G. (1992) Long-term prednisone and azathioprine treatment of a patient with idiopathic pulmonary hemosiderosis. *Pediatr. Pulmonol.* **13(3)**, 176–180.

- Saeed, M. M., Woo, M. S., MacLaughlin, E. F., Margetis, M. F., Keens, T. G. (1999) Prognosis in pediatric idiopathic pulmonary hemosiderosis. *Chest* **116(3)**, 721–725.
- Saha, B. K. (2020) Idiopathic pulmonary hemosiderosis: A state of the art review. *Respir. Med.* **176**, 106234.
- Saha, B. K. (2021) Is it time to call idiopathic pulmonary hemosiderosis by the correct name: Immune-mediated pulmonary hemosiderosis? *Am. J. Med. Sci.* **361(6)**, 809–811.
- Saha, B. K., Chong, W. H. (2021) Diffuse alveolar hemorrhage in cardiac diseases. *Lung* **199(2)**, 103–112.
- Saha, B. K., Chong, W. H. (2022) Lung transplant to manage end-stage lung disease due to idiopathic pulmonary hemosiderosis: A review of the literature. *Respir. Investig.* **60(1)**, 82–89.
- Saha, B. K., Milman, N. T. (2021a) Idiopathic pulmonary hemosiderosis: A review of the treatments used during the past 30 years and future directions. *Clin. Rheumatol.* **40(7)**, 2547–2557.
- Saha, B. K., Milman, N. T. (2021b) Short review of liposteroid: A novel targeted glucocorticoid preparation for treatment of autoimmune and inflammatory diseases. *Prague Med. Rep.* **122(4)**, 257–268.
- Saha, B. K., Chong, W. H., Milman, N. T. (2022) Differentiation of idiopathic pulmonary hemosiderosis from rheumatologic and autoimmune diseases causing diffuse alveolar hemorrhage: Establishing a diagnostic approach. *Clin. Rheumatol.* **41(2)**, 325–336.
- Saha, S., Chong, W. H., Saha, B. K. (2021) Unilateral diffuse alveolar hemorrhage due to selective directionality of mitral regurgitant jet in a patient with severe aortic stenosis. *Cureus* **13(4)**, e14714.
- Sakamoto, R., Matsumoto, S., Mitsubuchi, H., Nakamura, K. (2018) Liposteroid and methylprednisolone combination therapy for a case of idiopathic lung hemosiderosis. *Respir. Med. Case Rep.* **24**, 22–24.
- Sakurai, Y., Kamisue, S., Shima, M., Yoshioka, A. (1995) Successful treatment with liposteroid in an infant case of idiopathic pulmonary hemosiderosis. *日本小児血液学会雑誌* **9**, 42–46. (in Japanese)
- Sakurai, Y., Ohkubo, Y., Miura, S., Mariko, M., Akazawa, H., Imanaka, Y., Kinoshita, S., Yoshioka, A. (1999) Liposteroid therapy for chronic childhood idiopathic thrombocytopenic purpura: Two case reports. *Int. J. Pediatr. Hematol.* **6**, 27–31.
- Schiffelers, R. M., Banciu, M., Metselaar, J. M., Storm, G. (2006) Therapeutic application of long-circulating liposomal glucocorticoids in auto-immune diseases and cancer. *J. Liposome Res.* **16(3)**, 185–194.
- Soergel, K. H., Sommers, S. C. (1962) Idiopathic pulmonary hemosiderosis and related syndromes. *Am. J. Med.* **32(4)**, 499–511.
- Stainer, A., Rice, A., Devaraj, A., Barnett, J. L., Donovan, J., Kokosi, M., Nicholson, A. G., Cairns, T., Wells, A. U., Renzoni, E. A. (2019) Diffuse alveolar haemorrhage associated with subsequent development of ANCA positivity and emphysema in three young adults. *BMC Pulm. Med.* **19(1)**, 185.
- Taytard, J., Nathan, N., de Blic, J., Fayon, M., Epaud, R., Deschildre, A., Troussier, F., Lubrano, M., Chiron, R., Reix, P., Cros, P., Mahloul, M., Michon, D., Clement, A., Corvol, H.; French RespiRare® Group (2013) New insights into pediatric idiopathic pulmonary hemosiderosis: The French RespiRare® cohort. *Orphanet J. Rare Dis.* **8**, 161.
- Thompson, G., Klecka, M., Roden, A. C., Specks, U., Cartin-Ceba, R. (2016) Biopsy-proven pulmonary capillaritis: A retrospective study of aetiologies including an in-depth look at isolated pulmonary capillaritis. *Respirology* **21(4)**, 734–738.
- Tobai, H., Yano, J., Sato, N., Amanuma, F., Takahashi, M., Endo, M., Ishimura, M., Ohga, S., Maruyama, H. (2020) Successful liposteroid therapy for a recurrent idiopathic pulmonary hemosiderosis with Down syndrome. *Case Rep. Pediatr.* **2020**, 5292977.
- Virchow, R. (1864) *Krankhaften Geshwülste*. Hirschwald, Berlin.
- Wakiguchi, H., Ohga, S. (2016) Clinical utility of the liposteroid therapy: Potential effects on the macrophage activation. *Nihon Rinsho Meneki Gakkai Kaishi* **39(3)**, 190–196.
- Wakiguchi, H., Hasegawa, S., Hirano, R., Kaneyasu, H., Wakabayashi-Takahara, M., Ohga, S. (2015) Successful

- control of juvenile dermatomyositis-associated macrophage activation syndrome and interstitial pneumonia: Distinct kinetics of interleukin-6 and -18 levels. *Pediatr. Rheumatol. Online J.* **13**, 49.
- Watanabe, H., Ayusawa, M., Kato, M., Chou, A., Komori, A., Abe, Y., Matsumura, M., Kamiyama, H., Izumi, H., Takahashi, S. (2015) Idiopathic pulmonary hemosiderosis complicated by Down syndrome. *Pediatr. Int.* **57(5)**, 1009–1012.
- Wroblewski, B. M., Stefanovic, C. R., McDonough, V. M., Kidik, P. J. (1997) The challenges of idiopathic pulmonary hemosiderosis and lung transplantation. *Crit. Care Nurse* **17(3)**, 39–44.
- Yanagihara, T., Yamamoto, Y., Hamada, N., Suzuki, K., Ogata-Suetsugu, S., Harada, E., Tagawa, T., Fujiwara, M., Hashisako, M., Fukuoka, J., Nakanishi, Y. (2018) Recurrent idiopathic pulmonary hemosiderosis after long-term remission presented with Sjogren's syndrome: Idiopathic no more? *Respir. Med. Case Rep.* **25**, 68–72.
- Yokoyama, K., Watanabe, M. (1996) Limethason as a lipid microsphere preparation: An overview. *Adv. Drug Deliv. Rev.* **20(2)**, 195–201.
- Yokoyama, K., Okamoto, H., Watanabe, M., Suyama, T., Mizushima, Y. (1985) Development of a corticosteroid incorporated in lipid microspheres (liposteroid). *Drugs Exp. Clin. Res.* **11(9)**, 611–620.
- Zhang, Y., Luo, F., Wang, N., Song, Y., Tao, Y. (2019) Clinical characteristics and prognosis of idiopathic pulmonary hemosiderosis in pediatric patients. *J. Int. Med. Res.* **47(1)**, 293–302.

Correlation between Platelet Profile (Mean Platelet Volume, Platelet Volume Distribution Width and Plateletcrit) with Procalcitonin and C-reactive Protein in Critically Ill Children

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Abstract: The early detection of sepsis can be highly beneficial for the treatment and prognosis of critically ill children. Bacterial culture is the gold standard of bacterial infection, but it takes considerable time to get the result. Some biomarkers had been used as infection markers in children, such as C-reactive protein (CRP), full blood count with differential count and procalcitonin level. Platelet profile had been linked to infection in many studies. Platelet profile consists of mean platelet volume, platelet volume distribution width and plateletcrit. Platelet profiles are relatively inexpensive and widely available examination. It is routinely measured by automated hematology analysers in routine full blood examination, but its clinical importance and application is still limited, especially in children. The aim of this study is to analyse the correlations between platelet profiles with procalcitonin and CRP in critically ill children. A cross sectional study was conducted at Haji Adam Malik Hospital Medan, Indonesia. Patients admitted to paediatric intensive care unit, aged 1 month to 18 years were recruited. Platelet profile, procalcitonin and CRP were measured within 24 hours after admission at PICU. The most common indication of PICU admissions were central nervous system (27.9%) and respiratory

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(25.3%) disorders. Both MPV ($r=0.217$; $p=0.045$) and PDW ($r=0.23$; $p=0.033$) had statistically significant correlation with procalcitonin, while none of platelet indicators had significant correlation with CRP. PDW and MPV had statistically significant correlation with procalcitonin as a marker of bacterial infection, their roles as an initial marker of bacterial infection needs further research.

Introduction

The high mortality rate of critically ill children depends mainly on the presence of sepsis or organ failure. Therefore, early detection of sepsis will give advantage in the treatment and prognosis of critically ill children (Elmoneim et al., 2018). Bacterial culture is the gold standard of bacterial infection, but it takes long time to get the result. Research by Safdar et al. in 2017 and Kossiva et al. in 2014 showed some biomarkers to be used as infection markers in children, such as C-reactive protein (CRP), full blood count with differential and procalcitonin. Procalcitonin levels have been shown to distinguish between bacteremia and non-infectious inflammatory states accurately and quickly in critically ill patients (Charles et al., 2008).

Platelet profile consist of mean platelet volume (MPV), platelet volume distribution width (PDW) and plateletcrit (PCT). MPV is average size of the platelets in blood. Platelet distribution width is an indication of variation in platelet size, while PCT is a measure of total platelet mass (Baig, 2015). Of those major platelet indices, MPV had been the most common variable studied in association with infection. Although some studies have shown different result, most studies reported increased MPV in sepsis. Platelet profile was linked to infection and prognosis of septic patients in many studies (Sayed et al., 2020). Our previous studies reported that MPV had positive correlation with PELOD-2 score (PELOD – pediatric logistic organ dysfunction) on septic patients (Puspitasari et al., 2018) and the difference between MPV on days 1 and 3 (Δ MPV) significantly correlated with the change of PELOD-2 score on critically ill children (Yanni and Saragih, 2020). Platelet indices examination is relatively inexpensive and widely available. It is routinely measured by automated hematology analysers in routine full blood examination, but its clinical importance and application is still limited, especially in children.

The aim of this study is to analyse the correlations between platelet profiles with procalcitonin and C-reactive protein in critically ill children.

Material and Methods

A cross sectional study was conducted at Haji Adam Malik Hospital Medan, Indonesia on May to November 2019. Patients admitted to pediatric intensive care unit (PICU), aged 1 months–18 years old were recruited. Platelet indices (MPV, PDW and PCT), procalcitonin and CRP were measured within 24 hours of PICU admission. We also recorded PELOD-2 score on the admission day. Patient were excluded if their parents or guardian refused the blood examination. This study was approved by the Health Research Ethical Committee of Medical Faculty

of Universitas Sumatera Utara with the number 665/TGL/KEPK FK USU-RSUP HAM/2019.

Results

Among 86 subjects recruited, 51 (59.3%) children were boys. The most common indication of PICU admissions were central nervous system (27.9%) and respiratory disorders (25.3%). Characteristics of subjects were shown in Table 1.

We found that MPV ($r=0.217$; $p=0.045$) and PDW ($r=0.23$; $p=0.033$) had statistically significant correlation with procalcitonin (Table 2), while none of platelet indices correlated with CRP (Table 3). Scatter plot of correlation between platelet profile and procalcitonin are shown in Figure 1.

Discussion

Procalcitonin and CRP are well-known biomarker of sepsis. Serum procalcitonin levels are associated with blood culture positivity in patients with sepsis, and procalcitonin appears to have higher specificity and sensitivity for predicting bacterial infection than other markers (Irvem and Aksaray, 2018). The platelet profiles used in this study are PDW, MPV, and PCT which are simple and economical that is easy to interpret and are routinely measured in suspected infected patient. In addition, these results can also be obtained faster than culture which is the gold standard of bacterial infection (Erdogan et al., 2015).

MPV reflects the average size of platelets. Young platelets are larger than old platelets. Increased number of young platelets indicates increased platelet

Table 1 – Characteristic of subjects

Variable	n=86
Age (year), median (min–max)	3 (0.08–17.91)
Sex, n(%)	
– Boys	51 (59.3)
– Girls	35 (40.7)
Underlying disease, n(%)	
– Pneumonia	15 (17.4)
– Sepsis	19 (22.1)
– Central nervous system infection	16 (18.6)
– Congestive heart failure	3 (3.5)
– Chronic kidney disease	9 (10.5)
– Post surgical	14 (16.3)
– Others	10 (11.6)
PELOD-2 score, median (min–max)	8 (2–21)
≥ 10, n(%)	26 (30.2)
< 10, n(%)	60 (69.8)
Mortality, n(%)	43 (50)

Table 2 – Correlation between platelet profile and procalcitonin

	Platelet profile median (range)	Procalcitonin median (range)	p	R
MPV	9.85 (7.5–33.3)	6.36 (0.10–641.4)	0.045	0.217
PDW	9.70 (7.0–21.8)		0.033	0.230
PCT	263.15 (1.8–1706)		0.070	-1.960

MPV – mean platelet volume; PDW – platelet volume distribution width; PCT – plateletcrit

Table 3 – Correlation between platelet profile and CRP

	Platelet profile median (range)	CRP median (range)	p	R
MPV	9.85 (7.5–33.3)	1.9 (0–22.4)	0.149	0.157
PDW	9.70 (7.0–21.8)		0.242	0.128
PCT	263.15 (1.8–1706)		0.135	-0.162

CRP – C-reactive protein; MPV – mean platelet volume; PDW – platelet volume distribution width; PCT – plateletcrit

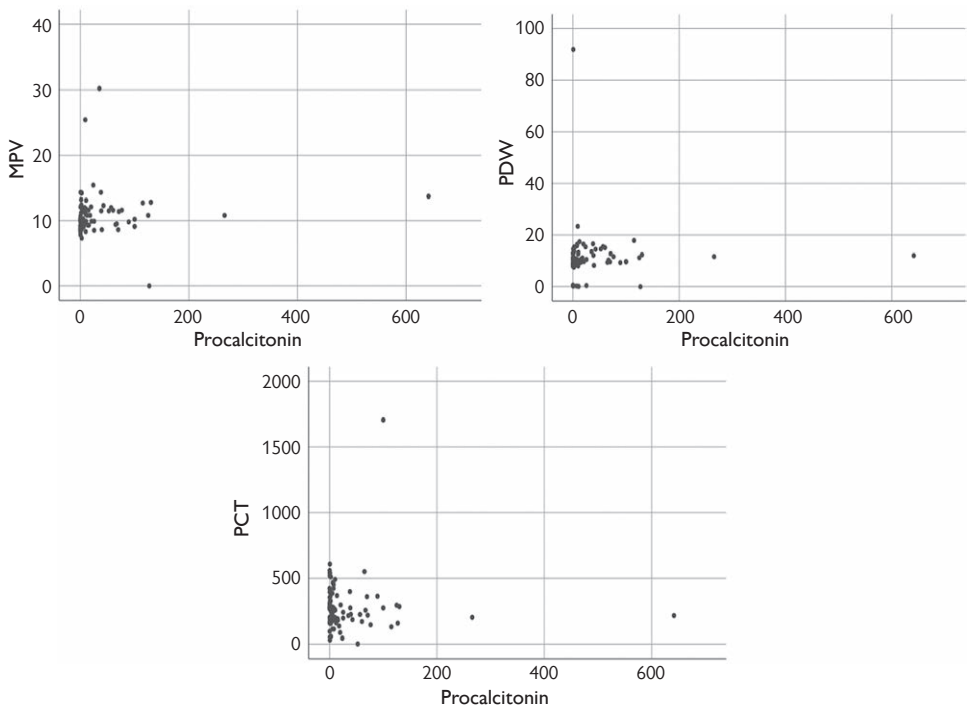


Figure 1 – Scatter plot of correlation between mean platelet volume (MPV) (A), platelet volume distribution width (PDW) (B), plateletcrit (PCT) (C) and procalcitonin levels.

production due to overconsumption induced by inflammation. Larger platelets are functionally, metabolically, and enzymatically more active than smaller ones. They contain more intracellular thromboxane A₂ and increased expression of procoagulant surface proteins such as p-selectin and glycoprotein IIIa, causing greater prothrombotic potential. Moreover, platelet-neutrophil interactions and platelet-endothelial interactions facilitate a variety of immune activation instances. Systematic review and meta-analysis on critically ill adult patients conclude that initial high MPV might not be used as a prognostic marker, while subsequent MPV changes might be meaningful (Tajarenmuang et al., 2016).

This study showed a significant correlation between MPV and PDW with procalcitonin level changes. The PDW shares similar behaviour to MPV during acute severe infection, it increases in platelet depletion when turnover is accelerated. PDW is an indicator of the heterogeneity in platelet size. A high value of PDW suggests a large range of platelet size due to swelling, destruction, and immaturity (Gao et al., 2014). We have not found any statistically significant correlation between plateletcrit and procalcitonin in this study. There were not many studies about plateletcrit, especially in critically ill children. Previous study on adults showed significant differences of plateletcrit when compared between groups based on their procalcitonin levels. Plateletcrit reflects the total mass of platelet in circulation within 1 unit of blood volume and it is said to be relevant to hematocrit towards erythrocyte (Djuang et al., 2018).

While procalcitonin has been considered a reliable biomarker for differentiating sepsis from non-infectious systemic inflammatory response syndrome (SIRS), C-reactive protein is a non-specific, acute-phase protein that increases after exposure to an inflammatory trigger. (Lanziotti et al., 2016). This study shows that platelet profile represents bacterial infection rather than inflammation since no significant correlation was found between platelet profile and CRP.

Mortality in this study was relatively high. Many factors can be related to mortality in critically ill children. The severity of disease on admission could be one possible explanation of high mortality in this study. Median value of PELOD-2 score on admission in this study was 8 (2–21), and 30.2% of subjects had PELOD-2 score ≥ 10 . Previous study reported that a PELOD-2 score > 10 is valid for predicting life-threatening organ dysfunction in pediatric patients with sepsis, with 75% sensitivity and 72% specificity (Suari et al., 2021), while other study in pediatric intensive care unit reported that the odds ratio for mortality with PELOD-2 score of ≥ 9 was 1.5 (95% CI 1.4–1.7) as compared to the score < 9 (El-Nawawy et al., 2017).

Conclusion

Our study concludes that PDW and MPV had statistically significant correlation with procalcitonin level as a marker of bacterial infection, their roles as an initial marker of bacterial infection needs further research.

References

- Baig, M. A. (2015) Platelet indices – Evaluation of their diagnostic role in pediatric thrombocytopenias (one year study). *Int. J. Res. Med. Sci.* **3(9)**, 2284–2289.
- Charles, P. E., Ladoire, S., Aho, S., Quenot, J., Doise, J., Prin, S., Olsson, N. O., Blettery, B. (2008) Serum procalcitonin in critically ill patients at the onset of bacteremia caused by either Gram negative or Gram positive bacteria. *BMC Infect. Dis.* **8**, 38.
- Djuang, M. H., Ginting, F., Hariman, H. (2018) Platelet indexes for bacterial sepsis severity assessment. *IJCPML* **24(3)**, 210–213.
- Elmoneim, A. A., Alhojaili, A. R., Banjer, N. R., Zolaly, M., El-Moneim, E. S. A. (2018) Changes in mean platelet volume in critically ill children and its relation to other clinical and laboratory findings. *Biomedical Research* **29(5)**, 882–888.
- El-Nawawy, A., Mohsen, A. A., Abdel-Malik, M., Taman, S. O. (2017) Performance of the pediatric logistic organ dysfunction (PELOD) and (PELOD-2) scores in a pediatric intensive care unit of a developing country. *Eur. J. Pediatr.* **176**, 849–855.
- Erdogan, S., Oto, A., Bosnak, M. (2015) The relation between mean platelet volume and mortality in critical pediatric patients. *Turk. J. Pediatr.* **57**, 605–610.
- Gao, Y., Li, Y., Yu, X., Guo, S., Ji, X., Sun, T., Lan, C., Lavergne, V., Ghannoum, M., Li, L. (2014) The impact of various platelet indices as prognostic markers of septic shock. *PLoS One* **9(8)**, e103761.
- Irvem, A., Aksaray, S. (2018) Procalcitonin, C-reactive protein, leukocyte, mean platelet volume levels in bloodstream infections. *J. Clin. Anal. Med.* **9(5)**, 391–395.
- Kossiva, L., Gourgiotis, D. I., Douna, B., Marmarinos, A., Sdogou, T., Tsentidis, C. (2014) Composite bacterial infection index in the evaluation of bacterial versus viral infections in children: A single centre study. *Pediatr. Therapeut.* **4**, 203.
- Lanziotti, V. S., Povoas, P., Soares, M., Silva, J. R. L., Barbosa, A. P., Salluh, J. I. F. (2016) Use of biomarkers in pediatric sepsis: Literature review. *Rev. Bras. Ter. Intensiva* **28(4)**, 472–482.
- Puspitasari, R., Saragih, R. A. C., Mutiara, E., Lubis, M. (2018) Correlation of mean platelet volume with PELOD-2 score as a prognostic factor of sepsis in children. *Pediatr. Crit. Care Med.* **19(6S)**, 36.
- Safdar, O. Y., Felemban, O. M., Alghamdi, A. A., Jastaniyyah, D. F., Abdouh, D. O., Alkindi, F. L., Saeedi, K. A., Barhameen, H. M., Al Turkistani, E. L., Bashammakh, D. S., Daali, S. M., Almsoudi, A. M., Sukkar, S. A., Al-Amoudi, S. O., Rayyis, L. A., Hadadi, A. A., Ibrahim, B. M. (2017) Serum procalcitonin as a diagnostic biomarker for bacterial infection in febrile children. *J. Med. Diagn. Meth.* **6**, 1.
- Sayed, S. Z., Mahmoud, M. M., Moness, H. M., Mousa, S. O. (2020) Admission platelet count and indices as predictors of outcome in children with severe sepsis: A prospective hospital-based study. *BMC Pediatr.* **20**, 387.
- Suari, N. R. M., Latief, A., Pudjadi, A. H. (2021) New PELOD-2 cut-off score for predicting death in children with sepsis. *Paediatrica Indonesiana* **61**, 39–45.
- Tajarernmuang, P., Phrommintikul, A., Limsukon, A., Pothirat, C., Chittawatanarat, K. (2016) The role of mean platelet volume as a predictor of mortality in critically ill patients: A systematic review and meta-analysis. *Crit. Care Res. Pract.* **2016**, 4370834.
- Yanni, G. N., Saragih, R. A. C. (2020) Platelet profile as prognostic factor in critically ill children. *Open Access Maced. J. Med. Sci.* **8(B)**, 966–968.

WALANT as an Optimal Approach in Hand Surgery during Pandemics

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Abstract: The emergence of the COVID-19 pandemic imposed fundamental changes in the field of surgery. Reorganization was made in order to adequately treat the patients during the pandemic. WALANT (Wide Awake Local Anesthesia No Tourniquet) approach was found to be a very convenient method in facilitating continuity in hand surgery with limited staff. A retrospective comparative study was performed between period of April 2020 till September 2021 at our clinic to evaluate advantages of WALANT approach. This study included 136 patients, from which 72 (53%) were operated with WALANT, compared to the control group of 64 (47%) patients without WALANT. Average hospital stay for the WALANT group was 2.2 days vs. 4.7 days for the control group. Average operating room personnel were 3.8 for WALANT and 6.2 for the control non-WALANT group. Intraoperative and postoperative VAS (visual analogue scale) score was evaluated. Due to its diversity, low cost and low complication rate, we recommend WALANT approach in acute and elective hand surgery.

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Introduction

The emergence of the COVID-19 pandemic imposed fundamental changes in everyday life. Due to the diversion of the staff and facilities for the treatment of patients with SARS-CoV-2, the elective cases for surgery were postponed for a period of time. That was not the case with emergency surgeries in our country that have been treated throughout the whole period since the pandemic has begun. For a more efficient functioning, reorganization was made in order to adequately treat the patients in the conditions imposed by the pandemic (DiFazio et al., 2020).

The capacity to perform urgent surgery has been reduced due to the diversion of anesthesiologists, theatre staff, anesthetic equipment and surgeons for management of the current crisis (DiFazio et al., 2020; Hobday et al., 2020). Furthermore, many departments are often and suddenly repurposed as COVID-19 intensive care units, increasing the demand for free hospital beds (Hobday et al., 2020; Khor et al., 2021). WALANT (Wide Awake Local Anesthesia No Tourniquet) approach was found to be a very convenient method in facilitating continuity in hand surgery with limited staff. Finding new strategies to get ahead was essential during this period.

Material and Methods

A retrospective comparative study was performed at the University Clinic for Plastic and Reconstructive Surgery in Skopje, North Macedonia. The aim of our study was to investigate the advantages of using Wide Awake Local Anesthesia No Tourniquet (WALANT), its applicability and overall outcome in hand trauma. In the period between April 2020 till September 2021, 304 patients with hand injuries were treated. Inclusion criteria for participation in the study were patients over 18 years old that were indicated for and consented to surgery with acute hand injuries. Acute hand injuries are defined as injuries of the hand that were inflicted suddenly by a traumatic force to the hand, that may result in lesion of anatomical structures and function and require immediate medical attention. Exclusion criteria were patients with mangled hand injuries with vascular trauma of the hand. Seventy-two patients were treated with WALANT by two surgeons in our department. Additionally, as a control group we designed a second group with patients from two other surgeons that did not use WALANT in the same time frame. This group counted 64 patients. All of the patients tested negative for COVID-19 with routine PCR tests on admission. Preoperatively, they received a single-shot antibiotic. Approximately 40 minutes before the procedure, WALANT solution containing lidocaine buffered with 8.4% sodium bicarbonate NaHCO_3 at a 5:1 ratio, was administered at the ward (Lalonde, 2017; Kurtzman et al., 2021). The solution we used was a mixture of 1% lidocaine with epinephrine in 1:100 000 ratio and 1 ml 8.4% bicarbonate in 10 ml of lidocaine-epinephrine solution to buffer lidocaine-induced acidity at the injection site. According to current guidelines, for surgery with longer duration (>2.5 hours) bupivacaine can be added to the mixture. Additionally, in case of epinephrine-induced ischemia phentolamine should be available as a reversal option

in a concentration of 1 g to 10 ml of 0.9% saline (Fish and Bamberger, 2021). The injection site was determined depending on the type and location of the injury.

We compared the two groups of patients by average hospital stay, pain using the visual analogue scale (VAS) score, the number of staff in the operating room per operation, conversion rate and complications. We assessed the COVID-19 transmission rate in the operating theatre and hospital ward. Patients were scheduled for regular follow-up 1 month postoperatively and questioned for pain levels and overall satisfaction with the procedure using VAS (Heller et al., 2016; Delgado et al., 2018). The results were statistically analysed.

Results

This study included 136 patients, from which 72 (53%) were operated with WALANT by two surgeons compared to the control group of 64 (47%) patients from two other surgeons from our department that did not use WALANT. The average age in the WALANT group was 46.8 (range 20–74 years), as for the other group the average age was 48.8 years (range 21–77 years) (Table 1).

The WALANT group counted 67 (93%) males and 5 (7%) females, whereas the non WALANT group were 56 (86%) males and 8 (14%) females. The total gender distribution was in favour for the male population (90%, n=123) compared to the females (10%, n=13) (Table 2).

Patients were divided according to the type of injury into several groups. Most of the patients were with combined injuries of the hand in both WALANT and

Table 1 – Age distribution of WALANT and Non-WALANT patients

Age	WALANT patients	Non-WALANT patients
20–29	13	11
30–39	16	14
40–49	11	9
50–59	12	10
60–59	9	7
70–79	11	13
Total	72 (average 46.8) (range 20–74)	64 (average 48.8) (range 21–77)

Table 2 – Gender distribution

Gender	Male	Female
WALANT	67 (93%)	5 (7%)
Non-WALANT	56 (86%)	8 (14%)
Total	123 (90%)	13 (10%)

Table 3 – Patients divided by their individual injuries

	Integument	Skeleton	Muscle/ tendon	Neuro- vascular	Combined
WALANT	3 (4%)	7 (10%)	28 (39%)	2 (2%)	32 (45%)
Non-WALANT	5 (8%)	3 (5%)	23 (36%)	1 (1%)	32 (50%)
Total	8 (6%)	10 (7%)	51 (38%)	3 (2%)	64 (47%)

Table 4 – Hospital days and number of staff needed in the operating room

	Hospital days	Operating room personnel
WALANT	2.2	3.8
Non-WALANT	4.7	6.2

non-WALANT group (47%, n=64). Patients with muscle or tendon injury were on second place with 38% (n=51). The rest of the patients were with skeleton (7%, n=10), integument (6%, n=8) and neurovascular (1%, n=1) injuries (Table 3).

Average hospital stay for the WALANT group was 2.2 days, as opposed to the control group 4.7 days, which is significantly less days in favour for the first group. Average operating room personnel were 3.8 for WALANT and 6.2 for the control non-WALANT group (Table 4). It required significantly less staff in the OR for the WALANT group compared to the other group.

Seventy three percent of the patients did not report pain in the first 6 hours after WALANT surgery. None of the patients required opioids for pain management. Only 1 patient with WALANT was converted to general anesthesia due to unexpected duration of surgery. There was no reported case of COVID-19 transmission in WALANT patients. No complications were reported as a result of the local anesthetic or adrenaline. The visual analogue scale was used to determine the pain intensity intraoperative and in the early postoperative phase. A numerical rating from 0 to 10 was used, with 10 being the least comfortable and 0 being the most comfortable (Heller et al., 2016; Delgado et al., 2018).

Table 5 – Visual analogue scale in both groups with intraoperative and postoperative score with statistically significant ($p<0.05$) in favour for the WALANT patients

	Intraoperative VAS	Postoperative VAS
WALANT	1.89 (SD 1.52)	1.20 (SD 1.10)
Non-WALANT	4.67 (SD 2.85)	3.85 (SD 1.55)

VAS – visual analogue scale; SD – standard deviation

Intraoperative the mean VAS for the WALANT group was 1.89 (SD [standard deviation] 1.52), whereas it was 4.67 (SD 2.85) for the other group, and the difference was statistically significant ($p < 0.05$).

Postoperatively, the mean VAS for the WALANT group was 1.20 (SD 1.10), whereas it was 3.85 (SD 1.55) for the other group, and the difference was statistically significant ($p < 0.05$) (Table 5).

Discussion

During COVID-19 pandemics our hospital underwent major reorganization of personnel and resources, all elective hand surgeries were cancelled, or postponed, and only acute hand injuries were treated. Our clinic encountered practicing medicine in unfamiliar and challenging circumstances, one of the biggest obstacles being lack of staff and hospital beds. As such, we started considering methods that required less engagement by healthcare workers and reduced the time of hospital stay. As a result of the scarcity of anesthesiology personnel, we started using Wide Awake Local Anesthesia No Tourniquet (WALANT). Introduced over a decade ago by Dr. Donald H. Lalonde, the WALANT technique was used for treating patients in the outpatient setting (Lalonde, 2017). Since then, the technique has gained popularity and its specter of use was broadened significantly (Turcotte et al., 2020; COVIDSurg Collaborative and GlobalSurg Collaborative, 2021). It is correlated with positive outcomes and patient satisfaction. In detail, local anesthetics, hemostatic agents and alkalizing agents are applied to the operative site through a local injection. It is believed that the alkalizing agent (lidocaine buffered with 8.4% sodium bicarbonate NaHCO_3 at a 5:1 ratio), acts beneficially for lowering pain associated with local anesthetic infiltration and further potentiates the hemostatic effect of epinephrine (Lalonde, 2017; Evangelista et al., 2019; Kurtzman et al., 2021). Current experiences show that it is convenient for performing common procedures of the hand or wrist, without the need of tourniquet and intravenous sedation. Thus, pain and discomfort experienced by the patient are significantly lower, while at the same time providing pain control and hemostasis, as well as intraoperative assessment of the hand function. Since the start of the pandemic, only a few authors have described the benefits of this type of anesthesia.

Alves et al. (2021) stated the benefits of WALANT during COVID-19 pandemic are essential. They state that the patients subjected to this technique did not feel any pain during the surgery, proving it to be effective in its purpose. Also, the possibility of evaluating the passive hand movement was a remarkable benefit. In addition, they emphasize the shorter post-surgical length of stay, thus, generating greater benefit to the patient's health.

In other publication, Nolan et al. (2020) clearly reviewed the best available evidence due to function, complications, cost, or patient-reported outcomes. They state that it is improved when flexor tendons are repaired using wide-awake

technique, because it will determine if this novel approach is superior to general or regional anesthesia.

The utilization of WALANT method has grown exponentially over time since its introduction. This anesthetic method has become an attractive option for both surgeons and patients. Its value has been demonstrated by achieving positive results in a broad extent of surroundings (Evangelista et al., 2019; Khor et al., 2021; Masterton and Talwar, 2021). Generally, patients undergoing wide-awake procedures are not succumbed to preoperative assessment for anesthesia clearance, such as laboratory tests and other diagnostic procedures (DiFazio et al., 2020; Khor et al., 2021). The technique is modest in consumption of healthcare resources and contributes to substantial health budget savings by reducing hospital stay, fewer office visits, personnel sparing, medication sparing, etc. Moreover, the properties of WALANT include no aerosolizing procedures, shorter recovery time, reduction of OR personnel and COVID-19 transmission consequently (Kurtzman et al., 2021). Perhaps the greatest advantage is the ability for intraoperative motion to assess the outcome of the repair or reconstruction of hand structures. The ability to assess active hand movement was a significant benefit. As far as tourniquet is concerned, not having an inflated tourniquet eliminates the possibility of ischemic pain, thus reducing the time restriction. Tourniquet inflicted ischemic pain also limits full motion of muscular system units, therefore disabling intraoperative evaluation of repair/reconstruction. The reperfusion pain should also be factored. Patient comfort is greatly enhanced, as there is no need for preoperative starving or stopping medication, anticoagulants included. This has great clinical significance as it lowers the risk for thrombotic occurrences. However, on the downside the duration of analgesia is shorter compared to regional blocks, the field is not as dry in comparison to using a pneumatic tourniquet, and it is recommended to wait at least twenty minutes between application and incision for maximal hemostatic effect. WALANT has diverse application in acute hand trauma surgery.

Conclusion

According to our experience, the WALANT method (Wide Awake Local Anesthesia No Tourniquet) was found to be a convenient and reliable method for treating urgent and non-urgent conditions in the domain of hand surgery. During COVID-19 pandemic we expanded the application of WALANT to more complex injuries with satisfactory results. The patients who were submitted to this approach did not experience any discomfort throughout the procedure, demonstrating that it is successful for its intended purpose. The many up-sides allowed for improved surgical success and as a result, a lower risk of the necessity of surgical re-approach, as well as a shorter post-surgical length of stay, and less healthcare personnel, all of which resulted in greater benefit to the patient's health and rational use of health resources.

We recommend WALANT for further use in acute hand trauma surgery, as well as in elective hand surgery, both in OR and outpatient settings due to its diversity, resulting in low cost and low complication rate.

References

- Alves, R. S., Consoni, D. A. P., Fernandes, P. H. O., Sasaki, S. U., Zaia, I. M., Santos, S. B. D., Sato, M. A. (2021) Benefits of the WALANT technique against the COVID-19 pandemic. *Acta Ortop. Bras.* **29(5)**, 274–276.
- COVIDSurg Collaborative, GlobalSurg Collaborative (2021) SARS-CoV-2 vaccination modelling for safe surgery to save lives: Data from an international prospective cohort study. *Br. J. Surg.* **108(9)**, 1056–1063.
- Delgado, D. A., Lambert, B. S., Boutris, N., McCulloch, P. C., Robbins, A. B., Moreno, M. R., Harris, J. D. (2018) Validation of digital visual analog scale pain scoring with a traditional paper-based visual analog scale in adults. *J. Am. Acad. Orthop. Surg. Glob. Res. Rev.* **2(3)**, e088.
- DiFazio, L. T., Curran, T., Bilaniuk, J. W., Adams, J. M., Durling-Grover, R., Kong, K., Nemeth, Z. H. (2020) The impact of the COVID-19 pandemic on hospital admissions for trauma and acute care surgery. *Am. Surg.* **86(8)**, 901–903.
- Evangelista, T. M. P., Pua, J. H. C., Evangelista-Huber, M. T. P. (2019) Wide-awake local anesthesia no tourniquet (WALANT) versus local or intravenous regional anesthesia with tourniquet in atraumatic hand cases in orthopedics: A systematic review and meta-analysis. *J. Hand Surg. Asian Pac. Vol.* **24(4)**, 469–476.
- Fish, M. J., Bamberger, H. B. (2021) *Wide-awake Local Anesthesia No Tourniquet (WALANT) Hand Surgery*. StatPearls (Internet).
- Heller, G. Z., Manuguerra, M., Chow, R. (2016) How to analyze the visual analogue scale: Myths, truths and clinical relevance. *Scand. J. Pain* **13**, 67–75.
- Hobday, D., Welman, T., O'Neill, N., Pahal, G. S. (2020) A protocol for wide awake local anaesthetic no tourniquet (WALANT) hand surgery in the context of the coronavirus disease 2019 (COVID-19) pandemic. *Surgeon* **18(6)**, e67–e71.
- Khor, W. S., Lazenby, D. J., Campbell, T., Bedford, J. D., Winterton, R. I. S., Wong, J. K., Reid, A. J. (2021) Reorganisation to a local anaesthetic trauma service improves time to treatment during the COVID-19 pandemic – Experience from a UK tertiary plastic surgery centre. *J. Plast. Reconstr. Aesthet. Surg.* **74(4)**, 890–930.
- Kurtzman, J. S., Etcheson, J. I., Koehler, S. M. (2021) Wide-awake local anesthesia with no tourniquet: An updated review. *Plast. Reconstr. Surg. Glob. Open* **9(3)**, e3507.
- Lalonde, D. H. (2017) Conceptual origins, current practice, and views of wide awake hand surgery. *J. Hand Surg. Eur. Vol.* **42(9)**, 886–895.
- Masterton, G., Talwar, C. (2021) WALANT in plastic surgery trauma – Why wait? *J. Plast. Reconstr. Aesthet. Surg.* **74(6)**, 1355–1401.
- Nolan, G. S., Kiely, A. L., Madura, T., Karantana, A. (2020) Wide-awake local anaesthesia no tourniquet (WALANT) vs regional or general anaesthesia for flexor tendon repair in adults: Protocol for a systematic review and meta-analysis. *Syst. Rev.* **9(1)**, 264.
- Turcotte, J. J., Petre, B. M., Jones, C. M., Gelfand, J. M. (2020) Maintaining access to orthopaedic surgery during periods of operating room resource constraint: Expanded use of wide-awake surgery during the COVID-19 pandemic. *J. Am. Acad. Orthop. Surg. Glob. Res. Rev.* **4(12)**, e20.00100.

The Manifestations of Covid-19 Infection. Manifestations in Patients with Temporomandibular Joint Disorders

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Abstract: The authors present a group of patients who were treated for exacerbation of temporomandibular joint disorders (TMD) following Covid-19 infection and who in the past had successfully undergone surgery of the temporomandibular joint (TMJ). The group consisted in total of 21 patients who relapsed after contracting Covid-19. There were 4 men and 17 women, the average age was 45.6 years (28–63). The most common complaint was pain. In all cases, the pain was located in the preauricular area, 4 patients had pain in the lateral side of the neck, 1 patient had pain of the nasal alae. During clinical examination, pain was present on palpation of the masseter muscle (19 patients), temporal muscle (4 patients) and the TMJ area (4 patients). In 4 cases, pain on palpation was present in the area of the nape and sternocleidomastoid muscles. Treatment in all cases was the same: thermotherapy, muscle relaxation massage and non-steroidal anti-inflammatory drugs. Symptoms subsided in all cases within 2 weeks. In light of the Covid-19 pandemic, it is also necessary to expect an increased number of patients with TMD. The authors recommend targeted patient histories regarding Covid-19 infection when examining patients with TMD symptoms – this will certainly facilitate determining the etiology of the pain.

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Introduction

Covid-19 infection was first reported in 2019 and during the following year became a worldwide pandemic. The disease is the result of infection by the SARS-CoV-2 coronavirus. Coronaviruses are RNA viruses classified as viruses that cause zoonotic infections. These respiratory viruses are transmitted mainly through contact with an infected person, specifically via droplets, infectious aerosols and contaminated objects and surfaces. Symptoms of Covid-19 infection most often include fever, dry cough, fatigue, shortness of breath, muscle and joint pain. Less common symptoms include nausea, pneumonia, cardiomyopathy, encephalitis and acute nephropathy (Feng et al., 2020; Huang et al., 2020; Li et al., 2020; Murat et al., 2021).

As of July 27th 2021, the number of Covid-19 cases reported worldwide was 194,723,719 (<https://coronavirus.jhu.edu/map.html>). At the time, 1,672,764 cases had been diagnosed in the Czech Republic (<https://koronavirus.mzcr.cz>).

Given that one of the symptoms of Covid-19 infection is involvement of the muscles and joints, the muscles of the head and jaw joint may also be affected (Asquini et al., 2021).

The aim of the study is to present a group of patients with chronic temporomandibular joint disorders (TMD), who underwent successful surgery but then experienced a deterioration in their condition following Covid-19 infection.

Material and Methods

The study evaluated patients who were treated for exacerbation of TMD following Covid-19 infection who in the past had undergone temporomandibular joint (TMJ) surgery: arthroscopy, arthroplasty, or total joint replacement. Only patients undergoing successful surgery were included.

A requirement was that these patients experienced at least 6 entirely problem-free months before contracting Covid-19. The group only included patients who had undergone unilateral TMJ surgery and who then experienced unilateral problems after Covid-19 infection. All patients included in the study had mild cases of Covid-19 infection not requiring hospitalization.

The characteristics of the patients' complaints were assessed: location of pain, subjective pain value (visual analogue scale 0–10, VAS), opening of mouth (maximal interincisal opening, MIO). The area of the joint, masticatory muscles, nape muscles and sternocleidomastoid muscles were examined by palpation. All patients (with the exception of those with total replacements) underwent ultrasound of the TMJ to compare the width of the joint space on the symptomatic side with the other unaffected side (using the Mindray DP-50, 7.5 MHz device, Shenzhen Mindray Bio-medical Electronics).

Results

The study included a total of 21 patients who had undergone TMJ surgery in the past and had experienced a relapse following Covid-19 infection. There were 4 men and 17 women, the average age was 45.6 years (28–63).

3 patients were originally diagnosed with Wilkes stage V (severe degenerative changes) – resulting in total joint replacement. 9 patients were originally diagnosed with Wilkes stage IV (disc dislocation, degenerative changes) – resulting in arthroscopic lysis and lavage (7 patients) and discectomy using subsequent free fat flap insertion (2 patients). 9 patients were originally diagnosed with Wilkes stage III (disc dislocation without repositioning) – resolved in all cases by arthroscopic lysis and lavage.

All patients had undergone surgery at least one year previously (mean of 14 months) and had been pain-free (mean VAS 0.25) and without limited jaw mobility (mean MIO 35.8 mm) for at least 6 months.

On average, these patients came in for treatment of TMJ problems 2 months after contracting Covid-19 (interval 1–8 months).

Pain was the dominant complaint.

The subjective pain value (VAS) was 4.5 (2–8). The VAS for these patients at the last check-up before Covid-19 infection was 0.25 (0–1).

In all cases, the patients reported pain in the preauricular area (5 patients concurrently reported ear pain, 8 patients reported toothache, 4 patients reported pain of the lateral side of the neck and 1 patient reported pain of the nasal alae).

During clinical examination, pain was present on palpation of the masseter muscle (19 patients), temporal muscle (4 patients) and the TMJ area (4 patients). In 4 cases, pain was present on palpation of the nape and sternocleidomastoid muscle.

In 18 patients, pain was also present in other joints and muscle groups of the body.

Jaw mobility was limited, MIO was 28.40 mm (25–38 mm). MIO in these patients at the last check-up before Covid-19 infection was 35.8 mm (30–47 mm).

Ultrasound of TMJ

4 patients were found to have a widening of the joint space on the affected side compared to the unaffected side.

In other patients, there was no widening of the joint space and no signs of effusion.

Treatment was the same in all cases: thermotherapy (application of dry heat 3× daily), muscle relaxation massage (several times daily) and non-steroidal anti-inflammatory drugs (Nalgesin 275 mg, naproxenum natricum 275 mg, Krka, Slovenia – 3 days 1 dose every 8 hours, 3 days every 12 hours). Within 2 weeks, the problems subsided in all cases. In the 4 patients where ultrasound revealed widening of the joint space, this disappeared after 2 weeks of treatment.

Discussion

The most common symptoms of Covid-19 infection include fever, dry cough, fatigue, shortness of breath and muscle and joint pain. In a meta-analysis, myalgia was the most common musculoskeletal symptom with a prevalence of 30–36% and headache was the most common neurological symptom with a prevalence of 58.33% among Covid-19 patients (Feng et al., 2020; Huang et al., 2020). In their retrospective study, Murat et al. (2021) assessed 210 patients with Covid-19, where pain was present in 69.3% (133 patients). 92 patients (69.2%) experienced myalgia/arthritis, 67 patients (50.4%) headache, 58 patients (43.6%) back pain, 44 patients (33.1%) lower back pain, 33 patients (25.0%) chest pain, 28 patients (21.1%) sore throat, and 18 patients (13.6%) abdominal pain. Chronic pain and fatigue have been observed after contracting Covid-19 (Moldofsky and Patcai, 2011). The predominant clinical symptoms in the present work were muscle pain, while objective changes in the TMJ (widening of the joint space confirmed by ultrasound) were only noted in 4 patients. 18 patients reported pain in multiple joints and muscle groups.

Covid-19 apparently affects the area of the jaw joint and masticatory muscles in two ways.

The first involves overexpression of proinflammatory cytokines, which affect the tissues of the masticatory muscles and synovial membrane of the TMJ. This leads to muscle and joint pain. At the same time, cytokines act in the cerebrospinal fluid, stimulating neurons of the trigeminal ganglia to produce calcitonin gene-related peptide (which plays a key role in migraines and affects arterial dilatation). This leads to headaches (Drożdżal et al., 2020).

Another effect of Covid-19 infection on joint structures and masticatory muscles is indirect and involves mental disorders and stress. Anxiety and depression are both consequences of Covid-19 infection. This is related to restricted physical activity due to forced quarantine, fear of infection, lack of information, financial loss, disruption of routine habits and loss of social contact (Drożdżal et al., 2020; Medeiros et al., 2020). Stress load is also increased by the fact that in many cases during the pandemic health care was limited and help and therapy were not provided soon enough (Drożdżal et al., 2020). Such stress results in a higher level of sympathetic activity and greater release of adrenocortical steroids (Almeida-Leite et al., 2020). Increased stress leads to poorer CNS (central nervous system) regulation of the prefrontal area of the cerebral cortex which regulates behaviour, thoughts and emotion, including inhibition of inappropriate motor reactions. Pathways in the hypothalamus and brain stem are activated (Quadri et al., 2015). This leads to an increase in parafunctional activity (clenching of teeth, maintaining the jaw in a rigid position, pressing of the tongue on teeth, playing with tongue on lips and cheeks). Polmann et al. (2019) report a 6× higher risk of sleep bruxism in patients with depression and anxiety. These parafunctional activities lead to repeated contraction of the masticatory muscles, muscle overexertion, local ischemia and muscle pain (Medeiros et al., 2020).

One must also consider the fact that patients with chronic TMD are more prone to experience pain and stress after Covid-19 infection (Asquini et al., 2021). This risk group includes the patients in our study, who all suffered from chronic TMD.

Patients with pain of the masticatory muscles and TMJ following Covid-19 infection can be successfully treated with physical therapy, thermotherapy and NSAIDs (non-steroidal anti-inflammatory drugs) (Drożdżał et al., 2020), as shown by this study as well.

Conclusion

In light of the Covid-19 pandemic it is necessary to expect an increased number of patients with TMD. The authors recommend targeted patient histories regarding Covid-19 infection when examining patients with TMD symptoms – this will certainly facilitate determining the etiology of the pain. One drawback of this study is, understandably, the small number of patients. The symptoms presented and therapy of these complications should therefore be taken as a guide and not an unequivocal recommendation. These will undoubtedly arise in the future from a larger and preferably multicenter study.

References

- Almeida-Leite, C. M., Stuginski-Barbosa, J., Rodrigues Conti, P. C. (2020) How psychosocial and economic impacts of COVID-19 pandemic can interfere on bruxism and temporomandibular disorders? *J. Appl. Oral Sci.* **28**, e20200263.
- Asquini, G., Bianchi, A. E., Borromeo, G., Locatelli, M., Falla, D. (2021) The impact of Covid-19-related distress on general health, oral behaviour, psychosocial features, disability and pain intensity in a cohort of Italian patients with temporomandibular disorders. *PLoS One* **16(2)**, e0245999.
- Drożdżał, S., Rosik, J., Lechowicz, K., Machaj, F., Szostak, B., Majewski, P., Rotter, I., Kotfis, K. (2020) COVID-19: Pain management in patients with SARS-CoV-2 infection-molecular mechanisms, challenges, and perspectives. *Brain Sci.* **10(7)**, 465.
- Feng, Y., Ling, Y., Bai, T. (2020) COVID-19 with different severities: A multicenter study of clinical features. *Am. J. Respir. Crit. Care Med.* **201(11)**, 1380–1388.
- Huang, X., Wei, F., Hu, L. (2020) Epidemiology and clinical characteristics of COVID-19. *Arch. Iran. Med.* **23(4)**, 268–271.
- Li, L., Huang, T., Wang, Y., Wang, Z., Liang, Y., Huang, T., Zhang, H., Sun, W., Wang, Y. (2020) COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. *J. Med. Virol.* **92**, 577–583.
- Medeiros, R. A., Vieira, D. L., Silva, E. V. F. D., Rezende, L. V. M. L., Santos, R. W. D., Tabata, L. F. (2020) Prevalence of symptoms of temporomandibular disorders, oral behaviors, anxiety, and depression in Dentistry students during the period of social isolation due to COVID-19. *J. Appl. Oral Sci.* **28**, e20200445.
- Moldofsky, H., Patcai, J. (2011) Chronic widespread musculoskeletal pain, fatigue, depression and disordered sleep in chronic post-SARS syndrome; a case-controlled study. *BMC Neurol.* **11**, 37.
- Murat, S., Karatekin, B. D., Icağasioglu, A., Ulasoglu, C., İçten, S., Incealtın, O. (2021) Clinical presentations of pain in patients with COVID-19 infection. *Ir. J. Med. Sci.* **190(3)**, 913–917.

- Polmann, H., Domingos, F. L., Melo, G., Stuginski-Barbosa, J., Guerra, E. N., Porporatti, A. L. (2019) Association between sleep bruxism and anxiety symptoms in adults: A systematic review. *J. Oral Rehabil.* **46(5)**, 482–491.
- Quadri, M. F., Mahnashi, A., Al Almutahhir, A., Tubayqi, H., Hakami, A., Arishi, M. (2015) Association of awake bruxism with khat, coffee, tobacco, and stress among Jazan University students. *Int. J. Dent.* **2015**, 84096.

Intervertebral Disc Degeneration: Functional Analysis of Bite Force and Masseter and Temporal Muscles Thickness

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Abstract: Intervertebral disc degeneration is a pathological condition associated with the intervertebral disc and is related to functional alterations in the human body. This study aimed to evaluate the maximum molar bite force and masseter and temporal muscles thickness in individuals with intervertebral disc degeneration. Thirty-two individuals were divided into two groups: those with degeneration of intervertebral discs (n=16) and those without degeneration (n=16). The maximum molar bite force (on the right and left sides) was measured using a dynamometer. Masseter and temporal muscle thickness during mandibular task rest and dental clenching in maximum voluntary contraction were analysed using ultrasound. Significant differences in the left molar bite force ($p=0.04$) were observed between the groups (Student's *t*-test, $p<0.05$). The intervertebral disc degeneration group

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had a lower maximum molar bite force. No significant differences in muscle thickness were observed between the masseter and temporal muscles in either group. However, based on clinical observations, the group with intervertebral disc degeneration presented less masseter muscle thickness and greater temporal muscle thickness in both mandibular tasks. Degenerative disease of the intervertebral discs promoted morphofunctional changes in the stomatognathic system, especially in maximum molar bite force and masticatory muscle thickness. This study provides insight into the interaction between spinal pathology and the stomatognathic system, which is important for healthcare professionals who treat patients with functional degeneration.

Introduction

Intervertebral discs are anatomical structures that form part of the vertebral column and are composed of the fibrous ring, nucleus pulposus, and cartilaginous end plates. They are indispensable in the union of adjacent vertebrae that allow flexion, extension, and rotation movements, without sacrificing a large amount of force, thus providing shock absorption within the spine (Prescher, 1998; Zvicer and Obradovic, 2018).

With aging, the spine, regardless of the region (cervical, thoracic, lumbar, or sacral), may face functional changes resulting from degeneration of the intervertebral discs, promoting postural imbalance with painful symptoms (Truszczyńska et al., 2016; Ji et al., 2020).

Degenerative disease of the intervertebral discs, considered progressive and chronic, is characterized by dehydration of the discs and alterations in the load distribution in the spine, promoting structural degradation of the healthy matrix that promotes the appearance of muscle dysfunction (Rustenburg et al., 2019; Cannata et al., 2020).

The etiopathogenesis is multifactorial and is mainly associated with genetic factors, smoking, aging, sedentary lifestyle, and obesity (Russo et al., 2017), affecting about 60 to 85% of adults at some stage of life, making them disabled with a negative impact on the quality of life, with great socioeconomic impact on the population (Cheung and Luk, 2019), and represents an important cause of morbidity and mortality in daily clinical practice (Kos et al., 2019).

Intervertebral disc degeneration is one of the main factors contributing to painful symptoms in the neck and back and is characterized by increased levels of pro-inflammatory cytokines secreted by intervertebral disc cells that promote extracellular matrix degradation, chemokine production, and changes in cell phenotype (Risbud and Shapiro, 2014; Abdollahzade et al., 2018).

When considering the degeneration of intervertebral discs, it is important to emphasize that the spine works as a single unit and its functional imbalance can provide compensatory changes in other levels of the spine itself or in other areas of the skeletal muscle system, demonstrating that the human organism is considered

a functional anatomical set, and any musculoskeletal impairment can affect other systems, such as the stomatognathic system (Spadaro et al., 2014).

Thus, the orofacial or adjacent dynamic structures of the stomatognathic system have become an important tool for functional assessment that can explain the relationship that exists between the systems of the human body when it is affected by chronic degenerative diseases (Donizetti Verri et al., 2019).

Therefore, the aim of this study was to evaluate the maximum molar bite force and masseter and temporal muscle thicknesses in individuals with intervertebral disc degeneration. The null hypothesis of this study was that the group with degeneration of the intervertebral discs would not present changes in relation to the maximum molar bite force and masseter and temporal muscle thickness when compared to the group without degenerative disease. This study presents two alternative hypotheses: 1) the group with degeneration of the intervertebral discs has a lower maximum molar bite force, and 2) a lower thickness of the masseter and temporal muscles compared to the group without degenerative disease.

Material and Methods

Study design and sample

This comparative cross-sectional observational study analysed the maximum molar bite force and masseter and temporal muscle thickness in subjects with and without intervertebral disc degeneration. Data for this study were collected at the Laboratory of Electromyography of the Department of Basic and Oral Biology, Faculty of Dentistry of Ribeirão Preto, University of São Paulo (FORP/USP). All subjects were informed about the purpose and stages of the research and signed a free and informed consent form approved by the FORP/USP ethics committee (process # 29014620.1.0000.5419).

G* Power 3.1.9.2 software (Franz Faul, Kiel University, Kiel, Germany) was used to calculate the sample size (a priori) considering $\alpha = 0.05$, effect size of 1.71, and power of 96% for the main result of the maximum left molar bite force by the pilot project of this study with five subjects. The minimum sample size obtained was $n=16$ for each group.

From a total of 80 evaluated subjects aged between 20 and 59 years, normal occlusion (Angle Class I), presence of all teeth (except third molars), absence of temporomandibular dysfunction (Research Diagnostic Criteria for Temporomandibular Disorders), and following the inclusion and exclusion criteria, 16 subjects (8 women and 8 men) were selected to compose the group with intervertebral disc degeneration (GI) with a mean age \pm standard deviation of 37.0 ± 8.3 years.

The diagnosis of intervertebral disc degeneration was confirmed by specialist physicians with the issuance of reports, clinical examinations, and images. The subjects in the group with degenerative intervertebral disc disease had vertebral dysfunction and instability.

Table 1 – Differences in characteristics (mean \pm standard deviation) between the group with intervertebral disc degeneration (GI) and the control group (GII)

Groups	Age	Body mass index
GI	37.0 \pm 8.3	25.9 \pm 3.4
GII	37.1 \pm 8.6	25.6 \pm 4.3
P-value	0.96	0.83

significant difference, Student's *t*-test (i.e. $p < 0.05$)

The control group consisted of 16 subjects without intervertebral disc degeneration (8 women and 8 men) (GII) with a mean age \pm standard deviation of 37.1 \pm 8.6 years. The groups were individually matched for sex, age, and body mass index (Table 1).

Subjects who presented with neurological and systemic pathologies, use of full or removable dentures, mental or physical discomfort during the assessments, congenital anomalies, previous spinal surgery, evidence of tumours on imaging examinations, spinal infection, fracture, and/or spinal deformities were considered ineligible. The participants in this study were selected from November 2019 to October 2021.

In this study, the analytical procedures were performed by a single researcher. Personal protective equipment was used in each examination: procedure gloves, laboratory coat, face shield, mask, and cap. Inter-examiner reliability was calculated using the intraclass coefficient (ICC). Reliability was considered acceptable for maximum molar bite force (ICC = 0.92) and muscle thickness (ICC = 0.99).

Bite force analysis

The recordings of the maximum right and left molar bite forces were performed with a digital dynamometer (Kratos, model IDDK, Equipamentos Industriais Ltda., Cotia, São Paulo, Brazil) adapted to the oral condition. The equipment consisted of two rods with Teflon discs at the ends on which the maximum bite force was captured.

The recorded molar bite force was displayed on the digital screen of the device in Newton (N). The molar bite force was measured with the subject seated in a comfortable chair, with the palms of the hands resting on the thighs. After each recording, the latex fingertips (Wariper-São Paulo, Brazil) were changed, and the device was cleaned with 70% alcohol. A biosafety protocol was applied for each protocol.

The subjects performed the tests by biting the equipment before the official collection of the bite force data to ensure the reliability of the procedure. Measurements were performed in the region of the right and left first permanent molars (Palinkas et al., 2010; Alam and Alfawzan, 2020). The subjects were asked

to bite the rods three times, with maximum effort, resting for 2 min between each recording and changing the right and left sides to avoid any influence of muscle fatigue (Bonjardim et al., 2009). The maximum molar bite force corresponding to the evaluated side was used as data.

Masseter and temporal muscles thickness analysis

Ultrasonographic images of the masseter and temporal muscles were obtained at rest and during dental clenching in maximal voluntary contraction while the subjects were sitting upright with their heads naturally positioned, using a portable ultrasound device (NanoMaxx; SonoSite Inc., Bothell, WA, USA) with a 13-MHz linear transducer (Bertram et al., 2003).

The location where the examination was performed was silent, with limited light for better visualization and capture of ultrasound images. Orientation was given to individuals participating in the study to remain calm during data collection. The location of the masticatory muscles was revealed by the application of digital palpation force (Palinkas et al., 2010; Gomes et al., 2022).

The linear transducer was coated with colourless conductive ultrasound gel to eliminate air between the device and the surface of the integumentary tissue, which could interfere with the capture of the ultrasound image. Considering that the belly of the masseter muscle is located approximately 2.75 cm above the mandibular angle, towards the upper eyelid and the anterior portion of the temporalis muscle, approximately 1.25 cm behind and above the external angle of the eye, the transducer was positioned transversely to the direction of the muscle fibres (da Silva et al., 2017).

Three ultrasound images were obtained from the masseter and temporal muscles during the mandibular tasks, with an interval of 2 min between each image (Righetti et al., 2020). In view of the three measurements obtained, the means were calculated, and the values obtained, in centimetres, and were used in the study.

Method error

To ensure the reliability of the results, Dahlberg's formula (Houston, 1983) was used. The bite force and muscle thickness were calculated using the records of five subjects and obtained during two different periods, with a period of 7 days. Small differences were observed in measurements between the first and second sessions for molar bite force, with the average of the three bites computed for the right and left sides (6.68%) and muscle thickness (5.22%).

Statistical analysis

Data were analysed using IBM SPSS 26.0 statistical software (IBM SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to demonstrate whether the variables maximal molar bite force and masseter and temporal muscles thickness at rest, and dental clenching in maximal voluntary contraction were normally distributed. As the

data showed a normal distribution, Student's *t*-test was applied to verify differences between variables. Statistical significance was set at $p < 0.05$.

Results

The maximum bite force and masseter and temporal muscle thickness of the two groups are shown in Table 2. Significant differences were observed in the maximum left molar force ($p=0.04$). The G1 group had a lower maximum molar bite force. No significant differences in muscle thickness were observed in the masseter and temporal muscles between the groups during the jaw and jaw tasks. However, from clinical observations, the G1 presented less thickness for the masseter muscles and greater thickness for the temporal muscles in both mandibular tasks.

Discussion

The null hypothesis of this study was rejected because there were significant differences between groups for maximum molar bite force, demonstrating the relationship between degeneration of anatomical structures and functionality of the human body systems.

One of our alternative hypotheses was that the group with degeneration of the intervertebral discs would have a lower bite force. This hypothesis was based on research demonstrating that degenerative diseases of the intervertebral discs release circulating inflammatory mediators, such as interleukin-6, which is associated with symptomatic disorders and is considered a harbinger of sarcopenia and

Table 2 – Differences in mean values (\pm standard deviations) of variables between groups

Variables	G1	GII	P-value
Bite force (N)			
Right	321.56 \pm 108.75	396.38 \pm 148.27	0.11
Left	337.15 \pm 102.18	420.70 \pm 125.91	0.04
Muscle thickness (cm)			
Rest			
RM	0.89 \pm 0.18	0.90 \pm 0.09	0.75
LM	0.87 \pm 0.15	0.90 \pm 0.16	0.59
RT	0.56 \pm 0.17	0.53 \pm 0.20	0.71
LT	0.58 \pm 0.19	0.57 \pm 0.20	0.86
MVC			
RM	1.22 \pm 0.15	1.30 \pm 0.20	0.18
LM	1.29 \pm 0.25	1.31 \pm 0.22	0.79
RT	0.67 \pm 0.19	0.64 \pm 0.18	0.64
LT	0.68 \pm 0.19	0.66 \pm 0.19	0.68

G1 – degeneration of intervertebral discs; GII – control; RM – right masseter; LM – left masseter; RT – right temporalis; LT – left temporalis; MVC – maximum voluntary contraction; significant difference, Student's *t*-test (i.e. $p < 0.05$)

changes in the functional capacity of skeletal striated musculature (Lin et al., 2021; Mehta et al., 2021). These factors can reduce muscle tissue and strength because of interleukin-6-induced atrophy in myotubes (Teixeira et al., 2021). Therefore, the first hypothesis of this study was accepted because GI showed a decrease in the maximum molar bite force with a significant difference on the left side compared to GII.

Continuous inflammation that affects anatomical structures, such as the intervertebral discs, resulting from an inadequate reaction against self-molecules, releases cytokines that transmit stimulating, modulating, and inhibitory signals to cells of the immune system (Podichetty, 2007). Activation of innate or adaptive immunity associated with degeneration episodes can provide inadequate repair responses that accompany disease progression and, consequently, can cause functional alterations in integrated systems (Waisman et al., 2015; Tezel, 2022). Circulating cytokines produced by macrophages, T cells, and monocytes interact with specific receptors on various types of cells and stimulate JAK-STAT signalling pathways that promote an inflammatory response involving cell adhesion, permeability, and apoptosis (Wang et al., 2012).

The inflammatory process is associated with increased plasma levels of cytokines, which damage the human body, and their release into the bloodstream contributes to the maintenance of inflammation, often promoting an increase in muscle fibrosis that causes fatigue and tissue changes, such as loss of muscle mass (Zhang et al., 2021). Several clinical conditions that circumvent signal propagation inside the cell can lead to apoptosis and reduced activation of satellite cells responsible for muscle regeneration, leading to the loss of muscle tissue (Ni and Yang, 2022).

These physiological conditions could explain the decrease in maximum molar bite force in the GI. In this study, circulating levels of cytokines in the human body were not quantified, and masticatory muscle fatigue was not measured.

Another important factor that may have influenced the lower molar bite force in the GI tract was the aging process that affects the spine, resulting from dehydration of the intervertebral discs, in which the ability to act as shock absorbers between the vertebrae is lost (Vital et al., 2021). The spine can undergo degenerative changes and morphological changes over the years (Prescher, 1998).

An understanding of the postural mechanism, which is expressed in countless positions, is obtained when the idea of the human body as a single functional unit, in which the ligaments and muscles act on the joints, promotes muscle tensions that they exert with equal power in both extremes of the muscle fibres, to produce momentary immobility of the body over the years (Tecco et al., 2007; Donizetti Verri et al., 2019).

When degenerative processes affect spinal structures, they can cause instability and consequently affect the stomatognathic system (Kielnar et al., 2021). The inappropriate position of the head, for example, due to degenerative diseases of the spine modifies the craniocervical and craniomandibular biomechanical relationships,

which can compromise the position of the mandible, modifying, for example, the occlusion with a direct impact on muscle strength (Lee et al., 2021). This may explain the lower molar bite force in the GI group. Postural assessments were not performed in this study.

Our second alternative hypothesis was that the GI presented smaller masseter and temporal muscle thickness during the analysis of mandibular tasks. This hypothesis is based on the aging of the human body, which includes adverse situations promoting organic dysfunction, such as mitochondrial dysfunction (Vergroesen et al., 2015), which triggers changes in the mitochondrial proteolytic system, dynamics, and mitophagy that induce the production of pathways that affect muscle tissue and functional performance (Romanello and Sandri, 2021).

The second hypothesis of this study was rejected because there were no significant differences between groups in the evaluation of masticatory muscle thickness during mandibular rest tasks and dental clenching during maximum voluntary contraction. These results corroborate the scientific finding that there were no significant differences between the groups with chronic degenerative disease and controls when comparing the masseter and temporal muscle thickness (Righetti et al., 2020).

However, in clinical observations, it was noted that GI had a smaller thickness for the masseter muscles and greater thickness for the temporal muscles in both mandibular tasks when compared to the control group. Degenerative diseases of the intervertebral discs may be related to changes in the composition of striated skeletal muscle tissue as a result of aging and physiological processes that affect the systems of the human body (Borisov and Carlson, 2000).

The masseter muscle is a dynamic anatomical structure that comprises the masticatory muscles, elevating the mandible against the maxilla and exerting masticatory force (Almukhtar and Fabi, 2019). As mentioned before, hypotheses would explain the smaller thickness of the masseter muscles of subjects with intervertebral disc degeneration when the activation of innate or adaptive immunity and the release of inflammatory mediators in the bloodstream are observed, producing morphological consequences (Waisman et al., 2015).

Another situation could explain the smaller thickness of the masseter muscles in the group with degeneration of intervertebral discs. We have to surmise that the systems interact with each other, and any functional modification can affect adjacent anatomical structures (Sun et al., 2021). Studies have reported that subjects who develop intervertebral disc degeneration can develop mitochondrial dysfunction that leads to cell energy failure, increased oxidative stress, and apoptosis, impacting homeostasis (Sabeti et al., 2021).

Oxidative stress contributes to muscle atrophy, increasing proteolysis and/or depressing protein synthesis (Shcherbik and Pestov, 2019). This could justify the smaller masseter muscle thickness in the group with intervertebral disc degeneration. The degree of oxidative stress was not measured (Powers et al., 2012).

We could also explain the clinically obtained results in relation to masseter muscle thickness in the group with intervertebral disc degeneration through the increase in apoptosis, mainly of the myonuclei, due to the inflammatory process during the development of degenerative diseases that contribute to the decrease in muscle tissue (Sudo and Kano, 2009).

Clinical evaluation of the temporal muscle thickness during mandibular rest tasks and dental clenching in maximum voluntary contraction showed greater thickness in GI than in GII. How can we explain this morphological pattern in the temporal muscles of the GI tract?

The analysis of the striated skeletal muscle tissue is an essential factor in the functional understanding of many diseases related to organic systems (Donizetti Verri et al., 2019; Katsuki et al., 2021) and observing the temporal muscle thickness can show the relationship with morphology, occlusion, and disorders of the temporomandibular joint, being a fundamental factor in the study of the stomatognathic system (Blicharz et al., 2021).

There is a hypothesis that could explain the reason for the greater thickness of the temporal muscles, assuming that neuronal control during movements of muscle groups is jointly controlled by the nervous system as a synergistic functional entity, where functional and morphological patterns are balanced to perform similar functions (Desrochers et al., 2019).

When a muscle is affected by morphological changes resulting from the degeneration of the human body, the synergistic muscle can be influenced by its function, promoting muscle hypertrophy resulting from the time of tension it is subjected to maintain its function (Joanisse et al., 2020). This can be used to interpret the greatest temporal muscle thickness.

This study had some limitations. One of the limitations was conducting this research during the coronavirus disease 2019 pandemic caused by the novel coronavirus (SARS-CoV-2), which rendered participation in this study difficult. Another limitation is failure in quantifying the circulating levels of cytokines in the human body, which could explain the reduction in muscle tissue and strength, as well as failure to measure the muscle fatigue process and the degree of oxidative stress that could justify the loss of muscle tissue.

Conclusion

The results of this study suggest that degenerative disease of the intervertebral discs alters the morphofunctionality of the stomatognathic system, with an emphasis on the lower maximum molar bite force, especially on the left side. Although the masseter and temporal muscle thickness did not differ significantly, there is clinical evidence that the degenerative disease can modify the morphology of the masseter and temporal muscles. This research allows us to guide new lines of scientific research on the stomatognathic system of subjects with degenerative intervertebral disc disease related to our hypotheses, which would explain the results of this study

and produce relevant information for health science. Therefore, future studies are needed to interpret the findings of this study, which show that there is still a gap between dentistry and medicine, considering the pathology that affects the spine and the dynamic structures of the stomatognathic system.

References

- Abdollahzade, S., Hanaei, S., Sadr, M., Mirbolouk, M. H., Fattahi, E., Rezaei, N., Khoshnevisan, A. (2018) Significant association of TNF- α , but not other pro-inflammatory cytokines, single nucleotide polymorphisms with intervertebral disc degeneration in Iranian population. *Clin. Neurol. Neurosurg.* **173**, 77–83.
- Alam, M. K., Alfawzan, A. A. (2020) Maximum voluntary molar bite force in subjects with malocclusion: multifactor analysis. *J. Int. Med. Res.* **48**, 300060520962943.
- Almukhtar, R. M., Fabi, S. G. (2019) The masseter muscle and its role in facial contouring, aging, and quality of life: a literature review. *Plast. Reconstr. Surg.* **143**, 39e–48e.
- Bertram, S., Brandlmaier, I., Rudisch, A., Bodner, G., Emshoff, R. (2003) Cross-sectional characteristics of the masseter muscle: An ultrasonographic study. *Int. J. Oral Maxillofac. Surg.* **32**, 64–68.
- Blicharz, G., Rymarczyk, M., Rogulski, M., Linek, P. (2021) Methods of masseter and temporal muscle thickness and elasticity measurements by ultrasound imaging: a literature review. *Curr. Med. Imaging* **17**, 707–713.
- Bonjardim, L. R., Lopes-Filho, R. J., Amado, G., Albuquerque, R. L. Jr., Goncalves, S. R. (2009) Association between symptoms of temporomandibular disorders and gender, morphological occlusion, and psychological factors in a group of university students. *Indian J. Dent. Res.* **20**, 190–194.
- Borisov, A. B., Carlson, B. M. (2000) Cell death in denervated skeletal muscle is distinct from classical apoptosis. *Anat. Rec.* **258**, 305–318.
- Cannata, F., Vadalà, G., Ambrosio, L., Fallucca, S., Napoli, N., Papalia, R., Pozzilli, P., Denaro, V. (2020) Intervertebral disc degeneration: A focus on obesity and type 2 diabetes. *Diabetes Metab. Res. Rev.* **36**, e3224.
- Cheung, J. P. Y., Luk, K. D. K. (2019) The relevance of high-intensity zones in degenerative disc disease. *Int. Orthop.* **43**, 861–867.
- da Silva, J. M., Pires, C. P. A. B., Rodrigues, L. A. M., Palinkas, M., de Luca Canto, G., de Vasconcelos, P. B., Rancan, S. V., Semprini, M., Siéssere, S., Regalo, S. C. H. (2017) Influence of mandibular tori on stomatognathic system function. *Cranio* **35**, 30–37.
- Desrochers, E., Harnie, J., Doelman, A., Hurteau, M. F., Frigon, A. (2019) Spinal control of muscle synergies for adult mammalian locomotion. *J. Physiol.* **597**, 333–350.
- Donizetti Verri, E., da Silva, G. P., Marianetti Fioco, E., Soares da Silva, N., Valin Fabrin, S. C., Augusto Bueno Zanella, C., Roberta Garrefa, C., Faria Júnior, M., Siéssere, S., Hallak, J. E. C., Palinkas, M., Chaves, T. C., Regalo, S. C. H. (2019) Effects of Parkinson's disease on molar bite force, electromyographic activity and muscle thickness of the masseter, temporal and sternocleidomastoid muscles: a case-control study. *J. Oral Rehabil.* **46**, 912–919.
- Gomes, G. G. C., Palinkas, M., da Silva, G. P., Gonçalves, C. R., Lopes, R. F. T., Verri, E. D., Fabrin, S. C. V., Fioco, E. M., Siéssere, S., Regalo, S. C. H. (2022) Bite force, thickness, and thermographic patterns of masticatory muscles post-hemorrhagic stroke. *J. Stroke Cerebrovasc. Dis.* **31**, 106173.
- Houston, W. J. (1983) The analysis of errors in orthodontic measurements. *Am. J. Orthod.* **83**, 382–390.
- Ji, Z. S., Yang, H., Yang, Y. H., Li, S. J., Luo, J. X., Zhang, G. W., Lin, H. S. (2020) Analysis of clinical effect and radiographic outcomes of Isobar TTL system for two-segment lumbar degenerative disease: a retrospective study. *BMC Surg.* **20**, 15.

- Joanisse, S., Lim, C., McKendry, J., Mcleod, J. C., Stokes, T., Phillips, S. M. (2020) Recent advances in understanding resistance exercise training-induced skeletal muscle hypertrophy in humans. *F1000Res*. **9**, F1000 Faculty Rev-141.
- Katsuki, M., Kakizawa, Y., Nishikawa, A., Yamamoto, Y., Uchiyama, T. (2021) Temporal muscle thickness and area are an independent prognostic factors in patients aged 75 or younger with aneurysmal subarachnoid hemorrhage treated by clipping. *Surg. Neurol. Int.* **12**, 151.
- Kielnar, R., Mika, A., Bylina, D., Sołtan, J., Stolarczyk, A., Pruszczyński, B., Rachenik, H., Szczegielniak, J., Królikowka, A., Oleksy, Ł. (2021) The influence of cervical spine rehabilitation on bioelectrical activity (sEMG) of cervical and masticatory system muscles. *PLoS One* **16**, e0250746.
- Kos, N., Gradisnik, L., Velnar, T. (2019) A brief review of the degenerative intervertebral disc disease. *Med. Arch.* **73**, 421–424.
- Lee, H. J., Jeon, D. G., Park, J. H. (2021) Correlation between kinematic sagittal parameters of the cervical lordosis or head posture and disc degeneration in patients with posterior neck pain. *Open Med. (Wars.)* **16**, 161–168.
- Lin, B., Bai, L., Wang, S., Lin, H. (2021) The association of systemic interleukin 6 and interleukin 10 levels with sarcopenia in elderly patients with chronic obstructive pulmonary disease. *Int. J. Gen. Med.* **14**, 5893–5902.
- Mehta, M., Louissaint, J., Parikh, N. S., Long, M. T., Tapper, E. B. (2021) Cognitive function, sarcopenia, and inflammation are strongly associated with frailty: A Framingham cohort study. *Am. J. Med.* **134**, 1530–1538.
- Ni, J. H., Yang, W. X. (2022) Extracellular and intracellular skeletons: How do they involve in apoptosis. *DNA Cell Biol.* **41**, 80–90.
- Palinkas, M., Nassar, M. S., Cecílio, F. A., Siéssere, S., Semprini, M., Machado-de-Sousa, J. P., Hallak, J. E., Regalo, S. C. (2010) Age and gender influence on maximal bite force and masticatory muscles thickness. *Arch. Oral Biol.* **55**, 797–802.
- Podichetty, V. K. (2007) The aging spine: The role of inflammatory mediators in intervertebral disc degeneration. *Cell. Mol. Biol. (Noisy-le-grand)* **53**, 4–18.
- Powers, S. K., Smuder, A. J., Judge, A. R. (2012) Oxidative stress and disuse muscle atrophy: Cause or consequence? *Curr. Opin. Clin. Nutr. Metab. Care* **15**, 240–245.
- Prescher, A. (1998) Anatomy and pathology of the aging spine. *Eur. J. Radiol.* **27**, 181–195.
- Righetti, M. A., Taube, O. L. S., Palinkas, M., Gonçalves, L. M. N., Esposito, D. S., de Mello, E. C., Regalo, I. H., Regalo, S. C. H., Siéssere, S. (2020) Osteoarthritis: Analyze of the molar bite force, thickness and masticatory efficiency. *Prague Med. Rep.* **121**, 87–95.
- Risbud, M. V., Shapiro, I. M. (2014) Role of cytokines in intervertebral disc degeneration: Pain and disc content. *Nat. Rev. Rheumatol.* **10**, 44–56.
- Romanello, V., Sandri, M. (2021) The connection between the dynamic remodeling of the mitochondrial network and the regulation of muscle mass. *Cell. Mol. Life Sci.* **78**, 1305–1328.
- Russo, V. M., Dhawan, R. T., Dharmarajah, N., Baudracco, I., Lazzarino, A. I., Casey, A. T. (2017) Hybrid bone single photon emission computed tomography imaging in evaluation of chronic low back pain: Correlation with modic changes and degenerative disc disease. *World Neurosurg.* **104**, 816–823.
- Rustenburt, C. M. E., Faraj, S. S. A., Ket, J. C. F., Emanuel, K. S., Smit, T. H. (2019) Prognostic factors in the progression of intervertebral disc degeneration: Which patient should be targeted with regenerative therapies? *JOR Spine* **2**, e1063.
- Saberi, M., Zhang, X., Mobasheri, A. (2021) Targeting mitochondrial dysfunction with small molecules in intervertebral disc aging and degeneration. *Geroscience* **43**, 517–537.

- Shcherbik, N., Pestov, D. G. (2019) The impact of oxidative stress on ribosomes: From injury to regulation. *Cells* **8**, 1379.
- Spadaro, A., Ciarrocchi, I., Masci, C., Cozzolino, V., Monaco, A. (2014) Effects of intervertebral disc disorders of low back on the mandibular kinematic: Kinesiographic study. *BMC Res. Notes* **7**, 569.
- Sudo, M., Kano, Y. (2009) Myofiber apoptosis occurs in the inflammation and regeneration phase following eccentric contractions in rats. *J. Physiol. Sci.* **9**, 405–412.
- Sun, K., Sun, X., Sun, J., Jiang, Y., Lin, F., Kong, F., Li, F., Zhu, J., Huan, L., Zheng, B., Wang, Y., Zou, W., Gao, L., Xu, X., Shi, J. (2021) Tissue renin-angiotensin system (tRAS) induce intervertebral disc degeneration by activating oxidative stress and inflammatory reaction. *Oxid. Med. Cell. Longev.* **2021**, 3225439.
- Tecco, S., Salini, V., Teté, S., Festa, F. (2007) Effects of anterior cruciate ligament (ACL) injury on muscle activity of head, neck and trunk muscles: A cross-sectional evaluation. *Cranio* **25**, 177–185.
- Teixeira, M. A., De Feudis, M., Reano, S., Raiteri, T., Scircoli, A., Zaggia, I., Ruga, S., Salvadori, L., Prodham, F., Marzullo, P., Molinari, C., Corà, D., Filigheddu, N. (2021) Cholecalciferol (vitamin D3) has a direct protective activity against interleukin 6-induced atrophy in C2C12 myotubes. *Aging (Albany NY)* **13**, 4895–4910.
- Tezel, G. (2022) Molecular regulation of neuroinflammation in glaucoma: Current knowledge and the ongoing search for new treatment targets. *Prog. Retin. Eye Res.* **87**, 100998.
- Truszczyńska, A., Dobrzyńska, M., Trzaskoma, Z., Drzał-Grabiec, J., Tarnowski, A. (2016) Assessment of postural stability in patients with lumbar spine chronic disc disease. *Acta Bioeng. Biomech.* **18**, 71–77.
- Vergroesen, P. P., Kingma, I., Emanuel, K. S., Hoogendoorn, R. J., Welting, T. J., van Royen, B. J., van Dieën, J. H., Smit, T. H. (2015) Mechanics and biology in intervertebral disc degeneration: A vicious circle. *Osteoarthritis Cartilage* **23**, 1057–1070.
- Vital, J. M., Sénégas, J., Bouloussa, H., Liguoro, D. (2021) Physiological aging spine. *Rev. Prat.* **71**, 497–508.
- Waisman, A., Liblau, R. S., Becher, B. (2015) Innate and adaptive immune responses in the CNS. *Lancet Neurol.* **14**, 945–955.
- Wang, X., Liu, Q., Ihsan, A., Huang, L., Dai, M., Hao, H., Cheng, G., Liu, Z., Wang, Y., Yuan, Z. (2012) JAK/STAT pathway plays a critical role in the proinflammatory gene expression and apoptosis of RAW264.7 cells induced by trichothecenes as DON and T-2 toxin. *Toxicol. Sci.* **127**, 412–424.
- Zhang, W. J., Chen, S. J., Zhou, S. C., Wu, S. Z., Wang, H. (2021) Inflammasomes and fibrosis. *Front. Immunol.* **12**, 643149.
- Zvicer, J., Obradovic, B. (2018) Bioreactors with hydrostatic pressures imitating physiological environments in intervertebral discs. *J. Tissue Eng. Regen. Med.* **12**, 529–545.

The Primary Brain Eosinophilic Angiocentric Fibrosis, A Rare Case Report

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Abstract: Eosinophilic angiocentric fibrosis (EAF) is a rare progressive fibrosing lesion involving the nasal cavity, paranasal sinuses, and the upper respiratory tract. There are few reports that it rarely involves the orbit; however, there is no report of intracranial involvement. Here, we report and share our experience with a rare case of primary intracranial EAF. A 33-year-old woman with a history of a suprasellar mass and unsuccessful surgical and medical treatment referred to us. Physical examination demonstrated right-sided blindness and ptosis, left-sided decreased visual acuity, and visual field defect. The brain imaging revealed an extra-axial intradural well-defined large suprasellar mass with parasellar (more on the right side) and retrosellar extension. Via pterional craniotomy and subfrontal approach, a very firm creamy-brownish well-defined fibrotic mass was encountered. The tumour texture was too firm to be totally resected. The microscope exited the surgical field off, and the tumour was incompletely resected using a rongeur. The histopathology finding favoured EAF. Further histopathology evaluation failed to show histologic features of IgG4-related disease. Although the preoperative diagnosis of EAF is impossible, in the setting of an indolent slow-growing lesion demonstrating hypointensity on the T2 image sequence of MRI (magnetic resonance imaging), EAF should be considered a differential diagnosis. In the setting of this diagnosis, the systemic and other organ involvement for a diagnosis of IgG4-RD should be evaluated. However, more cases are needed to illustrate the relation between these two entities.

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Introduction

Eosinophilic angiocentric fibrosis (EAF) is a rare progressive fibrosing lesion involving the nasal cavity, paranasal sinuses, and the upper respiratory tract (Ahn and Flanagan, 2018). There are few reports that it rarely involves the orbit; however, there is no report of intracranial involvement (Radhakrishnan et al., 2015; Ahn and Flanagan, 2018). Here, we report and share our experience with a rare case of primary intracranial EAF.

Case report

A 33-year-old woman referred to us due to progressive visual loss. Her medical history demonstrated previous craniotomy and biopsy, glucocorticosteroid prescription, and radiation therapy for a suprasellar mass. However, all treatments failed to be effective. The physical examination demonstrated right-sided blindness and ptosis, left-sided decreased visual acuity, and visual field defect, which was confirmed on perimetry. The brain imaging revealed a large intradural but extra-axial well-defined suprasellar mass with parasellar (more on the right side) and retrosellar extension. The lesion was hyperdense on a CT (computed tomography) scan (Figure 1). On MRI (magnetic resonance imaging), the lesion was isointense on T1, and hypointense on T2, and it showed inhomogeneous moderate enhancement on T1 with Gd injection (Figure 2). The laboratory data and hypophysial laboratory profiles were normal except for secondary hypothyroidism. Considering the progressive symptoms, surgical intervention was scheduled. Regarding the history of craniotomy, failure of all therapeutic modalities, and radiologic features of the lesion, we anticipated an unusual pathology and decided to carry out craniotomy again to achieve total resection. Via pterional craniotomy and subfrontal approach, a very firm creamy-brownish well-defined fibrotic mass was encountered. The tumour texture was too firm to be totally resected. The microscope exited the surgical field off, and the tumour was incompletely resected using a rongeur (Figure 3). The histopathology report was as follows: concentric proliferation of small vessels with obliteration of their luminal, which were confluent with extensive hyaline fibrosis and foci of fibrinoid necrosis. The involved area was surrounded by

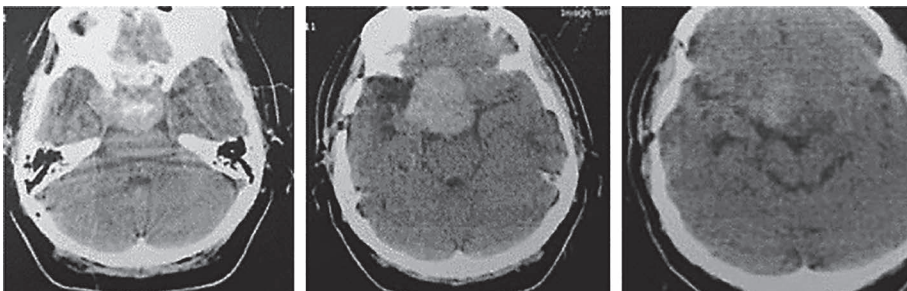


Figure 1 – The patient's brain computed tomography scan.

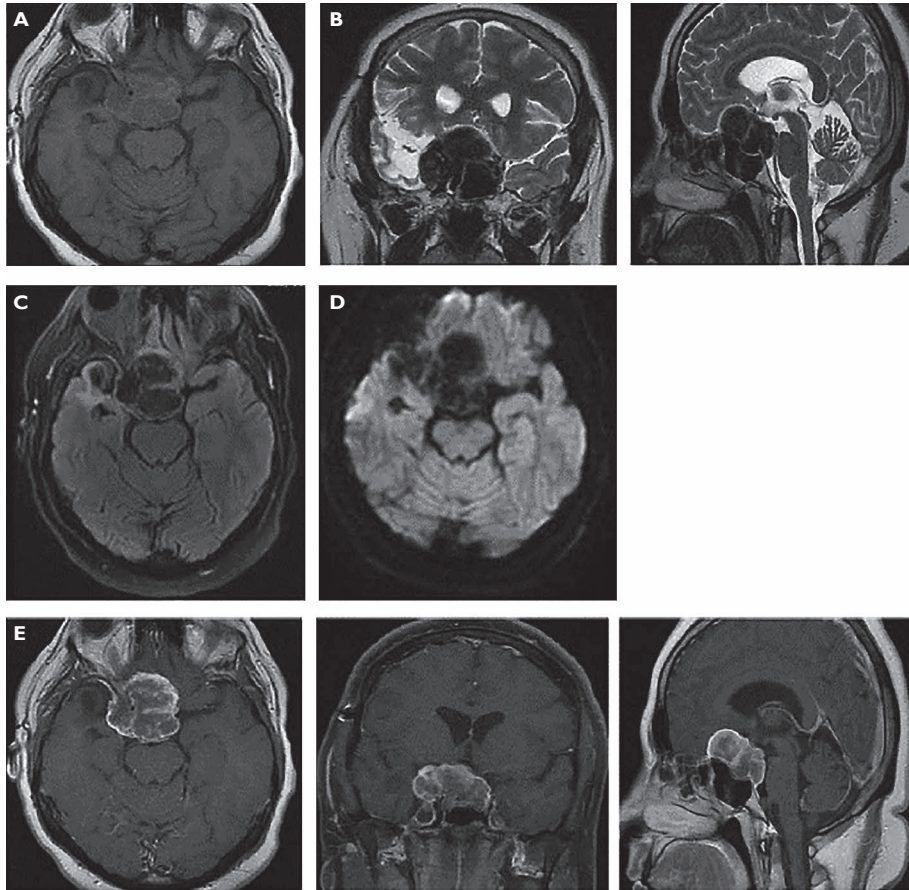


Figure 2 – The brain magnetic resonance imaging demonstrated a well-defined lobulated suprasellar mass with parasellar and retrosellar extension. The lesion is isointense on T1 (A), hyperintense on T2 (B) and FLAIR (C), and hypointense on DWI (no restriction effusion) (D), and exhibits inhomogeneous moderate enhancement on T1 with Gd injection images sequences (E).

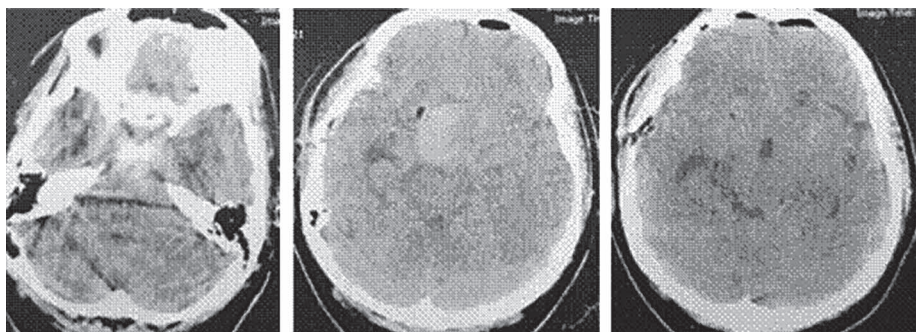


Figure 3 – The postoperative brain computed tomography scan demonstrated incomplete resection.

some lymphoplasmacell infiltration and considerable eosinophils. This finding was in favour of EAF. Further histopathology evaluation revealed that IgG4 was positive in less than 20% of IgG4 plasma cells, and immunostaining for IgG and IgG4 failed to exhibit histologic features of IgG4-related disease (IgG4-RD). Another expert and skilled neuropathologist reviewed the pathology specimen to avoid misdiagnosis.

Considering the course of the lesion (incomplete resection, no response to the medical therapy including corticosteroid and radiotherapy), the patient was referred to a radiation oncologist for monoclonal antibody therapy (Rituximab).

Discussion

EAF is a rare, benign, slow-growing but progressive lesion with an unknown etiology. It involves the nasal cavity, paranasal sinuses, and the upper respiratory tract. It has an indolent course, and patients present commonly with a prolonged history of nasal obstruction, nasal deformity, and epistaxis. Men are more involved than women are, and the mean age of involvement is 48 years old with a range of 16–79 years old (Karlígiotiis et al., 2014; Ahn and Flanagan, 2018).

Tumour grossly is a tan to white fleshy, firmly submucosal lesion with varying size and extension. Orbital involvement occurs rarely and can result in epiphora, proptosis, and diplopia (Lloyd et al., 2015; Ahn and Flanagan, 2018).

The etiology of the disease is unknown. Some researchers showed its association with allergic and atopic disorders, and recently, some others have noted that it is an IgG4-RD. In addition, there are reports of its association with the granuloma faciale and granulomatosis with polyangiitis (GPA) (previously Wegener's granulomatosis). Furthermore, some maintain that it is a progressive fibrotic reaction rather than a true separate disease (Lloyd et al., 2015; Radhakrishnan et al., 2015; Ahn and Flanagan, 2018).

CT scan and MRI may be non-specific. On CT scan, the lesion is usually homogenous in a dense mass. Calcification is a rare finding. The lesion is isointense on T1 and hypointense on T2 images sequences and exhibits moderate inhomogeneous enhancement on T1 with Gd injection (Jin et al., 2016).

The histologic finding in the early stage includes patchy eosinophilic vasculitis in submucosa small vessels, eosinophils aggregation, migration through the vessel wall and evidence of degranulation, and a variable number of plasma cells and lymphocytes. At the mature stage, the histology demonstrated the foci of early fibrosis, spindle-shape fibroblasts resulting in the pseudo granulomatosis appearance, and reactive lymphoid follicles. True granulomatosis reaction and cytologic atypia are absent. The histologic features of the late-stage are as follows: dense fibrosis thickening in the subepithelial stroma, plasma cells, lymphocytes progressive loss, prominence of the eosinophils, concentric lamellar collagen deposition giving the onionskin fibrosis appearance (a characteristic feature of EAF), and no necrosis, mitotic activity, and true granuloma (Ahn and Flanagan, 2018).

After histologic confirmation of EAF, the immunohistochemistry for IgG and IgG4 should be performed. The diagnostic histologic features for IgG-RD include morphologic appearance, storiform type fibrosis, increased number of IgG4 plasma cells (more than 50 cells in each high-power field), and a ratio of plasma-cells IgG4/IgG more than 40%. In this situation, other pathologies associated with increased IgG should be evaluated and ruled out, such as primary sclerosing cholangitis, rheumatoid arthritis, and lymphoma (Radhakrishnan et al., 2015; Ahn and Flanagan, 2018).

The broad spectrum of the disease accounted for the differential diagnosis of EAF. These include reactive conditions (Wegener's granulomatosis, sarcoidosis, Sjogren's disease, Kimura's disease, erythema elevatum diutinum, granuloma faciale, granulomatosis with polyangiitis, Churg-Strauss syndrome, and angio lymphoid hyperplasia with eosinophilia), neoplastic lesions, such as neurogenic tumours (schwannoma), vascular tumours (angiofibroma, hemangiomas), and mesenchymal tumours (fibroma, fibrosarcoma, nodular fasciitis, and fibromatosis). Many of these diseases can be ruled out by using history and physical examination, autoimmune serology and other laboratory data, as well as the absence of granuloma on histology (Radhakrishnan et al., 2015; Ahn and Flanagan, 2018).

Considering the treatment of EAF, there are some challenges. First, there is no definite treatment. Second, the recurrence rate is high, being up to approximately 70% recurrence rate, despite the total resection. Third, multiple surgical resections are needed due to the high recurrence rate. Fourth, both surgical and medical treatment may fail to treat the disease. The treatment options include surgery, systemic corticosteroid, intralesional corticosteroid, monoclonal antibody (Rituximab), and their combination. Furthermore, the laser has been used with limited long-term success (Karligkiotis et al., 2014; Jin et al., 2016; Ahn and Flanagan, 2018).

The treatment of choice is surgery, and the goal of surgery should be gross total resection. Corticosteroid is used for growth control, but it does not affect disease progression. Steroid-free agents are also used with inconclusive results and include mycophenolate mofetil, azathioprine, hydroxychloroquine, dapsone, anti-fibrotic agents like tamoxifen, and antihistamines. The role of radiotherapy is not obvious. Considering the side effects profile of radiotherapy and its potential for the malignant transformation, it does not appear to be a suitable treatment for this benign lesion, but it should be considered in a special situation (Jin et al., 2016).

Although the lesion demonstrates a benign, indolent, slow-growing natural history, and there is some evidence that the lesion may stabilize over time, most researchers report the high recurrence rate despite the optimal treatment. The prognosis is the most favourable in cases with complete surgical resection, despite the need for multiple surgical resections. There are no reports of fatality, but a high recurrence rate results in significant morbidity (Karligkiotis et al., 2014; Jin et al., 2016; Ahn and Flanagan, 2018).

It should be noted that if EAF is considered a separate entity, the present case is interesting and unique, since the suprasellar and intradural involvement were not reported previously. A review of the literature demonstrated only orbital and extradural involvement other than the sinonasal cavity and the upper respiratory tract. Moreover, in the present case, some points can be explained by the pathology of EAF. First, the patient suffers from an indolent, slow-growing tumour. Second, two sessions of surgical interventions fail to achieve complete resection due to the extremely firm texture of the tumour. Third, the lesion did not respond to corticosteroids and radiation therapy.

Recently, some authors considered the relation between EAF and IgG-RD, and some explained that EAF might be a type of IgG-related disease (Deshpande et al., 2011; Gallo et al., 2017; Ahn and Flanagan, 2018). Diagnostic criteria for IgG4-RD include histologic finding (as noted earlier), presence of a tumefactive lesion, multiple classic organ involvement, subacute onset, elevated serum IgG4, and plasma cells, and rapid response to immunosuppressive treatment (Radhakrishnan et al., 2015; Ahn and Flanagan, 2018).

Therefore, if this case is considered IgG4-RD (IgG4 related hypophysitis), some issues are encountered. First, although the level of IgG4 was elevated in our case, the ratio of IgG4 was less than 20% (although the elevated IgG4 is a criterion for the diagnosis of IgG4-RD, it is not pathognomonic and can be elevated in other conditions). Second, systemic evaluation could not show any evidence of systemic disease or other organ involvement. Third, there are many reports of IgG4-related hypophysitis presented radiologically as a macroadenoma. Radiologically, they usually demonstrate enlargement of the hypophysis or a macroadenoma with or without somewhat suprasellar extension, and occasional enlargement of the pituitary stalk. However, in the present case, the MRI finding does not support the diagnosis of a macroadenoma with suprasellar extension and appears to be primarily an extra-axial, intradural suprasellar mass. Fourth, a good diagnostic criterion for IgG4-RD is a favourable response to glucocorticoid therapy; however, the present case did not respond. Fifth, almost all cases of IgG4-related hypophysitis were treated by surgery or glucocorticosteroid; however, both treatments failed in our case.

Conclusion

Although the preoperative diagnosis of EAF is impossible, in the setting of an indolent slow-growing lesion demonstrating hypointensity on the T2 image sequence of MRI, EAF should be considered a differential diagnosis. In the setting of this diagnosis, the systemic and other organ involvement for the diagnosis of IgG4-RD should be evaluated. However, more cases are needed to illustrate the relation between the two entities.

References

- Ahn, J., Flanagan, M. (2018) Eosinophilic angiocentric fibrosis: A review and update of its association with immunoglobulin G4-related disease. *Arch. Pathol. Lab. Med.* **142(12)**, 1560–1563.
- Deshpande, V., Khosroshahi, A., Nielsen, G., Hamilos, D., Stone, J. (2011) Eosinophilic angiocentric fibrosis is a form of IgG4-related systemic disease. *Am. J. Surg. Pathol.* **35(5)**, 701–706.
- Gallo, J., Pisoni, C., Cueto, D., Stone, J., Pairs, S. (2017) Intranasal eosinophilic angiocentric fibrosis associated with IgG4-related disease. *Rev. Colomb. Reumatol.* **24(3)**, 189–192.
- Jin, C. J., Perez-Ordóñez, B., Witterick, I. (2016) Eosinophilic angiocentric fibrosis of the sinonasal tract. *BJR Case Rep.* **2(2)**, 20150419.
- Karligkiotis, A., Volpi, L., Ferrel, F., Cerati, M., Kagkeleri, E., Meloni, F., Castelnovo, P. (2014) Primary orbital eosinophilic angiocentric fibrosis with intranasal extension. *Head Neck* **36(1)**, E8–E11.
- Lloyd, A. P., Benzimra, J. D., Warfield, A. T., Prasad, B. T. S., Matthews, T. D., Ahmed, S. K. (2015) Optic neuropathy secondary to eosinophilic angiocentric fibrosis. *J. Neuroophthalmol.* **35(1)**, 45–47.
- Radhakrishnan, S., Adulkar, N., Rangarajan, V. (2015) Eosinophilic angiocentric fibrosis of the orbit: A case report and review of the literature. *Indian J. Pathol. Microbiol.* **58(1)**, 77–79.

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 Teich, N. (1984) Taxonomy of retroviruses. In: *RNA Tumor Viruses*, eds. Weiss, R., Teich, N., Varmus, H., Coffin, J., pp. 25–207, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
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