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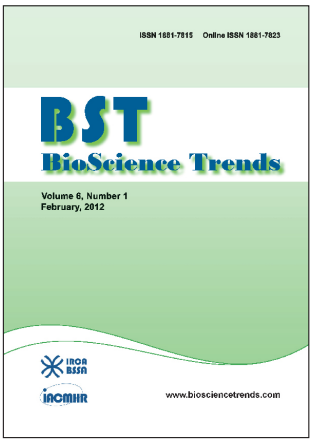

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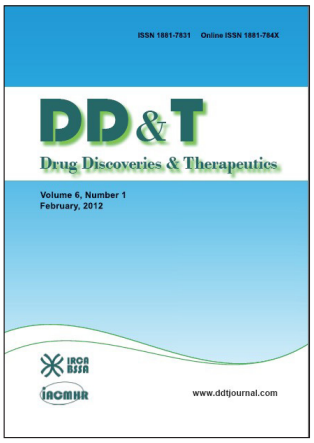

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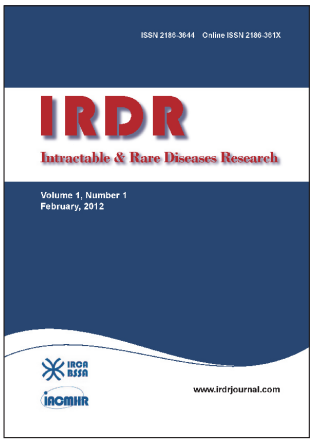

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Review

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Current treatment of atypical hemolytic uremic syndrome

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Summary **Tremendous advances have been made in understanding the pathogenesis of atypical Hemolytic Uremic Syndrome (aHUS), an extremely rare disease. Insights into the molecular biology of aHUS resulted in rapid advances in treatment with eculizumab (Soliris®, Alexion Pharmaceuticals Inc.). Historically, aHUS was associated with very high rates of mortality and morbidity. Prior therapies included plasma therapy and/or liver transplantation. Although often life saving, these were imperfect and had many complications. We review the conditions included under the rubric of aHUS: *S. pneumoniae* HUS (SpHUS), inborn errors of metabolism, and disorders of complement regulation, emphasizing their differences and similarities. We focus on the clinical features, diagnosis, and pathogenesis, and treatment of aHUS that results from mutations in genes encoding alternative complement regulators, SpHUS and HUS associated with inborn errors of metabolism. Mutations in complement genes, or antibodies to their protein products, result in unregulated activity of the alternate complement pathway, endothelial injury, and thrombotic microangiopathy (TMA). Eculizumab is a humanized monoclonal antibody that inhibits the production of the terminal complement components C5a and the membrane attack complex (C5b-9) by binding to complement protein C5a. This blocks the proinflammatory and cytolytic effects of terminal complement activation. Eculizumab use has been reported in many case reports, and retrospective and prospective clinical trials in aHUS. There have been few serious side effects and no reports of tachphylaxis or drug resistance. The results are very encouraging and eculizumab is now recognized as the treatment of choice for aHUS.**

Keywords: Alternate pathway of complement, atypical hemolytic uremic syndrome, DGPE deficiency, eculizumab, hemolytic uremic syndrome, *S. pneumoniae* hemolytic uremic syndrome, Cbl deficiency, thrombotic microangiopathy

1. Introduction

The hemolytic uremic syndromes (HUS) (*1,2*) as defined by Gasser *et al*. consist of the triad of acute hemolytic anemia with fragmented red blood cells (microangiopathic hemolytic anemia), thrombocytopenia, and acute kidney injury. The histopathological lesions in the kidneys and other organs are referred to as a thrombotic microangiopathy (TMA). The term "atypical" was first used to differentiate patients with diarrhea-associated HUS from those without prodromal diarrhea (*3*). We think that the term aHUS should now be used for the 10% of HUS cases not caused by inborn errors of metabolism, Shiga toxin *Escherichia Coli* (STEC) or *S. pneumoniae*. HUS (SpHUS). HUS is not a discrete entity but a group of conditions (*1*) with many causes and pathogenic mechanisms. Although it is now clear that HUS and thrombotic thrombocytopenic purpura (TTP) are completely different clinico-pathological entities, there are cases, more often in adults than in children that can be difficult to distinguish clinically (*4*). However, it is important to note that patients with TTP usually have ≤ 5% of normal ADAMTS13 levels (*5*). HUS may be may be acquired or inherited; some patients have an environmental trigger and a genetic mutation (Table 1).

The most important acquired causes of HUS include serotypes of *Enterohemorrhagic Escherichia coli* O157:H7 (*6*) and O104:H4 (*7*). Approximately 90% of

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Table 1. Causes of HUS

Acquired causes
E. escherichia coli 0157:H7; O104:H4 (*6,7*)
Shigella dysenteriae type 1 (*8*)
Invasive *S. pneumoniae* infection (*9*)
HIV infection (*10*)*
Cyclosporine, quinine, ticlopidine, clopidogrel (*10*)
Systemic lupus (*10*)
Malignancies (*10*)
Solid organ transplants (*10*)
Hematopoietic stem cell transplants (*11*)
Inherited causes
Atypical HUS with demonstrated mutations
Mutations in genes encoding complement regulators -CFH, CFI, and MCP (*12,13*)
Gain-of-function mutations in genes encoding complement activators C3 and complement factor B (*14*)
Mutations in thrombomodulin (*15*)
Antibodies to CHF - DEAP HUS (*16*)
Atypical HUS without demonstrated mutations - approximately 30% of cases
Inborn abnormalities of metabolism
Cobalamin C deficiency (*17*)
Methionine synthase deficiency (*18*)
Coenzyme Q (succinate coenzyme Q reductase (complex II) deficiency (*19*)
Folate deficiency (*20*)
Mutations in DGKE (encoding diacylglycerol kinase ε) (*21*)
Combined abnormalities
E. coli 0157:H7 and CHF, MCP mutations (*22*)
S. pneumoniae and CFI, CFH, THBD mutations (*23*)
Hematopoietic stem cell transplants (*11*)
CblC deficiency and CFH mutation (*24*)
Cisplatin-induced aHUS and a heterozygous *CD46* splice site mutation (*25*)

* Summarized in reference (*10*).

pediatric cases of HUS follow a diarrheal illness caused by shiga-like toxin producing bacteria [STEC] that result in STEC HUS (*4*). This was previously called diarrheal, D+ or typical HUS. The acute mortality rate is under 5% in children, and, although about 70% achieve full long-term recovery a small number progress to end-stage kidney disease (ESKD) (*26*). In contrast to aHUS, there is no known genetic basis for STEC HUS *per se* and STEC HUS does not typically recur before or after renal transplantation. However, a yet unknown number of patients with definite STEC HUS may also have CFH or membrane cofactor protein (MCP) mutations and therefore are at risk for post-transplant recurrences of HUS (*22*). In addition, the increasing evidence that the alternate pathway of complement may have a role in the pathogenesis of STEC HUS (*27*) is beyond the scope of this review.

2. *S. pneumoniae* HUS

Although SpHUS does not clearly fit under the rubric of aHUS, new studies may change this perception because complement dysregulation may be occurring in at least some cases of SpHUS (*23*). SpHUS (*9,28*) can be defined as acute hemolytic anemia, thrombocytopenia and acute kidney injury in a patient with invasive *S. pneumoniae* infection. The most accepted theory of the pathogenesis of SpHUS involves exposure of the Thomsen-Freidrenreich antigen (TF antigen) (*28*). Neuraminidase can also disrupt complement factor H (CFH) binding sites by desialyation. As a result, CFH cannot bind properly to C3 convertase on cell surfaces, leading to complement activation and cell injury (*29*). Abnormal CFH activity is another potential mechanism (*30*). Serotype 3 expresses Hic protein, a binding inhibitor for Factor H and serotype 2 produces pneumococcal surface protein C which may bind CFH and complement (*30*). Therefore, if CFH cannot inhibit the activity of the alternative complement cascade, there may be unchecked complement activation and cell damage. In addition, some patients also have mutations in complement factor I (CFI), CFH, and thrombomodulin (*THBD*) genes that result in severe complement dysregulation (*6*). Plasmapheresis theoretically removes a causative factor such as neuraminidase or anti-TF antibodies or replaces a deficient factor but there is insufficient evidence to support the use of plasmapheresis in SpHUS. There are no rigorous studies on the use of eculizumab in the treatment of SpHUS (*31*) although the theoretical risk of inhibiting complement activity in patients with invasive pneumococcal disease cannot be overlooked.

3. Inborn errors of metabolism

Inborn errors of metabolism complicated by HUS

(Table 1) are even more rare than aHUS and have a different pathogenesis. However, the report of chronic HUS associated with CblC deficiency as well as a mutation in the CFH gene indicates that both should be screened for in certain cases (*17*). The importance of differentiating these etiologies is exemplified in a report of Cbl deficiency associated with HUS in a 20-year-old (*32*). This is an informative case not only because of the age of presentation but also because eculizumab was used and Cbl deficiency was only diagnosed when he failed to respond to eculizumab.

CblC HUS disease: Inheritance is autosomal recessive inheritance. Usually presents in the neonatal period with vomiting, poor sucking, failure to thrive, lethargy, hypotonia, thrombocytopenia, microangiopathic hemolytic anemia and renal injury (*17*). It can also present during the first year of life, in childhood, and rarely in adults who have ataxia, cognitive impairment, and psychosis. CblC HUS may result from endothelial damage induced by hyperhomocysteinemia, impairment of the nitric oxide-dependent inhibition of platelet aggregation, or the procoagulant state of the endothelium leading to the formation of microthrombi. Blood homocysteine levels are high, urinary levels of homocystine and methylmalonic acid are increased, and there are increased propionylcarnitine levels and an increased C3/acetylcarnitine ratio. Treatment with parenteral hydroxycobalamin in combination with folic acid and betaine can reverse the renal injury (*17*).

Diacylglycerol kinase ε (DGKE) and HUS: Recessive loss-of-function mutations in the gene DGKE can cause HUS (*21,33,34*) not associated with activation of alternate complement pathway activation. Recessive mutations in DGKE were detected by exome sequencing in nine unrelated kindreds (*21*). Twenty-two percent of the siblings of the affected probands also had HUS and carried two affected alleles, demonstrating high penetrance. The HUS usually presented in the first year with multiple episodes and often progressed to ESKD by the second decade. DGKE mutations accounted for 27% of HUS cases in the first year of life and 50% of familial forms in this age group. Three patients developed nephrotic syndrome within 3 to 5 years of diagnosis (*33*). Renal biopsies showed chronic TMA, a membranoproliferative pattern, and podocyte foot process effacement, consistent with nephrotic syndrome. Of note, a membranoproliferative glomerulopathy also occurs in aHUS with the CFH mutation (*35*). Patients with DGKE mutations did not benefit from eculizumab or plasma treatments. Six patients had renal transplants without recurrences of HUS.

4. aHUS caused by dysregulation of the alternate complement pathway

aHUS is diagnosed by clinical and laboratory features and by the exclusion of other causes of HUS and TTP (Table 1). The incidence of aHUS is about 1 or 2 cases per 1,000,000/year. In the future it is likely that the term aHUS will be restricted to this subset of HUS caused by mutations in genes that regulate the alternate complement pathway. Most importantly, these are the patients that may benefit from eculizumab. Patients with aHUS present at any time of year, and may have had a family member with aHUS with or without a similar age of onset (*36-39*). They rarely have bloody diarrhea, often have an insidious onset, and tend to have severe arterial hypertension and a relapsing course. Extrarenal manifestations, mainly of the myocardium and central nervous system occur in a fifth of aHUS patients (*40-42*). Peripheral gangrene (*40*), gangrenous areas of the skin (*41*), retinal involvement, and cerebral artery stenosis and stroke (*42*) are serious but rare complications of aHUS. Most patients have an inexorable progression to ESKD. In those with a CFH mutation, 60-70% die or develop ESKD after their first presentation (*39*). The disease frequently recurs after renal transplantation (*43*).

Pathogenesis of aHUS: Acute and/or chronic uncontrolled dysregulation and/or excessive activation of the alternative pathway of complement is central to the pathogenesis of the sporadic and familial forms of aHUS (*12,13,44*). Activation of the complement cascade through the alternative pathway results in the generation of C3 convertase complexes that mediate the cleavage of C3 to C3a and C3b (*45*). The alternative pathway is initiated by deposition of preformed C3b on substrates such as bacteria and cell membranes, including erythrocytes. C3b is continuously available due to the interaction of C3 with water, a process called complement tick-over. C3b is necessary for the amplification and progression of the complement cascade through all pathways of activation and serves as a key immunoprotective and immunoregulatory molecule. The components of complement upstream of C5 are essential for microbial opsonization and immune complex clearance. All pathways of complement activation converge at the cleavage of the terminal complement protein C5 leading to the generation of molecules with pro-inflammatory and cell lytic properties. Targeted blockade at C5 with eculizumab therefore prevents the deleterious properties of terminal complement activation while preserving the immunoprotective and immunoregulatory functions of proximal complement (*46*).

The pathogenesis of aHUS is associated with dysregulation of the alternative complement pathway, with predisposing mutations, copy number variations, or polymorphisms in complement genes (*47*). Endothelial injury is also central to the pathogenesis of aHUS with exposure of the subendothelial matrix that becomes a target for complement activation. CFH from plasma may play a role in down-regulating complement activation on extracellular matrix and endothelial cells (*13*).

Approximately 70% of aHUS cases are associated with excessive complement activation in the microvasculature caused by known abnormalities affecting components of the alternative complement pathway (*13*). Normally, CFH, complement factor I (CFI), and membrane cofactor protein (MCP) regulate the activity of the C3 convertase on the cell surface and extracellular membranes and inhibit complement amplification. aHUS that is caused by loss-of-function mutations in CFH, MCP, and CFI are nonsense or missense mutations.

Mutations in the CFH gene occur in approximately 15-20% of aHUS patients, and autoantibodies against CFH are detected in approximately 10% of the patients (*48*). Most described mutations and autoantibodies affect short consensus repeats (SCR) 19-20 of CFH and disturb the physiological interaction of CFH with its ligands, in particular with C3b and endothelial cells (*49*). Five CFH-related genes (*CFHR1* to *CFHR5*) are located adjacent to the *CFH* gene on the long arm of chromosome 1 (*49*). This gene cluster is prone to rearrangements because of sequence homologies, and such genomic rearrangements may lead to hybrid genes or deletion of *CFHR1*, *CFHR3*, or *CFHR4*, all of which have been associated with aHUS. The deletion of CFHR1 and/or CFHR3 is strongly associated with the development of autoantibodies in aHUS. CFHR1 binds to C3b and C5 and regulates the C5 convertase and the terminal complement pathway. The CFHR1*B variant is associated with an increased risk for aHUS. The aHUS phenotype can result from antibodies to Factor H, but DEAP-HUS (deficiency of CFHR plasma proteins and factor H) refers specifically to the combination of an acquired autoantibody to Factor H and a genetic factor which, in most cases, is absence of the CFHR1 and CFHR3 proteins in plasma (*49*).

Pentraxin 3 (PTX3) is a soluble pattern recognition molecule expressed by endothelial cells and upregulated under inflammatory conditions. PTX3 activates complement, but it also binds CFH. Native CFH, factor H-like protein 1, and factor H-related protein 1 (CFHR1) bind to PTX3 and that PTX3-bound CFH and factor H-like protein 1 maintain their complement regulatory activities (*47*). PTX3, when bound to extracellular matrix, recruited functionally active CFH. aHUS-associated CFH mutations in the binding site caused reduced CFH binding to PTX3. Seven of nine analyzed anti-factor H autoantibodies isolated from aHUS patients inhibited the interaction between CFH and PTX3, and five autoantibodies also inhibited PTX3 binding to CFHR1. In addition, the aHUS-associated CFHR1*B variant showed reduced binding to PTX3 in comparison with CFHR1*A. Therefore, the interactions of PTX3 with complement regulators were impaired by certain mutations and autoantibodies affecting CFH and CFHR1. Kopp *et al* suggested that this could result in enhanced local complement-mediated inflammation, endothelial cell activation, and damage in aHUS (*47*).

The prevalence of CFH autoantibodies was studied in the Newcastle cohort of 142 aHUS patients (*50*). CFH autoantibodies were found in 13 individuals less than 11 years of age. In ten patients there were no copies of *CFHR1*, and in three patients there were two. In three patients with no copies of *CFHR1*, there was one copy of *CFHR3*, and these individuals had a novel deletion incorporating *CFHR1* and *CFHR4*. Mutations were identified in five patients: one in CFH, one in CFI, one in CD46, and two in C3. This emphasizes that multiple concurrent factors may be necessary in individual patients for disease manifestation. A high prevalence of deletions in CFH-related genes 3 and 1 (*delCFHR3-CFHR1*) and CFH autoantibodies were found in patients with HSCT-TMA; these were not detected in HSCT without TMA (*11*).

In addition to the inactivating mutations in genes encoding complement regulators (CFH, CFI, and MCP) there are also gain-of-function mutations in genes encoding the complement activators (C3 and complement factor B [CFB]) (*44*). aHUS mutations in CFB and C3 cause enhanced formation of C3 convertase or an increase in its resistance to inactivation by complement regulators. Mutations in the gene encoding thrombomodulin, a membrane-bound glycoprotein with anticoagulant properties that modulates complement activation on cell surfaces, also result in aHUS (*15*). About 20% of aHUS patients have mutations in more than one gene.

Clinical aHUS does not necessarily develop in all patients with a genetic mutation. Half of the family members with a mutation in one of the genes do not have clinical aHUS. Therefore, a second hit may be required to develop aHUS. This may be a trigger such as an infection, vaccinations, or pregnancy; or it may be an additional genetic variant (modifier) that increases the risk of developing the disease. Common at-risk genetic variants (single nucleotide polymorphisms and haplotype blocks) in *CFH*, *CD46*, and *CFHR1* might act as susceptibility factors for the development of aHUS. Therefore, the presence of a rare genetic variant (mutation), a common at-risk genetic variant (single nucleotide polymorphisms and haplotype blocks), and a trigger, may be necessary for the disease to occur. Modifiers such as CD46 and FHL-1 may determine the kidney phenotype of patients who present with homozygous CFH deficiency (*51*). On the other hand, patients with STEC HUS who do poorly or who have an unusual outcome such as a recurrence of anemia, thrombocytopenia, and neurological involvement may have CFH or other mutations (*22*).

Although the prognosis of aHUS is usually poor, some patients with the clinical features of aHUS but without known complement abnormalities have a favorable prognosis similar to diarrhea-associated disease (*52*). Patients with CFH mutations have the worst long-term prognosis with 73% progressing to

ESKD five years after diagnosis. For patients with *CFI* and *MCP* mutations, the percentage of ESKD is 50% and 38%, respectively. Thirty two percent of patients with aHUS with no identified genetic mutations progress to ESKD within five years (*53*). The post-transplantation recurrence rate is 76% in aHUS patients with CFH mutations, and 80% lose their grafts within one year of transplantation (*54*). Patients with CFI mutations also do poorly. Post-transplant recurrence occurs in 20% of patients with MCP-mutations. The risk of recurrences is not known for aHUS patients with CFB or C3 mutations. In the absence of identification of mutations in any of these genes, the risk of recurrence is 30%.

Supportive treatment of aHUS: Treatment includes blood transfusions, dialysis if indicated, and blood pressure control. Patients on dialysis may develop malignant hypertension, and bilateral nephrectomy may be needed to achieve blood pressure control in some of these patients. Traditionally, it is important to start plasma exchange (PE) or plasma infusion (PI) within 24 hours of diagnosis pending the results of mutation analysis (*55*). Many clinicians now advocate starting with eculizumab in cases in which the diagnosis of aHUS is more certain (a non-simultaneous family history of HUS, recurrent HUS, or hypocomplementemia at presentation). The rationale for PE and PI is to replace absent or mutated circulating complement regulators, such as CFH. However, the pathogenesis of HUS induced by the CFH mutation is incompletely understood. Many mutations are heterozygous, suggesting either a dominant negative effect or haplotype insufficiency. PI is likely to overcome the latter but not the former. PE also has the advantage of removing antibodies to CFH if they are the source of the problem. At least two patients with an isolated MCP (CD46) dysfunction have responded to plasma exchange (*56*). However, mutated CFB that permits excessive complement activation may be removed by PE. The exact frequency and duration of PE or PI are arbitrary and depend on the clinical responses.

The discovery of the abnormal molecular mechanisms that cause aHUS has enabled the development of a classification based on pathogenetic mechanisms as well as the clinical phenotypes. The genetic basis of aHUS may result in a new nomenclature, for example, CFH HUS, CFI HUS, DEAP HUS, *etc*. More importantly, the discovery of the molecular mechanisms has resulted in the use of eculizumab, a novel and specific therapy. Increased understanding of the molecular mechanisms responsible for the development of aHUS are the basis for the development of national and international guidelines for the investigation and treatment of this disease (*57*).

5. Management of aHUS (Table 2)

Eculizumab treatment of aHUS: Eculizumab (Soliris®, Alexion Pharmaceuticals Inc. Cheshire, CT, USA) is a humanized monoclonal antibody that inhibits the production of the terminal complement components and the membrane attack complex C5b-9 by binding to complement C5. This blocks the proinflammatory and cytolytic effects of terminal complement activation (*46*). Ideal outcome criteria for the use of eculizumab would be cessation of acute hemolysis, normalization of low platelet counts, stabilization or improvement in renal function, prevention of recurrences prior to and after renal transplant, reduction in mortality rate, normalization of complement proteins, and drug safety. Eculizumab treatment achieved all these outcomes in nearly all patients in the trials

Table 2. Current management of aHUS

Fresh frozen plasma and apheresis
While awaiting confirmatory tests
If TTP is a plausible diagnosis
Eculizumab
Treatment of choice if available
Liver and kidney transplant
If eculizumab is unavailable
May not be successful
Liver and kidney transplant plus short-term eculizumab
Precise criteria need to be established
If cost of long-term eculizumab is prohibitive

FDA approval of eculizumab for the treatment of aHUS was based on data from two prospective Phase 2 open-label clinical trials in adolescent and adult patients with aHUS, and a third retrospective study in children, adolescents, and adults with aHUS. Legendre *et al* (*58*) reported the combined results of two prospective trials. A total of 37 patients (17 in trial 1 and 20 in trial 2) received eculizumab for a median of 64 and 62 weeks, respectively. Eculizumab resulted in increases in the platelet count and 80% of the patients achieved a TMA event-free status. Eculizumab was associated with time-dependent increases in the estimated glomerular filtration rate (eGFR). Importantly, in trial 1, dialysis was discontinued in four of five patients. Furthermore, earlier intervention with eculizumab was associated with significantly greater improvement in the eGFR. Eculizumab was also associated with improvement in health-related quality of life. There were no cumulative toxicity of therapy, or meningococcal infections, through the follow-up period. Long-term eculizumab treatment over three years demonstrated ongoing inhibition of TMA with few side-effects in pediatric patients (*59*).

The largest prospective trial to-date of eculizumab in adult patients with aHUS was an open-label, single-arm, multinational trial that enrolled 41 adult patients (*60*).

Data from clinical trials were presented at the annual meeting of the American Society of Nephrology in 2013. Each trial demonstrated the clinical benefits

of eculizumab for the treatment of aHUS. There were no deaths in any of the trials during the duration of treatment. Two patients had meningococcal infections. Adverse events were headache, diarrhea and peripheral edema. The largest prospective trial of eculizumab was an open-label, single-arm, multinational trial of 41 adult patients (*60*). Eculizumab significantly improved renal function with a mean increase in eGFR from baseline of 29 mL/min/1.73 m^2. Most importantly, 20 of the 24 patients who were on dialysis at baseline discontinued dialysis by week 26.

A three-year update of the results of eculizumab in 20 aHUS patients with a long duration of disease and chronic kidney damage who had previously received prolonged PE/PI showed promising results. Despite long disease duration and CKD eculizumab led to improvements in hematologic and renal function over years (*61*).

We previously reviewed 32 case reports of eculizumab treatment of aHUS (*62*) to highlight important clinical observations that may be missed in epidemiological reviews. We reviewed the reports of 20 children 18 days to 17 years, and 12 adults 18 to 50 years. Twelve patients had no identified mutations or DEAP. Mutations were detected for CFH in 15, CFI in two, C3 in two, and MCP in one patient respectively. Eculizumab was given for up to 36 months. Renal function improved in four patients who received a single infusion of eculizumab, but each subsequently progressed to ESKD. Eculizumab was used in five cases aHUS relapse in native kidneys. In each case there was hematological recovery within 7-10 days. In three cases, there was also recovery in renal function, and these patients avoided dialysis. Eculizumab was continued as maintenance therapy. In the fourth case, there was improvement in renal function after a single dose of eculizumab. Eculizumab was used again in a subsequent relapse but there was progression to anuria, and eculizumab was stopped after hemodialysis was started. Eculizumab was used successfully in patients with aHUS refractory to, or allergic to plasma therapy. Eculizumab appeared to alter the course of a 28-day-old with aHUS refractory to plasma therapy. This infant was on mechanical ventilation and continuous renal replacement therapy and had multiple intestinal perforations and leg skin necrosis (*63*). Within 48 hours the patient recovered from acute kidney injury with complete hematologic remission and was disease-free after 14 months while on eculizumab, 300 mg every 3 weeks. No genetic cause was found for the aHUS.

Eculizumab has successfully treated post-transplant recurrences of aHUS (*64-66*). TMA was treated post-transplant in 14 patients and at the time of transplant in three cases. Eculizumab was used in a 34-year-old female who presented seven days post-simultaneous pancreas and kidney transplant with acute renal allograft dysfunction, thrombocytopenia, and microangiopathic hemolytic anemia. Renal biopsy revealed acute antibody-mediated rejection (AMR) and TMA. The clinical and laboratory manifestations partly responded to treatment with daily PE and intravenous immunoglobulin but resolved rapidly and completely on eculizumab (*67*). Eculizumab has been used pre-emptively for renal transplant in aHUS (*68*) and prophylactically after renal transplantation (*43,54,69*). The best results in terms of normalizing renal function were obtained when eculizumab was given as soon as possible after onset of aHUS (*43*). Eculizumab also successfully treated skin necrosis in aHUS (*41*).

The optimum maintenance doses and dosing schedules have not been determined, but severe aHUS was maintained in remission with sustained improved renal function on a reduced dose of eculizumab, 600 mg every 2 weeks (*70*). Eculizumab rescued a highly sensitized 13-year-old female who developed severe steroid-, ATG- and plasmapheresis-resistant AMR with TMA one week after a second kidney transplant despite previous desensitization therapy with immunoglobulin infusions (*71*).

Treatment of DEAP-HUS: There is no consensus regarding the optimal treatment of DEAP-HUS. DEAP-HUS is associated with a diarrheal prodrome in up to 53% of patients, and therefore initiation of appropriate therapies is frequently delayed. Despite delay in initiating plasma therapy, three cases all remitted with plasma therapy and normal renal function was restored (*16*). Long-term remission has also been achieved in cases of aHUS with anti-factor H antibodies treated with cyclophosphamide (*72*). Eculizumab rescue therapy resulted in a dramatic improvement in a patient deficient in the CFH-related protein 3/1 (CFHR3/1) involved in the pathogenesis of aHUS caused by CFH autoantibodies (*71*).

Eculizumab in pregnancy: Eculizumab was used safely in a 26-year-old woman with aHUS caused by a homozygous mutation in CFH who relapsed at 17 weeks of gestation. Eculizumab was started at 26 weeks, was well tolerated, induced a remission, and resulted in the delivery of a healthy neonate (*73*).

Safety information: Life-threatening and fatal meningococcal infections have occurred in patients treated with eculizumab. The prescribing physician must comply with the most current Advisory Committee on Immunization Practices (ACIP) recommendations for meningococcal vaccination in patients with complement deficiencies. Patients must be immunized with a meningococcal vaccine at least two weeks prior to administering the first dose of eculizumab unless the risks of delaying eculizumab therapy outweigh the risks of developing a meningococcal infection. Patients must be monitored for early signs of meningococcal infections and immediately evaluated and treated if infection is suspected. Bouts *et al* noted that according to the medication guide of the U.S. Food

and Drug Administration, a tetravalent unconjugated polysaccharide vaccine (serogroups A, C, Y, W135) must be provided at least two weeks before the first dose of eculizumab but that this approach is not sufficient for prevention in many countries, because none of the available vaccines contains a serogroup B antigen (*74*). A humoral immune response to conjugate Men C vaccination may be mounted and maintained despite chronic kidney disease, kidney transplantation, immunosuppressive drugs, and eculizumab. However, the authors stressed it was unclear whether serologically defined protective serum bactericidal antibody (SBA) titers mediate true protection from invasive *meningococcal* disease in an immunocompromised patient especially with treatment with a complement inhibitor, and that close monitoring of SBA titers seemed mandatory in their patient (*75*).

Dosing schedules and laboratory monitoring: Eculizumab is administered as an intravenous infusion. The recommended dosing for adult patients with aHUS is 900 mg weekly for the first 4 weeks, followed by 1200 mg weekly one week later, and 1200 mg every 2 weeks thereafter. The dosage regimen for pediatric patients is based upon body weight. Early signs of TMA include a decrease in platelet count and increases in serum LDH and serum creatinine levels. Patients should be followed for signs of TMA by monitoring serial platelet counts, serum LDH, and creatinine during eculizumab therapy and following discontinuation of eculizumab.

Adverse reactions: Administration of eculizumab may result in infusion reactions, including anaphylaxis or other hypersensitivity reactions. In clinical trials, no patients experienced an infusion reaction that required discontinuation of eculizumab. Eculizumab infusion should be interrupted and appropriate supportive measures should be instituted if signs of cardiovascular instability or respiratory compromise occur. The most frequently reported adverse reactions in aHUS single arm, prospective trials, (≥ 15% combined per patient incidence) were hypertension, upper respiratory tract infection, diarrhea, headache, anemia, vomiting, nausea, urinary tract infection, and leukopenia. The effect of withdrawal of anticoagulant therapy during eculizumab treatment has not been established. Therefore, treatment with eculizumab should not alter anticoagulant management.

Alternative therapies for aHUS: No other medications or monoclonal drugs are currently available for the prevention or treatment of aHUS. Genetic counseling should be offered. Pre-implantation diagnosis should be discussed. Plasma therapy, either with regular infusions of fresh frozen plasma and or PE has been the mainstay of treatment prior to the use of eculizumab (*76*) and may be helpful especially in patients with no identified complement mutation or in whom TTP is suspected (*5*). Plasma therapy has reduced the high mortality rate and prevented relapses but is far from optimal. There are no randomized controlled trials but there are guidelines for its use (*55*). Initial treatment with intensive PE should be initiated as soon as possible. The aim is to remove mutant complement proteins and factor H autoantibodies, and to provide fresh frozen plasma containing normal CFH as replacement fluid. Guidelines for chronic treatment are based on the risk of uncontrolled aHUS recurrence. The half-life of CFH is six days, and this provides a rationale for giving regular infusions every 1-2 weeks. Some patients become refractory to plasma, or have allergic reactions or have complications from the procedure. Renal transplant is associated with a very high incidence of recurrent aHUS and graft loss despite the use of plasma therapy (*43*).

Liver transplant alone or combined liver-kidney transplants have been used successfully in patients with aHUS, as CFH is synthesized in the liver. In contrast to the poor results of isolated kidney transplantation prior to eculizumab, the outcome of isolated liver or combined liver-kidney transplantation have been

Table 3. What is the optimum long-term treatment of aHUS?

Long-term eculizumab alone	Isolated kidney with chronic eculizumab	Combined liver – kidney transplant
Lower short-term risk	Lower short-term risk	Higher short-term mortality
Few known side-effects Long-term side-effects unknown	Long-term outcomes unknown	Long-term outcomes stable over 10-20 years
Long-term dependence on eculizumab to prevent recurrences of aHUS	Long-term dependence on eculizumab to prevent recurrences of aHUS	aHUS recurrence unlikely
No need for immunosuppressive agents	Complications of immunosuppressive agents	? Fewer complications of immunosuppressive agents
	Chronic rejection	? Reduced risk of chronic rejection
IV infusion every 2 weeks	IV infusion every 2 weeks	Better lifestyle with no infusions
Limited worldwide availability	Limited worldwide availability	More widely available But scarce liver plus kidney availability
Extremely expensive	Extremely expensive	Less expensive

Table 4. Summary of the value of eculizumab in aHUS

Positive effects
No deaths in any trial
Few serious side effects
Stabilizes hematological abnormalities
Can improve neurological abnormalities
Stabilizes renal abnormalities
Can reverse acute renal injury
Can improve eGFR
Patients may become dialysis-independent
Effective regardless of the type of detected mutation
Effective in aHUS without detected mutation
Effective in cases with DEAP
Prevents recurrent episodes pre-transplant
Prevents post-transplant recurrence
Rescues critically ill patients before and after renal transplant
Success in plasmapheresis-resistant AMR
Can be used safely in pregnancy
Negative effects
Rare cases of meningitis
Expensive
Not universally available

successful (*77*). It is likely that aHUS patients with CFI, CFB, or C3 mutations, which are also synthesized in the liver, could be treated similarly. However, combined liver-kidney transplantation may be associated with a poor outcome as a result of premature liver failure secondary to uncontrolled complement activation; concurrent treatment with eculizumab has overcome this problem (*78*). In fact, liver-kidney transplantation should not be carried out without a preparative regimen for complement regulation such as plasma therapy or eculizumab. All five children known to have undergone liver transplant without such preparation suffered fatal complications (*79*). Alternatively, a simple transplant with eculizumab therapy could be offered as a less invasive option. This concept was implemented by using eculizumab and PE in successful combined liver-kidney transplantation in a patient with CFH aHUS (*79*). The

Table 5. A tentative approach to investigation and treatment
(A). Neonates and infants

Causes	Investigations	Management
Alternate pathway of complement mutations	Serum C3 Serum CH50 (CHF, CHI, *etc*. if available)	Start plasma infusions immediately Start eculizumab as soon as possible If C3 is low and CH50 is high continue eculizumab and stop plasma
DGKe mutation	DGKe mutation if available	Stop plasma and eculizumab if no response (*81*) No specific treatment
Congenital TTP	ADAMTS13	Stop eculizumab if ADAMTS 13 < 5% Continue plasma infusions
CblC	Plasma amino acids Plasma homocystein Blood methylmalonic acid or plasma acylcarnitine	Stop plasma infusions and eculizumab if positive for CblC Low protein diet, betaine, methionine, and subcutaneous vitamin B12

(B). Children, adolescents and adults

Causes	Investigations	Management
STEC HUS (Management is clearer if there are severe bloody diarrhea and an epidemic of HUS)	Positive *E. coli* or shiga toxin – no further investigations	Dialysis if indicated No proven indications for plasma or plasmapheresis
TTP (Management is clearer if there is severe central nervous system disease and less severe renal disease)	ADAMTS13 ADAMTS 13 antibodies	Start plasma infusions and plasmapheresis immediately Continue plasma alone if ADAMTS 13 < 5% and negative for antibodies Continue plasmapheresis if ADAMTS 13 < 5% and positive antibodies to ADAMTS13
Alternate pathway of complement mutations (Management is clearer if there is minimal diarrhea, previously affected family member or a recurrence)	Serum C3 Serum CH50 (CHF, CHI, *etc*. if available)	Start plasma infusions immediately Start eculizumab as soon as possible If C3 is low and CH50 is high continue eculizumab and stop plasma Stop plasma once eculizumab is started Pre-emtive ue of eculizumab if a renal transplant is done See Table 3
DEAP HUS	Serum C3 Serum CH50 (CHF, CHI, *etc*. if available) Antibodies to CFH	Start plasma infusions immediately Start eculizumab as soon as possible If antibodies are detected, continue eculizumab and stop plasmapheresis
SpHUS (Management is clearer if there is invasive disease – pneumonia, meningitis)	Positive culture for *S. pneumoniae* Invasive *S. pneumoniae* infection	Dialysis if indicated

pros and cons of chronic treatment with eculizumab alone, kidney transplant plus chronic eculizumab treatment, and a combined liver and kidney transplant (with preparatory eculizumab treatment, are summarized in Table 3 (modified from *Ref. 80*).

Living-related kidney donation is contraindicated for aHUS patients with mutations in CFH, CFI, CFB, and C3 and in patients with aHUS in whom no mutations have been detected.

Conclusions

There has been demonstrable success of eculizumab treatment of aHUS in many case reports, and in retrospective and prospective clinical trials (Table 4). Eculizumab can stabilize hematological and renal abnormalities of aHUS. It is equally efficacious in aHUS cases regardless of demonstration of a detected mutation, but this statement needs to be tempered in cases of apparent aHUS who have an inborn error of metabolism. Eculizumab has also been efficacious in aHUS cases with DEAP.

The results of the prospective trials are increasingly encouraging. Patients with severe or chronic renal TMA have a poorer outcome than those with early and less severe TMA but may benefit from the drug. It is not established whether family members, without clinical evidence for aHUS but with a demonstrated mutation, should be given eculizumab. However, a compelling argument can be made for administering eculizumab to pre-symptomatic pregnant females with a known mutation or an affected family member. This also applies to a post-renal transplant patient with aHUS.

Traditionally, PE/PI was instituted immediately in all patients with an aHUS phenotype and this can still be done while awaiting the results of tests or availability of eculizumab. However, eculizumab should be started in patients with no response to PE/PI within a few days or in those cases in which the presumptive diagnosis of aHUS is more certain. The optimum doses and dosing schedules are established and it is apparent that long-term use is mandatory.

After much consideration we offer a tentative approach to the management of patients with HUS or TTP (Tables 5A and 5B). We recognize that eculizumab and sophisticated investigations are not universally available and that the cost of the drug may be prohibitive. We also recognize that some patients with STEC HUS (those with alternate pathway mutations) may benefit from eculizumab but this is beyond the scope of this review. Indications for eculizumab use in SpHUS need to be established. The discovery of the inherited cause of HUS caused by recessive mutations in DGKE (*11,12*) is extremely important because this condition does not respond to treatment with eculizumab. Equally important are the unknown number of cases of SpHUS (*18*), Cbl HUS (*29*) and STEC HUS (*16,51*) that are associated with mutations in alternate complement pathway regulating genes.

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Original Article

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Radiographic characteristics of the hand and cervical spine in fibrodysplasia ossificans progressiva

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Summary **Fibrodysplasia ossificans progressiva (FOP) is a disabling heritable disorder of connective tissue characterized by progressive heterotopic ossification in various extraskeletal sites. Early correct diagnosis of FOP is important to prevent additional iatrogenic harm or trauma. Congenital malformation of the great toes is a well-known diagnostic clue, but some patients show normal-appearing great toes. The thumb shortening and cervical spine abnormalities are other skeletal features often observed in FOP. This study aimed to address the quantitative assessment of these features in a cohort of patients with FOP, which potentially helps early diagnosis of FOP. Radiographs of the hand and cervical spine were retrospectively analyzed from a total of 18 FOP patients (9 males and 9 females) with an average age of 13.9 years (range 0.7-39.3 years). The elevated ratio of the second metacarpal bone to the distal phalanx of the thumb (> +1SD) was a consistent finding irrespective of the patient's age and gender. Infant FOP patients, in addition, exhibited an extremely high ratio of the second metacarpal bone to the first metacarpal bone (> +3SD). The height/depth ratio of the C5 vertebra increased in patients over 4 years of age (> +2SD). Additionally, the ratio of (height+depth) of the C5 spinous process to the C5 vertebral depth was markedly elevated in young patients (> +2SD). We quantitatively demonstrated the hand and cervical spine characteristics of FOP. These findings, which can be seen from early infancy, could be useful for early diagnosis of FOP even in patients without great toe abnormalities.**

Keywords: Fibrodysplasia ossificans progressiva, early diagnosis, radiographic characteristics

1. Introduction

Fibrodysplasia ossificans progressiva (FOP) is a severely disabling genetic disorder of connective tissues characterized by congenital malformations of the great toes and progressive heterotopic ossification (HO) in various extraskeletal sites including muscles, tendons, ligaments, fascias, and aponeuroses. FOP is caused by a recurrent activating mutation (c.617G > A, p.R206H) in the gene encoding activin receptor IA/activin-like kinase 2 (ACVR1/ALK2), a bone morphogenetic protein (BMP) type I receptor (*1*). HO typically begins to form during the first decade of life preceded by painful soft tissue swelling and inflammation (flare-ups), which are sometimes mistaken for aggressive fibromatosis or musculoskeletal tumors. Surgical resection of HO leads to explosive new bone formation (*2*). Since there is no definitive treatment to prevent progressive HO in FOP to date (*3*), early correct diagnosis is necessary to maintain their mobility by preventing additional iatrogenic harm (*4*).

Malformations of the great toes, such as hallux valgus, deformed proximal phalanges and shortened first metatarsal bones, are well-known pre-osseous features of FOP (*5*). A reported incidence of these deformities is 95%, suggesting that there exists rare FOP cases without the great toe abnormalities (*6*). We demonstrated additional early radiographic signs of FOP including shortening of the first metacarpal bones and hypertrophy of the posterior element of the cervical

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spine (*7*). Clinical awareness of these deformities can aid clinicians in making early diagnosis of FOP, but quantitative assessment of these deformities has not yet been determined.

In this study, we retrospectively examined radiographs of the hand and cervical spine in FOP patients and demonstrated various abnormal radiographic parameters helpful for early diagnosis of this specific disorder.

2. Materials and Methods

2.1. *Demographics*

This study represents a retrospective case-control study consisting of Japanese FOP patients followed up at health care facilities where members of the Research Committee on Japanese Fibrodysplasia Ossificans Progressiva practiced. After approval from the Institutional Review Board of the Nagoya University Hospital, we collected the hand and/or cervical spine radiographs from 18 FOP patients (9 males and 9 females) with an average age of 13.9 years (range 0.7-39.3 years) at the time of this study. The patients were diagnosed clinically and radiographically based on various characteristic findings of FOP including deformities of the great toes, extraskeletal HO, joint contractures, cervical fusions, broad femoral necks, and osteochondroma-like lesions. Molecular testing was performed on fourteen patients. Thirteen showed the common *ACVR1/ALK2* mutation within the glycine/serine-rich regulatory (GS) domain (c.617G > A, p.R206H), and one patient had an atypical mutation within the protein kinase domain (c.774G > T, p.R258S). Molecular studies were not conducted for the remaining 4 patients who showed characteristic skeletal features of FOP. We examined anteroposterior (AP) radiographs of the hands and lateral radiographs of the cervical spine in each individual. The earliest hands and cervical spine films were analyzed using image processing and analysis software ImageJ®.

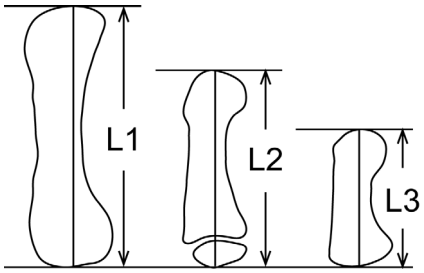

Figure 1. A schematic diagram illustrating the measurement method of bone length in the hand. Bone length was defined as the distance between the tangents drawn to each end of the bone, which were perpendicular to the bone axis. The entire bone length was measured for adults (L1), children (L2), and infants (L3).

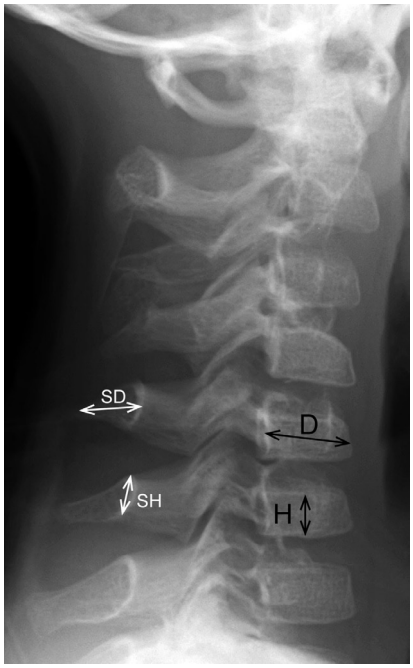

Figure 2. A radiograph depicting the measurements of the bone length in the cervical spine. The height (H) and depth (D) of the C5 vertebral body was measured at the midportion of the body. The height of the C5 spinous process (SH) was defined as the distance from the cranial to the caudal rim at the juxta-laminar zone. The depth of the spinous process (SD) was measured from the midpoint of the anterior wall to that of the posterior rim.

2.2. *Radiographic assessment of the hand*

According to the measurement method by Poznanski *et al*. (*8*), the length of each phalanx and metacarpal bone was measured. In brief, the tangent lines were drawn at both ends of each bone, which were perpendicular to the bone axis, and a bone length was defined as the distance between these two lines (Figure 1). We measured a length of the distal (D1) and proximal (P1) phalanges of the thumb as well as that of the first and second metacarpal bones (MET1 and MET2), and calculated the following bone length ratios, MET2/MET1, MET2/P1, MET2/D1, MET1/P1, MET1/D1, and P1/D1. Radiographs of both hands from one patient were separately analyzed to obtain the average value of the measurements. Reference ranges of these measurements in different ages and genders were used based on the literature from Poznanski *et al*. (*8*). The control data of these measurements in infant (n = 21) were determined by the radiographic database in Nagoya University Hospital.

2.3. *Radiographic assessment of the cervical spine*

According to the measurement method proposed by Remes *et al*. (*9*), the height and depth of the C5 vertebral body were measured. Briefly, vertebral body height (H) was measured at the midpoint of the vertebra, perpendicular to the lower end plate. The vertebral body depth (D) was measured at the midpoint of the body from the anterior wall to the posterior wall (Figure 2). The H/D ratios of the C5 vertebra were then calculated

Table 1. Characteristics and quantitative indices for the study population

Patient	Sex	ALK2 mutation	Age at X-ray (yrs)	Deviation of the bone length ratios (SD)			
			Hand/Cervical spine	MET2/D1	MET2/D1	H/D	(SH+SD)/D
1	M	R206H	0/0	1.0	1.0	0.6	0.1
2	M	R206H	0/0	2.4	2.4	0.6	7.1
3	M	R206H	1/3	3.1	3.1	0.9	2.8
4	F	R206H	5/6	2.8	2.8	3.3	8.3
5	M	R206H	8/7	6.2	6.2	2.8	3.7
6	M	R206H	12/18	4.1	4.1	1.9	1.5
7	F	R206H	17/17	4.0	4.0	4.1	NA
8	F	R206H	20/NA	2.2	2.2	NA	NA
9	M	R206H	29/NA	2.7	2.7	NA	NA
10	M	R206H	34/NA	3.5	3.5	NA	NA
11	F	R206H	36/NA	1.0	1.0	NA	NA
12	M	R206H	39/16	1.9	1.9	3.0	1.8
13	F	R206H	NA/18	NA	NA	0.6	2.4
14	F	R258S	14/14	1.7	1.7	4.9	NA
15	M	ND	NA/4	NA	NA	3.2	8.8
16	F	ND	NA/8	NA	NA	9.2	7.9
17	F	ND	NA/16	NA	NA	4.4	NA
18	F	ND	5/5	5.3	5.3	5.3	5.4

M denotes male; F, female; ND, not determined; NA, not applicable; SD, standard deviation.

and compared to normal reference values established by Remes *et al*. in different age and gender groups (*9*). In addition, we measured the height and depth of the C5 spinous process. The height of the spinous process (SH) was defined as the distance from the cranial to caudal margin at the junction of the spinous process and lamina. The depth of spinous process (SD) was measured from the midportion of the anterior wall to that of the posterior rim demarcating a thick cortex shadow (Figure 2). The sum of SH and SD measurements was used for the evaluation of spinous process size, then the (SH + SD)/D ratio of the C5 vertebra was calculated. Reference values of the (SH + SD)/D ratio were established from the radiographic database of normal controls in Nagoya University Hospital.

3. Results

3.1. *Characteristics of the study cohort*

Patients' characteristics and quantitative indices of the measurements are shown in Table 1. Deviation of the bone length ratios in the hand and cervical spine was calculated based on age-matched reference values.

3.2. *Radiographic characteristics of the hand*

Mean and standard deviation of the MET2/D1 and MET2/MET1 ratio in control infants (n = 21) are 2.9 ± 0.29 and 1.64 ± 0.08, respectively. Twenty-six hand radiographs from 14 patients (8 males and 6 females) were available. Regardless of age and gender, all FOP patients showed a MET2/D1 ratio larger than +1SD of normal controls (Figure 3A and 3B). In infant patients without an epiphyseal ossification center of the first metacarpal bone, the MET2/MET1 ratio was extremely

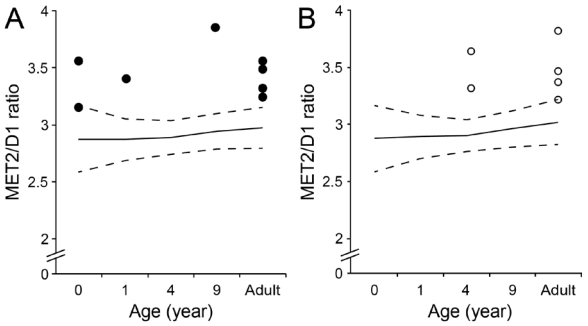

Figure 3. Scatter plots showing the bone length ratio of the second metacarpal bone (MET2) to the distal phalanx of the thumb (D1) in male (A) and female (B) patients with FOP. Solid and dash lines denote the normal value and the standard deviation (SD) of the MET2/D1 ratio, respectively.

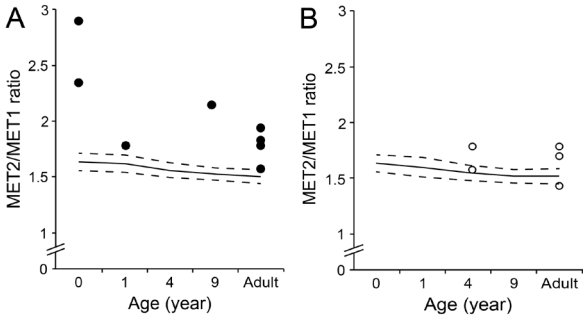

Figure 4. Scatter plots showing the bone length ratio of the second metacarpal bone (MET2) to the first metacarpal bone (MET1) in male (A) and female (B) patients with FOP. Solid and dash lines denote the normal value and the standard deviation (SD) of the MET2/MET1 ratio, respectively.

large (> +3SD of normal controls) (Figure 4A and 4B). The MET2/P1 ratio was higher in infant patients, but it scattered around the mean value with increasing age (data not shown). There were no characteristic features in the values of the MET1/P1, MET1/D1, and P1/D1 ratios in FOP patients, although the MET1/P1 and MET1/D1

Table 2. Mean and standard deviation of normal controls for the (SH+SD)/D ratio of the C5 vertebra

Age group	<1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21
Mean	1.05	1.10	1.09	1.20	1.21	1.43	1.37	1.47	1.33	1.47	1.50	1.53	1.51	1.57	1.69	1.86	1.76	1.73	1.71	1.78	1.86
SD	0.13	0.15	0.15	0.13	0.11	0.18	0.18	0.17	0.19	0.18	0.16	0.18	0.20	0.19	0.12	0.16	0.22	0.22	0.23	0.24	0.25
N	11	21	17	13	6	13	25	19	20	17	16	17	14	20	16	21	23	31	28	38	20

SD denotes standard deviation; N, number of control subjects; SH, height of the spinous process; SD, depth of the spinous process; D, depth of the vertebral body.

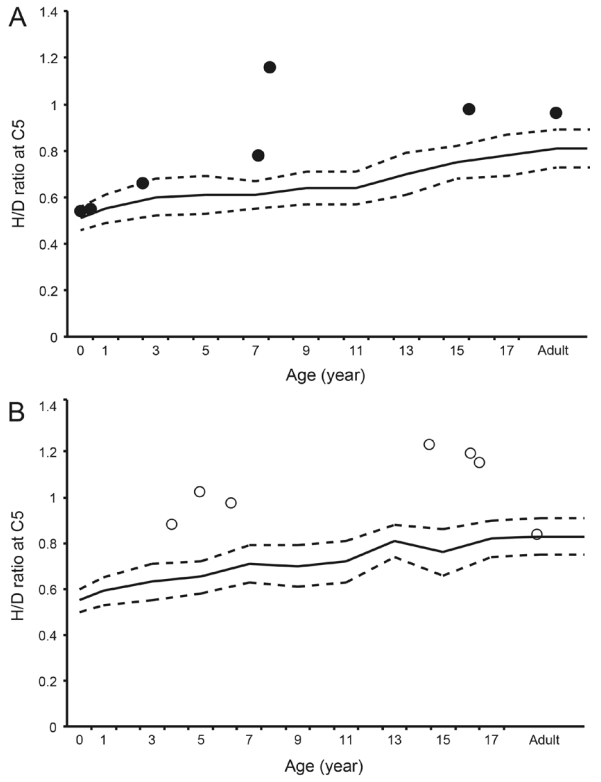

Figure 5. Scatter plots showing the bone length ratio of the C5 vertebral height (H) to depth (D) in male (A) and female (B) patients with FOP. Solid and dashed lines denote the normal value and the standard deviation (SD) of the H/D ratio, respectively.

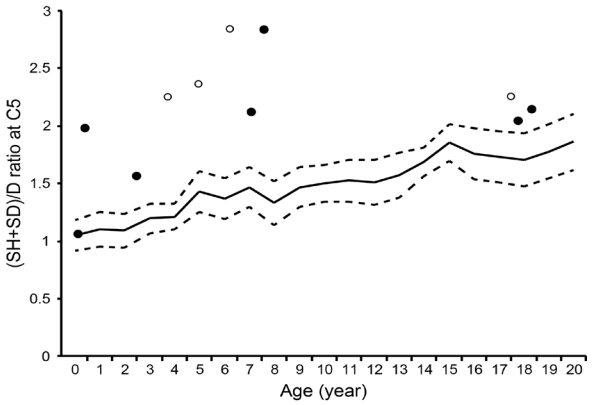

Figure 6. Scatter plots showing the bone length ratio of the C5 spinous process height (SH) + depth (SD) to the C5 vertebral depth (D). Solid and open circles indicate male and female, respectively. Solid and dashed lines denote the normal value and the standard deviation (SD) of the (SH+SD)/D ratio, respectively.

ratios were relatively small (< -1SD of normal controls) in infant FOP patients (data not shown).

3.3. *Radiographic characteristics of the cervical spine*

Reference values of the (SH + SD)/D ratio of the C5 vertebra are shown in Table 2. There were 14 (7 males and 7 females) cervical spine radiographs available for analysis. Among them, three radiographs were excluded from analysis of the (SH + SD)/D ratio for insufficient resolution. The H/D ratio of the C5 vertebra exceeded +2SD of normal controls in patients over 4 years of age except one female adult patient (Figure 5A and 5B). Similarly, the (SH + SD)/D ratio of the C5 vertebra was larger than +2SD of normal controls in young patients except one male infant (Figure 6).

4. Discussion

In the present study, we quantitatively proved the hand and cervical spine abnormalities in FOP including shortened thumbs as well as tall and narrow vertebral bodies and hypertrophic posterior elements of the cervical spine (*7,10*). Especially in young patients, shortening of the first metacarpal bone and enlargement of the cervical spinous processes were pathognomonic findings useful for early diagnosis of FOP before the appearance of HO.

Previous studies have reported that thumb shortening was seen in 50% of FOP patients (*6*). In the present study, all patients had a MET2/D1 ratio larger than +1SD of normal controls, and 85% (11/13) of the patients showed an increased MET2/MET1 ratio. The thumb shortening, therefore, seems to be more common than previous reports in FOP. Furthermore, an extremely high MET2/MET1 ratio in infant patients suggested that disproportionate shortening of the first metacarpal bone was an important early radiographic finding in FOP (Figure 7).

It is an intriguing feature of FOP that thumb morphogenesis is exclusively disrupted in the development of digit formation (*11*). The thumb is the last digit in the autopod to form, and it is different from other digits in terms of its relative position, shape, size, and number of phalanges. These unique thumb identities may be attributed to the expression profile of *HoxD* genes, which are pivotal transcriptional factors regulating limb patterning and growth (*12*). All four *HoxD10* to *D13* genes are expressed in the future digit II-V area in the autopod during the hand plate formation, whereas sole expression of the

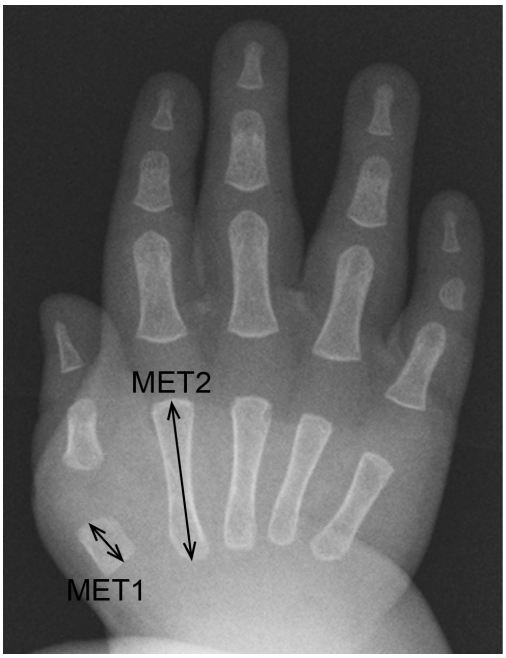

Figure 7. An anteroposterior radiograph of the right hand of Patient 1 at the age of eight months showing marked shortening of the first metacarpal bone. The MET2/MET1 ratio and the corresponding SD value is 2.9 and 16.3, respectively.

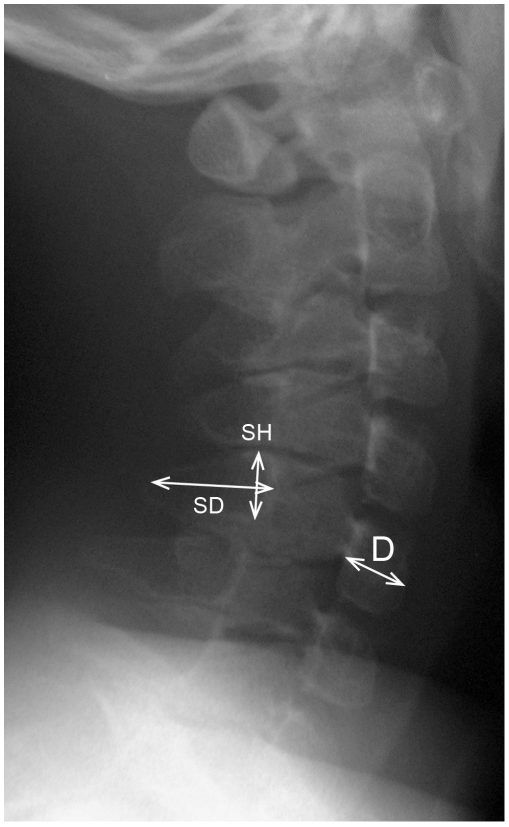

Figure 8. A lateral radiograph of the cervical spine of Patient 16 at the age of eight years showing enlarged spinous process of the C5 vertebra. The (SH+SD)/D ratio and the corresponding SD value is 2.8 and 7.9, respectively.

HoxD13 gene in the presumptive digit I area is of great significance (*13*). Mutations in the homeodomain of the *HoxD13* gene cause brachydactyly type D that is characterized by variable shortening of the distal phalanx of the thumb. This mutated HoxD13 proteins responsible for its decreased affinity for the double-stranded DNA target containing a cognitive sequence of the homeodomain (*14*). Interestingly, previous research has revealed that BMP signaling-dependent Smad1/4 proteins prevented HoxD10 and HoxD13 from binding to DNA targets (*15*). Constitutively-activated BMP signaling in FOP thus is likely to impair HoxD13-mediated transcriptional regulation by direct interactions between BMP-induced Smads and HoxD13. Mesenchymal condensation and chondrocyte proliferation of the presumptive digit I area could be suppressed by down-regulated HoxD13 function, whereas in presumptive digits II to V areas, it could be preserved by compensating expressions of other *HoxD* genes (*HoxD11* and *HoxD12*). Dysregulated BMP signal transduction during embryogenesis seems to cause relative shortening of the first metacarpals and distal phalanges of the thumb in FOP.

More than 90% of adult FOP patients showed fusion of the facet joints, which is a type of orthotopic ossification (*6*). To our knowledge, however, there are no reports delineating the precise prevalence of tall and narrow vertebral bodies and enlarged posterior elements of the cervical vertebrae. Here we demonstrated that the H/D and (SH + SD)/D ratios in the C5 vertebrae were larger than +2SD of normal values in 64% and 73% of patients, respectively (Figure 8). In addition to neck stiffness, which seemed to be an important early clinical sign before the appearance of HO (*6*), tall and narrow vertebrae and hypertrophic spinous processes of the cervical spine are radiographic characteristics in young FOP patients.

In a previous *in vivo* study, genetically-engineered overexpression of BMP-2/4 both dorsally and laterally to the neural tube manifested combined phenotypes of hypertrophic spinous processes and large deletion of the lateral and ventral parts of vertebral bodies (*16*). Thus, mesenchymal condensations at the paraxial mesoderm in FOP, where BMP-2 signaling is aberrantly activating, could be responsible for both enlarged spinous processes and relatively tall vertebral bodies.

The common *ACVR1/ALK2* mutation (c.617G > A, p.R206H) shows a homogeneous phenotype including congenital malformation of the great toes and the skeletal features in the thumb and cervical spine (*17*). In contrast, several atypical mutations in the *ALK2/ACVR1* gene, such as L196P, R258S, R375P, G328R, and P197_F198 del insL, have been identified in patients who showed normal-appearing great toes (*18*). In this study, one patient (Patient 14) with an atypical mutation (c.774G > C, p.R258S) showed normal-appearing great toes. She also lacked the shortened thumb but exhibited exceptionally tall and narrow vertebral bodies. Another patient (Patient 4) who showed neither malformed great

toes nor shortening of the first metacarpal bone also manifested distinctive features of the cervical spine in spite of the common *ACVR1/ALK2* mutation. We believe that radiographic characteristics of the cervical spine are potent diagnostic clues for FOP especially in cases without typical deformities of the great toes.

Acknowledgements

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Case Report

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Seizures caused by pyridoxine (vitamin B6) deficiency in adults: A case report and literature review

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Summary **Pyridoxine (vitamin B6) deficiency is a recognised cause of intractable seizures in neonates. However, pyridoxine deficiency related seizures in adults were rarely reported. This article reports a case of a 79 year old lady who suffered from new-onset seizures and was successfully treated with vitamin B6. The patient had chronic renal disease and weight loss due to anepithymia following a pelvic fracture. This article also reviews literatures of seizures caused by pyridoxine deficiency in adults. Seizures caused by vitamin B6 deficiency in adults may result from dietary deficiency, liver disease, pregnancy and certain medications and can be easily treated by vitamin B6 with excellent outcome. Clinicians should consider vitamin B6 deficiency as a potential aetiology of seizures, even in patients who suffer from other underlying diseases which can cause seizures.**

Keywords: Adult, literature review, pyridoxine, seizure, vitamin B6

1. Introduction

Pyridoxine (vitamin B6) deficiency is a recognised cause of intractable seizures and most reported cases of this condition are in neonates (*1,2*). Very few adult cases of pyridoxine deficiency related seizures have been reported in the English literature (*3-7*). This article reports a case of an elderly lady who suffered from new-onset seizures and was successfully treated with vitamin B6, includes a literature review of seizures caused by pyridoxine deficiency in adults.

2. Case Report

A 79 year old lady, a family member of the author, suffered from Parkinson's disease, and stage 4 chronic renal failure and was not on dialysis. She experienced a sudden onset of seizure-like attacks with upper limb jerking, head tilting back and eyeballs rolling up. She had five episodes within 24 hours. In two of the five episodes, she was responsive initially and then briefly unresponsive for one to two minutes. She had never experienced such episodes previously. Five weeks prior to the onset, the patient had fall which resulted in a pelvic fracture and she was slowly recovering at home. She lost 5 kg (from 33 kg to 28 kg) due to anepithymia during the 5 weeks.

The patient was taken to Box Hill Hospital in Melbourne by ambulance after the 5th episode. Her renal function tests showed Urea 14.6 mmol/L, Creatinine 440 umol/L and eGFR 8 mL/min/1.73m^2 which were no worse than the results of five weeks earlier (28.0, 485, 8). A brain CT scan was unremarkable. She had another episode in the Emergency Department (ED) just after the CT scan when she was observed by an ED doctor to be initially responsive, with jerking of upper limbs, and then becoming tonic and unresponsive. Her systolic blood pressure and heart rate increased to 230 mmHg and 150/min respectively during the episode. It lasted three minutes and resolved spontaneously. The conclusion of the neurologist's consultation in ED was that her seizure-like episodes were secondary to chronic kidney disease, despite a decrease in her urea level compared to test results five weeks ago. The neurologist prescribed oral clonazepam 0.25 mg twice a day, increasing to 0.5 mg if necessary.

The patient was then admitted to the renal ward of the hospital and did not experience any further episodes on the first day of the admission. She had a mild episode in the early morning of the second day in the hospital and underwent an EEG in the afternoon which was unremarkable. She was seizure free on the third day and did not receive a second dose of clonazepam

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in the evening. The patient had an episode similar to the one in the ED in the afternoon on the 4th day and was given clonazepam 1mg intravenously. She was given clonazepam 0.25 mg at night for the next two days and had one episode each day. She was then given clonazepam 0.25 mg twice a day on 7th day and had one episode on that day. She had two episodes in the morning of the 8th day and the second clonazepam dose was increased from 0.25 mg to 0.5 mg after a neurology review.

The patient was discharged from hospital on day 9 of admission and doctors advised the family to adjust the clonazepam dose at home. On the day of discharge, the patient had two episodes of seizure-like attacks in the hospital and two more episodes at home. She was given clonazepam 0.25 mg twice during that day. On the following day of discharge, she had five episodes within 12 hours and was given clonazepam 0.25 mg in the morning and 0.5 mg in the evening. The author reviewed the literature on seizures, and learned that vitamin B6 deficiency could be a potential cause of seizures. The patient was given vitamin B6 10 mg that night, and then four times the following day. Vitamin B6 10 mg three times a day was given thereafter. The clonazepam dose was gradually reduced and stopped 14 days post discharge. The patient has not experienced any seizure activity since vitamin B6 was introduced and has remained seizure free (Table 1).

3. Vitamin B6 and vitamin B6 deficiency

The term pyridoxine connotes the six vitamers of vitamin B6: the alcohol pyridoxine, the aldehyde pyridoxal, the amine pyridoxamine, and their respective 5'-phosphorylated esters (pyridoxine phosphate, pyridoxal phosphate and pyridoxamine phosphate) (*8*). Both pyridoxine phosphate and pyridoxamine phosphate are converted into the active cofactor pyridoxal phosphate (PLP) by pyridox(am)ine 5'-phosphate oxidase. PLP plays numerous roles in over 140 metabolic reactions making up at least 4% of all classified enzyme activities including transamination of amino acids, decarboxylation reactions, modulation of activity of asteroid hormones and regulation of gene expression (*9,10*). From a neurological perspective, pyridoxine homeostasis is important in dopaminergic, serotonergic, glutaminergic and gabaergic neurotransmission (*8*).

Vitamin B6 is widely available in animal and plant derived food, including meat, nuts and whole grain products. As such, clinical vitamin B6 deficiency states are rare (*11,12*). Nevertheless, vitamin B6 deficiency may occur in patients with inadequate dietary intake. The elderly, alcoholics, renal patients undergoing dialysis, patients with liver disease, rheumatoid arthritis, women with type 1 diabetes, and those infected with HIV have an increased risk of vitamin B6 deficiency, despite adequate dietary intakes (*13-15*). The availability of vitamin B6 in the body can be affected by certain medications such as anticonvulsants and corticosteroids (*16*). Isoniazid, a medication used in the treatment of tuberculosis, and cycloserine, penicillamine, and hydrocortisone may interfere with vitamin B6 metabolism. These medications may form a complex with vitamin B6 that is inhibitory for pyridoxal kinase, or they may positively displace PLP from binding sites (*17*). The classic clinical syndrome for vitamin B6 deficiency is a seborrhoeic dermatitis-like eruption, atrophic glossitis with ulceration, angular cheilitis, conjunctivitis, intertrigo, and neurologic symptoms of somnolence, confusion, and neuropathy.

4. Vitamin B6 deficiency and seizures

As mentioned, the active form of vitamin B6, *i.e.*, PLP – a coenzyme that is necessary for the synthesis and metabolism of amino acids, is an important cofactor for many processes including neurotransmitter formation. Specially, PLP is an essential factor in the process of

Table 1. 30 days of seizure activities and treatment in a 79 years old lady

Day	Home/Hospital	No of seizure episode(s)	Oral clonazepam (time)		Oral vitamin B6 (time)
			Morning	Evening	
Day 1	Home	4	-	-	-
Day 2	Hospital admission	2	0.25 mg	0.25 mg	-
Day 3	Hospital	1	0.25 mg	0.25 mg	-
Day 4	Hospital	0	0.25 mg	-	-
Day 5	Hospital	1	1.00 mg intravenously (5 pm)		-
Day 6	Hospital	1	0.25 mg	0.25 mg	-
Day 7	Hospital	1	0.25 mg	0.25 mg	-
Day 8	Hospital	1	0.25 mg	0.25 mg	-
Day 9	Hospital	2	0.25 mg	0.50 mg	-
Day 10	Discharged to home	4	0.25 mg	0.25 mg	-
Day 11	Home	5	0.25 mg	0.50 mg	10 mg (10 pm)
Day 12	Home	0	-	0.125 mg	10 mg × 4
Day 13	Home	0	0.125 mg	0.125 mg	10 mg × 3
Days 14-22	Home	0	-	0.125 mg	10 mg × 3
Days 23-30	Home	0	-	-	10 mg × 3

decarboxylation of glutamine into Gamma-Animobutytic Acid (GABA), a major inhibitory neurotransmitter. A deficiency of PLP leads to decreased GABA concentration in the brain, thereby increasing the risk for seizures (*18*).

Seizures caused by vitamin B6 deficiency was first described in 1954 by Hunt *et al* (*1*). They reported a case of a newborn with pharmacoresistant seizures that eventually came under control after treatment with a multivitamin preparation and subsequently deduced that pyridoxine was the factor responsible for controlling the infant's epileptic seizures. The disorder has been known as pyridoxine-dependent seizures or pyridoxine-dependent epilepsy. Since then, more than 100 cases of neonatal pyridoxine-dependent seizures have been reported worldwide (*8,19-21*). More recently, mutations in the *ALDH7A1* gene which encodes the protein antiquitin, an aldehyde dehydrogenase that functions within the cerebral lysine catabolism pathway, were found to be responsible for the biochemical abnormalities underlying PDS (*8,22,23*).

5. Seizures caused by vitamin B6 deficiency in adults

Reported cases of vitamin B6 deficiency related seizures in adults were much less than that in neonates. Table 2 summarises cases of seizures caused by pyridoxine deficiency in adults reported in the English literature.

5.1. *Therapeutic doses of isoniazid*

Isonicotinic acid hydrazide (INH) is an effective and widely used medication in tuberculosis treatment. Severe acute neurotoxicity of INH overdose is characterised by recurrent seizures, profound metabolic acidosis, coma and even death (*24-30*). INH is thought to cause seizures by interfering with γ-aminobutyric acid synthesis. Specifically, INH inhibits glutamic acid decarboxylase by inhibiting pyridoxal 5 phosphate, a co-factor for glutamic acid decarboxylase enzyme. The consequent reduction in GABA levels increases susceptibility to seizures.

In addition to INH overdose, therapeutic doses of INH have been reported to induce seizures which were successfully treated with pyridoxine (*3,5*). It is important to be aware that possible isoniazid neurotoxicity may occur in patients with chronic renal failure or even in healthy individuals when recommended preventive doses of isoniazid are used.

5.2. *Pregnancy*

It is recognised that there is an increased demand for vitamin B6 during pregnancy which may lead to low vitamin B6 levels. Schulze-Bonhage and colleagues (*4*) reported a case of development of seizures and status epilepticus during pregnancy with low vitamin B6 levels. The patient had pyridoxine-dependent epileptic seizures during early childhood, but had been completely seizure free for 23 years with oral pyridoxine hydrochloride supplementation therapy (100 mg/day). The lack of response to antiepileptic medication and rapid improvement with parenteral administration of vitamin B6 makes a relation between low vitamin B6 levels and the development of seizures and status epilepticus highly probable. Although it is unknown whether decreased vitamin B6 levels that occur during pregnancy can precipitate seizures in otherwise healthy women with no history of epilepsy due to a disturbed metabolism of pyridoxine, pregnancy may be potential risk factor for seizures caused by vitamin B6 deficiency.

5.3. *Levodopa/carbidopa intestinal gel infusion (LCIG)*

Skodda and Müller (*7*) reported a cachectic case of seizures after the initiation of treatment with Levodopa/carbidopa intestinal gel infusion (LCIG). Vitamin B6 level in serum was markedly reduced (2.7 μg/L). The seizures ceased within 2 days of pyridoxine treatment and her serum pyridoxine level was normal (23.3 μg/L) after successful treatment. A relationship between vitamin B6 deficiency and LCIG therapy was suspected because of the chronological sequence of increasing LCIG dosage and the first manifestation of seizures although mechanisms by which how LCIG therapy might influence vitamin B6 levels are not clear. Vitamin B6 deficiency might be also aggravated by suboptimal dietary intakes in the case as her BMI was only 15.8.

5.4. *Dietary deficiency of vitamin B6*

The case of new onset of seizures described in this report was successfully treated with small doses of pyridoxine (40 mg for 24 h and then 30 mg daily). A hypothesis for this case is that the patient had insufficient dietary intake due to anepithymia following the fall and pelvic fracture which was evident by weight loss (from 33 kg to 28 kg within 5 weeks). Insufficient dietary intakes may cause vitamin B6 deficiency which subsequently induces seizures. Gerlach and colleagues (*6*) reported three cases of refractory seizures consequent to vitamin B6 deficiency which may be aggravated by alcoholism or liver disease (*31-35*). Low PLP concentration in those patients may occur over time owing to a dietary deficiency coupled with intact aldehyde oxidase activity.

6. Conclusion

Seizures caused by vitamin B6 deficiency in adults are rarely reported and may be underdiagnosed and underreported. This condition may result from dietary deficiency, liver disease, pregnancy and certain medications and can be easily treated by vitamin B6

Table 2. Reported cases of seizures related to pyridoxine deficiency in adults

Author/s (year)	Age (Gender)	Medical history prior to seizures	Failed seizure treatment	Pyridoxine Pre (Post) pyridoxine treatment	Pyridoxine (Vitamin B6) treatment and response
Asnis DS, *et al* (*3*) (1993)	66 (F)	• Oral administration of isoniazid 300 mg/day for 4 days following PPD test positive • Just began peritoneal dialysis training program	–	–	Oral pyridoxine 50 mg/day: Seizure ceased within 24 hours
Schulze-Bonhage A, *et al* (*4*) (2004)	30 (F)	• History of early childhood pyridoxine-dependent epileptic seizures • Seizure free for 23 years with oral pyridoxine hydrochloride 100 mg per day • Status epilepticus during week 14 of pregnancy	Days 1 and 2: parenteral phenytoin 750 mg/day Days 3 and 4: parenteral phenytoin 500 mg/day and phenobarbitone 450 mg/day→ continuous benzodiazepine infusion (bolus of 4 mg and subsequent infusion rate of 1 mg/h)	PLP(pyridoxal 5'-phosphate): 2.96 ng/mL* (19 ng/mL) *Normal range: 4.3-17.5 ng/mL)	Parenteral pyridoxine hydrochloride 100 mg/day for a week and oral pyridoxine 100 mg/day: Disappearance of epileptic discharges on the first EEG recording obtained 3 days after intravenous administration of pyridoxine and regained consciousness after antiepileptic drug was tapered off
Vasu and Saluja (*5*) (2006)	45 (F)	• Isonicotinic acid hydrazide 300 mg/day for 2 months after being found to be PPD positive	Intravenous diazepam 5 mg, lorazepam 4 mg and fosphenytoin 1200 mg	–	Intravenous pyridoxine 5 g: Seizure ceased
Gerlach, *et al* (*6*) (2011)	54 (M)	• Advanced alcoholic cirrhosis and encephalopathy • Hepatic transplantation • Intolerance of enteral nutrition	Intravenous phenytoin 1000 mg followed by intravenous phenytoin 100 mg every 8 hours and then intravenous phenyton 350mg every 24 hours	PLP: 2 mcg/L* (6 mcg/L) *Normal range: 5-50 mcg/L)	Intravenous pyridoxine 200 mg every 24 hours: Seizures ceased within 2 days Oral pyridoxine 100 mg daily: Seizure free
	59 (M)	• End-stage renal disease and intermittent hemodialysis • Hepatitis C • Gastroesophageal reflux disease • Evacuation of subdural hematoma	Intravenous fosphenyotoin 1000 mg followed by intravenous phenytoin 100 mg every 8 hours and enteral levetiracetam 250 mg every 12 hours then levetiracetam 500 mg every 12 hours via nasogastric tube Phenobarbital 600 mg x 2 then enteral phenobarbital 20 mg twice daily	PLP: 3 mcg/L* (5 mcg/L) *Normal range: 5-50 mcg/L)	Intravenous pyridoxine 100 mg twice daily: Seizures ceased following day Oral Pyridoxine 100 mg daily: Seizure free
	78 (M)	• Intraventricular hemorrhage • History of alcoholism	Intravenous phenytoin 1000 mg followed by intravenous phenytoin 100 mg every 8 hours	PLP: 4 mcg/L* (26 mcg/L) *Normal range: 5-50 mcg/L)	Intravenous pyridoxine 100mg every 12 hours: Seizures ceased within 24 hours Oral Pyridoxine 100 mg daily: Seizure free
Skodda and Müller (*7*) (2013)	74 (F)	• Parkinson's disease treated with Levodopa/carbidopa intestinal gel infusion for 3 month • Cachectic (BMI = 15.8)	Levetiracetam (500 mg/day) and lorazepam (2 mg/day) Intravenous lorazepam 0.5-1.0 mg Levetiracetam (maximum daily dose of 4000 mg) successively supplemented by lorazepam (maximum daily dose of 4 mg) and phenytoin (from 1500 mg/day to 400 mg/day)	Pyridoxine: 2.7 µg/l* (23.3 µg/L) *Normal range: 5-30 µg/L)	Pyridoxine 100 mg twice daily: Seizures ceased within 2 days
Tong (current)	79 (F)	• Stage 4 chronic renal disease • Weight loss (from 33 kg to 28 kg in 5 weeks) due to anepithymia following a fall and pelvic fracture	Oral clonazepam 0.25 mg - 0.75 mg/daily	–	Oral pyridoxine 50 mg for 24 hours and then 30 mg daily: seizures ceased within 24 hours and remained seizure free since

with excellent outcome. Clinicians should consider vitamin B6 deficiency as a possible aetiology in patients presenting with seizures, even in those who suffer from other underlying disease which can cause seizures.

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Case Report

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Histiocytic meningioma: A distinctive subtype of meningioma?

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Summary **Meningioma with extensive histiocytic changes is rare. We describe a case of histiocytic meningioma which occurred in a 55-year-old woman. The patient had a progressive headache and a decline in fine motor coordination and memory for the past four years. Magnetic resonance imaging demonstrated a well-demarcated, dura-based and contrast-enhancing mass lesion in the right superior frontoparietal region. Histopathologically, the tumor showed neoplastic meningothelial proliferation with extensive and multifocal histiocytic infiltration. The histiocytic component constituted approximately half of the entire tumor. Immunohistochemically, both meningothelial and histiocytic cells showed immunoreactivity for epithelial membrane antigen (EMA), while the histiocytic cells were also positive for CD4 and CD68. In addition, there were scattered S100-positive histiocytes throughout the tumor. Proliferative index highlighted by Ki67 immunostain was 1.6%. There were no high-grade changes such as frequent mitoses, necrosis, or brain parenchymal invasion in the specimen. With review of the literature, we propose that this type of meningioma should be considered as a separate subtype of meningioma. The biological basis and differential diagnosis are discussed.**

Keywords: Meningioma, Histiocyte, Brain tumor

1. Introduction

Meningioma is the most common primary intracranial tumor and shows a significant histological diversity. The Word Health Organization (WHO) Classification of Tumours of the Central Nervous System recognizes multiple variants. Meningioma with extensive histiocytic changes is rare, and the biologic basis of this type of tumor is unclear. We present a case of a histiocytic meningioma and discuss the possible biologic origins and its differential diagnosis.

2. Case report

2.1. *Clinical history*

The patient was a 55-year old female who presented with a progressive headache, blurring vision and progressive decline in fine motor function and memory for four years. The patient previously had a CT scan after a head trauma more than four years ago, and a "very small" and clinically suspicious meningioma was noted at that time. The current magnetic resonance imaging demonstrated a well-demarcated, dura-based and contrast-enhancing mass measuring 4 cm in greatest dimension in the right superior frontoparietal region (Figure 1). The tumor was surgically removed subsequently

2.2. *Macroscopy and microscopy*

The excised brain specimen was grossly examined after fixation in 10% buffered formalin. The tissue was then embedded in paraffin. Sections with 4 μm thickness were generated. The sections were stained with hematoxylin and eosin as well as utilizing a immunohistochemical technique, an avidin-biotin-complex immunoperoxidase method. The immunohistochemical study used antibodies against epithelial membrane antigen (EMA), CD4, CD68, S-100

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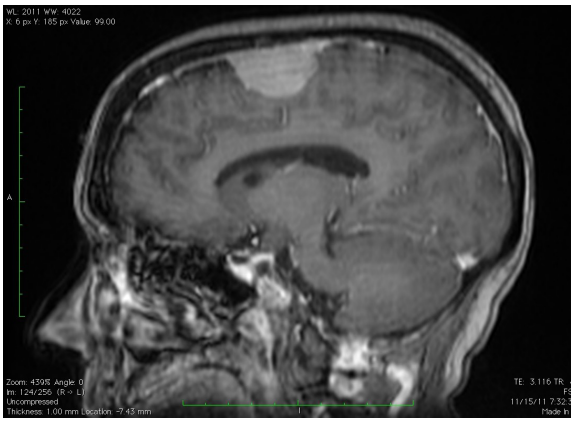

Figure 1. The T1-weighted MRI with contrast demonstrates a well-demarcated, dura-based and contrast-enhancing mass lesion of 4 cm in greatest dimension in the right superior frontoparietal region.

and Ki67. The tissue sections were blocked with 0.3% hydrogen peroxidase, washed in PBS for 30 minutes, and then mounted using 1% goat normal serum in PBS for 30 minutes. Subsequently, the primary specific antibody was added at a dilution of 1:100, and the sections were incubated at 4°C overnight. The stained sections were reviewed by two pathologists.

2.3. *Results*

Gross pathologic examination revealed multiple fragments of tan tissue. Under the microscope, the tumor showed atypical meningothelial proliferation with extensive and multifocal histiocytic infiltration (Figure 2A). The histiocytic component constituted approximately half of the entire tumor. There were no high-grade changes such as frequent mitoses, necrosis and brain parenchymal invasion in the specimen. Immunohistochemically, both meningothelial cells and histiocytic cells showed immunoreactivity to EMA (Figure 2B). The histiocytes also expressed both CD68 (Figure 2C) and CD4 (Figure 2D). In addition, there were few scattered S100-positive histiocytes throughout the tumor (Figure 2E). Proliferative index highlighted by Ki67 immunostain was 1.6%. Based on the morphologic and immunohistochemical features of this tumor, a diagnosis of histiocytic meningioma, WHO Grade I, was made.

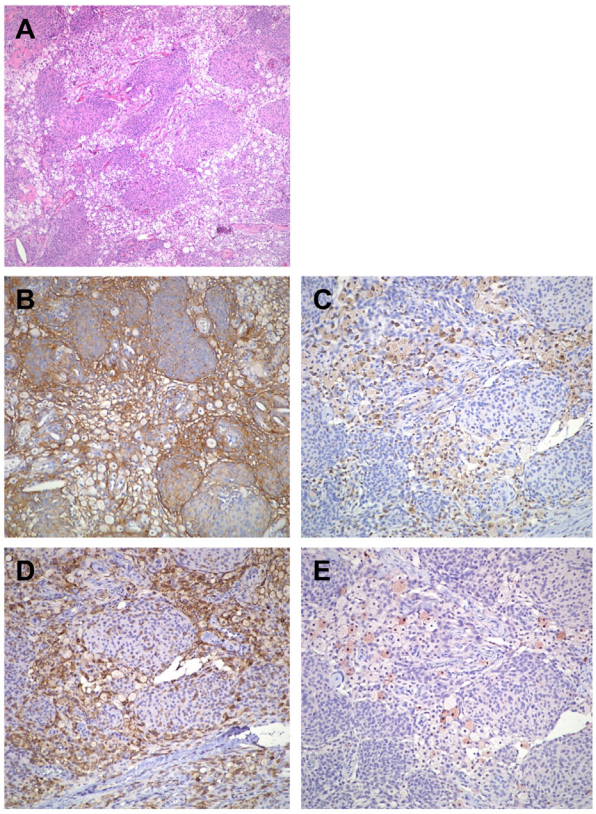

Figure 2. The tumor exhibits atypical meningothelial proliferation with extensive and multifocal histiocytic infiltration. The histiocytic component constitutes approximately half of the entire tumor **(A)**. Both meningothelial cells and histiocytic cells show immunoreactivity to EMA **(B)**. The histiocytic cells are positive for CD68 **(C)** and CD4 **(D)**. Scattered S100-positive histiocytes are seen throughout the tumor **(E)**. (**A**: hematoxylin and eosin stain, 100×; **B**: EMA stain, 200×; **C**: CD68 stain, 200×; **D**: CD4 stain, 200×; **E**: S100 stain, 200×).

3. Discussion

We present a case of a meningothelial meningioma that contained a significant amount of histiocytes. The biological origin of histiocytic cells in meningioma is not clear. There are two major hypotheses on the development of such tumors. The first is that the histiocytic cells represent a metaplastic change. Immunohistochemical and ultrastructural studies demonstrated that the histiocytes express epithelial membrane antigen and have ultrastructural features similar to the meningothelial cells (*1,2*). This theory considers that the histiocytic changes are secondary to the metabolic abnormality of neoplastic meningothelial cells, which results in cellular degeneration. In our case, all of the histiocytic cells express EMA, which signifies that they may share the same cell origin with meningothelium. An alternate hypothesis is that the histiocytic cells originate from monocytic lineage. A dura-based xanthogranuloma was reported in the literature by Husain *et al* (*3*). The histiocytes in that tumor expressed monocytic/histiocytic biomarkers CD68 and S100, but not meningothelial biomarker EMA, which indicates that the histiocytes derived from monocytic origin rather than meningothelium. Furthermore, Ikota *et al* (*4*) described a case they deemed a "xanthomatous meningioma" that exhibited a typical meningothelial meningioma with xanthomatous changes. In their case, some of the xanthomatous cells expressed EMA, while others were only positive for the histiocytic biomarkers, suggesting that both metaplasia and monocytic origination might play roles in the pathogenesis of that tumor. However, in this case, it is yet to be determined whether this histiocytic infiltration

is neoplastic or inflammatory. It is worthy to note that our patient had a history of head trauma four years prior to this presentation. It has been known that xanthomas can be secondary to trauma (*5*). Perhaps, traumatic events or other tissue insults facilitate the histiocytic infiltration in otherwise typical meningiomas.

Histiocytic meningioma should be distinguished from other primary intracranial tumors such as microcystic meningioma and hemangioblastoma as well as from other intracranial histiocytic disorders such as xanthoma and Rosai-Dorfman disease. Microcystic meningiomas are generally hypocellular tumors characterized by a lacey and vacuolated appearance due to both clear cytoplasm and extracellular fluid filled spaces (*6*). The cytoplasm of these tumor cells is evenly clear and not foamy as in histiocytic meningioma. Furthermore, the neoplastic cells of microcystic meningiomas are reactive for EMA but not CD68. In contrast to histiocytic meningiomas that have two distinct cell populations, meningothelial cells and histiocytes, hemangioblastomas are composed of vascular and stromal components. The stromal component is characteristic of large and vacuolated stromal cells that are morphologically indistinguishable from foamy histiocytes. However, the stromal cells express inhibin and brachyury that are not expressed in the histiocytes of histiocytic meningiomas. In addition, EMA is not expressed in hemangioblastomas (*7*). Dura-based xanthomas or xanthogranulomas have been described as either primary disease or secondary to Histiocytosis X (*8*). They are characterized by nodular encapsulated infiltrates of histiocytes with lipid-filled clear cytoplasm mixed with small lymphocytes. However, a typical neoplastic meningothelial component is not present. Intra-cranial Rosai-Dorfman disease (RDD) is rare and may occur in the presence or absence of lymph node involvement and present as a dural mass (*9*). RDD is characterized by a polymorphic infiltration of histiocytes, lymphocytes and plasma cells. Emperipolesis, the hallmark of this disease, is often present. The histiocytes in RDD only express histiocytic biomarkers, but not epithelial markers, indicating their non-meningothelial origin. Although rare, it is important to be aware of histiocytic meningioma when encountering a dura-based lesion with infiltrates of histiocytic cells, because different histiocytic or histiocyte-containing diseases have different pathogenesis and prognosis and they require different treatment.

The WHO classifies the meningiomas containing a non-epithelial component such as fat, bone, cartilage or myxoid tissue as metaplastic meningioma (*10*). Whether the histiocytic meningioma should be grouped into the metaplastic meningioma category or considered as an independent entity needs more cases for further investigation. Since not all histiocytes in this type of tumor have the same biological features as meningothelial cells, we believe that it may be reasonable to separately classify this group of tumors as histiocytic meningioma. Our case does not exhibit atypical or anaplastic histopathological features and is graded as WHO grade I.

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Commentary

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Iron deficiency, ischaemic strokes, and right-to-left shunts: From pulmonary arteriovenous malformations to patent foramen ovale?

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Summary **Has the recent identification of iron deficiency as a risk factor for ischaemic stroke in patients with pulmonary arteriovenous malformations (AVMs) unmasked a new paradigm for stroke/infarct pathogenesis? This commentary reviews evidence that spans associations between iron deficiency and ischaemic strokes, iron deficiency enhancement of platelet aggregation in response to serotonin/5HT, settings in which plasma 5HT is elevated, and clinical trial confirmation that 5HT receptor antagonists prevent ischaemic stroke. The critical leap which directs attention away from atherothrombotic events at the neurovascular wall is that ischaemic strokes due to pulmonary AVMs are attributable to compromised pulmonary capillary bed filtration of venous blood. Right-to-left shunting is continuous through pulmonary AVMs, but also occurs intermittently in approximately 30% of the general population with intracardiac shunts such as patent foramen ovale (PFO). The testable hypothesis presented is that paradoxical embolism of venous platelet-based aggregates may constitute part of the causal chain between iron deficiency and ischaemic stroke, not only in the rare disease state of pulmonary AVMs, but also in major subgroups of the general population.**

Keywords: Iron deficiency, 5HT (5 hydroxytryptamine, serotonin), right-to-left shunt, pulmonary capillary filter, paradoxical embolism, patent foramen ovale, platelet aggregation

1. Introduction

Strokes are the fourth ranked cause of death, and each year, leave millions of people severely disabled. The most common are ischaemic strokes which occur when a region of the brain is deprived of oxygenated blood supply, usually due to direct occlusion of arterial vessels. There is Class I, Level A evidence that ischaemic strokes can be prevented by anti-platelet agents (*1*).

The conventional list of ischaemic stroke risk factors is headed by smoking, hypertension, hypercholesterolaemia, obesity and diabetes, with a focus on atherosclerotic-based mechanisms. However, these risk factors do not explain the very high burden of ischaemic stroke in low income countries (*2*).

The rare disease state of pulmonary arteriovenous malformations (PAVMs) provides an alternative perspective. PAVMs are abnormal dilated vascular channels that provide a direct communication between pulmonary arteries and pulmonary veins, and hence a right-to-left shunt (Figure 1). In cross-sectional studies, approximately one in eight patients with PAVMs experience a clinical stroke (*3,4*) with imaging revealing a far higher ischaemic burden: in a study performed using 20th century scanners, 34/67 (51%) patients with median age 41 year had evidence of cortical or subcortical infarcts (*5*).

The general presumption was that the infarcts/strokes in PAVM patients developed as a result of paradoxical emboli of venous thromboemboli (VTE), and that stroke risk would increase with the severity of PAVMs. Surprisingly however, a prospective series of 219 patients published in 2008 demonstrated no clear link between ischaemic stroke and severity of

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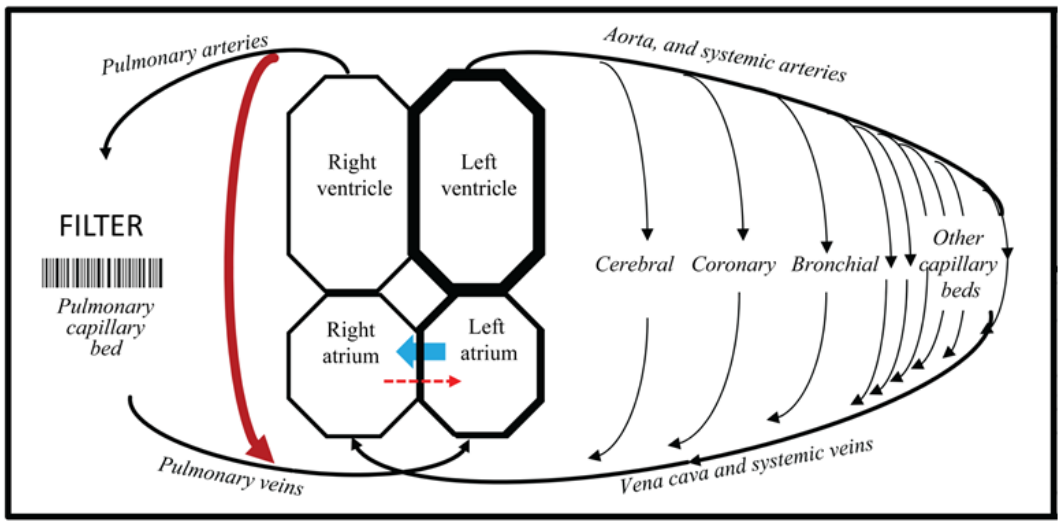

Figure 1. The pulmonary capillary filter and right-to-left shunts. Cartoon of the circulations, indicating the pulmonary capillary bed and the mechanical filter through which venous blood should pass before returning to the systemic arterial tree. Red arrows represent right-to-left shunts which allow venous blood to return to systemic arteries having bypassed filtration, gas exchange, and other pulmonary capillary functions. Right-to-left shunting is continuous through pulmonary AVMs, but only intermittent through intracardiac shunts such as patent foramen ovale, when flow is predominantly left-to-right (blue arrow, see text). Note that the lungs have a second arterial supply from the systemic circulation (bronchial vessels indicated) that can preserve arterial supply to lung tissue if the pulmonary circulation is compromised.

PAVMs, venous thromboemboli, or conventional stroke risk factors (*3*). Further exploration of factors that contributed to the risks of these strokes was recommended (*3*).

2. Iron deficiency and ischaemic strokes

Understanding which PAVM patients are at higher risk of ischaemic stroke is important for patients, because while treatment by embolisation reduces stroke risk (*3*), individual PAVMs are often too small for embolisation, and most treated patients are left with residual right-to-left shunts. Additionally, it seemed plausible that the stroke risks for patients with right-to-left shunts due to PAVMs may also be relevant to individuals with other right-to-left shunts such as patent foramen ovale (*3*) which persist in approximately 30% of adults in the general population (*6*) (Figure 1).

2.1. *Iron deficiency and ischaemic strokes due to PAVMs*

Earlier this year, a study in 497 consecutive adults with PAVMs identified low serum iron levels, and high levels of fibrinogen (the predominant plasma protein for platelet adhesion), as new risk factors for ischaemic strokes in the population (*4*).The age/gender adjusted odds ratio of 0.96 (95% CI: 0.92, 1.00), per µmol/L increase in serum iron, implied that a modest reduction in serum iron would be associated with approximately double the risk of stroke. In the PAVM patients, iron deficiency was attributed to inadequate replacement of haemorrhagic iron losses due to underlying hereditary haemorrhagic telangiectasia (HHT), particularly nosebleeds (*7*).

As in the previous study, there were no associations between ischaemic stroke and known vascular risk factors: almost half of all strokes (29/61, 47.5%) occurred in lifelong non-smokers without any documented conventional stroke risk factors (hypertension, hypercholesterolemia, diabetes, or arrhythmias) (*3,4*). There was also no association between stroke risk and markers or outcomes of conventional venous thromboemboli (often considered responsible for paradoxical embolic events), or with platelet number or haemoglobin that are commonly cited as possible mediators of iron deficiency risks (*4*). There was an overlap with myocardial infarction, attributed to a common paradoxical process (*4*).

2.2. *Iron deficiency and ischaemic stroke in the general population*

This was not the first report to link iron deficiency to ischaemic stroke. Case reports and small series have generally focused on paediatric populations (*8*, and references), but major epidemiological studies in adults also support a link between iron deficiency and ischaemic stroke, operating independently to known stroke risk factors (*9,10*, and references). Iron deficiency would offer a plausible missing risk factor for stroke pathogenesis, because it affects in excess of 1 billion individuals worldwide, and is a particular problem in countries with restricted diets, and where iron losses are increased, for example due to hookworm and/or urinary schistosomiasis (*7,11*).

3. Iron deficiency and platelets

Iron deficiency has multiple effects that could be implicated in stroke pathogenesis, including anaemia/ reduced blood oxygen content, high cardiac output with lower systemic vascular resistance, increased blood viscosity, and thrombocythaemia (*12,13*). Many were discussed in the respective stroke manuscripts (*8-10* and references), but do not really stand up to careful scrutiny as likely primary mechanisms for focal ischaemic strokes (*4*). Given the proven efficacy of anti-platelet agents in stroke prevention (*1*), it seems surprising that a study linking iron deficiency to exuberant platelet aggregation(*14*) has been overlooked until now (*4*).

Four decades ago, the Oxford-based authors demonstrated increased platelet aggregation to serotonin (5-hydroxytryptamine, 5HT) (*14*). As confirmed and recently reviewed (*4*), the platelet response is relatively specific, with no difference observed in platelet aggregation responses to adenosine diphosphate (ADP). In many countries, 5HT is considered a minor platelet agonist, but evidence particularly from Japan suggests otherwise: Plasma 5HT concentrations increase after platelet activation, and in a number of pathologies and disease states including atherosclerosis (*4,15,16*). Critically, antagonists to the receptor 5HT2A result in dose-dependent inhibition of platelet aggregation in ischemic stroke patients (*15*), and achieved therapeutic equivalence with aspirin in secondary prevention of ischaemic stroke (*16*). Conversely, cardiovascular side effects are recognised for drugs that increase extracellular 5HT/serotonin, such as serotonin reuptake inhibitors (SSRIs) (*17*).

Taken together, these data suggest that for individuals with concurrent iron deficiency and a state associated with elevated plasma 5HT, exuberant platelet aggregation might be predicted.

4. PAVMs and compromised pulmonary capillary filtration

The 5HT/platelet data could be relevant to platelet aggregation during atherothrombotic events at arterial walls. However, multiple studies implicate iron deficiency in ischaemic stroke pathogenesis in children too young to have accumulated a significant atherosclerotic load (*8* and references). Furthermore, as noted above, conventional atherosclerotic-based risk factors were not a feature of the PAVM patients with ischaemic strokes (*3,4*). A different paradigm seems to be needed.

4.1. *The pulmonary capillary filter*

After forming or entering the venous circulation, particulate matter and multicellular aggregates should lodge safely in pulmonary capillaries/arterioles. In man, morphometric, perfusion, and echocardiographic studies indicate that the cut off size for pulmonary capillary transit just exceeds the 7μm diameter of erythrocytes (*4*). The filter is exploited by conventional nuclear perfusion scans performed to diagnose pulmonary emboli: technetium-labelled albumin macroaggregates are injected intravenously, and impact in pulmonary capillaries receiving pulmonary arterial flow.

4.2. *PAVMs allow blood-bourne particles to bypass pulmonary capillary filtration*

If the pulmonary capillary filter were breached, for example if venous blood could pass through the right-to-left shunts of PAVMs, it would be expected that a proportion of venous particulate matter would impact not in the lungs, but in next (systemic) capillary bed. This is observed if perfusion scans are performed in patients with PAVMs, with striking cerebral images (*4*). The final clinical outcome following neurovascular impaction is more difficult to predict, and will depend on end organ thrombo-inflammatory and other vascular/ tissue responses -clearly very few impactions result in a clinical stroke.

5. Patent foramen ovale (PFO) and intracardiac shunts

Could intracardiac shunts that affect at least 1 in 3 of the general population, provide a rationale for the iron deficiency- ischaemic stroke associations in children and adults? Recent AHA guidelines detail management strategies for ischaemic strokes associated with PAVMs in the same section as patent foramen ovale (PFO), recommending anti-platelet agents for secondary prevention in both conditions (Class IIa, Level B Evidence) (*1*).

In contrast to PAVMs, only a small proportion of individuals with PFO suffer ischemic strokes, but stroke rates are higher in the subgroup of PFO patients with permanent right-to-left shunts (*18*).

The discrepant stroke rates make intuitive sense in the light of physiological comparisons of right-to-left shunting through pulmonary AVMs, compared to intracardiac defects such as PFOs. Pulmonary AVMs provide almost continuous right-to-left shunts because the pressure in the pulmonary artery generally exceeds that of the pulmonary vein: shunt quantifications are highly reproducible within the same patient (*4,12*). PFOs and other intracardiac septal defects normally exhibit left-to-right flow, due to the higher pressure at equivalent points in the systemic compared to pulmonary circulation (Figure 1). At the end of valsalva manouvres however, pressure changes result in reversal of flow across such septal defects, and a transient right-to-left shunt (*18*).

This is important because valsalva manouvres occurs surprisingly frequently during daily life, for example during nasal/sinus clearance and strained bowel evacuations (*18*). Times when PFO right-to-left shunts would be in operation also include sleep apnoea, now recognised to be associated with ischaemic stroke and other adverse cardiovascular events (*19,20*). Associated pressure changes are well recognised, but valsalva provocation of right-to-left shunting, allowing the particulate constituents of venous blood to bypass the mechanical filter provided by the pulmonary capillary bed, has not been emphasised to date.

6. Future studies

Examining whether paradoxical embolism of venous platelet-based aggregates is likely to be contributing to ischaemic stroke risks in the general population could be relatively easy to address, particularly given the lead through iron deficiency.

First, future epidemiological studies of associations between iron deficiency and ischaemic stroke could test the null hypothesis that the presence of a PFO, or any form of right-to-left shunt, does not modify the odds ratio for stroke attributable to iron deficiency. It may be possible to address this retrospectively using subgroups of published series in which contrast echocardiographic studies have been undertaken (*8-10*).

Prospective studies could also test whether exuberant platelet aggregation to 5HT is associated with enhanced risk of ischaemic stroke, and whether contribution of iron deficiency to the stroke model is reduced once adjusted for the platelet aggregation phenotype.

Most importantly, it would seem wise that for future randomised controlled trials examining the potential efficacy of prevention/treatment of iron deficiency in stroke prevention, additional assessments should be incorporated in order to allow appropriate risk stratifications of physiological groupings. Suggestions include contrast echocardiographic studies to evaluate right-to-left shunts, capturing a history of valsalva-precipitating clinical events in study populations, and concurrent assessments of platelet 5HT aggregation responses. For secondary prevention when it would be unethical to withhold anti-platelet therapy, further comparisons of the relative efficacy of 5HT receptor antagonists versus compounds such as aspirin or clopidogrel could be made, capturing whether any differences were more or less evident in subgroups stratified by iron deficiency or echocardiographic evidence of shunting.

7. Conclusion

For society and individuals, the ultimate burden of ischaemic strokes both directly, and through contributions of small ischaemic strokes to vascular dementia, is profound. The identification of iron deficiency as a risk factor for ischaemic strokes in the rare disease of PAVMs appears to introduce new paradigms for stroke pathogenesis. Given the current evidence base, and prevalence of both compromised pulmonary capillary filtration and iron deficiency, it seems appropriate to now test the platelet-serotonin-shunt hypothesis, and establish the extent to which the pulmonary capillary filter prevents ischaemic strokes and other infarct-mediated pathologies such as myocardial infarction.

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