Congenital Cytomegalovirus and Autoimmune Neutropenia: Cause or Coincidence?

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Abstract

Background:

Congenital cytomegalovirus (CCMV) accounts for high rates of infant morbidity and mortality. Neutropenia is a common finding in CCMV infection, of which the age of presentation overlaps with autoimmune neutropenia (AIN). AIN represents one of the most common forms of chronic neutropenia in childhood.

Methods:

A literature search exploring biological associations between CCMV and AIN was conducted: PubMed (MEDLINE), Ovid and Web of Science. We further describe 2 cases of concurrent CCMV and AIN. Both cases were confirmed with the indirect granulocyte immunofluorescence test and alternative etiologies for neutropenia excluded.

Results:

Our 2 patients represent confirmed cases of AIN in infants with CCMV. One patient demonstrated neutropenia while undergoing treatment with Valganciclovir, while the other was never treated. With interruption of Valganciclovir in infant A, neutrophil counts (ANC) did not improve and upon resumption of treatment ANC remained static.

Conclusions:

Further studies examining a possible biologic link between CCMV and AIN are advocated for. We encourage clinicians to actively consider AIN in the differential diagnosis of all infants with CCMV presenting with neutropenia.

Introduction

The worldwide prevalence of congenital cytomegalovirus (CCMV) has been estimated at 0.2%-2%, the most common of the congenital infections [1]. CCMV is associated with high rates of morbidity and mortality including congenital neurologic and ophthalmic sequelae, sensorineural hearing loss, and hepatic and haematologic dysfunction [2]. Although neutropaenia was not reported in historical cohorts of children with CCMV [3-5], neutropaenia as a result of the primary disease process has been recognised [6, 7]. Neutropaenia is also a well-recognised side effect of Ganciclovir/Valganciclovir and often complicates disease management in infancy [8, 9].

Autoimmune neutropaenia (AIN) is described as one of the most common causes of chronic neutropaenia of infancy and exhibits similarities in both clinical manifestations and timing of symptom onset to that of CCMV; AIN typically occurs after the neonatal period which overlaps with the time course of CCMV treatment [10, 11]. AIN is thought to be commonly caused by viral triggers, and differs from neonatal autoimmune neutropenia (NAN) in that it typically presents outside of the immediate neonatal period (median age of presentation, 8 months) with normal neutrophils at birth. Nevertheless, timing is dependent on exposure to the triggering agent and subsequent presentation to medical attention [12]. In contrast, NAN results from the transplacental transfer of antibodies directed against foetal neutrophils.

Given the overlapping natural histories of CCMV and AIN along with CMV's known immune modulating effects, a plausible biological link warrants further exploration. We describe two cases of concurrent AIN and CCMV presenting to a large tertiary care hospital infectious diseases service. Both patients were diagnosed with CCMV and presented with neutropaenia (absolute neutrophil count [ANC] $<1.5 \times 10^3/mm^3$) in the infant period, having had a normal ANC

documented near birth. To our knowledge, this is the first description of confirmed AIN in infants with CCMV infection.

Methods

A systematic search of the literature was conducted from: PubMed (MEDLINE), Ovid (EMBASE), and Web of Science (S1). Case findings were evaluated and information gathered though a search of online medical records containing laboratory results, radiographic findings, clinic notes, and email communications between primary care providers and specialist paediatric infectious disease services. Parental consent for publication was sought by the authors. In both patients AIN was diagnosed based on the presence of granulocyte specific IgM antibodies in the serum detected by indirect granulocyte immunofluorescence test (GIFT) [13].

Case 1

Infant A, of white Caucasian ethnicity, presented at birth with respiratory distress and a presumptive diagnosis of sepsis after a term delivery (40 weeks gestation) with meconium stained amniotic fluid. The infant was found to be positive for CMV in the blood (PCR: 5060 copies/ml) as well as urine (866,000 copies/ml) and CSF (non-quantitative). Retrospectively, maternal booking serologies were negative for CMV (IgM/IgG) denoting primary maternal seroconversion in pregnancy. Infant A was found to have a birth weight between the 25-50 percentiles, normocephalic, and had normal audiological and ophthalmologic assessments. Brain MRI showed inflammatory white matter changes. Thrombocytopaenia (platelets $61x10^3/mm^3$) and abnormal liver function tests (LFTs) were noted at birth. ANC was $8.75x10^3/mm^3$ (WCC:$12.5x10^3/mm^3$) at birth and fell to $0.8x10^3/mm^3$ approximately one months after the start of Valganciclovir (16mg/kg/dose BD). Valganciclovir was subsequently held due to persistent neutropaenia, falling to a nadir of $0.23x10^3/mm^3$. Despite withholding Valganciclovir for 6 weeks, the ANC continued to be well below the normal limit for age. Alternative aetiologies

for persistent neutropaenia were investigated inclusive of autoimmune and connective tissue autoantibodies, HIV, syphilis, vitamin B12 and folate. A bone marrow examination was not performed. Secondary to a rising CMV viral load, Valganciclovir was restarted despite persistently low ANC; a total six-month course was completed. A GIFT test done at 3.5 months of age demonstrated anti-neutrophil antibodies to HNA-1b. The patient remained in stable condition without any clinically significant infections despite ongoing neutropaenia. ANC recovery began by 6 months of age and continues to be followed clinically with no recrudescence of neutropaenia up to 15 months of age. *Figure 1*

Case 2

Infant B, born at term (39 weeks gestation) and of South Asian ethnicity, presented at birth with intrauterine growth restriction (birth weight 2-9%) and was found to be CMV positive in both blood (<1500 copies/ml) and urine (340,000 copies/ml) at three days of age. The infant's father was diagnosed with CMV colitis at 28 weeks gestation at which point the mother's serology was negative for both IgM and IgG. There was subsequent evidence of antenatal seroconversion. A foetal MRI at 32 weeks gestation and at 10 days of age was normal. A newborn head ultrasound was also normal. Infant B was found to be normocephalic at birth with normal audiological and ophthalmic assessments. Baseline platelets and LFTs at birth were within normal limits. Infant B did not meet national or European criteria for treatment with Valganciclovir. ANC at birth was $3.2x10^3/mm^3$ (WCC: $6.9x10^3/mm^3$). Neutropaenia was noted at approximately one month of age with a nadir ANC measuring $0.4x10^3/mm^3$. Similar to Infant A, alternative aetiologies for persistent neutropaenia were investigated and found to be normal/negative. Again, a bone marrow examination was not performed. A GIFT test done at 2.5 months of age demonstrated non-typable anti-neutrophil antibodies. The patient remained clinically well without any significant infections although this patient was treated with prophylactic Azithromycin. Neutrophil count recovery began by 4 months although remained

marginally low in the first year of life. Patient B continues to be followed clinically without recrudescence of neutropaenia up to 13 months of age. *Figure 2*

Discussion

CMV, a virus of the *Herpesviridae* family, exhibits several innate viral characteristics lending it to potential induction of autoimmune disease. Its ability to manipulate both adaptive and innate immune pathways and its pervasive local and systemic replication throughout the human lifespan, along with its large coding capacity has implicated the virus in the pathogenicity of autoimmune diseases [14]. Furthermore, a recombinant CMV vaccine candidate has been shown to induce autoantibodies to the anti-ribonucleoprotein response system implicated in Systemic Lupus Erythmatosis (SLE) and mixed connective tissue disease, whilst also inducing low levels of rheumatoid factor and anti-double stranded DNA antibodies [15]. Nevertheless, the same viral features: ubiquitous human prevalence, lifelong viral persistence, and ability to exist in latent and active replication phases, make for difficulty in determining a causal relationship between CMV and autoimmune disease.

Our two cases support CMV's propensity to induce autoimmune phenomena. CMV infection has previously been linked to several autoimmune diseases in paediatric populations inclusive of: idiopathic thrombocytopaenic purpura (ITP) [16], systemic lupus erythromatosis (SLE) and SLE disease activity [17], Guillan-Barré syndrome [18], autoimmune thyroid disease [19], type 1 diabetes [20], and autoimmune haemolytic anaemia [21, 22]. Nevertheless, data in paediatric populations is sparse and causation is lacking. The pathogenesis of severe, yet benign, primary neutropaenia has also been associated with other viral agents, particularly hepatitis C [23] and viruses of the *Herpesviridae* family (EBV, CMV, HHV6, HSV, varicella) [24], whereas parvovirus

[25] and HIV infection [26] have been specifically implicated in the development of autoimmune antibodies to neutrophils.

Infant A exhibited antibodies to the HNA-1b neutrophil antibody whereas Infant B showed non-specific anti-neutrophil antibodies. Both *Bux et al.* and Bruin *et al.* determined that autoantibodies to HNA-1a and HNA-1b are the most common anti-neutrophil autoantibodies found in AIN [10, 12] whereas neutrophil antibodies directed against adhesion glycoproteins HNA-4a/b, CD 35 and FcyIIb are much less frequently isolated [27, 28]. Although the origin of the aforementioned neutrophil autoantibodies is largely unknown, molecular mimicry as a result of exposure to viral or other microbial antigens is hypothesised. These autoantibodies not only result in a decrease in neutrophil number but may also inhibit neutrophil function. Nonetheless, AIN typically runs a benign, self-limiting course [29].

Increased recognition of CCMV and its treatment with Ganciclovir/Valganciclovir in the neonatal period may coincide with the onset of AIN. Whether early CMV exposure plays a role in the development of neutrophil autoantibodies or whether the natural history of the two conditions occur in parallel remains to be determined. Recognition of AIN in the context of symptomatic CCMV is of particular importance as treatment with Ganciclovir/Valganciclovir are themselves associated with neutropaenia. In the sentinel Kimberlin *et al*. study grade 3 and 4 neutropaenia occurred in 19% and 21% of those treated with 6 weeks and a further 4.5 months of Valganciclovir respectively, whilst 27% whom only received 6 weeks of treatment remained neutropaenic after discontinuing treatment at 6 weeks. Three patients required discontinuation of Valganciclovir temporarily secondary to clinically significant neutropaenia (ANC <0.5 $x10^3/mm^3$). Rates of neutropaenia in the previous CASG study utilising Ganciclovir were significantly higher (63% and 38% at 6 weeks and 6 months respectively) [30]. Thus, differentiating between drug-induced neutropaenia, neutropaenia associated with CCMV

disease, and alternative aetiologies (inclusive of primary AIN) is of utmost importance. Notably, this is critical in avoiding unnecessary and/or lengthy interruption of CCMV treatment which aims to prevent further hearing deterioration or a worsening of systemic, and/or primary neurologic manifestations.

Our patients establish the detection of persistent neutropaenia with self-antibodies directed at neutrophils in both an asymptomatic, and thus untreated individual, and in a symptomatic infant treated with Valganciclovir, both of whom had normal ANC documented at birth. Both infants had persistence of neutropaenia for more than 6 months without any severe infections. Prophylactic antibiotics where administered in one patient. Neutropaenia did not recover when Valganciclovir was withheld in Patient A and neutropaenia continued after 6 months when Valganciclovir treatment was completed. Granulocyte Colony Stimulating Factor was not given in either cases. Alterative aetiologies for neutropaenia: infectious, rheumatologic, metabolic, malignant, and nutritional, were explored in both patients and found to be non-contributory.

Conclusions

Prompt recognition of AIN in infants with CCMV infection is advised in order to avoid delay or interruption of treatment. Although drug induced neutropaenia with Ganciclovir/Valganciclovir is common, alternative aetiologies for neutropaenia require investigation, particularly when neutrophil counts fail to improve with treatment interruption or in those not currently undergoing treatment [31]. Further studies investigating a causal link between CMV infection in the perinatal period and its propensity to induce autoimmune disease are required.

Conflict of Interest

The authors declare no conflicts of interest.

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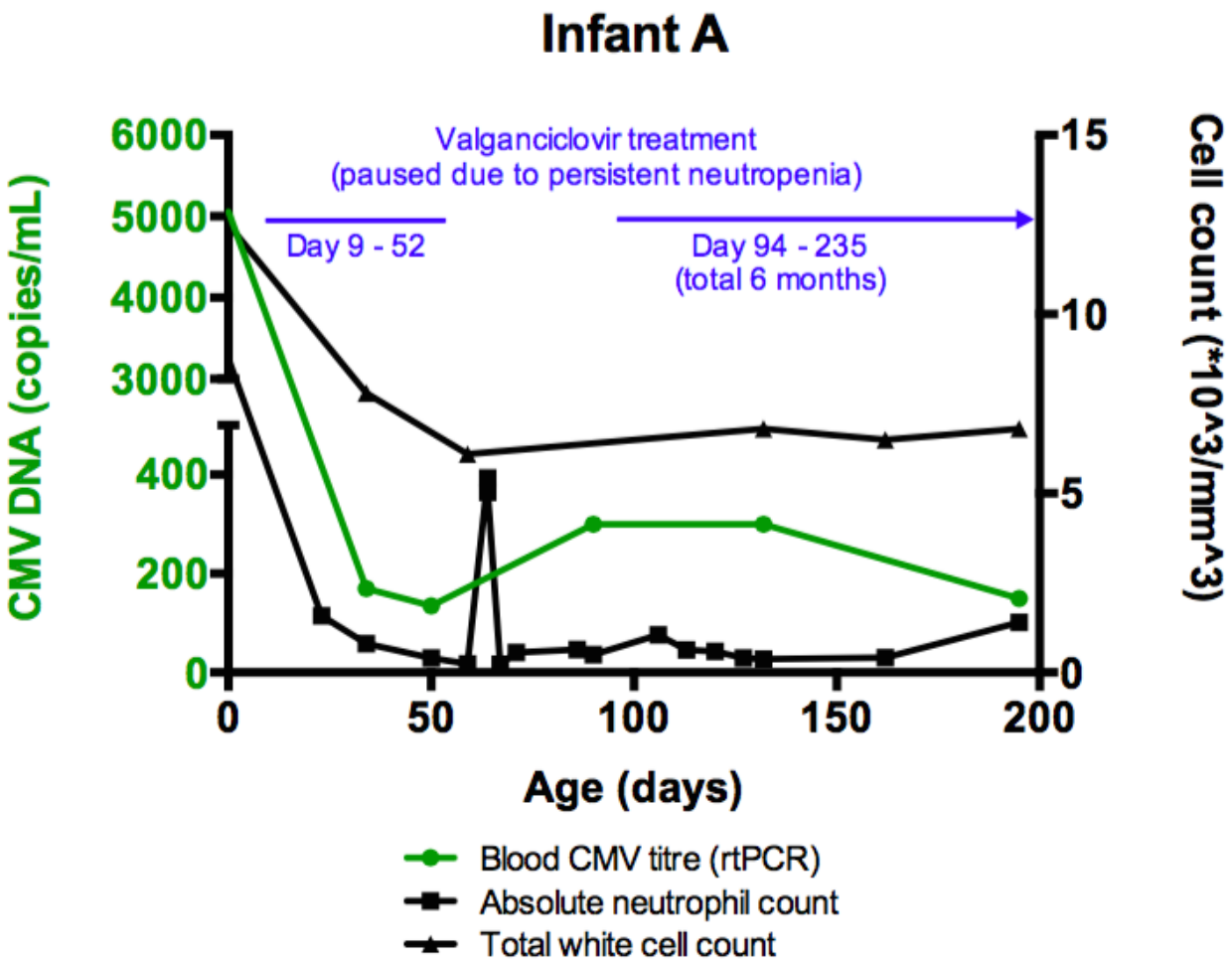

Figure 1: Neutrophil counts and CMV viral load over time for Patient A

Note: Spike in neutrophil count between day 50-100 secondary to febrile vaccine response

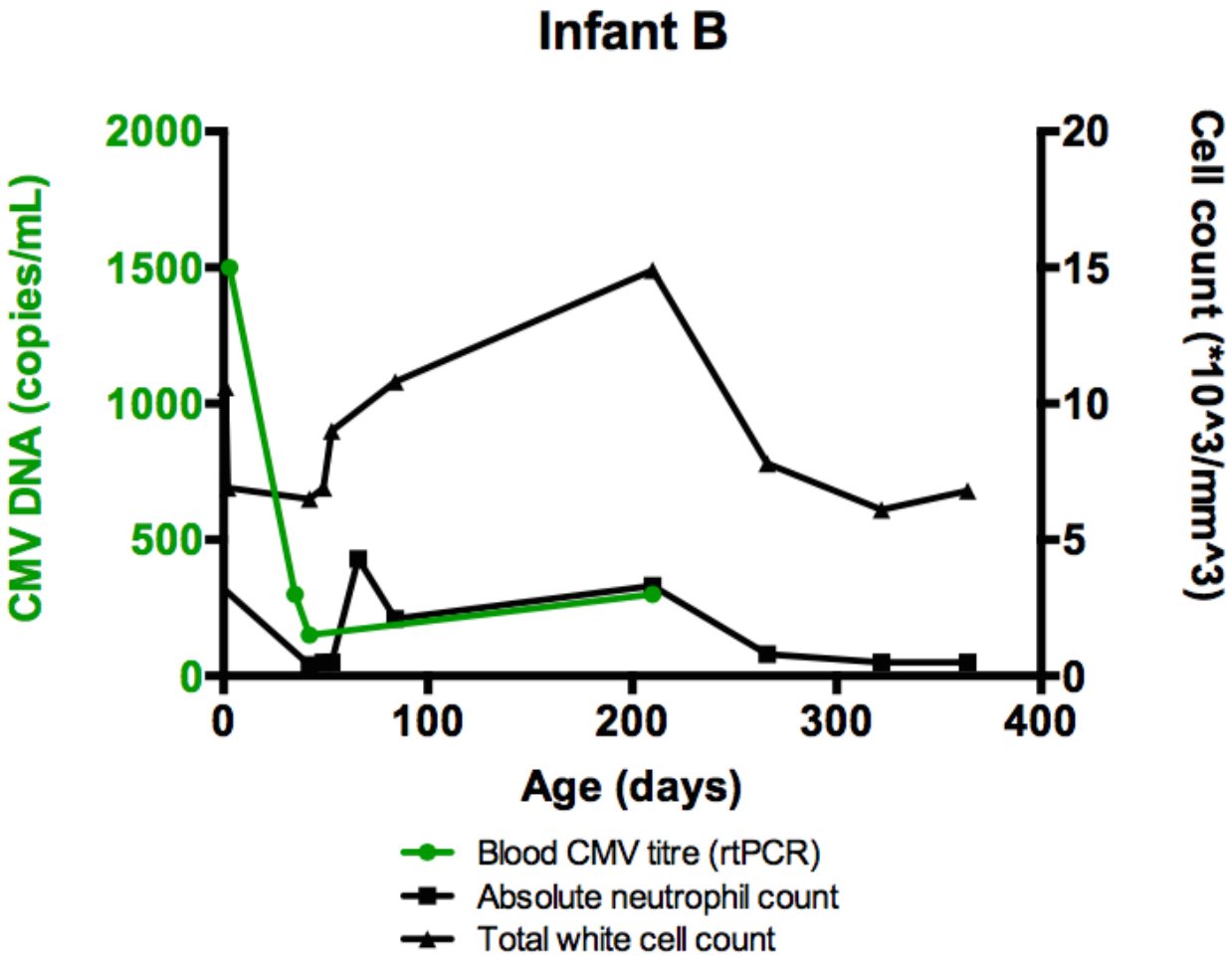

Figure 2: Neutrophil counts and CMV viral load over time for Patient B