HK J Paediatr (new series) 2010;**15**:289-298

Case Reports

An Infant with Severe Congenital Neutropenia Presenting with Persistent Omphalitis: Case Report and Literature Review

PPW ***Lee***, TL ***Lee***, MHK ***Ho***, PCY ***Chong***, CC ***So***, YL ***Lau***

Abstract Severe congenital neutropenia (SCN) is a rare, heterogeneous group of inherited disorders of neutrophil precursors presenting with pyogenic infections and severe neutropenia in infancy. We hereby describe an infant with persistent omphalitis and severe neutropenia. The diagnosis of SCN was confirmed by typical bone marrow findings. However, the response to usual dose of granulocyte colony-stimulating factor (G-CSF) was suboptimal. The requirement of high dose G-CSF suggests a higher risk of malignant transformation into myelodysplastic syndrome or acute myeloid leukaemia, and close monitoring for clonal changes and progression to frank leukaemia is needed. In this article we discussed the clinical approach to omphalitis, a condition commonly encountered by paediatricians, as well as differential diagnosis of neutropenia in neonates and infants. We also summarise the molecular etiologies and pathogenesis underlying SCN, which has only been unraveled in the past few years. While majority of patients with SCN respond to G-CSF which significantly reduces the risk of infections and mortality, dosage of G-CSF should be carefully titrated. Patients requiring high-dose G-CSF should be closely monitored for developing malignant transformation, which is uniformly associated with poor prognosis despite chemotherapy and hematopoietic stem cell transplantation.

Key words Severe congenital neutropenia

Case Report

A male neonate was admitted for umbilical swelling and discharge on day 16 of life. He was the second child of the family, and was born full term by elective Caesarean section with a birth weight of 3.22 kg. Antenatal ultrasound showed moderate hydronephrosis of the right kidney and mild hydronephrosis on the left, otherwise his antenatal and perinatal course was uneventful. Parents were not consanguineous. There was no family history of blood disease or autoimmune disease. He had been started on trimethoprim prophylaxis.

On admission his general condition was satisfactory and was afebrile. Examination showed a swollen umbilicus with erythema and yellowish discharge. Examination of other organ systems was unremarkable. Blood investigations were as follows: white cell count 9.6 x 10^9/L, absolute neutrophil count (ANC) 0.1 x 10^9/L, absolute lymphocyte count (ALC) 4.7 x 10^9/L, monocyte 4.4 x 10^9/L (normal range 0.2-1.2 x 10^9/L), haemoglobin 14.3 g/dL and platelet 570 x 10^9/L. The patient did not have any dysmorphic features, abnormal skin pigmentation, skeletal dysplasia or hepatosplenomegaly. Intravenous cloxacillin and gentamicin were commenced after sepsis workup, and the

Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong, China

PPW **Lee** (李珮華) *MBBS, FHKCPaed, FHKAM*
TL **Lee** (李子良) *MBBS, FHKCPaed, FHKAM*
MHK **Ho** (何學工) *MBBS, FHKCPaed, FHKAM*
PCY **Chong** (莊俊賢) *MBBS, FHKCPaed, FHKAM*
YL **Lau** (劉宇隆) *MD(Hon), FRCPCH, HKCPaed*

Department of Pathology, Queen Mary Hospital, The University of Hong Kong, Pokfulam, Hong Kong, China

CC **So** (蘇志釗) *MBBS, FRCPath(UK), FHKCPath*

Correspondence to: Prof. YL **Lau**

Received February 20, 2010

umbilical swab yielded heavy growth of methicillin sensitive *Staphylococcus aureus* and Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*. Gentamicin was switched to amikacin according to sensitivity results. The combination of cloxacillin and amikacin was given as a 10-day course resulting in clinical improvement, and the patient was discharged on day 32 of life.

However, his condition deteriorated after antibiotics were stopped, and he was re-admitted on day 35 of life because of recurrent umbilical redness and discharge. He also had reduced oral intake and was noted to have abdominal distension. He had low grade fever upon admission. There was erythema, induration and tenderness around the periumbilical region. Intravenous cloxacillin and amikacin were commenced. However, there was persistent umbilical discharge and progressive increase in abdominal distension. In addition, a firm, tender swelling beneath the umbilicus was noted. Abdominal X-ray showed dilated bowel loops but there was no abnormal gas density. Ultrasound of the abdomen showed mild cutaneous thickening at the periumbilical region, and there was no abscess formation. Umbilical swab taken prior to previous hospital discharge yielded *E. coli* of ESBL strain with intermediate sensitivity to amikacin, but fully sensitive to imipenem. In addition, scanty growth of Enterococcus species was identified which was sensitive to ampicillin. The antibiotic regimen was therefore changed to intravenous ampicillin, high dose cloxacillin and imipenem. Umbilical swab repeated 6 days after the second admission became sterile, but there was still persistent discharge and peri-umbilical erythema (Figure 1). Anti-microbial therapy was changed to intravenous meropenem for an addition of 14 days, and signs of inflammation gradually resolved.

Serial monitoring of complete blood count (Figure 2) revealed persistent neutropenia and monocytosis. Haemoglobin, total white count and platelet count remained normal. Blood smear showed profound neutropenia and the few neutrophils seen had normal morphology. Anti-neutrophil antibodies were negative. Bone marrow examination showed highly cellular marrow particles (Figure 3). A fair number of myeloid cells were seen and granulopoiesis was prominently left-shifted with predominance of neutrophil precursors. Neutrophilic maturation beyond myelocyte stage was absent. Blasts were not increased and dysgranulopoietic features were not present. Monocytosis was evident. Erythropoiesis was normoblastic and megakaryocytes were mildly increased and normal in morphology. There was no abnormal infiltrate. The overall clinical and haematological findings were consistent with severe congenital neutropenia.

Granulocyte colony stimulating factor (G-CSF) at 25 μg daily (6 μg/kg/day) was started on day 46, and was gradually stepped up to 65 mg daily (15.4 μg/kg/day) because of suboptimal response (Figure 2). There was a surge of ANC to 3.21 x 10^9/L, and G-CSF was spaced out to alternate day dosing, but ANC soon dropped to 0.23 x 10^9/L and the patient developed fever 1 week later. G-CSF was resumed at 65 μg daily and he was treated with 1-week course of antibiotics. There was transient improvement of ANC to 2.94 x 10^9/L, but later remained persistently below 0.5 x 10^9/L despite increasing the dose to 75 μg daily since day 79 (13.6 μg/kg/day). Furthermore, he suffered from an episode of retroauricular abscess caused by methicillin-sensitive *Staphylococcus aureus* infection, and G-CSF was further increased to 20 μg/kg/day because of persistent severe neutropenia. According to literature reports, the mean dose of G-CSF to maintain ANC >0.5 x 10^9/L was 11.9 μg/kg/day (range 1-120 μg/kg/day). While we continue to step up the dose of G-CSF hoping to achieve a sustained ANC rise, the overall impression was a suboptimal response to G-CSF. In view of the high risk of myelodysplastic syndrome and leukaemia, the option of haematopoietic stem cell transplantation was discussed with parents. Human leucocyte antigen (HLA) typing was performed, but the patient was not HLA matched with his parents and elder brother. Bone marrow examination at 4 months of age did not show features of myelodysplasia, and cytogenetics examination did not show abnormal clonal changes. Search for match-unrelated donor identified potential cord blood and bone marrow donors, and confirmatory typing is in progress.

Mutation Analysis

Genomic DNA was isolated from peripheral blood and PCR direct sequencing, including all the coding sequence and flanking splice sites of the 5 exons of neutrophil elastase (*ELA2*) gene and 7 exons of the *HAX1* gene was performed. Homology analysis with *ELA2* and *HAX1* reference sequence was performed using the *NCBI* program BLAST (http://www.ncbi.nlm.gov/BLAST/). A heterozygous missense mutation p.C151Y caused by G>A at nucleotide position 452 of *ELA2* gene was identified. No mutation in the *HAX1* gene was found. Mutation of G-CSF receptor (*CSF3R*) gene, which is a risk factor for malignant transformation, was negative.

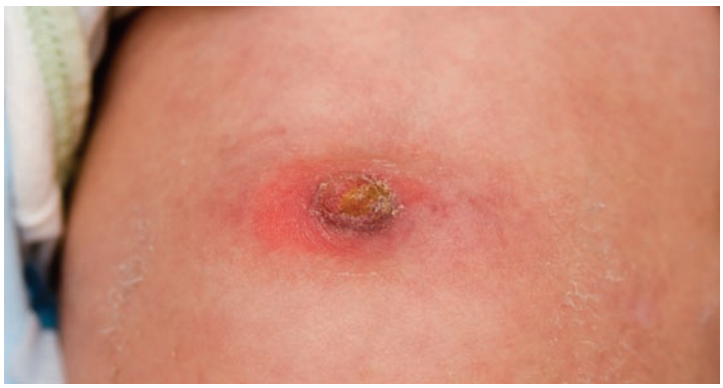

Figure 1 Residual peri-umbilical erythema in the patient with severe congenital neutropenia after repeated courses of antibiotics.

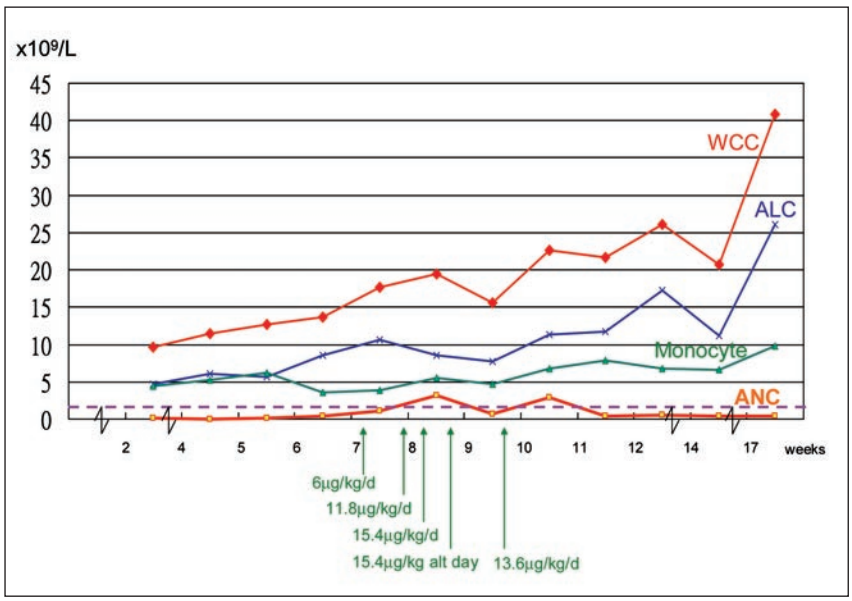

Figure 2 Blood counts before and after commencement of G-CSF.

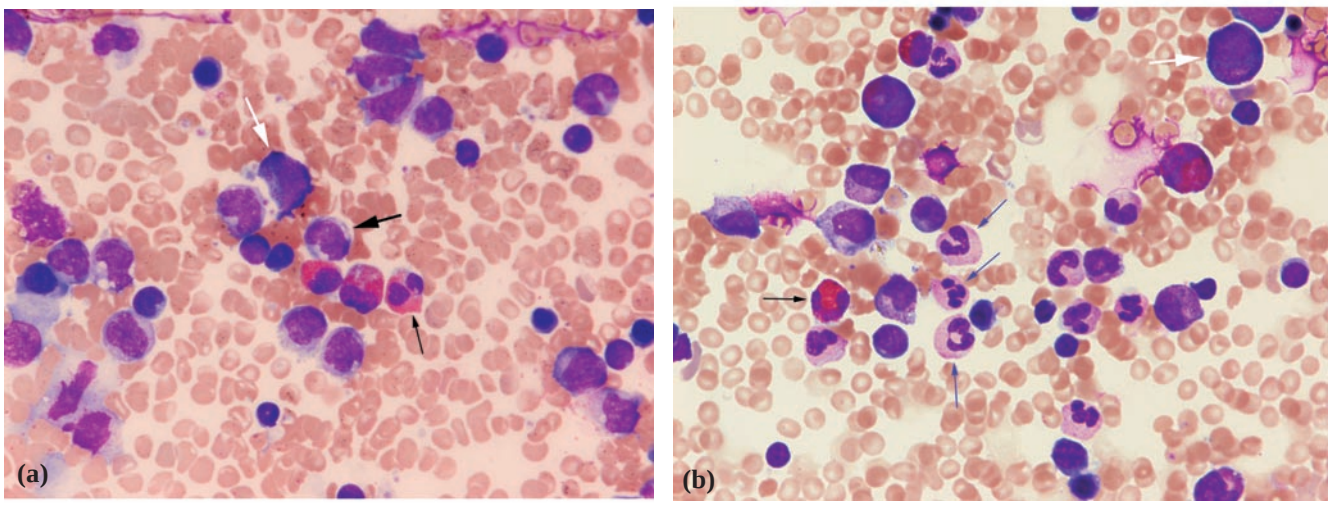

Figure 3 (a) Bone marrow aspirate of our patient at diagnosis (x80 Wright-Giemsa). Many myeloid precursors are present. Promyelocytes (white arrow) are found. Eosinophilic maturation (thin black arrow) is evident, but neutrophilic maturation is absent. Monocytosis is also detected (thick black arrow). (b) Bone marrow aspirate of a normal child for comparison. Normal granulocytic differentiation is seen, including presence of promyelocytes (white arrow), eosinophils (thin black arrow) and neutrophils (blue arrows).

Cysteine at amino acid position 151 is a highly evolutionary conserved residue. In general substitution by other amino acids will be poorly tolerated. At the amino acid level, cysteine is a small, polar residue while tyrosine is a big, non-polar residue, such change is predicted to cause significant alteration in the local structure. In addition, C151 residue is involved in stability-maintaining disulfide bridges. A substitution at a predicted stabilising residue is considered to destabilise structure.[1,2]

C151Y was previously reported in a kindred with 2 affected children with severe congenital neutropenia (SCN). However, the same mutation was also found in the father who was phenotypically normal, including peripheral blood neutrophil count and bone marrow morphology.[3] However, based on the above bioinformatics analysis, there are sufficient reasons to regard C151Y as a pathogenic mutation in our patient. The apparent disparity between genotype and phenotype is likely due to other unrecognised disease-modifying factors.

Omphalitis in the Neonate: Diagnostic Approach

We diagnosed a patient with severe congenital neutropenia who presented with persistent omphalitis in the neonatal period. Separation of the umbilical cord normally occurs between 5 to 8 days after birth. The process of umbilical cord separation involves thrombosis of the umbilical vessels, contraction of the vessel wall, collagenase activity and necrosis of the stump which is mediated by phagocytic action, followed by epithelialisation of the cord stump. In developed countries, the incidence of omphalitis in newborns delivered in the hospital setting was estimated to be 0.5-1.0%.[4] Infection of the cord delays the process of umbilical vessel obliteration, and common risk factors for omphalitis include non-sterile delivery, maternal genital tract infection, prolonged rupture of membrane, prematurity, low birth weight, umbilical catheterisation, and inappropriate care and handling such as application of ash to the cord stump in some cultural rituals.[5,6] Anatomical defects such as patent urachus and immunological defects such as phagocytic dysfunction such as leucocyte adhesion defect or neutropenia, should be considered for neonates with protracted, severe omphalitis or failure of umbilical cord separation beyond 2 weeks of life.

The signs of omphalitis include periumbilical edema, erythema, tenderness and discharge. Common pathogens include skin flora such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and group A streptococcus, as well as group B streptococcus acquired perinatally. Enteric organisms such as *Escherichia coli*, Klebsiella and Pseudomonas may be implicated as well. In developing countries, tetanus may be a causative organism when the cord is contaminated with soil. Clinicians should be vigilant of the signs of complications, such as cellulitis and lymphangitis of the abdominal wall indicating spreading infection. Inflammation extending to the subcutaneous fat and deep fascia leads to periumbilical necrotising fasciitis and systemic sepsis, usually caused by mixed aerobic and anaerobic infection, and results in high mortality rate (50-87.5%). Other complications include peritonitis, intra-abdominal abscess, ascending infection leading to portal vein thrombosis and hepatic abscess.[7]

Neutropenia in Neonates and Infants

The normal range of absolute neutrophil count (ANC) varies with age. Within the first 24 hours of life, neutrophils constitute 60-70% of total white cell count and the lower limit of normal is taken to be 5.0×10^9/L. As the total white cell count gradually lowers after the first few days of life, the lower limit of ANC is taken as 1.5×10^9/L during the first week, and 1.0×10^9/L from the second week to 6 months. After the first year the ANC is normally greater than 1.5×10^9/L. ANC of $1.0\text{-}1.5 \times 10^9$/L is regarded as mild neutropenia and $0.5\text{-}1.0 \times 10^9$/L as moderate neutropenia. Severe neutropenia is defined as ANC $\leq 0.5 \times 10^9$/L, and predicts a substantial risk for pyogenic infection when the duration lasts for more than 2 to 3 months.[8]

Causes of neutropenia can be broadly classified into intrinsic disorders of myeloid and stem cells, and secondary to extrinsic factors such as infection (viral, bacterial, fungal, protozoan), alloimmune or autoimmune phenomenon, drug-induced, hypersplenism and malignancy.[9] Neutropenia may also be present in a number of primary immunodeficiency disorders, such as X-linked agammaglobulinemia, X-linked hyeprIgM syndrome and Wiskott-Aldrich syndrome.[10] Neonatal alloimmune neutropenia occurs when there has been maternal sensitisation to fetal antigens, leading to production of antibodies against neutrophils *in utero* manifesting as immune-mediated neutrophil destruction in the newborn. Babies born to mothers who have immune neutropenia themselves may develop neutropenia because of passively transferred maternal anti-neutrophil IgG antibodies. These are transient events and the condition is

expected to resolve by 2-3 months of age when such maternal IgG antibodies disappear.[11]

Approach to Neonates and Infants with Neutropenia

Disorders of proliferation and maturation of myeloid cells leading to neutropenia usually manifest in the neonatal or early infancy period. The degree of neutropenia is usually profound, often <0.2 x 10^9/L in severe congenital neutropenia. On evaluation, all infective episodes should be carefully documented, including the onset, site, severity, duration of symptoms and response to antimicrobial therapy. Perinatal course and drug history should be reviewed, and family history of recurrent infection, neutropenia, haematological and immunological disorders should be elicited.

Neonates and young infants with neutropenia and febrile illness may have life-threatening sepsis and should be immediately attended. Physical examination aims at identifying 1) sites of infection and 2) features suggestive of syndromal disorders (Table 1). The oral cavity should be examined for oral candidiasis and gingivostomatitis. Infants with neutropenia are predisposed to cutaneous pyogenic infections such as skin pustules, cellulitis and cutaneous abscess. There may be persistent inflammation and drainage of the umbilical area, and the buttock should be examined for perianal inflammation and abscess. Clinicians should also be aware of invasive infections such as deep organ abscess, meningitis and bloodstream infection.[8,9] Patients with chronic, recurrent infections may have poor nutritional status and failure to thrive. Infants may have phenotypic features suggestive of specific

Table 1 Neutropenia syndromes: genetic aetiology, inheritance and clinical features

Condition	Inheritance	Gene mutation and pathogenesis	Phenotype features
Severe congenital neutropenia	AD, sporadic	ELA2; accelerated apoptosis of neutrophil precursors	Severe neutropenia ANC <0.5x10^9/L
	AR	HAX1; accelerated apoptosis of neutrophil precursors	Associated cardiac and urogenital anomalies in G6PC3 mutations
	AR	G6PC3; accelerated apoptosis of neutrophil precursors	Low T and B cells in GFI1 mutations
	AR	GFI1; defective haematopoietic stem cell differentiation	
	X-linked	WAS; gain-of-function mutation, enhanced and delocalised actin polymerisation, ↑ neutrophil apoptosis	
Cyclic neutropenia	AD	ELA2	Neutropenia lasts for 3-6 days per 21-day cycle
Bone marrow failure syndromes			
Fanconi syndrome	AR	FANC; defects in DNA repair	Pancytopenia, dysplastic thumbs
Dyskeratosis congenita	X-linked AR, AD	DKC1; telomerase defect TREC, TERT; ribosomal dysfunction	Leukoplakia, abnormal skin pigmentation, short, stature, dystrophic nails, dental caries, epiphora hyperhidrosis, may progress to pancytopenia
Diamond-Blackfan syndrome	Sporadic (75%) AR, AD	RPS19; ribosomal protein defect	Erythroid failure, neutropenia in 25-40%; craniofacial and thumb anomalies
Pearson's syndrome	Sporadic	Mitochondrial respiratory chain dysfunction	Refractory sideroblastic anemia, neutropenia, thrombocytopenia, exocrine pancreatic insufficiency, vacuolization of erythroid and myeloid precursors in bone marrow

(continued on page 294)

Table 1 Neutropenia syndromes: genetic etiology, inheritance and clinical features (con't)

Condition	Inheritance	Gene mutation and pathogenesis	Phenotype features
Neutropenia associated with skeletal dysplasia			
Cartilage hair hypoplasia	AR	RMRP; abnormal mitochondrial RNA processing, defective granulopoiesis	Short-limb dwarfism, fine hypopigmented hair, CD4 & CD8 lymphopenia, recurrent viral infections
Shwachman-Diamond syndrome	AR	SBDS, bone marrow stem cell defect, impaired neutrophil production	Pancreatic exocrine insufficiency, skeletal anomalies, intermittent neutropenia (2/3) or persistent neutropenia (1/3), anaemia (40-60%), thrombocytopenia (30%)
Cohen syndrome	AR	VPS13B	Obesity, hypotonia, mental retardation, craniofacial anomalies, limb and spinal anomalies, large incisor
Neutropenia associated with metabolic disorder			
Glycogen storage disease type 1b	AR	SLC37A4; defective transport of G6P from cytosol to ER	Hypoglycaemia, dyslipidemia, hepatomegaly, ↑ uric acid, lactic acidemia, neutropenia, neutrophil dysfunction (defective oxidative burst & chemotaxis)
Barth syndrome	X-linked recessive	TAZ; defect in cardiolipin essential to mitochondrial integrity	Dilated cardiomyopathy, skeletal myopathy, mild neutropenia, carnitine deficiency, growth delay
Neutropenia as a manifestation of PID			
Agammaglobulinaemia CVID/selective IgA deficiency	X-linked, AR, variable	BTK (X-linked), Igα, Igβ, λ5, BLNK (AR) ICOS, TACI, other unknown genes; variable inheritance	Recurrent infections related to antibody deficiency Cellular immunodeficiency, autoimmunity & malignancy in CVID, neutropenia? Immune-mediated
Hyper-IgM syndrome	X-linked AR	CD40L CD40, AID, UNG	Recurrent sinopulmonary & gastrointestinal infections, autoimmunity
Wiskott-Aldrich syndrome	X-linked	WAS	Recurrent sinopulmonary infections, thrombocytopenia, eczema
WHIM syndrome	AD	CXCR4; excessive neutrophil apoptosis	Warts, hypogammaglobulinaemia, recurrent infections, myelokathexis
Reticular dysgenesis	AR	AK2; stem cell failure in lymphoid and myeloid development	Severe combined immunodeficiency, pancytopenia
SCN / neutrophil dysfunction associated with hypopigmentation			
Chédiak-Higashi syndrome	AR	LYST; abnormal protein trafficking, impaired neutrophil chemotaxis	Intermittent neutropenia, albinism, prolonged bleeding time, neuropathy, ↓NK/T cell function
Griscelli syndrome type 2	AR	RAB27A; impaired lytic granule release	Partial albinism, intermittent neutropenia, thrombocytopenia, haemophagocytosis, T-cell defect
Hermansky-Pudlak syndrome type 2	AR	AP3B1; impaired lysosomal protein traffic	Hypopigmentation, prolonged bleeding times, dysfunction NK, NK-T and antigen-presenting cells
p14 deficiency	AR	p14 gene; endosomal dysfunction	Hypopigmentation, short stature, hypogammaglobulinemia, ↓B cells, defective neutrophil killing and function of cytotoxic T cells

neutropenia syndromes, such as hypopigmentation (Chédiak-Higashi syndrome and Griscelli syndrome), skeletal dysplasia (Shwachman-Diamond syndrome, cartilage hair hypoplasia, Cohen syndrome, Schimke immuno-osseous dysplasia) and exocrine pancreatic insufficiency (Shwachman-Diamond syndrome, Pearson syndrome). Glycogen storage disorder is suspected when there is hypoglycemia and hepatomegaly. Dyskeratosis congenita would be suspected when the patient has dysplastic nails and skin changes.[12,13] We previously diagnosed a girl with dyskeratosis congenita, who had pancytopenia (severe neutropenia <0.5 x 10^9/L), dystrophic nails, increased skin pigmentation and smooth tongue mucosa. She underwent successful bone marrow transplantation from her HLA-matched sister in 1995.[14,15] She is currently 25 years old and remains in remission.

Full blood count should be accompanied by direct blood smear in all patients with neutropenia. If leucopenia, anaemia and thrombocytopenia are present, marrow failure syndromes or marrow infiltrative diseases should be considered. In reticular dysgenesis, total white cell count including the differentials will all be reduced. Monocytes, eosinophils and basophils are commonly elevated in severe congenital neutropenia. Pathognomonic features for specific diagnoses may be present, such as pyknotic nuclei of neutrophils in myelokathexis, or abnormally large neutrophil granules in Chédiak-Higashi syndrome. Bone marrow aspirates from patients with myelokathexis show myeloid hypercellularity with increased numbers of granulocytes at all stages of differentiation. Most patients with myelookathexis have warts, hypogammaglobulinaemia and various types of infections, termed WHIM syndrome.

Serial monitoring of blood counts should be made, and if there is persistent neutropenia for more than 1 to 2 weeks, detailed diagnostic evaluation and referral to a paediatric haematologist is warranted. Further investigations include viral serology (e.g. cytomegalovirus, Epstein-Barr virus, parvovirus as clinically indicated), anti-neutrophil antibodies and immunoglobulin pattern.[9] Cyclic neutropenia is characterised by 21-day cycles of oscillating neutrophil count, with neutropenia spanning 3-6 days. The classical approach to establish the diagnosis is to obtain complete blood count 2-3 times per week for 4-6 weeks.[8] Bone marrow examination should be considered when SCN, bone marrow failure syndromes and malignancy are suspected. Bone marrow finding of SCN is characterised by maturation arrest of neutrophil precursors, monocytosis, normal erythropoietic and thrombopoietic lineages.

For our patient, the ANC was extremely low (<0.2 x 10^9/μL) upon presentation, and on one occasion neutrophil was actually absent on direct blood smear review. There was elevated blood monocytes (2-4 times of normal) and mild thrombocytosis. Neutrophil count remained persistently low on serial testing and no cyclic pattern could be observed. Bone marrow aspirate showed failure of maturation beyond the promyelocyte/myelocyte stage. All these findings were classical of severe congenital neutropenia.

Severe Congenital Neutropenia

Severe congenital neutropenia is characterised by maturation arrest of myelopoiesis at the level of promyelocyte/myelocyte stage in the bone marrow, resulting in paucity of mature neutrophils in the peripheral blood. In general, a minimum of 3 documented ANC <0.5 x 10^9/L over a 3-month period and onset of neutropenia within the first few months of life are required for diagnosis.[16] The incidence is estimated at 1/200,000 live births with equal distribution for gender. Approximately 60% are inherited in an autosomal dominant manner, while the rest are autosomal recessive. With an annual birth rate of 15 million in China, it is estimated that 75 babies with SCN are born every year.

An increasing number of genes accounting for congenital neutropenia are identified, as listed in Table 1.[8,12,13,17] Heterozygous mutations in the *ELA2* gene are the most common genetic abnormality, accounting for approximately 50-60% of patients with SCN. Over 70 distinct mutations of *ELA2* gene have been identified in patients with SCN or cyclic neutropenia.[18] Both autosomal dominant inheritance and sporadic cases have been described. With a few exceptions, most *ELA2* mutations are specifically associated with either SCN or cyclic neutropenia, suggesting a genotype-phenotype correlation. Mutations in *HAX1*, *GFI1*, *G6PC3* and *WAS* are rare. It was recommended that genotyping of *HAX1* and *G6PC3* should be considered in patients in whom *ELA2* mutation was not found, or with family history suggestive of autosomal recessive SCN, especially if associated with neurological abnormalities (such as cognitive defects, mental retardation and epilepsy in *HAX1*) or cardiac and urogenital anomalies (*G6PC3*).[18] In 25-40% of patients with SCN, no known mutation could be identified.[16,17]

Treatment of Severe Congenital Neutropenia

As SCN is such a rare disorder, pooled data analysis from different centers worldwide is needed to establish the natural disease course, treatment response and outcomes. The Severe Congenital Neutropenia International Registry (SCNIR) was established in 1994 and collected data on more than 700 patients.[16] More than 90% responded to G-CSF treatment with elevation of ANC to more than 1.0 x 10^9/L, and required significantly fewer antibiotics and days of hospitalisation.[19] Before the availability of G-CSF in 1987, 42% of patients with SCN died within the first 2 years of life. The cumulative incidence of death from sepsis was reported to be 8% after receiving 10 years of G-CSF therapy.[20] G-CSF acts by stimulating neutrophil production and delaying their apoptosis. The most important parameter for the risk of bacterial infections is the neutrophil count, therefore G-CSF dose titration is aimed at maintaining ANC above 1000/μL. The mean and median dose of G-CSF to maintain ANC above 1000/μL was 11.9 μg/kg/day and 5.4 μg/kg/day respectively, and most patients responded to G-CSF dose below 25 μg/kg/day.[20] G-CSF is usually commenced at a starting dose of 5 μg/kg/day, then stepped up to 10 μg/kg/day and then by increments of 10 μg/kg/day at 14-day intervals if ANC remains <0.5 x 10^9/L. The dose is maintained once ANC remains steady at >1.0 x 10^9/L.[21]

Myelodysplastic Syndrome and Leukaemia in Severe Congenital Neutropenia

Patients with SCN are at risk for developing myelodysplastic syndrome (MDS) and leukaemia, with a peak incidence during the second decade of life.[22] The cumulative incidence of leukaemia in 374 patients registered in the SCNIR (enrolled from 1987-2000) was 21% after 10 years.[20] The predominant type of leukaemia was acute myeloid leukaemia, but acute lymphoid leukaemia, chronic myelomonocytic leukaemia and bi-phenotypic leukaemia were also reported. The risk was higher among those who require a higher dose of G-CSF (40% in those requiring >8 μg/kg/day vs 11% requiring <8 μg/kg/day after 20 years of treatment). The investigators concluded that poor response to G-CSF predicted adverse outcome in terms of leukaemia development and survival.

Development of leukaemia in patients with SCN is associated with acquired genetic abnormalities, such as mutations in *CSF3R*, *RAS*, monosomy 7 and trisomy 21.[16] Approximately 80% of patients with leukaemic transformation were found to have point mutations in *CSF3R* in their marrow cells, independent of their SCN genotypes. In contrast, only 30% of patients who have not yet developed leukaemia had *CSF3R* mutations.[23] All mutations involved a stop codon predicted to cause truncation of the intracellular portion of the G-CSF receptor protein, producing an exaggerated hyperproliferative response to G-CSF and favours clonal expansion.[24,25] Leukaemic transformation was recognised in patients with SCN in the pre-GCSF era, and *CSF3R* mutations could be detected in some patients before commencement of G-CSF, suggesting that G-CSF is not responsible for these mutations. In many cases an increasing number of different *CSF3R* mutations from the original or different clones accumulate throughout the disease course, suggestive of genetic instability of the *CSF3R* gene.[19,24,26] How *CSF3R* mutations were acquired in the disease course and how they contribute to malignant transformation is not fully understood. It was suggested that *CSF3R* analysis may be a useful marker to predict the risk of malignant transformation. It is recommended that bone marrow examination and cytogenetic studies should be performed annually, or whenever a falling blood count is present, to detect morphological and clonal abnormalities (e.g. monosomy 7, trisomy 21) suggestive of malignant transformation.[19]

When frank MDS / leukaemia develops, G-CSF should be stopped and some patients may have spontaneous remission. The outcome of chemotherapy in treating MDS/AML in SCN is poor and long-term remission could hardly be achieved. Expedient arrangement for HSCT should be made. For our patient, HLA-typing of the parents and child was arranged in the early course of disease as the dose of G-CSF required was rather high, suggestive of a poor-risk category. Match-unrelated donor (MUD) search was initiated once we knew that the child's HLA-type was not matched with his parents and elder brother.

Management of patient with significant chromosomal abnormalities or clonal disease but without evidence of MDS/AML is more controversial. The advantage of proceeding with HSCT in these patients is that the curative rate would be higher with a lower malignancy burden and good general health status. However, the tempo and definitive risk to develop frank MDS is unpredictable, and the option of close monitoring of marrow status versus early HSCT opens for discussion.[27]

Haematopoietic Stem Cell Transplantation for Severe Congenital Neutropenia

Haematopoietic stem cell transplantation is the only currently available treatment for patients who develop refractoriness to G-CSF or malignant transformation. There are 3 published series reporting the indication and outcome of patients with SCN undergoing HSCT, as summarised in Table 2.[28-30] Overall, the prognosis of HSCT for patients with malignant transformation was poor. There were possible reasons: (1) the use of chemotherapy to treat MDS/AML led to increased risk of treatment-related mortality during transplantation; (2) the need for expedient transplant often compromised the opportunity to identify a better HLA-matched donor, if match-related donor is not available and (3) intrinsically poor prognosis of patients

Table 2 Outcome of haematopoietic stem cell transplant in patients with severe congenital neutropenia

	SCN International registry[28]	**French SCN registry[29]**	**2-center retrospective study (USA)[30]**
Year	1978-1998	1993-2003	1997-2001
Number of patients	29	9	6
Indications for HSCT and outcome	(out of 304 patients registered)	(out of 101 patients registered)	
1. Malignant transformation	**n=18, only 3 survived**	**n=4, 3 survived** (MUD, UCB, MRD), 1 had severe chronic GVHD and died of septic shock on D+258 (UCB)	**n=6 (all were malignant transformation)** ◆ 2 patients transplanted for **MDS** remained alive (one MRD and one MUD), no pre-HSCT chemotherapy
2. Refractory / partial response to G-CSF therapy	**n=8** ◆ **5 with good outcome** without major HSCT-related complications, all received HSCT from HLA-identical sibling ◆ **Chronic GVHD** in 1 patient receiving BMT from 1-Ag mismatched MUD ◆ **Severe haemorrhagic cystitis** requiring cystectomy in 1 patient ◆ **Multi-organ failure and death** in 1 patient receiving haploidentical HSCT	**n=4, 3 survived** (MUD, UCB, MRD), 1 had EBV-PTLD and died of septic shock 42 days after second HSCT for primary non-engraftment (MUD)	◆ 4 patients with AML received induction chemotherapy before BMT, all died ❖ Chronic GVHD (n=2, MUD BMT) ❖ Primary graft failure (1-Ag mismatched MUD BMT) ❖ Failed engraftment and died of relapsed AML (1-Ag mismatched MUD BMT)
3. Other indications	**1. Pancytopenia + *CSF3R* mutation (n=1)** Received haploidentifcal HSCT, died of severe GVHD and sepsis **2. *CSF3R* mutation (n=1)** BM from HLA-identical sibling, acute grade III skin and gut GVHD, chronic skin GVHD **3. HSCT before the availability of G-CSF (n=1)** BM from HLA-identical sibling, graft rejection, good response with G-CSF after its availability	**Pancytopenia** UCB 6/10 matched, died of aspergillosis on D+374	

Ag, antigen; AML, acute myeloid leukaemia; BM, bone marrow; BMT, bone marrow transplantation; EBV, Ebstein-Barr virus; GVHD, graft-versus-host disease; HLA, human leucocyte antigen; HSCT, haematopoietic stem cell transplant; MRD, match-related donor; MUD, match-unrelated donor; PTLD, post-transplant lymphoproliferative disorder UCB, unrelated cord blood.

who had malignant transformation from SCN. An older age at the time of HSCT was associated with worse outcome.[28] Molecular studies for detecting poor prognostic indicators such as *CSF3R* mutations should be performed, so that early HSCT from HLA-identical siblings can be considered and initiation of MUD search can be arranged. The use of reduced intensity conditioning regimen was successful in some patients with SCN,[31,32] and may be further explored to see if a broader application of HSCT in SCN can be recommended.

For our patient, we favor early transplantation once a good-match donor is identified. His high G-CSF requirement is a poor prognostic factor for malignant transformation, and the success of transplantation is higher before he suffers from significant organ damage because of recurrent infections. The family has been counseled in detail about the indications and outcomes of HSCT.

References

1. Thusberg J, Vihinen M. Bioinformatic analysis of protein structure-function relationships: case study of leukocyte elastase (ELA2) missense mutations. Hum Mutat 2006;27:1230-43.
2. Thusberg J, Vihinen M. Pathogenic or Not? And If So, Then How? Studying the effects of missense mutations using bioinformatics methods. Hum Mutat 2009;30:703-14.
3. Germeshausen M, Schulze H, Ballmaier M, Zeidler C, Welte K. Mutations in the gene encoding neutrophil elastase (ELA2) are not sufficient to cause the phenotype of congenital neutropenia. Br J Haematol 2001;115:222-4.
4. Fraser N, Davies BW, Cusack J. Neonatal omphalitis: a review of its serious complications. Acta Paediatr 2006;95(5):519-22.
5. Sawardekar KP. Changing spectrum of neonatal omphalitis. Pediatr Infect Dis J 2004;23:22-6.
6. Mason WH, Andrews R, Ross LA, Wright HT. Omphalitis in the newborn infant. Pediatr Infect Dis J 1989;8:521-5.
7. Ameh EA, Nmadu PT. Major complications of omphalitis in neonates and infants. Pediatr Surg Int 2002;18:413-6.
8. Segel GB, Halterman JS. Neutropenia in pediatric practice. Pediatr Rev 2008;29:12-23.
9. James RM, Kinsey SE. The investigation and management of chronic neutropenia in children. Arch Dis Child 2006;91:852-8.
10. Cham B, Bonilla MA, Winkelstein J. Neutropenia associated with primary immunodeficiency syndromes. Semin Hematol 2002; 39:107-12.
11. Palmblad JE, von dem Borne AE. Idiopathic, immune, infectious, and idiosyncratic neutropenias. Semin Hematol 2002;39:113-20.
12. Boxer L, Dale DC. Neutropenia: causes and consequences. Semin Hematol 2002;39:75-81.
13. Klein C. Congenital neutropenia. Hematology Am Soc Hematol Educ Program 2009:344-50.
14. Ha SY, Lee ACW, Chan CF, Liang RHS, Yuen HL, Lau YL. Successful bone marrow transplant for congenital bone marrow failure syndromes. HK J Paediatr (New Series) 1996;1:53-5.
15. Lau YL, Ha SY, Chan CF, Lee AC, Liang RH, Yuen HL. Bone marrow transplant for dyskeratosis congenita. Br J Haematol 1999;105:571.
16. Welte K, Zeidler C. Severe congenital neutropenia. Hematol Oncol Clin N Am 2009;23:207-20.
17. Schäffer AA, Klein C. Genetic heterogeneity in severe congenital neutropenia: how many aberrant pathways can kill a neutrophil? Curr Opin Allergy Clin Immunol 2007;7:481-94.
18. Xia J, Bolyard AA, Rodger E, et al. Prevalence of mutations in ELANE, GFI1, HAX1, SBDS, WAS and G6PC3 in patients with severe congenital neutropenia. Br J Haematol 2009;147:535-42.
19. Zeidler C, Germeshausen M, Klein C, Welte K. Clinical implications of ELA2-, HAX1-, and G-CSF-receptor (CSF3R) mutations in severe congenital neutropenia. Br J Haematol 2008; 144:459-67.
20. Rosenberg PS, Alter BP, Bolyard AA, et al. Severe Chronic Neutropenia International Registry. The incidence of leukemia and mortality from sepsis in patients with severe CN receiving long-term G-CSF therapy. Blood 2006;107:4628-35.
21. Zeidler C, Welte K. Kostmann syndrome and severe congenital neutropenia. Semin Hematol 2002;39:82-8.
22. Luna-Fineman S, Shannon KM, Lange BJ. Childhood monosomy 7: epidemiology, biology, and mechanistic implications. Blood 1995;85:1985-99.
23. Germeshausen M, Ballmaier M, Welte K. Incidence of CSF3R mutations in severe CN and relevance for leukemogenesis: results of a long-term survey. Blood 2007;109:93-9.
24. Dong F, Brynes RK, Tidow N, Welte K, Löwenberg B, Touw IP. Mutations in the gene for the granulocyte-colony stimulating factor receptor in patients with acute myeloid leukemia preceded by severe CN. N Engl J Med 1995;333:487-93.
25. Hunter MG, Avalos BR. Granulocyte colony-stimulating factor receptor mutations in severe congenital neutropenia transforming to acute myelogenous leukemia confer resistance to apoptosis and enhance cell survival. Blood 2000;95:2132-7.
26. Germeshausen M, Ballmaier M, Welte K. Incidence of CSF3R mutations in severe CN and relevance for leukemogenesis: results of a long-term survey. Blood 2007;109:93-9.
27. Freedman MH, Alter BP. Risk of myelodysplastic syndrome and acute myeloid leukaemia in congenital neutropenia. Semin Hematol 2002;39:128-33.
28. Zeidler C, Welte K, Barak Y, et al. Stem cell transplantation in patients with severe congenital neutropenia without evidence of leukemic transformation. Blood 2000;95:1195-8.
29. Ferry C, Ouachée M, Leblanc T, et al. Hematopoietic stem cell transplantation in severe congenital neutropenia: experience of the French SCN register. Bone Marrow Transplant 2005;35:45-50.
30. Choi SW, Boxer LA, Pulsipher MA, et al. Stem cell transplantation in patients with severe congenital neutropenia with evidence of leukemic transformation. Bone Marrow Transplant 2005;35:473-7.
31. Nakazawa Y, Sakashita K, Kinoshita M, et al. Successful unrelated cord blood transplantation using a reduced-intensity conditioning regimen in a 6-month-old infant with congenital neutropenia complicated by severe pneumonia. Int J Hematol 2004;80:287-90.
32. Fukano R, Nagatoshi Y, Shinkoda Y, et al. Unrelated bone marrow transplantation using a reduced-intensity conditioning regimen for the treatment of Kostmann syndrome. Bone Marrow Transplantation 2006;38:635-6.