A novel syndrome of congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers and developmental delay (SIFD)

Daniel H. Wiseman 1, Alison May 2, Stephen Jolles 3, Philip Connor 4, Colin Powell 5, Matthew M. Heeney 6, Patricia J. Giardina 7, Robert J. Klaassen 8, Pranesh Chakraborty 8, Michael T. Geraghty 8, Nathalie Major-Cook 9, Caroline Kannengiesser 9, Isabelle Thuret 10, Alexis A. Thompson 11, Laura Marques 12, Stephen Hughes 13, Sylvia S. Bottomley 14, Mark D. Fleming 15, Robert F. Wynn 1.

1 Department of Haematology, Royal Manchester Children’s Hospital, Manchester, UK
2 Department of Haematology, Cardiff University School of Medicine, Cardiff, UK
3 Department of Immunology, University Hospital of Wales, Cardiff, UK
4 Department of Paediatrics, Children’s Hospital for Wales, Cardiff, UK
5 Institute of Molecular and Experimental Medicine, School of Medicine, Cardiff University, Cardiff, UK
6 Division of Hematology/Oncology, Boston Children’s Hospital, Boston, MA, USA
7 Division of Hematology-Oncology, Children’s Cancer and Blood Foundation Laboratories, Weill Cornell Medical College, New York, NY, USA
8 Department of Pediatrics, Children’s Hospital of Eastern Ontario, University of Ottawa, Ottawa, ON, Canada
9 INSERM UMR773, Université Paris Diderot; Assistance Publique des Hospitaux de Paris (AP-HP), Département de Génétique, Hôpital Xavier Bichat, Paris, France
10 Department of Pediatric Hematology, Hôpital de la Timone, Marseilles, France
11 Department of Pediatrics, Northwestern University Feinberg School of Medicine; Ann & Robert H. Lurie Children’s Hospital, Chicago, IL, USA
12 Paediatric Department, Infectious Diseases and Immunodeficiencies Unit, Centro Hospitalar do Porto, Porto, Portugal
13 Department of Immunology, Royal Manchester Children’s Hospital, Manchester, UK
14 Department of Medicine, Hematology-Oncology Section, University of Oklahoma College of Medicine, Oklahoma City, OK, USA
15 Department of Pathology, Boston Children’s Hospital, Boston, MA, USA

Corresponding Author:
Daniel H. Wiseman
Department of Haematology, Royal Manchester Children’s Hospital
Oxford Road, Manchester M13 9WL, UK
E-mail: dwiseman@picr.man.ac.uk
Phone: +44 (0)161 276 3000

Running Title: Sideroblastic anemia with immunodeficiency
Key Points

1) A novel clinical syndrome of congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers and developmental delay is described.
2) Bone marrow transplant resulted in complete and durable resolution of the hematologic and immunologic manifestations.

Abstract

Congenital sideroblastic anemias (CSAs) are a heterogeneous group of inherited disorders identified by pathological erythroid precursors with perinuclear mitochondrial iron deposition in bone marrow. An international collaborative group of physicians and laboratory scientists collated clinical information on cases of CSA lacking known causative mutations, identifying a clinical subgroup of CSA associated with B immunodeficiency, periodic fevers and development delay. Twelve cases from ten families were identified. Median age at presentation was 2 months. Anemia at diagnosis was sideroblastic, typically severe (median Hb 7.1g/dL) and markedly microcytic (median MCV 62.0fL). Clinical course involved recurrent febrile illness and gastrointestinal disturbance, lacking an infective cause. Investigation revealed B cell lymphopenia (CD19+ range 0.016–0.22x10^9/L) and panhypogammaglobulinemia in most cases. Children displayed developmental delay alongside variable neurodegeneration, seizures, cerebellar abnormalities, sensorineural deafness and other multi-system features. Most required regular blood transfusion, iron chelation and IVIg replacement. Median survival was 48 months, with seven deaths caused by cardiac or multi-organ failure. One child underwent bone marrow transplantation aged 9 months, with apparent cure of the hematologic and immunologic manifestations. We describe and define a novel CSA and B cell immunodeficiency syndrome with additional features resembling a mitochondrial cytopathy. The molecular etiology is under investigation.
Introduction

Sideroblastic anemia (SA) describes a heterogeneous group of acquired and inherited disorders of erythropoiesis characterized by the presence in bone marrow (BM) of erythroid precursors with pathological, perinuclear mitochondrial iron deposition ("ringed sideroblasts"). During the past two decades the genetic bases of several distinct congenital SA (CSA) disorders have been defined. Some affect mainly or exclusively erythroid cells; other 'syndromic' forms occur within multi-system disorders with extensive non-hematopoietic manifestations. The genes responsible for these phenotypes encode proteins involved in mitochondrial heme biosynthesis (e.g. ALAS2; SLC25A38), iron-sulfur (Fe-S) cluster biogenesis/transport (ABCB7; GLRX5) and mitochondrial translation (PUS1; YARS2; mitochondrial deletions)1-3. Anemia can be microcytic, normocytic or macrocytic; vary from mild to transfusion-dependent; present at any age up to the ninth decade; and may or may not be associated with spontaneous iron overloading. However, ≥40% of CSA cases remain unexplained at the molecular level4.

As with other rare disorders, collection and collaborative review of patient data by expert centers allows for classification based on clinical characteristics that may aid discovery of novel, genetically-defined disorders4-6. Here we describe a novel syndrome identified amongst a group of mutation-negative CSA patients, associated with B cell immunodeficiency, periodic fevers, developmental delay and other recurring multi-system features. Although the molecular basis remains under investigation, detailed review of these cases through an international collaborative effort has revealed a consistent phenotype strongly suggesting a shared etiology.

Methods

Case histories and diagnostic samples from children with CSA were sent to reference laboratories in the UK and North America for molecular studies. Subsets of mutation-negative patients presenting with co-existent B-cell lymphopenia and panhypogammaglobulinemia were identified independently by each group. Informal communication between groups acknowledged a recurring phenotype, prompting further communication internationally and subsequent recognition of a larger cohort of similar cases at other referral centers. Exhaustive literature searches failed to identify any previous reports of this combination of features. Recognition of a potential novel syndrome prompted formation of an International Collaborative Group, comprising all physicians and scientists involved in treating or investigating these cases. Additional cases were sought by retrospective review of case histories within local registries, focusing on those lacking an identified genetic abnormality. Where potentially relevant cases were found direct communication with referring teams sought additional clinical information to support or refute a common clinical phenotype.

The aims of the collaborative group were to: (i) collate clinical information to characterize the clinical phenotype and natural history of the putative syndrome; (ii) propose provisional diagnostic criteria;
and (iii) collect patient samples for molecular investigation (coordinated at Boston Children’s Hospital). A data collection tool was designed and completed for each case from retrospective review of medical records. Details were collated centrally and clinical summaries were produced. Diagnostic criteria emerged during the course of the study and were agreed upon by consensus opinion.

Genetic testing for CSA (ALAS2, SLC25A38, GLRX5, ABCB7, PUS1, YARS2, mtDNA deletion), periodic fever syndromes (MEFV, TNFRSF1A, MVK, NLRP3) or defects in B cell maturation (BTK, λ5/14.1) had been variously performed in different diagnostic and research laboratories involving standard techniques (sequence analysis of coding regions, exon-intron boundaries and important 5’ and 3’ regulatory regions of the genes; full mtDNA sequence analysis and Southern blot analysis for mtDNA deletion detection). In some cases muscle biopsy cytochrome oxidase staining and other investigations for altered respiratory chain activities were performed, employing standard techniques.

Results

We identified 11 children (5 male; 6 female) from 10 families resident in Europe and North America (3 UK; 1 France; 1 Portugal; 4 USA; 2 Canada) with the clinically distinctive combination of CSA and B-lymphopenia and/or panhypogammaglobulinemia. An additional case (deceased brother of child #3) was added on recognition of the index child’s family history and review of historical case records. Multiple ethnicities were represented, including North European Caucasian, Pakistani and Spanish/Hispanic. All were born to apparently healthy parents following uncomplicated pregnancies. Selected demographics and results are presented in Table 1. Summaries of individual clinical courses are provided online as supplemental material.

Family History

Consanguineous parentage was present in only three families and most children had several healthy/unaffected siblings. However, the cohort did include two sibling pairs. Children #1 (male) and #2 (female) were born to consanguineous parents and shared the described phenotype. Pedigree analysis was complicated by presence of other siblings unaffected by severe anemia or immunodeficiency, but who displayed a different cluster of syndromic features (consistent with the Cenani-Lenz syndrome) not shared with the affected children, indicating another recessive condition in this family (Figure 1). Child #3 (female) conclusively displayed CSA with documented B-immunodeficiency, having had an older brother (#4) almost certainly affected by the same condition. This sibling had been diagnosed with a severe congenital dyserythropoietic anemia many years earlier, and had died before 5 years of age. Although sideroblastic anemia was not specifically documented, retrospective review revealed a clinical course including hypogammaglobulinemia, periodic fevers, developmental delay, seizures, lymphadenopathy, brittle hair and avascular necrosis. Autopsy was remarkable only for adrenal hemorrhage. Clinical and laboratory details are scant and no patient material is available; however, consensus opinion was that he almost certainly shared the
same multi-system CSA disorder as his fully investigated younger sister. No other familial relationships exist between any of the other included children, and no family history of sideroblastic (or other) anemia was evident in any pedigrees analyzed. In aggregate the pedigree information is most consistent with autosomal recessive inheritance of this condition.

**Clinical Presentation**

Two-thirds of cases (8/12) presented in the neonatal period or within first 3 months of life; all had done so by 7 months except child #1, who presented at 18 months. Diagnosis of SA preceded laboratory confirmation of immunodeficiency in all cases. The manner of initial presentation was similar for most cases. Typically, it involved a febrile illness characterized by elevated inflammatory markers, poor feeding and gastrointestinal upset, for which no infective agent could be identified and during which anemia was detected. Two children (#3 and #4) were diagnosed when severe pallor was noted at birth, before the first febrile illness. Another (#9) suffered cyclical vomiting with metabolic acidosis but without prominent fevers.

**Anemia**

At presentation anemia was generally severe (median Hb 7.1g/dL; range 4.8–8.3) and markedly microcytic (median MCV 62.0fL; range 53.6–73.2). Peripheral blood smears typically revealed hypochromasia, microcytosis, variable schistocytosis, basophilic stippling and frequent nucleated erythrocytes. All children underwent investigation for common causes of congenital anemia, yielding identification of sickle cell trait in one (#8) and heterozygous α+ thalassemia in another (#1); however, none had significant hemoglobinopathies to explain the clinical presentation. Iron deficiency, red blood cell (RBC) membrane defects, enzymopathies and porphyrias were consistently excluded.

Bone marrow (BM) examination revealed plentiful ringed sideroblasts in all cases tested, typically representing >45-50% of developing erythroblasts (particularly the latest forms; Figure 2). The exception was child #4, in whom Perls’ stain was not performed/reported (as discussed above). Erythroid hyperplasia and dyserythropoiesis were universal features, with deficient cytoplasmic hemoglobinization consistently reported. Other myeloid lineages appeared unaffected. BM cytogenetics showed a normal karyotype for every case.

Most children displayed hyperferritinemia that was often marked. This was in part ascribed to the inflammatory nature of presenting episodes, but also likely reflected secondary iron overload as documented on BM examination. Child #1 underwent more extensive ferrokinetic investigation that revealed rapid uptake of iron into BM but suboptimal incorporation into RBCs, together with increased serum transferrin receptor levels, indicative of dysfunctional iron metabolism and ineffective erythropoiesis. Elevated transferrin receptor was noted in at least one other case (#12). Two children (#6; #8) had grossly elevated transferrin saturation on first biochemical iron studies.
Both had only been transfused to a modest degree and iron loading was attributed at least in part to increased gut absorption, as observed in certain other CSAs.

**Periodic Fevers**

A recurrent fever syndrome requiring multiple hospitalizations through infancy and early childhood was documented in at least eleven cases. Episodes were characterized by high fevers, systemic upset and elevated inflammatory markers, with repeated investigation failing to attribute an infective cause; vomiting and diarrhea were prominent features. In some individuals the documented frequency and duration of episodes were remarkably consistent, suggesting a periodic fever syndrome. For example, child #11 was hospitalized for fever at predictable 3–4 week intervals, with resolution within 5–7 days apparently uninfluenced by antibiotics. Periodicity varied between individuals and in some cases declined or increased in frequency over time. Child #2, for instance, initially suffered febrile episodes every 6 days, each lasting 4–5 days, but over several years the interval between attacks extended to >2 months.

**Immunodeficiency**

Recurrent inflammatory episodes prompted screening for co-existent immunodeficiency, which was investigated in different ways and at different stages across the cohort. Some individuals also suffered episodes of sinopulmonary bacterial infection, clinically distinct from the periodic fever episodes. Eleven children examined displayed significant B-cell lymphopenia and hypogammaglobulinemia. Serum immunoglobulin (Ig) levels at diagnosis varied considerably, reflecting differences in both severity of clinical phenotype and age when first tested (3 months to 5 years), given the different age-specific reference ranges and potentially confounding influence of maternal IgG transfer. When first tested, IgG levels ranged from severely deficient to normal, with frank panhypogammaglobulinemia developing later in the more mildly affected children. Where IgG subclasses were quantified, IgG1 and IgG3 levels were relatively lower than IgG2 and IgG4. Review of serial Ig measurements is further confounded by the influence of regular intravenous (IV) Ig replacement therapy in most cases; even so, a progressive decline in serum Ig levels over time was typically observed.

The pattern of hypogammaglobulinemia was mirrored by peripheral blood B cell (CD19+) quantification. B-lymphopenia was a unifying feature (range 0.016–0.22 x10^9/L; normal ~0.6–3.0), in the context of an initially normal total lymphocyte count. In several cases B-cell numbers fluctuated, dropping to nadir levels during inflammatory crises but partially recovering between attacks. While B-cell numbers were significantly reduced, other lymphocyte classes were initially preserved but progressively fell, resulting in profound B-, T- and NK-lymphopenia (Figure 3). The degree of hypogammaglobulinemia and B-lymphopenia at presentation broadly correlated with anemia severity and prognosis, irrespective of when in the disease course these were first measured. Additionally,
several children failed to mount durable serological response to both conjugated and unconjugated vaccinations.

The nature of child #11’s immunodeficiency was investigated in particular detail. In addition to low circulating B-cells and panhypogammaglobulinemia, skewed B-cell maturation was identified in peripheral blood: analysis of B-cell maturation showed the vast majority of circulating B-cells (~90%) to be naïve CD27−/IgD+, with class switched, CD27+/IgD− B-cells representing only 4%, suggesting a relatively early defect in B-cell maturation. Detailed maturation analysis by flow cytometry of BM samples revealed the presence of early B-precursors but with a ‘leaky’ maturation arrest before the cytoplasmic Igμ+ pre-B-II stage (Figure 4).

The only child without documented hypogammaglobulinemia or B-lymphopenia was #12, in whom both parameters tested early in life were in the low-normal range. However, he has suffered unexplained and severe recurrent febrile episodes from infancy, in the context of a severe microcytic, mutation-negative CSA. His inclusion within the cohort was carefully considered but agreed upon by consensus on review of clinical records. Currently 3 years of age he is being recalled for repeat immunology investigation.

Developmental Delay

Developmental delay was observed in all children except #11, who underwent bone marrow transplantation (BMT) at 9 months having yet to demonstrate any significant delay; he continues to meet normal milestones post-transplant. Although variably manifested and documented, delay was typically significant and sufficiently alarming to prompt further neurological investigation. Generalized and truncal hypotonia were recurring features, often severe, progressive and associated with gross motor developmental delay. Comprehension and communication were profoundly impaired in many children. In keeping with his milder immunodeficiency phenotype child #12’s developmental delay was relatively modest, essentially confined to delayed speech development; at latest follow up he remains developmentally normal but under close observation.

Additional Syndromic Manifestations

The similarities in clinical presentation demonstrate a convincing link between the cases described. In addition, most members of the cohort displayed other, variable but recurring multi-system abnormalities (summarized in Table 2):

Sensorineural hearing impairment. At least five children developed severe bilateral sensorineural deafness. One received bilateral cochlear implants.

Recurrent seizures. Five children suffered recurrent seizures early in life. These ranged from partial complex to generalized tonic-clonic, displaying a variety of related EEG abnormalities.
Other neurological/neuromuscular abnormalities. In addition to those features already mentioned, gross ataxia and other cerebellar signs occurred in several cases. Neuroimaging variously revealed cerebral atrophy; delayed cortical white matter myelination; abnormal enhancement of external capsule and thalamus; communicating hydrocephalus; and cerebellar abnormalities including decreased perfusion.

Nephrocalcinosis/renal tubular dysfunction. Three children developed renal calculi at an early age; one (#9) underwent thorough metabolic investigation and was found to have significant hypercalciuria (3.0mol/mol Cr; normal range 0.08–0.6). A fourth (#3) was diagnosed with renal tubular Fanconi syndrome, a renal tubular acidosis frequently associated with nephrocalcinosis; this resulted in chronic hypokalemia and hypophosphatemia although no actual calculi were documented during life. Most children, however, were not specifically evaluated for these entities.

Aminoaciduria and other metabolic abnormalities. Six children displayed excessive generalized aminoaciduria, with increased urinary metabolites of the tricarboxylic acid pathway additionally detected in child #2. One (#8) had grossly elevated plasma alanine levels, which have decreased over time. In no cases were abnormalities diagnostic of any specific inborn error of metabolism. Most displayed further nonspecific metabolic and biochemical abnormalities but with no clear pattern, including hyperglycemia and chronic hypokalemia, hypophosphatemia and hypocalcemia. Metabolic acidosis was a common feature, particularly during acute febrile crises.

Cardiomyopathy: Two children were diagnosed with moderate or severe, symptomatic dilated cardiomyopathy during second year of life; notably, both had expressed a particularly severe hematologic/immunologic phenotype. Moreover, cardiac failure was the primary or a contributing cause of death in at least five of the seven deceased children. Monitoring revealed only modest cardiac and systemic iron overload, indicating that cardiac disease (typically diagnosed within the first two years, or causing death within five years of life) was not directly attributable to pathological effects of iron overload.

Pigmentary retinitis. At least four children were diagnosed with a retinitis resembling retinitis pigmentosa (RP); in one this manifested as the atypical RP variant retinitis punctata albescens. However, again most were not screened for this.

Other features. Several other abnormalities were noted in isolated cases and their relevance to the syndrome remains unclear. Hepatosplenomegaly was documented in four children but when present was typically mild. Brittle hair was noted in three cases. Another (#1) displayed chronic ichthyotic skin changes, punctuated with eruptions of erythema and/or hypopigmentation. Skin biopsy revealed a perivascular lymphohistiocytic infiltrate within papillary dermis but lacked specific diagnostic features; electron microscopy showed small foci of fibrillar material resembling amyloid. One child (#11) had detailed investigation of mitochondrial respiration in muscle tissue, revealing moderately reduced
activity of respiratory chain complex I with borderline low complex II and IV activity. Muscle histology revealed accentuated staining for lipid and the oxidative enzymes cytochrome oxidase, NADH and SDH, suggesting a nonspecific metabolic myopathy.

**Clinical Interventions**

Ten children required regular or intermittent RBC transfusional support from infancy or early childhood. This resulted in secondary transfusional iron overload necessitating iron chelation therapy in surviving cases. The other two displayed a milder phenotype in which anemia remained stable without clinical indication for regular transfusion. Pyridoxine was administered to most children on recognition of CSA, with no discernible impact on Hb, symptoms or transfusion requirement. Other dietary supplements (e.g. thiamine; riboflavin) were attempted in certain cases, without clinical benefit. Ten children received regular or intermittent IV Ig replacement therapy. This was observed in some cases to reduce bacterial sinopulmonary infections but had minimal influence on the ‘sterile’ periodic fever episodes. One (#1) received anakinra, which was successful in alleviating the febrile episodes; however, development of allergy necessitated treatment discontinuation.

One child (#11) underwent myeloablative allogeneic BMT from an unrelated donor at 9 months of age. He was heavily transfusion-dependent since presentation at 7 weeks and subsequently suffered debilitating periodic fevers requiring frequent hospitalization. Recognition of other cases forming this cohort suggested an unacceptably poor life expectancy. Moreover, at 9 months of age organ function remained good, permitting myeloablative transplantation with busulfan, cyclophosphamide and alemtuzumab conditioning. Full donor chimerism was confirmed by day +28 and is maintained beyond three years post-transplant. Around day +100 he became unwell with an episode initially mimicking his pre-transplant ‘sterile’ inflammatory crises; on this occasion *Enterobacter cloacae* was isolated from blood cultures and he responded to appropriate antibiotics. He remains clinically well with normal blood count and serum immunoglobulin levels, normal growth and development, and no admissions for unexplained inflammatory illness since transplant (Figure 5). However, 32 months post-transplant a pigmentary retinitis was detected on routine surveillance. He has yet to declare any other syndromic manifestations.

**Outcome**

The pace of progression and prognosis reflected the considerable heterogeneity of the disorder, with distinctly more and less severe phenotypes observed. At time of publication, seven of the children have died (58%), at median 4 years of age (range 16 months–14 years). Those more severely affected failed to survive beyond the third year of life, having suffered severe transfusion-dependent anemia and very low B-cell numbers and Ig levels from diagnosis, with cardiomyopathy seemingly associated with poorer prognosis and early death. Cases #3 and #1 survived until age 7 and 14 years respectively, despite both suffering significant neurodegenerative complications from early childhood. All deaths were from cardiac or multi-organ failure, most in the context of sepsis.
unresponsive to broad-spectrum antibiotics. Of the five surviving children, three have displayed a noticeably milder phenotype: two (#2; #9) without regular transfusion requirement who remain alive in eighth and sixth years of life respectively, and another (#12) with a noticeably milder immunodeficiency. The longest surviving child remains alive aged 19 years, but with significant neurological impairment and transfusional iron overload. Child #11 remains alive into fifth year of life, 41 months post BMT; the hematological and immunological manifestations have apparently been completely corrected. Interventions and outcomes are summarized in Table 3.

Discussion

We describe a novel syndrome of CSA, B-cell lymphopenia, panhypogammaglobulinemia, periodic fevers and developmental delay, with additional recurring syndromic features. The similarities in phenotype suggest a probable shared etiology. Following recognition of these features the search for additional cases has been remarkably successful, suggesting the likelihood that further cases exist.

The defining hematologic feature is that of severe microcytic CSA, usually presenting in infancy and conferring transfusion dependence. SAs are a heterogenous group of inherited or acquired anemias with the shared hallmark of the ringed sideroblast in BM. Ringed sideroblasts are pathological erythroblasts with a ring of perinuclear, iron-positive granules that represent iron-laden mitochondria, reflecting disrupted iron metabolism. Genetically defined CSAs are uncommon or extremely rare, have a variety of inheritance patterns and may result from an erythroid-specific defect or as part of a multi-system syndrome. None of the previously defined causes of CSA is associated with immunodeficiency.

The archetypal ‘pure’ SAs result from defects in heme biosynthesis, causing mitochondrial siderosis, microcytic anemia and secondarily progressive systemic iron overload. X-linked SA (XLSA) is the most common and results from mutations in \( ALAS2 \), with new mutations continuing to be described. Nevertheless, XLSA accounts for <40% of cases of CSA and was an unlikely explanation in our cohort given the purely erythroid-specific expression of \( ALAS2 \). Nonetheless \( ALAS2 \) mutations were excluded in all cases. A more attractive candidate was \( SLC25A38 \), which encodes a putative inner mitochondrial membrane glycine importer. Recessive \( SLC25A38 \) mutations cause a phenotypically similar SA, with failure to deliver glycine having a similar impact on heme synthesis as \( ALAS2 \) deficiency. Intriguingly, the second highest tissue expression (after the erythron) is in CD19+ B-cells. However, \( SLC25A38 \) sequencing was normal in our cohort.

Mitochondria also serve as a major site of Fe-S biogenesis. Functional defects in this system cause severe mitochondrial iron overload, defective activity of Fe-S-dependent enzymes and oxidative damage, explaining several syndromic forms of SA. Autosomal recessive \( GLRX5 \) defects can cause a microcytic SA, although the single described case had far milder anemia and fewer
ringed sideroblasts than our cohort, presenting in the fifth decade of life\textsuperscript{13,14}. Missense mutations of \textit{ABCB7} result in the X-linked SA and ataxia syndrome (XLSA/A)\textsuperscript{15-17}, of potential interest given the cerebellar abnormalities observed in several of our cases. However, neither gene has any reported impact on lymphopoiesis and mutations were excluded by sequence analysis.

Another defining feature of our syndrome was clinically significant immunodeficiency with panhypogammaglobulinemia and/or B-lymphopenia, often profound and present from an early age. Since this is not a feature of any described CSA syndrome, coincidental occurrence of a separate cause for immunodeficiency was considered. However, the reduction in mature CD19+ B-cells in peripheral blood and early onset argue against co-incidental common variable immunodeficiency, the most common cause of selective B-immunodeficiency, which generally presents later in life with normal circulating mature B-cell numbers\textsuperscript{18}. The ‘leaky’ maturation block within the B-precursor compartment at the pre-B-II stage (Figure 4) most closely resembles that observed in X-linked agammaglobulinemia, which results from mutations in Bruton’s tyrosine kinase (Btk) and accounts for 85% of patients with defects in early B-cell development\textsuperscript{19}. However, direct sequencing of \textit{BTK} gene coding exons, splice sites and \textit{BTK} transcript products failed to identify any mutations; likewise for the \textit{λ5/14.1} gene, which encodes a component of the pre-B-receptor complex transiently expressed by B-precursors, deficiency of which causes a similar phenotype\textsuperscript{20,21}. Pertinently, SA has not been described with any congenital immunodeficiency syndrome. Moreover, the progressive decline in T- and NK-cells seen in several cases suggests a more general impact on lymphopoiesis than a purely selective B-cell maturation defect.

Another recurring feature was periodic episodes of fever and inflammation, clinically resembling acute sepsis but typically lacking a causative organism and associated with gastrointestinal upset. Several cases documented remarkably consistent periodicity, cycling every 2–4 weeks and resolving within 5–7 days. This pattern resembles that observed in the periodic fever syndromes: auto-inflammatory disorders arising from mutations (usually autosomal recessive) in genes regulating IL-1β secretion and other elements of innate immunity\textsuperscript{22-24}. However, these have no documented (or intuitive) impact on B-cell maturation and direct sequencing of relevant genes excluded familial Mediterranean fever (\textit{MEFV}), TNF-receptor associated period fever (\textit{TNFRSF1A}), hyperimmunoglobulin D syndrome (\textit{MVK}) and the cryopyrinopathies (\textit{NLRP3}) in several cases.

The patients displayed additional non-hematologic/immunologic manifestations. Most common was progressive neurodegeneration and developmental delay, often with other neurologic, cardiologic, metabolic and other multi-system abnormalities. These disparate features recall syndrome complexes associated with the mitochondrial cytopathies (MCs), a diverse group of disorders characterized by impaired mitochondrial respiratory chain function and energy production. Mitochondrial proteins are the products of both nuclear DNA (nDNA) and separately inherited mitochondrial DNA (mtDNA), with the latter displaying up to 100-fold higher mutation rate\textsuperscript{25}. mtDNA contains 37 genes encoding 13 mitochondrial proteins (subunits of respiratory chain enzyme complexes), 22 transfer RNA (tRNA) species and 2 ribosomal RNAs\textsuperscript{26,27}. Mutations of mtDNA and
related nDNA genes can cause major disruption of mitochondrial biogenesis and function, resulting in distinct multi-system disorders with protean (but overlapping) clinical features\textsuperscript{28-30}. Neurological involvement is frequent, including neurodegeneration, ataxia, sensorineural deafness and seizures\textsuperscript{26}. Lactic acidosis, hyperalaninemia, renal tubular dysfunction, nephrocalcinosis, RP and brittle hair are also common features, resembling the spectrum of abnormalities seen in our cohort.

Hematologic manifestations are less prominent but do feature in some MCs\textsuperscript{31}, including rare syndromic CSAs. Large mtDNA deletions are responsible for Pearson marrow-pancreas syndrome, characterized by exocrine pancreatic dysfunction with SA and BM failure\textsuperscript{2,32}. Nearly half display heteroplasmia for a canonical 4977-bp mtDNA deletion, although others have different, occasionally nonoverlapping deletions removing tRNA species required for mitochondrial translation\textsuperscript{33}. However, the anemia is typically macrocytic, with other cytopenias and characteristic vacuolization of early erythroid/myeloid precursors. Moreover, pancreatic abnormalities were not prominent in our cohort. Large mtDNA deletions can alternatively (or eventually) cause the distinct Kearns-Sayre syndrome, characterized by progressive ophthalmoplegia, RP, cardiac conduction defects, cerebellar ataxia, deafness, myopathy and endocrinopathies. SA can feature, but despite phenotypic overlap with our cohort the anemia is typically normo-/macrocytic and less severe. Significant mtDNA deletions, mutations and rearrangements were excluded by direct sequencing of the entire mitochondrial genome in most of our cases. Furthermore, immunodeficiency is not a typical feature of most MC syndrome complexes.

However, precedent for MCs involving perturbed immune function does exist, with a neonatal-onset mitochondrial respiratory chain disease associated with mtDNA depletion and progressive T cell immunodeficiency described. Additional features included cytopenias, psychomotor retardation, axial hypotonia, hypoplasia of corpus callosum and impaired myelination\textsuperscript{34}. Moreover, the lack of overt mtDNA depletion in our cases does not exclude a MC. Most genes involved in mitochondrial protein synthesis, expression and function are nuclear in origin, with >100 nDNA genes implicated in various MCs\textsuperscript{29}. Indeed, nDNA defects account for >80% of pediatric MC cases (>50% in adults)\textsuperscript{35}. A pertinent association between defective mitochondrial protein expression and CSA is provided by the mitochondrial myopathy with lactic acidosis and sideroblastic anemia (MLASA) syndrome. This was first identified in patients of Persian-Jewish descent with recessive mutations of \textit{PUS1} (on chromosome 12q24.33), resulting in decreased pseudouridylation of mitochondrial tRNAs, reduced tRNA stability/function and impaired protein translation\textsuperscript{36,37}. Clinical expression is variable but typically manifests with myopathy, lactic acidosis, SA, and occasionally with mental retardation and dysmorphia. Biochemical investigation may reveal decreased activity of respiratory chain complexes I–IV\textsuperscript{2}. A similar syndrome is described in patients with mutations of \textit{YARS2} (chromosome 12p11.21), which encodes an enzyme (tyrosyl-tRNA synthetase) that catalyses binding of tyrosine to its cognate tRNA. Reduced enzymatic activity results in decreased mitochondrial protein synthesis and mitochondrial respiratory chain dysfunction involving complexes I, III and IV\textsuperscript{38}. Some patients developed cardiomyopathies. Neither \textit{PUS1} nor \textit{YARS2} mutations were found in our cases, and important phenotypic differences remain: notably, immunodeficiency is not described in MLASA.
However, alternative nuclear genes important in the synthesis, function and maintenance of tRNA and mitochondrial protein translation seem logical candidates and are currently being investigated. The primary defect in our syndrome must embrace both B-cell development and mitochondrial iron metabolism, in an as yet-undefined manner.

This study is limited by its retrospective nature. The patients had been diagnosed and investigated sporadically, in different centers and countries, most long before initiation of this project. Consequently a standardized, consistent approach to investigating all aspects of this multi-system disorder was impossible. Moreover, given that some historical cases were managed at multiple centers, comprehensive and specific clinical details were sometimes difficult to obtain. This resulted in considerable variation in amount and quality of information gathered. Although these cases cannot yet be formally unified by a shared genetic insult, all have undergone detailed central review with consensus agreement of sufficient similarity to warrant collation and reporting as a single clinical entity at this time. Laboratory investigation on patient material is ongoing.

Until the molecular basis of this syndrome is formally established we propose naming the syndrome “Sideroblastic anemia with Immunodeficiency, Fevers and developmental Delay” (SIFD), with the following diagnostic criteria:

**Required:**

(i) Sideroblastic anemia: which is severe, microcytic and of early onset

(ii) B-cell immunodeficiency: panhypogammaglobulinemia and/or absolute reduction in mature CD19+ B-cells in peripheral blood

(iii) Fevers: which are recurrent and associated with systemic inflammation in the absence of an identifiable infective cause

(iv) Developmental delay

**Additional features that may be present:**

(i) Growth retardation

(ii) Sensorineural deafness

(iii) Seizures

(iv) Cerebellar signs

(v) Cerebral hemisphere &/or cerebellar neuroimaging abnormalities

(vi) Dilated cardiomyopathy

(vii) Nephrocalcinosis

(viii) Aminoaciduria

(ix) Retinitis pigmentosa

(x) Brittle hair

(xi) Hepatosplenomegaly
Conclusion

We describe a novel syndromic form of early onset, severe CSA associated with B-immunodeficiency, periodic fevers and developmental delay. Additional features reveal a multi-system disorder reminiscent of a mitochondrial cytopathy but without evidence of mtDNA depletion and with some atypical features. Regular blood transfusion, IVIg replacement and iron chelation are likely required treatments but have had little impact on quality of life determinants. Mortality within the first decade of life was high, typically in the context of acute sepsis without positive microbiology and unresponsive to broad-spectrum antibiotics. This might implicate the ‘cytokine storm’ as contributing to these terminal events, suggesting a potential role for alternative interventions (e.g. IL-1 blockade, employed with some success in one case). However, there is evidence of variable penetrance. Two affected siblings have demonstrated somewhat different clinical outcomes, and some cases have shown a distinctly milder phenotype with survival into teenage years and/or less intensive transfusion requirement. The only child to receive allogeneic BMT did so after careful consideration of his severe disease expression, and engraftment of donor hematopoiesis has successfully reversed the hematologic and immunologic manifestations. He has since developed retinitis but remains otherwise well more than three years post-transplant. This provides rationale for considering early BMT in other cases and emphasizes the importance of early recognition/diagnosis. To what extent multi-system involvement is preventable by BMT remains unknown.

Although a rare syndrome, the relative ease with which 12 cases have been identified in a short period of time suggests numerous other children may be affected. Diagnosis of this multi-system disorder requires specialist hematology and immunology investigations (e.g. Perls’ examination of BM; B-cell enumeration) and given the diverse features, with variable expression, it might present to a range of specialists. Awareness of SA as a potential explanation for the anemia is particularly important, since it may not be routinely considered alongside more familiar causes of microcytosis. Moreover, certain features (e.g. retinitis; aminoaciduria) may not be clinically apparent and without specific investigations would remain undetected. Our study represents a first step in raising this awareness. We have established a database and welcome correspondence from colleagues recognizing similar cases. Work is ongoing to establish the molecular basis of the disorder using primary patient material and cell lines derived from these children.
Acknowledgements

Authors wish to acknowledge grant support from the US National Institutes of Health (NIH R01 DK087892) to M.D.F; the US Department of Veterans Affairs and the Oklahoma Center for Advancement of Science and Technology to S.S.B; and a Fellowship from the UK National Institute for Social Care and Health to to S.J. We thank Mark Layton from Imperial College, London, UK, for organizing serum transferrin receptor and ferrokinetic measurements on child #1 and his permission to include the data here; in addition, it was Mark Layton who made the initial diagnosis of sideroblastic anemia in child #1 and referred the sample to Cardiff for molecular investigation of ALAS2. We thank Mirjam van der Burg and Jacques J.M. van Dongen from Erasmus Medical Center, Rotterdam, Netherlands, for performing the B cell maturation analysis on child #11’s bone marrow. We also thank all the physicians and other healthcare professionals involved in the care of the children included in this study.

Authorship

Contribution: R.F.W and D.H.W. conceived the project and identified cases referred from European centers, in collaboration with A.M. Concurrently, M.D.F. identified a similar pattern of cases referred from several North American centers and compiled a similar database, in collaboration with the referring physicians. All authors were involved in the initial establishment of the International Collaborative Group. D.H.W. created the data collection proforma, provided clinical information for case #11, collated and analyzed returned information for all cases, and wrote the final manuscript and clinical vignette for case #11. All other authors contributed cases with which they had direct clinical involvement, providing information through completion of a data collection proforma, drafting of clinical vignettes and direct communication with D.H.W. M.D.F. and A.M. were primarily responsible for molecular analysis for known CSA mutations on samples from the North American and European children respectively. M.D.F. is coordinating the central database of putative SIFD cases and the work investigating its molecular basis using patient-derived cell lines and other primary material from these cases. All authors reviewed and provided revisions of the full manuscript in the drafting process.

Conflict-of-interest disclosure: None of the authors declare any competing financial interests.
References


Tables

Table 1. Selected demographics and laboratory parameters for the 12 children identified with congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers and developmental delay. Laboratory results are the earliest recorded results available, unless otherwise stated. Missing data (indicated by ?) resulted from inability to retrospectively retrieve laboratory records despite best efforts of treating physicians; where possible a qualitative indication is instead provided, based on available documentation and correspondence and physician recall of the case/s. Cases were numbered in the order they were included for analysis. († and § indicate sibling pairs; ND: not done; RS: ringed sideroblasts; BM: bone marrow; Igs: immunoglobulins; IVlg: intravenous immunoglobulin replacement therapy; *: first iron studies performed (or retrievable) were measured after commencing regular transfusion program.
<table>
<thead>
<tr>
<th>Case #</th>
<th>Country of Birth</th>
<th>Year of Birth</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Consanguinity?</th>
<th>Age at Presentation</th>
<th>Blood Count at Diagnosis</th>
<th>RS (% of BM erythroblasts)</th>
<th>Iron Studies (Pre-transfusion unless indicated by *)</th>
<th>Serum Igs at Diagnosis (Pre-IVlg unless indicated by *)</th>
<th>B cell Numbers at Diagnosis (x10^9/L) / [ %total lymphs ]</th>
<th>Notes</th>
</tr>
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<td>1†</td>
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<td>1996</td>
<td>F</td>
<td>Pakistani</td>
<td>Yes</td>
<td>18 months</td>
<td>7.2</td>
<td>53.6</td>
<td>52-65%</td>
<td>19</td>
<td>139</td>
<td>352</td>
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<td>41290*</td>
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<td>Low Normal Low Low</td>
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<td>2†</td>
<td>Wales</td>
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<td>M</td>
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<td>95</td>
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<td></td>
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<td>1988**</td>
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<td>7.0</td>
<td>61.0</td>
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<td>1998*</td>
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<tr>
<td>7</td>
<td>France</td>
<td>2003</td>
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<td>7 months</td>
<td>6.0</td>
<td>62.0</td>
<td>&gt;40%</td>
<td>ND</td>
<td>729</td>
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<td>90%*</td>
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<td>7 months</td>
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<td>11</td>
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<td>7 weeks</td>
<td>4.8</td>
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<td>139</td>
<td>35</td>
<td>62</td>
</tr>
<tr>
<td>12</td>
<td>USA</td>
<td>2009</td>
<td>M</td>
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<td>57.0</td>
<td>40%</td>
<td>30%</td>
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Table 2. Summary of clinical phenotype for each of the cases in the cohort. Detailed clinical vignettes describing each child’s clinical course are provided online as supplementary material. (+ denotes feature confirmed during life; - denotes feature confirmed to be absent during life or at latest follow up; +/- denotes borderline/mild presence of abnormality or clinical feature; blank cells denote insufficient information, usually as feature was not specifically investigated or considered during life; ND: BM ringed sideroblastosis not confirmed but highly likely given similarities to older sibling with confirmed CSA (discussed in text); § clinical course characterized by recurrent inflammatory episodes with gastrointestinal upset and raised inflammatory markers, although high fevers were not recalled as a prominent feature; * low normal results when tested early in life; see main text for explanation of rationale for child’s inclusion within cohort).

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<th>Case Number:</th>
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<tr>
<td>Sideroblastic anemia</td>
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<td>ND</td>
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<tr>
<td>B lymphopenia &amp;/or Hypogamma-globulinemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
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<td>Recurrent inflammatory episodes</td>
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<tr>
<td>Seizures</td>
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<td>+</td>
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<tr>
<td>Neuroimaging abnormalities</td>
<td>Cerebral atrophy;</td>
<td>Cerebral atrophy</td>
<td>Cerebral atrophy; Decreased cerebellar perfusion</td>
<td>Communicating hydrocephalus; Macrocephaly</td>
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<td>-</td>
<td>Cerebral atrophy; Abnormal enhancement of external capsule &amp; thalamus</td>
<td>Delayed white matter myelination</td>
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<tr>
<td>Nephrocalcinosis</td>
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<td>+</td>
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<tr>
<td>Aminoaciduria +/- Hyperalaninemia</td>
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<td>+</td>
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<tr>
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<td>+/-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Lactic acidosis</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Brittle hair</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Other features</td>
<td>Ichthyotic skin changes; Abnormal high amplitude slow activity on EEG</td>
<td>Increased urinary TCA intermediates</td>
<td>Renal tubular Fanconi syndrome</td>
<td>Malabsorption; Small bowel villous atrophy; Avascular necrosis (hip)</td>
<td>Pancreatic insufficiency</td>
<td>Hypercalciuria</td>
<td>Torticollis</td>
<td>Hypertension</td>
<td>Nonspecific metabolic myopathy on muscle biopsy</td>
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</table>

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Table 3. Summary of selected interventions attempted and outcome for each of the children within the cohort. (IVIg: intravenous immunoglobulin; *child #11’s transfusion-dependence and immunodeficiency fully resolved after bone marrow transplant).

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<th>Case #</th>
<th>INTERVENTIONS</th>
<th>OUTCOME</th>
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<tr>
<td>12</td>
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</table>
Figure Legends

Figure 1. Pedigree for cases #1 and #2 with descriptions of phenotypes displayed. The other siblings unaffected by sideroblastic anemia and immunodeficiency exhibited a different cluster of features consistent with the Cenani-Lenz syndrome.

Figure 2. Photomicrographs of bone marrow smears, demonstrating presence of ringed sideroblasts and erythroid hyperplasia with dyserythropoietic features. Row A: case #1 (Perl’s stain); Row B: case #11 (H&E); Row C: case #11 (Perl’s stain).

Figure 3. Graph showing decline in circulating B, NK and T cell numbers in case #1 with increasing age. This child died during 15th year of life, at which point she displayed profound lymphocytopenia of all three lymphocyte classes. (Units are in cells/μL)

Figure 4. Detailed B cell maturation analysis performed on bone marrow from case #11. A) Bar chart displays proportion of B cells at different stages of maturation within the bone marrow compartment as determined by flow cytometric analysis. Lower bar is from child #11 aged 8 months, demonstrating a leaky maturation block before the cytoplasmic Igμ-positive pre-B-II cell stage. Middle bar shows repeat analysis in the same patient 10 months after bone marrow transplant, at which time the pattern had reverted to that observed in age-matched normal controls (upper bar). B) Relative proportions of B cells at different maturation stages in bone marrow samples before and after bone marrow transplant. (Analysis performed by M van der Burg and J.J.M. van Dongen at Erasmus Medical Center, Rotterdam, Netherlands).

Figure 5. Graphical representation of selected clinical events in child #11’s early disease course. Line graph traces serial C-reactive protein levels, illustrating recurrent inflammatory episodes occurring with periodicity of 4-6 week intervals; red lines represent periods of hospitalization. Other than a single early episode of Enterobacter septicemia, bone marrow transplant appears to have effectively cured the immunologic and fever components of the disease as well as the sideroblastic anemia.
Figure 1

Still birth 33wk


Syndactyly  Syndactyly; Radius/ulnar fusion;
Congenital sideroblastic anemia; Recurrent fevers;
B cell lymphopenia; Hypogamma-
Hypogammaglobulinemia; globulinemia;
Recurrent fevers; Developmental
developmental
delay; delay;
Cerebral atrophy; Cerebral atrophy;
Sensorineural Sensorineural
default; deafness; deafness;
Nephrocalcinosis; Nephrocalcinosis;
Ichthyotic skin Pigmentary retinitis
changes;

[borderline
low/normal Hb and
MCV, slightly
decreased MCH,
alpha thalassemia
not yet tested for, no
recurrent fevers]
Figure 2
Figure 3
Figure 4

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<th>% Positivity Post-Transplant</th>
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</tr>
<tr>
<td>- Stage 3</td>
<td>2.5%</td>
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<td>- Stage 4</td>
<td>6.9%</td>
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<td>- Stage 5</td>
<td>39.0%</td>
<td>16.6%</td>
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<td>- Stage 6</td>
<td>16.7%</td>
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<td>- Stage 7</td>
<td>11.5%</td>
<td>30.2%</td>
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<td><strong>Immature B-cell (SmIgM+/SmIgD–)</strong></td>
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<td>- Stage 8</td>
<td>5.5%</td>
<td>19.6%</td>
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<td><strong>Mature B-cell (SmIgM+/IgD+)</strong></td>
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<tr>
<td>- Stage 9</td>
<td>14.4%</td>
<td>16.8%</td>
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</table>
Figure 5

- ICU admission with Enterobacter cloacae septicaemia
- No further episodes of periodic inflammatory illness since BMT
A novel syndrome of congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers and developmental delay (SIFD)

Daniel H. Wiseman, Alison May, Stephen Jolles, Philip Connor, Colin Powell, Matthew M. Heeney, Patricia J. Giardina, Robert J. Klaassen, Pranesh Chakraborty, Michael T. Geraghty, Nathalie Major-Cook, Caroline Kannengiesser, Isabelle Thuret, Alexis A. Thompson, Laura Marques, Stephen Hughes, Sylvia S. Bottomley, Mark D. Fleming and Robert F. Wynn

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