

Research Articles

Alter, B. P. (2005). "Bone marrow failure: a child is not just a small adult (but an adult can have a childhood disease)." Hematology Am Soc Hematol Educ Program: 96-103.

Aplastic anemia may be inherited or acquired. The distinction between these lies not in the age of the patient, but in the clinical and laboratory diagnoses. Adult hematologists must consider adult presentations of the inherited disorders, in order to avoid incorrect management of their patients. Physicians for adult patients must also realize that children with inherited disorders now survive to transition into their care. The major inherited bone marrow failure syndromes associated with development of pancytopenia include Fanconi anemia, dyskeratosis congenita, Shwachman-Diamond syndrome, and amegakaryocytic thrombocytopenia. The ages at presentation are highly variable, but often include individuals of adult age who have previously undiagnosed Fanconi anemia or dyskeratosis congenita. Many of the genes responsible for these disorders have been identified (12 Fanconi anemia genes, 3 dyskeratosis congenita genes, and 1 each for Shwachman-Diamond syndrome and amegakaryocytic thrombocytopenia). A high index of suspicion and specific testing of children or adults with what appears to be acquired aplastic anemia may identify inherited disorders. Correct classification of patients with aplastic anemia of any age is mandatory for their appropriate management.

Alter, B. P. (2007). "Diagnosis, genetics, and management of inherited bone marrow failure syndromes." Hematology Am Soc Hematol Educ Program 2007: 29-39.

The inherited bone marrow failure syndromes are traditionally considered to be pediatric disorders, but in fact, many of the patients now are diagnosed as adults, and many diagnosed as children now live to reach adulthood. The most common of these rare disorders include Fanconi anemia, dyskeratosis congenita, Shwachman-Diamond syndrome and amegakaryocytic thrombocytopenia, which often develop aplastic anemia and may evolve into myelodysplastic syndrome and acute myeloid leukemia; and Diamond-Blackfan anemia, severe congenital neutropenia, and thrombocytopenia absent radii, single cytopenias that rarely if ever become aplastic but have increased risks of leukemia. In addition, the first three syndromes have high risks of solid tumors: head and neck and anogenital squamous cell carcinoma in Fanconi anemia and dyskeratosis congenita, and osteogenic sarcoma in Diamond-Blackfan anemia. Diagnosis of a marrow failure syndrome requires recognition of characteristic physical abnormalities when present, and consideration of these disorders in the differential diagnosis of patients who present with "acquired" aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia, or atypically early cancers of the types seen in the syndromes. Ultimate proof will come from identification of pathogenic mutations in genes associated with each syndrome.

Research Articles

Bagby, G. C., J. M. Lipton, et al. (2004). "Marrow failure." Hematology Am Soc Hematol Educ Program: 318-36.

New discoveries in cell biology, molecular biology and genetics have unveiled some of the pathophysiological mysteries of some of the bone marrow failure syndromes. Many of these discoveries have revealed why these syndromes show so much clinical overlap and some hold the potential for influencing the development of new therapies. In children and adults with pancytopenia and hypoplastic bone marrows proper differential diagnosis requires that some attention be directed toward defining molecular and cellular pathogenetic mechanisms because, once identified, some of these mechanisms will clearly suggest rational therapeutic approaches, treatment options that should be avoided, or both. In Section I, Drs. Jeffrey Lipton and Grover Bagby review the approach to diagnosis and management of patients with the inherited bone marrow failure syndromes, Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, and the Shwachman-Diamond syndrome. Extraordinary progress has been made in identifying the genes bearing pathogenetically relevant mutations in these disorders, but slower progress has been made in defining the precise functions of the proteins these genes encode in normal cells, in part because it is increasingly obvious that the proteins are multifunctional. In practice, it is clear that in patients with dyskeratosis congenita and Fanconi anemia, the diagnosis must be considered not only in children but in adults as well. In Section II, Dr. Elaine Sloand outlines a very practical and evidence-based approach to diagnosis and management of acquired hypoplastic states emphasizing overlap between non-clonal and clonal hematopoiesis in such conditions. The pathogenesis of T lymphocyte-mediated marrow failure is presented as a clear-cut rationale for use of immunosuppressive therapy and stem cell transplantation. Practical management of patients with refractory disease with and without evidence of clonal evolution (either paroxysmal nocturnal hemoglobinuria [PNH] or myelodysplasia [MDS]) is presented. In Section III, the challenge of hypoplastic MDS is reviewed by Dr. Charles Schiffer. After reviewing the most up-to-date classification scheme, therapeutic options are reviewed, focusing largely on agents that have most recently shown some promising activity, including DNA demethylating agents, thalidomide and CC5013, arsenic trioxide, and immunosuppressive therapy. Here are also outlined the rationale and the indications for choosing allogeneic bone marrow transplantation, the only therapy with known curative potential.

Beharry, S., L. Ellis, et al. (2002). "How useful is fecal pancreatic elastase 1 as a marker of exocrine pancreatic disease?" J Pediatr 141(1): 84-90.

OBJECTIVES: To evaluate the role of fecal elastase 1 (E1) as a marker of exocrine pancreatic insufficiency (PI). **STUDY DESIGN:** Fecal E1 was measured in patient groups with (1) failure to thrive but no pancreatic or intestinal disease (disease control patients); (2) PI; (3) pancreatic sufficiency; and (4) steatorrhea caused by a variety of intestinal diseases. **RESULTS:** Fecal E1 in all disease control patients exceeded 200 microg/g stool. Only 1 (2%) of 50 patients with PI

Research Articles

exceeded the minimum reference value of 100 microg/g stool. In contrast, 3 (11%) of 28 patients with pancreatic sufficiency (with Shwachman-Diamond syndrome) had fecal E1 concentrations <100 microg/g stool, as did 5 (20%) of 25 patients with steatorrhea from intestinal causes, all of whom had diluted feces caused by short gut. **CONCLUSIONS:** Fecal E1 is a useful noninvasive screening test of PI in childhood. A negative test (>100 microg/g stool) had 99% predictive value for ruling out PI. However, a positive test in those with short gut or Shwachman-Diamond syndrome must be interpreted with caution.

Bhatla, D., S. M. Davies, et al. (2008). "Reduced-intensity conditioning is effective and safe for transplantation of patients with Shwachman-Diamond syndrome." Bone Marrow Transplant.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative treatment for the BM dysfunction seen in patients with Shwachman-Diamond syndrome (SDS). Historically, these patients have fared poorly with intensive conditioning regimens with increased regimen-related toxicity especially involving the heart and lungs. We report our institutional experience with a reduced-intensity-conditioning protocol in seven patients with SDS and BM aplasia or myelodysplastic syndrome/AML. The preparative regimen consisted of Campath-1H, fludarabine and melphalan. Four patients received matched related marrow and three received unrelated stem cells (two PBSCs and one marrow). All but one was 8 of 8 allele HLA matched. All patients established 100% donor-derived hematopoiesis. No patient in this cohort developed grades III-IV GVHD. One patient had grade II skin GVHD that responded to systemic corticosteroids and one had grade I skin GVHD, treated with topical corticosteroids. Two out of seven patients developed bacterial infections in the early post transplant period. Viral infections were seen in four out of seven patients and were successfully treated with appropriate antiviral therapy. All patients are currently alive. These data indicate that HSCT with reduced-intensity conditioning is feasible in patients with SDS and associated with excellent donor cell engraftment and modest morbidity. *Bone Marrow Transplantation* advance online publication, 26 May 2008; doi:10.1038/bmt.2008.151.

Boocock, G. R., J. A. Morrison, et al. (2003). "Mutations in SBDS are associated with Shwachman-Diamond syndrome." Nat Genet 33(1): 97-101.

Shwachman-Diamond syndrome (SDS; OMIM 260400) is an autosomal recessive disorder with clinical features that include pancreatic exocrine insufficiency, hematological dysfunction and skeletal abnormalities. Here, we report identification of disease-associated mutations in an uncharacterized gene, SBDS, in the interval of 1.9 cM at 7q11 previously shown to be associated with the disease. We report that SBDS has a 1.6-kb transcript and encodes a predicted protein of 250 amino acids. A pseudogene copy (SBDSP) with 97% nucleotide sequence identity resides in a locally duplicated genomic segment of 305 kb. We found recurring mutations resulting from gene conversion in 89% of

Research Articles

unrelated individuals with SDS (141 of 158), with 60% (95 of 158) carrying two converted alleles. Converted segments consistently included at least one of two pseudogene-like sequence changes that result in protein truncation. SBDS is a member of a highly conserved protein family of unknown function with putative orthologs in diverse species including archaea and eukaryotes. Archaeal orthologs are located within highly conserved operons that include homologs of RNA-processing genes, suggesting that SDS may be caused by a deficiency in an aspect of RNA metabolism that is essential for development of the exocrine pancreas, hematopoiesis and chondrogenesis.

Boxer, L. and D. C. Dale (2002). "Neutropenia: causes and consequences." Semin Hematol 39(2): 75-81.

Neutrophils play a critical role in the acute inflammatory response and host-defenses against bacterial infections. Neutropenia, a deficiency of these cells, predisposes to infection, chiefly by organisms resident on body surfaces. The risk of infection is greatest with severe neutropenia, defined by an absolute blood neutrophil count (ANC) less than $0.5 \times 10^9/L$. Severe chronic neutropenia, lasting for more than a few weeks, can be caused by congenital marrow defects, as well as intrinsic and acquired disorders. Evaluation of patients begins with confirmation of neutropenia and examination of a blood smear. A careful review of the patient's medical history, family history, and physical examination is extremely important. Most severely neutropenic patients have a history of oral ulcers and inflammation and recurrent skin infections. Examination of a bone marrow aspirate and/or biopsy and cytogenetic testing are primary for diagnostic evaluation.

Calado, R. T., S. A. Graf, et al. (2007). "Mutations in the SBDS gene in acquired aplastic anemia." Blood 110(4): 1141-6.

Shwachman-Diamond syndrome (SDS; OMIM 260400), an inherited bone marrow failure syndrome, is caused by mutations in both alleles of the SBDS gene, which encodes a protein of unknown function. Here we report heterozygosity for the 258 + 2 T>C SBDS gene mutation previously identified in SDS patients in 4 of 91 patients with apparently acquired aplastic anemia (AA) but not in 276 ethnically matched controls (Fisher exact test, $P < .004$). Affected patients were young and had a poor outcome; they had reduced SBDS expression but no evidence of the pancreatic exocrine failure or skeletal abnormalities typical of SDS. Length of telomeres in granulocytes of SBDS heterozygous patients was short for their age, and in SDS patients with both SBDS alleles affected further analyzed, granulocytes' telomeres were even shorter, correlating in length with SBDS expression. Higher heterogeneity in telomere length also was observed in SDS patients. Telomerase activity of SBDS-deficient patients' lymphocytes was comparable with controls, and no physical interaction between SBDS protein and telomerase complex components (TERT or TERC) was established. We propose that heterozygosity for the 258 +

Research Articles

2 T>C SBDS mutation predisposes to AA by accelerating telomere shortening of leukocytes via a telomerase-independent mechanism.

Cesaro, S., R. Oneto, et al. (2005). "Haematopoietic stem cell transplantation for Shwachman-Diamond disease: a study from the European Group for blood and marrow transplantation." Br J Haematol 131(2): 231-6.

This report assessed the results of allogeneic stem cell transplantation (allo-SCT) in 26 patients with Shwachman-Diamond disease (SDS) and severe bone marrow abnormalities. The conditioning regimen was based on busulphan (54%), total body irradiation (23%), fludarabine (15%) or other chemotherapy combinations (8%). Standard prevention of graft versus host disease (GVHD) with cyclosporin +/- methotrexate was adopted in 54% of the patients whilst in vivo or in vitro T-cell depletion was used in 17 and four patients respectively. Neutrophil and platelet engraftment were achieved in 21 (81%) and 17 (65%) of 26 patients after a median time of 18 days and 29 days respectively. The incidence of grade III and IV acute GVHD was 24% and of chronic GVHD 29%. Nine patients died after a median time of 70 d, post-SCT. After a median follow-up of 1.1 years, the transplant-related mortality was 35.5% (95% CI 17-54) whilst the overall survival was 64.5% (95% CI 45.7-83.2). Allo-SCT was found to be successful in more than half of SDS patients with severe bone marrow dysfunction. Further improvements would be anticipated by a better definition of the optimum time in the course of disease to transplant and by the adoption of less toxic conditioning regimens.

Cipolli, M. (2001). "Shwachman-Diamond syndrome: clinical phenotypes." Pancreatology 1(5): 543-8.

The clinical phenotype of Shwachman-Diamond syndrome (SDS) is extremely heterogeneous, showing a wide range of abnormalities and symptoms. The main characteristics of the syndrome are exocrine pancreatic dysfunction, haematologic abnormality and growth retardation. At diagnosis, especially when made in infancy, symptoms of pancreatic insufficiency are always present. This condition could be considered as a transient pancreatic insufficiency. In fact, several studies have shown that, with advancing age, about 40-60% of patients become pancreatic sufficient. Observations on the evolution of pancreatic activity lead us to believe that the diagnosis of SDS must be considered even in the absence of signs and symptoms of pancreatic insufficiency. Intermittent neutropenia is the most common haematological finding in SDS, but more of the bone marrow cellular elements can be involved. In recent years, recombinant human granulocyte colony-stimulating factor has been used in some SDS subjects with severe neutropenia and frequent infection. The major haematological problem in the disease is the appearance of acute myeloid leukaemia; however, its prevalence is difficult to establish. Growth retardation is

Research Articles

a typical manifestation. Weight and length are deficient at birth and remain below normal over time. Some studies show that SDS patients present short stature rather than malnutrition and this would suggest an inherent growth problem. A broad spectrum of skeletal abnormalities has been found to be associated with this syndrome. Short ribs with broadened anterior ends and metaphyseal dyschondroplasia of the long bone are the most common findings. Elevated liver enzymes and hepatomegaly are present in the first years of life with subsequent improvement without complications. Developmental delay, learning disorders and attention deficit disorders are also reported.

Costa, E. and R. Santos (2008). "Hematologically important mutations: Shwachman-Diamond syndrome." Blood Cells Mol Dis 40(2): 183-4.

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, bone marrow dysfunction, and skeletal abnormalities. The Shwachman-Bodian-Diamond syndrome (SBDS) gene was identified as a causative gene for SDS in 2003, and genetic analyses of SDS have been performed. Over the last 4 years, a number of different mutations affecting the SBDS gene have been described. In this report, a summary of documented SDS associated mutations is provided.

Cunningham, J., M. Sales, et al. (2002). "Does isochromosome 7q mandate bone marrow transplant in children with Shwachman-Diamond syndrome?" Br J Haematol 119(4): 1062-9.

We report on nine children with Shwachman-Diamond syndrome (SDS), eight of whom had clonal abnormalities of chromosome 7. Seven children had an isochromosome 7 [i(7)(q10)] and one a derivative chromosome 7, all with an apparently identical (centromeric) breakpoint. Children with SDS are predisposed to myelodysplasia (MDS) and acute myeloid leukaemia (AML) often with chromosome 7 abnormalities. Allogeneic transplants have been used to treat these children, however, they are a high-risk transplant group and require careful evaluation. Three of the children were transplanted but only one survived, who to our knowledge remains the longest surviving SDS transplant patient (4.5 years +). The six non-transplanted children are well. In classic MDS, chromosome 7 abnormalities are associated with rapid progression to acute leukaemia; however, we present evidence to suggest that isochromosome 7q may represent a separate disease entity in SDS children. This is a particularly interesting finding given that the SDS gene has recently been mapped to the centromeric region of chromosome 7. Our studies indicate that i(7)(q10) is a relatively benign rearrangement and that it is not advisable to offer allogeneic transplants to SDS children with i(7)(q10) alone in the absence of other clinical signs of disease progression.

Research Articles

Dhar, S. and J. M. Anderton (1994). "Orthopaedic features of Shwachman syndrome. A report of two cases." J Bone Joint Surg Am 76(2): 278-82.

Dokal, I. and T. Vulliamy (2008). "Inherited aplastic anaemias/bone marrow failure syndromes." Blood Rev 22(3): 141-53.

The inherited aplastic anaemias/bone marrow (BM) failure syndromes are a heterogeneous group of disorders characterized by BM failure usually in association with one or more somatic abnormality. The BM failure often presents in childhood but this may not be until adulthood in some cases highlighting the need for the adult haematologist to be aware of these disorders. Indeed some patients initially labelled as "idiopathic aplastic anaemia" are cryptic presentations of these genetic syndromes. Since 1992, when the first Fanconi anaemia (FA) gene was cloned there have been considerable advances in the genetics of these syndromes. These advances are beginning to provide a better understanding of normal haemopoiesis and how this might be disrupted in patients with BM failure. They have also provided important insights into some fundamental biological pathways: DNA repair-FA/BRCA pathway; telomere maintenance- dyskeratosis congenita related genes; ribosome biogenesis- Shwachman Diamond syndrome and Diamond-Blackfan anaemia genes. Additionally, as these disorders are usually associated with developmental abnormalities and an increased risk of cancer they are providing new insights into human development and the genesis of cancer. These advances have led to improved diagnosis of patients with these disorders. They may now also provide the platform for developing new treatments.

Donadieu, J., T. Leblanc, et al. (2005). "Analysis of risk factors for myelodysplasias, leukemias and death from infection among patients with congenital neutropenia. Experience of the French Severe Chronic Neutropenia Study Group." Haematologica 90(1): 45-53.

BACKGROUND AND OBJECTIVES: The two main complications of severe chronic neutropenia are fatal sepsis and myelodysplasia/acute leukemia (MDS/AL). Granulocyte colony-stimulating factor (G-CSF) therapy has significantly reduced the frequency and severity of infections, but its possible influence on the risk of malignancy is not known. **DESIGN AND METHODS:** The French Severe Chronic Neutropenia (SCN) Registry has prospectively collected data since 1994 on 231 patients with various forms of SCN, namely severe congenital neutropenia (n=101), cyclic neutropenia (n=60), glycogen storage disease type Ib (GSDIb) (n=15) and Shwachman-Diamond syndrome (SDS)(n=55). The median overall follow-up is 11.1 years. Parameters of exposure to G-CSF therapy, such as the time averaged dose, follow up after first use of G-CSF, and the cumulative dose, have been recorded. **RESULTS:** Eight septic deaths occurred, of which 6 among patients with severe congenital neutropenia and 2 in patients with cyclic neutropenia; none of these 8 patients

Research Articles

was receiving G-CSF therapy. No septic deaths occurred during G-CSF therapy. Thirteen cases of MDS/AL were recorded. The cumulative incidence of MDS/AL was 2.7% (SD 1.3%) at 10 years and 8.1% (SD 2.7%) at 20 years.

INTERPRETATION AND CONCLUSIONS: Risk factors for MDS/AL were the diagnostic category, the severity of neutropenia, younger age at diagnosis, and strong exposure to G-CSF. MDS/AL only occurred in patients with severe congenital neutropenia and SDS. Owing to their particular susceptibility to infections, patients with severe congenital neutropenia had the strongest exposure to G-CSF; the risk of leukemia increased with the degree of G-CSF exposure in this subgroup.

Donadieu, J., G. Michel, et al. (2005). "Hematopoietic stem cell transplantation for Shwachman-Diamond syndrome: experience of the French neutropenia registry." Bone Marrow Transplant 36(9): 787-92.

Our objective was to study the outcome of allogeneic hematopoietic stem cell transplantation (HSCT) for Shwachman-Diamond Syndrome (SDS). Among 71 SDS patients included in the French Severe Chronic Neutropenia Registry, 10 received HSCT between 1987 and 2004 in five institutions. The indications were bone marrow failure in five cases, and myelodysplastic syndrome (MDS) or leukemia in five cases. The median follow-up of patients who survived without relapse is 6.9 years (3.1-16.8 years). The conditioning regimen consisted of a busulfan-cyclophosphamide combination (n=6) or total body irradiation plus chemotherapy (n=4). Six patients received stem cells from unrelated donors and four from identical siblings. Engraftment was complete in eight patients and unassessable in two patients. These latter two patients died of infections 32 and 36 days after HSCT, with grade IV graft-versus-host disease and multiorgan dysfunction. A third patient died from an acute respiratory distress syndrome 17 months after HSCT with progressive granulocytic sarcoma. One patient had an MDS relapse 4 months after HSCT and died 10 months later. The overall 5-year event-free survival rate is 60+/-15%. We conclude that HSCT is feasible for patients with SDS who develop bone marrow failure or malignant transformation.

Dror, Y. (2005). "Shwachman-Diamond syndrome." Pediatr Blood Cancer 45(7): 892-901.

Shwachman-Diamond syndrome (SDS) is an inherited marrow failure disorder with varying cytopenia, pancreatic dysfunction, and metaphyseal dysostosis. SDS is also characterized by a risk of myelodysplasia and leukemia in up to one third of the patients. Over the last 5 years, major advances have been made in understanding the bone marrow phenotype. The gene associated with the disease, SBDS, has recently been identified. Herein we provide an update on the clinical features, the hematopoietic defects, and the genetics of the disease as they are currently understood. We also review the diagnostic and therapeutic approaches to the hematological complications in the syndrome.

Research Articles

Dror, Y., P. Durie, et al. (2002). "Clonal evolution in marrows of patients with Shwachman-Diamond syndrome: a prospective 5-year follow-up study." Exp Hematol 30(7): 659-69.

OBJECTIVES: Shwachman-Diamond syndrome (SDS) is characterized by varying degrees of marrow failure. Retrospective studies suggested a high propensity for malignant myeloid transformation into myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The study's aims were to determine the cellular and molecular characteristics as well as the clinical course of malignant myeloid transformation and clonal marrow disease in patients with SDS. **METHODS:** This is a longitudinal prospective study of 14 patients recruited for annual hematological evaluations. Results of baseline and serial hematological assessments for up to 5 years are reported. **RESULTS:** Clonal marrow cytogenetic abnormalities (CMCA) were detected in 4 patients (29%) on first testing or at follow-up. The abnormalities were del(20q) in two patients, i(7q) in one, and combined del(20q) and i(7q) in one. The following tests did not distinguish patients with CMCA from other SDS patients: severity of peripheral cytopenia, fetal hemoglobin levels, percentage of marrow CD34+ cells, colony growth from marrow CD34+ cells, cluster-to-colony ratio, marrow stromal function, percentage of marrow apoptosis cells, and granulocyte colony-stimulating factor receptor expression. RAS and p53 mutation analysis and AML blast colony assays were uniformly negative. No patients showed progression into more advanced stages of MDS or into AML. In one patient, the abnormal clone became undetectable after 2 years of follow-up. **CONCLUSIONS:** We conclude that although CMCA in SDS is high, progression into advanced stages of MDS or to overt AML may be slow and difficult to predict. Treatment should be cautious since some abnormal clones can regress.

Federman, N. and K. M. Sakamoto (2005). "The genetic basis of bone marrow failure syndromes in children." Mol Genet Metab 86(1-2): 100-9.

The bone marrow failure syndromes consist of a number of rare diseases, in which there is ineffective hematopoiesis by the bone marrow. Subsequently, absent or decreased production of a single cell line, single cytopenia, or of all cell lines, and pancytopenia, develops. The mechanisms of hematopoiesis and the defects that result in bone marrow failure are beginning to be better understood. This paper will review the genetic and molecular basis of several important bone marrow failure syndromes in children, Fanconi anemia, Shwachman-Diamond syndrome, Diamond-Blackfan anemia, congenital amegakaryocytic thrombocytopenia, dyskeratosis congenita, and severe congenital neutropenia, and the recent discoveries that have enhanced our understanding of the pathogenesis of these diseases.

Research Articles

Freedman, M. H. and B. P. Alter (2002). "Risk of myelodysplastic syndrome and acute myeloid leukemia in congenital neutropenias." Semin Hematol 39(2): 128-33.

Granulocyte colony-stimulating factor (G-CSF) has had a major impact on the management of "severe chronic neutropenia" (SCN), a collective term referring to congenital, idiopathic, or cyclic neutropenia. Almost all patients respond to G-CSF with increased neutrophils, reduced infections, and improved survival. Some responders with congenital neutropenia and Shwachman-Diamond syndrome (SDS) have developed myelodysplastic syndrome and acute myeloid leukemia (MDS/AML), which raises the question of the role of G-CSF in pathogenesis. The issue is complicated because both disorders have a propensity for MDS or AML as part of their natural history. To address this, the Severe Chronic Neutropenia International Registry (SCNIR) used its large database of chronic neutropenia patients treated with G-CSF to determine the incidence of malignant myeloid transformation in the two disorders, and its relationship to treatment and to other patient characteristics. No statistically significant relationships were found between age at onset of MDS or AML and patient gender, G-CSF dose, or duration of G-CSF therapy. What was observed, however, was the multistep acquisition of aberrant cellular genetic changes in marrow cells from patients who transformed, including activating ras oncogene mutations, clonal cytogenetic abnormalities, and G-CSF receptor mutations. In murine models, the latter produces a hyperproliferative response to G-CSF, confers resistance to apoptosis, and enhances cell survival. Since congenital neutropenia and SDS are inherited forms of bone marrow failure, G-CSF may accelerate the propensity for MDS/AML in the genetically altered stem and progenitor cells, especially in those with G-CSF receptor and ras mutations (82% and 50% of patients who transform, respectively). Alternatively, and equally plausible, G-CSF may simply be an "innocent bystander" that corrects neutropenia, prolongs patient survival, and allows time for the malignant predisposition to declare itself. In patients who transform to overt MDS or AML, hematopoietic stem cell transplantation is the only chance for cure. In those with "soft" signs of MDS, such as an isolated clonal cytogenetic change but without other evidence of MDS, or with an isolated G-CSF receptor mutation, there is room for conservative management. One option is to reduce the G-CSF dosage as much as possible, and observe the tempo of progression, if any, to more overt signs of malignancy.

Gohring, G., A. Karow, et al. (2007). "Chromosomal aberrations in congenital bone marrow failure disorders--an early indicator for leukemogenesis?" Ann Hematol 86(10): 733-9.

As chromosomal instability may contribute to leukemogenesis in patients with congenital bone marrow failure (CBMF) disorders, it was the aim of this study to characterize chromosomally aberrant clones that arise during the clinical course of disease by means of R-banding and fluorescence in situ hybridization (FISH) analyses. In addition, multicolor-FISH and array-comparative genomic

Research Articles

hybridization (CGH) were applied to characterize clonal chromosome aberrations in more detail. Between January 2004 and December 2005, we prospectively analyzed 90 samples of 73 patients with proven or suspected CBMF disorders enrolled in a German Study Network of CBMF diseases. Clonal aberrations could be identified in four of 73 patients examined. In one child with congenital thrombocytopenia, Jacobsen syndrome [del(11)(q24)c] was diagnosed, and thus a CBMF could be excluded. In a girl with Shwachman-Diamond syndrome, two independent clones, one with an isochromosome i(7)(q10), another with a complex aberrant karyotype, were identified. Simultaneously, transition into a myelodysplastic syndrome (MDS) occurred. The brother, who was also afflicted with Shwachman-Diamond syndrome, showed an isochromosome i(7q) as a single aberration. In the fourth patient with severe congenital neutropenia, an add(21)(q22) marker containing a low-level amplification of the AML1 gene was identified at the time point of transition into acute myelogenous leukemia (AML). In summary, we suggest that follow-up of patients with CBMF using chromosome and FISH analyses will be helpful for the early detection of transition into MDS or AML and thus should be an integral part of the clinical management of these patients.

Grinspan, Z. M. and C. A. Pikora (2005). "Infections in patients with Shwachman-Diamond syndrome." Pediatr Infect Dis J 24(2): 179-81.

Shwachman-Diamond syndrome (OMIM 260400) is a multisystemic disorder characterized by pancreatic insufficiency, bone marrow dysfunction, skeletal abnormalities and immune dysfunction. Prompted by the case of a 13-year-old girl with Shwachman-Diamond syndrome who presented with pneumonia attributable to *Pseudomonas aeruginosa*, we review infectious complications of this disease. Pneumonia, recurrent otitis media and skin infections/abscesses constitute the majority of infections among these children.

Hall, G. W., P. Dale, et al. (2006). "Shwachman-Diamond syndrome: UK perspective." Arch Dis Child 91(6): 521-4.

So much has been added to our knowledge of Shwachman-Diamond syndrome (SDS) since it was last reviewed in this journal some 25 years ago, that there is now an urgent need to bring the condition to the attention of a new generation of paediatricians. SDS, although a rare autosomal recessive disorder, demands wide attention because it features in the differential diagnosis of a number of important childhood diseases. It can be diagnosed in children of all ages, or in adults. SDS most commonly presents in infancy with features of exocrine pancreatic insufficiency, bone marrow dysfunction, and short stature.

Research Articles

Ho, W., C. Cheretakis, et al. (2007). "Prevalence of oral diseases in Shwachman-Diamond syndrome." Spec Care Dentist 27(2): 52-8.

The aim of this study was to determine the prevalence and severity of oral diseases in patients with ShwachmanDiamond syndrome (SDS). Thirty-five persons with SDS were compared to 20 healthy controls. A cross-sectional survey was carried out using self-reporting questionnaires and dental radiographs collected from the subjects and their dentists. Overall, oral diseases were more prevalent among subjects with SDS when compared to controls ($p < 0.001$). Persons with SDS also had more caries in both primary ($p < 0.03$) and permanent dentitions ($p < 0.01$), and also had delayed dental development ($p < 0.04$). Oral soft tissue pathoses, such as recurrent oral ulcerations ($p < 0.00$) and gingival bleeding upon brushing ($p < 0.00$), were significantly more prevalent in subjects with SDS. Pain on eating was also more frequent amongst persons with SDS ($p < 0.008$) and was often associated with oral ulcerations ($p < 0.002$). In conclusion, based on self-completed subject and dentist questionnaires, diseases of oral hard and soft tissues were more prevalent and severe in persons with SDS when compared with healthy controls.

Ip, W. F., A. Dupuis, et al. (2002). "Serum pancreatic enzymes define the pancreatic phenotype in patients with Shwachman-Diamond syndrome." J Pediatr 141(2): 259-65.

OBJECTIVE: To evaluate the role of serum enzymes for defining the pancreatic phenotype in Shwachman-Diamond syndrome (SDS), an inherited multisystem condition. **STUDY DESIGN:** Serum pancreatic trypsinogen and isoamylase were measured in 164 patients known or presumed to have SDS. The diagnosis was confirmed in 90 patients. Among 74 unconfirmed cases, 35 ("probable SDS") had hematologic dysfunction but lacked documented pancreatic dysfunction, whereas 39 patients ("improbable SDS") lacked both documented pancreatic and hematologic dysfunction. Classification and regression tree (CART) analysis was performed in 90 patients with SDS and 134 control patients to establish a rule for defining the pancreatic phenotype of SDS; the rule was then applied to the patients with unconfirmed diagnosis. **RESULTS:** In the control patients, serum trypsinogen showed little variation with age, whereas serum isoamylase values rose from birth on, attaining adult values by 3 years. For patients with SDS, serum trypsinogen values were low in young patients and tended to increase with age, whereas serum isoamylase values remained low at all ages. The CART rule combined results from both enzymes and classified the pancreatic phenotype in all but one SDS patient, who was <3 years of age. Excluding patients <3 years of age, CART identified the pancreatic phenotype in 82% and 7% of the "probable SDS" and "improbable SDS" cases, respectively. **CONCLUSIONS:** Serum pancreatic enzymes are useful for determining the pancreatic phenotype and confirming the diagnosis of SDS.

Research Articles

Kuijpers, T. W., M. Alders, et al. (2005). "Hematologic abnormalities in Shwachman Diamond syndrome: lack of genotype-phenotype relationship." Blood 106(1): 356-61.

Shwachman-Diamond syndrome (SDS) is an autosomal-recessive disorder characterized by short stature, exocrine pancreatic insufficiency, and hematologic defects. The causative SBDS gene was sequenced in 20 of 23 unrelated patients with clinical SDS. Mutations in the SBDS gene were found in 75%, being identical in 11 patients. Hematologic parameters for all 3 lineages were determined over time such as absolute neutrophil counts (ANCs), granulocyte functions, and erythroid and myeloid colony formation (erythroid burst-forming unit [BFU-E] and granulocyte-monocyte colony-forming unit [CFU-GM]) from hematopoietic progenitor cells, percentage of fetal hemoglobin (HbF), and platelet counts. Persistent neutropenia was present in 43% in the absence of apoptosis and unrelated to chemotaxis defects (in 65%) or infection rate. Irrespective of the ANC in vivo, abnormal CFU-GM was observed in all patients with SDS tested (14 of 14), whereas BFU-E was less often affected (9 of 14). Cytogenetic aberrations occurred in 5 of 19 patients in the absence of myelodysplasia. One child died during allogeneic bone marrow transplantation. In conclusion, neutropenia and defective chemotaxis did not result in severe clinical infection in SDS. CFU-GMs were impaired in all patients tested. From the SBDS sequence data, we conclude that in patients with genetically proven SDS a genotype-phenotype relationship in SDS does not exist in clinical and hematologic terms.

Liu, J. M. and S. R. Ellis (2006). "Ribosomes and marrow failure: coincidental association or molecular paradigm?" Blood 107(12): 4583-8.

Gene products mutated in the inherited bone marrow failure syndromes dyskeratosis congenita (DC), cartilage-hair hypoplasia (CHH), Diamond-Blackfan anemia (DBA), and Shwachman-Diamond syndrome (SDS) are all predicted to be involved in different aspects of ribosome synthesis. At this moment, however, it is unclear whether this link indicates a causal relationship. Although defective ribosome synthesis may contribute to each of these bone marrow failure syndromes (and perhaps others), precisely which feature of each disease is a consequence of failure to produce adequate amounts of ribosomes is obscured by the tendency of each gene product to have extraribosomal functions. Delineation of the precise role of each gene product in ribosomal biogenesis and in hematopoietic development may have both therapeutic and prognostic importance and perhaps even direct the search for new bone marrow failure genes.

Mack, D. R. (2002). "Shwachman-Diamond syndrome." J Pediatr 141(2): 164-5.

Research Articles

Mack, D. R., G. G. Forstner, et al. (1996). "Shwachman syndrome: exocrine pancreatic dysfunction and variable phenotypic expression." Gastroenterology 111(6): 1593-602.

BACKGROUND & AIMS: Shwachman syndrome is an inherited condition with multisystemic abnormalities, including exocrine pancreatic dysfunction. The aim of this study was to evaluate the occurrence and progression of features in a large cohort of patients. METHODS: Clinical records of 25 patients with Shwachman syndrome were reviewed. RESULTS: Mean birth weight (2.92 +/- 0.51 kg) was at the 25th percentile. However, by 6 months of age, mean heights and weights were less than the 5th percentile. After 6 months of age, growth velocity was normal. Severe fat maldigestion due to pancreatic insufficiency was present in early life (fecal fat, 26% +/- 17% of fat intake; age, < 2 years). Serial assessment of exocrine pancreatic function showed persistent deficits of enzyme secretion, but 45% of patients showed moderate age-related improvements leading to pancreatic sufficiency. Neutropenia was the most common hematologic abnormality (88%), but leukopenia, thrombocytopenia, and anemia were also frequently encountered. Patients with hypoplasia of all three bone marrow cellular lines (n = 11) had the worst prognosis; 5 patients died, 2 of sepsis and 3 of acute myelogenous leukemia. Other findings included hepatomegaly and/or abnormal liver function test results and skeletal abnormalities. CONCLUSIONS: A wide and varied spectrum of phenotypic abnormalities among patients with Shwachman syndrome is described. Pancreatic acinar dysfunction is an invariable abnormality. Patients with severe bone marrow involvement may have a guarded prognosis.

Makitie, O., L. Ellis, et al. (2004). "Skeletal phenotype in patients with Shwachman-Diamond syndrome and mutations in SBDS." Clin Genet 65(2): 101-12.

Pancreatic exocrine and bone marrow dysfunctions are considered to be universal features of Shwachman-Diamond syndrome (SDS) whereas the associated skeletal dysplasia is variable and not consistently observed. The genetic defect in SDS has recently been identified; causative mutations have been shown in the SBDS gene. The aims of this study were to characterize the nature, frequency, and age-related changes of radiographic skeletal abnormalities in patients with SBDS mutations and to assess genotype-phenotype correlation. Fifteen patients (mean age 9.7 years) with a clinical diagnosis of SDS and documented SBDS gene mutations were included. Review of their skeletal radiographs showed abnormalities in all patients. The skeletal changes were variable, even in patients with identical genotypes. The typical features were (1) delayed appearance of secondary ossification centers, (2) variable widening and irregularity of the metaphyses in early childhood, followed by progressive thickening and irregularity of the growth plates, and (3) generalized osteopenia. There was a tendency towards normalization of the epiphyseal maturation defect and progression of the metaphyseal changes with

Research Articles

age. The results suggest that the characteristic skeletal changes are present in all patients with SDS and SBDS mutations, but their severity and localization varies with age. No phenotype-genotype correlation was observed.

Niemeyer, C. M. and I. Baumann (2008). "Myelodysplastic syndrome in children and adolescents." Semin Hematol 45(1): 60-70.

Myelodysplastic syndromes (MDS) are clonal disorders characterized by ineffective hematopoiesis and subsequent frequent development of acute myeloid leukemia (AML). In children and adolescents, MDS are uncommon disorders, accounting for less than 5% of hematopoietic malignancy, with great heterogeneity in presentation and clinical course. The genetic changes predisposing children to MDS are largely obscure. Monosomy 7 is the most common chromosomal abnormality, often occurring as a sole abnormality. The recent pediatric modification of the World Health Organization (WHO) classification has greatly facilitated the diagnostic process. Refractory cytopenia (RC) is the most common MDS subtype in children, occurring in about half of all MDS cases. There is consensus that the relationship between MDS with increased blast count and de novo AML is better defined by biological and clinical features than by blast count. Because monosomy 7 is the only chromosomal abnormality strongly suggestive of MDS, children presenting with a low blast count and other chromosomal aberrations or normal karyotype must be closely observed before a diagnosis of MDS can be established. With an increasing number of children surviving primary cancer with chemotherapy or radiation therapy, the incidence of secondary therapy-related MDS is rising. The MDS risk is also increased in patients with inherited bone marrow failure disorders; this relationship provides valuable insights into MDS biology. Allogeneic hematopoietic stem cell transplantation (HSCT) from a matched related or suitable unrelated donor is the choice for most children with MDS and can rescue a large proportion of patients.

Rosenberg, P. S., B. P. Alter, et al. (2006). "The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy." Blood 107(12): 4628-35.

In patients with severe congenital neutropenia (SCN), sepsis mortality is reduced by treatment with granulocyte colony-stimulating factor (G-CSF), but myelodysplastic syndrome and acute myeloid leukemia (MDS/AML) have been reported. We studied 374 patients with SCN and 29 patients with Shwachman-Diamond syndrome (SDS) on long-term G-CSF enrolled in the Severe Chronic Neutropenia International Registry. In SCN, sepsis mortality was stable at 0.9% per year. The hazard of MDS/AML increased significantly over time, from 2.9% per year after 6 years to 8.0% per year after 12 years on G-CSF. After 10 years, the cumulative incidence was 8% for sepsis mortality and 21% for MDS/AML. A subgroup of SCN patients (29%) received more than the median dose of G-CSF (> or = 8 microg/kg/d), but achieved less than the median absolute neutrophil

Research Articles

count (ANC) response (ANC < 2.188 x 10⁹/L [2188/microL] at 6-18 months). In these less-responsive patients, the cumulative incidence of adverse events was highest: after 10 years, 40% developed MDS/AML and 14% died of sepsis, compared with 11% and 4%, respectively, of more responsive patients whose ANC was above the median on doses of G-CSF below the median. Risk of MDS/AML may be similar in SDS and SCN. In less-responsive SCN patients, early hematopoietic stem cell transplantation may be a rational option.

Rothbaum, R., J. Perrault, et al. (2002). "Shwachman-Diamond syndrome: report from an international conference." J Pediatr 141(2): 266-70.

Ruggiero, A., F. Molinari, et al. (2008). "MRI findings in Shwachman diamond syndrome." Pediatr Blood Cancer 50(2): 352-4.

Shwachman Diamond Syndrome (SDS) is a rare congenital disorder characterized by pancreatic insufficiency, bone marrow dysfunction, and skeletal changes. Because of the heterogeneous clinical presentation and the limits of laboratory tests that assess pancreatic insufficiency, the diagnosis of SDS can be challenging. Pancreatic lipomatosis, a typical feature of this syndrome, is also difficult to assess by direct tissue sampling. In these circumstances, magnetic resonance imaging (MRI) provides a readily available, noninvasive tool to evaluate the pancreatic fat content. We report a case of a 12-month-old male in which abdominal MRI was used to confirm the clinical diagnosis of SDS.

Rujkijyanont, P., J. Beyene, et al. (2007). "Leukaemia-related gene expression in bone marrow cells from patients with the preleukaemic disorder Shwachman-Diamond syndrome." Br J Haematol 137(6): 537-44.

Shwachman-Diamond syndrome (SDS) is an inherited bone marrow failure disorder with cytopenia and a high propensity for myelodysplastic syndrome (MDS) and leukaemia, particularly acute myeloid leukaemia. The mechanism of leukaemogenesis in SDS is unknown. In accordance to the multi-hit theory of carcinogenesis, it is likely that several molecular and cellular hits occur before MDS/leukaemia become apparent. This study used oligonucleotide microarray to identify gene expression patterns, which were shown to be associated with leukaemogenesis, in marrow mononuclear cells of nine SDS patients without overt transformation compared to healthy controls. Among 154 known leukaemia-related genes, several oncogenes were found to be upregulated, including LARG, TAL1 and MLL, and of several tumour suppressor genes were downregulated, including DLEU1, RUNX1, FANCD2 and DKC1. Real time polymerase chain reaction confirmed statistically higher expression of LARG and TAL1 in SDS marrows. We conclude that SDS marrow mononuclear cells exhibit abnormal gene expression patterns, which might result in continuous stimulation

Research Articles

favouring evolution or progression of malignant clones. Additional molecular and cytogenetic events are probably necessary for the malignant process to be irreversible and complete.

Sauer, M., C. Zeidler, et al. (2007). "Substitution of cyclophosphamide and busulfan by fludarabine, treosulfan and melphalan in a preparative regimen for children and adolescents with Shwachman-Diamond syndrome." Bone Marrow Transplant 39(3): 143-7.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only definitive treatment for severe bone marrow dysfunction and clonal disorders in patients diagnosed with Shwachman-Diamond syndrome (SDS). In an attempt to minimize regimen-related toxicity (RRT), we have initiated a fludarabine/treosulfan/melphalan-based pilot protocol avoiding the combination of busulfan and cyclophosphamide. Median age at transplantation was 9.6 years (range 1.5-17 years). All three patients received conditioning with fludarabine (30 mg/m²/day x 6), treosulfan (12 g/m²/day x 3) and melphalan (140 mg/m²/day x 1). CAMPATH-1H (0.1 mg/kg x 2) was added in two cases, while rabbit ATG (Genzyme; 3 x 2.5 mg/kg) was given to the cord blood recipient. One patient was transplanted with a non-manipulated marrow graft from an HLA-identical sibling, one with a marrow graft from a 10/10 matched unrelated donor, and one with a 9/10 matched unrelated umbilical cord blood (UCB) unit. Mean cell doses given were 3.6 x 10⁸ nucleated cells/kg BW for the bone marrow recipients and 4.2 x 10⁷ nucleated cells/kg BW for UCB recipient. Overall, two of three patients are alive and display 100% donor chimerism. Acute graft-versus-host disease grade II was seen in one patient, while no GVHD exceeding grade I occurred in the remaining two.

Schibli, S., M. Corey, et al. (2006). "Towards the ideal quantitative pancreatic function test: analysis of test variables that influence validity." Clin Gastroenterol Hepatol 4(1): 90-7.

BACKGROUND & AIMS: Although pancreatic stimulation tests quantify acinar and ductal exocrine pancreatic function, no standard methodology exists. We evaluated the impact of several variables on test accuracy. **METHODS:** We performed a retrospective analysis of pancreatic stimulation tests, which involved continuous stimulation with cholecystikinin and secretin, 3 sampling periods (20-min each), and perfusion markers to correct for intestinal losses. Results were recalculated using the following variables: no correction for losses; shortened sampling time (20-min); no correction and shortened sampling time; and enzyme concentration. We examined how these variables influenced measurements of pancreatic secretion and classification of pancreatic function status (sufficient or insufficient). **RESULTS:** We analyzed 363 tests in control patients (20), and patients with cystic fibrosis (137), Shwachman-Diamond syndrome (40), or other pancreatic or intestinal disorders (166). Recovery of pancreatic juice varied

Research Articles

markedly between tests (median, 59%; range, 4%-106%) and was significantly poorer during the first 20-minute period compared with the 2 subsequent periods ($P < .01$). Failure to correct for intestinal losses underestimated secretory capacity (median trypsin output reduced by $>50\%$, $P < .0001$) and shortened sampling time increased test variability. Both variables together resulted in greater discrepancies. More than 25% of the pancreatic-sufficient patients with impaired pancreatic function were misclassified as pancreatic insufficient when uncorrected output plus a shortened sampling time or enzyme concentration were used to define categories. **CONCLUSIONS:** Pancreatic function tests using brief aspiration periods without marker perfusion or measures of concentration greatly underestimate pancreatic secretory capacity and misclassify the clinical status of an unacceptably large number of patients.

Shimamura, A. (2006). "Inherited bone marrow failure syndromes: molecular features." Hematology Am Soc Hematol Educ Program: 63-71.

Recent advances resulting from the identification of the genes responsible for four inherited marrow failure syndromes, Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome, are reviewed. The interpretation of genetic testing should be guided by an understanding of the limitations of such testing for each disorder. The possibility of an inherited basis for marrow failure must be considered for adults as well as children with aplastic anemia. Shared molecular themes are emerging from functional studies of the genes underlying the different inherited disorders. Genomic instability may result from impaired DNA repair in Fanconi anemia or telomere dysregulation in dyskeratosis congenita. Mutations affecting ribosome assembly or function are associated with Diamond-Blackfan anemia, dyskeratosis congenita, and Shwachman-Diamond syndrome. These findings raise new questions about the molecular mechanisms regulating hematopoiesis and leukemogenesis. Clinical implications arising from these molecular studies are explored.

Shimamura, A. (2006). "Shwachman-Diamond syndrome." Semin Hematol 43(3): 178-88.

Shwachman-Diamond syndrome (SDS) is an autosomal recessive marrow failure syndrome associated with exocrine pancreatic insufficiency and leukemia predisposition. Bone marrow failure typically manifests with neutropenia, but anemia, thrombocytopenia, or aplastic anemia may also develop. Additional organ systems, such as liver or bone, may also be affected. Clonal cytogenetic abnormalities, particularly those involving chromosome 7 such as monosomy 7 or isochromosome 7, may develop. Mutations in the SBDS gene are found in approximately 90% of patients meeting clinical diagnostic criteria. SBDS is a highly conserved gene of unknown function. Studies of the yeast orthologue YLR022c and structurally related proteins suggest a role in RNA metabolism. In human cells, the SBDS protein localizes to both the cytoplasm and the nucleus,

Research Articles

and shuttles in and out of the nucleolus in a cell cycle-dependent manner. A discussion of diagnostic workup, medical management, and treatment is presented.

Smith, O. P. (2002). "Shwachman-Diamond syndrome." Semin Hematol 39(2): 95-102.

Shwachman-Diamond syndrome (SDS), described just under 40 years ago, is a rare, autosomal-recessive disorder usually manifest in infancy and characterized by exocrine pancreatic insufficiency, short stature, and bone marrow dysfunction. Additional clinical features include metaphyseal dysostosis, epiphyseal dysplasia, immune dysfunction, liver disease, growth failure, renal tubular defects, insulin-dependent diabetes mellitus, and psychomotor retardation. Hematological manifestations other than neutropenia include anemia, raised fetal hemoglobin (HbF) levels, thrombocytopenia, impaired neutrophil chemotaxis, and aplastic anemia; as with other constitutional bone marrow failure syndromes, there is a predilection to malignant myeloid transformation. No unifying pathogenetic mechanism(s) has yet been shown to be responsible for SDS, although new insights into the molecular, genetic, and cellular basis of this rare disease have recently been described.

Steele, J. M., L. Sung, et al. (2006). "Disease progression in recently diagnosed patients with inherited marrow failure syndromes: a Canadian Inherited Marrow Failure Registry (CIMFR) report." Pediatr Blood Cancer 47(7): 918-25.

BACKGROUND: Inherited bone marrow failure syndromes (IMFSs) are genetic disorders characterized by defective single-lineage or multi-lineage hematopoiesis. IMFS patients are at risk for severe cytopenias, development of marrow cytogenetic abnormalities (MCA), myelodysplasia (MDS), and malignancy. The rate of disease progression and proportion of patients at risk for these complications is currently unclear. We examined recently diagnosed IMFS patients to determine distribution of diagnoses, disease progression and development of significant outcomes. **METHODS:** The CIMFR is a prospective multi-center study established in 2001 to register all IMFS patients in Canada. Analysis was restricted to patients diagnosed after November 30, 1997. Summary statistics were used to depict the study population while survival was described using the Kaplan-Meier method. **RESULTS:** 74 CIMFR patients were considered recently diagnosed. Median age at diagnosis was 2.7 years (range, birth to 40.6). Annual follow-up data were available for 53 (72%) patients. The five most prevalent diagnoses were Fanconi anemia (FA), Shwachman-Diamond syndrome (SDS), Diamond-Blackfan anemia (DBA), dyskeratosis congenita (DKC), and Kostmann's neutropenia (KS). Eighteen (24%) patients were unclassifiable. Twenty-eight (53%) follow-up patients had disease progression as indicated by new or worsening cytopenias, new marrow changes, or initiation of

Research Articles

transfusion support and/or medical therapy. Fourteen (19%) fulfilled minimal diagnostic criteria for myelodysplasia. Eleven patients had hematopoietic stem cell transplantation (HSCT) by first follow-up. Five patients have died. Survival at 36 months is 89.8 +/- 5.7%. CONCLUSIONS: IMFS patients are often diagnosed at a young age. The relative distribution of diagnoses is similar to previous reviews of published cases; however, 25% of patients are currently unclassifiable. Disease progression has occurred in approximately 50% of follow-up patients. Early mortality is noted. Continued prospective observation of these patients is warranted.

Stepanovic, V., D. Wessels, et al. (2004). "The chemotaxis defect of Shwachman-Diamond Syndrome leukocytes." Cell Motil Cytoskeleton 57(3): 158-74.

Shwachman-Diamond Syndrome (SDS) is a rare autosomal recessive, multisystem disorder presenting in childhood with intermittent neutropenia and pancreatic insufficiency. It is characterized by recurrent infections independent of neutropenia, suggesting a functional neutrophil defect. While mutations at a single gene locus (SBDS) appear to be responsible for SDS in a majority of patients, the function of that gene and a specific defect in SDS neutrophil behavior have not been elucidated. Therefore, employing 2D and 3D computer-assisted motion analysis systems, we have analyzed the basic motile behavior and chemotactic responsiveness of individual polymorphonuclear leukocytes (PMNs) of 14 clinically diagnosed SDS patients. It is demonstrated that the basic motile behavior of SDS PMNs is normal in the absence of chemoattractant, that SDS PMNs respond normally to increasing and decreasing temporal gradients of the chemoattractant fMLP, and that SDS PMNs exhibit a normal chemokinetic response to a spatial gradient of fMLP. fMLP receptors were also distributed uniformly through the plasma membrane of SDS PMNs as in control PMNs. SDS PMNs, however, were incapable of orienting in and chemotaxing up a spatial gradient of fMLP. This unique defect in orientation was manifested by the PMNs of every SDS patient tested. The PMNs of an SDS patient who had received an allogenic hematopoietic stem cell transplant, as well as PMNs from a cystic fibrosis patient, oriented normally. These results suggest that the defect in SDS PMNs is in a specific pathway emanating from the fMLP receptor that is involved exclusively in regulating orientation in response to a spatial gradient of fMLP. This pathway must function in parallel with additional pathways, intact in SDS patients, that emanate from the fMLP receptor and regulate responses to temporal rather than spatial changes in receptor occupancy.

Toiviainen-Salo, S., O. Makitie, et al. (2008). "Shwachman-Diamond syndrome is associated with structural brain alterations on MRI." Am J Med Genet A 146A(12): 1558-64.

Shwachman-Diamond syndrome (SDS) is an autosomal recessive condition that

Research Articles

results from mutations in the SBDS gene, at chromosome 7q11. Main features include exocrine pancreatic failure, neutropenia and skeletal dysplasia. This study investigated brain structures by magnetic resonance imaging (MRI) in patients with SDS. MRI of the brain was performed in nine patients (7 males, age range 7-37 years) with SDS and mutations in the SBDS gene and in 18 age- and gender-matched controls. MRI images were assessed visually, and volumetric analyses of the brain matter and structural midsagittal measurements were performed. Eight out of nine SBDS mutation-verified patients reported learning difficulties. Patients with SDS had smaller occipitofrontal head circumferences than the controls (Z-score -1.3 vs. +0.3, $P = 0.021$), and decreased global brain volume (1.74 L vs. 1.94 L, $P = 0.019$); both gray matter ($P = 0.042$) and white matter ($P = 0.007$) volumes were reduced. Patients with SDS had no macroscopic brain malformations, but they had significantly smaller age- and head size-adjusted areas of posterior fossa ($P = 0.006$), vermis ($P = 0.002$), corpus callosum ($P = 0.020$), and pons ($P = 0.002$), and significantly larger cerebrum-vermis ratio ($P < 0.0001$) than the healthy controls. SDS patients had structurally smaller posterior fossa and cerebellar vermis, corpus callosum, and brainstem than the healthy controls. The MRI findings may be related to the neuropsychological features described in SDS.

Toiviainen-Salo, S., M. K. Mayranpaa, et al. (2007). "Shwachman-Diamond syndrome is associated with low-turnover osteoporosis." *Bone* 41(6): 965-72.

INTRODUCTION: Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency and bone marrow dysfunction. These result in malabsorption and hematological abnormalities. A skeletal dysplasia is also an integral feature of SDS. The present study assessed prevalence and determinants of osteopenia and osteoporosis in patients with SDS and disease-causing mutations in the SBDS gene. **MATERIALS AND METHODS:** Eleven patients (8 males) aged from 5 to 37 years (median 16.7 years) with a genetically confirmed diagnosis of SDS were assessed for fracture history, bone mineral content (BMC), lean tissue mass (LTM) and bone mineral density (BMD) (Hologic Discovery A), osteoporotic vertebral changes, and for blood biochemistry and hematological parameters. Iliac crest bone biopsies were obtained from four patients for histology and histomorphometry. **RESULTS:** The main findings were: (1) markedly reduced BMD Z-scores at the lumbar spine (median -2.1, range -4.4 to -0.8), proximal femur (median -1.3, range -2.2 to -0.7) and, whole body (median -1.0, range -2.8 to +0.6), and reduced Z-scores for height-adjusted BMC/LTM ratio (median -0.9, range -3.6 to +1.1); (2) vertebral compression fractures in three patients; and (3) blood biochemistry suggestive of mild vitamin D and vitamin K deficiency. Bone biopsies in four patients showed significant low-turnover osteoporosis with reduced trabecular bone volume, low numbers of osteoclasts and osteoblasts, and reduced amount of osteoid. **CONCLUSIONS:** The results suggest that in addition to the skeletal dysplasia, SDS is associated with a more generalized

Research Articles

bone disease characterized by low bone mass, low bone turnover and by vertebral fragility fractures. Osteoporosis may result from a primary defect in bone metabolism, and could be related to the bone marrow dysfunction and neutropenia.

Toiviainen-Salo, S., M. Raade, et al. (2008). "Magnetic resonance imaging findings of the pancreas in patients with Shwachman-Diamond syndrome and mutations in the SBDS gene." J Pediatr 152(3): 434-6.

Pancreatic MRI was evaluated in 14 patients with a clinical diagnosis of Shwachman-Diamond syndrome, and the findings were correlated with Shwachman-Bodian-Diamond gene (SBDS) genotype. The findings suggest that patients with mutations in the SBDS gene have a characteristic magnetic resonance imaging pattern of fat-replaced pancreas and that SBDS mutations are unlikely in patients without this pattern.

Vibhakar, R., M. Radhi, et al. (2005). "Successful unrelated umbilical cord blood transplantation in children with Shwachman-Diamond syndrome." Bone Marrow Transplant 36(10): 855-61.

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by pancreatic insufficiency and variable degrees of neutropenia. SDS patients are at risk of developing myelodysplasia, aplastic anemia, and leukemic transformation. The role and timing of allogeneic hematopoietic stem cell transplantation (HSCT) in SDS remain controversial. We report three SDS patients with severe aplasia transplanted using unrelated umbilical cord blood (UCB). Patients received melphalan (180 mg/m²), etoposide (1200 mg/m²), anti-thymocyte globulin (90 mg/kg), and total lymphoid irradiation (500 cGy); graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and prednisone. Myeloid engraftment occurred promptly with absolute neutrophil count >500 cells/mm³ on day 15 +/- 5 and all patients displayed 100% donor chimerism by 2 months post transplant. The major complication of transplant was GVHD, with all patients developing grade II or III acute GVHD, one progressing to chronic extensive GVHD. Patients are alive 309, 623, and 2029 days post transplant. Factors important in HSCT outcome for SDS may include transplantation at a young age, avoidance of cyclophosphamide, and adequate GVHD prophylaxis. Importantly, these cases also suggest that unrelated UCB, in the absence of a matched family member, is an excellent alternative stem cell source for SDS patients undergoing HSCT.

Woloszynek, J. R., R. J. Rothbaum, et al. (2004). "Mutations of the SBDS gene are present in most patients with Shwachman-Diamond syndrome." Blood 104(12): 3588-90.

Shwachman-Diamond Syndrome (SDS) is a rare multisystem disorder

Research Articles

characterized by exocrine pancreatic insufficiency, bone marrow dysfunction, and metaphyseal chondrodysplasia. Recent studies show that mutations of SBDS, a gene of unknown function, are present in the majority of patients with SDS. In the present study, we show that most, but not all, patients classified based on rigorous clinical criteria as having SDS had compound heterozygous mutations of SBDS. Full-length SBDS protein was not detected in leukocytes of SDS patients with the most common SBDS mutations, consistent with a loss-of-function mechanism. In contrast, SBDS protein was expressed at normal levels in SDS patients without SBDS mutations. These data confirm the absence of SBDS mutations in this subgroup of patients and suggest that SDS is a genetically heterogeneous disorder. The presence (or absence) of SBDS mutations may define subgroups of patients with SDS who share distinct clinical features or natural history.

Zhang, S., M. Shi, et al. (2006). "Loss of the mouse ortholog of the shwachman-diamond syndrome gene (Sbds) results in early embryonic lethality." *Mol Cell Biol* 26(17): 6656-63.

Mutations in SBDS are responsible for Shwachman-Diamond syndrome (SDS), a disorder with clinical features of exocrine pancreatic insufficiency, bone marrow failure, and skeletal abnormalities. SBDS is a highly conserved protein whose function remains largely unknown. We identified and investigated the expression pattern of the murine ortholog. Variation in levels was observed, but *Sbds* was found to be expressed in all embryonic stages and most adult tissues. Higher expression levels were associated with rapid proliferation. A targeted disruption of *Sbds* was generated in order to understand the consequences of its loss in an *in vivo* model. Consistent with recessive disease inheritance for SDS, *Sbds*(+/-) mice have normal phenotypes, indistinguishable from those of their wild-type littermates. However, the development of *Sbds*(-/-) embryos arrests prior to embryonic day 6.5, with muted epiblast formation leading to early lethality. This finding is consistent with the absence of patients who are homozygous for early truncating mutations. *Sbds* is an essential gene for early mammalian development, with an expression pattern consistent with a critical role in cell proliferation.