LANZKOWSKY'S MANUAL OF PEDIATRIC HEMATOLOGY AND ONCOLOGY

SIXTH EDITION

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Dedication

This book is dedicated

To our parents,
Abe and Lily Lanzkowsky, Thelma and Al Lipton,
and Vicky and Lawrence Fish, who instilled in us the importance of integrity,
the rewards of industry and the primacy of being a mensch.

To our wives,
Rhona Lanzkowsky, Linda Lipton, and Leah Fish,
who understand that the study of medicine is a
lifelong and consuming process.

To our children and grandchildren,
our pride and joy.

And

To our patients,
students, pediatric house staff,
fellows in pediatric hematology and oncology,
and to our colleagues who have taught us so much over the years.

Today he can discover the errors of yesterday
and tomorrow he may obtain new light
on what he thinks himself sure of today

Moses Maimónides

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DR PHILIP LANZKOWSKY

PHILIP LANZKOWSKY

Dr Philip Lanzkowsky was born in Cape Town on March 17, 1932 and graduated high school from the South African College and obtained his MBChB degree from the University of Cape Town School of Medicine in 1954 and his Doctorate degree in 1959 for his thesis on *Iron Deficiency Anemia In Children.* He completed a pediatric residency at the Red Cross War Memorial Children's Hospital in Cape Town in 1960. In the same year, he received the Diploma in Child Health (DCH) from the Royal College of Physicians and Surgeons of London, and in 1961 was made member of the prestigious Royal College of Physicians of Edinburgh (RCPE). After working in Pediatrics at the University of Edinburgh and at St Mary's Hospital of the University of London, Dr Lanzkowsky did a pediatric Hematology-Oncology fellowship at Duke University School of Medicine and at the University of Utah.

In 1963 he was appointed Consultant Pediatrician and Pediatric Hematologist to the Red Cross War Memorial Children's Hospital at the University of Cape Town and introduced Pediatric Hematology and Oncology as a distinct discipline. In 1965 he was appointed Director of Pediatric Hematology and Associate Professor of Pediatrics at the New York Hospital-Cornell University School of Medicine.

In 1970 he was appointed Professor of Pediatrics and Chairman of Pediatrics at Long Island Jewish Medical Center and established a division of Pediatric Hematology-Oncology which he directed until 2000. He was the founder of the Schneider Children's Hospital (presently named Steven and Alexandra Cohen Children's Medical Center of New York), which he developed, planned, and was the hospital's Executive Director and Chief of Staff from its inception in 1983 until 2010.

Dr Lanzkowsky has received numerous honors and awards and has lectured extensively at various institutions and medical schools in the United States and around the world. In 1973 he was appointed Fellow of the Royal College of Physicians of Edinburgh, and in 1994 he received a Doctor of Science Degree (Honoris Causa) from St Johns University in New York for "his notable contribution to the field of pediatric medicine and to the children of the world". Among many other awards he was the recipient of the Joseph Arenow Prize for original postgraduate research in the field of Science, Medicine and Applied Science from the University of Cape Town, the John Adams Memorial Traveling fellowship administered by the Nuffield Foundation, the Hill-Pattison-Struthers Bursary from the Royal College of Physicians of Edinburgh and the Sonia Mechanick Traveling Fellowship from the South African College of Medicine. In addition to having been the editor of five editions of the *Manual of Pediatric Hematology and Oncology* used by clinicians worldwide, he is the author of *How It All Began: The History of a Children's Hospital* and over 280 scientific papers, abstracts, monographs, and book chapters.

Dr Lanzkowsky's medical writings have been prodigious. His seminal contributions to the medical literature have included the first description of the relationship of pica to iron-deficiency anemia (*Arch. Dis Child*., 1959), Effects of timing of clamping of umbilical cord on infant's hemoglobin level (*Br. Med. J*., 1960), Normal oral D-xylose test values in children (*New Engl. J. Med*., 1963), Normal coagulation factors in women in labor and in the newborn (*Thromboses at Diath. Hemorr*., 1966), Erythrocyte abnormalities induced by malnutrition (*Br. J. Haemat*., 1967), Radiologic features in iron deficiency anemia (*Am. J. Dis. Child*., 1968), Isolated defect of folic acid absorption associated with mental retardation (*Blood*, 1969; *Am. J. Med*, 1970), Disaccharidase levels in iron deficiency (*J. Pediat*., 1981) and Hexokinase "New Hyde Park" in a Chinese kindred (*Am. J. Hematol*., 1981).

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Preface to the Sixth Edition

The sixth edition of *Lanzkowsky's Manual of Pediatric Hematology and Oncology* has significant changes from previous editions. The title of the book, the editors, and its content have changed but the objective has remained unchanged and every effort has been made to retain the original style and clarity which have become the hallmark of the previous editions.

The title has changed to include the name of the original and sole editor of the book in its various editions for the past 45 years. In addition, the list of editors has increased from single editorship to include two additional hematologist-oncologists to reflect advances in pediatric hematology and oncology over the years. Jeffrey Lipton MD, PhD and Jonathan Fish MD have been selected as coeditors.

The book has been expanded from 33 chapters in the fifth edition to 36 in the present edition. A chapter has been added on the burgeoning subject of diagnostic, molecular, and genomic methodologies for the hematologist-oncologist and a new chapter has been added on transfusion medicine. Lymphoproliferative disorders and myelodysplasia have been assigned separate chapters and lymphoid and myeloid leukemia have also been assigned distinct chapters.

A number of new experts in particular fields have been added to the contributing-author panel.

Despite these significant changes the book has retained the original objective, format, and clarity of the founding editor. It remains a practical, concise, up-to-date guide to all professional staff treating children with hematological and oncologic diseases. The book is replete with detailed tables, practical algorithms, and flow diagrams useful for teaching housestaff, fellows, nursing staff, and practicing physicians and essential for the day-to-day investigation and management of patients with hematologic and oncologic conditions.

I would like to pay tribute to Drs Gungor Karayalcin and Ashok Shende, my close associates for over 40 years who retired after a lifelong career in clinical practice and research in Pediatric Hematology-Oncology, for their major contribution to the first four editions of the book which formed the very foundation of all subsequent editions.

Philip Lanzkowsky
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Royalty payments accrued to Dr Lanzkowsky for all future editions of this book will be donated to the Division of Pediatric Hematology-Oncology at the Cohen Children's Medical Center of New York for children with cancer whose families have financial difficulties.

Preface to the Fifth Edition

The fifth edition of the *Manual of Pediatric Hematology and Oncology* differs considerably from previous editions but has retained the original intent of the author to offer a concise manual of predominantly clinical material culled from personal experience and to be an immediate reference for the diagnosis and management of hematologic and oncologic diseases. I have resisted succumbing to the common tendency of writing a comprehensive tome which is not helpful to the practicing hematologist-oncologist at the bedside. The book has remained true to its original intent.

The information included at all times keeps "the eye on the ball" to ensure that pertinent, up-to-date, practical clinical advice is presented without extraneous information, however interesting or pertinent this information may be in a different context.

The book differs from previous editions in many respects. The number of contributors has been considerably expanded drawing on the expertise of leaders in different subjects from various institutions in the United States. Increased specialization within the field of hematology and oncology has necessitated including this large a number of contributors in order to bring to the reader balanced and up-to-date information for the care of patients. In addition, the number of chapters has increased from 27, in the previous edition, to 33. The reason for this is that many of the chapters, such as hemolytic anemia and coagulation, had become so large and the subject so extensive that they were better handled by subdividing the chapter into a number of smaller chapters. An additional chapter on the psychosocial aspects of cancer for children and their families, not present in previous editions, has been added.

Some chapters have been extensively revised and re-written where advancement in knowledge has dictated this approach, for example, Hodgkin lymphoma, neuroblastoma and rhabdomyosarcoma and other soft-tissue sarcomas, whereas other chapters have been only slightly modified. In nearly all the chapters there has been significant change in the management and treatment section reflecting advances that have occurred in these areas.

This edition has retained the essential format written and developed decades ago by the author and, with usage over the years, has proven to be highly effective as a concise, practical, up-to-date guide replete with detailed tables, algorithms and flow diagrams for investigation and management of hematologic and oncologic conditions. The tables and flow diagrams included in the book have been updated using the latest information and the most recent protocols of treatment, which have received general acceptance and have become the standard of care, have been included. In a book with so many details, errors inevitably occur. I do not know where they are because if I did they would have been corrected. I apologize in advance for any inaccuracies that may have crept in inadvertently.

The four previous editions of this book were published when the name of the hospital was the Schneider Children's Hospital. Effective April 1, 2010 the name of the hospital was changed to the Steven and Alexandra Cohen Children's Medical Center of New York.

I would like to acknowledge Morris Edelman, MB, BCh, BSc (Laboratory Medicine) for his contribution in reviewing the pathology on Hodgkin disease.

I thank Rose Grosso for her untiring efforts in the typing and coordination of the various phases of the development of this edition.

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Preface to the Fourth Edition

This edition of the *Manual of Pediatric Hematology and Oncology* is the fourth edition and the sixth book written by the author on pediatric hematology and oncology. The first book written by the author 25 years ago was exclusively on pediatric hematology and its companion book, exclusively on pediatric oncology, was written 3 years later. The book reviewers at the time suggested that these two books be combined into a single book on pediatric hematology and oncology and the first edition of the *Manual of Pediatric Hematology and Oncology* was published by the author in 1989.

It is from these origins that this 4th edition arises—the original book written in its entirety by the author was 456 pages—has more than doubled in size. The basic format and content of the clinical manifestations, diagnosis and differential diagnosis has persisted with little change as originally written by the author. The management and treatment of various diseases have undergone profound changes over time and these aspects of the book have been brought up-to-date by the subspecialists in the various disease entities. The increase in the size of the book is reflective of the advances that have occurred in both hematology and oncology over the past 25 years. Despite the size of the book, the philosophy has remained unchanged over the past quarter century. The author and his contributors have retained this book as a concise manual of personal experiences on the subject over these decades rather than developing a comprehensive tome culled from the literature. Its central theme remains clinical as an immediate reference for the practicing pediatric hematologist-oncologist concerned with the diagnosis and management of hematologic and oncologic diseases. It is extremely useful for students, residents, fellows and pediatric hematologists and oncologists as a basic reference assembling in one place, essential knowledge required for clinical practice.

This edition has retained the essential format written and developed decades ago by the author and, with usage over the years, has proven to be highly effective as a concise, practical, up-to-date guide replete with detailed tables, algorithms and flow diagrams for investigation and management of hematologic and oncologic conditions. The tables and flow diagrams have been updated with the latest information and the most recent protocols of treatment, that have received general acceptance and have produced the best results, have been included in the book.

Since the previous edition, some 5 years ago, there have been considerable advances particularly in the management of oncologic disease in children and these sections of the book have been completely rewritten. In addition, advances in certain areas have required that other sections of the book be updated. There has been extensive revision of certain chapters such as on Diseases of the White Cells, Lymphoproliferative Disorders, Myeloproliferative Disorders and Myelodysplastic Syndromes and Bone Marrow Failure. Because of the extensive advances in thrombosis we have rewritten that entire section contained in the chapter on Disorders of Coagulation to encompass recent advances in that area. The book, like its previous editions, reflects the practical experience of the author and his colleagues based on half a century of clinical experience. The number of contributors has been expanded but consists essentially of the faculty of the Division of Hematology Oncology at the Schneider Children's Hospital, all working together to provide the readers of the manual with a practical guide to the management of the wide spectrum of diseases within the discipline of pediatric hematology-oncology.

I would like to thank Laurie Locastro for her editorial assistance, cover design, and for her untiring efforts in the coordination of the various phases of the production of this edition. I also appreciate the efforts of Lawrence Tavnier for his expert typing of parts of the manuscript and would like to thank Elizabeth Dowling and Patrician Mastrolembo for proof reading of the book to ensure its accuracy.

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Preface to the Third Edition

This edition of the *Manual of Pediatric Hematology and Oncology*, published 5 years after the second edition, has been written with the original philosophy in mind. It presents the synthesis of experience of four decades of clinical practice in pediatric hematology and oncology and is designed to be of paramount use to the practicing hematologist and oncologist. The book, like its previous editions, contains the most recent information from the literature coupled with the practical experience of the author and his colleagues to provide a guide to the practicing clinician in the investigation and up-to-date treatment of hematologic and oncologic diseases in childhood.

The past 5 years have seen considerable advances in the management of oncologic diseases in children. Most of the advances have been designed to reduce the immediate and long-term toxicity of therapy without influencing the excellent results that have been achieved in the past. This has been accomplished by reducing dosages, varying the schedules of chemotherapy, and reducing the field and volume of radiation.

The book is designed to be a concise, practical, up-to-date guide and is replete with detailed tables, algorithms, and flow diagrams for investigation and management of hematologic and oncologic conditions. The tables and flow diagrams have been updated with the latest information, and the most recent protocols that have received general acceptance and have produced the best results have been included in the book.

Certain parts of the book have been totally rewritten because our understanding of the pathogenesis of various diseases has been altered in the light of modern biological investigations. Once again, we have included only those basic science advances that have been universally accepted and impinge on clinical practice.

I thank Ms Christine Grabowski, Ms Lisa Phelps, Ms Ellen Healy and Ms Patricia Mastrolembo for their untiring efforts in the coordination of the writing and various phases of the development of this edition. Additionally, I acknowledge our fellows, Drs Banu Aygun, Samuel Bangug, Mahmut Celiker, Naghma Husain, Youssef Khabbase, Stacey Rifkin-Zenenberg, and Rosa Ana Gonzalez, for their assistance in culling the literature.

I also thank Dr Bhoomi Mehrotra for reviewing the chapter on bone marrow transplantation, Dr Lorry Rubin for reviewing the sections of the book dealing with infection, and Dr Leonard Kahn for reviewing the pathology.

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Preface to the Second Edition

This edition of the *Manual of Pediatric Hematology and Oncology*, published 5 years after the first edition, has been written with a similar philosophy in mind. The basic objective of the book is to present useful clinical information from the recent literature in pediatric hematology and oncology and to temper it with experience derived from an active clinical practice.

The manual is designed to be a concise, practical, up-to-date book for practitioners responsible for the care of children with hematologic and oncologic diseases by presenting them with detailed tables and flow diagrams for investigation and clinical management.

Since the publication of the first edition, major advances have occurred, particularly in the management of oncologic diseases in children, including major advances in recombinant human growth factors and bone marrow transplantation. We have included only those basic science advances that have been universally accepted and impinge on clinical practice.

I would like to thank Dr Raj Pahwa for his contributions on bone marrow transplantation, Drs Alan Diamond and Leora Lanzkowsky-Diamond for their assistance with the neuro-radiology section, and Christine Grabowski and Lisa Phelps for their expert typing of the manuscript and for their untiring assistance in the various phases of the development of this book.

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Preface to the First Edition

The *Manual of Pediatric Hematology and Oncology* represents the synthesis of personal experience of three decades of active clinical and research endeavors in pediatric hematology and oncology. The basic orientation and intent of the book is clinical, and the book reflects a uniform systematic approach to the diagnosis and management of hematologic and oncologic diseases in children. The book is designed to cover the entire spectrum of these diseases, and although emphasis is placed on relatively common disorders, rare disorders are included for the sake of completion. Recent developments in hematology-oncology based on pertinent advances in molecular genetics, cytogenetics, immunology, transplantation, and biochemistry are included if the issues have proven of value and applicability to clinical practice.

Our aim in writing this manual was to cull pertinent and useful clinical information from the recent literature in pediatric hematology and oncology and to temper it with experience derived from active clinical practice. The result, we hope, is a concise, practical, readable, up-to-date book for practitioners responsible for the care of children with hematologic and oncologic diseases. It is specifically designed for the medical student and practitioner seeking more detailed information on the subject, the pediatric house officer responsible for the care of patients with these disorders, the fellow in pediatric hematology-oncology seeking a systemic approach to these diseases and a guide in preparation for the board examinations, and the practicing pediatric hematologist-oncologist seeking another opinion and approach to these disorders. As with all brief texts, some dogmatism and "matters of opinion" have been unavoidable in the interests of clarity. The opinions expressed on management are prudent clinical opinions; and although they may not be accepted by all, pediatric hematologists-oncologists will certainly find a consensus. The reader is presented with a consistency of approach and philosophy describing the management of various diseases rather than with different managements derived from various approaches described in the literature. Where there are divergent or currently unresolved views on the investigation or management of a particular disease, we have attempted to state our own opinion and practice so as to provide some guidance rather than to leave the reader perplexed.

The manual is not designed as a tome containing the minutiae of basic physiology, biochemistry, genetics, molecular biology, cellular kinetics, and other esoteric and abstruse detail. These subjects are covered extensively in larger works. Only those basic science advances that impinge on clinical practice have been included here. Each chapter stresses the pathogenesis, pathology, diagnosis, differential diagnosis, investigations, and detailed therapy of hematologic and oncologic diseases seen in children.

I would like to thank Ms Joan Dowdell and Ms Helen Witkowski for their expert typing and for their untiring assistance in the various phases of the development of this book.

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Introduction: Historic Perspective

Philip Lanzkowsky

REFLECTION ON 60 YEARS OF PROGRESS IN PEDIATRIC HEMATOLOGY-ONCOLOGY

As the sixth edition of the *Manual of Pediatric Hematology-Oncology* is published, I have reflected on the advances that have occurred since I began practicing hematology-oncology almost 60 years ago and since my first book on the subject was published by McGraw Hill in 1980. The present edition is more than double the size of the original book.

Our understanding of hematologic conditions has advanced considerably with the explosion of molecular biology and the management of most hematologic conditions has kept pace with these scientific advances. Our understanding of the basic science of oncology, molecular biology, genetics, and the management of oncologic conditions has undergone a seismic change. The previous age of dismal and almost consistent fatal outcomes for most childhood cancers has been replaced by an era in which most childhood cancers are cured. This has been made possible not only because of advances in oncology but because of the parallel development of radiology, radiologic oncology, and surgery as well as supportive care such as the pre-emptive use of antibiotics and blood component therapy. It has been a privilege to be a witness and participant in this great evolution over the past 60 years. Yet we still have a long way to go as current advances are superseded by therapy based upon the application of knowledge garnered from an accurate understanding of the fundamental biology of cancer.

In the early days of hematology-oncology practice, hematology dominated and occupied most of the practitioner's time because most patients with cancer had a short lifespan and limited therapeutic modalities were available.

Automated electronic blood-counting equipment has enabled valuable red cell parameters such as mean corpuscular volume (MCV) and red cell distribution width (RDW) to be applied in routine clinical practice. This advance permitted the reclassification of anemias based on MCV and RDW. Previously these parameters were determined by microscopy with considerable observer variability. The attempt at a more accurate determination of any one of these parameters was a laborious, time-consuming enterprise relegated only as a demonstration in physiology laboratories.

Rh hemolytic disease of the newborn and its management by exchange transfusion, which occupied a major place in the hematologists' domain, has now become almost extinct in developed countries due to the use of Rh immunoglobulin.

The description of the various genetic differences in patients with vitamin B_{12} deficiency has opened up new vistas of our understanding of cobalamin transport and metabolism. Similar advances have occurred with reference to folate transport and metabolism.

Gaucher and other similar diseases have been converted from crippling and often disabling disorders to ones where patients can live a normal and productive life thanks to the advent of enzyme replacement therapy.

Aplastic anemia has been transformed from a near death sentence to a disease with hope and cure in 90% of patients thanks to immunosuppressive therapies, hematopoietic stem cell transplantation, and advanced supportive care. The emergence of clonal disease years later in patients treated medically with immunosuppressive therapy, however, does remain a challenge. The discovery of the various genes responsible for Fanconi anemia and other inherited bone marrow failure syndromes has revealed heretofore unimaginable advances in our understanding of DNA repair, telomere maintenance, ribosome biology and other new fields of biology. The relationship of these syndromes to the development of various cancers may hold the key to our better understanding of the etiology of cancer as well as birth defects.

The hemolytic anemias, previously lumped together as a group of congenital hemolytic anemias, can now be identified as separate and distinct enzyme defects of the Embden–Meyerhof and hexose monophosphate

pathways in intracellular red cell metabolism as well as various well-defined defects of red cell skeletal proteins due to advances in molecular biology and genetics. With improvement in electrophoretic and other biochemical as well as molecular techniques, hemoglobinopathies are being identified which were not previously possible.

Diseases requiring a chronic transfusion program to maintain a hemoglobin level for hemodynamic stability, such as in thalassemia major, frequently had marked facial characteristics with broad cheekbones and developed what was called "bronze diabetes," a bronzing of the skin along with organ damage and failure, particularly of the heart, liver, beta cells of the pancreas, and other tissues due to secondary hemochromatosis because of excessive iron deposition. The clinical findings attributed to extramedullary hematopoiesis are essentially of historic interest because of the development and widespread use of proper transfusion and chelation regimens. However, the full potential of the role of intravenous and oral chelating agents is yet to be realized due to the problems of compliance with difficult treatment regimens and also due to failure of some patients to respond adequately. Advances in our understanding of the biology of iron absorption and transport at the molecular level hold out promise for further improvement in the management of these conditions. Curative therapy in thalassemia major and other conditions by hematopoietic stem cell transplantation in suitable cases is widely available today. Gene therapy looms on the horizon but will not, for some time, be available to patients in the developing world requiring the development of other approaches.

In the treatment of idiopathic thrombocytopenic purpura, intravenous gamma globulin and anti-D immunoglobulin as well as thrombopoietin mimetic agents have been added to the armamentarium of management and are useful in specific indications in patients with this disorder.

Major advances in the management of hemophilia have included the introduction of commercially available products for replacement therapy which has saved these patients from a life threatened by hemorrhage into joints, muscles, and vital organs. Surgery has become possible in hemophilia without the fear of being unable to control massive hemorrhage during or after surgery. The devastating clinical history of tragic hemophilia outcomes has been relegated to the pages of medical history. Patients with inhibitors, however, still remain a clinical challenge. The whole subject of factors associated with inherited thrombophilia such as mutations of factor V, prothrombin G20210A and 5,10-methylenetetrahydrofolate reductase as well as the roles of antithrombin, protein C and S deficiency, and antiphospholipid antibodies in the development of thrombosis has opened new vistas of understanding of thrombotic disorders. Notwithstanding these advances, the management of these patients still presents a clinical challenge.

There are few diseases in which advances in therapy have been as dramatic as in the treatment of childhood leukemia. In my early days as a medical student, the only available treatment for leukemia was blood transfusion. Patients never benefitted from a remission and died within a few months. Steroids and single-agent chemotherapy, first with aminopterin, demonstrated the first remissions in leukemia and raised hope of a potential cure; however, relapse ensued in almost all cases and most patients died within the first year of diagnosis. In most large pediatric oncology centers there were few patients with leukemia as the disease was like a revolving door—diagnosis and death. The development of multiple-agent chemotherapy for induction, consolidation, and maintenance, CNS prophylaxis and supportive care ushered in a new era of cure for patients with leukemia. These principles were refined over time by more accurate classification of acute leukemia using morphological, cytochemical, immunological, cytogenetic, and molecular criteria which replaced the crude microscopic and highly subjective characteristics previously utilized for the classification of leukemia cells. These advances paved the way for the development of specific protocols of treatment for different types of leukemia. The management of leukemia was further refined by risk stratification, response-based therapy, and identification of minimal residual disease, all of which have led to additional chemotherapy or different chemotherapy protocols, resulting in an enormous improvement in the cure rate of acute leukemia. The results have been enhanced by modern supportive care including antibiotic, antifungal, antiviral therapy, and blood component therapy. Those patients whose leukemia is resistant to treatment or who have recurrences can be successfully treated by advances that have occurred with the development of hematopoietic stem cell transplantation. The challenge of finding appropriate, unrelated transplantation donors has been ameliorated by molecular HLA-typing techniques and the development of large, international donor registries. Emerging targeted and pharmacogenetic therapies hold great promise for the future. Already dramatic results are being reported exploiting surface antigens targeted by engineered cytotoxic T-cells.

Hodgkin disease, originally defined as a "fatal illness of the lymphatics," is a disease that is cured in most cases today. Initially, Hodgkin disease was treated with high-dose radiation to the sites of identifiable disease resulting in some cures but with major life-long radiation damage to normal tissues because of the use of cobalt machines and higher doses of radiation than is currently used. The introduction of nitrogen mustard early on,

as a single-agent chemotherapy, improved the prognosis somewhat. A major breakthrough occurred with the staging of Hodgkin disease and the use of radiation therapy coupled with multiple-agent chemotherapy (MOPP). With time this therapeutic approach was considerably refined to include a reduction in radiation dosage and field and a modification of the chemotherapy regimens designed to reduce toxicity of high-dose radiation and of some of the chemotherapeutic agents. These major advances in treatment ushered in a new era in the management and cure of most patients with this disease. The management of Hodgkin disease, however, did go through a phase of staging laparotomy and splenectomy with a great deal of unnecessary surgery and splenectomies being performed. There were considerable surgical morbidity and post-splenectomy sepsis, occasionally fatal, which occurred in some cases. With the advent of MRI and PET scans, surgical staging, splenectomy, and lymphangiography have become unnecessary.

Non-Hodgkin lymphoma, previously considered a dismal disease, is another success story. Improvements in histologic, immunologic, and cytogenetic techniques have made the diagnosis and classification more accurate. The development of a staging system and multiagent chemotherapy was a major step forward in the management of this disease. This, together with enhanced supportive care, including the successful management of tumor lysis syndrome, have all contributed to the excellent results that occur today.

Brain tumors were treated by surgery and radiation therapy with devastating results due to primitive neurosurgical techniques and radiation damage. The advent of MRI scans has made the diagnosis and the determination of the extent of disease more accurate. Major technical advances in neurosurgery such as image guidance, which allows 3D mapping of tumors, functional mapping, and electrocorticography, which allow pre- and intraoperative differentiation of normal and tumor tissue, the use of ultrasonic aspirators and neuroendoscopy, have all improved the results of neurosurgical intervention and has resulted in less surgical damage to normal brain tissue. These neurosurgical advances, coupled with the use of various chemotherapy regimens, have resulted in considerable improvements in outcome for some. This field, however, still remains an area begging for a better understanding of the optimum management of these devastating and often fatal tumors. Improved radiation techniques, including proton beam therapy, have led to more precise radiation fields, sparing normal brain.

In the early days of pediatric oncology Wilms tumor in its early stages was cured with surgery followed by radiation therapy. The diagnosis was made with an intravenous pyelogram and inferior venocavogram and chest radiography was employed to detect pulmonary metastases. The diagnosis and extent of disease were better defined when CT of the abdomen and chest became available. The development of the clinicopathological staging system and the more accurate definition of the histology into favorable and unfavorable histologic types, allowed for more focused treatment with radiation and multiple chemotherapy agents, for different stages and histology of Wilms tumor, resulting in the excellent outcomes observed today. The success of the National Wilms Tumor Study Group (NWTSG), more than any other effort, provided the model for cooperative group therapeutic cancer trials, which in large measure have been responsible for advances in the treatment of Wilms tumor.

The diagnosis of neuroblastoma and its differentiation histologically from other round blue cell tumors such as rhabdomyosarcoma, Ewing sarcoma, and non-Hodgkin lymphoma was difficult before neurone-specific enolase cytochemical staining, Shimada histopathology classification, N-myc gene status, VMA and HVA determinations, and MIBG scintigraphy were introduced. In the future, new molecular approaches will offer diagnostic tools to provide even greater precision for diagnosis. The existing markers coupled with a staging system have enabled neuroblastoma to be assigned to various risk group categories with specific multimodality treatment protocols for each risk group, which has improved the prognosis in this disease. Improvements in diagnostic radiology determining extent of disease and modern surgical techniques have enhanced the advances in chemotherapy in this condition. With these advances and the addition of targeted immunologic approaches and radiopharmaceutical-linked therapy to our armamentarium the progress for disseminated neuroblastoma appears to be improving.

Major advances have occurred in rhabdomyosarcoma treatment over the years. Early on treatment of this disease was characterized by mutilating surgery including amputation and a generally poor outcome. More accurate histologic diagnosis, careful staging, judicious surgery, combination chemotherapy, and radiotherapy have all contributed a great deal to the improved cure rates with significantly less disability.

Malignant bone tumors had a terrible prognosis. They were generally treated by amputation of the limb with the primary tumor; however, this was usually followed by pulmonary metastases and death. The major advance in the treatment of this disease came with the use of high-dose methotrexate and leukovorin rescue which, coupled with limb salvage treatment, has resulted in improved survival and quality-of-life outcomes.

Of note however, improvement in outcomes for pediatric sarcomas have not kept pace with those for leukemia, lymphoma, and other tumors. The advances in the treatment of hepatoblastoma were made possible by safer

anesthesia, more radical surgery, intensive postoperative management together with multiagent chemotherapy and more recently the increased use of liver transplantation. These advances have allowed many patients to be cured compared to past years.

Histiocytosis is a disease that has undergone many name changes from Letter–Siwe disease, Hand–Schüller–Christian disease and eosinophilic granuloma to the realization that these entities are one disease, renamed histiocytosis X (to include all three entities) to its present name of Langerhans cell histiocytosis (LCH) due to the realization that these entities have one pathognomonic pathologic feature that is the immunohistochemical presence of Langerhans cells defined in part by expression of CD1a or langerin (CD207), which induces the formation of Birbeck granules. Advances have occurred in the management of this disease by an appreciation of risk stratification depending on number and type of organs involved in this disease process as well as by early response to therapy. Once this was established, systemic therapy was developed for the various risk groups which led to appropriate and improved primary and salvage therapy and the introduction of new agents with better overall results. Recent advances describing stereotypic mutations in LCH offer hope for new targeted approaches.

Until a final prevention or cure for cancer in children is at hand, hematopoietic stem cell transplantation must be viewed as a major advance. Improved methods for tissue typing, the use of umbilical and peripheral blood stem cells, improved preparative regimens, including intensity-reduced approaches and better management of graft-versus-host disease (GVHD) has made this an almost routine treatment modality for many metabolic disorders, hemoglobinopathies, and malignant diseases following ablative chemotherapy in chemotherapy-sensitive tumors. Post-transplantation support with antibiotic, antifungal, antiviral, hematopoietic growth factors and judicious use of blood component therapy has made this procedure safer than it was in years gone by.

The recognition of severe and often permanent damage to organs and life-threatening complications from chemotherapy and radiation therapy has, over the years, led to regimens consisting of combination chemotherapy at reduced doses and reduction in dose and field of radiation with improved outcome. An entire new scientific discipline, survivorship, has arisen because of the near 80% overall cure rate for childhood cancer. Focusing on the improvement of the quality of life of survivors coupled with research in this new discipline gives hope that many of the remaining long-term effects of cancer chemotherapy in children will be mitigated and possibly eliminated.

Major advances have occurred in the management of chemotherapy-induced vomiting and pain management because of the greater recognition and attention to these issues and the discovery of many new, effective drugs to deal with these symptoms. The availability of symptom control and palliative care has provided a degree of comfort for children undergoing chemotherapy, radiation, and surgery that did not exist only a few years ago.

Hematologist-oncologists today are privileged to practice their specialty in an era in which most oncologic and many hematologic diseases in children are curable and at a time when national and international cooperative groups are making major advances in the management of these diseases and when basic research is at the threshold of making major breakthroughs. The present practice is grounded in evidence-based research that has been and is still being performed by hematologist-oncologists and researchers that form the foundation for ongoing advances. Today we stand on the shoulders of others, which permits us to see future advances unfold to benefit generations of children. While we bask in the glory of past achievements, we should always be cognizant that much work remains to be done until the permanent cure of all childhood malignancies and blood diseases is at hand.

This book encompasses the advances in the management of childhood cancer which have been accomplished to date and which have become the standard of care.

CHAPTER

1

Diagnostic Molecular and Genomic Methodologies for the Hematologist/Oncologist

Vijay G. Sankaran

Over the past several years, molecular diagnostic testing in patients with hematologic and oncologic disorders has become increasingly sophisticated and prevalent. While in the past focused genetic tests were performed, in recent years the widespread use of genomic and molecular approaches in both research and clinical settings has shown potential to refine our understanding of pediatric blood disorders and cancer. This chapter provides an overview of the currently used molecular and genomic methods. In that way, the format for this chapter differs from those that describe specific disease entities. Thus the chapter can be read in its entirety as essential background for the modern practice of pediatric hematology/oncology. We will primarily focus on those genetic methods that are currently in use in clinical settings. Undoubtedly, in the coming years, the use of certain methods will evolve and new methods will become available. With this in mind, there are two goals in this chapter. We first aim to provide an overview of the types of currently used clinical genetic testing methods and specifically attempt to examine their utility in detecting specific changes at the molecular level that underlie both congenital and acquired conditions that are commonly seen by pediatric hematologists and oncologists. This overview will be important for clinicians to better understand how newly developed methods could supplant the currently used approaches in the coming years. The second goal of this chapter is to provide a basic understanding of the limitations that exist for the most common molecular and genomic methods in use, so that clinicians who receive these results can be sufficiently versed in these methods and avoid misinterpreting the results obtained from these tests.

CLINICAL MOLECULAR AND GENOMIC METHODOLOGIES

Despite the large range of approaches that have been developed, all of the methods remain focused on the goal of identifying patients' molecular lesions that underlie their disease. The methods can be broadly classified into two categories:

1. Direct testing: These approaches look for the presence of genetic mutations that directly contribute to disease.
2. Indirect testing: This category includes approaches that compare genomic or molecular markers in multiple affected individuals to unaffected individuals. These approaches often identify markers that may segregate with a disease, but the markers themselves may not cause the disease itself.

There may be overlap between these categories and certain methods may identify causal genetic mutations in some instances (direct testing), while only identifying segregating markers (indirect testing) in other cases.

Table 1.1 lists the commonly used genetic testing methodologies, along with the types of molecular lesions that they are able to identify.

Linkage Analysis

While most methods are now focused on identifying the precise molecular cause of disease, indirect tests can be quite useful, particularly for mapping causes of a disease in a family. For example, testing for markers such as

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TABLE 1.1 Overview of Molecular and Genomic Diagnostic Methodologies

Method	Common point mutations	Rare point mutations	Copy number variants	Uniparental disomy	Balanced inversions or translocations	Repeat expansions	Examples of use in pediatric hematology/oncology
Linkage analysis (using markers such as short tandem repeats)	X		X				Family pedigree with history of hereditary spherocytosis and interest in identifying causal gene
Fluorescent *in situ* hybridization			X		X		Acquired monosomy in myelodysplastic syndrome
Array comparative genomic hybridization			X	X			Testing for microdeletion in patient with hematologic and syndromic phenotype
Genome-wide single nucleotide polymorphism microarrays	X		X				Testing for small copy number variants in pediatric leukemia
Targeted polymerase chain reaction analysis	X	X				X	Testing for JAK2 V617F mutation in patient with a myeloproliferative disorder
Sanger sequencing	X	X					Molecular diagnosis of a patient with pyruvate kinase deficiency
Multiplex ligation-dependent probe amplification			X			X	Deletions in α- or $\delta\beta$- thalassemia cases
Gene panel sequencing	X	X					Severe congenital neutropenia
Whole-genome or -exome sequencing	X	X	X				Unknown bone marrow failure syndrome

single nucleotide polymorphisms (SNPs) or short tandem repeats (STRs, which are 2–5 base long repetitive elements with varying numbers of repeats) that are found throughout the genome can be extremely useful as a way to identify likely causal genes, particularly in diseases where multiple possible causal genes have been implicated. For example, in hereditary spherocytosis a number of genes including *ANK1*, *SPTB*, *SPTA*, *SLC4A1*, and *EBP42* are implicated in the disease. Many of these genes are quite large and while sequencing a panel of genes is certainly possible, in a large family, the use of either SNP-based methods or other markers such as STRs can identify the likely causal locus to help focus targeted sequencing efforts. These methods are commonly used for diagnostic mapping in resource-poor settings where whole-genome sequencing (WGS) methods may not be available and these methods can also be extremely useful in other settings. For example, if a family is being followed with a known disease, but no coding mutations are identified on targeted sequencing, these approaches can help validate that there is linkage to a specific gene and they may assist in the efforts to identify mutations in regulatory regions of the implicated gene. Specifically, segregation of markers that are in linkage with the causal mutation should only be found in affected family members and would suggest that the causal mutation is located nearby. These methods are also commonly used as an initial screen in families where possible cancer predisposition syndromes may exist and can help focus in-depth analysis on certain regions of the genome. Even in cases where whole-exome or -genome sequencing is performed, linkage can provide an excellent indirect approach to focus on marker genes that segregate appropriately in individuals who have a particular disease.

Fluorescent *In Situ* Hybridization

Fluorescent *in situ* hybridization (FISH) was developed in the 1980s and uses fluorescently labeled DNA probes to query whether entire chromosomes or parts of a chromosome may be duplicated or deleted in cells. The fluorescently labeled DNA probes are complementary to the region of interest on a chromosome and therefore specifically hybridize only to this region and not to others. FISH is commonly used to assess for gain or loss

of chromosomes or large parts of chromosomes in patients with hematologic malignancies. Typically, a number of cells from the bone marrow are tested for the presence of such chromosomal aberrations, which can have important roles both in terms of disease diagnosis and prognosis. FISH has a benefit in that it is a cytogenetic method and therefore individual cells are assessed rather than a population of cells in aggregate, which is the case for other methods that examine copy number variation, including array comparative genomic hybridization (CGH) or genome-wide SNP microarrays. In addition, FISH remains the best clinically available method to detect classic cytogenetic changes that are diagnostic and implicated in a number of pediatric cancers, such as translocations that are frequently seen in leukemia and certain solid tumors.

Array CGH

FISH lacks the sensitivity to detect smaller chromosomal deletions or duplications, which often represent important DNA copy number variations found in disease. Array CGH takes advantage of microarrays that have oligonucleotide probes at varying densities to detect differences in DNA copy number by comparing a sample genome with a reference sample (or group of reference samples) and examining whether there is an increase or decrease in signals at a particular genomic region in comparison with the control, which would be indicative of duplications or deletions, respectively. This method has significant sensitivity to detect DNA copy number changes, particularly smaller changes, in a variety of different samples. This can be applied to congenital disorders, where copy number changes can cause disease when present in the germline. In addition, in acquired hematologic or other malignancies, there can be acquired copy number changes. This increased sensitivity to detect smaller copy number changes has led to an increased detection of copy number changes in genomes of unclear significance. A number of resources are cataloging such changes in humans, although nonuniform deposition of deletion information into such databases makes the ongoing interpretation of either germline or acquired somatic copy number changes difficult in some cases. It is likely that as these databases grow with more phenotype information available, there will be increased insight into whether a deletion or duplication may be pathogenic.

Genome-Wide or Focused SNP Arrays

Microarrays provide the opportunity to genotype SNPs in a large-scale and potentially genome-wide manner. These approaches can be used for several applications. Similar to array CGH, these methods can be used to detect copy number variation that is either found in the germline or that is acquired. The resolution of the deletions detected using such approaches can be as good or in some cases, depending upon SNP or probe density, better than the resolution achieved with array CGH methods. In many cases, SNP arrays are used in place of array CGH in many clinical labs to detect copy number changes routinely. Indeed, the use of these SNP arrays is particularly widespread in the diagnostic evaluation of hematologic malignancies. Another application of such arrays is to genotype common SNPs in the genome, either for linkage mapping, as discussed above, or for identification of a common variation that confers risk of having certain diseases. While this has proven useful in some diseases, it is important to bear in mind that most such associations are probabilistic and not deterministic of acquiring disease. The clinical utility of this application is not clear, although a number of direct-to-consumer services offer such genotyping and will report relative risk information to individuals who request such services.

As more disease-associated mutations are being identified, there have been efforts to develop focused SNP arrays for specific phenotypes or diseases. In the future, such approaches may have clinical utility. However this application may be surpassed by large-scale genome sequencing as it becomes affordable (discussed below). One limitation of this approach is that there is continuous discovery of new causal alleles and genes in many diseases, which limits the clinical utility of such arrays.

Multiplex Ligation-Dependent Probe Amplification

Multiplex ligation-dependent probe amplification (MLPA) is a molecular approach that involves annealing of two adjacent oligonucleotides to a segment of genomic DNA followed by quantitative polymerase chain reaction (PCR) amplification to characterize copy number or other changes in the DNA. A series of MLPA probes can together screen and map deletions that occur in a particular region. In contrast to array CGH or SNP arrays, this approach is best applied to detect DNA copy number alterations in specific focused regions and this approach

can allow such alterations to be finely mapped. For example, MLPA is commonly used to map deletions that commonly occur in the α-globin gene locus in cases of α-thalassemia. Traditionally, this mapping was done using Southern blotting (to determine a specific DNA sequence), but this is now rarely done for clinical purposes and in most instances MLPA is used for such applications in clinical labs. When results from MLPA are reported, it is important to bear in mind that the resolution will depend upon the number of probes used and, in general, precise deletion coordinates will not be defined using MLPA alone. Often to better map deletion sites, PCR-based Sanger sequencing approaches are used to map breakpoints once general coordinates have been defined using MLPA (discussed below).

Targeted PCR Analysis

Often a single mutation confers significant disease risk or occurs in the majority of cases of a disease. In these cases, PCR approaches can be used for amplification and separation of different alleles. A number of approaches to separate different alleles using PCR have been developed and since these methods are largely specific to individual platforms, the details of specific methods will not be covered here. These approaches are commonly used for detection of mutations, such as factor V Leiden that confers an increased risk of venous thrombosis and is found in several percent of the general population. In certain hematologic malignancies, such as myeloproliferative diseases, there are common mutations such as JAK2 V617F that occurs in many cases and focused genotyping of this variant is often performed as a clinical test. These tests can be done at low cost and relatively rapidly because of their focused nature and output of the presence/absence of a single mutation. However, using this approach it is impossible to detect relevant variants that have not been genotyped in a gene of interest.

Focused Sanger and Gene Panel Sequencing

Traditional DNA sequencing has relied upon the chain terminator method developed by Frederick Sanger, where a chain-terminating dideoxynucleotide is coupled to a fluorescent dye and this can allow sequencing in a single reaction by detecting terminated DNA fragments of various sizes. A chromatogram obtained after capillary-based separation displays the sequentially elongated fragments that each end in a specific fluorescent terminating dideoxynucleotide, allowing identification of the sequence of DNA. This approach is often applied to a series of reactions using PCR that cover a gene or in some instances a panel of genes implicated in disease. A limitation is that a single reaction can only assess a single sequence of several hundred bases and therefore multiple reactions will need to be run for most genes. It should be kept in mind that while Sanger sequencing can be very sensitive to detect point mutations, copy number or structural changes in genes will often be missed using this approach. Therefore, targeted sequencing of a disease gene can often be complemented using array CGH or SNP array-based approaches to look for deletions that may be implicated in a subset of cases of a particular disease.

Whole-Genome or -Exome Sequencing

While Sanger sequencing methods were once the primary method to map DNA sequences, the development of high-throughput next-generation sequencing (NGS) platforms over the past several years has allowed rapid and low-cost sequencing of large portions of the genome. NGS takes advantage of various technologies to sequence millions to billions of DNA strands in parallel to yield substantially more throughput than Sanger sequencing approaches. Moreover, NGS approaches bypass fragment-cloning steps that were necessary for genome sequencing using traditional Sanger sequencing approaches. In NGS, DNA is broken into short fragments, a subset of fragments may be enriched (such as with exome sequencing where sequences that encode regions overlapping with exons are enriched), and the sequences of all the fragments are then read on NGS platforms. The details of these approaches vary depending upon the technology or platform used, but in general all NGS methods use similar principles.

The use of NGS platforms to sequence the entire genome or exome of a patient with a particular disorder for clinical purposes has only begun to emerge and these technologies are currently being primarily used in the research setting. Studies are beginning to address the diagnostic yield of these approaches. One important consideration in using these approaches is that since the entire genome (or a substantial portion) is sequenced, potentially pathogenic variants may be identified that may not contribute to the disease that initially motivated the

WGS. At the current time, there are varying opinions on the types of circumstances in which such incidental findings can be reported to patients and studies are exploring the impact of delivering such information to patients. There has been substantial debate in the community regarding the delivery and broad impact of the information derived from NGS on patients, physicians, and society. Undoubtedly, this is an area that will evolve considerably in the coming years.

Currently WGS or whole-exome sequencing (WES) are ordered in the clinical setting for detection of rare variants in patients with a phenotype that is suspected to be due to a single-gene disorder, after known single-gene candidates have either been eliminated or when a multigene testing approach is prohibitively expensive. Before such tests are ordered, it is important that clinicians gather a thorough family history, fully evaluate a patient's phenotype, and obtain appropriate informed consent. There is little doubt that as WGS and WES are routinely employed in clinical settings, specific guidelines for when it is best to use these tests for patients with particular groups of diseases will emerge.

It is important for clinicians to be aware of the significant limitations of WGS and WES. NGS approaches can currently only sequence nonrepetitive regions of the genome and some diseases occur in repetitive DNA (i.e., fragile X syndrome) and thus diagnoses will be missed using such approaches. In addition, NGS approaches cannot currently detect most copy number variants, insertion−deletion variants, or chromosomal translocations. It is likely that with improvements in technology to allow for longer reads in NGS and with better computational methods, these types of variants could be detected using these approaches in the future.

Since numerous potential causal variants may be identified using such broad-based sequencing approaches, the American College of Medical Geneticists has developed recommendations regarding categories to which variants should be assigned and these are helpful for clinicians to be aware of when interpreting results from WES or WGS (although they apply to other sequencing approaches as well).

- Disease-causing: A sequence variant that has previously been reported as a cause of a disorder
- Likely disease-causing: A sequence variant that has not been previously reported, but is of a type that would be expected to cause disease
- Possibly disease-causing: A sequence variant that has not been previously reported and is of the type that may or may not cause a particular disorder
- Likely not disease-causing: Sequence variation that has not been reported and is not likely of the type that would cause a particular disorder
- Not disease-causing: Sequence variation has previously been reported and is a recognized neutral variation
- Variant of unknown clinical significance: Sequence variation is not known or expected to be causative of disease, but is found to be associated with a clinical presentation.

While these categories are useful when results are reported, they are largely dependent upon databases of prior variants. As more sequencing data are being reported, it is important to bear in mind that some variants previously thought to be pathogenic are being reclassified as benign. It is very likely that the categorization for a particular variant may evolve over time and therefore it is useful to evaluate the prior reports of any genetic variants identified in such studies on an individual basis. In some cases, it is important to bear in mind that in many single-gene disorders, variable penetrance or expressivity (where patients with a particular mutation may or may not have the disease or may have varying severities of the disease) may have a significant and underestimated impact.

INTERPRETING AND EVALUATING THE RESULTS FROM CLINICAL GENETIC TESTING

A key role of any pediatric hematologist/oncologist will be interpreting and evaluating the results obtained from clinical diagnostic genetic testing. As newer methods are used, different limitations of each approach need to be addressed. While for each diagnostic approach we have addressed some specific limitations, we also want to present a general framework for evaluating the validity of such testing in general. Table 1.2 presents some considerations that are applicable for any test and provides examples of complications that may need to be kept in mind.

For each particular test, specific test sensitivities and specificities will exist. In addition, for a particular test and diagnosis there will also be certain clinical sensitivities and specificities that need to be accounted for.

TABLE 1.2 Overview of Terms Used to Evaluate the Validity of Genetic Tests

Term	Definition	Calculation	Examples of complications
Test sensitivity	Proportion of assays with the genetic change that have a positive test result	True positives/ (true positives + false negatives)	Impaired amplification of mutant allele in mosaic setting due to selection
Test specificity	Proportion of assays without the genetic change that have a negative test result	True negatives/ (true negatives + false positives)	Technical impairment in MLPA leading to false appearance of deletion will lower test specificity
Clinical sensitivity	Proportion of people with a disease who have a positive test result	True positives/ (true positives + false negatives)	Multiple genes involved in a disease may lead to some patients not having a particular mutation or set of mutations
Clinical specificity	Proportion of people without a disease who have a negative test result	True negatives/ (true negatives + false positives)	Variable penetrance of disease (i.e., carriers of Diamond–Blackfan anemia mutations without symptoms)
Positive predictive value	The likelihood that a patient has a disease, given a positive test result	True positives/ (true positives + false positives)	Variable penetrance or expressivity can complicate this
Negative predictive value	The likelihood that a patient does not have a disease, given a negative test result	True negatives/ (true negatives + false negatives)	Multiple genes involved in a disease may complicate this

Finally, the positive and negative predictive values for any test should also be considered. Depending upon the genetic lesion under consideration, this may alter what tests are performed. For example, while large chromosomal alterations or translocations are readily detected using FISH, smaller deletions may only be detected using array CGH or SNP arrays. Therefore, depending upon the type of lesion expected, the various tests may be used in different ways or combinations. Another example is that while WES or WGS can be useful for looking for many causes of a disease, they may miss deletions that could result in a subset of cases of a particular disease. Therefore, it may be useful to both run WES and a SNP array on a particular patient if the disease can be caused by deletions in some cases.

One limitation currently faced in interpreting the results of WGS is that even when a mutation is identified in a regulatory region of a gene, its affect on the gene implicated in that disease may not be immediately apparent. If other affected and unaffected family members are available, linkage information can help demonstrate that a particular region is associated with the disease and support the presence of a nearby causal allele. For example, in a few cases of sideroblastic anemia, mutations were identified in regulatory regions of the *ALAS2* gene, which normally harbors coding mutations in most other cases of this disease. Appropriate identification of these mutations required a combination of linkage analysis and functional follow-up tests. This work was primarily done as part of a research study, but it is possible that in the future as more such noncoding mutations are identified, that these may be found to be important contributors to a variety of disorders seen in patients.

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CHAPTER

2

Hematologic Manifestations of Systemic Illness

Lawrence C. Wolfe

A variety of systemic illnesses including acute and chronic infections, neoplastic diseases, connective tissue disorders, and storage diseases are associated with hematologic manifestations. The hematologic manifestations are the result of the following mechanisms:

- Bone marrow dysfunction
  - Anemia or polycythemia
  - Thrombocytopenia or thrombocytosis
  - Leukopenia or leukocytosis
- Hemolysis
- Immune cytopenias
- Alterations in hemostasis
  - Acquired inhibitors to coagulation factors
  - Acquired von Willebrand disease
  - Acquired platelet dysfunction
- Alterations in leukocyte function

HEMATOLOGIC MANIFESTATIONS OF DISEASES RELATED TO VARIOUS ORGANS

Heart

Microangiopathic hemolysis occurs with prosthetic valves or synthetic patches utilized for correction of cardiac defects, particularly when there is failure of endothelialization ("Waring Blender" syndrome) or rarely after endoluminal closure of a patent ductus arteriosus. Microangiopathic hemolysis has the following characteristics:

- Hemolysis is secondary to fragmentation of the red cells as they are damaged against a distorted vascular surface.
- Hemolysis is intravascular and may be associated with hemoglobinemia and hemoglobinuria.
- Iron deficiency occurs secondary to the shedding of hemosiderin within renal tubular cells into the urine (hemosiderinuria).
- Thrombocytopenia secondary to platelet adhesion to abnormal surfaces.
- Autoimmune hemolytic anemia may occasionally occur after cardiac surgery with the placement of foreign material within the vascular system.

Cardiac Anomalies and Hyposplenism

Cardiac anomalies, particularly *situs inversus*, may be associated with hyposplenism and the blood smear may show Howell–Jolly bodies, Pappenheimer bodies, and elevated platelet counts.

Infective Endocarditis

Hematologic manifestations include anemia (due to immune hemolysis or chronic infection), leucopenia, or leukocytosis and rarely thrombocytopenia and pancytopenia.

Lanzkowsky's Manual of Pediatric Hematology and Oncology.
DOI: http://dx.doi.org/10.1016/B978-0-12-801368-7.00002-8

Coagulation Abnormalities

- A coagulopathy exists in some patients with cyanotic heart disease. The coagulation abnormalities correlate with the extent of the polycythemia. Hyperviscosity may lead to tissue hypoxemia, which could trigger disseminated intravascular coagulation (DIC).
- Marked derangements in coagulation such as DIC, thrombocytopenia, thrombosis, and fibrinolysis can accompany surgery involving cardiopulmonary bypass. Heparinization must be strictly monitored.

Platelet Abnormalities

Quantitative and qualitative platelet abnormalities are associated with cardiac disease:

- Thrombocytopenia occurs secondary to microangiopathic hemolysis associated with prosthetic valves.
- Cyanotic heart disease can produce polycythemia, thrombocytopenia, prolonged bleeding time, and abnormal platelet aggregation.
- Patients with chromosome 22q11.2 deletion (DiGeorge syndrome) can have platelet abnormalities including the Bernard–Soulier-like syndrome due to haploinsufficiency of the gene for GP1BB and thrombocytopenia due to autoimmunity.

Polycythemia

- The hypoxemia of cyanotic heart disease produces a compensatory elevation in erythropoietin and secondary polycythemia.
- Patients are at increased risk for cerebrovascular accidents secondary to hyperviscosity.
- Patients are also at risk for symptomatic hypoglycemia (especially in the neonatal period).
- The use of partial exchange transfusion has been suggested, although the long-term value of exchange has been challenged.

Gastrointestinal Tract

Esophagus

- Iron-deficiency anemia may occur as a manifestation of gastroesophageal reflux.
- Endoscopy may be required in unexplained iron deficiency.

Stomach

- The gastric mucosa is important in both vitamin B_{12} and iron absorption.
- Chronic atrophic gastritis produces iron deficiency. There may be an associated vitamin B_{12} malabsorption.
- Gastric resection may result in iron deficiency or in vitamin B_{12} deficiency due to lack of intrinsic factor.
- Zollinger–Ellison syndrome (increased parietal cell production of hydrochloric acid) may cause iron deficiency through mucosal ulceration.
- *Helicobacter pylori* infection, in addition to causing chronic gastritis, has been implicated in the initiation of iron-deficiency anemia, vitamin B_{12} deficiency, autoimmune thrombocytopenia, and platelet aggregation defects (ADP-like defect).

Small Bowel

- Celiac disease or tropical sprue may cause malabsorption of iron and folate. Table 2.1 lists the various hematologic manifestations of celiac disease.
- Inflammatory bowel disease (IBD) may cause anemia of chronic inflammation and iron deficiency from blood loss.
- Eosinophilic gastroenteritis can produce peripheral eosinophilia.
- Diarrheal illnesses of infancy can produce life-threatening methemoglobinemia.

Lower Gastrointestinal Tract

- Ulcerative colitis is often associated with iron-deficiency anemia.
- Peutz–Jeghers syndrome (intestinal polyposis and mucocutaneous pigmentation) predisposes to adenocarcinoma of the colon.
- Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease) may produce iron deficiency, platelet dysfunction, and hemostatic defects.

TABLE 2.1 Hematologic Manifestations of Celiac Disease

Problem	Frequency	Comments
Anemia: iron deficiency, folate deficiency, vitamin B_{12} deficiency, and other nutritional deficiencies	Common	The anemia is most commonly secondary to iron deficiency but may be multifactorial in etiology. Low serum levels of folate and vitamin B_{12} without anemia are frequently seen. Anemia due to other deficiencies appears to be rare
Thrombocytopenia	Rare	May be associated with other autoimmune phenomena
Thrombocytosis	Common	May be secondary to iron deficiency or hyposplenism
Thromboembolism	Uncommon	Etiology is unknown but may be related to elevated levels of homocysteine or other procoagulants
Leukopenia/neutropenia	Uncommon	Can be autoimmune or secondary to deficiencies of folate, vitamin B_{12}, or copper
Coagulopathy	Uncommon	Malabsorption of vitamin K
Hyposplenism	Common	Rarely associated with infections
IgA deficiency	Common	May be related to anaphylactic transfusion reactions
Lymphoma	Uncommon	The risk is highest for intestinal T-cell lymphomas

From: Halfdanarson et al. (2007), with permission.

Pancreas

- Hemorrhagic pancreatitis produces acute normocytic, normochromic anemia. It may also be associated with DIC.
- Shwachman–Diamond syndrome (SDS; see Chapter 13) is characterized by congenital exocrine pancreatic insufficiency, metaphyseal bone abnormalities, and neutropenia. There may also be some degree of anemia and thrombocytopenia.
- Cystic fibrosis produces malabsorption of fat-soluble vitamins (e.g., vitamin K) with impaired prothrombin production.
- Pearson syndrome is characterized by exocrine pancreatic insufficiency and severe sideroblastic anemia (see Chapter 13).

Liver

Anemia

Anemias of diverse etiologies occur in acute and chronic liver disease. Red cells are frequently macrocytic (mean corpuscular volume (MCV) of 100–110 fl). Target cells and acanthocytes (spur cells) are frequently seen. Some of the pathogenic mechanisms of anemia include:

- Shortened red cell survival and red cell fragmentation (spur cell anemia) in cirrhosis often occur in later-stage cirrhosis in the presence of dyslipidemia.
- Hypersplenism with splenic sequestration in the presence of secondary portal hypertension.
- Iron-deficiency anemia secondary to blood loss from esophageal varices in portal hypertension.
- Chronic hemolytic anemia in Wilson disease secondary to copper accumulation in red cells. Hemolytic anemia may be the presenting symptom in this disease.
- Aplastic anemia following acute hepatitis (usually seronegative) in certain immunologically predisposed hosts.
- Megaloblastic anemia secondary to folate deficiency in malnourished individuals.

Coagulation Abnormalities

The liver is involved in the synthesis of most of the coagulation factors. Liver dysfunction can be associated with either hyper- or hypocoagulable states because both procoagulant and natural anticoagulant synthesis are impaired. Table 2.2 lists the various coagulation abnormalities seen in liver disease and Table 2.3 lists the tests to differentiate between the coagulopathy of liver disease and other etiologies.

TABLE 2.2 Coagulation Abnormalities in Liver Disease

Hemorrhage	Thrombosis
(1) Thrombocytopenia/platelet dysfunction due to hypersplenism, altered TPO production	(1) Decreased anticoagulant—AT-III Protein C and S
(2) Decreased liver synthesis of procoagulant factors	(2) Portal hypertension-portal vein thrombosis
(3) Impaired carboxylation of vitamin K factors	
(4) Dysfibrinogenemia	
(5) Hyperfibrinolysis due to increased tPA and decreased PAI, α_2 antiplasmin	

TPO, thrombopoietin; tPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor.

TABLE 2.3 Tests to Differentiate Coagulopathies of Different Etiologies

Procoagulant factors	Liver	Vitamin K	DIC
F V	Decreased (late)	Normal	Decreased
F VII	Decreased (early)	Decreased	Decreased
F VIII	Normal/increased	Normal	Decreased

Factor I (Fibrinogen)

Fibrinogen levels are generally normal in liver disease. Low levels may be seen in fulminant acute liver failure.

Factors II, VII, IX, and X (Vitamin K-Dependent Factors)

These factors are reduced in liver disease secondary to impaired synthesis. Factor VII is the most sensitive.

Factor V

Factor V does not require vitamin K for synthesis and is highly representative of actual liver function. Factor V levels at 36 h post liver injury have been used as a standalone marker for the possible need for transplant in patients with early liver failure.

Factor VIII

The procoagulant activity of Factor VIII is generally normal in liver disease. This makes Factor VIII an important factor to measure in distinguishing between DIC and severe liver disease in a patient with abnormal coagulation tests and thrombocytopenia. If there is associated DIC, factor VIII will be markedly depressed, whereas in severe liver disease Factor VIII remains close to or normal. Traditionally, the Factor VII and Factor VIII levels are measured along with the PT, PTT, and fibrinogen to distinguish liver disease from DIC.

Protein C, Protein S, and Antithrombin III

These natural anticoagulants are decreased in liver disease. Proteins C and S are most sensitive to vitamin K deficiency. In many cases this fall in the levels of natural anticoagulant creates a sensitive balance between loss of procoagulant activity and natural anticoagulant activity. Bleeding or thrombosis may appear quickly when additional illness (e.g., infection) may upset the balance.

Tissue plasminogen activator (TPA) and alpha-2-antiplasmin

Tissue plasminogen activator is cleared by the liver and as liver disease progresses TPA activity increases. Alpha-2-antiplasmin is also suppressed by liver disease, creating increased plasmin activity and ultimately the syndrome of hyperfibrinolysis with a tendency toward severe bleeding.

α_2-Macroglobulin and plasmin activator inhibitor

These opponents of plasma activity are still present in liver disease.

Kidneys

Renal disease may affect red cells, white cells, platelets, and coagulation.

Severe renal disease with renal insufficiency is frequently associated with chronic anemia (and sometimes pancytopenia). This type of anemia is characterized by:

- Hemoglobin as low as 4–5 g/dl
- Normochromic and normocytic red cell morphology unless there is associated microangiopathic hemolytic anemia (as in the hemolytic-uremic syndrome (HUS)), in which case schistocytes and thrombocytopenia are seen
- Reticulocyte count low
- Decreased erythroid precursors in bone marrow aspirate.

The following mechanisms are involved in the pathogenesis of this type of anemia:

- Erythropoietin deficiency is the most important factor (90% of erythropoietin synthesis occurs in the kidney)
- Shortened red cell survival is secondary to uremic toxins or in HUS secondary to microangiopathic hemolysis
- Renal failure itself inhibits erythropoiesis and in conjunction with decreased erythropoietin levels produces a hypoplastic marrow
- Increased blood loss from a hemorrhagic uremic state and into a hemodialysis circuit causes iron deficiency.
- Dialysis can lead to folic acid deficiency.

Treatment

- Recombinant human erythropoietin (rHuEPO):[1]
  - Determine the baseline serum erythropoietin and ferritin levels prior to starting rHuEPO therapy. If ferritin is less than 100 ng/ml, give ferrous sulfate 6 mg/kg/day aimed at maintaining a serum ferritin level above 100 ng/ml and a threshold transferrin saturation of 20%. With the advent of less immunoreactive forms of intravenous iron, prophylactic strategies utilizing intravenous iron infusion at the end of dialysis have been very successful. These are usually well tolerated compared to oral iron on a daily basis.
  - Start with rHuEPO treatment in a dose of 50–100 units/kg/day subcutaneously (SC) three times a week.
  - Monitor blood pressure closely (increased viscosity produces hypertension in 30% of cases) and perform complete blood count (CBC) weekly.
  - Titrate the dose:
    - If no response, increase rHuEPO up to 300 units/kg/day SC three times a week
    - If hematocrit (Hct) reaches 40%, stop rHuEPO until Hct is 36% and then restart at 75% dose
    - If Hct increases very rapidly (>4% in 2 weeks), reduce dose by 25%.

  Figure 2.1 shows a flow diagram, in greater detail, for the use of erythropoietin-stimulating agents in patients with chronic kidney disease.
- Folic acid 1 mg/day is recommended because folate is dialyzable
- Packed red cell transfusion is rarely required.

Endocrine Glands

Thyroid

Anemia is frequently present in hypothyroidism. It is usually normochromic and normocytic. The anemia is sometimes hypochromic because of associated iron deficiency and occasionally macrocytic because of vitamin B_{12} deficiency. The bone marrow is usually fatty and hypocellular and erythropoiesis is usually normocytic. The finding of a macrocytic anemia and megaloblastic marrow in children with hypothyroidism should raise the possibility of an autoimmune disease with antibodies against parietal cells as well as against the thyroid, leading to vitamin B_{12} deficiency (juvenile pernicious anemia with polyendocrinopathies).

[1]Thrombosis of vascular access occurs in 10% of cases treated with rHuEPO.

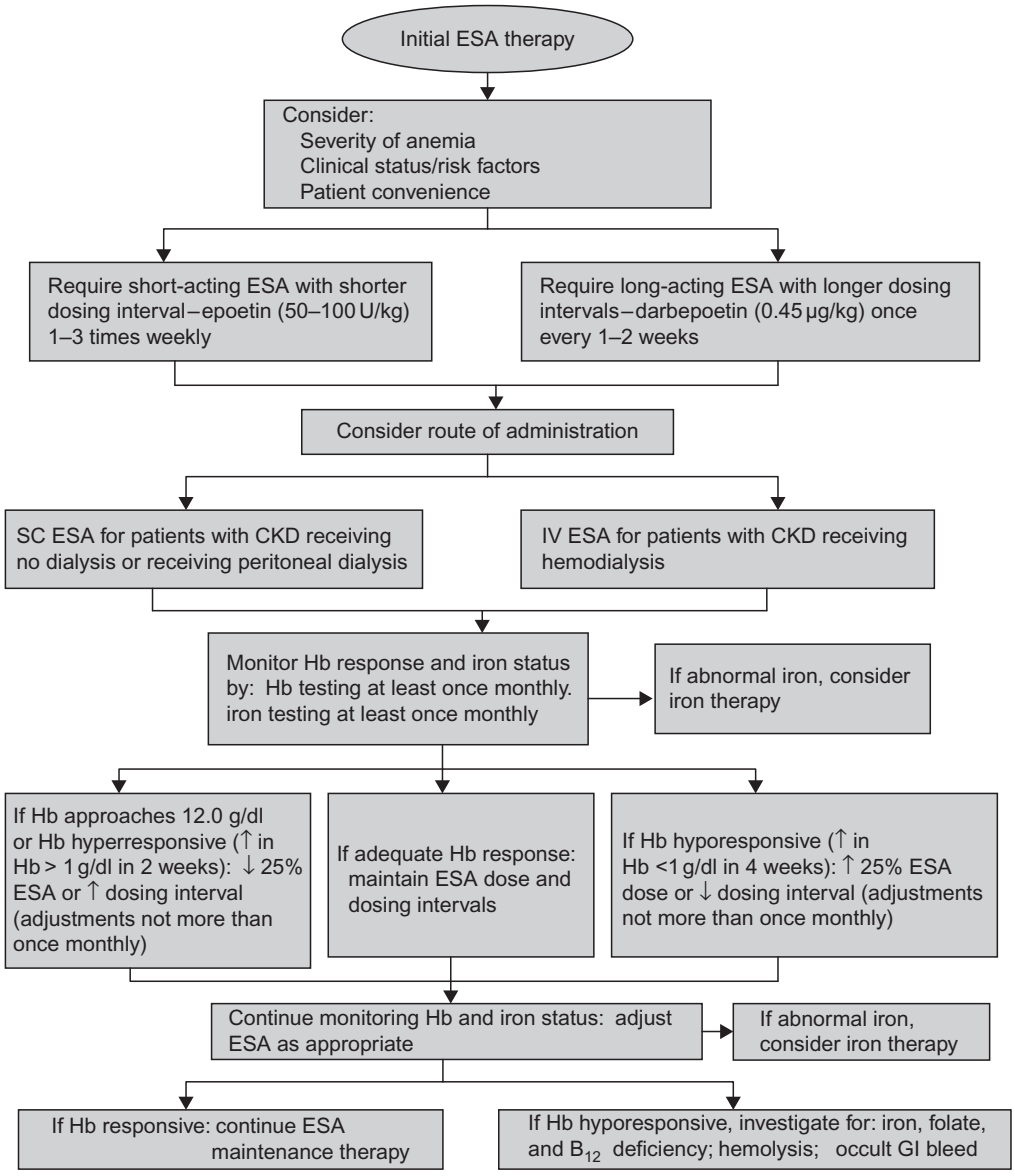

FIGURE 2.1 Recommended erythropoietin-stimulating agent (ESA) treatment in patients with chronic kidney disease. SC, subcutaneous; IV, intravenous; CKD, chronic kidney disease; ↑, increase; ↓, decrease. Source: *From Wish and Coyne (2007), with permission.*

Adrenal Glands

- Androgens stimulate erythropoiesis.
- Conditions of androgen excess, such as Cushing syndrome and congenital adrenal hyperplasia, can produce secondary polycythemia.
- In Addison disease, some degree of anemia is also present but may be masked by coexisting hemoconcentration. The association between Addison disease and megaloblastic anemia raises the possibility of an inherited autoimmune disease directed against multiple tissues, including parietal cells (juvenile pernicious anemia with polyendocrinopathies) (see Chapter 7).

Lungs

- Hypoxia secondary to pulmonary disease results in secondary polycythemia.
- Idiopathic pulmonary hemosiderosis is a chronic disease characterized by recurrent intra-alveolar microhemorrhages with pulmonary dysfunction, hemoptysis, and hemosiderin-laden macrophages, resulting

in iron-deficiency anemia. A precise diagnosis can be established by the presence of siderophages in the gastric aspirate. Apart from a primary idiopathic type, there is also a variant associated with hypersensitivity to cows' milk and one that occurs with a progressive glomerulonephritis (Goodpasture syndrome). Treatment is controversial and may involve:

- Corticosteroids
- Withdrawal of cow's milk
- Use of oral or IV iron when indicated
- Packed red cell transfusions when indicated.

Skin

Mast Cell Disease

Mast cell disease or mastocytosis is associated with an abnormal accumulation of mastocytes (more closely related to monocytes or macrophages rather than to basophils) in the dermis (cutaneous mastocytosis) or in an internal organ (systemic mastocytosis). The systemic form is rare in children. In children, this condition is more common under 2 years of age. It usually presents either as a solitary cutaneous mastocytoma or, more commonly, as urticaria pigmentosa. Involvement beyond the skin is unusual in children, but splenomegaly and bone lesions have been reported. No reports of bone marrow disease in either acquired or congenital mastocytosis have been reported.

Eczema and Psoriasis

Patients with extensive eczema and psoriasis commonly have anemia. The anemia is usually normochromic and normocytic (anemia of chronic disease) and mild in most cases, but severely affected individuals can have hemoglobin levels less than 9 g/dl.

Dermatitis Herpetiformis

- Macrocytic anemia secondary to malabsorption.
- Hyposplenism: Howell–Jolly bodies may be present on blood smear.

Dyskeratosis Congenita

This disease is characterized by ectodermal dysplasia and aplastic anemia (see Chapter 8). The aplastic anemia is associated with high MCV, thrombocytopenia, and elevated fetal hemoglobin. This may occur before the onset of skin manifestations.

Hereditary Hemorrhagic Telangiectasia

This autosomal dominant disorder is associated with a bleeding disorder. Easy bruisability, epistaxis, and respiratory and gastrointestinal bleeding may be caused by telangiectatic lesions.

Ehlers–Danlos Syndrome

This condition may be associated with platelet dysfunction: reduced aggregation with ADP, epinephrine, and collagen. An unusual sensitivity to aspirin is described in type IV Ehlers–Danlos syndrome (see Chapter 14).

CHRONIC ILLNESS

Chronic illnesses such as cancer, IBD, connective tissue disease, and chronic infection are associated with anemia. The anemia has the following characteristics:

- Normochromic, normocytic, occasionally microcytic
- Usually mild, characterized by decreased plasma iron and normal or increased reticuloendothelial iron
- Impaired flow of iron from reticuloendothelial cells to the bone marrow
- Decreased sideroblasts in the bone marrow.

The tests to differentiate the anemia of chronic illness from iron-deficiency anemia are listed in Table 2.4 and therapeutic options for the treatment of anemia in chronic disease are outlined in Table 2.5.

TABLE 2.4 Laboratory Tests to Differentiate Anemia of Chronic Disease from Iron-Deficiency Anemia[a]

Variable (serum levels)	Anemia of chronic disease	Iron-deficiency anemia	Both conditions[b]
Iron	Reduced	Reduced	Reduced
Transferrin	Reduced to normal	Increased	Reduced
Transferrin saturation	Normal to mildly reduced	Reduced	Reduced
Ferritin	Normal to increased	Reduced	Reduced to normal
Soluble transferrin receptor	Normal	Increased	Normal to increased
Cytokine levels	Increased	Normal	Increased

[a]*Relative changes are given in relation to the respective normal values.*
[b]*Patients with both conditions include those with anemia of chronic disease and true iron deficiency.*
Modified from: Weiss and Goodnough (2005), with permission.

TABLE 2.5 Therapeutic Options for the Treatment of Anemia of Chronic Disease

Treatment	Anemia of chronic disease	Anemia of chronic disease with true iron deficiency
Treatment of underlying disease	Yes	Yes
Transfusions[a]	Yes	Yes
Iron supplementation	No[b]	Yes[c]
Erythropoietin agents	Yes	Yes, in patients who do not have a response to iron therapy

[a]*This treatment is for the short-term correction of severe or life-threatening anemia. Potentially adverse immunomodulatory effects of blood transfusions are controversial.*
[b]*Although iron therapy is indicated for the correction of anemia of chronic disease in association with absolute iron deficiency, no data from prospective studies are available on the effects of iron therapy on the course of underlying chronic disease.*
[c]*Overcorrection of anemia (hemoglobin >12 g/dl) may be potentially harmful to patients; the clinical significance of erythropoietin-receptor expression on certain tumor cells needs to be investigated.*
From: Weiss and Goodnough (2005), with permission.

In inflammatory diseases, cytokines released by activated leukocytes and other cells exert multiple effects that contribute to the reduction in hemoglobin levels. The pathophysiology of anemia of chronic disease is shown in Figure 2.2:

1. Interleukins (IL), especially IL-6 along with endotoxin, induce hepcidin synthesis in the liver. Hepcidin in turn binds to Ferroportin located both in the GI tract and the reticuloendothelial system. Hepcidin binding induces the degradation of Ferroportin, sequestering oral intake iron from the GI tract and reticuloendothelial iron from storage sites. Hence in the classic patient with the anemia of chronic illness, the serum iron will be low, but there will also be a low level of transferrin iron-binding capacity secondary to a suppression of protein synthesis. This leads to a normal, or slightly diminished, iron saturation. The serum ferritin is then paradoxically elevated secondary to the hepcidin-induced sequester. The adult literature recognizes gray zone cases with normal or lower iron saturations and a low normal ferritin. In these situations laboratory testing for soluble transferrin receptor (which is elevated directly in response to iron deficiency) is used to direct iron treatment. These patients might also be simply given an intravenous iron challenge which will demonstrate improvement over 7–10 days if iron deficiency is present.
2. Inhibition of erythropoietin release from the kidney (especially by IL-1β and tumor necrosis factor-alpha (TNF-α)) leads to reduced erythropoietin-stimulated hematopoietic proliferation.
3. Direct inhibition of the proliferation of erythroid progenitors (especially by TNF-α, interferon-gamma (IFN-γ) and IL-1β).
4. Increased erythrophagocytosis by reticuloendothelial macrophages.

Treatment involves treating the underlying illness.

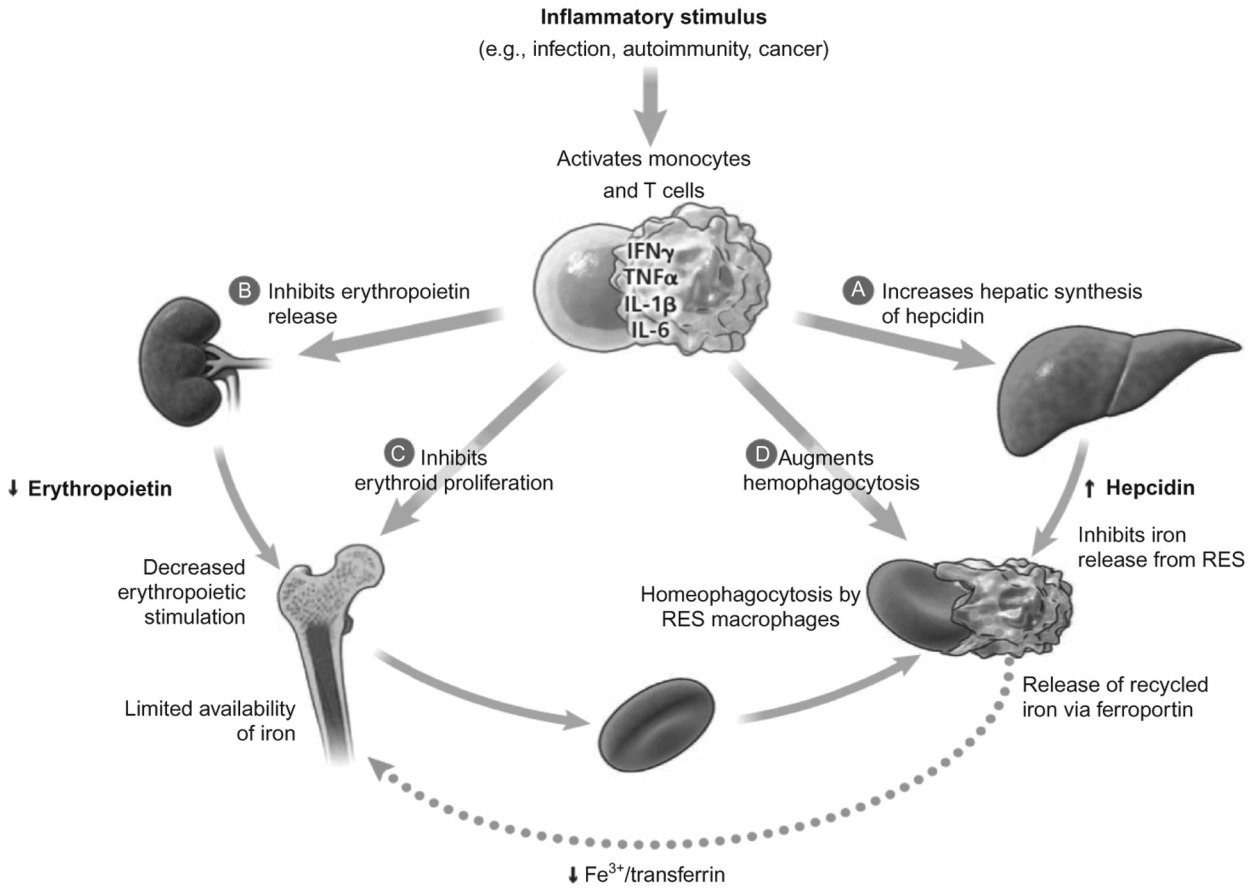

FIGURE 2.2 Pathophysiology of anemia of chronic disease. RES, Reticuloendothelial system. Source: *From Zarychanski and Houston (2008), with permission.*

Inflammatory Bowel Disease as a Model for Anemia of Chronic Illness

- In both Crohn's disease and ulcerative colitis the anemia of chronic illness is often seen—sometimes before gastrointestinal symptomatology manifests.
- It is often associated with concomitant iron deficiency due to bleeding from the involved bowel.
- The patient may present with mild normochromic anemia or severe microcytic anemia.

In an older child or adolescent presenting with iron deficiency a detailed history of gastrointestinal symptoms must be pursued and any suggestion of anemia of chronic illness may alter the type and route of iron medication. If a patient with IBD presents with anemia, iron saturation and ferritin should be assessed prior to the initiation of treatment. The anemia of chronic illness is not iron-deficient erythropoiesis. It is a balance between the effect of elevated hepcidin in sequestering iron and a direct effect of cytokines slowing down erythropoiesis—and hence diminishing the erythropoietic call for iron. If the patient has a very low iron saturation and an elevated ferritin, oral iron is likely to have less effect given the mucosal block to iron in the anemia of chronic illness. Intravenous iron preparations will bypass that block and also diminish the additional gastrointestinal toxicity of oral iron. The administration of iron alone may not ameliorate the situation as patients may have such a severe effect of inflammation on erythropoiesis that they may require simultaneous administration of erythropoietin in pharmacologic doses along with iron. These considerations are identical to those faced in other conditions with major ongoing inflammation (e.g., juvenile rheumatoid arthritis).

Connective Tissue Diseases

Rheumatoid Arthritis

- Anemia of chronic illness (normocytic, normochromic)
- High incidence of iron deficiency

- Leukocytosis and neutropenia common in exacerbations of juvenile rheumatoid arthritis (JRA)
- Thrombocytosis associated with a high level of IL-6 occurs in many patients, although there may be transient episodes of thrombocytopenia.

Felty Syndrome

- Triad of rheumatoid arthritis, splenomegaly, and neutropenia.
- Patients may be at risk for life-threatening bacteremia. Splenic dysfunction resulting in infection with encapsulated organisms has been observed.
- Treatment involves controlling the rheumatoid arthritis, which often leads to improvement in the anemia. Parenteral antibiotics, with coverage for encapsulated organisms, for febrile episodes is recommended.
- G-CSF may be used in urgent situations although but there are concerns about splenic rupture with the use of this G-CSF because of case reports of spontaneous rupture of the spleen in Felty syndrome.

Systemic Lupus Erythematous

- Two types of anemia are common: anemia of chronic illness (normocytic, normochromic) and acquired, autoimmune direct antiglobulin (DAT)-positive hemolytic anemia.
- Neutropenia is common as a result of decreased marrow production and immune-mediated destruction.
- Lymphopenia with abnormalities of T-cell function.
- Immune thrombocytopenia: Antiphospholipid antibodies may be present which prolong the aPTT but are associated with severe thrombosis (lupus anticoagulant).

Polyarteritis Nodosa

- Microangiopathic hemolytic anemia may be associated with renal disease or hypertensive crises.
- Prominent eosinophilia.

Wegener Granulomatosis

This autoimmune disorder is rare in children. Hematological features include:

- Anemia: normocytic; RBC fragmentation with microangiopathic hemolytic anemia
- Leukocytosis with neutrophilia
- Eosinophilia
- Thrombocytosis.

Kawasaki Syndrome

This syndrome is characterized by:

- Mild normochromic, normocytic anemia with reticulocytopenia
- Leukocytosis with neutrophilia and toxic granulation of neutrophils and vacuoles
- Decreased T-suppressor cells
- High C_3 levels
- Increased cytokines IL-1, IL-6, IL-8, interferon-α, and TNF
- Marked thrombocytosis (mean platelet count 700,000/mm^3)
- DIC.

Henoch–Schönlein Purpura

Henoch–Schönlein purpura (HSP), also called anaphylactoid purpura, is associated with systemic vasculitis characterized by unique palpable, erythema multiforma-like purpuric lesions, transient arthralgias or arthritis (especially affecting knees and ankles), colicky abdominal pain, and nephritis.

- Anemia occasionally occurs as a result of GI bleeding or decreased RBC production caused by renal failure.
- Transient decreased Factor XIII activity may occur, which may play a role in either gastrointestinal bleeding or HSP.
- Vitamin K deficiency from severe vasculitis-induced intestinal malabsorption has been reported.

Infections

Anemia

- Chronic infection is associated with the anemia of chronic illness.
- Acute infection, particularly viral infection, can produce transient bone marrow aplasia or selective transient erythrocytopenia.
- Parvovirus B19, with tropism for the developing red cell, infection in patients with an underlying hemolytic disorder (such as sickle cell disease, hereditary spherocytosis) can produce a rapid fall in hemoglobin and an erythroblastopenic crisis marked by anemia and reticulocytopenia. There may be an associated neutropenia and less commonly, thrombocytopenia.
- Many viral and bacterial illnesses may be associated with hemolysis.

White Cell Alterations

- Viral infections can produce leukopenia and neutropenia. Neutrophilia with an increased band count and left shift frequently results from bacterial infection.
- Neonates, particularly premature infants, may not develop an increase in white cell count in response to infection. Neonatal neutropenia may be serious and requires investigation and treatment. G-CSF has been used and found to be helpful in randomized clinical trials.
- Eosinophilia may develop in response to parasitic infections.

Clotting Abnormalities

Severe infections, for example Gram-negative sepsis, can produce DIC. Polymicrobial sepsis (including both aerobic and anaerobic organisms) in the head and neck region may cause thrombosis of major vessels. When this occurs in the jugular veins it leads to a constellation of findings called Lemierre's syndrome (suppurative thrombophlebitis with inflammation starting in the pharynx and spreading to the lateral parapharyngeal tissues in association with jugular vein thrombosis).

Thrombocytopenia

Infection can produce thrombocytopenia through decreased marrow production, immune destruction, or DIC.

Viral and Bacterial Illnesses Associated with Marked Hematologic Sequelae

Parvovirus

Parvovirus B19 has a peculiar predilection for red cell precursors in the bone marrow. It has preference for the red cell precursors because it uses P antigen as a receptor. This viral infection is associated with a transient erythroblastopenic crisis, particularly in individuals with an underlying hemolytic disorder. In addition, it can produce thrombocytopenia, neutropenia, and a hemophagocytic syndrome. In immunocompromised individuals, parvovirus B19 infection can produce prolonged aplasia. Bone marrow aspirate shows decreased or arrested maturation of erythroid precursors and the pathognomonic "giant pronormoblasts."

Epstein–Barr Virus

Epstein–Barr Virus (EBV) infection is associated with the following hematologic manifestations:

- Atypical lymphocytosis
- Acquired immune hemolytic anemia
- Agranulocytosis
- Aplastic anemia, rarely
- Lymphadenopathy and splenomegaly
- Immune thrombocytopenia.

EBV infection also has immunologic and oncologic associations (see Chapter 16). Some of the EBV-associated lymphoproliferative disorders are given in Table 2.6.

TABLE 2.6 EBV-Associated Lymphoproliferative Disorders

EBV-ASSOCIATED B-CELL LYMPHOPROLIFERATIVE DISORDERS
1. Classic Hodgkin lymphoma
2. Burkitt lymphoma
3. Posttransplantation lymphoproliferative disorders
4. HIV-associated lymphoproliferative disorders
a. Primary CNS lymphoma
b. Diffuse large B-cell lymphoma, immunoblastic
c. HHV-8-positive primary effusion lymphoma
d. Plasmablastic lymphoma
EBV-ASSOCIATED T/NK-CELL LYMPHOPROLIFERATIVE DISORDERS
1. Peripheral T-cell lymphoma, unspecified
2. Angioimmunoblastic T-cell lymphoma
3. Extranodal nasal T/NK-cell lymphoma

EBV, Epstein–Barr virus; HHV-8, human herpes virus-8; NK, natural killer.
Modified from: Carbone et al. (2008), with permission.

Human Immunodeficiency Virus

The main pathophysiology of human immunodeficiency virus (HIV) infection is a constant decline in CD4 lymphocytes, leading to immune failure and death. The other bone marrow cell lines also decline as HIV disease (acquired immunodeficiency syndrome (AIDS)) progresses.

HIV infection has the following hematologic manifestations.

Thrombocytopenia

Thrombocytopenia occurs in about 40% of patients with AIDS. Initially, the clinical findings resemble those of immune thrombocytopenic purpura (ITP). Some degree of splenomegaly is common and the platelet-associated antibodies are often in the form of immune complexes that may contain antibodies with anti-HIV specificity. Megakaryocytes are normal or increased and production of platelets is reduced in the bone marrow.

Thrombotic thrombocytopenic purpura (TTP) is also associated with HIV disease. This occurs in advanced AIDS.

Anemia and Neutropenia

HIV-infected individuals develop progressive cytopenia as immunosuppression advances. Anemia occurs in approximately 70–80% of patients and neutropenia in 50%. Cytopenias in advanced HIV disease are often of complex etiology and include the following:

- A production defect in the marrow appears to be most common.
- Antibody and immune complexes associated with red and white cell surfaces may contribute. Up to 40% have erythrocyte-associated antibodies. Specific antibodies against i and U antigens have occasionally been noted. About 70% of patients with AIDS have neutrophil-associated antibodies. The pathogenesis of the hematologic disorders includes:
  - Infections: Myelosuppression is frequently caused by involvement of the bone marrow by infecting organisms (e.g., mycobacteria, cytomegalovirus (CMV), parvovirus, fungi and, rarely, *Pneumocystis jiroveci*).
  - Neoplasms: Non-Hodgkin lymphoma (NHL) in AIDS patients is associated with infiltration of the bone marrow in up to 30% of cases. This is particularly prominent in the small non-cleaved histologic subtype of NHL.
  - Medications: Widely used antiviral agents in AIDS patients are myelotoxic, for example, zidovudine (AZT) causes anemia in approximately 29% of patients. Ganciclovir and trimethoprim/sulfamethoxazole or pyrimethamine/sulfadiazine may cause neutropenia. In general, bone marrow suppression is related to the drug dosage and to the stage of HIV disease. Importantly, the other nucleoside analogs of anti-HIV compounds (dideoxycytidine (ddC), dideoxyinosine (ddI), stavudine (d4T), or lamivudine (3TC)), are usually not associated with significant myelotoxicity.
  - Nutrition: Poor intake is common in advanced HIV disease and is occasionally accompanied by poor absorption. Vitamin B_{12} levels may be significantly decreased in HIV infection although vitamin B_{12} is not effective in treatment. The reduction in serum vitamin B_{12} levels is due to vitamin B_{12} malabsorption and abnormalities in vitamin B_{12}-binding proteins.

TABLE 2.7 AIDS-Related Neoplasms in Children

1. Classic Hodgkin lymphoma (lymphocyte depleted)
2. Non-Hodgkin lymphoma
   a. Burkitt lymphoma
   b. Central nervous system lymphoma
   c. Diffuse large B-cell lymphoma
   d. Mucosa-associated lymphoid tissue (MALT)-type lymphoma
3. Leiomyoma and leiomyosarcoma
4. Kaposi's sarcoma
5. Acute leukemias
6. Miscellaneous tumors—isolated cases of hepatoblastoma, fibrosarcoma of liver, embryonal rhabdomyosarcoma of biliary tree, Ewing's tumor of the bone

Modified from: Balarezo and Joshi (2002), with permission.

TABLE 2.8 Spectrum of Systemic Lymphoproliferative Lesions in Children with AIDS

1. Hyperplasia involving
   a. Lymph nodes
   b. Peyer's patches of ileum
   c. Lymphoid nodules in esophagus and colon
   d. Thymus
   e. Pulmonary lymphoid hyperplasia (PLH)
2. Lymphoplasmacytic infiltrates in
   a. Lungs (lymphoid interstitial pneumonitis (LIP))
   b. Salivary glands
   c. Liver
   d. Thymitis and multilocular thymic cyst
3. Polyclonal polymorphic B-cell lymphoproliferative disorder (PBLD) involving
   a. Lungs
   b. Liver, spleen, lymph nodes
   c. Kidneys
   d. Salivary glands
   e. Muscle, periadrenal fat
4. Myoepithelial sialadenitis
5. Myoepithelial sialadenitis with focal lymphoma
6. MALT lymphoma (involving nodal and extranodal sites)
7. Non-MALT lymphoma (involving nodal and extranodal sites)

Modified from: Balarezo and Joshi (2002), with permission.

Coagulation Abnormalities

The following abnormalities occur:

- Dysregulation of immunoglobulin production may affect the coagulation cascade through antibody-mediated effects. The dysregulation of immunoglobulin production may also occasionally result in beneficial effects, as in the resolution of anti-Factor VIII antibodies in HIV-infected patients with hemophilia.
- Lupus-like anticoagulant (antiphospholipid antibodies) or anticardiolipin antibodies occur in 82% of patients. The titers or specificities have not led to thrombosis in most patients.
- Thrombosis may occur secondary to protein S deficiency. Low levels of protein S occur in 73% of patients.

Role of Hematopoietic Growth Factors in Acquired Immunodeficiency Syndrome

- rHuEPO results in a significant improvement in Hct and reduces transfusion requirements while the patient is receiving AZT. rHuEPO therapy should be initiated if the erythropoietin level is less than 500 IU/l.
- G-CSF in a dose of 5 mg/kg/day SC is the most widely used growth factor in neutropenia.
- GM-CSF at doses starting at 250 μg/day is also effective but has more side effects than G-CSF and is used less often.

Cancers in Children with Human Immunodeficiency Virus Infection

Malignancies in children with HIV infection are not as common as in adults. Table 2.7 lists the AIDS-related neoplasms in children with HIV infection and Table 2.8 lists the spectrum of lymphoproliferative lesions in children with AIDS.

NHL is the most common malignancy secondary to HIV infection in children. It is usually of B-cell origin as in Burkitt's (small non-cleaved cell) or immunoblastic (large cell) NHL. The mean age at presentation of malignancy in congenitally transmitted disease is 35 months, with a range of 6–62 months. In transfusion-transmitted disease, the latency from the time of HIV seroconversion to the onset of lymphoma is 22–88 months. The CD4 lymphocyte count is less than 50/mm^3 at the time of diagnosis of the malignancy.

The presenting manifestations include:

- Fever
- Weight loss
- Extranodal manifestations (e.g., hepatomegaly, jaundice, abdominal distention, bone marrow involvement, or central nervous system (CNS) symptoms). Some patients will already have had lymphoproliferative diseases such as lymphocytic interstitial pneumonitis or pulmonary lymphoid hyperplasia. These children usually have advanced (stage III or IV) disease at the time of presentation. Children with CNS lymphomas present with developmental delay or loss of developmental milestones or encephalopathy (dementia, cranial nerve palsies, seizures, or hemiparesis). Differential diagnosis includes infections such as toxoplasmosis, cryptococcosis, or tuberculosis. Contrast-enhanced computed tomography (CT) studies of the brain show hyperdense mass lesions that are usually multicentric or periventricular. CNS lymphomas in AIDS are fast-growing and often have central necrosis and a "rim of enhancement" as in an infectious lesion. A stereotactic biopsy will provide a definitive diagnosis.

Treatment of HIV Infection-Related Lymphomas Treatment consists of standard protocols as described in Chapter 22 on NHL. In addition, a concomitant approach to improving HIV viral load is critical in achieving positive survival outcomes in infected patients. Treatment of CNS lymphomas is more difficult. Intrathecal therapy is indicated even for those without evidence of meningeal or mass lesions at diagnosis of NHL. Radiation therapy may be a helpful adjunct for CNS involvement.

The following are more favorable prognostic features in NHL secondary to AIDS:

- CD4 lymphocyte count above 100/mm^3
- Normal serum LDH level
- No prior AIDS-related symptoms
- Good Karnofsky score (80–100).

Proliferative Lesions of Mucosa-Associated Lymphoid Tissue Mucosa-associated lymphoid tissue (MALT) shows reactive lymphoid follicles with prominent marginal zones containing centrocyte-like cells, lymphocytic infiltration of the epithelium (lymphoepithelial lesion), and the presence of plasma cells under the surface epithelium. These lesions may be associated with the mucosa of the gastrointestinal tract, Waldeyer's ring, salivary glands, respiratory tract, thyroid, and thymus. Proliferative lesions of MALT can be benign or malignant (such as lymphomas). The proliferative lesions arising from MALT form a spectrum or a continuum extending from reactive to neoplastic lesions. The neoplastic lesions are usually low grade, but may progress into high-grade MALT lymphomas. MALT lymphomas characteristically remain localized, but if dissemination occurs, they are usually confined to the regional lymph nodes and other MALT sites. MALT lesions represent a category of pediatric HIV-associated disease that may arise from a combination of viral etiologies, including HIV, EBV, and CMV.

Treatment of Low-Grade MALT Lymphoma

1. α-Interferon: 1 million units/m^2 SC three times a week (continued until regression of disease or severe toxicity occurs).
2. Rituxan (monoclonal antibody-anti-CD20): 375 mg/m^2 IV weekly for 4 weeks (courses may be repeated as clinically indicated). Some patients may not require any treatment because of the indolent nature of the disease.

Leiomyosarcomas and Leiomyomas

Malignant or benign smooth muscle tumors, leiomyosarcomas (LS) and leiomyomas (LM), are the second most common type of tumor in children with HIV infection. The incidence in HIV patients is 4.8% (in non-HIV children, it is 2 per million). The most common sites of presentation are the lungs, spleen, and gastrointestinal tract. Patients with endobronchial LM or LS often have multiple nodules in the pulmonary parenchyma. Bloody diarrhea, abdominal pain, or signs of obstruction may signal intraluminal bowel lesions.

These tumors are clearly associated with EBV infection. *In situ* hybridization and quantitative polymerase chain reaction studies of LM and LS demonstrated that high copy numbers of EBV are present in every tumor cell. The EBV receptor (CD21/C3d) is present on tumor tissue at very high concentrations but it is present at lower concentrations in normal smooth muscle or control LM/LS that had no EBV DNA in them. In AIDS patients, the EBV receptor may be unregulated, allowing EBV to enter the muscle cells and cause their transformation.

Treatment:

- Complete surgical resection where possible
- Radiation therapy
- Decreasing viral load in patients if they present with an acceleration
- Possible use of immunomodulators or chemotherapy.

Kaposi Sarcoma

Kaposi Sarcoma (KS) is rare in children and constitutes the third most common malignancy in pediatric AIDS patients; it occurs in 25% of adults with AIDS. KS occurs only in those HIV-infected children who were born to mothers with HIV. The lymphadenopathic form of KS is seen mostly in Haitian and African children and may represent the epidemic form of KS unrelated to AIDS. The cutaneous form is a true indicator of the disease related to AIDS. Visceral involvement has not been pathologically documented in children with AIDS. The incidence is falling in patients with early HAART treatment as KS is an AIDS-defining cancer.

Leukemias

Almost all HIV-associated leukemia is of B-cell origin. They represent the fourth most common malignancy in children with AIDS. The clinical presentation and biologic features are similar to those found in non-HIV children. Treatment involves chemotherapy designed for B-cell leukemia and lymphomas, as well as lowering viral load where necessary.

Miscellaneous Tumors

There is no increase in Hodgkin disease in children with AIDS. Children with AIDS rarely develop hepatoblastoma, embryonal rhabdomyosarcoma, fibrosarcoma, and papillary carcinoma of the thyroid. The occurrence of these tumors is probably unrelated to the HIV infection.

Infections

Torchs

This is a group of congenital infections including toxoplasma, rubella, CMV, herpes simplex virus (HSV), and syphilis. They can all cause neonatal anemia, jaundice, thrombocytopenia, and hepatosplenomegaly. They have significant sequelae so prevention, early identification and treatment are required.

Salmonella typhi

Typhoid fever usually produces profound leukopenia and neutropenia in the initial stages of the illness and is often accompanied by thrombocytopenia. Bone marrow examination may show marrow suppression but also hematophagocytosis. Diminished absolute eosinophil counts may be a clue to the diagnosis.

Acute Infectious Lymphocytosis

Acute infectious lymphocytosis is caused by a *Coxsackie* virus and is a rare benign, self-limiting childhood condition. It is associated with a low-grade fever, diarrhea, and marked lymphocytosis (50,000/mm^3). Lymphocytes are mainly CD4 T-cells. The condition resolves in 2−3 weeks without treatment.

Bartonellosis

Bartonellosis is caused by a Gram-negative bacillus *Bartonella bacilliformis* confined to the mountain valleys of the Andes. The vector is a local sand fly. Infection from this organism causes a fatal syndrome of severe hemolytic anemia with fever (Oroya fever). Another species of Bartonella, *B. hensele* causes cat scratch fever. It is associated with a regional lymphadenitis following a scratch by a cat. Thrombocytopenia may occur in this condition.

Tuberculosis

Tuberculosis is caused by *Mycobacterium tuberculosis*. Hematologic manifestations include leukemoid reaction mimicking CML, monocytosis, and rarely pancytopenia from diffuse granulomatous marrow infiltration (often associated with leukoerythroblasosis).

Leptospirosis (Weil Disease)

This disease is caused by a leptospira, *L. icterohemorrhagiae*. A coagulopathy occurs which is complex and can be corrected with vitamin K administration. Thrombocytopenia commonly occurs and is the cause of bleeding when this occurs.

Parasitic Illnesses Associated with Marked Hematologic Sequelae

Malaria

The etiology of anemia in acute infections is multifactorial:

- Intracellular parasite metabolism alters negative charges on the RBC membrane, which causes altered permeability with increased osmotic fragility. Spleen removes the damaged RBC or the parasites are "pitted" during the passage from spleen which results in microspherocytes of RBC.
- Autoimmune hemolytic anemia may also occur. An IgG antibody is formed against the parasite and resulting immune complex attaches nonspecifically to RBC, complement is activated and cell destruction occurs. Positive Coombs test due to IgG is found in 50% of patients with *Plasmodium falcifarum* malariae.
- Thrombocytopenia without DIC is common. IgG antimalarial antibody bonds to the platelet-bound malaria antigen and the IgG platelet parasite complex is removed by the reticuloendothelial system.

Babesiosis

Babesiosis is caused by several species from the genus *Babesia* that colonize erythrocytes. It is a zoonotic disease transmitted by the *Ixodid* tick and has similar clinical features to malaria. The clinical features include fever, myalgia and arthralgia with hepatosplenomegaly and hemolysis. Blood film may reveal intraerythrocytic trophozoites arranged in the form of a "Maltese cross."

Leishmaniasis

The protozoal species *Leishmania* causes progressive splenomegaly and subsequent pancytopenia (anemia, neutropenia, and thrombocytopenia). The bone marrow is usually hypercellular with hemophagocytosis. Some children may show coagulopathy.

Hookworm

Worldwide hookworm is a major cause of iron-deficiency anemia. Two species infest humans:

- *Ancylostoma duodenale* is found in the Mediterranean region, North Africa, and the west coast of South America.
- *Necator americanus* is found in most of Africa, Southeast Asia, Pacific islands, and Australia.

Hookworms penetrate exposed skin, usually soles of bare feet and migrate through the circulation to the right side of the heart, then lungs (causing hypereosinophilic syndrome), through the airway down to the esophagus. They mature in the small intestine and attach their mouthparts to the mucosa. They suck blood, with each adult *A. duodenale* consuming about 0.2 ml/day. Heavily infested children may present with profound iron-deficiency anemia, hypoproteinemia, and marked eosinophilia.

Tapeworm

Diphyllobothrium latum is a fish tapeworm. It is acquired by eating uncooked freshwater fish. This worm infestation in the intestine results in vitamin B_{12} deficiency.

Trypanosomiasis

This disease may cause immune-mediated anemia and less often thrombocytopenia and neutropenia. More importantly, as making the diagnosis is so important for survival, the trypanosomes are more likely to be seen early in the illness on classic thick smears.

LEAD INTOXICATION

The most striking hematologic feature of lead intoxication is basophilic stippling (coarse basophilia) of red cells. It is caused by precipitation of denatured mitochondria secondary to inhibition of prymidine-5′-nucleotidase. Lead also produces ring sideroblasts in the marrow and it is associated with hypochromic microcytic anemia and markedly elevated free erythrocyte protoporphyrin levels.

NUTRITIONAL DISORDERS

Protein-Calorie Malnutrition

Protein deficiency in the presence of adequate carbohydrate caloric intake (kwashiorkor) is associated with mild normochromic, normocytic anemia secondary to reduced RBC production despite normal or increased erythropoietin levels as well as reduced red cell survival. Protein-calorie malnutrition is also associated with impaired leukocyte function.

Scurvy

Mild anemia is common. There is a bleeding tendency due to loss of vascular integrity which may result in petechiae, subperiosteal, orbital or subdural hemorrhages. Hematuria and melena may occur.

Anorexia Nervosa

Anorexia nervosa is associated with hematologic changes which may be helpful in diagnosis:

- Red cell morphology is striking for the unusual morphology of acanthocytosis. This occurs due to acquired hypobetalipoproteinemia secondary to nutritional failure
- Mild anemia (macrocytic), neutropenia, and thrombocytopenia
- Mild predisposition to infection associated with neutropenia
- Gelatinous changes of bone marrow which may become severely hypoplastic.

BONE MARROW INFILTRATION

The bone marrow may be infiltrated by non-neoplastic disease (storage disease), granulomata from infection, sarcoid or rheumatologic disease, or neoplastic disease. In storage disease, a diagnosis is made on the basis of the family history, clinical picture, enzyme assays of white cells or cultured fibroblasts, and bone marrow aspiration revealing the characteristic cells of the disorder. Mutation analysis, where available, is the standard for diagnosis. Differential diagnosis of granulomatous conditions begins with recognition of the granulomas in bone marrow morphology (with culture if suspicious at the time of bone marrow examination). Neoplastic disease may arise *de novo* in the marrow (leukemias) or invade the marrow as metastases from solid tumors (neuroblastoma or rhabdomyosarcoma). Table 2.9 lists the diseases that may infiltrate the marrow.

Gaucher Disease

Gaucher disease is the most common lysosomal storage disease, resulting from deficient activity of β-glucocerebrosidase. It is inherited in an autosomal-recessive manner. There are more than 200 mutations identified in the β-glucocerebrosidase gene located on 1q21, including point mutations, crossovers, and recombinations,