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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Only in a few cases have names of disorders been changed or altered. The DISEASE DIAGNOSIS portion carries one such medical entity in this issue. The disorder described by Thomas Hodgkin in 1832 was earlier known as Hodgkin's Disease but is currently known as Hodgkin's Lymphoma (HL). The disease occurrence shows two peaks: the first in young adulthood (age 15–35) and the second in those over 55 years old. The annual incidence of Hodgkin's lymphoma is about 1 in 25,000 people, and the disease accounts for slightly less than 1% of all cancers worldwide. The incidence of Hodgkin's lymphoma is increased in patients with HIV infection. Also, patients with a history of infectious mononucleosis due to Epstein-Barr virus may have an increased risk of HL.

The 10-year overall survival rate is more than 90% for any stages, early stage may help more (stage I or II) Hodgkin's lymphoma. Since many patients are young, they often live 40 years or more after treatment. Classical Hodgkin's lymphoma (excluding nodular lymphocyte predominant Hodgkin's lymphoma) can be subclassified into 4 pathologic subtypes based upon Reed-Sternberg cell morphology and the composition of the reactive cell infiltrate seen in the lymph node biopsy specimen (the cell composition around the Reed-Sternberg cell(s). Complete clinico-diagnostic approach can be had within the covers of this communique.

Aminoaciduria is the presence of amino acids in the urine. Small amounts of amino acids are also present in normal urine. Increased total urine amino acids may result from metabolic disorders, chronic liver disease or renal disorders. Aminoacidurias can be divided into primary and secondary aminoacidurias. An attempt was made in the previous issue to cover the clinically important aminoacidurias in the previous issue, however, as the issue has now gained vast significance, so we have attempted to cover it in greater depth. Hence, the overflow from the previous issue has been presented in this issue. INTERPRETATION segment in this issue too discusses the remainder of aminoaciduria portion.

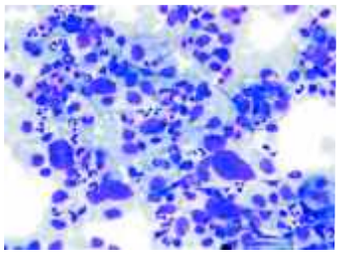
Likewise, TROUBLE SHOOTING section, in this issue, is also carrying the leftover portion from the last issue that brought to your notice – Large Scale Lab Errors and how to handle them. Space constraints do sometimes come in our way of presenting the whole article in one issue alone.

BOUQUET is lurking somewhere inside with all the three classic components that it is known for - Humour, Wisdom and Mental Delights (as brain teasers).

DISEASE DIAGNOSIS

HODGKIN'S LYMPHOMA

Introduction: Hodgkin's lymphoma, previously known as Hodgkin's disease, is a type of lymphoma, which is a cancer originating from white blood cells called lymphocytes. It was named after Thomas Hodgkin, who first described abnormalities in the lymph system in 1832. Hodgkin's lymphoma is characterized by the orderly spread of disease from one lymph node group to another and by the development of systemic symptoms with advanced disease. When Hodgkins cells are examined microscopically, multinucleated Reed-Sternberg cells (RS cells) are the characteristic histopathologic finding. Hodgkin's lymphoma may be treated with radiation therapy, chemotherapy or Hematopoietic stem cell transplantation, the choice of treatment depending on the age and sex of the patient and the stage, bulk and histological subtype of the disease. The disease occurrence shows two peaks: the first in young adulthood (age 15–35) and the second in those over 55 years old. The 10-year overall survival rate is more than 90% for any stages, early stage may help more (stage I or II) Hodgkin's lymphoma. Since many patients are young, they often live 40 years or more after treatment. However, few studies follow patients as long as 25 years, and those studies are of older treatments with more life-threatening adverse effects, so it is impossible to predict long-term outcomes of newer, less-harmful treatments. Radiation treatments, and some chemotherapy drugs, pose a risk of causing potentially fatal secondary cancers, heart disease, and lung disease 40 or more years later. Modern treatments greatly minimize the chances of these late effects. Patients with a history of infectious mononucleosis due to Epstein-Barr virus may have an increased risk of HL.



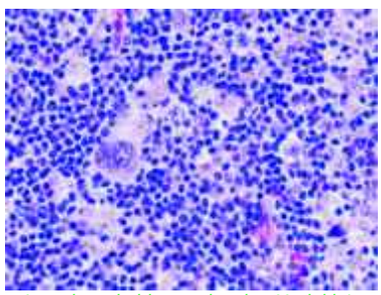
Micrograph showing Hodgkin's lymphoma

History: Hodgkin's lymphoma was first described in an 1832 report by Thomas Hodgkin, although Hodgkin noted that perhaps the earliest reference to the condition was provided by Marcello Malpighi in 1666. While occupied as museum curator at Guy's Hospital, Hodgkin studied seven patients with painless lymph node enlargement. Of the seven cases, two were patients of Richard Bright, one was of Thomas Addison, and one was of Robert Carswell. Carswell's report of this seventh patient was accompanied by numerous illustrations that aided early descriptions of the disease. Hodgkin's report on these seven patients, entitled "On some morbid appearances of the absorbent glands and spleen", was presented to the Medical and Chirurgical Society in London in January 1832 and was subsequently published in the society's journal, Medical-Chirurgical Society Transactions. Hodgkin's paper went largely unnoticed, however, even despite Bright highlighting it in an 1838 publication. Indeed, Hodgkin himself did not view his contribution as particularly significant. In 1856, Samuel Wilks independently reported on a series of patients with the same disease that Hodgkin had previously described. Wilks, a successor to Hodgkin at Guy's Hospital, was unaware of Hodgkin's prior work on the subject. Bright made Wilks aware of Hodgkin's contribution and in 1865, Wilks published a second paper, entitled "Cases of enlargement of the lymphatic glands and spleen", in which he called the disease "Hodgkin's disease" in honor of his predecessor. Theodor Langhans and WS Greenfield first described the microscopic characteristics of Hodgkin's lymphoma in 1872 and 1878, respectively. In 1898 and 1902, respectively, Carl Sternberg and Dorothy Reed independently described the cytogenetic features of the malignant cells of Hodgkin's lymphoma, now called Reed-Sternberg cells. Tissue specimens from Hodgkin's seven patients remained at Guy's Hospital for a number of years. Nearly 100 years after Hodgkin's initial publication, histopathologic reexamination confirmed Hodgkin's lymphoma in only three of seven of these patients. The remaining cases included non-Hodgkin lymphoma, tuberculosis, and syphilis. Hodgkin's lymphoma was one of the first cancers which could be treated using radiation therapy and, later, it was one of the first to be treated by combination chemotherapy.

Epidemiology: Unlike some other lymphomas, whose incidence increases with age, Hodgkin's lymphoma has a bimodal incidence curve; that is, it occurs most frequently in two separate age groups, the first being young adulthood (age 15–35) and the second being in those over 55 years old although these peaks may vary slightly with nationality. Overall, it is more common in males, except for the nodular sclerosis variant, which is slightly more common in females. The annual incidence of Hodgkin's lymphoma is about 1 in 25,000 people, and the disease accounts for slightly less than 1% of all cancers worldwide. The incidence of Hodgkin's lymphoma is increased in patients with HIV infection. In contrast to many other lymphomas associated with HIV infection it occurs most commonly in patients with higher CD4 T cell counts.

Classification: Types: Classical Hodgkin's lymphoma (excluding nodular lymphocyte predominant Hodgkin's lymphoma) can be subclassified into 4 pathologic subtypes based upon Reed-Sternberg cell morphology and the composition of the reactive cell infiltrate seen in the lymph node biopsy specimen (the cell composition around the Reed-Sternberg cell(s)). Nodular sclerosing CHL: Is the most common subtype and is composed of large tumor nodules showing scattered lacunar classical RS cells set in a background of reactive lymphocytes, eosinophils and plasma cells with varying degrees of collagen fibrosis/sclerosis. Mixed-cellularity subtype: Is a common subtype and is composed of numerous classic RS cells admixed with numerous inflammatory cells including lymphocytes, histiocytes, eosinophils, and plasma cells. without sclerosis. This type is most often associated with EBV infection and may be confused with the early, so-called 'cellular' phase of nodular sclerosing CHL. Lymphocyte-rich or Lymphocytic predominance: Is a rare subtype, show many features which may cause diagnostic confusion with nodular lymphocyte predominant B-cell Non-Hodgkin's Lymphoma (B-NHL). This form also has the most favorable prognosis. Lymphocyte depleted: Is a rare subtype, composed of large numbers of often pleomorphic RS cells with only few reactive lymphocytes which may easily be confused with diffuse large cell lymphoma. Many cases previously classified within this category would now be reclassified under anaplastic large cell lymphoma.

Unspecified: Nodular lymphocyte predominant Hodgkin's lymphoma expresses CD20, and is not currently considered a form of classical Hodgkin's. For the other forms, although the traditional B cell markers (such as CD20) are not expressed on all cells, Reed-Sternberg cells are usually of B cell origin. Although Hodgkin's is now frequently grouped with other B cell malignancies, some T cell markers (such as CD2 and CD4) are occasionally expressed. However, this may be an artifact of the ambiguity inherent in the diagnosis. Hodgkin's cells produce Interleukin-21 (IL-21), which was once thought to be exclusive to T cells. This feature may explain the behavior of classical Hodgkin's lymphoma, including clusters of other immune cells gathered around HL cells (infiltrate) in cultures.



Lymph node biopsy showing Hodgkin's lymphoma, mixed-cellularity type.

Staging: The staging is the same for both Hodgkin's as well as non-Hodgkin's lymphomas. After Hodgkin's lymphoma is diagnosed, a patient will be staged: that is, they will undergo a series of tests and procedures that will determine what areas of the body are affected. These procedures will include documentation of their histology, a physical examination, blood

magnetic resonance imaging (MRI) scans of the chest, abdomen and pelvis, and a bone marrow biopsy. Positron emission tomography (PET) scan is now used instead of the gallium scan for staging. In the past, a lymphangiogram or surgical laparotomy (which involves opening the abdominal cavity and visually inspecting for tumors) were performed. Lymphangiograms or laparotomies are very rarely performed, having been supplanted by improvements in imaging with the CT scan and PET scan. On the basis of this staging, the patient will be classified according to a staging classification (the Ann Arbor staging classification scheme is a common one): Stage I is involvement of a single lymph node region (I) (mostly the cervical region) or single extralymphatic site (Ie); Stage II is involvement of two or more lymph node regions on the same side of the diaphragm (II) or of one lymph node region and a contiguous extralymphatic site (IIe); Stage III is involvement of lymph node regions on both sides of the diaphragm, which may include the spleen (IIIs) and/or limited contiguous extralymphatic organ or site (IIIe, IIIes); Stage IV is disseminated involvement of one or more extralymphatic organs. The absence of systemic symptoms is signified by adding 'A' to the stage; the presence of systemic symptoms is signified by adding 'B' to the stage. For localized extranodal extension from mass of nodes that does not advance the stage, subscript 'E' is added.

Signs and symptoms: Patients with Hodgkin's lymphoma may present with the following symptoms: Night Sweats. Unexplained weight loss. Lymph nodes: the most common symptom of Hodgkin's is the painless enlargement of one or more lymph nodes. The nodes may also feel rubbery and swollen when examined. The nodes of the neck and shoulders (cervical and supraclavicular) are most frequently involved (80–90% of the time, on average). The lymph nodes of the chest are often affected, and these may be noticed on a chest radiograph. Splenomegaly: enlargement of the spleen occurs in about 30% of people with Hodgkin's lymphoma. The enlargement, however, is seldom massive and the size of the spleen may fluctuate during the course of treatment. Hepatomegaly: enlargement of the liver, due to liver involvement, is present in about 5% of cases. Hepatosplenomegaly: the enlargement of both the liver and spleen caused by the same disease. Pain following alcohol consumption: classically, involved nodes are painful after alcohol consumption, though this phenomenon is very uncommon. Back pain: nonspecific back pain (pain that cannot be localized or its cause determined by examination or scanning techniques) has been reported in some cases of Hodgkin's lymphoma. The lower back is most often affected. Red-coloured patches on the skin, easy bleeding and petechiae due to low platelet count (as a result of bone marrow infiltration, increased trapping in the spleen etc. – i.e. decreased production, increased removal). Systemic symptoms: about one-third of patients with Hodgkin's disease may also present with systemic symptoms, including low-grade fever; night sweats; unexplained weight loss of at least 10% of the patient's total body mass in six months or less, itchy skin (pruritus) due to increased levels of eosinophils in the bloodstream; or fatigue (lassitude). Systemic symptoms such as fever, night sweats, and weight loss are known as B symptoms; thus, presence of fever, weight loss, and night sweats indicate that the patient's stage is, for example, 2B instead of 2A. Cyclical fever: patients may also present with a cyclical high-grade fever known as the Pel-Ebstein fever, or more simply "P-E fever". However, there is debate as to whether or not the P-E fever truly exists.

Cause: There are no guidelines for preventing Hodgkin's lymphoma because the cause is unknown or multifactorial. A risk factor is something that statistically increases your chance of getting a disease or condition. Risk factors include: Sex: male. Ages: 15–40 and over 55. Family history. History of infectious mononucleosis or infection with Epstein-Barr virus, a causative agent of mononucleosis. Weakened immune system, including infection with HIV or the presence of AIDS. Prolonged use of human growth hormone. Exotoxins, such as Agent Orange. The etiology of Hodgkin lymphoma is believed to be multifactorial. Several studies have documented a link between Hodgkin lymphoma and Epstein-Barr virus (EBV). EBV DNA can be identified in HRS cells in approximately 50% of patients in developed countries and in = 90% of patients in developing countries. Advances in techniques to isolate HRS cells, immunohistochemical and molecular biology techniques, have helped to clearly identify 2 immunophenotypes for HRS cells. Immunophenotype I is characterized by CD20 positivity, J-chain rearrangements, and, in general, CD30 and CD15 negativity, which is typical of nodular lymphocyte predominant Hodgkin lymphoma. Immunophenotype II is CD30 positive and has absence of J chains with frequent expression of CD15, a pattern consistent with classic Hodgkin lymphoma histological subtypes as defined by the World Health Organization classification of lymphomas. The clinical manifestations of Hodgkin lymphoma may be a direct result of cytokine production by HRS cells or the surrounding cells within the affected lymph nodes. Systemic symptoms have been attributed to the production of interleukin (IL)-6, whereas some of the histopathological characteristics such as eosinophilia and collagen sclerosis have been attributed to cytokine production, such as IL-4, IL-5 exotoxin, IL-6, IL-7, tumor necrosis factor (TNF), lymphotoxin, transforming growth factor β (TNF-β), and basic fibroblast growth factor. The constitutive translocation of nuclear factor κB (NF-κB) to the nucleus of HRS cells is essential for the malignant transformation of HRS cells. A paracrine activation of NF-κB in Hodgkin lymphoma is observed; both HRS cells and the surrounding supporting cells produce cytokines that upregulate several members of the TNF receptor superfamily including CD30, CD40, or EBV latent membrane protein-1 (LMP-1). The production of the ligand for these receptors is responsible for the phosphorylation and translocation to the nucleus of NF-κB, which, in turn, leads to inhibition of apoptosis, proliferation, and secretion of proinflammatory cytokines. Other factors include the following: Genetic predisposition: Clustering in families suggests a genetic predisposition, with an increased incidence especially among same-sex siblings, monozygotic twins, and parent-child pairs. Familial Hodgkin lymphoma has been associated with specific human leukocyte antigens (HLAs). Familial cases account for 4.5% of all cases. Infectious agents: EBV is found in approximately 50% of cases of Hodgkin lymphoma in the United States and in western Europe. Socioeconomic factors: Usually internationally, parental income and parental education level are inversely related to the incidence of Hodgkin lymphoma. Immune dysregulation: Patients may have T-cell immunodeficiency, human immunodeficiency syndrome (HIV) infection or acquired immunodeficiency syndrome (AIDS), or congenital immunodeficiency syndromes. Diet: At present, no conclusive association is recognized between dietary habits and the development of Hodgkin disease. Environment: Clustering of cases in families or racial groups has supported the idea of a common environmental link.

Pathophysiology: Hodgkin lymphoma is a B-cell malignant disorder that affects the reticuloendothelial and lymphatic systems. Invasion can affect other organs and systems, predominantly the lungs, bone, bone marrow, liver parenchyma, and, rarely, the CNS. Epidemiologic data suggest that environmental, genetic, and immunologic factors are involved in the development of Hodgkin lymphoma. Clustering of cases in families or racial groups supports the idea of a genetic predisposition or a common environmental factor. In identical twins of patients with Hodgkin lymphoma, the risk of developing Hodgkin lymphoma is higher than that of other first-degree relatives. Subjects with acquired or congenital immunodeficiency disorders also have an increased risk of developing Hodgkin lymphoma. Findings from several epidemiologic studies have suggested links between Hodgkin lymphoma and certain viral illnesses. The strongest case to date is a relationship to Epstein-Barr virus (EBV) in that EBV viral DNA can be found in Hodgkin-Reed-Sternberg (HRS) cells. Infants and children aged 0-14 years with Hodgkin disease have EBV DNA in their HRS cells more often than young adults aged 15-39 years with Hodgkin lymphoma. In addition, the prevalence of EBV-positive classic Hodgkin lymphoma tumors geographically differs. The rate of EBV positivity is 50% in Great Britain, Jordan, Egypt, and South Africa; 91% in Greece; and 100% in Kenya. In general, EBV is most common in mixed-cellularity Hodgkin lymphoma, in young children, and in developing countries. In EBV-positive Hodgkin

lymphoma, EBV-encoding genes play a role in preventing apoptosis. Latent membrane protein-1 (LMP-1) expressed in EBV-positive HRS cells mimics an activated CD40 receptor, activating the antiapoptotic nuclear factor (NF)–kappa-B pathway.

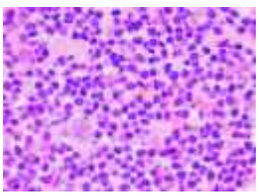
Differential Diagnoses: Acute Lymphoblastic Leukemia: Lymphadenopathy. Brucellosis: Lymphoproliferative Disorders. Catscratch Disease: Mononucleosis and Epstein-Barr Virus Infection. Cytomegalovirus Infection: Non-Hodgkin Lymphoma. Histoplasmosis: Toxoplasmosis. Lymph Node Disorders: Tuberculosis. Lymphadenitis. Other Problems to Be Considered: Fibrosing mediastinitis, Atypical mycobacteria, AIDS.

Diagnosis: Hodgkin's lymphoma must be distinguished from non-cancerous causes of lymph node swelling (such as various infections) and from other types of cancer. Definitive diagnosis is by lymph node biopsy (usually excisional biopsy with microscopic examination). Blood tests are also performed to assess function of major organs and to assess safety for chemotherapy. Positron emission tomography (PET) is used to detect small deposits that do not show on CT scanning. PET scans are also useful in functional imaging (by using a radiolabeled glucose to image tissues of high metabolism). In some cases a Gallium Scan may be used instead of a PET scan.

Workup: Laboratory Studies: Hematological and blood chemistry evaluation may reveal nonspecific findings in patients with Hodgkin disease (Hodgkin's disease) that may be associated with disease extent. Several of these findings have been used as prognostic factors. In addition to stage and male sex, the international prognostic factors for advanced Hodgkin lymphoma (HL) include certain laboratory findings as poor prognostic factors. In addition to the following findings, an erythrocyte sedimentation rate of more than 50 may also be a poor prognostic factor: Hemoglobin concentration less than 10.5 g/dL. WBC count of 15,000/µL or less. Absolute lymphocyte count less than 800/µL. Albumin level less than 4 g/dL. The CBC count may reveal the following: Hemolytic anemia (Coombs positive), anemia of chronic disease, anemia secondary to involvement of the bone marrow. Leukocytosis, lymphopenia, eosinophilia, monocytosis. Thrombocytopenia and/or an idiopathic thrombocytopenia purpura–type picture. Assessment of acute-phase reactants may involve the erythrocyte sedimentation rate and C-reactive protein, serum copper, and ferritin levels.

A full serum chemistry panel may aid in evaluating levels of serum electrolytes; lactate dehydrogenase levels (LDH), which reflects bulk of disease; alkaline phosphatase, which indicates bony metastasis; as well as liver and kidney function. Urinalysis may reveal proteinuria. Nephrotic syndrome may be associated with Hodgkin lymphoma. Imaging Studies: Chest radiography is performed with anteroposterior and lateral projections to assess the bulk of the mediastinal mass. Mediastinal mass with a thoracic ratio of 33% or greater is of prognostic importance. CT or MRI of neck, chest, abdomen, and/or pelvis may be indicated to assess sites of disease (nodal and extranodal) as well as to assess liver and spleen involvement. Ultrasonography can be used to assess the abdominal and pelvic structures in centers with limited resources in which CT scanning or MRI is not available. On positron emission tomography (PET), uptake of the radioactive glucose analog 2-[18F]fluoro-2-deoxy-D-glucose (FDG) is correlated with proliferative activity in tumors undergoing anaerobic glycolysis. PET scans are used with increasing frequency to identify the extent of disease at diagnosis and for follow up. After two cycles of therapy with doxorubicin (Adriamycin), bleomycin, vinblastine, and dacarbazine (ABVD), a positive PET scan finding may be predictive of poor outcome. Gallium scanning is rarely used and has been replaced by PET scanning. Bone scan is necessary only when bony metastases are suspected because of an elevated alkaline phosphatase level. However, the same information may be obtained with PET scanning. Procedures: Staging laparotomy is no longer advocated in pediatric Hodgkin lymphoma. Lymph node biopsy findings may be helpful. Histopathologic studies consist of hematoxylin and eosin staining and special immunohistochemical staining for surface markers such as CD15, CD20, CD30, and CD45. Consider other immunohistochemical staining to ensure that they are negative and to rule out non-Hodgkin lymphoma, such as CD3 and anaplastic lymphoma kinase (ALK). Fine-needle aspiration is not recommended because of lack of stromal tissue and the difficulty of classifying the Hodgkin lymphoma into one of the classic subtypes versus the nodular lymphocyte–predominant (NLP) subtype. Bilateral bone marrow biopsy is necessary in all patients with suspected involvement of the bone marrow and in those with stage IIB, III, or IV disease.

Pathology: Macroscopy: Affected lymph nodes (most often, laterocervical lymph nodes) are enlarged, but their shape is preserved because the capsule is not invaded. Usually, the cut surface is white-grey and uniform; in some histological subtypes (e.g. nodular sclerosis) a nodular aspect may appear. A fibrin ring granuloma may be seen. Microscopy: Micrograph showing a "popcorn cell", the Reed-Sternberg cell variant seen in nodular lymphocyte predominant Hodgkin lymphoma. H&E stain. Microscopic examination of the lymph node biopsy reveals complete or partial effacement of the lymph node architecture by scattered large malignant cells known as Reed-Sternberg cells (RSC) (typical and variants) admixed within a reactive cell infiltrate composed of variable proportions of lymphocytes, histiocytes, eosinophils, and plasma cells. The RSC are identified as large often bi-nucleated cells with prominent nucleoli and an unusual CD45-, CD30+, CD15+/- immunophenotype. In approximately 50% of cases, the RSC are infected by the Epstein-Barr virus. Characteristics of classic RSC include large size (20–50 micrometres), abundant, amphophilic, finely granular/homogeneous cytoplasm; two mirror-image nuclei (owl eyes) each with an eosinophilic nucleolus and a thick nuclear membrane (chromatin is distributed at the cell periphery). Variants: Hodgkin cell (atypical mononuclear RSC) is a variant of RS cell, which has the same characteristics, but is mononucleated. Lacunar RSC is large, with a single hyperlobated nucleus, multiple, small nucleoli and eosinophilic cytoplasm which is retracted around the nucleus, creating an empty space ("lacunae"). Pleomorphic RSC has multiple irregular nuclei. "Popcorn" RSC (lympho-histiocytic variant) is a small cell, with a very lobulated nucleus, small nucleoli. "Mummy" RSC has a compact nucleus, no nucleolus and basophilic cytoplasm. Hodgkin's lymphoma can be sub-classified by histological type. The cell histology in Hodgkin's lymphoma is not as important as it is in non-Hodgkin's lymphoma: the treatment and prognosis in classic Hodgkin's lymphoma usually depends on the stage of disease rather than the histotype.



Micrograph of a classic Reed-Sternberg cell.

Management: Patients with early stage disease (IA or IIA) are effectively treated with radiation therapy or chemotherapy. The choice of treatment depends on the age, sex, bulk and the histological subtype of the disease. Patients with later disease (III, IVA or IVB) are treated with combination chemotherapy alone. Patients of any stage with a large mass in the chest are usually treated with combined chemotherapy and radiation therapy.

Prognosis: Treatment of Hodgkin's disease has been improving over the past few decades. Recent trials that have made use of new types of chemotherapy have indicated higher survival rates than have previously been seen. In one recent European trial, the 5-year survival rate for those patients with a favorable prognosis was 98%, while that for patients with worse outlooks was at least 85%. In 1998, an international effort identified seven prognostic factors that accurately predict the success rate of conventional treatment in patients with locally extensive or advanced stage Hodgkin's lymphoma. Freedom from progression (FFP) at 5 years was directly related to the number of factors present in a patient. The 5-year FFP for patients with zero factors is 84%. Each additional factor lowers the 5-year FFP rate by 7%, such that the 5-year FFP for a patient with 5 or more factors is 42%. The adverse prognostic factors identified in the international study are: Age ≥ 45 years, Stage IV disease, Hemoglobin < 10.5 g/dl, Lymphocyte count <600/µl or <8%, Male, Albumin <4.0g/dl, White blood count ≥ 15,000/µl.

TROUBLESHOOTING

LARGE SCALE LAB ERRORS (continued from previous issue)

CASE 2: INCORRECT HIV SCREENING RESULTS

In 2006, a technologist at Laboratory Y was reviewing HIV1 and HIV2 enzyme-linked immunosorbent assay screening results and noted that the laboratory had reported a negative result 2 days previously for a patient who had tested positive several weeks earlier. The technologist brought the matter to her supervisor who informed Laboratory Y's medical and technical directors. The medical director had the patient's negative specimen retested twice, and on both retests, the result was positive. As a matter of policy, the laboratory tested 3 levels of controls with each run, and review of control results showed expected levels of absorbance on the day the incorrect negative patient result had been obtained. Laboratory Y's medical director had the negative patient result corrected to positive and called the director of the laboratory that had referred the specimen to Laboratory Y. Until the cause of the problem could be determined and corrected, the medical director of Laboratory Y also instructed testing personnel to have all HIV specimens tested twice before results were released and to release patient results only if both tests produced the same result. The decision to test all specimens twice was made on the day the issue was first noted, as was correction of the erroneous result. The laboratory used an automated system for testing antibodies to HIV1 and HIV2 that had been cleared by the appropriate authorities (AA) under the review paradigm. An understanding of the likely cause of the error developed during the next 2 weeks as additional clues were discovered. Two days after the initial error, a technologist noticed that one of the patient wells in the HIV microtiter tray contained less fluid than the other wells. The instrument was stopped, and examination of the tray revealed that patient specimen had not been dispensed into the well in question by the instrument's automated pipettor but had been properly dispensed into all of the other wells in the tray. The batch was discarded, and testing was resumed (again in duplicate). The technologist was instructed to watch the instrument as it divided the patient specimen to ensure that specimen was dispensed into each reaction well. The service representative from the instrument manufacturer was summoned to investigate the issue. The representative told Laboratory Y's section manager that technologists should check for bubbles in pipette tips and check each pipette tip to make sure it had a hole in the end. Neither of these instructions was in the AA cleared product insert. During the next week 1 additional "nondispense event" was observed, in which the automated dispensing arm did not dispense patient specimen into a test well. Visual examination of the disposable pipette tip revealed no bubble or obvious defects. The director of product service at the instrument manufacturer was contacted. The instrument manufacturer committed to cooperate in investigating the problem and offered to pay for all reagents associated with retesting until the problem was resolved. Based on the nondispense events that had been observed, Laboratory Y's management estimated that approximately 1 in 400 specimens was not being properly dispensed into test wells. A nondispense event had the potential to produce a false-negative result, but not a false-positive result, and would only cause incorrect results in truly positive specimens. Because 99% of specimens tested by Laboratory Y were negative, management estimated that approximately (1 in 400 tests) × (1 in 100 specimens) or 1 in 40,000 test results reported by the laboratory would be incorrect. Because the date on which erratic dispensing started was unknown, management considered all results produced on the test instrument to be potentially impacted. Approximately 30,000 specimens had been tested since the instrument was first placed in service, 8 months before the index error became known. The manufacturer of the HIV test system denied receiving other reports of nondispense events. Nevertheless, the director of Laboratory Y reported the problem to the AA Office of In Vitro Diagnostic Device Evaluation and Safety and posted the issue on the AAs Web-based voluntary reporting system. Laboratory Y was not contacted by the AA in follow-up. Laboratory Y was a reference laboratory that received specimens from other clinical laboratories. The director of Laboratory Y determined that a look-back and retesting program should be instituted to notify caregivers and patients about the small but real possibility of false-negative HIV test results. In implementing the look-back program, a number of issues surfaced: (1) As a reference facility, Laboratory Y did not have the addresses of tested patients and, in a number of cases, did not know the names of ordering physicians. Laboratory Y offered to assist client laboratories in notifying ordering physicians and their patients and to defray the costs of printing self-addressed patient letters that could be mailed to ordering physicians. (2) Although it was clear that ordering physicians needed to be informed that the problem potentially impacted HIV test results, it was not clear whether patient letters should include the name of the test because a misunderstanding might cause patients anxiety out of proportion to actual risk. (3) Some people were of the opinion that patients needed to be informed directly, irrespective of the wishes of the patients' ordering physicians. Others believed that the risk of harm (1 in 40,000 results) was too low to require direct patient communication and that communication with the patients' caregiver was adequate. The question of whether retesting should be recommended or simply offered was also debated. (4) There was controversy about whether the name of the instrument manufacturer should be included in letters to referring laboratories or physicians. (5) A special process needed to be created to facilitate no-charge retesting. (6) Debate ensued about whether laboratory charges should be reversed, given the low risk of error in any given specimen. (7) It was not clear whether caregiver notification letters should be signed by the director of Laboratory Y or the director of the referring laboratory or be unsigned. The risk management department at several hospitals that referred specimens to Laboratory Y contacted Laboratory Y management for information and updates. Some risk management departments prepared press releases, but no statements were released to the press. When 2 subsequent nondispense events were detected, Laboratory Y revised its risk estimate and calculated that the risk of an incorrect result might be as high as 1 in 10,000 tests. One hospital risk management department wanted Laboratory Y to send follow-up letters to caregivers with the revised risk estimate, since a risk of approximately 1 in 40,000 tests had been previously reported. Three months after the incident, Laboratory Y replaced its HIV test system with another system that was programmed to detect dispensing failures. The manufacturer of the original test system paid for all reagent expenses associated with testing and part of the look-back and notification expenses and subsequently modified its own HIV test system to automatically detect dispensing failures. Six months after the incident, only 1 patient who had initially tested negative subsequently tested positive. This patient had initially tested negative on 5 occasions, was known to be engaging in high-risk behavior, and was being tested on a regular basis because of his risk profile. The patient was considered to have seroconverted and not to have been subject to a false-negative result.

DISCUSSION

Laboratories use a portfolio of "controls" or "good laboratory practices" to prevent errors and ensure the integrity of laboratory processes and the

accuracy of test results. These practices range from contemporaneous quality control testing to periodic employee competency assessment and from preproduction validation of new test methods to external proficiency testing and on-site laboratory inspection. Some controls used in laboratories are prescribed by legislation, regulation, or health care accrediting agencies (eg, CAP etc), others are adopted by laboratory management in response to local imperatives or perceived vulnerabilities. An example of the latter might include the decision by laboratory management to double check the output of a printer that has shown variable function in the past. Although the application of good laboratory practices undoubtedly reduces the frequency of mistakes or "nonconformances," laboratory errors nevertheless occur. As is the case in other branches of medicine, most laboratory errors do not impact patient outcomes. As case 1 illustrates, only a small fraction of laboratory errors affect management of a patient's condition, and only a subset of these errors cause injury to patients. Nevertheless, the potential to injure patients is implicit in most laboratory errors, and for that reason every laboratory error deserves some sort of investigation and response. When automated testing produces laboratory errors on a large scale, the risk of adverse patient impact is increased. Large-scale laboratory errors vary considerably with respect to their cause and potential consequences. The 2 case reports presented in the article concern errors related to execution of calibration procedures and instrument design. The cause of other systematic errors known to us have ranged from defects in reagent quality, variation in manufacture of blood collection tubes, and defects in automated flow cytometry gating software or computer screens used to collect gestational age information for prenatal testing. The diverse causes and consequences of large-scale testing errors make us hesitant to recommend a standard "cookie cutter" approach to error recovery. Incidents that involve blood specimens and chronic diseases offer the opportunity for retesting. Incidents involving irreplaceable specimens or testing that occurs in acute high-stakes settings reduce recovery options.

BOUQUET

In Lighter Vein

A young blonde was on vacation in the depths of Louisiana. She wanted a pair of genuine alligator shoes in the worst way, but was very reluctant to pay the high prices the local vendors were asking. After becoming very frustrated with the “no haggle” attitude of one of the shopkeepers, the blonde shouted, “Maybe I’ll just go out and catch my own alligator so I can get a pair of shoes at a reasonable price!”
The shopkeeper said, “By all means, be my guest. Maybe you’ll luck out and catch yourself a big one!” Determined, the blonde turned and headed for the swamps, set on catching herself an alligator.
Later in the day, the shopkeeper was driving home, when he spotted the young woman standing waist deep in the water, shotgun in hand. Just then, he saw a huge 9-foot alligator swimming quickly toward her. She took aim, killed the creature, and with a great deal of effort hauled it on to the swamp bank. Lying nearby were several more of the dead creatures. The shopkeeper watched in amazement. Just then the blonde flipped the alligator on its back, and frustrated, shouts out, “Damn it, this one isn’t wearing any shoes either!”

Last year I replaced all the windows in my house with that expensive double-pane energy efficient kind, and today, I got a call from the contractor who installed them. He was complaining that the work had been completed a whole year ago and I still hadn’t paid for them.
Hellloooo,……..just because I’m blonde doesn’t mean that I am automatically stupid.
So, I told him just what his fast talking sales guy had told me last year, that in ONE YEAR these windows would pay for themselves!
Hellllooooo? It’s been a year, I told him!
There was only silence at the other end of the line, so I finally just hung up. He never called back. I bet he felt like an idiot.

A lawyer and two friends--a Rabbi, and a Hindu holy man--had car trouble in the countryside and asked to spend the night with a farmer.
The farmer said, "There might be a problem. You see, I only have room for two to sleep in the house. So one of you must sleep in the barn.”
"No problem," chimed the Rabbi. "My people wandered in the desert for forty years. I am humble enough to sleep in the barn for one evening." With that he departed to the barn, and the others bedded down for the night.
Moments later a knock was heard at the door; the farmer opened the door. There stood the Rabbi from the barn. "What's wrong?" asked the farmer. He replied, "I am grateful to you, but I just can't sleep in the barn. There is a pig in the barn, and my faith believes that is an unclean animal.”
His Hindu friend agrees to swap places with him. But a few minutes later the same scene reoccurs. There is a knock on the door. "What's wrong?" the farmer asks. The Hindu holy man replies, "I, too, am grateful for your helping us out, but there is a cow in the barn. In my country cows are considered sacred and I can't sleep on holy ground!”
That left only the lawyer to make the change. He grumbled and complained, but went out to the barn. Moments later there was another knock on the farmer's door. Frustrated and tired, the farmer opens the door, and there stood the pig and the cow.

Wisdom Whispers

- "Hope and expectation are a fool's income."
- "It is better to bend than break."
- "Making money selling manure is better than losing money selling musk."
- "He who wants to be rich in a year comes to the gallows in half a year."
- "A deaf husband and a blind wife are always a happy couple."
- "The poor-houses are filled with the honestest people."
- "Hope is a good breakfast, but a bad supper."
- "Good will, like a good woman, is hard to get and easy to lose."
- "Who excuses himself without being accused makes his fault manifest."

Brain Teasers

Identify the following human parasites

1 

2 

3 

4 

Answers: 1. Ascaris lumbricoides 2. Guinea worm 3. Liver fluke 4. Tapeworm (long)

INTERPRETATION

AMINOACIDURIAS AND INBORN ERRORS OF METABOLISM (continued from previous issue)

CYSTINOSIS AND HOW TO SCREEN FOR THIS DISORDER: There are three clinical forms of cystinosis. The infantile (in first decade of life) and juvenile forms (second decade of life) will lead to renal involvement and insufficiency. The third form, the adult form, is benign and is not included in this objective. Diagnosis is confirmed by quantitatively determining the cystine concentration in leukocytes or cultured fibroblasts. Urinary findings in the laboratory are polyuria, pyelonephritis, decreased GFR, aminoaciduria, hyperphosphaturia, glucosuria, and kaliuresis. Urine odor resembles that of sulfur.

5-HYDROXYINDOLEACETIC ACID (5-HIAA) AND HOW TO SCREEN FOR THIS DISORDER: 5-HIAA is a metabolite in the metabolic pathway of tryptophan. In the healthy individual, any 5-HIAA in urine is the natural metabolic by-product of serotonin degradation. If a carcinoid tumors (contains argentaffin cells) are present, then also is the enzyme "tryptophan hydroxylase" which catalyzed the formation of 5-hydroxytryptophan (5-HPT). This 100 fold increase in 5-HPT is in turn is oxidized to 5-HIAA, which is rapidly excreted into the urine. Patients who are to be tested for 5-HIAA will collect a 24-hour urine (use HCL as a preservative) and are to be instructed to NOT eat bananas, pineapples, or tomatoes (have high levels of serotonin). Such patients are to be medication free for 72 hours. The urinary screening tests; ferric chloride (blue-green color produced), nitroso-naphthol (dark violet or purple color produced).

MELANURIA AND HOW TO SCREEN FOR THIS DISORDER: Melanin is a metabolite out of an alternate pathway of tyrosine that produces melanin, protein, thyroxine, and tyrosine sulfate. Melanin is produced by cells called melanocytes. If melanin is deficient, albinism is resultant. If melanin is being overproduced, the increased amounts will be excreted in the urine. Over production may be due to a melanoma. If a urine specimen (containing melanin) is allowed to sit in the lab, it will darken to a black or brown color. The following are urinary screening test. If they are positive, further patient evaluation is necessary. [1] Ferric chloride: gray-black (possibly with precipitate). [2] Acetest tablet (sodium nitroprusside): red. [3] Ehrlich's test: red.

TYROSYLURIA AND HOW TO SCREEN FOR THIS DISORDER: Tyrosyluria simply means that there is an increased quantity of tyrosine derived metabolites in the urine. If there is tyrosyluria, then tyrosinemia is present. Tyrosinuria is the consequence of excess amino acids spilling in the urine from tyrosinemia. The most common form of tyrosinemia a transitory condition observed in infants and is due to the lack of development of the liver. As the liver mature, the disorder disappears. If a patient has a severe liver disease, tyrosinemia may be resultant. There are two hereditary forms of tyrosinemia and in either case, the patient usually dies in the first decade of life. Tyrosine can be screened for in the urinary lab with the following: [1] Ferric chloride test: transient green color. [2] Nitroso-naphthol test: red color. [3] Millon's test: red color produced.

INDICANURIA AND HOW TO SCREEN FOR THIS DISORDER: Indicanuria is associated with an increased intestinal absorption of indole, which is converted to indican in the liver. Indican is secreted in its colorless form into the urine. Air oxidation will covert the indican to indigo blue which can stain diapers and clothing. Early diagnosis can be made if blue stains are noted in the diapers. This phenomenon is associated with Hartnup's disease. Hartnup's disease is an autosomal recessive trait characterized by pellagra-like skin lesions, neural disorders, 10 fold loss of monoaminomonocarboxylic amino acids in the urine along with the an intestinal defect that prevents the absorption of these amino acids. This disease can be treated with a high protein diet and nicotinamide supplement. Urine can be screened for indican using ferric chloride. A blue-violet color is produced that can be extracted into chloroform.

ALKAPTONURIA AND HOW TO SCREEN FOR THIS DISORDER: Alkaptonuria is a rare disorder of tyrosine metabolism with a deficiency in the enzyme "homogentisic acid oxidase". The consequence of this disease is the accumulation of homogentisic acid in the connective tissue leaving the tissue darkly pigmented. This pigmented condition is called "ochronosis" and the patient will develop degenerative arthritis. Homogentisic acid (dihydroxyphenylacetic acid) accumulates and is excreted in the urine. Urine will readily darken with standing and exposure to air or sunlight. It is easy to miss the urine changes and "ochronosis" may be identified through x-rays, intermittent but acute arthritis, brown scleral pigment, or thickened ears. Urine screening procedures include: [1] ferric chloride: transient blue color. [2] clinitest tablet: orange-red. [3] silver nitrate: black. [4] ammonium hydroxide: will alkalinize the urine, turning it black. This disorder is usually not diagnosed until middle-age. If dark stains are noted in the diapers of neonates, then the disorder may be diagnosed earlier. At this present time there is no satisfactory treatment for alkaptonuria.

MUCOPOLYSACCHARIDOSES AND HOW TO SCREEN FOR THIS DISORDER: Mucopolysaccharidoses lysosomal storage diseases and are inherited disorders of connective tissue. There are seven types (I - VII), with 13 subtypes, and each is due to a deficiency in an enzyme that cannot effectively degrade dermatin sulfate, keratan sulfate, heparan sulfate, and/or chondroitin sulfate (mucopolysaccharides). The affected mucopolysaccharides will accumulate in the organs and tissues, affecting their function. Urinary screening tests include: [1] Cetyltrimethylammonium bromide (CTAB): Add 1.0 mL of 5% citrate buffered CTAB to 5.0 mL centrifuge urine. If positive, the white flocculation will occur. [2] A filter paper may be prepared by soaking in 2% Azure A dye. Add a drop of urine to the dried paper. If positive, a blue spot will appear. Confirm the test by soaking the paper in a solution of 0.5% acetic acid in 100% methanol. If mucopolysaccharides are present, the spot will remain. Only Types MPS I-S, MPS II (mild), MPS-IV B, MPS VI (mild), and possibly MPS VII have a good prognosis. The other eight types/subtypes have very poor prognosis.

CYSTINURIA AND HOW TO SCREEN FOR THIS DISORDER: Cystinuria is an inherited autosomal recessive disorder and is characterized by increased amounts of cystine in the urine. This is because the renal tubules cannot reabsorb this amino acid from the glomerular filtrate. Other amino acids affected by this disorder are ornithine, lysine, and arginine. Cystine has low solubility in urine, hexagonal crystals are routinely observed and kidney stones are a constant concern. Urinary screening tests for cystine are: [1] cyanide-nitroprusside: red-purple color. [2] clinitest tablet: red-orange. False positive can occur if ketones or homocystine are present. If a patient is suspected of having cystinuria, look for RBC's in the sediment. This may indicate irritation from kidney stones. Chromatography tests will confirm the presence of the amino acids associated with this disorder.

MAPLE SYRUP URINE DISEASE (MSUD) AND HOW TO SCREEN FOR THIS DISORDER: This rare autosomal recessive disorder is also called "branched-chain ketonuria" or "branched-chain aminoaciduria. Leucine, isoleucine, and valine accumulate in the blood and a keto-acidosis results. The ketoacids are excreted in the urine and give the urine its characteristic "maple syrup" odor. Infants with this disorder demonstrate problems one week after birth. The disorder, once identified, can be controlled by diet and monitoring the production of ketoacids. This disease should be confirmed by liquid chromatography technology. Urine screening tests include: [1] ferric chloride: green-gray color. [2] nitroso-naphthol: red color. [3] 2,4-dinitrophenylhydrazine: yellow. [4] Acetest: purple.

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