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CONTENTS

Editorial

There are a few diseases that are said to be associated with ROYALTY. No, they have nothing to do with the status of the individual (sufferer) but rather with the genetic lineage of the individual in question. They are passed on in families. The first written account of the disease we are talking about occurred in the second century in the Babylonian Talmud. In it Rabbi Judah haNasi, redactor of the Mishneh, wrote: "If she circumcised her first child and he died, and a second one also died, she must not circumcise her third child." This passage refers to both the prolonged bleeding caused by circumcision and to the maternal inheritance of the disease. Perhaps, you have guessed it right – We are talking about Haemophilia. In 1803, Dr. John Conrad Otto, a Philadelphian physician, wrote an account about "a hemorrhagic disposition existing in certain families" in which he called the affected males "bleeders." He recognized that the disorder was hereditary and that it affected mostly males and was passed down by healthy females. In 1947, Pavlosky, a doctor from Buenos Aires, found haemophilia A and haemophilia B to be separate diseases by doing a lab test. Queen Victoria passed haemophilia A on to many of her descendants. Queen Victoria passed the mutation to her son Leopold and, through several of her daughters, to various royals across the continent, including the royal families of Spain, Germany, and Russia. In Russia, Tsarevich Alexei Nikolaevich, son of Nicholas II, was a descendant of Queen Victoria through his mother Empress Alexandra and suffered from haemophilia.

In Spain, Queen Victoria's youngest daughter, Princess Beatrice, had a daughter Victoria Eugenie of Battenberg, who later became Queen of Spain. Two of her sons were haemophiliacs and both died from minor car accidents: Her eldest son, Prince Alfonso of Spain, Prince of Asturias, died at the age of 31 from internal bleeding after his car hit a telephone booth. Her youngest son, Infante Gonzalo, died at age 19 from abdominal bleeding following a minor car accident where he and his sister hit a wall avoiding a cyclist. Neither appeared injured or sought immediate medical care and Gonzalo died two days later from internal bleeding.

There are numerous such instances. Just flip this page to know more! This is what DISEASE DIAGNOSIS is talking about in the issue that you are now holding.

HIV has become more of a chronic disease for practitioners to manage, requiring careful, but routine, clinical monitoring. Laboratory markers, such as the HIV-1 RNA viral load and CD4 cell count, are regularly used for patient management in addition to predicting disease progression and/or treatment outcomes. INTERPRETATION portion delves deep into the CD4 cell counts and there relevance in managing HIV/AIDS.

Breast cancer is one of the first malignancies for which the use of targeted therapy has become a routine and life-extending practice. For more than 3 decades, management of breast cancer has largely been determined by the measurement of the estrogen receptor (ER), primarily because of the substantial benefit that endocrine therapy provides for patients with ER-positive but not ER-negative tumors. Progesterone receptor (PgR), a product of the interaction of estrogen with ER, is also commonly measured but has value mostly as a prognostic marker. The human epidermal growth factor receptor-2 gene ERBB2 (commonly referred to as HER2), which is amplified in approximately 15%-20% of breast cancers, is also a significant biomarker in breast cancer. Evaluating ER, PR and HER2/Neu Immunohistochemistry markers can have a direct bearing on the treatment and hence life expectancy of the patient. TROUBLE SHOOTING segment outlines the problems encountered while evaluating these markers and how to overcome them.

BOUQUET will tease your brain, make you laugh and make you think and look back at life – all at the same time. Go ahead try this new intellectual recipe.

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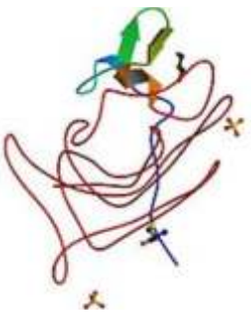
DISEASE DIAGNOSIS

Haemophilia

Deficiency in coagulation factor VIII is the cause of haemophilia A.



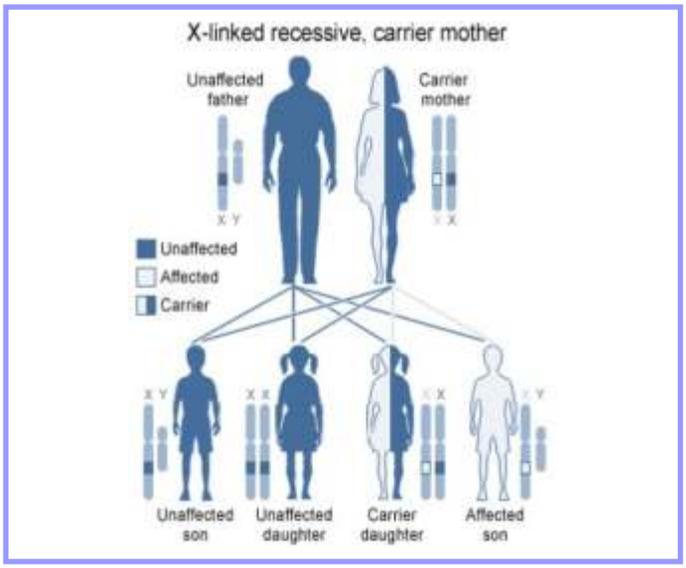
Deficiency in coagulation factor VIII is the most common cause of haemophilia. Haemophilia (also spelled hemophilia in North America, from the Greek haima 'blood' and philia 'friend' is a group of hereditary genetic disorders that impair the body's ability to control blood clotting or coagulation, which is used to stop bleeding when a blood vessel is broken. Haemophilia A (clotting factor VIII deficiency) is the most common form of the disorder, occurring at about 1 in 5,000–10,000 male births. Haemophilia B (factor IX deficiency) occurs at about 1 in about 20,000–34,000 male births. Similarly to most recessive sex-linked, X chromosome disorders, haemophilia is more likely to occur in males rather than females. This is because females have two X chromosomes while males have only one, lacking a 'back up' copy for the defective gene; meaning, the defective gene is guaranteed to manifest in any male who carries it. Because females have two X chromosomes and because haemophilia is rare, the chance of a female having two defective copies of the gene is very low, thus females are almost exclusively asymptomatic carriers of the disorder. Female carriers may inherit the defective gene from either their mother, father, or it may be a new mutation. Only under rare circumstances do females actually have haemophilia. Haemophilia lowers blood plasma clotting factor levels of the coagulation factors needed for a normal clotting process. Thus when a blood vessel is injured, a temporary scab does form, but the missing coagulation factors prevent fibrin formation, which is necessary to maintain the blood clot. Thus a haemophiliac does not bleed more intensely than a normal person, but can bleed for a much longer amount of time. In severe haemophiliacs even a minor injury could result in blood loss lasting days, weeks, or not ever healing completely. In areas such as the brain or inside joints, this can be fatal or permanently debilitating.

Scientific discovery: The first written account of haemophilia occurred in the second century in the Babylonian Talmud. In it Rabbi Judah haNasi, redactor of the Mishneh, wrote: "If she circumcised her first child and he died, and a second one also died, she must not circumcise her third child." This passage refers to both the prolonged bleeding caused by circumcision and to the maternal inheritance of the disease. The first medical professional to describe a disease was Albucasis. In the tenth century he described families whose males died of bleeding after only minor traumas. While many other such descriptive and practical references to the disease appear throughout historical writings, scientific analysis did not begin until the start of the nineteenth century. In 1803, Dr. John Conrad Otto, a Philadelphian physician, wrote an account about "a hemorrhagic disposition existing in certain families" in which he called the affected males "bleeders." He recognized that the disorder was hereditary and that it affected mostly males and was passed down by healthy females. His paper was the second paper to describe important characteristics of an X-linked genetic disorder (the first paper being a description of color blindness by John Dalton who studied his own family). Otto was able to trace the disease back to a woman who settled near Plymouth in 1720. The idea that affected males could pass the trait onto their unaffected daughters was not described until 1813 when John Hay published an account in The New England Journal of Medicine. The term "haemophilia" is derived from the term "haemorrhaphilia" which was used in a description of the condition written by Friedrich Hopff in 1828, while he was a student at the University of Zurich. In 1937, Patek and Taylor, two doctors from Harvard, discovered anti-hemophilic globulin. In 1947, Pavlosky, a doctor from Buenos Aires, found haemophilia A and haemophilia B to be separate diseases by doing a lab test. This test was done by transferring the blood of one haemophiliac to another haemophiliac. The fact that this corrected the clotting problem showed that there was more than one form of haemophilia.

European royalty: Queen Victoria passed haemophilia A on to many of her descendants. Haemophilia has featured prominently in European royalty and thus is sometimes known as "the royal disease". Queen Victoria passed the mutation to her son Leopold and, through several of her daughters, to various royals across the continent, including the royal families of Spain, Germany, and Russia. In Russia, Tsarevich Alexei Nikolaevich, son of Nicholas II, was a descendant of Queen Victoria through his mother Empress Alexandra and suffered from haemophilia. Ryan White was an American haemophiliac who became infected with HIV/AIDS through contaminated blood products. It was claimed that Rasputin was successful at treating the Tsarevich's haemophilia. At the time, a common treatment administered by professional doctors was to use aspirin, which worsened rather than lessened the problem. It is believed that, by simply advising against the medical treatment, Rasputin could bring visible and significant improvement to the condition of Alexei. In Spain, Queen Victoria's youngest daughter, Princess Beatrice, had a daughter Victoria Eugenie of Battenberg, who later became Queen of Spain. Two of her sons were haemophiliacs and both died from minor car accidents: Her eldest son, Prince Alfonso of Spain, Prince of Asturias, died at the age of 31 from internal bleeding after his car hit a telephone booth. Her youngest son, Infante Gonzalo, died at age 19 from abdominal bleeding following a minor car accident where he and his sister hit a wall avoiding a cyclist. Neither appeared injured or sought immediate medical care and Gonzalo died two days later from internal bleeding.

Occurrence: Haemophilia is rare, with only about 1 instance in every 10,000 births (or 1 in 5,000 male births) for haemophilia A and 1 in 50,000 births for haemophilia B.

Genetics



Causes: Haemophilia A is a recessive X-linked genetic disorder involving a lack of functional clotting Factor VIII and represents 80% of haemophilia cases. Haemophilia B is a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. It comprises approximately 20% of haemophilia cases. Haemophilia C is an autosomal genetic disorder (i.e. not X-linked) involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive: heterozygous individuals also show increased bleeding.

Severity: There are numerous different mutations which cause each type of haemophilia. Due to differences in changes to the genes involved, patients with haemophilia often have some level of active clotting factor. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1-5% active factor have moderate haemophilia, and those with mild haemophilia have between 5-40% of normal levels of active clotting factor.

Symptoms: Characteristic symptoms vary with severity. In general symptoms are internal or external bleeding episodes, which are called "bleeds". Patients with more severe hemophilia suffer more severe and more frequent bleeds, while patients with mild haemophilia typically suffer more minor symptoms except after surgery or serious trauma. Moderate haemophiliacs have variable symptoms which manifest along a spectrum between severe and mild forms. Prolonged bleeding and re-bleeding are the diagnostic symptoms of haemophilia. Internal bleeding is common in people with severe haemophilia and some individuals with moderate haemophilia. The most characteristic type of internal bleed is a joint bleed where blood enters into the joint spaces. This is most common with severe haemophiliacs and can occur spontaneously (without evident trauma). If not treated promptly, joint bleeds can lead to permanent joint damage and disfigurement. Bleeding into soft tissues such as muscles and subcutaneous tissues is less severe but can lead to damage and requires treatment. Children with mild to moderate haemophilia may not have any signs or symptoms at birth especially if they do not undergo circumcision. Their first symptoms are often frequent and large bruises and hematomas from frequent bumps and falls as they learn to walk. Swelling and bruising from bleeding in the joints, soft tissue, and muscles may also occur. Children with mild haemophilia may not have noticeable symptoms for many years. Often, the first sign in very mild haemophiliacs is heavy bleeding from a dental procedure, an accident, or surgery. Females who are carriers usually have enough clotting factors from their one normal gene to prevent serious bleeding problems, though some may present as mild haemophiliacs.

Differential diagnosis: Haemophilia A can be mimicked by von Willebrand disease. von Willebrand Disease type 2A, where decreased levels of von Willebrand Factor can lead to premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 2A is inherited in an autosomal dominant fashion. von Willebrand Disease type 2N, where von Willebrand Factor cannot bind Factor VIII, autosomal recessive inheritance. (i.e.; both parents need to give the child a copy of the gene). von Willebrand Disease type 3, where lack of von Willebrand Factor causes premature proteolysis of Factor VIII. In contrast to haemophilia, vWD type 3 is inherited in an autosomal recessive fashion. Additionally, severe cases of vitamin K deficiency can present similar symptoms to heamophilia. This is due to the fact that vitamin K is necessary for the human body to produce several protein clotting factors. This vitamin deficiency is rare in adults and older children but is common in newborns. Infants are born with naturally low levels of vitamin K and do not yet have the symbiotic gut flora to properly synthesize their own vitamin K. Bleeding issues due to vitamin K deficiency in infants is known as "haemorrhagic disease of the newborn," to avoid this complication newborns are routinely injected with vitamin K supplements.

Diagnosis: If hemophilia is suspected, or if one appears to have a bleeding problem, the doctor will take personal and family medical histories. This will reveal whether the patient or anyone in the family has a history of frequent and/or heavy bleeding and bruising. Besides, physical examination and blood tests are used to diagnose hemophilia. Blood tests are used to find out: How long it takes for blood to clot, Whether blood has low levels of any of the clotting factors, Whether one of the clotting factors is completely missing from blood. The test results will show whether one has hemophilia, if yes, of which type and how severe it is. Hemophilia A and B are classified as mild, moderate, or severe, depending on the amount of clotting factor VIII or IX in the blood.

The severity of symptoms can overlap between the categories. For example, some people who have mild hemophilia may have bleeding problems almost as often or as problematic as some people who have moderate hemophilia. Severe hemophilia can cause serious bleeding problems in babies. Therefore, children who have severe hemophilia usually are diagnosed during the first year of life. People who have milder forms of hemophilia may not be diagnosed until they're adults. The bleeding problems of hemophilia A and hemophilia B are the same. Only special blood tests can tell which type of the disorder a person has. Knowing which type is important because the treatments are different. Pregnant women who are known hemophilia carriers can have the disorder diagnosed in their unborn children as early as 10 weeks into their pregnancies. Women who are hemophilia carriers also can have "preimplantation diagnosis" to have children who don't have hemophilia. For this process, women have their eggs removed and then fertilized by sperm in a laboratory. The embryos that result from this fertilization are then tested for hemophilia. Only embryos that lack the condition will be implanted in the womb.

Investigations: A history of prolonged bleeding after trauma or surgery (including dental extractions) or of episodes of spontaneous bleeding into muscles or joints usually indicates some defect in the hemostatic mechanism. Specific coagulation factor assays can diagnose the type and severity of hemophilia. A positive family history can also help diagnose hemophilia, but 20% of all cases have no family history.

Characteristic findings in hemophilia A include: factor VIII assay 0% to 30% of normal, prolonged partial thromboplastin time (PTT), normal platelet count and function, bleeding time, and prothrombin time.

Characteristics of hemophilia B include: deficient factor IX-C, baseline coagulation results similar to those in hemophilia A, with normal factor VIII.

In hemophilia A or hemophilia B, the degree of factor deficiency determines severity: mild hemophilia — factor levels 5% to 40% of normal, moderate hemophilia — factor levels 1% to 5% of normal, severe hemophilia — factor $\leq$ 1%.

Complications: Severe complications are much more common in severe and moderate haemophiliacs. Complications may be both directly from the disease or from its treatment: Deep internal bleeding, e.g. deep-muscle bleeding, leading to swelling, numbness or pain of a limb. Joint damage, potentially with severe pain and even destruction of the joint and development of arthritis. Transfusion transmitted infection from blood transfusions that are given as treatment. Adverse reactions to clotting factor treatment, including the development of an immune inhibitor which renders factor replacement less effective. Intracranial hemorrhage is a serious medical emergency caused by the buildup of pressure inside the skull. It can cause disorientation, nausea, loss of consciousness, brain damage, and death.

Treatment: Though there is no cure for haemophilia, it can be controlled with regular infusions of the deficient clotting factor, i.e. factor VIII in haemophilia A or factor IX in haemophilia B. Factor replacement can be either isolated from human blood serum, recombinant, or a combination of the two. Some haemophiliacs develop antibodies (inhibitors) against the replacement factors given to them, so the amount of the factor has to be increased or non-human replacement products must be given, such as

porcine factor VIII. Commercially produced factor concentrates such as "Advate", a recombinant Factor VIII produced by Baxter International, come as a white powder in a vial which must be mixed with sterile water prior to intravenous injection. If a patient becomes refractory to replacement coagulation factor as a result of circulating inhibitors, this may be partially overcome with recombinant human factor VII (NovoSeven), which is registered for this indication in many countries. In early 2008, the US Food and Drug Administration (FDA) approved Xyntha (Wyeth) anti-haemophilic factor, genetically engineered from the genes of Chinese hamster ovary cells. Since 1993 (Dr. Mary Nugent) recombinant factor products (which are typically cultured in Chinese hamster ovary (CHO) tissue culture cells and involve little, if any human plasma products) have been available and have been widely used in wealthier western countries. While recombinant clotting factor products offer higher purity and safety, they are, like concentrate, extremely expensive, and not generally available in the developing world. In many cases, factor products of any sort are difficult to obtain in developing countries. In Western countries, common standards of care fall into one of two categories: prophylaxis or on-demand. Prophylaxis involves the infusion of clotting factor on a regular schedule in order to keep clotting levels sufficiently high to prevent spontaneous bleeding episodes. On-demand treatment involves treating bleeding episodes once they arise. In 2007, a clinical trial was published in the New England Journal of Medicine comparing on-demand treatment of boys (< 30 months) with haemophilia A with prophylactic treatment (infusions of 25 IU/kg body weight of Factor VIII every other day) in respect to its effect on the prevention of joint-diseases. When the boys reached 6 years of age, 93% of those in the prophylaxis group and 55% of those in the episodic-therapy group had a normal index joint-structure on MRI. Prophylactic treatment, however, resulted in average costs of $300,000 per year. The author of an editorial published in the same issue of the NEJM supports the idea that prophylactic treatment not only is more effective than on demand treatment but also suggests that starting after the first serious joint related hemorrhage may be more cost effective than waiting until the fixed age to begin. This study resulted in the first (October 2008) FDA approval to label any Factor VIII product to be used as prophylactically. As a result, the factor product used in the study (Bayer's Kognate) is now labeled for use to prevent bleeds, making it more likely that insurance carries in the US will reimburse consumers who are prescribed and use this product prophylactically. Despite Kognate only recently being "approved" for this use in the US, it and other factor products have been well studied and are often prescribed to treat Haemophilia prophylactically to prevent bleeds, especially joint bleeds.

Preventive exercises: It is recommended that people affected with haemophilia do specific exercises to strengthen the joints, particularly the elbows, knees, and ankles. Exercises include elements which increase flexibility, tone, and strength of muscles, increasing their ability to protect joints from damaging bleeds. These exercises are recommended after an internal bleed occurs and on a daily basis to strengthen the muscles and joints to prevent new bleeding problems. Many recommended exercises include standard sports warm-up & training exercises such as stretching of the calves, ankle circles, elbow flexions, and quadriceps sets.

Alternative and complementary treatments: While not a replacement for traditional treatments, preliminary scientific studies indicate that hypnosis and self-hypnosis can be effective at reducing bleeds and the severity of bleeds and thus the frequency of factor treatment. Herbs which strengthen blood vessels and act as astringents may benefit patients with haemophilia, however there are no peer reviewed scientific studies to support these claims. Suggested herbs include: Bilberry (Vaccinium myrtillus), Grape seed extract (Vitis vinifera), Scotch broom (Cytisus scoparius), Stinging nettle (Urtica dioica), Witch hazel (Hamamelis virginiana), and yarrow (Achillea millefolium).

Contraindications: Anticoagulants such as Heprin and Warfarin are contraindicated for people with haemophilia as these can aggravate clotting difficulties. Also contraindicated are those drugs which have "blood thinning" side effects. For instance, medications which contain aspirin, ibuprofen, or naproxen sodium should not be taken because they are well known to have the side effect of prolonged bleeding. Also contraindicated are activities with a high likelihood of trauma, such as motorcycling and skateboarding. Popular sports with very high rates of physical contact and injuries such as American football, hockey, boxing, wrestling, and rugby should be avoided by people with haemophilia. Other active sports like soccer, baseball, and basketball also have a high rate of injuries, but have overall less contact and should be undertaken cautiously and only in consultation with a doctor. Females possess two X-chromosomes, males have one X and one Y chromosome. Since the mutations causing the disease are recessive, a woman carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other chromosome should express itself to produce the necessary clotting factors. However, the Y-chromosome in men has no gene for factors VIII or IX. If the genes responsible for production of factor VIII or factor IX present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome, so the deficient gene is not masked by the dominant allele and he will develop the illness. Since a male receives his single X-chromosome from his mother, the son of a healthy female silently carrying the deficient gene will have a 50% chance of inheriting that gene from her and with it the disease; and if his mother is affected with haemophilia, he will have a 100% chance of being a haemophiliac. In contrast, for a female to inherit the disease, she must receive two deficient X-chromosomes, one from her mother and the other from her father (who must therefore be a haemophiliac himself). Hence haemophilia is far more common among males than females. However, it is possible for female carriers to become mild haemophiliacs due to lyonisation (inactivation) of the X chromosomes. Haemophiliac daughters are more common than they once were, as improved treatments for the disease have allowed more haemophiliac males to survive to adulthood and become parents. Adult females may experience menorrhagia (heavy periods) due to the bleeding tendency. The pattern of inheritance is criss-cross type. This type of pattern is also seen in colour blindness. A mother who is a carrier has a 50% chance of passing the faulty X chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters. A son cannot inherit the defective gene from his father. Genetic testing and genetic counselling is recommended for families with haemophilia. Prenatal testing, such as amniocentesis, is available to pregnant women who may be carriers of the condition. As with all genetic disorders, it is of course also possible for a human to acquire it spontaneously through mutation, rather than inheriting it, because of a new mutation in one of their parents' gametes. Spontaneous mutations account for about 33% of all cases of haemophilia A. About 30% of cases of haemophilia B are the result of a spontaneous gene mutation.

Likelihood: If a female gives birth to a haemophiliac child, either the female is a carrier for the disease or the haemophilia was the result of a spontaneous mutation. Until modern direct DNA testing, however, it was impossible to determine if a female with only healthy children was a carrier or not. Generally, the more healthy sons she bore, the higher the probability that she was not a carrier. If a male is afflicted with the disease and has children with a female who is not even a carrier, his daughters will be carriers of haemophilia. His sons, however, will not be affected with the disease. The disease is X-linked and the father cannot pass haemophilia through the Y chromosome. Males with the disorder are then no more likely to pass on the gene to their children than carrier females, though all daughters they sire will be carriers and all sons they father will not have haemophilia (unless the mother is a carrier).

TROUBLESHOOTING

Proper Handling of Breast Cancer Specimens

Assessing Hormone Receptor Status: Breast cancer is one of the first malignancies for which the use of targeted therapy has become a routine and life-extending practice. For more than 3 decades, management of breast cancer has largely been determined by the measurement of the estrogen receptor (ER), primarily because of the substantial benefit that endocrine therapy provides for patients with ER-positive but not ER-negative tumors. Large overviews of randomized clinical trials have confirmed that women with ER-negative invasive breast cancers do not derive benefit from endocrine treatments. The clinical significance of this biomarker has rendered the assessment of the ER status of primary invasive breast cancer mandatory. Progesterone receptor (PgR), a product of the interaction of estrogen with ER, is also commonly measured but has value mostly as a prognostic marker. In the early 1990s, immunohistochemical (IHC) testing of ER and PgR receptor-specific antibodies was developed for use on sections of frozen tissue; subsequent development of new antigen retrieval methods and the development of new antibodies allowed ER testing to be performed on formalin-fixed and paraffin-embedded material, as well. Despite significant experience with ER and PgR assessments in most laboratories, confirmation studies associated with recent breast cancer clinical trials have shown that there is a 5%-10% false-negative rate and a 5% false-positive rate when institutional results are compared with central laboratory determinations. Although some of this discrepancy is attributable to differences in threshold values, the percentages of error are nevertheless very alarming. In addition, significantly discrepant results in ER testing have been uncovered in several Canadian provinces, where variations in tissue handling, testing methods, training, and staffing were all implicated.

Determining HER2 Overexpression: The human epidermal growth factor receptor-2 gene ERBB2 (commonly referred to as HER2), which is amplified in approximately 15%-20% of breast cancers, is also a significant biomarker in breast cancer. Gene amplification results in overexpression of a breast cancer tumor cell surface receptor protein, which possesses tyrosine kinase activity and potentiates tumor cell growth. HER2 protein overexpression and HER2 gene amplification are associated with poor clinical outcomes in patients with breast cancer. However, in the 1990s, trastuzumab, a new human monoclonal antibody that targeted this protein, was developed. When used in HER2-overexpressing metastatic breast cancer, trastuzumab, either alone or added to chemotherapy, has been found in clinical trials to reduce the risk for disease recurrence by 50% and the risk for mortality by 30%.It is also highly effective in the adjuvant treatment of early-stage breast cancer. Recent results with a small-molecule HER1/HER2 tyrosine kinase inhibitor, lapatinib, demonstrate that the oral agent, added to capecitabine, improves clinical outcomes in patients with advanced disease. HER2 overexpression determined by IHC testing is now accepted as a strong predictive marker for clinical benefit in both the metastatic and adjuvant settings when drugs targeting the HER2 receptor protein are used. The trials that provided evidence of benefit with these therapies, however, also showed a false-positive rate of 15%-18% in institutionally performed assays relative to those done in a central laboratory. There was also a significant false-negative rate of up to 5%.

Specimen Handling: Many of the factors leading to poor test accuracy are related to specimen handling before the actual test is performed. Data are emerging on the impact of this variation on testing results. To understand the contribution of specimen handling to breast cancer testing inaccuracy, researchers conducted a retrospective trial involving review of 5077 patient records from 1999 to 2003. It was found a significant discrepancy in the rate of ER-negative tumors when those removed Sunday through Thursday were compared with those removed on the weekend (Friday/Saturday). The testing was performed in a central laboratory with standardized processes and 3 trained pathologists. Although it was concluded that variable specimen handling explained the results, the exact cause of the variation was not known. Researchers therefore conducted a prospective trial comparing facilities that recorded time of tumor removal and time into formalin (test group) with those that did not record those times. It was found that the mean time from removal of tumor to specimen fixation in the test group was 18 minutes. The PgR- (but not the ER-) negative rate was significantly lower in facilities that did not record the time, suggesting that merely documenting the specimen handling is an effective intervention for controlling variation. Frequency of ER-negative test results on breast cancer specimens by hospital and by specimen handling group. *Designates the hospital where the tests were performed. Number of samples included was 5077. Other compelling data about the impact of variable fixation practices come from researchers in far eastern countries where, until recently, all breast cancer was considered ER negative, based on results obtained after prolonged specimen handling. Researchers have published data establishing that the ER-positive rate for breast cancer tumors in far eastern women is identical to that observed in western women when tissue is properly collected and fixed promptly. Previous observations about the lack of ER positivity was attributed to delays in appropriate fixation of tumor specimens.

Warm and Cold Ischemia Time: The interval from the interruption of the tumor blood supply to the initiation of tissue fixation is widely accepted as an important variable in the analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. Warm ischemia time is the time from the interruption of the blood supply to the tumor by the surgeon to the excision of the tissue specimen; cold ischemia time is the time from excision of the tumor to the initiation of tissue fixation. Numerous studies and articles have discussed the progressive loss of activity of these labile molecules following the surgical interruption of blood flow, which leads to tissue ischemia, acidosis, and enzymatic degradation. Although there are data about the impact of variation in cold ischemic time on protein expression in tissues, the contribution of the warm ischemic interval to this macromolecular degradation is currently under study. The standardization of the cold ischemia time is an important step to help ensure that differences in levels of protein expression for clinically relevant targets such as ER are biologically meaningful and are not artifacts related to the manner in which the tissue was handled.

Optimal Fixation Processes: Timing and documentation. The breast tumor specimen should be fixed quickly in an adequate volume of fixative (optimally 10-fold greater than the volume of the specimen). The time of tissue collection (defined as the time that the tissue is removed from the surgical field) and the time the tissue is placed in fixative both must be recorded on the tissue specimen requisition to document the cold ischemic time. Ideally, when pathologists and surgeons communicate effectively with OR staff, the appropriate recording process (Table given below) becomes routine.

Table. Pathology Responsibilities for Documenting Fixation Intervals

Grossing Room:	Ensure adequate documentation of specimen removal, fixation start, and duration of fixation times Include specimen removal and fixation start and fixation duration times as part of the dictation
Transcription:	Ensure that times are included as part of the dictation Transcribe times to be included in pathology report
Pathologist:	Ensure times are documented and included as part of the pathology report Inquire about cases with missing fixation times and ensure proper documentation Evaluate test results taking fixation interval documentation into account

Fixation techniques. It is also critical that specimens be adequately fixed in 10% neutral buffered formalin (NBF). To ensure proper fixation, specimens must be promptly examined and sectioned by the pathologist so that fixative will penetrate all the areas of tumor to be microscopically examined. In situations where excision specimens are obtained remotely from the gross examination laboratory, the pathologists should work with operating suite personnel to ensure that the sample is bisected through the tumor and promptly placed in NBF before transport. The time to insertion of tumor sample into fixative, as well as the time of tumor removal from the patient, should be noted on the specimen requisition by the personnel in the surgical suite. Although less optimal than immediate gross examination of the fresh sample by the pathologist, this process is preferable to storage of the sample in the refrigerator unfixed or in fixative without sectioning. Only 10% NBF should be used as the fixative for breast tissue specimens; higher or lower concentrations of NBF are not acceptable. This recommendation is based on published literature regarding the expected or characteristic immunoreactivity of NBF for ER, PgR, and HER2 in breast cancer, which has been accrued over many years and has been clinically validated with patient

outcomes in numerous clinical trials. In addition, the US Food and Drug Administration (FDA) approval for assay kits analyzing ER, PgR, and HER2 explicitly states that formalin fixation should be used and that FDA approval for the kits is not applicable if an alternative fixative is used. If the laboratory uses a formalin alternative for fixation, the assay must be validated against tissue fixed in NBF to assure that the alternative fixative yields identical results. The laboratory director assumes responsibility for the validity of assay results when nonstandard procedures are followed. Breast tissue specimens must be fixed in 10% NBF for no less than 6 hours and no more than 72 hours before processing. Formalin, which is aqueous, completely dissolved formaldehyde, penetrates tissue at a rate of approximately 1 mm/hr. Breast excision samples must be incised in a timely fashion to initiate formalin fixation throughout the tissue; fixation does not begin until formaldehyde has penetrated into the tissue. However, permeation of tissue by formalin is not the same as the chemical reaction of fixation, which involves protein cross-linking by formaldehyde. Chemical fixation requires time, with the rate-limiting step being the equilibrium between formaldehyde and methylene glycol in solution, which is time dependent and can be measured in hours ("clock reaction"). Although complete tissue fixation usually requires 24 hours, published studies have documented that breast samples require a minimum of 6-8 hours of formalin fixation to obtain consistent IHC assay results for ER and HER2. Underfixation of breast tissue may lead to false-negative ER results and false-positive HER2 results. In these situations, the tissue is actually fixed in 100% ethanol, which is used to dehydrate the specimens after fixation. Overfixation is likely to be less problematic than underfixation but potentially could also lead to false-negative results due to excessive protein cross-linking by formaldehyde. Standard antigen retrieval protocols are optimized for 24 hours of fixation time. These recommendations apply also to needle biopsy specimens and cytology specimens.

Improving the Process: Although the new guidelines for ER, PgR, and HER2 specify ideal processes and conditions, both OR and pathology personnel will have to cooperate to implement these practices and make them routine. OR staff, encouraged by surgeons, must routinely record the time of specimen removal from the patient. Samples must be promptly transported to the gross examination room of the pathology laboratory so that they may be promptly examined and fixed. This process change must be explained and enforced by the pathologist. Both pathologists and surgeons must be alert to the potential for nonroutine handling of specimens on Friday afternoons and Saturdays, or at remote locations, and should be vigilant in following up on processes used under these circumstances. Armed with adequate information about the specimen handling conditions for each breast cancer sample, the pathologist who observes an unexpected ER, PgR, or HER2 result will be able to do some problem solving to discover potential reasons for that unusual finding and guide the surgeon and patient correctly. Such unexpected findings might include ER-negative and/or PgR-negative and/or HER2-positive results on low-grade breast cancer specimens, or HER2-negative results on high-grade ER-negative or ER-positive breast cancer specimens. Targeted therapies are highly effective when applied in appropriate patients. Without accurate testing, patients whose tumors will not respond may nevertheless face treatment with expensive and potentially toxic drugs, whereas patients whose lives may be prolonged by effective treatment may be denied those options. In the early days of ER measurement, virtually all US laboratories froze and shipped breast cancer samples to properly qualified laboratories that performed these assays in a controlled manner. It is not unreasonable to expect the same type of uniform compliance with conditions required for today's breast cancer specimen analyses of ER, PgR, and HER2 status. This reality must be kept in mind as healthcare providers face the challenges of altering "business as usual" to comply with new requirements and conditions. Patients will be the ultimate beneficiaries of these changes.

BOUQUET

In Lighter Vein

A new business was opening and one of the owner's friends wanted to send him flowers for the occasion. They arrived at the new business site and the owner read the card,.... "Rest in Peace."The owner was angry and called the florist to complain. After he had told the florist of the obvious mistake and how angry he was, the florist replied,"Sir, I'm really sorry for the mistake, but rather than getting angry, you should imagine this: somewhere, there is a funeral taking place today, and they have flowers with a note saying,... 'Congratulations on your new location!'"

A hillbilly went hunting one day in Oklahoma and bagged three ducks. He put them in the bed of his pickup truck and was about to drive home when he was confronted by an ornery game warden who didn't like hillbillies. The game warden ordered the hillbilly to show his hunting license, and the hillbilly pulled out a valid Oklahoma hunting license.

The game warden looked at the license, then reached over and picked up one of the ducks, sniffed its butt, and said "This duck ain't from Oklahoma. This is a Kansas duck. You got a Kansas huntin' license, boy?"

The hillbilly reached into his wallet and produced a Kansas hunting license.

The game warden looked at it, then reached over and grabbed the second duck, sniffed its butt, and said "This ain't no Kansas duck. This duck's from Arkansas. You got a Arkansas license?"

The hillbilly reached into his wallet and produced an Arkansas hunting license.

The warden then reached over and picked up the third duck, sniffed its butt, and said This ain't no Arkansas duck. This here duck's from South Carolina. You got a South Carolina huntin' license?"

Again the hillbilly reached into his wallet and brought out a South Carolina hunting license.

The game warden was extremely frustrated at this point, and he yelled at the hillbilly "Just where the hell are you from?"

The hillbilly turned around, bent over, dropped his pants, and said "You tell me, expert."

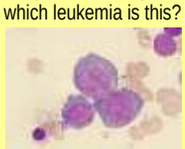
Wisdom Whispers

- "Better when birds sing than where irons ring."
- "Listen or your tongue will keep you deaf."
- "The herb one knows one should bind to one's finger."
- "Ill begun, ill done."
- "A wound never heals so well that the scar cannot be seen."
- "To conceal disease is fatal."
- "Three women, three geese, and three frogs, make a fair."
- "Joy and sorrow are next door neighbours."
- "Better be the head of a dog than the tail of a lion."
- "Labour warms, sloth harms."
- "Let him not complain of being cheated who buys cloth by the pattern."
- "A kitchen-dog is never a good rabbit-hunter."
- "Wear not out your welcome."
- "Some who mean only to warm, burn themselves."

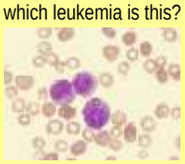
Brain Teasers

Provide the likely diagnosis by looking at the pictures given below

1. Peripheral smear phtomicrograph, which leukemia is this?



2. Peripheral smear, which leukemia is this?



3. Peripheral smear, identify the marked cell

4. Identify the marked cell

Answers: Fig.1. Acute Erythroid Leukemia (M6), 2. Acute Promyelocytic leukemia, 3. Eosinophilic band form, 4. Atypical immature plasma cell

INTERPRETATION

CD4 COUNTS, HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) AND HIV MANAGEMENT

As a result of successful antiretroviral treatment over the last 20 years, HIV has become more of a chronic disease for practitioners to manage, requiring careful, but routine, clinical monitoring. Laboratory markers, such as the HIV-1 RNA viral load and CD4 cell count, are regularly used for patient management in addition to predicting disease progression and/or treatment outcomes. The HIV viral load is considered to be the gold standard for evaluating treatment success, although it is often limited by the cost. Furthermore, in certain cases, there is a mismatch between an undetectable viral load (< 50 copies/ml) and the absence of immune reconstitution, which can be confusing to both the treatment provider and patient. In this review, the utility of the CD4 count as a predictor for HIV disease progression in patients not on therapy is evaluated, as well as a method for monitoring a patient's response to therapy. Its use in predicting immune reconstitution in patients initiating antiretrovirals is also identified. We hope to aid the clinician by examining the most recent literature and discussing the added value of the CD4 count in the management of a person with HIV infection.

Introduction: Morbidity and mortality from HIV/AIDS has declined both in the USA, as well as other developed countries, and in developing nations since the advent of HAART. In clinical trials, different HAART regimens have been found to produce viral suppression in up to 80–90% of subjects. However, the long-term durability of potent HAART in clinical practice is not entirely clear. Success rates of clinical trials are not easily translated into clinical practice, and treatment outcomes in a clinic setting have been shown to be worse than those in research trials. Treatment failure, whether attributable to virologic failure, stopping HAART or loss to follow-up, leads to increases in morbidity and mortality. Having a reliable marker to evaluate disease progression and predict treatment outcomes would be useful for the practitioner and patient alike. Since the introduction of HAART, much has been studied regarding which factors best predict a patient's success on HAART. Previously described predictors of treatment failure include poor adherence to medications, one or more missed visits in the previous year, prior virologic failure, a regimen consisting only of nucleosides, higher baseline viral loads and lower baseline CD4 cell counts. Scoring systems have been designed and validated to assess the incidence of clinical disease progression among patients receiving HAART. In a model designed by the EuroSIDA study group, the most recent CD4 cell count, viral load and hemoglobin level were independently related to the risk of disease progression, as was a late presentation of persons with advanced disease, before the start of HAART. Several cohort studies and clinical trials have shown that the CD4 count is the strongest predictor of subsequent disease progression and survival. The use of the CD4 count as an independent and reliable marker for treatment outcome is attractive from various aspects. First, CD4 counts are already the most important factor in deciding whether to initiate antiretroviral therapy and opportunistic prophylaxis – all HIV-positive patients in high-income countries, and an increasing number of patients in low-income countries have a baseline CD4 count at entry into care. Second, the CD4 count is a relatively objective and simple marker to follow. Finally, the cost of CD4 counts has become more affordable, including in developing countries. This article further evaluates the use of the CD4 count in assessing the clinical status of HIV-infected individuals, in making informed decisions regarding the initiation of antiretroviral therapy and in monitoring the success of such therapy.

What is an adequate CD4 response on HAART?

An adequate CD4 response for most patients on therapy is defined as an increase in the range of 50–150 cells/mm3 per year with an accelerated response in the first 3 months of treatment. In general, CD4 counts should be checked every 3–4 months to determine when to start antiretroviral therapy, to assess immunologic response to therapy and to evaluate the need for initiation or discontinuation of prophylaxis for opportunistic infections (OIs). Patients with good virologic control average approximately 50–100 cells/mm3 per year until a steady-state level is reached. A clinically significant change between CD4 counts approximates a 30% change in the absolute count or an increase or decrease in CD4 percentage by 3%. For those patients who adhere to therapy with sustained viral suppression and are clinically stable for more than 2–3 years, the frequency of CD4 count monitoring may be extended to every 6 months. In cases of discordant CD4 and viral-load results, the clinician should first exclude a laboratory error and consider retesting the patient. Absolute CD4 counts may fluctuate among individuals or be influenced by factors that affect the total white blood cell count and lymphocyte percentages. Bone marrow-suppressive medications and IFN-α may reduce the absolute CD4 count. Acute infection, sepsis, malaria and TB can decrease both absolute CD4 counts and percentages. In turn, a splenectomy or co-infection with human T-cell leukemia virus type 1 may cause misleadingly elevated absolute CD4 counts. Although it does not appear to yield clinical effects, administration of IL-2 has been shown to increase CD4 counts; and steroids can both increase and decrease CD4 counts. Sex, race and psychological and physical stress typically have a minimal effect on CD4 counts. Pregnancy can lead to hemodilution with a small decline in CD4 count but no decline in percentage. In many of these cases, the CD4 percentage remains stable and may be a more appropriate parameter to assess the patient's immune function.

Baseline CD4 count as a predictor of disease progression & treatment outcome: Numerous studies have demonstrated that the baseline CD4 count serves as a significant prognostic indicator for treatment outcome. In one study, patients starting therapy with a CD4 count below 200 cells/mm3 were almost twice as likely (HR: 1.90) to fail treatment, compared with those starting with a CD4 count higher than 200 cells/mm study showed an inverse relationship between the CD4 count at baseline and a risk of progression to AIDS or death. This effect was quite dramatic: the adjusted HR for progression to AIDS or death was 0.24 (95% CI: 0.20–0.30) for patients starting HAART with a baseline CD4 count of 200–350 cells/mm3, compared with patients with a CD4 count below 50?cells/mm3. Recent data support the prognostic value at higher CD4 cell-count levels. In a large cohort study, patients initiating HAART with CD4 counts of 350–500?cells/mm3 had a 94% increased risk of death, relative to those with baseline CD4 counts above 500 cells/mm. Equally, this and another study documented an increased risk of death when HAART was deferred until the CD4 count fell below 350 cells/mm3. studies and others have renewed and validated the impetus for earlier treatment of HIV. A similarly strong association has been observed between the baseline CD4 count and the subsequent CD4 response on HAART therapy. These studies demonstrate that individuals with the highest baseline CD4 count at HAART initiation have the best chance for full immune reconstitution and restoration of a near-normal CD4 count and support a higher CD4 threshold for HAART initiation. In the ACTG 384 study, immune reconstitution was evaluated for five different baseline CD4 strata (< 50 to > 500 cells/mm3). Although absolute CD4 count increases were similar in all strata, only patients in the higher baseline strata achieved close to normal CD4 values following 3 years of HAART. In addition, abnormalities in CD4 naive-memory cell-count ratios and T-cell activation markers were clearly more pronounced in the lower CD4 strata, although immune imbalances remained among all patients. The ACTG 384 study suggests that T-cell subsets and ratios may give more detailed prognostic information on immune recovery on HAART than absolute CD4 counts. Importantly, some of these activation markers have already been strongly linked with disease progression. Several recent studies have focused on the prognostic value of inflammatory (e.g., high-sensitivity C-reactive protein and IL-6) and coagulation markers (e.g., d-dimer). Increasingly, it is becoming clear that elevated levels of these markers are predictive of overall mortality and AIDS- and non-AIDS-related events, independent of CD4 counts or viral-load values.

(To be continued....)

TULIP NEWS

Setting a New Trend in Rapid Testing

Combiquic

HIV/HCV

Combiquic is a rapid qualitative advanced Flow through (Immunoconcentration) assay for the simultaneous and differential detection of antibodies to HIV 1 & 2 and HCV in Human Serum/plasma

Evaluated by NIB
Approved by DCGI

Performance of COMBIQUIC

Comparison with Licensed EIA

Parameter	HIV 1 & 2	HCV
Sensitivity	100.00 %	100.00 %
Specificity	100.00 %	99.61 %

NIB Evaluation

Parameter	HIV 1 & 2	HCV
Sensitivity	100.00 %	99.00 %
Specificity	99.58 %	

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