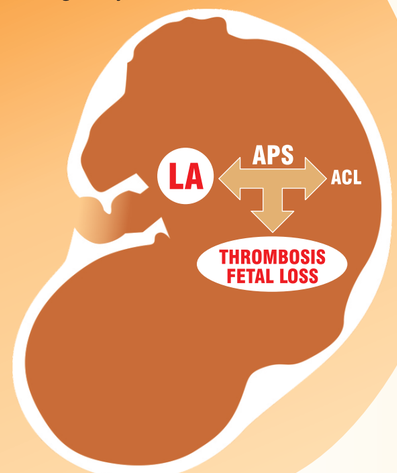
TULIP NEWS

ALLEVIATE

THE AGONY OF MISCARRIAGES

LA's
(Lupus anticoagulants)
play an important role in recurrent Pregnancy loss



The screening of APA's* both with ACL's* for sensitivity and **LA for SPECIFICITY** reinforces the diagnosis.

LA - PREVALENCE

- 3-48% LA prevalence in recurrent pregnancy loss.
- Pregnancy loss occurred in 24 of the 55 pregnancies in patients with persistent LA positivity compared with 8 of 67 pregnancies in LA negative pregnancies.
- Greater pregnancy loss in women with repeated LA positivity.

APA's - Anti Phospholipid Antibodies ACL's - Anti Cardiolipin Antibodies

SCREEN WITH
- **LIQUICELIN - E/LUPAKCT**
  APTT Test / KCT Test
- **LA Screen**
  DRVVT Test

CONFIRM WITH
- **LA Confirm**
  DRVVT Test

DETECT LA PROTECT PREGNANCIES

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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Early pregnancy loss is a frustrating and heart-wrenching experience for both the patient and the physician. Early pregnancy loss is unfortunately the most common complication of human gestation, occurring in as many as 75% of all women trying to conceive. Most of these losses are unrecognized and occur before or with the next expected menses. Of those that are recognized, 15-20% result in spontaneous abortions (SABs) or ectopic pregnancies. Approximately 5% of couples trying to conceive have 2 consecutive miscarriages, and approximately 1% of couples have 3 or more consecutive losses.

Early pregnancy loss is defined as the termination of pregnancy before 20 weeks gestation or with a fetal weight of < 500 g. Most investigators agree that both ectopic and molar pregnancies should not be included in the definition.

Most studies demonstrate a spontaneous miscarriage rate of 10-15%. However, the true rate of early pregnancy loss is close to 50% because of the high number of chemical pregnancies that are not recognized in the 2-4 weeks after conception. Most of these pregnancy failures are due to gamete failure (eg, sperm or oocyte dysfunction). In a classic study, 221 women were followed up during 707 total menstrual cycles. A total of 198 pregnancies were achieved. Of these, 43 (22%) were lost before the onset of menses, and another 20 (10%) were clinically recognized losses.

The likelihood for an SAB increases with each successive miscarriage. Data from various studies indicate that after 1 SAB, the baseline risk of a couple having another SAB is approximately 15%. However, if 2 SABs occur, the subsequent risk increases to approximately 30%. The rate is higher for women who have not had at least 1 liveborn infant. Several groups have estimated that the risk of pregnancy loss after 3 successive abortions is 30-45%, which is comparable to the risk in those who had 2 SABs. This data prompted a controversy regarding the timing of diagnostic evaluation, with many specialists preferring to begin after 2 losses rather than 3. The **DISEASE DIAGNOSIS** segment takes up detail all issues related to Spontaneous Abortions.

A procedure that is often conducted by Pathologists and sometimes leads to a wasted effort on account of wrong procedure or even if the procedure is correct the sample preparation may be faulty. Sometimes the clinical cause may not permit the proper collection of the sample. Any guesses? We are talking about bone marrow aspiration/ biopsy. The complete correct procedure, sample preparation, post-procedure care and complications are outlined for your consumption. The **TROUBLESHOOTING** section details the process in ample detail.

The **INTERPRETATION** portion discusses for you the Antiphospholipid Antibodies and Miscarriages. The whole approach to Lupus Anticoagulants is outlined in an easy to understand schematic flow chart.

Amidst all the above-mentioned clinical components of the issue, the non-clinical component entitled **BOUQUET** has not been forgotten.

Tulip Group

DISEASE DIAGNOSIS

RECURRENT EARLY PREGNANCY LOSS

Overview

Early pregnancy loss is a frustrating and heart-wrenching experience for both the patient and the physician. Early pregnancy loss is unfortunately the most common complication of human gestation, occurring in as many as 75% of all women trying to conceive. Most of these losses are unrecognized and occur before or with the next expected menses. Of those that are recognized, 15-20% result in spontaneous abortions (SABs) or ectopic pregnancies. Approximately 5% of couples trying to conceive have 2 consecutive miscarriages, and approximately 1% of couples have 3 or more consecutive losses. Early pregnancy loss is defined as the termination of pregnancy before 20 weeks gestation or with a fetal weight of < 500 g. Most investigators agree that both ectopic and molar pregnancies should not be included in the definition. Table 1 provides specific definitions.

Table 1: Terms Used to Describe Pregnancy Loss

Term	Definition
Chemical pregnancy loss	Loss of a biochemically evident pregnancy
Early pregnancy loss	Abortion of the first trimester, loss of a histologically recognized pregnancy, or a loss based on ultrasonographic findings
SAB	Pregnancy loss before 20 weeks gestation, as based on last menstrual period
Habitual or recurrent abortion	2 or more consecutive SABs*
Stillbirth	Pregnancy loss after 20 weeks gestation (Neonatal loss is the death of a liveborn fetus.)

Incidence

Most studies demonstrate a spontaneous miscarriage rate of 10-15%. However, the true rate of early pregnancy loss is close to 50% because of the high number of chemical pregnancies that are not recognized in the 2-4 weeks after conception. Most of these pregnancy failures are due to gamete failure (eg, sperm or oocyte dysfunction). In a classic study by Wilcox et al in 1988, 221 women were followed up during 707 total menstrual cycles. A total of 198 pregnancies were achieved. Of these, 43 (22%) were lost before the onset of menses, and another 20 (10%) were clinically recognized losses. The likelihood for an SAB increases with each successive miscarriage. Data from various studies indicate that after 1 SAB, the baseline risk of a couple having another SAB is approximately 15%. However, if 2 SABs occur, the subsequent risk increases to approximately 30%. The rate is higher for women who have not had at least 1 liveborn infant. Several groups have estimated that the risk of pregnancy loss after 3 successive abortions is 30-45%, which is comparable to the risk in those who had 2 SABs. This data prompted a controversy regarding the timing of diagnostic evaluation, with many specialists preferring to begin after 2 losses rather than 3.

Etiology

The etiology of early pregnancy loss is varied and often controversial. More than 1 etiologic factor is often present. The most common causes of recurrent miscarriages are as follows:

Genetic causes: Aneuploidy, Somatic, Sex chromosome, Mendelian disorders, Multifactorial disorders, Parental chromosomal abnormalities (translocations), Chromosomal inversions.

Immunologic causes: Autoimmune causes, Alloimmune causes.

Anatomic causes: Uterine müllerian anomaly, Uterine septum (the anomaly most commonly associated with pregnancy loss), Hemiuterus (unicornuate uterus), Bicornuate uterus, Diethylstilbestrol-linked condition, Acquired defects (eg, Asherman syndrome), Incompetent cervix, Leiomyomas, Uterine polyps.

Infectious causes: Environmental causes, Smoking, Excessive alcohol consumption, Caffeine.

Endocrine factors: Diabetes mellitus, Antithyroid antibodies, Luteal phase deficiency.

Hematologic disorders: The gestational age at the time of the SAB can provide clues about the cause. For instance, nearly 70% of SABs in the first 12 weeks are due to chromosomal anomalies. However, losses due to antiphospholipid syndrome (APS) and cervical incompetence tend to occur after the first trimester.

Genetic Causes

Prevalence and Types:

Most spontaneous miscarriages are caused by an abnormal (aneuploid) karyotype of the embryo. At least 50% of all first-trimester SABs are cytogenetically abnormal. This figure does not include abnormalities caused by single genetic disorders, such as Mendelian disorders, or mutations at several loci. Some examples that may not be detected by evaluating karyotypes are polygenic or multifactorial disorders. The highest rate of cytogenetically abnormal conception occurs earliest in gestation, with rates declining after the embryonic period (>30 mm crown-rump length). The rate of normal (euploid) and abnormal (aneuploid) abortuses increases with maternal age. Recurrent miscarriage may result from 2 types of chromosomal abnormalities: (1) the recurrence of a numerical abnormality (aneuploidy) in the embryo, which is usually not inherited or (2) a structural abnormality derived from 1 parent.

Aneuploidy: Cytogenetically abnormal embryos are usually aneuploid because of sporadic events, such as meiotic nondisjunction, or polyploid from fertilization abnormalities.

Autosomal trisomy: Autosomal trisomy is involved in 50% of the cytogenetically abnormal abortuses in the first trimester. It may arise de novo because of meiotic nondisjunction during gametogenesis in parents with a normal karyotype. Autosomal trisomy results from maternal meiosis I errors (either complete trisomies or monosomies).

Specific trisomies: Trisomy 16, which accounts for 30% of all trisomies, is the most common. Viable trisomies have been observed for chromosomes 13, 16, and 21. Approximately one third of fetuses with Down syndrome (trisomy 21) survive to term.

Autosomal monosomies: Autosomal monosomies are rarely, if ever, observed.

Monosomy X (Turner syndrome): Turner syndrome is frequently observed and is the most common chromosomal abnormality observed in SABs. Turner syndrome accounts for 20-25% of cytogenetically abnormal abortuses.

Triploidy and tetraploidy: Triploidy and tetraploidy are related to abnormal fertilization and are not compatible with life. Triploidy is found in 16% of abortions, with fertilization of a normal haploid ovum by 2 sperm (dispermy) as the primary pathogenic mechanism. Tetraploidy occurs in approximately 8% of chromosomally abnormal abortions, resulting from failure of an early cleavage division in an otherwise normal diploid zygote.

Parental Chromosomal Abnormalities

Structural chromosomal abnormalities occur in approximately 3% of cytogenetically abnormal abortuses. These abnormalities are thought to be most commonly inherited from the mother. Structural chromosomal problems found in men often lead to low sperm concentrations, male infertility, and, therefore, a reduced likelihood of pregnancy and miscarriage. Translocations are the most common types of structural abnormalities and can be balanced or unbalanced. Slightly more than one half of unbalanced rearrangements result from abnormal segregation of Robertsonian

translocations (when 2 acrocentric chromosomes fuse near the centromere region with loss of the short arms), and the rest arise de novo during gametogenesis. In reciprocal translocations, there is an exchange of material between nonhomologous chromosomes. The offspring created from parental gametes with the abnormality may have normal or carrier karyotypes. Adjacent segregation results in unbalanced distribution of the chromosomes involved in the translocation, leading to partial trisomy for 1 chromosome and partial monosomy for the other chromosome. The severity of the phenotype depends on the chromosomes involved and on the positions of their breakpoints. Other structural rearrangements, such as inversions or ring chromosomes, are relatively rare. These chromosomal abnormalities can be associated with congenial malformations and mental retardation, as well as SAB.

Genetic Abnormalities/Mendelian Disorders

Certain genetic mutations, such as the autosomal dominant disorder leading to myotonic dystrophy, may predispose a patient to infertility or even miscarriage. The cause of the abortion in this disease is unknown, but it may be related to abnormal gene interactions combined with disordered uterine function and implantation defects. Other presumed autosomal dominant disorders associated with SAB include lethal skeletal dysplasias, such as thanatophoric dysplasia and type II osteogenesis imperfecta. Maternal disease associated with increased fetal wastage includes connective tissue disorders, such as Marfan syndrome, Ehlers-Danlos syndrome, homocystinuria, and pseudoxanthoma elasticum. Hematologic abnormalities associated with recurrent pregnancy loss include dysfibrinogenemia, factor XIII deficiency, congenital hypofibrinogenemia and afibrinogenemia, and sickle cell anemia. Women with sickle cell anemia are at increased risk for fetal loss, possibly because of placental-bed microinfarcts.

Management

For couples who have had an SAB due to a suspected genetic cause, the standard of care is to offer genetic counseling. Because advanced age increases the risk of an abnormal karyotype in a conceptus, amniocentesis is routinely offered for all pregnant women of advanced maternal age, which is defined as women older than 35 years. A woman's risk of having an aneuploid fetus is 1 per 80 when she is older than 35 years; this is far greater than the risk of fetal loss after amniocentesis, which is 1 per 200. A study by Warburton et al indicated that routine karyotype analysis after 1 miscarriage is not cost-effective or prognostic. However, after 2 SABs, analysis of the abortuses is useful. In 1990, Drugan et al examined 305 women with 2 or more miscarriages and found an increased risk for fetal aneuploidy in these couples with chorionic villus sampling or amniocentesis. Therefore, couples with recurrent miscarriage should undergo karyotype evaluation by means of amniocentesis or chorionic villus sampling during a subsequent pregnancy. Because karyotype analysis does not help in detecting abnormalities caused by single gene mutations or mutations at several loci (small structural deletions and rearrangements), different techniques, such as fluorescence in situ hybridization (FISH), are being used to complement standard cytogenetics. If a parental chromosome abnormality is found, this should be the starting point for familial testing, and proper family counseling is recommended. If an increased risk for future pregnancies is identified, all alternatives should be discussed, including foregoing any attempts at further conception, adopting, trying to conceive again with early prenatal testing, using donor gametes, or performing preimplantation genetic diagnosis (PGD). The concept of preimplantation genetic screening (PGS) has been recently introduced. This involves using FISH to screen the removed blastomere for aneuploidy in older women and in those with recurrent SABs. PGS and FISH can be used to accurately detect common aneuploidies accounting for 70% of aneuploidic first trimester losses (chromosomes 13, 15, 16, 17, 18, 21, X, and Y), but these methods are criticized for their inability to detect all chromosomal abnormalities. Theoretically, selection of chromosomally normal embryos for uterine transfer increases the likelihood for implantation, but the reports in the literature have been conflicting in regards to the efficacy of PGS in this setting. In 2006, a retrospective analysis by Munne et al of women older than 40 years showed a decrease in the SAB rates from 40% to 22% in the group that underwent PGD. However, efficacy of PGS in decreasing SAB rates was challenged in other studies. A randomized trial of 408 women of advanced maternal age undergoing a total of 836 cycles concluded that the ongoing pregnancy rate, as well as live birth rate, were significantly lower in the women assigned to the PGS group compared with those without PGS. The authors theorized the possibility that biopsy of a blastomere on day 3 hampers the potential of an embryo to successfully implant. Another reason for their result could be the flaws inherent in the FISH procedure, such as inability to detect aneuploidy in all chromosomes or examining mosaic cells. Based on the practice guidelines published in Fertility and Sterility in 2007, available evidence does not support the use of PGS to increase live birth rates in women of advanced maternal age. Also, available evidence does not currently support the use of PGS for patients with recurrent pregnancy loss because it does not improve ongoing pregnancy or live birth rates and does not decrease miscarriage rates in such women. However, couples in whom pregnancy loss can be attributed to a balanced translocation may benefit from specific genetic testing by PGD. Reported disadvantages of PGD include misdiagnosis of chromosomal normality, possible lowering of implantation rates with embryonic biopsy, and poor suitability of tested embryos for cryopreservation.

Immunologic Causes

Autoimmune Abnormalities:

Recurrent pregnancy loss is associated with several autoimmune diseases. One such disease is antiphospholipid antibody syndrome (APS), also known as lupus anticoagulant syndrome and Hugh syndrome. This disorder is characterized by the presence of APL antibodies, which are frequently linked to pregnancy losses in the pre-embryonic (< 6 wk), embryonic (6-9 wk), and fetal (≥10 wk gestation) time periods. 10-20% of women with early losses are positive for the anti-phospholipid antibodies, and an unusually high proportion of pregnancy losses occur in the fetal period compared to unselected population.(NEJM 2002). Three classes of clinically significant APL antibodies have been identified: anticardiolipin (aCL), lupus anticoagulant (LAC), and anti-β_2 glycoprotein I antibodies. In addition, biologically false-positive serologic test results for syphilis may have similar clinical significance. APS is diagnosed when medical, obstetric, and appropriate laboratory findings are present. Diagnosis of APS requires the presence of at least 1 of the clinical criteria and at least 1 of the laboratory criteria: Clinical criteria: Vascular thrombosis. Pregnancy morbidity. 3 or more unexplained consecutive miscarriages with anatomic, genetic, and hormonal causes excluded. 1 or more unexplained death(s) of a morphologically normal fetus at or after the 10 weeks gestation. 1 or more premature birth(s) of a morphologically normal neonate at or before 34 weeks gestation, associated with severe preeclampsia or severe placental insufficiency. Laboratory criteria: aCL: Immunoglobulin G (IgG) and/or immunoglobulin M (IgM) isotype is present in medium or high titer on 2 or more occasions, 6 or more weeks apart. Demonstration of a prolonged phospholipid-dependent coagulation on screening tests (eg, activated partial thromboplastin time, kaolin clotting time, dilute Russell viper venom time, dilute prothrombin time, Textarin time). Failure to correct the prolonged screening test result by mixing with normal platelet-poor plasma. Shortening or correction of the prolonged screening test result with the addition of excess phospholipids. Exclusion of other coagulopathies as clinically indicated (eg, factor VIII inhibitor) and heparin. These antibodies can be demonstrated with enzyme-linked immunosorbent assay (ELISA) or a coagulation result positive for LAC. Notably, the presence of the antibodies alone in the absence of other clinical symptoms does not define the syndrome. Patients with the combination of high APLA titers and the IgG isotype have a prognosis worse than those with the combination of low titers and the IgM isotype. However, the type of APLA (aCL, LAC, or anti–beta-2 glycoprotein I) does not influence the prognosis. APLAs are found in fewer

than 2% of apparently healthy pregnant women, in fewer than 20% of apparently healthy women with recurrent fetal loss, and in more than 33% of women with systemic lupus erythematosus (SLE).

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is by far the most common disease associated with APS. Patients with SLE have a 12-30% prevalence for ACL antibodies, and 15-34% for LAC antibodies. SLE, as associated with antiphospholipid antibodies, has been linked to increased rates of miscarriage and late pregnancy loss since 1954. Patients with SLE have a median miscarriage rate of 10%, which is similar to the general population. However, the 8% median rate of late pregnancy loss among these patients is considerably higher than in their healthy counterparts. The higher late pregnancy loss rate is related to increased incidence of fetal death in the second and third trimesters in patients with SLE, and most of these are associated with the presence of APLAs. Three factors are predictive of adverse obstetric outcome in patients with SLE. Disease before conception, Onset of SLE during pregnancy, Underlying renal disease. Other obstetric and medical conditions associated with APLAs are listed below. Obstetric conditions associated with APLAs: Preeclampsia, Intrauterine growth restriction, Abnormal fetal heart rate tracings, Preterm deliveries, Pregnancy wastage. Medical conditions associated with APLAs: Arterial and venous thrombosis, Autoimmune thrombocytopenia, Autoimmune hemolytic anemia, Livedo reticularis, Chorea, Pulmonary hypertension, Chronic leg ulcers.

Antinuclear antibodies

Antinuclear antibodies (ANAs) have also been associated with recurrent pregnancy loss, even in patients without evidence of overt autoimmune disease. In most published studies, the ANA titers in women with recurrent miscarriages were only mildly elevated. However, these mild elevations are nonspecific and common in the general population (even in those with no history of pregnancy loss). Therefore, extrapolating this as a cause is difficult. Further studies are needed to prove or disprove ANA as a causal agent in recurrent miscarriages, and measuring ANAs is not recommended as part of an evaluation of recurrent miscarriage.

Antithyroid antibodies

Unlike ANA, antithyroid antibodies are known as independent markers for an increased risk of miscarriage. In 1990, Stagnaro-Green et al observed 500 consecutive women for thyroid-specific autoantibodies (specifically, antithyroglobulin and/or antithyroid peroxidase) in the first trimester of pregnancy. Women with a positive result for thyroid autoantibodies had a 17% rate of pregnancy loss compared with 8.4% for women without evidence of thyroid autoantibodies. None of the women with thyroid autoantibodies had clinically evident thyroid disease, and the increase in pregnancy loss was not due to changes in thyroid hormone levels or APLA. The pathophysiology involved in this phenomenon is unclear and probably represents a generalized autoimmune defect rather than a thyroid-induced abnormality. However, available data do not support the use of thyroid autoantibody testing in women with recurrent pregnancy loss.

Alloimmune Abnormalities

Miscarriage may occur when the maternal immune response to antigens of placental or fetal tissues is abnormal. Human leukocyte antigen (HLA) sharing has been reported as such an alloimmune response. HLA sharing is a condition in which the normal process that allows for the creation of maternal blocking antibodies in pregnancy is decreased. However, studies to date have proven no association between recurrent pregnancy loss and HLA.

Anatomic Causes

Anatomic uterine defects are known to cause obstetric complications, including recurrent pregnancy loss, preterm labor and delivery, and malpresentation, although many women with such defects may have uncomplicated pregnancies. Most commonly, the complications result from impaired vascularization and fetal growth restriction. The incidence of uterine anomalies is estimated to be 1 per 200-600 women, depending on the method used for diagnosis. When manual exploration is performed at the time of delivery, uterine anomalies are found in approximately 3% of women. However, uterine abnormalities are present in approximately 27% of women with a history of pregnancy loss.

Uterine müllerian anomalies

The most common uterine defects include septate, unicornuate, bicornuate, and didelphic uteri. Of these, the unicornuate uterus is least common, but can result in malpresentation and fetal growth restriction. The highest rate of reproductive losses are found in bicornuate uteri (47%) compared with unicornuate uteri (17%), but both are frequently associated with second trimester loss and preterm delivery. Women with unicornuate and didelphys uteri have the highest rate of abnormal deliveries, while women with uterine septa have a 26% risk of reproductive loss. In addition to müllerian anomalies, other anatomic causes of recurrent pregnancy loss to consider for include diethylstilbestrol exposure related-anomalies, Asherman syndrome, incompetent cervix, leiomyomas, and uterine polyps. Controversies exist among these listed uterine anatomic abnormalities as causes for pregnancy loss. They are suggested but not scientifically proven potential causes.

Management

Accurate diagnosis of mullerian anomalies is essential. Imaging studies of choice include hysteroscopy, hysterosalpingography (HSG), sonohysterograms, and vaginal ultrasonography. Findings may be confirmed with MRI. For instance, a banana-shaped cavity with a single fallopian tube is the most common finding in a unicornuate uterus. Prophylactic cervical cerclage should be considered in patients with a unicornuate uterus. Some authors support expectant management in these patients, with serial assessments of cervical lengths by using digital and ultrasonographic examinations. Surgical correction of uterine anatomic abnormalities has not been shown to benefit pregnancy outcomes in a prospective controlled trial. However, data from uncontrolled retrospective reviews have suggested that resection of the uterine septum increases delivery rates (70-85% in 1 study).

Infectious Causes

The theory that microbial infections can cause miscarriage has been presented in the literature as early as 1917 when DeForest et al observed recurrent abortions in women exposed to farm animals with brucellosis. Numerous organisms have been implicated in sporadic causes of miscarriage, but common microbial causes of RPL have not been confirmed. In fact, infection is viewed as a rare cause of recurrent miscarriage. A recent review failed to show sufficient evidence for the notion that any type of infection can be identified as a causal factor for recurrent miscarriage. Most patients with a history of recurrent miscarriage do not benefit from an extensive infection workup. Listeria Monocytogenes, Chlamydial Genitourinary Infections, Ureaplasma Infection, Mycoplasma Infections, Bacterial Vaginosis, Cytomegalovirus, Rubella, Herpes Simplex, HIV Disease, Parvovirus B19 Infection, Toxoplasmosis, Treponematosis (Endemic Syphilis), Lyme Disease, Malaria.

Environmental Causes

Environmental causes of human malformation account for approximately 10% of malformations, and fewer than 1% of all human malformations are related to exposures to prescription drugs, chemicals, or radiation.

Isotretinoin (Accutane)

Isotretinoin is a retinoic acid used to treat severe acne and is associated with SAB.

Anesthetic gases

The relationship between exposure to trace concentrations of waste anesthetic gases in the operating room and the possible development of adverse health effects has been a concern for many years. However, the studies that did show an increased incidence of miscarriage and congenital anomalies had many flaws. A meta-analysis from 1997 pooled data from 19 reports and concluded that in the prescavenging era, the relative risk of spontaneous abortion from exposure to anesthetic gas was 1.9. Since then,

most operating rooms use ventilation systems to minimize occupational exposure to the gases.

Tobacco

Maternal exposure to tobacco and its effect on reproductive outcomes has been the subject of many studies. Cigarette smoke contains hundreds of toxic compounds. Nicotine is thought to reduce placental and fetal circulation through its vasoactive actions. Carbon monoxide depletes both fetal and maternal oxygen supply, and lead is a known neurotoxin. Despite the many harmful effects to a woman's health, maternal smoking appears to only slightly increase the risk of SABs.

Alcohol

Maternal exposure to excess alcohol has been reported to be associated with an increased risk for SAB.

Coffee consumption

Coffee consumption has been the subject of much debate since the 1980s. Studies have demonstrated conflicting results, some finding that moderate coffee consumption (< 350 mg/d) is not related to the risk of SABs , whereas others claim that the risk of SAB increases even at this level of exposure. In 2008, a large cohort study of 1063 patients by Weng et al demonstrated that caffeine consumption had a dose-dependent increase in the risk of miscarriage at all levels of consumption. Patients with caffeine intake of less than 200 mg/d were 1.42 times more likely to have an early miscarriage, whereas in those with intake of 200 mg/d or greater, the risk increased to 2.23 times compared with patients with no caffeine use. In addition, the magnitude of the association appeared to be stronger among women without a history of miscarriage than that among women with such a history.

Endocrine Causes

Ovulation, implantation, and the early stages of pregnancy depend on an intact maternal endocrine regulatory system. Most attention was historically directed at maternal systemic endocrine disorders, luteal phase abnormalities, and hormonal events that follow conception, particularly progesterone levels in early pregnancy.

Diabetes mellitus:

Women with diabetes mellitus who have good metabolic control are no more likely to miscarry than women without diabetes. However, women with poorly controlled diabetes, as evidenced by high glycosylated HgA1c levels in the first trimester, are at a significantly increased risk of both miscarriage and fetal malformation. The SAB rate increases 2-3 fold in these women compared with the general population. Screening for occult diabetes in asymptomatic women is not necessary unless the patients present with an elevated random glucose level or exhibit other clinical signs of diabetes mellitus or if there is an unexplained loss in the second trimester.

Thyroid dysfunction:

No direct evidence suggests that thyroid disease is associated with recurrent miscarriages. However, the presence of antithyroid antibodies (2 thyroid antigens: thyroglobulin and thyroid peroxidase) may represent a generalized autoimmune abnormality, which could be a contributing factor in miscarriages. Screening for thyroid disease is not useful unless the patient is symptomatic.

Low progesterone levels:

Progesterone is the principal factor responsible for the differentiation of proliferative endometrium to secretory, rendering the endometrium receptive to embryo implantation. Since Allen and Corner published their classic results on physiologic properties of the corpus luteum in 1929, low progesterone levels have been assumed to be associated with miscarriage. Luteal support remains critical until approximately 7 weeks gestation, at which time the placental trophoblast has acquired enough steroidogenic ability to support the pregnancy. In patients in whom the corpus luteum is removed before 7 weeks, miscarriage results. If progesterone is given to these patients, the pregnancy is salvaged. Recent experience with RU486 (an antiprogestin) has shown that this treatment can effectively terminate a pregnancy up to 56 days from the last menstrual period.

Luteal phase defects:

In 1943, Jones first discussed the concept of insufficient luteal progesterone resulting in either infertility or early pregnancy loss. This disorder was defined as inadequate endometrial maturation resulting from a qualitative or quantitative disorder in corpus luteal function. Methods used to diagnose luteal phase defects (LPDs) include records of basal body temperature, evaluation of progesterone concentrations, and histologic dating of endometrial biopsy specimens. The criterion standard in diagnosis of LPD is the histological characteristics of a luteal phase endometrial biopsy being more than 2 days behind the findings expected in a normal cycle. However, substantial inter- and intra-observer discrepancies occur even when the standard histologic criterion is applied, which has lead to the controversy surrounding this disorder. Furthermore, although LPD has been reported in 23-60% of women with recurrent miscarriage, as many as 31% of normally fertile women have an LPD according to the results from serial endometrial biopsy procedures. However, since no reliable method is available to diagnose this disorder, controversy exists regarding both the definition and the diagnosis itself. An additional factor that accounts for many of the discrepancies in the literature is the frequent use of the patient's subsequent menses as a reference point for determining when she had ovulated, which assumes a normal 28-day cycle. In 1 of the few prospective studies on this subject, endometrial biopsy was performed in women with 3 or more consecutive miscarriages. The pathologist then accurately dated the biopsy samples using LH assays to pinpoint the time of ovulation. LPD was believed to be the cause in 17% of these recurrent miscarriages. The authors also examined luteal-phase serum progesterone levels, and noted that they were normal in the women with LPD. Thus, luteal phase deficiency was most likely the result of an abnormal response of the endometrium to progesterone rather than a subnormal production of progesterone by the corpus luteum. This finding is corroborated by other studies, showing that as many as 50% of women with histologically defined LPD have normal serum progesterone levels. The physician must be selective in deciding who should be screened for LPD, since there is no definitive treatment to make a difference in pregnancy outcomes. Only 1 randomized trial has shown that treatment with progesterone supplementation has a beneficial effect on pregnancy outcomes , while most other studies failed to demonstrate that any type of support (eg, progesterone, human chorionic gonadotropin) results in a significant difference. So although it is known that postimplantation failure or an early nonviable pregnancy are associated with low serum progesterone levels, there is no evidence that progesterone supplementation in patients with LPD would restore the normal hormonal profile. Therefore, one approach is to screen only patients with either a history of recurrent miscarriages or recurrent failures with infertility therapy. In addition, the best accuracy is achieved if the same pathologist reviews the histologic findings, and if the day of ovulation is based on LH levels rather than subsequent menses.

Endocrine modulation of decidual immunity:

The transformation of endometrium to decidual affects all cell types present in the uterine mucosa. These morphologic and functional changes facilitate implantation, but they also help control trophoblast migration and prevent overinvasion in maternal tissue. Attention focuses on the interaction between the extravillous trophoblast and the leukocyte populations infiltrating the uterine mucosa. Most of these cells are large granular lymphocytes (LGLs) and macrophages; few T and B cells are present. The LGL population is unusual, staining strongly for natural killer (NK) cell marker CD56, but the cells do not express the CD16 and CD3 NK markers. NK cells with this distinct phenotype are found in high numbers, primarily in the progesterone-primed endometrium of the uterus. The number of CD56 cells, which is low in the proliferative-phase endometrium, increases in the midluteal phase, and peaks in the late secretory phase, suggesting that recruitment of LGLs is under hormonal control. Progesterone is essential in this process because LGLs are not found before menarche, after menopause, or in conditions associated with unopposed estrogen (eg,

endometrial hyperplasia, carcinoma). In women who have undergone oophorectomy, LGLs appear only after treatment with both estrogen and progesterone. The increase in the number of NK cells at the implantation site in the first trimester suggests their role in pregnancy maintenance. They preferentially kill target cells with little or no HLA expression. The extravillous trophoblast (which expresses modified forms of 1 HLA) is resistant to lysis by decidual NK cells under most circumstances, allowing the invasion needed for normal placentation. These CD56 cells probably differentiate in utero from precursor cells because serum levels are negligible. The only cytokine that has been able to induce proliferation of these cells is IL-2. IL-2 also transforms NK cells into lymphokine-activated killer (LAK) cells, which can lyse first-trimester trophoblast cells in vitro. As expected, IL-2 has not been found in vivo at uterine implantation sites; otherwise, stimulation of decidual NK cells would cause widespread destruction of the trophoblast. Trophoblast HLA expression is increased by interferon, a phenomenon that may offer protection from LAK cell lysis. Therefore, an equilibrium exists between the level of HLA expression on the trophoblast and the amount of lymphokine activation of NK cells, leading to the concept of fine regulation of trophoblast invasion.

Hematologic Defects

Hematologic changes and pregnancy:

Many recurrent miscarriages are characterized by defective placentation and microthrombi in the placental vasculature. In addition, certain inherited disorders that predispose women to venous and/or arterial thrombus formation are associated with thrombophilic causes for pregnancy loss. Various components of the coagulation and fibrinolytic pathways are important in embryonic implantation, trophoblast invasion, and placentation. Because the association between APLA and recurrent miscarriage is now firmly established, interest has been garnered in the possible role of other hemostatic defects in pregnancy loss.

Normal pregnancy-associated hypercoaguable state:

In normal pregnancy, there is an increase in the levels of procoagulant factors, such as factors VII, VIII, X, and fibrinogen, as early as 12 weeks' gestation. However, this thrombogenicity is not balanced by an increase in naturally occurring anticoagulants (ie, antithrombin III, proteins C and S). In fact, protein S levels decrease by 40-50%, while antithrombin III and protein C levels remain constant. Fibrinolytic activity is also decreased, with progressively increasing levels of plasminogen activator inhibitor-1 (PAI-1), produced by endothelial cells, and plasminogen activator inhibitor-2 (PAI-2), produced by the trophoblast, during pregnancy. The effects of PAI-1 and PAI-2 are localized to the invasive trophoblast, which is seemingly regulated to some extent by the balance between plasminogen activators and inactivators. Platelet activation and increased production of thromboxane, as well as decreased sensitivity to the antiaggregation effects of prostacyclin, increases the prothrombic state of pregnancy. Vasorelaxation and the resulting stasis of the venous blood flow further favors coagulation. Urokinase plasminogen activator (uPA), which is active around the time of implantation, triggers the localized production of plasmin, which in turn catalyzes the destruction of the extracellular matrix, thus facilitating implantation. uPA is also found in the maternal venous sinuses, and, therefore, plays a role in maintaining the patency of these channels. uPA receptors are also expressed on first-trimester human trophoblast cells, acting to limit deposition of fibrin in the intervillous spaces.

Changes associated with abnormal pregnancy:

Compelling evidence suggests that women with a history of recurrent miscarriage are in a procoagulant state even when they are not pregnant. Abnormal gestations are associated with production of certain factors (eg, cytokines) that may convert a thromboresistant endothelium to one that is more thrombogenic. Abnormal gestations have abnormal fibrin distribution in chorionic villi that make allogenic contact with maternal tissue. Endothelial cells in these areas appear to be deficient in the thrombin-thrombomodulin anticoagulant pathway, making the area more prone to clot formation. Defective trophoblast invasion of the spiral arteries has been found when placental-bed biopsies are performed on women after a miscarriage and on those patients with preeclampsia or intrauterine growth restriction. A large study of 116 nonpregnant women with recurrent miscarriages who tested negative for LAC and aCLs showed that 64% had at least 1 abnormal fibrinolysis-related result, most commonly a high PAI-1 level. No defects were found in the control group, which consisted of 90 fertile women with no history of miscarriage. In 1994, Patrassi and colleagues found that 67% of patients, regardless of whether or not they were aCL positive, had a defect in their fibrinolytic pathway. Evidence also suggests that just before a miscarriage, defects are present in hemostatic variables. In 1991, Tulppala and coworkers revealed that women with a history of recurrent miscarriages have an abundance of thromboxane production at 4-6 weeks gestation and a decrease in prostacyclin production at 8-11 weeks gestation, as compared with women without such a history. This shift in the thromboxane-to-prostacyclin ratio can lead to vasospasms and platelet aggregation, causing microthrombi and placental necrosis. levels of protein C and fibrinopeptide A seem to decrease just before a miscarriage occurs, suggesting activation of the coagulation cascade. In 2005, a review of the literature from the previous 10 years revealed that only 3 types of thrombophilia may be related to recurrent pregnancy loss: elevated homocysteine levels, factor V Leiden or APC resistance (associated with second trimester loss), and antiphospholipid antibodies (associated with second trimester loss). Most studies report that 5-20% of women with recurrent pregnancy loss have positive test results for antiphospholipid antibodies. In a cohort of 76 women with antiphospholipid antibodies, 50% of pregnancy losses occurred after the first trimester compared with 10% in women without antiphospholipid antibodies.

Activated protein C resistance (Factor V Leiden):

Factor V is a coagulation factor that is normally cleaved and inactivated by activated protein C (APC). Patients with a single point mutation in the gene coding for factor V produce a mutated factor V (called Factor V Leiden) that is resistant to inactivation by APC, resulting in increased thrombin production and a hypercoagulable state. This mutated gene is inherited as an autosomal dominant trait and is the most common cause of thrombosis and familial thrombophilia, with a prevalence of 3-5% in the general population. In patients with a history of venous thrombosis, the prevalence rate is as high as 40%. In normal pregnancies, APC resistance naturally decreases. However, women with APC resistance before pregnancy tend to have an even greater degree of resistance. In 1995, Rai and colleagues evaluated 120 women with a history of recurrent miscarriages. None of the women had a history of thrombosis, LAC, or aCL antibodies. The prevalence of APC resistance was higher in women who had a second-trimester miscarriage than in those with a first-trimester loss (20% vs 5.7%). The best way to detect APC resistance is both coagulation-based assay and DNA testing to detect the actual mutation.

Coagulation inhibitors:

Little data exist evaluating deficiencies of antithrombin III, protein S, or protein C and pregnancy loss.

Specific coagulation factor deficiencies:

The deficiency of factor XII (Hageman) is associated with both systemic and placental thrombosis, leading to recurrent miscarriage in as many as 22% of patients evaluated in 1 study. Overall, however, the data on deficiency of this factor are limited.

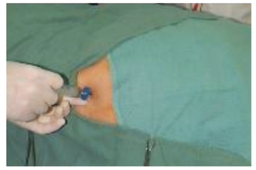
Abnormal homocysteine metabolism:

Homocysteine is an amino acid formed during the conversion of methionine to cysteine. Hyperhomocystinemia, which may be congenital or acquired, is associated with thrombosis and premature vascular disease. This condition is also associated with pregnancy loss. In 1 study, 21% of women with a history of elevated homocysteine levels had recurrent pregnancy loss. The gene for the inherited form is transmitted in an autosomal recessive form. The most common acquired form is due to folate deficiency. In these patients, folic acid replacement helps achieve normal homocysteine levels within few days.

TROUBLESHOOTING

BONE MARROW ASPIRATION/ BIOPSY

Overview



Bone marrow aspiration.

The procedure known as trepanning, or trephination, of bone is the oldest surgical practice that continues to have clinical relevance in modern times. The method dates as far back as the Neolithic period and initially entailed the drilling of cranial bones as a form of medical intervention for headaches and mental illnesses. However it was not until 1905, when the Italian physician Pianese reported bone marrow infiltration by the parasite *Leishmania*, that this procedure was applied toward clinical evaluation. In the present day, inspection of the bone marrow is considered one of the most valuable diagnostic tools to evaluate hematologic disorders. Indications have included the diagnosis, staging, and therapeutic monitoring for lymphoproliferative disorders such as chronic lymphocytic leukemia (CLL), Hodgkin and Non-Hodgkin lymphoma, hairy cell leukemia, myeloproliferative disorders, and multiple myeloma. Furthermore, evaluation of cytopenia, thrombocytosis, leukocytosis, anemia, and iron status can be performed. The application of bone marrow analysis has grown to incorporate other, nonhematologic, conditions. For example, in the investigation for fever of unknown origin (FUO), specifically in those patients with autoimmune deficiency syndrome (AIDS), the marrow may reveal the presence of microorganisms, such as tuberculosis, *Mycobacterium avium intracellulare* (MAI) infections, histoplasmosis, leishmaniasis, and other disseminated fungal infections. Furthermore, the diagnosis of storage diseases (eg. Niemann-Pick disease and Gaucher disease), as well as the assessment for metastatic carcinoma and granulomatous diseases (eg, sarcoidosis) can be performed. Bone marrow analysis can also be performed in patients with idiopathic thrombocytopenia purpura (ITP), incidental elevated serum paraprotein levels, iron deficiency anemia, B_{12}/folate deficiency, polycythemia vera, or infectious mononucleosis; but these conditions are more appropriately diagnosed by routine laboratory. Bone marrow consists of stem cells, which are large, "primitive," undifferentiated cells supported by fibrous tissue called stroma. There are 2 main types of stem cells and, therefore, the bone marrow consists of 2 types of cellular tissue. One type of stem cell is involved in producing blood cells and the other is involved in producing stromal cells, which are responsible for the supporting stroma. Sampling of the marrow consists of either aspiration of the cellular component and/or acquirement of tissue fragments. Aspiration of the marrow, as shown below, has been primarily utilized for cytologic assessment, with analysis directed toward morphology and obtainment of a differential cell count. Further sampling allows for material to be directed toward other ancillary test such as cytogenetics, molecular studies, microbiologic cultures, immunohistochemistry, and flow cytometry. Biopsies, on the other hand, allow for studies of the marrow's overall cellularity, detection of focal lesions, and extent of infiltration by various pathologic entities.

For patient education information, visit eMedicine's Osteoporosis and Bone Health Center and Cancer Screening Center, as well as Bone Marrow Biopsy.

Preliminary Assessment

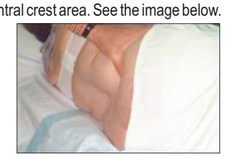
An initial review of the patient's clinical background is necessary to determine whether a bone marrow evaluation is warranted.

Medical history: Travel history: exposure to parasites (leishmaniasis), fungi (histoplasmosis, *Cryptococcus*), mycobacteria. Immune compromise or immune deficiency status: This may contribute to a high infection risk, such as in patients with human immunodeficiency virus (HIV) infection, underlying autoimmune deficiency (eg, Wiskott Aldrich Syndrome), and/or the use of immunosuppressive agents. Risk of bone fragility: Previous surgeries, chemotherapy, and radiation therapy can increase the risk of bone fragility, as well as pathologic processes that may contribute to bone resorption (eg, osteoporosis, multiple myeloma). Previous diagnosis of malignancies: These are a risk for metastasis to bone. Glycogen storage diseases. Risk for hematologic anomalies: Contributing factors include a patient's nutrition status, alcoholism, medications, and history of a coagulation factor deficiency. Allergies: Testing and/or knowledge of a patient's allergy status are preventive measures to the potential allergens exposed during bone marrow sampling, such latex, anesthetics (eg, lidocaine), antiseptics (eg, povidone-iodine).

Clinical presentation: Perform a thorough physical examination to assess the patient for signs of malignancy, infections, lesions associated with hemorrhagic injury, as well as disorders of hemostasis and coagulation. Laboratory tests should initially include complete blood cell (CBC) counts, a reticulocyte count, peripheral blood smears, prothrombin time/international normalized ratio (PT/INR), and activated partial thromboplastin time (aPTT). Other studies take into account the clinical presentation and may consist of the following: serum iron studies, serum ferritin study, vitamin B_{12} and folate levels, erythrocyte sedimentation rate (ESR), serum protein electrophoresis, platelet function studies, coagulation mixing study, fibrin D-dimers, serum fibrinogen levels, serum bilirubin levels, and radiographs. Obtain informed patient consent that provides procedural information and potential complications (eg, hemorrhage, infections, pain). This will minimize any apprehension that the patient may have.

Collection Site

The safe and preferred sites for bone marrow aspiration and/or biopsy are described below. **Aspiration and biopsy:** Posterior superior iliac crest: This is the most commonly employed site for reasons of safety, a decreased risk of pain, and accessibility. The posterior superior iliac crest site is localized to the central crest area. See the image below.



Patient position (posterior superior iliac crest).

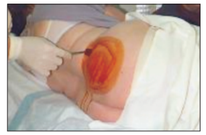
Anterior superior iliac crest: This is an alternative site when the posterior iliac crest is unapproachable or not available due to infection, injury, or morbid obesity. The anterior superior iliac crest site is localized to the center prominence, under the lip of the crest. This location is generally not preferred due to the dense cortical layer, which makes obtaining samples more difficult and smaller in size, as well as creates a risk for an increased painful event.

Aspiration only: The sternum is sampled only as a last resort in those older than 12 years and in those who are morbidly obese, but it should be avoided in highly agitated patients. To decrease the risk of penetrating the underlying soft-tissue organs, the sternal site is limited to a region that spans between the second and third intercostal spaces. The tibia is sampled only for infants younger than 1 year, and the procedure is conducted under general anesthesia. This site is localized to the proximal anteromedial surface, below the tibial tubercle. The tibial location is not utilized in older patients because the marrow cellularity is not consistent.

Procedure

A marrow biopsy of the posterior/anterior iliac crest is generally performed before aspiration sampling due to the fact the biopsy technique induces elevated thromboplastic substances. The consequence of this is a reduction in the effectiveness of an aspiration sampling. However, as many clinical requests are for an aspiration sample only, this technique is described first.

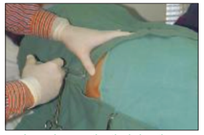
Aspiration: The patient is placed in the lateral decubitus position, with the top leg flexed and the lower leg straight. Palpate the iliac crest, and mark the preferred sampling site with a pen. Aseptic technique is employed, including sterile gloves and gown. The site is prepared with an antiseptic (eg, povidone-iodine or chlorhexidine gluconate), scrubbed, and draped, exposing only the site to be sampled. See the images below.



Skin preparation.

Site preparation.

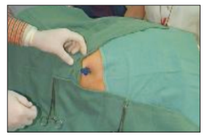
The skin and the underlying tissue to the periosteum are infiltrated with a local anesthetic (eg, approximately 10 mL of 1% Xylocaine [lidocaine]). A 10-mL syringe with a 25-gauge needle is used to inject an initial 0.5 mL directly under the skin, raising a wheal. A 22-gauge needle is used to penetrate deeper into the subcutaneous tissue and the underlying periosteum, an area roughly 1 cm in diameter.



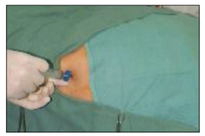
Local anesthetic injection.

Adequacy of the anesthesia is tested by gently prodding the periosteum with the tip of the needle and questioning the patient for any painful sensation. It is important to be aware of changes in the patient's comfort level throughout the procedure to not only decrease the patient's anxiety level, but to minimize movements that may affect the efficacy of the procedure. Having a family member present may help to alleviate the patient's anxiety. To ensure sufficient pain control is being managed well, the person performing the procedure should talk to the patient, discuss the steps taken throughout the process, and listen to the manner as well as the content of the patient's response.

A skin incision is made with a small surgical blade, through which the bone marrow aspiration needle, with a stylet locked in place, is inserted.



Aspiration needle placement.



Bone marrow aspiration.

Once the needle contacts the bone, it is advanced by slowly rotating clockwise and counterclockwise until the cortical bone is penetrated and the marrow cavity is entered. Contact with the marrow cavity is usually noted by a sudden reduction in pressure. The depth of the penetration should not extend beyond an initial 1 cm.

Once within the marrow cavity, the stylet is removed. Using a 20 mL syringe, approximately 0.3 mL of bone marrow is aspirated. A volume greater than 0.3 mL may dilute the sample with peripheral blood and thus is not recommended. The material collected for bone marrow slides is generally not mixed with an anticoagulant, and it is processed immediately by a technologist; this avoids any cellular morphologic artifacts. If there is to be a delay in slide preparation, place the sample in an EDTA (ethylenediaminetetraacetic acid) anticoagulant-containing tube, preferably a pediatric-sized tube to avoid exposure to excess anticoagulant.

If additional marrow is needed for ancillary studies, subsequent specimens are obtained by attaching a separate syringe, collecting 5 mL at a time. The samples are then transferred into an anticoagulant-containing tube that is appropriate to the requested study: heparin for cytogenetic analysis; either heparin or EDTA for immunophenotyping; formalin for a Cytoblock preparation; and, glutaraldehyde for ultrastructural examination.

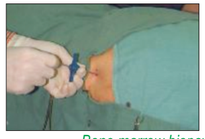
The marrow needle is removed, and pressure is applied to the aspiration site with gauze until any bleeding has stopped (see Postprocedure Care).

Once the aspiration is completed, the specimen is processed by the hematopathology technician.

Bone marrow biopsy: Any of several needle models can be utilized; however, the Jamshidi needle is considered the most popular. This disposable needle is tapered at the distal end to help retain the specimen for improved extraction.

Patient preparation is to be followed in the manner previously described for bone marrow aspiration.

The needle, with stylet locked in place, is held with the palm and index finger and repositioned so that a new insertion site is created for biopsy sampling. Once the needle touches the bone surface, the stylet is removed.



Bone marrow biopsy. Jamshidi needle placement.

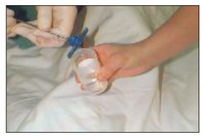
Using firm pressure, slowly rotate the needle in an alternating clockwise-counterclockwise motion, and advance it into the bone marrow cavity to obtain an adequate bone marrow specimen measuring approximately 1.6-3 cm in length.

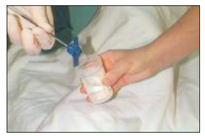
Rotate the needle along its axis to help loosen the sample, pull back approximately 2-3 mm, and advance the needle again slightly, at a different angle, to help secure the specimen.

Following this procedure, slowly pull the needle out, while rotating in an alternating clockwise and counterclockwise motion.

Remove the specimen from the needle and introduce a probe through the distal cutting end. If the aspirate was unsuccessful (ie, a "dry tap"), the core biopsy may be used to make touch preparations (see Slide Preparation). This must be performed before placing the specimen in formalin.

Place the specimen in formalin solution for histologic processing.





Bone marrow biopsy specimen.

Bone marrow biopsy specimen in fixative solution.

The marrow needle is removed, and pressure is applied to the site with gauze until any bleeding has stopped (see Postprocedure Care).

The Sternum

Note: With this site, only aspiration is to be performed, and it is only to be performed on adolescent and adult patient populations.

The second to third intercostal level of the sternum is palpated, and the selected sample site is marked with a pen. Note: The area chosen should be

to one side of the midline as the marrow cellularity is considered to be diminished at that location.

The designated area is prepared with an antiseptic scrub and draped.

Aseptic technique is employed, including sterile gloves and gown.

Local anesthetic is used to infiltrate from the skin to the periosteum.

After small cut is made in the skin with a surgical blade, the aspiration needle with the stylet locked in place, is inserted until the needle touches the bone.

With the same technique described in the above section (see Procedure: Posterior/Anterior Iliac Crest), advance the needle into the marrow cavity, obtain the specimen, and remove the needle. Note: Unlike other sites, the attached guard is not to be removed; rather, it is adjusted to allow for the maximum depth of needle penetration to 0.5 cm. This prevents needle slippage that can result in injury to the underlying mediastinal organs.

Core biopsies are not to be performed from the sternum.

Unilateral Versus Bilateral Iliac Crest Biopsy

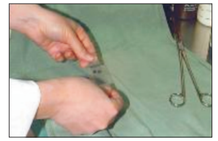
Controversy exists in the application of bilateral iliac biopsies. However, recent studies have indicated that this technique increases the probability of detecting focal lesions, such as in the case of carcinoma and lymphoma staging, where 11-16% of cases may be missed with unilateral biopsies. Wang et al reported an improvement in identifying bone malignancy in the following pathologic cases : Hodgkin disease by 19.5%, sarcomas by 14%, carcinomas by 11.5%, and non-Hodgkin lymphoma by 4.6%. Unilateral iliac sampling was considered sufficient in patients diagnosed with multiple myeloma, chronic myeloproliferative disorders, and myelodysplastic syndromes.

Postprocedure Care

After the procedure, firm pressure is applied for 5 minutes to several layers of sterile gauze placed over the wound site. Remove residual antiseptic to avoid further skin irritation by the solution. If hemorrhage from the wound persists, then place the patient in the supine position, with gauze over the wound site, so that consistent pressure can be applied for a minimum of 30 minutes. Rarely, bleeding may be present; if that is the case, consider placing a pressure dressing, again with the patient in a supine position, for an additional 1 hour. The patient is to be discharged with orders that the wound dressing is to be maintained in a dry state for 48 hours. The wound site is to be checked frequently, and if persistent bleeding or worsening pain occurs, these findings are to be reported to the clinician's office.

Slide Preparation

This stage in bone marrow preparation should be performed by trained personnel, such as a hematopathology technician. Thin-spread preparations of aspiration-collected samples, placed onto glass slides, can be prepared in numerous ways, all of which have the aim to retain and evaluate marrow particles. These spicules of fat droplets (not prominently seen in pediatric cases) and fragmented bone are likely to have adherent cellular material and thus be a target for morphologic evaluation. An aspirate smear is the most simplistic of the methods, similar in presentation as a peripheral blood smear. A drop of the acquired specimen is placed 1 cm from the edge that opposes the frosted "labeled" end and, with a second glass slide placed at a 30º angle, the sample is pushed toward the opposing side in one rapid smooth stroke. Excess sample can be removed by tilting the glass slide onto gauze or pipetting the extraneous fluid. Squash preparations are prepared on glass slides by placing marrow particles on a slide and pressing the particles with another slide. These preparations are used to better observe cellular interactions as the architecture of the marrow unit is preserved. The cover slip method produces samples that have been concentrated more than the squash preparation. The aspirate particles are selected from a petri dish and directly placed onto a glass cover slip. In a manner similar to the squash method, a second cover slip is gently applied to crush the sample. Each cover slip is then stained individually. Thus, enhanced removal of contaminating peripheral blood is performed, again with retention of the marrow unit architecture. At times, biopsy touch prints are useful, especially if the aspirate is dry and the only sample available is the bone marrow biopsy. In touch preparations, the hematopathology technician gently touches the tissue fragment onto a glass slide; this can provide morphologic details similar to that of an aspirate. Marrow particles can be collected in aggregate as a clot and processed in a similar manner to that of tissue. The solid component is concentrated by placing the specimen in a finely meshed bag that retains the tissue fragments, but which allows excess fluid to escape.



Bone marrow aspiration and biopsy slide preparation.

Bone marrow aspiration and biopsy slides before staining.

Standard stains used for the initial evaluation include Wright or May-Grunwald-Giemsa staining which enhance cytologic detail. Other special stains can be utilized for various purposes such as Prussian blue for iron in cases of suspected hemosiderosis or for the ringed sideroblasts of myelodysplastic syndromes. Myeloperoxidase, Sudan Black B, and leukocyte alkaline phosphatase are used in the categorization of acute myeloid leukemias. Periodic acid-Schiff (PAS) stain enhances depiction of cells that are implicated in glycogen storage diseases.

Morbidity/Mortality

In 2002 the British Society of Haematology initiated an annual survey to assess the various types and incidence of bone marrow biopsy adverse events. Bain summarized results of a 7-year (1995 to 2001) retrospective study and identified 26 adverse events among approximately 54,890 biopsies, with an overall annual incidence of 0.05%. The most common side effects in order of decreasing frequency were the following: hemorrhage, needle breakage, and infections. Risk factors for hemorrhage included concurrent anticoagulation therapy or underlying myeloproliferative/myelodysplastic syndrome, in which platelet function was affected. Two cases were fatal and were attributed to sepsis and massive hemorrhage. Four years later, a prospective study by Bain revealed 15 adverse events in a single year, with an overall incidence of 0.07%, not significantly different from the previous study's results. However, although hemorrhage was still considered the most commonly encountered side effect, this study revealed that pain, anaphylactic reaction, and fractures were prominent secondary consequences. Two fatality cases, attributed to laceration of blood vessels, were reported from 20,323 bone marrow aspiration and biopsy procedures.

Special Concerns

General anesthesia is required for pediatric cases, some sternal bone marrow sampling cases, and in those patients who are highly anxious. Sternal bone marrow aspiration has a higher risk of complications than other sites due to the delicate bone structure (approximately 1 cm thick in adults). Penetration of the underlying mediastinal organs can result in mediastinitis, pulmonary embolism, pneumothorax, cardiac tamponade, and cardiac tissue injury. For these reasons, biopsies are not to be performed from the sternum. Awareness of anatomic variations and pathologies that may affect bone density (eg, osteoporosis, multiple myeloma) can prevent further complications and injuries. Thrombocytopenia is not a contraindication to bone marrow aspiration and biopsy. Corrective action is required for coagulation disorders before bone marrow sampling. Application of sterile

techniques is required in the prevention of infections. Dry tap, or the lack of specimen obtainment during the aspiration sampling process, is most commonly due to technical problems such as misalignment of the needle. Other conditions that should be considered and may contribute to the decision of obtaining a biopsy are recent radiation therapy exposure, aplastic anemia, myelofibrosis, or bone infiltrative neoplasm. Knowing that tissue shrinkage can occur at an approximate rate of 25% after processing, the desired biopsy sampling size should initially be greater than 1.5 cm, preferably 2-3 cm in length (pediatric samples may be as small as 0.5 cm). Such a size will allow for the evaluation of 5 or 6 intertrabecular spaces, which is considered sufficient sampling for a diagnosis. Rarely, chronic pain may occur at the site of bone marrow sampling, thus requiring further clinical management.

Medical-Legal Pitfalls

Failure to prevent, recognize, or initiate rapid response to excessive bleeding or, rarely, to an anaphylaxis anesthetic event during the bone marrow sampling procedure . Failure to use proper safety techniques, such as having a guard device to prevent needle slippage, specifically during sternal aspiration. Failure to identify complications in sampling an iliac crest that results in penetration of the underlying gastrointestinal tract as well as blood vessels—the latter which runs the risk of the development of massive retroperitoneal hemorrhage and gluteal compartment syndrome.

BOUQUET

In Lighter Vein

---A plane is on its way to Toronto, when a blonde in economy class gets up, and moves to the first class section and sits down. The flight attendant watches her do this, and asks to see her ticket she then tells the blonde that she paid for economy class, and that she will have to sit in the back.
The blonde replies, "I'm blonde, I'm beautiful, I'm going to Toronto and I'm staying right here."
The flight attendant goes into the cockpit and tells the pilot and the co-pilot that there is a blonde bimbo sitting in first class, that belongs in economy, and won't move back to her seat.
The co-pilot goes back to the blonde and tries to explain that because she only paid for economy she will have to leave and return to her seat. The blonde replies, "I'm blonde, I'm beautiful, I'm going to Toronto and I'm staying right here."
The co-pilot tells the pilot that he probably should have the police waiting when they land to arrest this blonde woman who won't listen to reason.
The pilot says, "you say she is a blonde? I'll handle this, I'm married to a blonde. I speak blonde."
He goes back to the blonde and whispers in her ear, and she says, "oh, I'm sorry." and gets up and goes back to her seat in economy.
The flight attendant and co-pilot are amazed and asked him what he said to make her move without any fuss.
"I told her, "first class isn't going to Toronto".

--- THE PERFECT HUSBAND

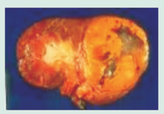
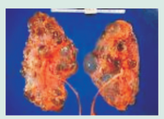
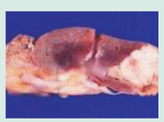
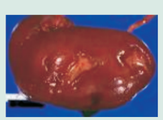
Several men are in the locker room of a golf club. A cellular phone on a bench rings and a man engages the hands-free speaker function and begins to talk. Everyone else in the room stops to listen.
MAN: "Hello"
WOMAN: "Hi Honey, it's me. Are you at the club?"
MAN: "Yes."
WOMAN: "I'm at the shops now and found this beautiful leather coat. It's only $2,000; is it OK if I buy it?"
MAN: "Sure, go ahead if you like it that much."
WOMAN: "I also stopped by the Lexus dealership and saw the new models. I saw one I really liked."
MAN: "How much?"
WOMAN: "$90,000." ;
MAN: "OK, but for that price I want it with all the options."
WOMAN: "Great! Oh, and one more thing... I was just talking to Janie and found out that the house I wanted last year is back on the market. They're asking $980,000 for it."
MAN: "Well, then go ahead and make an offer of $900,000. They'll probably take it. If not, we can go the extra eighty-thousand if it's what you really want."
WOMAN: "OK. I'll see you later! I love you so much!"
MAN: "Bye! I love you, too."
The man hangs up. The other men in the locker room are staring at him in astonishment, mouths wide open.
He turns and asks, "Anyone know who's phone this is?"

Wisdom Whispers

- When you were born, you were crying and everyone around you was smiling. Live your life so that when you die, You're the one who is smiling And everyone around you is crying.
- The happiest of people Don't necessarily have the best of everything; They just make the most of everything that comes along their way.
- It's true that we don't know What we've got until it's gone, But it's also true that we don't know What we've been missing until it arrives.
- The best kind of friends, Is the kind you can sit on a porch and swing with, Never say a word, And then walk away feeling like it was the best conversation you've ever had.

Brain Teasers

Match the following Gross Pictures with corresponding disease entities.

1. 
2. 
3. 
4. 

A. Polycystic kidney
B. Renal cell carcinoma
C. Renal infarct
D. Renal tuberculosis

Answers : 1. B, 2. A, 3. D, 4. C.

INTERPRETATION

INTERPRETING ANTIPHOSPHOLIPIDS ANTIBODIES AND MISCARRIAGES

The Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the clinical presence of thromboembolic disease or pregnancy complications (that include fetal loss, fetal growth restriction, preeclampsia) associated with demonstration of persistent antiphospholipid antibodies. APS is characterized by an autoimmune process that is separate in many women from systemic lupus erythromatosus (SLE) and other connective diseases. Lupus anticoagulants (LA) and Anticardiolipin antibodies (ACA) are clinically important antiphospholipid antibodies (APA) that belong to a heterogeneous group of antibodies directed against negatively charged phospholipids. LA and ACL predispose to clotting in vivo, predominantly by interfering with the antithrombotic role of platelets and therefore are associated with clinical thrombosis. LA and ACL are therefore considered to be important markers for pregnancy loss and intrauterine fetal demise. A greater pregnancy loss was observed in women who were repeatedly positive for LA. In a study by *Donald I Feinstein*, a history of pregnancy loss occurred in 24 of the 55 pregnancies in patients with persistent LA positivity compared with 8 of 67 pregnancies in LA negative pregnancies. Recently LA's and ACA's have been recognized as having a role in recurrent pregnancy loss even in women with no clinically diagnosed autoimmune disease. The diagnosis of APS is based on both clinical and laboratory criteria. The clinical criteria includes vascular thrombosis or pregnancy morbidity. The laboratory criteria includes a positive test for lupus anticoagulants, anticardiolipin antibodies, or anti-β-2-gycloprotein I antibodies on two or more occasions at least 12 weeks apart. Since treatment with anti-aggregants, high dose intravenous immunoglobulins and plasmapheresis can lead to successful pregnancies it would be wise to monitor closely cases with APA positivity.

Lupus anticoagulants:

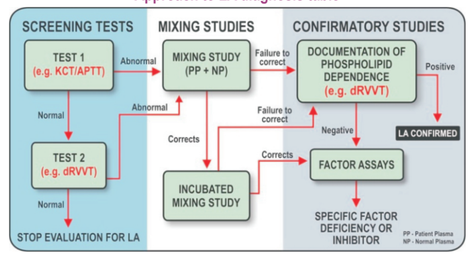
First described in 1952, the Lupus anticoagulant (LA) an immunoglobulin, is an immediate acting coagulation inhibitor which appears to be directed specifically against the phospholipids moiety of prothrombinase complex formed by interaction of factors Xa, Va, platelet phospholipids and calcium. LA binds to phospholipid active coagulation factors which slows down the rate of thrombin generation and therefore retards clot formation in vitro, but promotes both venous and arterial thrombosis in vivo. Its strong association with thromboembolic phenomenon, spontaneous miscarriage and stillbirth has been established in several studies. Several areas of concern have been identified in the diagnosis of LA including the pre-analytical variables, analytical variables, biologic homogeneity of LAs, and their relationship to other phospholipid dependant antibodies.

In its 1991 report The Lupus anticoagulant / Antiphospholipid subcommittee (Subcommittee) recommended four criteria for diagnosis of LA: Prolongation of phospholipid dependant clotting assay. Evidence if inhibition demonstrated by mixing studies. Evidence of phospholipid dependence. Lack of specific inhibition of any one coagulation factor.

The following recommendations and guidelines serves to be useful for LA diagnosis: Both patient and normal plasma should be as platelet-free as possible; the platelet count should be less than 10 X 10^9/L. Two or more tests should be used to screen for a LA before the diagnosis is excluded. At least one of these tests should be based on a low phospholipid concentration (eg. KCT, dRVVT). The assays should represent different assay principles; (for eg. APTT and dRVVT). Inhibitor activity should be documented by the effect of patient plasma on pooled normal plasma. This step can be incorporated into the initial screening procedure. A diagnosis of LA should not be made on the basis of multiple abnormal screening assays and mixing studies alone. Confirmatory studies need to be performed to document the phospholipid dependence of the inhibitor. Confirmatory assays must be based on the method giving an abnormal screening assay. For eg. If dRVVT (LA screen) is abnormal, then a confirmatory assay based on dRVVT (LA confirm) must be used. Routine clotting tests such as PT and APTT should be performed to evaluate the possibility of other coagulation disorders that may interfere in LA methodology. Solid phase assays for anti-phospholipid assays (Anticardiolipin antibodies), although frequently positive in patients with LA should not be considered as a confirmatory procedure for LA activity.

Based on these recommendations an approach to LA diagnosis has been charted as described below:

Approach to LA diagnosis table



Establishing a routine approach to LA diagnosis would lead to improved management of these patients. A number of integrated systems for diagnosis of LA's have been developed. These systems incorporate a screening assay, a mixing study and confirmatory procedure into a single battery. A simplified method for performing the DRVVT has been introduced. In this method the venom, calcium, heparin neutralizer and phospholipids are combined into a single reagent (DRVVT screen). The reagent has been further modified to show phospholipids dependence by increasing the phospholipids and modifying the factor X activator (DRVVT confirm). The baseline dRVVT can be performed on the patient plasma and/or 1:1 mixture of patient and normal plasma. If an abnormal time is obtained, the confirmatory assay can be performed. Wisloff and colleagues have introduced another integrated approach to the diagnosis of LAs. APTT's and dRVVTs are performed on normal plasma and a test mixture of patient and normal plasma in a ratio of 1:1 at low and high concentrations of phospholipids. The ratio of clotting times of the test mixture at low and high concentrations of phospholipid is then compared to the ratio of clotting times of the normal plasma at the low and high concentrations of phospholipids.

The Anticardiolipin Antibody

The ACA on the other hand are immunoglobulins (IgG, IgM and IgA class) directed against a complex consisting of β-2 gycloprotein I (β-2 GPI) bound to cardiolipin, an anionic phospholipid. Anticardiolipin antibodies were the first antiphospholipid antibodies identified by immunoassay. Originally the antigenic epitope on cardiolipin was thought to reside on cardiolipin itself, but it has been subsequently shown that the antigen detected by cardiolipin antibodies resides within a protein cardiolipin complex usually involving β-2 GPI as protein partner. Current immunoassays for ACA are therefore performed in the presence of β-2 GPI. The anticardiolipin antibodies are usually detected by solid phase immunoassays such as ELISA based methods and employ cardiolipin and β-2 GPI(as a cofactor) on solid phase.

Conclusion

Recurrent fetal loss is responsible in causing great physiological, mental and social agony to women. ACA and LA are the clinically important APAs contributing to this enigma. The simplicity of the anticardiolipin antibody test and the belief that it is always associated with LA has been responsible in reducing the cases of fetal loss to some extent. However several cases of recurrent miscarriages are associated with LA but not ACL and the complexity of LA detection has be a hurdle in reducing the pregnancy morbidity associated with APS. The availability of proper guidelines for diagnosis of LA and the improvement in the assays for its detection would definitely be responsible in fostering the diagnosis of lupus anticoagulants thereby alleviating the agony of miscarriages in women.