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The Crux

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CONTENTS

Editorial

Glomerulonephritis, also known as **glomerular nephritis**, is a term used to refer to several renal diseases (usually affecting both kidneys). Many of the diseases are characterised by inflammation either of the glomeruli or small blood vessels in the kidneys, hence the name, but not all diseases necessarily have an inflammatory component.

As it is not strictly a single disease, its presentation depends on the specific disease entity: it may present with isolated hematuria and/or proteinuria (blood or protein in the urine); or as a nephrotic syndrome, a nephritic syndrome, acute renal failure, or chronic renal failure.

They are categorized into several different pathological patterns, which are broadly grouped into non-proliferative or proliferative types. Diagnosing the pattern of GN is important because the outcome and treatment differs in different types. Primary causes are intrinsic to the kidney. Secondary causes are associated with certain infections (bacterial, viral or parasitic pathogens), drugs, systemic disorders (SLE, vasculitis), or diabetes.

Here in this issue, under the "DISEASE DIAGNOSIS" segment we present to you Acute Glomerulonephritis with all its clinico-diagnostic details. Often, if the acute phase can be taken care of, things can revert back to normal, as if nothing had happened!

Under the "INTERPRETATION" section, we submit the art and science of Cervical Cytology Assessment. All classifications, methodologies and diagnostic approaches as related to the ages of the patients are outlined in ample detail.

The most important histopathology sub branch currently is Immunohistochemistry. Often, it is fraught with false negative, false positive results or atypical results. "TROUBLE SHOOTING" portion simply tells you how to avoid the above-mentioned unwanted results.

We do not any longer have the space constraint, hence, we shall be providing exhaustive, full length coverage of the articles under consideration. Happy Reading !!!

DISEASE DIAGNOSIS

ACUTE GLOMERULONEPHRITIS.

Background

Bright initially described acute glomerulonephritis (GN) in 1927. Acute poststreptococcal glomerulonephritis (PSGN) is the archetype of acute GN. Acute nephritic syndrome is the most serious and potentially devastating form of the various renal syndromes. Acute GN comprises a specific set of renal diseases in which an immunologic mechanism triggers inflammation and proliferation of glomerular tissue that can result in damage to the basement membrane, mesangium, or capillary endothelium. Hippocrates originally described the manifestation of back pain and hematuria, which lead to oliguria or anuria. With the development of the microscope, Langhans was later able to describe these pathophysiologic glomerular changes. Acute GN is defined as the sudden onset of hematuria, proteinuria, and red blood cell (RBC) casts. This clinical picture is often accompanied by hypertension, edema, (ie, decreased glomerular filtration rate [GFR]), and renal salt and water retention. Acute GN can be due to a primary renal disease or to a systemic disease. Most original research focuses on acute PSGN. Treatment of PSGN is mainly supportive, because there is no specific therapy for renal disease. When acute GN is associated with chronic infections, the underlying infections must be treated.

Pathophysiology

Glomerular lesions in acute GN are the result of glomerular deposition or in situ formation of immune complexes. On gross appearance, the kidneys may be enlarged up to 50%. Histopathologic changes include swelling of the glomerular tufts and infiltration with polymorphonucleocytes. Immunofluorescence reveals deposition of immunoglobulins and complement. Except in PSGN, the exact triggers for the formation of the immune complexes are unclear. In PSGN, involvement of derivatives of streptococcal proteins has been reported. A streptococcal neuraminidase may alter host immunoglobulin G (IgG). IgG combines with host antibodies. IgG/anti-IgG immune complexes are formed and then collect in the glomeruli. In addition, elevations of antibody titers to other antigens, such as antistreptolysin O or antihyaluronidase, DNAase-B, and streptokinase, provide evidence of a recent streptococcal infection.

Structural and functional changes

Acute GN involves both structural changes and functional changes. Structurally, cellular proliferation leads to an increase in the number of cells in the glomerular tuft because of the proliferation of endothelial, mesangial, and epithelial cells. The proliferation may be endocapillary (ie, within the confines of the glomerular capillary tufts) or extracapillary (ie, in the Bowman space involving the epithelial cells). In extracapillary proliferation, proliferation of parietal epithelial cells leads to the formation of crescents, a feature characteristic of certain forms of rapidly progressive GN. Leukocyte proliferation is indicated by the presence of neutrophils and monocytes within the glomerular capillary lumen and often accompanies cellular proliferation. Glomerular basement membrane thickening appears as thickening of capillary walls on light microscopy. On electron microscopy, this may appear as the result of thickening of basement membrane proper (eg, diabetes) or deposition of electron-dense material, either on the endothelial or epithelial side of the basement membrane. Electron-dense deposits can be subendothelial, subepithelial, intramembranous, or mesangial, and they correspond to an area of immune complex deposition. Hyalinization or sclerosis indicates irreversible injury. These structural changes can be focal, diffuse or segmental, or global. Functional changes include proteinuria, hematuria, reduction in GFR (ie, oligoanuria), and active urine sediment with RBCs and RBC casts. The decreased GFR and avid distal nephron salt and water retention result in expansion of intravascular volume, edema, and, frequently, systemic hypertension. **Poststreptococcal glomerulonephritis:** Streptococcal M-protein was previously believed to be responsible for PSGN, but the studies on which this belief was based have been discounted. Nephritis-associated streptococcal cationic protease and its zymogen precursor (nephritis-associated plasmin receptor [NAPlr]) have been identified as a glyceraldehyde-3-phosphate dehydrogenase that functions as a plasmin(ogen) receptor. Immunofluorescence staining of the renal biopsy tissues with anti-NAPlr antibody revealed glomerular NAPlr deposition in early-phase acute PSGN, and glomerular plasmin activity was almost identical to NAPlr deposition in renal biopsy tissues of acute PSGN patients. These data suggest that NAPlr may contribute to the pathogenesis of acute PSGN by maintaining plasmin activity. Antibody levels to NAPR are elevated in streptococcal infections (of group A, C, and G) associated with GN but are not elevated in streptococcal infections without GN, whereas anti-streptolysin-O titers are elevated in both circumstances. These antibodies to NAPR persist for years and perhaps are protective against further episodes of PSGN. In a study in adults, the 2 most frequently identified infectious agents were streptococci (27.9%) and staphylococci (24.4%).

Etiology

The causal factors that underlie acute GN can be broadly divided into infectious and noninfectious groups. **Infectious:** The most common infectious cause of acute GN is infection by Streptococcus species (ie, group A, beta-hemolytic). Two types have been described, involving different serotypes: Serotype 12 - Poststreptococcal nephritis due to an upper respiratory infection, occurring primarily in the winter months. Serotype 49 - Poststreptococcal nephritis due to a skin infection, usually observed in the summer and fall and more prevalent in southern regions of the United States. PSGN usually develops 1-3 weeks after acute infection with specific nephritogenic strains of group A beta-hemolytic streptococcus. The incidence of GN is approximately 5-10% in persons with pharyngitis and 25% in those with skin infections. Nonstreptococcal postinfectious GN may also result from infection by other bacteria, viruses, parasites, or fungi. Bacteria besides group A streptococci that can cause acute GN include diplococci, other streptococci, staphylococci, and mycobacteria. Salmonella typhosa, Brucella suis, Treponema pallidum, Corynebacterium bovis, and actinobacilli have also been identified. Cytomegalovirus (CMV), coxsackievirus, Epstein-Barr virus (EBV), hepatitis B virus (HBV), rubella, rickettsiae (as in scrub typhus), and mumps virus are accepted as viral causes only if it can be documented that a recent group A beta-hemolytic streptococcal infection did not occur. Acute GN has been documented as a rare complication of hepatitis A. Attributing glomerulonephritis to a parasitic or fungal etiology requires the exclusion of a streptococcal infection. Identified organisms include Coccidioides immitis and the following parasites: Plasmodium malariae, Plasmodium falciparum, Schistosoma mansoni, Toxoplasma gondii, filariasis, trichinosis, and trypanosomes. **Noninfectious:**

Noninfectious causes of acute GN may be divided into primary renal diseases, systemic diseases, and miscellaneous conditions or agents. Multisystem systemic diseases that can cause acute GN include the following: Vasculitis (eg, Wegener granulomatosis) – This causes glomerulonephritis that combines upper and lower granulomatous nephritides). Collagen-vascular diseases (eg, systemic lupus erythematosus [SLE]) – This causes glomerulonephritis through renal deposition of immune complexes). Hypersensitivity vasculitis – This encompasses a heterogeneous group of disorders featuring small vessel and skin disease. Cryoglobulinemia – This causes abnormal quantities of cryoglobulin in plasma that result in repeated episodes of widespread purpura and cutaneous ulcerations upon crystallization. Polyarteritis nodosa – This causes nephritis from a vasculitis involving the renal arteries. Henoch-Schönlein purpura – This causes a generalized vasculitis resulting in glomerulonephritis. Goodpasture syndrome – This causes circulating antibodies to type IV collagen and often results in a rapidly progressive oliguric renal failure (weeks to months). Primary renal diseases that can cause acute GN include the following: Membranoproliferative glomerulonephritis (MPGN) - This is due to the expansion and proliferation of mesangial cells as a consequence of the deposition of complements. Type I refers to the granular deposition of C3; type II refers to an irregular process. Berger disease (IgG-immunoglobulin A [IgA] nephropathy) - This causes GN as a result of diffuse mesangial deposition of IgA and IgG. "Pure" mesangial proliferative GN. Idiopathic rapidly progressive glomerulonephritis - This form of GN is characterized by the presence of glomerular crescents. Three types have been distinguished: Type I is an antiglomerular basement membrane disease, type II is mediated by immune complexes, and type III is identified by antineutrophil cytoplasmic antibody (ANCA). Miscellaneous noninfectious causes of acute GN include the following: Guillain-Barré syndrome, Irradiation of Wilms tumor, Diphtheria-pertussis-tetanus (DPT) vaccine, Serum sickness, Epidermal growth factor receptor activation and possibly to its inhibitor cetuximab.

Epidemiology

International statistics: Worldwide, Berger disease is the most common cause of GN. With some exceptions, the incidence of PSGN has fallen in most Western countries. PSGN remains much more common in regions such as Africa, the Caribbean, India, Pakistan, Malaysia, Papua New Guinea, and South America. In Port Harcourt, Nigeria, the incidence of acute GN in children aged 3-16 years was 15.5 cases per year, with a male-to-female ratio of 1.1:1; the current incidence is not much different. Geographic and seasonal variations in the prevalence of PSGN are more marked for pharyngeally associated GN than for cutaneously associated disease. **Age-, sex-, and race-related demographics:** Postinfectious GN can occur at any age but usually develops in children. Most cases occur in patients aged 5-15 years; only 10% occur in patients older than 40 years. Outbreaks of PSGN are common in children aged 6-10 years. Acute nephritis may occur at any age, including infancy. Acute GN predominantly affects males (2:1 male-to-female ratio). Postinfectious GN has no predilection for any racial or ethnic group. A higher incidence (related to poor hygiene) may be observed in some socioeconomic groups. **Prognosis:** Most epidemic cases follow a course ending in complete patient recovery (as many as 100%). The mortality of acute GN in the most commonly affected age group, pediatric patients, has been reported at 0-7%. Sporadic cases of acute nephritis often progress to a chronic form. This progression occurs in as many as 30% of adult patients and 10% of pediatric patients. GN is the most common cause of chronic renal failure (25%). In PSGN, the long-term prognosis generally is good. More than 98% of individuals are asymptomatic after 5 years, with chronic renal failure reported 1-3% of the time. Within a week or so of onset, most patients with PSGN begin to experience spontaneous resolution of fluid retention and hypertension. C3 levels may normalize within 8 weeks after the first sign of PSGN. Proteinuria may persist for 6 months and microscopic hematuria for up to 1 year after onset of nephritis. Eventually, all urinary abnormalities should disappear, hypertension should subside, and renal function should return to normal. In adults with PSGN, full recovery of renal function can be expected in just over half of patients, and prognosis is dismal in patients with underlying diabetic glomerulosclerosis. Few patients with acute nephritis develop rapidly progressive renal failure. Approximately 15% of patients at 3 years and 2% of patients at 7-10 years may have persistent mild proteinuria. Long-term prognosis is not necessarily benign. Some patients may develop hypertension, proteinuria, and renal insufficiency as long as 10-40 years after the initial illness. Immunity to type M protein is type-specific, long-lasting, and protective. Repeated episodes of PSGN are therefore unusual. The prognosis for nonstreptococcal postinfectious GN depends on the underlying agent, which must be identified and addressed. Generally, the prognosis is worse in patients with heavy proteinuria, severe hypertension, and significant elevations of creatinine level. Nephritis associated with methicillin-resistant Staphylococcus aureus (MRSA) and chronic infections usually resolves after treatment of the infection. Other causes of acute GN have outcomes varying from complete recovery to complete renal failure. The prognosis depends on the underlying disease and the overall health of the patient. The occurrence of cardiopulmonary or neurologic complications worsens the prognosis. **Patient Education:** Counsel patients about the need for the following measures: Salt restriction during the acute phase to control edema and volume-related hypertension, Blood pressure monitoring at periodic intervals, Ongoing long-term monitoring of patients with persistent urinary abnormalities and elevated blood pressure, Consideration of protein restriction and angiotensin-converting enzyme (ACE) inhibitors (in patients who show evidence of persistent abnormalities or in those who develop late evidence of progressive disease), Early antibiotic treatment of close contacts.

History

A thorough history should be obtained, focusing on the identification of an underlying systemic disease (if any) or recent infection. Most often, the patient is a boy, aged 2-14 years, who suddenly develops puffiness of the eyelids and facial edema in the setting of a poststreptococcal infection. The urine is dark and scanty, and the blood pressure may be elevated. Nonspecific symptoms include weakness, fever, abdominal pain, and malaise. Ask the patient about the onset and duration of the illness. Symptom onset is usually abrupt. In the setting of acute postinfectious glomerulonephritis (GN), a latent period of up to 3 weeks occurs before onset of symptoms. However, the latent period may vary; it is typically 1-2 weeks for postpharyngitis cases and 2-4 weeks for cases of postdermal infection (ie, pyoderma). The onset of nephritis within 1-4 days of streptococcal infection suggests preexisting renal disease. Identify a possible etiologic agent (eg, streptococcal throat infection [pharyngitis], skin infection [pyoderma]). Recent fever, sore throat, joint

pains, hepatitis, travel, valve replacement, and/or intravenous drug use may be causative factors. Rheumatic fever rarely coexists with acute PSGN. Assess the consequences of the disease process (eg, uremic symptoms). Inquire about loss of appetite, generalized itching, tiredness, listlessness, nausea, easy bruising, nosebleeds, facial swelling, leg edema, and shortness of breath. Inquire about symptoms of acute glomerulonephritis, including the following: Hematuria - This is a universal finding, even if it is microscopic. Gross hematuria is reported in 30% of pediatric patients, often manifesting as smoky-, coffee-, or cola-colored urine. Oliguria. Edema (peripheral or periorbital) - This is reported in approximately 85% of pediatric patients; edema may be mild (involving only the face) to severe, bordering on a nephrotic appearance. Headache - This may occur secondary to hypertension; confusion secondary to malignant hypertension may be seen in as many as 5% of patients. Shortness of breath or dyspnea on exertion - This may occur secondary to heart failure or pulmonary edema; it is usually uncommon, particularly in children. Possible flank pain secondary to stretching of the renal capsule. Ask about symptoms specific to an underlying systemic disease that can precipitate acute GN (see Etiology). Classic presentations include the following: Triad of sinusitis, pulmonary infiltrates, and nephritis, suggesting Wegener granulomatosis, Nausea and vomiting, abdominal pain, and purpura, observed with Henoch-Schönlein purpura, Arthralgias, associated with systemic lupus erythematosus (SLE), Hemoptysis, occurring with Goodpasture syndrome or idiopathic progressive glomerulonephritis, Skin rashes, observed with hypersensitivity vasculitis or SLE; also possibly due to the purpura that can occur in hypersensitivity vasculitis, cryoglobulinemia, and Henoch-Schönlein purpura.

Physical Examination

The following description does not address all of the physical findings that can be associated with the nonnephrotic features of an infectious process, renal disorder, or systemic disease that causes acute GN; to do so would be beyond the scope of this article. Patients often have a normal physical examination and blood pressure; most frequently, however, patients present with a combination of edema, hypertension, and oliguria. The physician should look for the following signs of fluid overload: Periorbital and/or pedal edema, Edema and hypertension due to fluid overload (in 75% of patients), Crackles (ie, if pulmonary edema), Elevated jugular venous pressure, Ascites and pleural effusion (possible). The physician should also look for the following: Rash (as with vasculitis, Henoch-Schönlein purpura, or lupus nephritis), Pallor, Renal angle (ie, costovertebral) fullness or tenderness, joint swelling, or tenderness, Hematuria, either macroscopic (gross) or microscopic, Abnormal neurologic examination or altered level of consciousness (from malignant hypertension or hypertensive encephalopathy), Arthritis. Other signs include the following: Pharyngitis, Impetigo, Respiratory infection, Pulmonary hemorrhage, Heart murmur (possibly indicative of endocarditis), Scarlet fever, Weight gain, Abdominal pain, Anorexia, Back pain, Oral ulcers.

Complications

Progression to sclerosis is rare in the typical patient; however, in 0.5-2% of patients with acute GN, the course progresses toward renal failure, resulting in kidney death in a short period. Abnormal urinalysis (ie, microhematuria) may persist for years. A marked decline in the glomerular filtration rate (GFR) is rare. Pulmonary edema and hypertension may develop. Generalized anasarca and hypoalbuminemia may develop secondary to severe proteinuria. A number of complications that result in relevant end-organ damage in the central nervous system (CNS) or the cardiopulmonary system can develop in patients who present with severe hypertension, encephalopathy, and pulmonary edema. Those complications include the following: Hypertensive retinopathy, Hypertensive encephalopathy, Rapidly progressive GN, Chronic renal failure, Nephrotic syndrome.

Diagnostic Considerations

The following 4 renal syndromes commonly mimic the early stage of acute glomerulonephritis (GN): Anaphylactoid purpura with nephritis, Chronic GN with an acute exacerbation, Idiopathic hematuria, Familial nephritis.

Postinfectious GN must be differentiated from the following conditions: Immunoglobulin A (IgA) nephritis - The latent period between infection and onset of nephritis is 1-2 days; alternatively, nephritis may be concomitant with upper respiratory tract infection (ie, "synpharyngitic nephritis," in contrast to the "postpharyngitic nephritis" seen in poststreptococcal GN [PSGN], which occurs 1-3 weeks later). Membranoproliferative GN (MPGN), types I and II - This is a chronic disease, but it can manifest with an acute nephritic picture with hypocomplementemia; failure of acute nephritis to resolve should prompt consideration of this possibility. Lupus nephritis - Gross hematuria is unusual in lupus nephritis. GN of chronic infection - This can manifest as acute nephritis. Unlike PSGN, in which the infection may have resolved by the time nephritis occurs, patients with nephritis of chronic infection have an active infection at the time nephritis becomes evident. Circulating immune complexes play an important role in the pathogenesis of acute GN in these diseases. Vasculitis - Nephritis of methicillin-resistant S aureus (MRSA) may be associated with vasculitic lesions of the lower extremities. Predominantly nonglomerular diseases- Thrombotic thrombocytopenic purpura (TTP), hemolytic-uremic syndrome (HUS), atheroembolic renal disease, and acute hypersensitivity interstitial nephritis may present with features of acute nephritic syndrome and should be differentiated.

Go to Emergent Management of Acute Glomerulonephritis and Acute Poststreptococcal Glomerulonephritis for complete information on these topics. Other problems to be considered include the following: Bacterial, viral, and fungal etiologies, Chronic GN, Idiopathic hematuria, IgA nephropathy, Irradiation of Wilms tumor, Trauma.

Differential Diagnoses

Acute Renal Failure, Amyloidosis, Familial Renal, Angioedema, Ascites, Cirrhosis, Diabetes Mellitus, Type 2, Glomerulonephritis, Crescentic, Glomerulonephritis, Diffuse Proliferative, Glomerulonephritis, Membranoproliferative, Glomerulonephritis, Poststreptococcal, Glomerulonephritis, Rapidly Progressive, Goodpasture Syndrome, Guillain-Barré Syndrome, Heart Failure, Hemolytic-Uremic Syndrome, Hypertensive Emergencies, Impetigo, Necrotizing Fasciitis, Nephritis, Interstitial, Nephritis, Lupus, Pediatrics, Fever, Pediatrics, Pharyngitis, Pharyngitis, Rheumatic Fever, Scarlet Fever, Serum Sickness, Systemic Lupus Erythematosus, Thrombocytopenic Purpura, Transplants, Renal.

Approach Considerations

Urinalysis and sediment examination are crucial in the evaluation of patients with acute nephritic syndrome. Look for protein, blood, red blood cells (RBCs), white blood cells (WBCs), dysmorphic RBCs, acanthocytes, cellular (ie, RBC, WBC) casts, granular casts, and oval fat

bodies. In some instances, marked sterile pyuria is present. The presence of RBC casts is almost pathognomonic of glomerulonephritis (GN). Urine electrolyte, urine sodium, and fractional excretion of sodium (FENa) assays are needed to assess salt avidity. Other tests should include the following: Complete blood count (CBC), Blood urea nitrogen (BUN), serum creatinine, and serum electrolytes (especially serum potassium), Erythrocyte sedimentation rate (ESR), Complement levels (C3, C4, CH50). Streptozyme testing may be useful. Imaging studies are helpful in some patients.

Initial Blood Tests: A CBC is performed. A decrease in the hematocrit may demonstrate a dilutional anemia. In the setting of an infectious etiology, pleocytosis may be evident. Electrolyte levels are measured (particularly the serum potassium), along with BUN and creatinine (to allow estimation of the glomerular filtration rate [GFR]). The BUN and creatinine levels will exhibit a degree of renal compromise. The ESR is usually increased.

Complement Levels: Differentiation of low and normal serum complement levels may allow the physician to narrow the differential diagnosis. Results are not readily available to the emergency physician but may be useful to the consultant. Low serum complement levels suggest the following systemic diseases: cryoglobulinemia, systemic lupus erythematosus (SLE), bacterial endocarditis, and shunt nephritis. Under the same conditions, renal diseases characteristic of membranoproliferative GN (MPGN) or poststreptococcal GN (PSGN) also may be considered. Normal serum complement levels suggest a visceral abscess, polyarteritis nodosa, Goodpasture syndrome, or Henoch-Schönlein purpura. In addition, normal complement levels suggest renal diseases such as immune complex disease, idiopathic rapidly progressive GN, and immunoglobulin G (IgG) or immunoglobulin A (IgA) nephropathy. Low C3 levels are found in almost all patients with acute poststreptococcal nephritis; C4 levels may be slightly low. Hypocomplementemia is noted in 73.9% of adult patients. Type III cryoglobulinemia may be present. If methicillin-resistant S aureus (MRSA) is the inciting agent, then hypocomplementemia is usually not present, but plasma immunoglobulins, especially IgA, are markedly elevated.

Urinalysis and 24-Hour Urine Study: The urine is dark. Its specific gravity is greater than 1020. RBCs and RBC casts are present. Proteinuria is observed. With the qualitative estimation of proteinuria, determination of high-molecular-weight (HMW) protein (eg, fractional excretion of IgG [FEIgG]) and low-molecular-weight (LMW) protein (eg, alpha-1-microglobulin), may help predict the clinical outcome and may help in guiding steroid and immunosuppressive therapy, especially in patients with primary glomerular diseases with nephrotic syndrome. The 24-hour urine protein excretion and creatinine clearance, though not indicated in the emergency department (ED) setting, may be helpful to document the degree of renal dysfunction and proteinuria. With this test, it is important to remember that creatinine clearance is a "steady-state" measurement. Because of rapidly changing renal function, the creatinine clearance may not reveal the true picture; therefore, it is better to wait until renal function has stabilized before performing creatinine clearance.

Streptozyme Test: The streptozyme tests test includes many streptococcal antigens that are sensitive for screening but are not quantitative, such as DNase, streptokinase, streptolysin O, and hyaluronidase. The antistreptolysin O (ASO) titer is increased in 60-80% of patients. The increase begins in 1-3 weeks, peaks in 3-5 weeks, and returns to normal in 6 months. ASO titer is unrelated to severity, duration, or prognosis of renal disease. With ASO titer or streptozyme titer, increasing titers confirm recent infection. In patients with skin infection, anti-DNase B (ADB) titers are more sensitive than ASO titers for infection with Streptococcus.

Blood and Tissue Culture: Blood culture is indicated in patients with fever, immunosuppression, intravenous (IV) drug use history, indwelling shunts, or catheters. Blood culture may indicate hypertriglyceridemia, decreased glomerular filtration rate, or anemia. Cultures of throat and skin lesions to rule out Streptococcus species may be obtained.

Other Laboratory Tests: With the antibody to nephritis-associated protease (NAPR), levels are elevated in streptococcal infections with GN but not in streptococcal infections without GN. The antinuclear antibody test is useful for patients with acute GN and symptoms of underlying systemic illness, such as systemic lupus erythematosus and polyarteritis nodosa. Other tests include anti-DNA antibodies, triglyceride levels, hepatitis B and C serologies, antineutrophil cytoplasmic antibody (ANCA), c-ANCA (ie, if Wegener granulomatosis is suspected).

Radiography and Computed Tomography: Chest radiography is needed in patients with a cough, with or without hemoptysis (eg, Wegener granulomatosis, Goodpasture syndrome, pulmonary congestion). Abdominal radiographic imaging (ie, computed tomography [CT]) is needed if visceral abscesses are suspected; also look for chest abscesses. CT scan of the head without contrast may be necessary in any patient with malignant hypertension or altered mental status.

Ultrasonography and Echocardiography

Bedside renal ultrasonography may be appropriate to evaluate kidney size, as well as to assess the echogenicity of the renal cortex, exclude obstruction, and determine the extent of fibrosis. A kidney size of less than 9 cm is suggestive of extensive scarring and a low likelihood of reversibility. Echocardiography may be performed in patients with a new cardiac murmur or a positive blood culture to rule out endocarditis or a pericardial effusion.

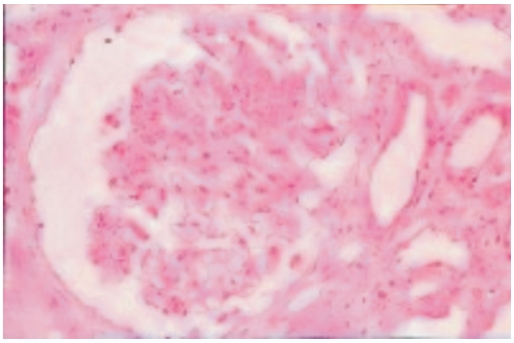
Renal Biopsy

Generally, a renal biopsy is not necessary for a diagnosis of acute PSGN; however, in most cases, it is important because histology guides both prognosis and therapy. Renal biopsy may be required for definitive diagnosis, particularly in primary renal diseases. Renal biopsy is not indicated as an ED procedure.

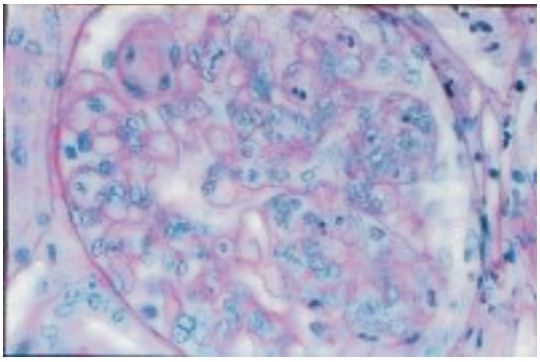
Candidates for biopsy are patients with an individual or family history of renal disease and patients with an atypical presentation, including massive proteinuria, nephrotic syndrome, or a rapid rise in creatinine level without resolution. In a study of the types and characteristics of GN found in patients with HIV infection, Nebuloni et al reviewed 73 renal biopsies and found that immune complex GNs predominated in the biopsied patients (40 cases), with mesangial proliferative and MPGN being the most common of these (10 and 8 cases, respectively). The authors also reported unusual characteristics in the immune complex GNs, including multiple-site deposits and frequent sclerotic tendencies.

Histologic Findings

Diffuse endocapillary proliferative changes are found. The most common histologic patterns are diffuse (72.1%), focal (12.8%), and mesangial (8.1%) proliferative GN in adults. In postinfectious GN, the glomerulus is hypercellular with marked cellular infiltration (ie, polymorphonuclear neutrophils, monocytes) (see the images).

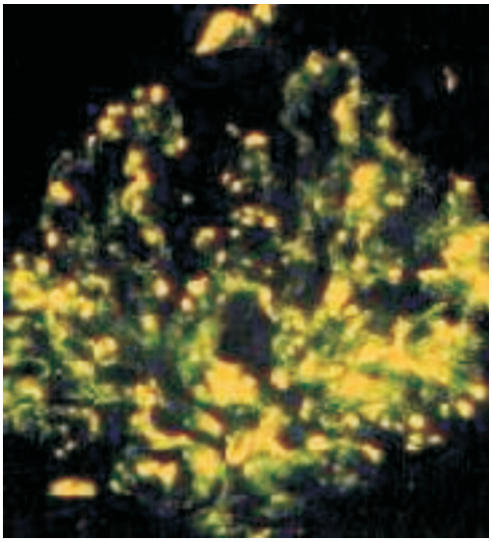


Light microscopy (hematoxylin and eosin stain X 25): Photograph showing enlargement of glomerular tuft with marked decrease of urinary space and hypercellularity. The hypercellularity is due to proliferation of endogenous cells and polymorphonuclear leukocyte infiltrate.

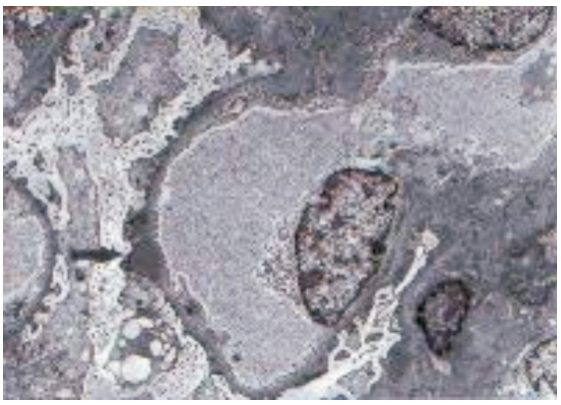


Light microscopy (periodic acid-Schiff stain X 40): Photograph showing enlargement of glomerular tuft with marked decrease of urinary space and hypercellularity. The hypercellularity is due to proliferation of endogenous cells and polymorphonuclear leukocyte infiltrate.

Immunofluorescence may show fine, granular deposits of IgG in a “starry sky” appearance (see the first image below). Large subepithelial deposits may be observed on electron microscopy (see the second image below). Crescents may be observed.



Immunofluorescence (X25): Fine granular deposits of immunoglobulin G (IgG) along the basement membrane and mesangium, with starry sky" appearance.



" Ultrastructure (electron microscopy): Photograph showing proliferation of endothelial cells and mesangial cells and leukocyte infiltrate associated with presence of large, subepithelial, electron-dense deposits (ie, "hump") (see arrow).

Approach Considerations

Treatment of acute poststreptococcal glomerulonephritis (PSGN) is mainly supportive, because there is no specific therapy for renal disease. When acute glomerulonephritis (GN) is associated with chronic infections, the underlying infections must be treated. The expertise available in the ICU may be needed for management of patients with hypertensive encephalopathy or pulmonary edema. Consultation with a nephrologist may be indicated. On an outpatient basis, renal function, blood pressure, edema, serum albumin, and urine protein excretion rate should be monitored.

Antibiotics: Antibiotics (eg, penicillin) are used to control local symptoms and to prevent spread of infection to close contacts. Antimicrobial therapy does not appear to prevent the development of GN, except if given within the first 36 hours. Antibiotic treatment of close contacts of the index case may help prevent development of PSGN.

Other agents: Loop diuretics may be required in patients who are edematous and hypertensive in order to remove excess fluid and to correct hypertension. Vasodilator drugs (eg, nitroprusside, nifedipine, hydralazine, diazoxide) may be used if severe hypertension or encephalopathy is present. Glucocorticoids and cytotoxic agents are of no value, except in severe cases of PSGN.

Diet and Activity

Sodium and fluid restriction should be advised for treatment of signs and symptoms of fluid retention (eg, edema, pulmonary edema). Protein restriction for patients with azotemia should be advised if there is no evidence of malnutrition. Bed rest is recommended until signs of glomerular inflammation and circulatory congestion subside. Prolonged inactivity is of no benefit in the patient recovery process.

Long-Term Monitoring: Long-term studies on children with PSGN have revealed few chronic sequelae. Results of such studies are controversial because homogenous populations suitable for proper epidemiologic analysis have not been assembled. Long-term studies show higher mortality rates in elderly patients, particularly those on dialysis. Patients may be predisposed to crescent formation.

Medication Summary: The goals of pharmacotherapy are to reduce morbidity, to prevent complications, and to eradicate the infection. Agents used include antibiotics, loop diuretics, vasodilators, and calcium channel blockers.

INTERPRETATION

CERVICAL CYTOLOGY - PAP'S SMEAR

Overview

Worldwide, approximately 500,000 new cases of cervical cancer and 274,000 deaths are attributable to cervical cancer yearly, making cervical cancer the second most common cause of death from cancer in women. Fortunately, the incidence of cervical cancer has decreased by more than 50% in the past 30 years, largely due to the increasing use of cervical cancer screening with cervical cytology. Although worldwide cervical cancer rates have decreased dramatically with the increase in screening efforts, incidence and prevalence in developing countries remains high due to lack of screening programs, with approximately 80% of all cervical cancer deaths occurring in the developing world. The mainstay of cervical cancer screening for the last 60 years has been the Papanicolaou test. The Papanicolaou test, also known as the *Pap test* or the *Pap smear*, was developed in the 1940s by Georgios Papanikolaou. It involves exfoliating cells from the transformation zone of the cervix to enable examination of these cells microscopically for detection of cancerous or precancerous lesions. In one newer technique, liquid-based cytology, these cells are released into a vial of liquid preservative that is then used in the cytology lab to produce a slide for microscopic evaluation of the cells. The older, traditional Pap technique involves direct transfer of the cervical cells to a microscope slide for evaluation. Although the traditional method may introduce confounders such as blood and other debris to the slide, which may make interpretation more difficult, both conventional cytology and liquid-based cytology have been shown to have similar sensitivity and specificity for moderate dysplasia or worse lesions when using a threshold of LSIL or higher. In addition, both types of cytological screening are considered acceptable by the American College of Obstetricians and Gynecologists. When abnormal cells are detected on the Pap Test, diagnostic testing in the form of colposcopy is often indicated. This testing may be followed by diagnosis of dysplasia via colposcopic biopsies. Subsequent cervical cancer may be prevented through the diagnosis and treatment of these cervical cancer precursors. Evidence shows that approximately 99-100% of cervical cancers are attributable to infection by high-risk types of the human papillomavirus (HPV). HPV represents a family of double-stranded, circular DNA viruses that can infect skin or mucosal cells, including the anogenital region and the oral cavity, and may be transmitted easily via sexual intercourse or direct contact. More than 100 types of HPV exist, 12 of which can involve the anogenital region and are considered "high risk" or oncogenic in nature. These include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Of these, HPV 16 is responsible for the largest number of CIN 3 and cervical cancer cases. Although HPV is a necessary factor in the development of cervical dysplasia that can eventually lead to cervical cancer, most women infected with HPV will not develop cervical dysplasia. The presence of high-risk HPV DNA is accompanied by cytologic abnormalities approximately one third of the time. Whether an HPV infection will progress relates to the persistence of the infection and also possibly to the immune response and smoking status of the woman.

Indications

The Pap test is indicated for screening for malignant and premalignant lesions of the cervix. The recommended age at initiation of cervical cancer screening has undergone significant revision over time as the natural history of HPV infection and subsequent cervical dysplasia has been elucidated. Although former guidelines recommended starting Pap smear screening at age 18 or the onset of sexual activity, these guidelines were revised in 2006 to recommend initiation 3 years after the onset of sexual activity or age 21, whichever comes first. In 2009, these were further revised to recommend that cervical cancer screening begin at age 21, regardless of sexual history. Abnormal cervical cytology is very common in young women, and most abnormal cytology resolves without treatment in adolescents. In addition, women under the age of 21 account for only 0.1% of all cervical cancers, and no evidence exists that cervical cancer screening in this age group reduces cervical cancer incidence, morbidity, or mortality. Recognizing these facts and the likelihood of cervical cancer screening leading to unnecessary and potentially harmful evaluation and treatment in women at very low risk for malignancy, the 2009 ACOG guideline revision recommended cervical cancer screening beginning at age 21 years of age, regardless of sexual history. Related international bodies agreed with the following recommendations and issued age-appropriate screening strategies for cytology (Pap tests) and HPV testing for cervical cancer screening. (see Table).

Table. Summary of 2012 Screening Guidelines from the American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology (Open Table in a new window)

Parameters	ACS Recommendations
Age to start screening	Begin screening with cytology at 21 years old, regardless of sexual history
Screening interval age 21–29	Screen with cytology alone every 3 years.* HPV testing should not be used for screening in this age group.
Screening interval age 30-65	Screen with a combination of cytology and HPV testing every 5 years (preferred) or cytology alone every 3 years. Screening by HPV testing alone is generally not recommended.*
Age to stop screening	Age 65, if the woman has adequate negative prior screening and is not otherwise at high risk for cervical cancer
Screening after hysterectomy	Not indicated for women without a cervix and without a history of a high-grade precancerous lesion (eg, CIN2 or CIN3) in the past 20 years or cervical cancer ever
HPV-vaccinated women	Screen according to the same recommendations as for unvaccinated women

These guidelines do not address special populations (eg, women with a history of cervical cancer, women who were exposed in utero to diethylstilbestrol, women who are immunocompromised) who may require more intensive or alternative screening.

*If abnormal test results are present, further testing and management should be performed according to the ASCCP Guidelines as noted below under Management of Abnormal Cytology.

The guidelines do not address special populations (eg, women with a history of cervical cancer, women who were exposed in utero to diethylstilbestrol (DES), women who are immunocompromised) who

may require more intensive or alternative screening. ACOG recommends annual screenings for immunocompromised patients; women with history of CIN2, CIN3, or cancer; and women who were exposed to DES in utero. HIV-positive women should be screened twice in the first year following diagnosis and yearly thereafter. Screening should continue for at least 20 years after treatment of CIN2 or more. Testing for the high-risk HPV types, often referred to as HPV DNA testing, has become increasingly used in cervical cancer screening and may be performed from any liquid-based cytology specimen. Currently, HPV DNA testing by the Hybrid Capture 2 HPV DNA Assay is approved by the FDA for reflex testing with ASC-US cytology and for co-testing with the Pap test in women age 30 years or older. Type-specific testing for HPV 16/18 (Cervista HPV HR), also known as HPV genotyping, is also FDA approved for the same indications; however, it is only recommended by the ASCCP for triage of women age 30 years and older who have negative cytology and are HPV DNA positive. Note that HPV DNA testing for the purpose of cervical cancer screening is NOT indicated in women younger than 30 due to the high prevalence of HPV infections in this age group as well as the transient nature of these infections. HPV DNA testing is also NOT recommended in women younger than 21 for any cytology result (and if performed should be ignored). In addition, testing for low-risk HPV types is never appropriate and should not be performed under any circumstances. Reviews conducted for the US Preventive Services Task Force support these indications. Studies havel confirmed the low incidence of cervical cancer among women younger than 20 years and underscored the difficulties of detection and the high frequency of false-positives in this age group. Further, incidence of and mortality rates from cervical cancer in women aged 65 years and older who have had a Pap smear within 3 years have decreased since 2000, and available evidence reinforces discontinuation of cervical cancer screening among these women who have had satisfactory screening and are not otherwise at high risk. In a systematic review, Whitlock et al found evidence supporting the use of liquid-based or conventional cytology for cervical cancer screening, but cautioned that more evidence is needed before adopting HPV-enhanced primary screening for women aged 30 years and older.

Preparation

Ideally, cervical screening should be scheduled when the patient is not menstruating. Avoid vaginal intercourse, douching, use of tampons, use of medicinal vaginal cream or contraceptive cream for 24-48 hrs prior to cervical screening. Ideally, pre-existing cervicitis should be treated prior to cervical screening. Screening, however, should proceed in the presence of bleeding or cervicitis, as these symptoms may be related to cervical dysplasia or neoplasm, which may be detected with cervical screening.

Equipment: Examination table with foot supports, Examination light, Metal or plastic speculum, Examination gloves, Cervical spatula and cytobrush, Liquid-based cytology container or glass slide and fixative.

Positioning: The patient should be supine, in dorsal lithotomy position to correctly perform a pap smear (see the image below). The coccyx of the patient must be at the edge of the examination table to provide adequate visualization of the cervix once the speculum is inserted.

Positioning and procedure.

CONVENTIONAL PAP SMEAR

Traditional Pap smear

Test Information

Conventional Pap smears are prepared by the provider by smearing the collection device on a glass slide labeled with the patient's name. Good collection techniques and immediately fixation are important in providing good quality specimens for evaluation by the laboratory. Some causes of less then optimal and/or unsatisfactory specimens are thick smears, excessive mucus, blood, inflammation, poor fixation, or improper use of lubricant.

Precaution: If you use lubricant, do not use an excessive amount. Avoid contaminating the sample site.

Material Required:

1. Glass Slides
2. Spray Fixative
3. Collection Device(s)
   Endocervical Brush & Plastic Spatula (preferred method)
   Broom (alternate method)

Method:

Conventional: Brush/Spatula Method (preferred)

1. Label end of a glass slide with patient's name.
2. Scrape whole circumference of the ectocervix with a plastic spatula.
3. Spread the material quickly and evenly onto the glass slide.
4. Fix immediately with spray-fixative.
5. Obtain an adequate sampling from the endocervix using an endocervical brush.
   Insert the brush into the cervix until only the bottom-most fibers are exposed.
   Slowly rotate ¼ or ½ turn in one direction – do not over-rotate.
6. Gently roll the brush across the slide (do not rub the brush back and forth).
7. Fix immediately with spray-fixative.

Broom Method

1. Label end of a glass slide with patient's name.
2. Obtain and adequate sampling from the cervix using a broom-like device.
   Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix.
   Push gently, and rotate the broom in a clockwise direction 5 times.
3. Transfer the sample to the glass slide with a single paint-stroke action.
   Apply first one side of the bristles.
   Turn brush over.
   Then paint the slide again in exactly the same area.
4. Fix immediately with spray-fixative.

Record Patient Information

1. Record patient's identification on the requisition card.
   A minimum of two identifiers are needed – pre-printed patient labels are preferred.
   Insure patient identification on glass slide matches form.
   For details on filling out requisition cards see "Instructions for Completing GYN Requisition Forms."

2. Place glass slide in slide holder.
3. Place slide holder containing slide and requisition card into a transport bag for delivery to the laboratory.

Reasons for Rejection:

1. Unlabeled or mislabeled glass slide.
2. Discrepancies between glass slide and requisition.
3. Specimen sent by unauthorized person.
4. Glass slide broken beyond repair.
5. Contaminated requisition (e.g. blood stained).

THINPREP PAP TEST

Synonyms: Liquid-based Pap test

Test Information:

The ThinPrep Pap test is a liquid-based test as opposed to a conventional Pap smear. The ThinPrep Pap test has a number of advantages over a smear. One of these advantages is that certain other tests (i.e. HPV, Chlamydia and Gonorrhea) can be run from the same specimen. Following proper collection procedures will help to ensure that an adequate specimen is collected for interpretation by the laboratory.

Precautions

1. For best results we recommend using the two-step brush and plastic spatula combination rather than the one-step broom device.
2. If you need to use lubricant, do not use any with ingredients that contain "carbomers" or "carbopol polymers." These products will interfere with processing and potential result in an unsatisfactory result.

Materials Required

1. ThinPrep Pap Collection Vial
2. Collection Devise(s)

   Endocervical Brush & Plastic Spatula (preferred method)

   Broom (alternate method)

Method

Brush/Spatula Method (preferred)

1. Obtain an adequate sampling from the ectocervix using a plastic spatula.
2. Rinse the spatula as quickly as possible into the preservative filled ThinPrep vial.

   Swirl the spatula vigorously in the vial 10 times.

   Discard the spatula.
3. Obtain an adequate sampling from the endocervix using an endocervical brush.

   Insert the brush into the cervix until only the bottom-most fibers are exposed.

   Slowly rotate ¼ or ½ turn in one direction – do not over-rotate.
4. Rinse the brush as quickly as possible in the preservative filled ThinPrep vial.

   Rotate the device 10 times while pushing against the vial wall.

   Swirl the brush vigorously to further release material.

   Discard the brush.
5. Tighten the cap so that the black line on the cap passes the black line on the vial.

Broom Method (alternate)

1. Obtain and adequate sampling from the cervix using a broom-like device.

   Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix.

   Push gently, and rotate the broom in a clockwise direction 5 times.
2. Rinse the broom as quickly as possible into the preservative filled ThinPrep vial.

   Push the broom into the bottom of the vial 10 times, forcing the bristles apart.

   Swirl the broom vigorously to further release material.

   Discard the broom.
3. Tighten the cap so that the black line on the cap passes the black line on the vial.

Record Patient Information

1. Record patient's identification on the ThinPrep vial and the requisition form.

   A minimum of two identifiers are needed – pre-printed patient labels are preferred.

   Insure patient identification on vial matches form.

   For details on filling out requisition cards see "Instructions for Completing GYN Requisition Forms."
2. Place ThinPrep vial and requisition card into a transport bag for delivery to the laboratory.

Reason for Rejection:

1. Unlabeled or mislabeled vial.
2. Discrepancies between vial and requisition.
3. Specimen sent by unauthorized person.
4. Empty vial (e.g. leaky vial).
5. Contaminated requisition (e.g. from leaky vial).

Complications

Complications are extraordinarily rare and include minor bleeding and infection. The patient must be educated on the likelihood of vaginal spotting immediately after a pap smear is performed, as this is considered normal.

Limitations

Although the Pap smear is one of the best screening tests in medicine and its implementation has decreased the incidence of cervical cancer by over 50%, it does have its limitations. First, the sensitivity of one Pap smear for cervical dysplasia ranges from 30-87%[21] (with the average approximately 58%). In addition, the intraobserver and interobserver reproducibility is poor and ranges from approximately 43-68% at best. Nearly half of all new cervical cancers are found in women who have never had cervical cytology screening prior to diagnosis. Unfortunately, however, false-negative Pap smears are associated with up to 30% of all new cervical cancer diagnoses. HPV DNA testing has improved sensitivity over cervical cytology but lower specificity. For women age 30 years and older, the sensitivity and specificity of the HPV DNA test for detecting CIN 2 or worse is roughly 95% and 87%, respectively.

Interpreting Cytology Results

Results from cervical cytology specimens are reported according to the 2001 Bethesda System Classification, as listed below

Negative for intraepithelial lesion or malignancy

Epithelial cell abnormality

Squamous cell

- Atypical squamous cells (ASC) of undetermined significance (ASC-US) or atypical squamous cells that cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesions (LSIL), includes human papillomavirus (HPV), mild dysplasia, and CIN 1
- High-grade squamous intraepithelial lesions (HSIL), includes moderate to severe dysplasia, carcinoma in situ, CIN 2, and CIN 3
- Squamous cell carcinoma

Glandular cell

- Atypical glandular cells (AGC), specify endocervical, endometrial, or not otherwise specified (NOS)
- Atypical endocervical cells, favor neoplastic, specify endocervical or NOS
- Endocervical adenocarcinoma in situ (AIS)
- Adenocarcinoma

Other

- Endometrial cells in a woman 40 years of age or older
- Management of Abnormal Cytology

The following section discusses management of abnormal cytology (2012 ASCCP Consensus Guidelines).

Management of women with ASC-US

An ASCUS (ASC-US or ASC) Pap smear is the most common type of abnormal Pap smear result. ASCUS is an acronym for atypical cells of undetermined significance and indicates mild cellular cervical changes with an unknown cause. While an ASCUS Pap smear result may sound alarming, it is considered mildly abnormal. There is no immediate cervical cancer risk in an ASCUS Pap smear result.

The Causes of ASCUS Pap Smear Results

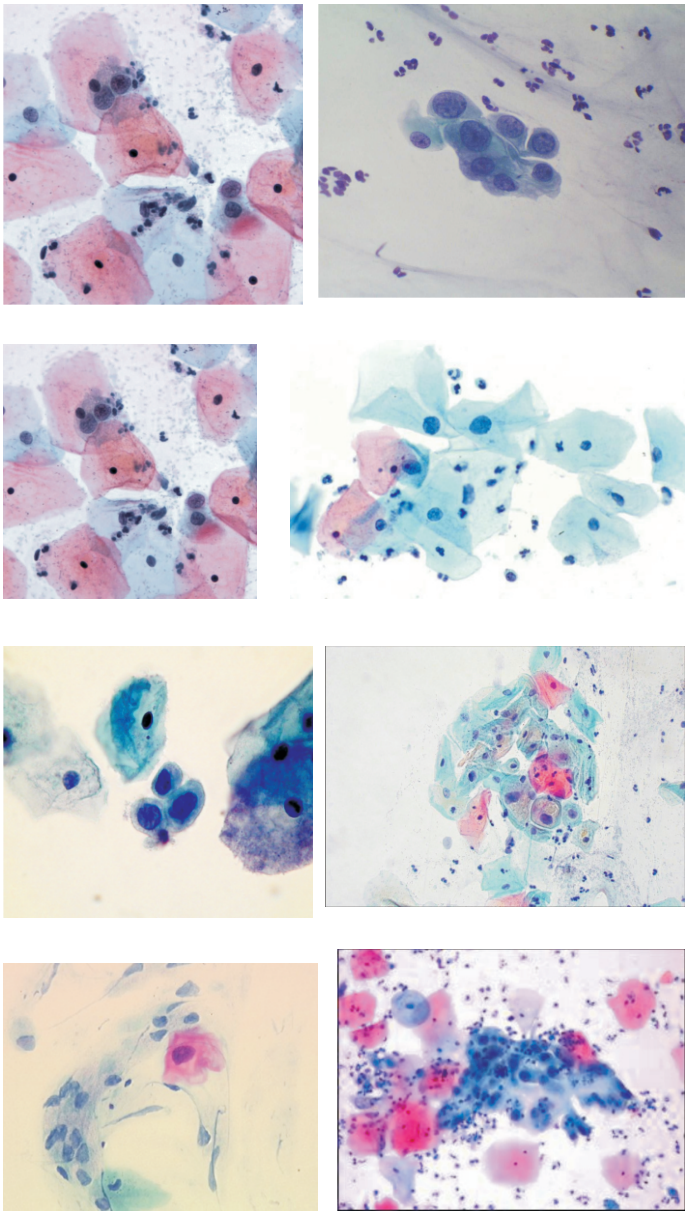
A common cause of ASCUS Pap smears are minor infection and cervical inflammation. Infection and inflammation can cause cervical cells to appear abnormal, but eventually return to a normal appearance. For some women, an ASCUS result is due to changes in the cervical cells caused by HPV infection. In most cases, these cervical changes do not progress to cervical cancer, but require further monitoring and possible treatment to prevent cervical cancer. Rarely, invasive cervical cancer may be detected through further examination and testing. For adolescents and young women, ASCUS Pap smear results are as often or more often caused by HPV infection than a vaginal infection or cervical inflammation. In older women, ASCUS results are more often because of vaginal infection or cervical inflammation, not an infection of HPV.

Managing an ASCUS Pap Smear Result

The method in which a doctor manages an ASCUS pap smear result varies. New guidelines set forth by American Society for Colposcopy and Cervical Pathology (ASCCP) recommend:

Adolescent Women (age 20 and younger): For young women with an ASCUS Pap smear result, the test is repeated in 12 months.

Adult Women: Adult women with an ASCUS Pap result will either have the Pap test repeated at 6 and 12 months or have a reflexive HPV DNA test. A reflexive HPV DNA test utilizes the sample used for the Pap smear and eliminates the need for another sampling. ASCCP guidelines favor HPV DNA testing for adult women with ASCUS Pap results.



ASCUS CELLS

An HPV DNA test is performed just like a Pap smear. The test detects the presence of a high risk HPV infection that could potentially lead to cervical pre-cancer or cancer if left unmonitored or untreated.

- HPV testing is preferred; if negative, repeat co-testing in 3 years; if positive, perform colposcopy
- Repeat cytology in 1 year is acceptable; if negative, repeat cytology in 3 years; if ASC or greater, perform colposcopy

Women aged 21-24 years with ASC-US

- Repeat cytology in 12 months
- If repeat cytology is ASC-H, AGC, or HSIL, perform colposcopy; otherwise, repeat cytology in another 12 months
- If repeat cytology at 24 months is negative, resume routine screening; otherwise, perform colposcopy
- Alternative is to perform HPV testing; if positive, repeat cytology in 12 and 24 months as above; if negative, resume routine screening.

Women aged 20 years or younger with ASC-US or LSIL

- HPV infection and minor abnormal cytology results common in adolescents, but invasive cancer is rare
- Per 2009 ACOG guidelines, Pap tests only recommended beginning at age 21 years, regardless of sexual history; however, Pap tests are still performed in the 20 years or younger age group in some cases because of lack of knowledge of current guidelines
- Conservative management preferred for this group because of the high likelihood of spontaneous resolution within 2 years of initial infection; abnormal cervical cytology in adolescents, therefore, should be followed according to the recommendations for women ages 21-24 years, as described above

Pregnant women with ASC-US

- Managed same as nonpregnant women
- Endocervical curettage (ECC) is contraindicated in pregnant women and should not be collected if colposcopy is performed
- Deferring colposcopy until at least 6 weeks postpartum is also acceptable

Management of women with ASC-H

- Perform colposcopy (regardless of HPV status)

Management of women with LSIL

Women aged 25 years or greater with LSIL

- Perform colposcopy
- If HPV co-testing was performed and negative, repeat co-testing in 1 year is preferred

Women aged 21-24 years with LSIL

- Repeat cytology in 12 and 24 months; follow guidelines for ASC-US

Pregnant women with LSIL

- Managed same as nonpregnant women
- ECC is contraindicated in pregnant women and should not be collected if colposcopy is performed
- Also acceptable to defer colposcopy until at least 6 weeks postpartum

Postmenopausal women with LSIL

- Acceptable options include reflex HPV testing, repeat Pap at 6 and 12 months, and colposcopy
- If HPV negative or no CIN on colposcopy, repeat cytology in 12 months; if HPV positive or repeat cytology is ASC or greater, perform colposcopy
- May return to routine screening if 2 consecutive negative cytology results

Management of women with HSIL

- Refer to colposcopy regardless of age
- Immediate loop electrosurgical excision is acceptable, except in patients younger than 25 years or who are pregnant

Pregnant women with HSIL

- Managed same as nonpregnant women
- ECC and immediate loop electrosurgical excision are contraindicated in pregnant women and should not be performed

Management of women with AGC

Women with AGC, including ASC-NOS, AGC-favor neoplasia, and AIS

- Refer to colposcopy with endocervical sampling
- If age 35 or greater or with other risk factors for endometrial neoplasia, endometrial sampling should also be performed.

Women with atypical endometrial cells

- Perform endometrial biopsy and endocervical sampling. If no pathology found, proceed with colposcopy.

Management of women with benign endometrial cells found in cervical cytology

- No additional evaluation is required in asymptomatic premenopausal women
- In postmenopausal women, perform endometrial biopsy

Management of women age 30 years and older who are Pap negative and HPV positive

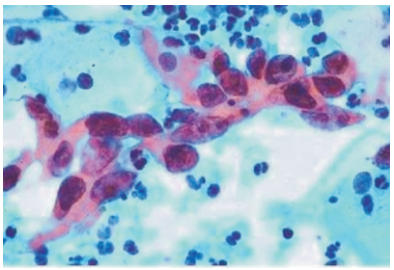
Repeat cytology and HPV DNA testing in 12 months

- If cytology negative, HPV negative, repeat co-testing in 3 years
- If cytology abnormal with any HPV result, follow ASCCP guidelines
- If cytology negative, HPV positive, perform colposcopy

Another option would be to perform HPV 16 and 18 testing

- If 16 or 18 positive, perform colposcopy
- If 16 and 18 negative, repeat co-testing in 12 months
- If cytology negative, HPV negative, repeat co-testing in 3 years
- If cytology abnormal with any HPV result, follow ASCCP guidelines
- If cytology negative, HPV positive, perform colposcopy
- In a traditional Pap (or Papanicolaou) test -- used since the 1040s -- the clinician obtains a specimen of cervical cells that are smeared on a slide and examined under a microscope. Using a newer method approved in 1996, known as liquid-based cytology (brand name ThinPrep), cervical cells are rinsed in a preservative solution before examination; the same sample can also be tested for the presence of HPV. Most U.S. gynecologists now use the ThinPrep test.
- Investigators from the Netherlands and Belgium conducted a study to assess the performance of liquid-based cytology compared with conventional Pap cytology for detecting histologically confirmed cervical intraepithelial neoplasia (CIN).
- This controlled trial included 89,784 women aged 30 to 60 years who participated in a Dutch cervical screening program at 246 family practice facilities. Between April 2004 and July 2006, 122 practices assigned to use liquid-based cytology screened 49,222 patients, while 124 practices assigned to use conventional Pap tests screened 40,562 patients.

Participants were followed for 18 months through the end of January 2008. The main outcome measures were CIN detection rates and positive predictive values using the 2 cytology systems.



Squamous cell carcinoma. Cervical cytology

BOUQUET

In Lighter Vein

A Marwari phones a dentist to inquire about the cost for a tooth extraction.
"Rs 850 for an extraction, sir" the dentist replied.
“Rs 850!!! Don't you have anything cheaper?”
"That's the normal charge," said the dentist.
“What about if you just don't use any anesthetic?”
"That's unusual, sir, but I could do it and knock Rs 150 off.
“What about if you just use one of your dentist trainees and still without anesthetic?”
"I can't guarantee their professionalism and it'll be painful. But the price could drop to Rs 400".
“How about if you make it a training' session, and one of your students do the extraction while the other students watch and learn?”
"It'll be good for the students", mulled the dentist.
"and it's going to be very traumatic, I can reduce the charge to Rs 200.
"Now you talking ! It's a deal," said the Marwari.
"Can I confirm an appointment for my wife for tomorrow then?

A woman is driving for 1st time on the highway.
Her husband calls & says: "Be careful love, It's just been on the radio, that someone is driving opposite to the traffic on the highway.."
She replies: "Someone..? These rascals are in hundreds.!!!

No English dictionary has been able to adequately explain the difference between the words COMPLETE and FINISHED.
However, in a recent linguistic conference supposedly held in London, England and attended by some of the best linguists in the world, Samsundar Balgobin, a Guyanese, was the clear winner...
His final challenge was this: "Some say there is no difference between the meaning of the words "COMPLETE" and "FINISHED".
Please explain the difference between COMPLETE and FINISHED in a way that is easy to understand."
Here is his answer:
"When you marry the right woman, you are COMPLETE;
but, when you marry the wrong woman, you are FINISHED.
And when the right one catches you with the wrong one, you are 'COMPLETELY FINISHED'!"
His answer was received with a standing ovation lasting over 5 minutes and it entitled him to receive an invitation to dine with Queen. His answer prompted her to call him after the contest.

An Old Lady was asked: "At your ripe age, what's preferable, Parkinson's or Alzheimer's?"
The wise Old Lady answered: "Definitely Parkinson's. Better to spill half a peg of scotch than to forget where you kept the bottle!"

Test By Puncture

Santa meets Banta in a hospital and expresses surprise, "What are you here for?"
Banta says, "I am here for blood test and these idiots are going to puncture my finger."
Santa started crying, "Oh my God, I am here for urine test and I am too young yet, what will happen to me?"

Gossiping Gurkhas

A Gurkha guard boasts to other, "You know, when I was small, that Victoria Tower fell down upon me."
So the second Gurkha guard inquired, "Hey why? Did it kill you then?"
The puzzled first one says, "I don't remember, I was too young then."

Wisdom Whispers

"One Of The Most Adventurous Things Left Us Is To Go To Bed. For No One Can Lay A Hand On Our Dreams."

"Throw Your Dreams Into Space Like A Kite, And You Do Not Know What It Will Bring Back, A New Life, A New Friend, A New Love, A New Country."

“Only The Dreamer Shall Understand Realities, Though In Truth His Dreaming Must Be Not Out Of Proportion To His Waking.”

“When Your Dreams Tire, They Go Underground And Out Of Kindness That's Where They Stay.”

“We've Removed The Ceiling Above Our Dreams. There Are No More Impossible Dreams.”

“If A Dream Affords The Dreamer Some Light On Himself, It Is Not The Person With Closed Eyes Who Makes The Discovery But The Person With Open Eyes Lucid Enough To Fit Thoughts Together. Dream--a Scintillating Mirage Surrounded By Shadows--is Essentially Poetry.”

“Where All Is But Dream, Reasoning And Arguments Are Of No Use, Truth And Knowledge Nothing.”

“A Dreamer Lives Forever, And A Toiler Dies In A Day.”

“In Bed My Real Love Has Always Been The Sleep That Rescued Me By Allowing Me To Dream.”

"To Believe In One's Dreams Is To Spend All Of One's Life Asleep."

"You See Things; And You Say "why?" But I Dream Things That Never Were; And I Say "why Not?""

"If You Have Built Castles In The Air, Your Work Need Not Be Lost; That Is Where They Should Be. Now Put The Foundations Under Them."

"Dreams Come True; Without That Possibility, Nature Would Not Incite Us To Have Them."

"Anyone Can Escape Into Sleep, We Are All Geniuses When We Dream, The Butcher's The Poet's Equal There."

Brain Teasers

CORRELATE THE FOLLOWING

1. Bronze	A. Diabetes
2. Asbestos	B. Mesothelioma lung
3. Sulphur	C. Mycetoma foot
4. Fluorosis	D. Teeth
5. Scarlet	E. Fever
6. Lyme	F. Disease
7. Black	G. Plague
8. Pink	H. Eye

Answers: 1.A, 2.B, C. 3.D, 4.E. 5.E, 6.F, 7.G, 8.H

TROUBLESHOOTING

IHC TROUBLESHOOTING TIPS

Detailed troubleshooting tips and techniques for IHC

Contents

No staining

The primary antibody and the secondary antibody are not compatible

Use secondary antibody that was raised against the species in which the primary was raised (e.g primary is raised in rabbit, use anti-rabbit secondary).

Not enough primary antibody is bound to the protein of interest

Use less dilute antibody, Incubate longer (e.g. overnight) at 4°C.

The antibody may not be suitable for IHC procedures which reveal the protein in its native (3D form)

Test the antibody in a native (non-denatured) WB to make sure it is not damaged.

The primary/secondary antibody/amplification kit may have lost its activity due to improper storage , improper dilution or extensive freezing/thawing

Run positive controls to ensure that the primary/secondary antibody is working properly.

The protein is not present in the tissue of interest

Run a positive control recommended by the supplier of the antibody.

The protein of interest is not abundantly present in the tissue

Use an amplification step to maximize the signal.

The secondary antibody was not stored in the dark

Always prevent the secondary antibody from exposure to light.

Deparafinization may be insufficient

Deparaffinize sections longer, change the xylene.

Fixation procedures (using formalin and paraformaldehyde fixatives) may be modifying the epitope the antibody recognizes

Use antigen retrieval methods to unmask the epitope, fix for less time.

The protein is located in the nucleus and the antibody (nuclear protein) cannot penetrate the nucleus

Add a permeabilizing agent to the blocking buffer and antibody dilution buffer.

The PBS buffer is contaminated with bacteria that damage the phosphate groups on the protein of interest

Add 0.01% azide in the PBS antibody storage buffer or use fresh sterile PBS.

High background

Blocking of non specific binding might be absent or insufficient

Increase the blocking incubation period and consider changing blocking agent. Abcam recommends 10% normal serum 1hr for sections or 1-5% BSA for 30 min for cells in culture.

The primary antibody concentration may be too high

Titrate the antibody to the optimal concentration, incubate for longer but in more dilute antibody (a slow but targeted binding is best).

Incubation temperature may be too high

Incubate sections or cells at 4°C.

The secondary antibody may be binding non-specifically (damaged)

Run a secondary control without primary antibody.

Tissue not washed enough, fixative still present

Wash extensively in PBS between all steps.

Endogenous peroxidases are active

Use enzyme inhibitors i.e. Levamisol (2 mM) for alkaline phosphatase or H_2O_2 (0.3% v/v) for peroxidase.

Fixation procedures (using formalin and paraformaldehyde fixatives) are too strong and modified the epitope the antibody recognizes

Change antigen retrieval method, decrease the incubation time with the antigen unmasking solution.

Too much amplification (amplification technique)

Reduce amplification incubation time and dilute the amplification kit.

Too much substrate was applied (enzymatic detection)

Reduce substrate incubation time.

The chromogen reacts with the PBS present in the cells/tissue (enzymatic detection)

Use Tris buffer to wash sections prior to incubating with the substrate, then wash sections/cells in Tris buffer.

Pemeabilization has damaged the membrane and removed the membrane protein (membrane protein)

Remove permeabilizing agent from your buffers.

Non specific staining

Primary/secondary antibody concentration may be too high

Try decreasing the antibody concentration and/or the incubation period. Compare signal intensity against cells that do not express the target.

Endogenous peroxidases are active

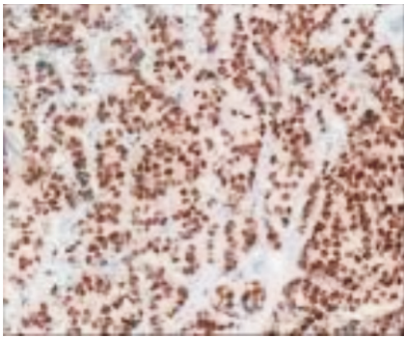
Use enzyme inhibitors i.e. Levamisol (2 mM) for alkaline phosphatase or H_2O_2 (0.3% v/v) for peroxidase.

The primary antibody is raised against the same species as the tissue stained (e.g mouse primary antibody tested on mouse tissue). When the secondary antibody is applied it binds to all the tissue as it is raised against that species

Use a primary antibody raised against a different species than your tissue.

The sections/cells have dried out

Keep sections/cells at high humidity and do not let them dry out.



ER positive Breast Biopsy Section

TULIP NEWS

Microxpress Introduces ULTRA PAP™

ULTRA-PAP Kit is modification of the classical PAP staining, formulated to give fast PAP staining of specimen smear with a simplified procedure thereby aiding clear nuclear and cytoplasmic staining.

Kit Contents :

ULTRAPAP – Nuclear Stain (100 ml), ULTRAPAP – Cyto-Stain A (55 ml), ULTRAPAP – Cyto-Stain B (55 ml), Scotts Tap Water Buffer (30 ml), Micro-Fix Fixative Spray (50 ml), Dehydrant (IPA) (3 x 100 ml), Xylene (2 x 100 ml), D. P. X. Mounting Medium (20 ml) and empty bottle (50ml) for preparing working cyto stain reagent.

Reagent Preparation :

As required make a Working Cyto-Stain by mixing equal amounts of ULTRAPAP Cyto - Stain A & B (An empty bottle is provided for the same). The Working Cyto- Stain is stable for at least 3 Months, provided contamination and hydration are avoided. The other contents are ready to use.

Pack Size : 250 Smears

Ultra Fast Papanicolaou Staining Kit !

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