In vitro diagnostics for the medical dermatologist. Part II: Hypercoagulability tests

Kelsey B. Nusbaum, BS,[a] Abraham M. Korman, MD,[b] Kelly H. Tyler, MD,[b] Jessica A. Kaffenberger, MD,[b] John C. Trinidad, MD,[b] Steven Dean, DO,[c] Spero Cataland, MD,[d] and Benjamin H. Kaffenberger, MD, MS[b]
Cincinnati and Columbus, Ohio

Learning objectives
After completing this learning activity, participants should be able to discuss the strengths and weaknesses of various laboratory tests used to test for hypercoagulability disorders and infectious causes of skin disease; become familiar with new laboratory tests for detecting and diagnosing hypercoagulability disorders and infectious causes of skin disease; and recognize potential sources for discordant laboratory testing.

Disclosures
Editors
The editors involved with this CME activity and all content validation/peer reviewers of the journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Authors
The authors involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

Planners
The planners involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s). The editorial and education staff involved with this journal-based CME activity have reported no relevant financial relationships with commercial interest(s).

The skin often provides initial clues of hypercoagulability with features such as livedo reticularis, livedo racemosa, retiform purpura, necrosis, and ulcerations. Because these cutaneous manifestations are nonspecific, laboratory testing is often needed to evaluate for underlying causes of hypercoagulability. Importantly, these disorders are reported to be the most common mimicker, resulting in an erroneous diagnosis of pyoderma gangrenosum. Understanding inherent properties of, and indications for, available tests is necessary for appropriate ordering and interpretation of results. Additionally, ordering of these tests in an indiscriminate manner may lead to inaccurate results, complicating the interpretation and approach to management. This second article in this continuing medical education series summarizes information on methodology, test characteristics, and limitations of several in vitro laboratory tests used for the work up of hypercoagulability and vasculopathic disease as it pertains to dermatologic disease. (J Am Acad Dermatol 2021;85:301-10.)

Key words: antiphospholipid antibodies; cryofibrinogenemia; cryoglobulinemia; D-dimer; diagnostic testing; factor deficiency; genetic thrombophilia; hereditary thrombophilia; hypercoagulability; medical dermatology; monoclonal gammopathy; multiple myeloma; pyoderma gangrenosum; retiform purpura; thrombogenic vasculopathy; thrombotic vasculopathy; ulcer.

From the University of Cincinnati College of Medicine[a]; Division of Dermatology[b]; Division of Cardiovascular Medicine[c]; and Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus.[d]
Funding sources: None.
IRB approval status: Not applicable.
Accepted for publication March 26, 2021.
Reprints not available from the authors.
Correspondence to: Benjamin H Kaffenberger, MD, MS, Department of Dermatology, Ohio State University Medical Center, 1328 Dublin Road, Suite 100, Columbus, OH 43212. E-mail: Benjamin.Kaffenberger@osumc.edu.
0190-9622/$36.00
© 2021 by the American Academy of Dermatology, Inc.
https://doi.org/10.1016/j.jaad.2021.03.108

Date of release: August 2021.
Expiration date: August 2024.

Scanning this QR code will direct you to the CME quiz in the American Academy of Dermatology's (AAD) online learning center where after taking the quiz and successfully passing it, you may claim *1 AMA PRA Category 1 credit*. NOTE: You must have an AAD account and be signed in on your device in order to be directed to the CME quiz. If you do not have an AAD account, you will need to create one. To create an AAD account: go to the AAD's website: www.aad.org.

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

Abbreviations used:

ELISA: enzyme-linked immunosorbent assay
IFE: immunofixation electrophoresis
Ig: immunoglobulin
FLC: free light chain
SPEP: serum protein electrophoresis
VTE: venous thromboembolism

INTRODUCTION

After blood vessel injury, the complex process of hemostasis is initiated with the formation of a platelet plug, followed by activation of clotting factors and formation of a fibrin mesh to stop the bleeding (Fig 1).[1] Although typically tightly regulated, disruption at any step in the process can lead to pathologic thrombosis or bleeding. Excess activity of thrombogenic factors or a deficiency of antithrombogenic factors predispose to the development of a hypercoagulable state, which along with stasis and endothelial injury comprise the Virchow triad.[2,3] Ranges of underlying inherited and acquired conditions are known to be associated with hypercoagulability and subsequent risk of thrombosis (Table I).[1,3-5]

This article focuses on cutaneous manifestations of hypercoagulability, including livedo reticularis, livedo racemosa, retiform purpura, necrosis, and ulcerations, often with severe and disproportionate pain. Skin findings are often variable and nonspecific, which may result in misdiagnosis. Highlighting the variability of appearances, hypercoagulable disease is the most common underlying etiology for misdiagnosed pyoderma gangrenosum.[6,7] Due to the nonspecificity of these cutaneous manifestations, the ordering of select laboratory tests is necessary to facilitate the diagnosis of underlying causes of hypercoagulability. We provide a guide for the work up of cutaneous hypercoagulability, discussing D-dimer, antiphospholipid antibodies, hereditary thrombophilia, monoclonal gammopathy, cryoglobulinemia, and cryofibrinogenemia.

SCREENING WITH D-DIMER

Scenario: A 55-year-old man is transferred to your hospital in respiratory failure secondary to COVID-19-related multifocal pneumonia. On presentation, purpura, necrosis, and bullae are noted on his bilateral upper and lower extremities. Hypercoagulability work up is pending. Which rapid test could be ordered to screen for small- or large-vessel thrombosis (Fig 2)?

Key points

- Patients with severe COVID-19 are at risk for a multiorgan vasculopathy mimicking an antiphospholipid antibody-like state.
- Scenario interpretation: Although nonspecific, D-dimer is an inexpensive, rapid test that screens for thrombosis in multiple diseases but is not specific for any particular diagnosis.

Although well established as a screening test for pulmonary embolus and deep vein thrombosis, D-dimer has been investigated as a screen for hypercoagulability in other medical conditions. With the onset of COVID-19, a broad hypercoagulable response has been reported to cause an antiphospholipid antibody-like state, resulting in necrosis, gangrene, stroke, and kidney injury, as well as arterial and venous thrombosis.[8] As in our case, critical patients are screened with D-dimer and prophylactic anticoagulation is considered.[9,10] D-dimer has also been found to identify the emergence of a hypercoagulable state in systemic lupus erythematosus and systemic sclerosis with a high sensitivity for predicting small- or large-vessel thrombosis, regardless of antiphospholipid antibody status (Table I).[11,12]

Time-consuming enzyme-linked immunosorbent assays (ELISAs) have been replaced by rapid, automated antibody-based assays that target epitopes on cross-linked D-dimer fragments.[13] Sensitivity is reduced in patients receiving anticoagulation.[14-16] Additionally, specificity of D-dimer for detecting hypercoagulable states is low. Common causes of false-positive results include increasing age, malignancy, pregnancy, recent surgery, and connective tissue disease.[17] Elevated D-dimer can also be observed in venous disease with or without ulceration.[18]

Recommended approach to ordering

D-dimer is a rapid, inexpensive test that may be used as an initial screen to rule out small- and large-vessel thrombotic events, such as unilateral extremity swelling, livedo racemosa, purpura fulminans, and ulcerations in the presence of livedo or purpura. Factors known to cause false-positive results should be considered when interpreting a positive D-dimer. Interpretation should be age-adjusted for patients older than 50 years of age, with the normal cut off estimated at age in years times 10 mcg/L.[19]

ANTIPHOSPHOLIPID ANTIBODIES

Scenario: A 28-year-old woman with a history of late-term pregnancy loss presents with a 3-week history of persistent leg discoloration. On examination, she has a reticulated, net-like, violaceous discoloration of the legs with necrotic eschars. An antiphospholipid antibody panel returns positive for lupus anticoagulant and anticardiolipin antibodies. When should testing be repeated? What is the significance of this patient's antibody profile (Fig 3)?

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

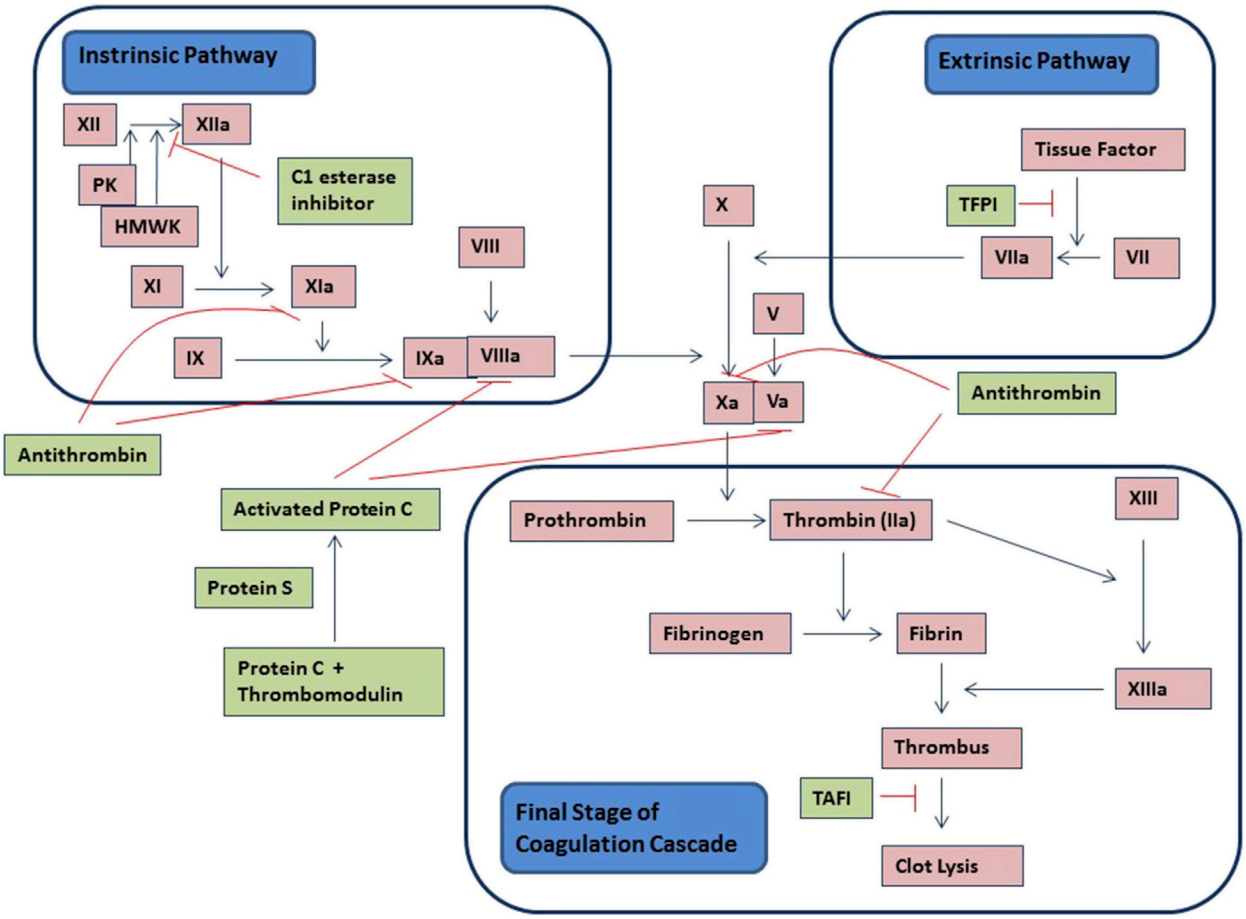

Fig 1. Extrinsic and intrinsic pathways converge on the common pathway with the activation of factor X to Xa. Factor Xa facilitates conversion of prothrombin to thrombin, which in turn facilitates the conversion of fibrinogen to fibrin. Fibrin is cross-linked to form a fibrin mesh that acts to stabilize the platelet plug and stop the bleeding. Inhibitory steps are in green, including the natural anticoagulants protein C, protein S, and antithrombin. Increased activity of coagulation factors or decreased activity of anticoagulants predispose to the formation of pathologic thrombi. Reprinted with permission from Chang Y, Dabiri G, Damstetter, et al: Coagulation disorders and their cutaneous presentations: Pathophysiology. *J Am Acad Dermatol*. 2016;74(5):783-792. *TAFI*, thrombin activatable fibrinolysis inhibitor; *TFPI*, tissue factor pathway inhibitor.

Key points

- Antiphospholipid antibody testing should be limited to patients with clinical features of antiphospholipid syndrome and not performed during an acute thromboembolic event or while on anticoagulation therapy.
- Scenario interpretation: To fulfill a diagnosis of antiphospholipid syndrome, testing should be repeated at least 12 weeks after a positive test and should include an assessment of lupus anticoagulant, anticardiolipin, and anti-β2 glycoprotein I to differentiate this diagnosis from transient antiphospholipid antibodies, which may appear with an acute thrombotic event. The presence of multiple antiphospholipid antibodies is associated with a higher risk of future thrombosis.

The diagnosis of antiphospholipid syndrome requires at least 1 laboratory criterion: presence of lupus anticoagulant, >99th percentile titer of anticardiolipin, or anti-β2 glycoprotein I antibodies.[20] Diagnosis also requires 1 clinical criterion: unprovoked venous thromboembolism (VTE) in patients younger than 50 years of age, thrombosis at unusual sites, late pregnancy loss, or pregnancy loss in patients with known autoimmune disease.[21]

Lupus anticoagulant

There are 3 phases to lupus anticoagulant testing: screening, mixing, and confirmation. Screening is performed with 2 phospholipid-dependent assays, diluted Russell viper venom time[22-24] and activated partial thromboplastin time, which detect the

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

Table I. Inherited and acquired causes of hypercoagulability

Inherited	Acquired
Anticoagulant deficiency	Smoking
Protein C*	Obesity
Protein S*	Pregnancy
Antithrombin III*	Oral contraception
Factor V Leiden*	Immobilization
Prothrombin G20210A*	Malignancy
Hyperhomocysteinemia	Calciphylaxis
Methyltetrahydrofolate reductase deficiency	Disseminated intravascular coagulation
Cystathione-β-synthase deficiency	Antiphospholipid syndrome*
Elevated factor VIII	Cryoglobulinemia*
Plasminogen deficiency	Cryofibrinogenemia*
Tissue factor pathway inhibitor deficiency	Thrombotic thrombocytopenic purpura

*Discussed in the current manuscript.

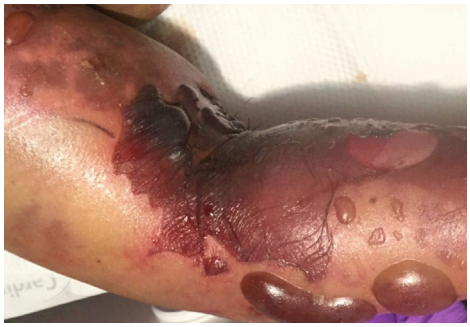

Fig 2. A middle-aged man with COVID-19-related pneumonia who developed purpura, necrosis, and bullae involving his upper and lower extremities.

prolonged clotting time expected with lupus anticoagulant.[24] Failure to correct prolonged clotting time with added normal plasma signifies that the prolonged test is due to an inhibitor as opposed to a factor deficiency. Finally, confirmation testing is performed by adding phospholipids to patient serum; correction of clotting time confirms that an antiphospholipid antibody is responsible for the abnormal test.

Anticardiolipin and anti-β2 glycoprotein I antibodies

Testing for anticardiolipin and anti-β2 glycoprotein I antibodies is performed with ELISAs and multiplex immunoassays. ELISA assays are more widely used with commercially available kits, but have variable sensitivity and specificity (Table II).[25-28] Multiplex immunoassays are automated with the advantage of being able to test for both antibodies simultaneously. Sensitivity and specificity are generally

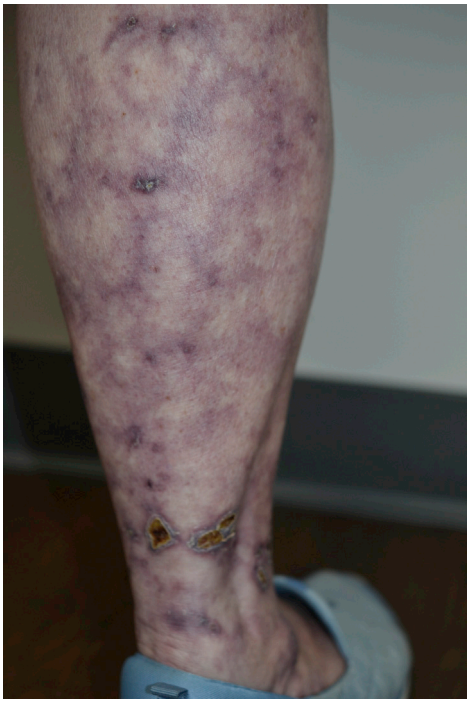

Fig 3. A young woman with a history of pregnancy loss who presented with new onset livedo racemosa.

higher with multiplex assays.[25,29] Although both assays can test for immunoglobulin (Ig)G, IgM, and IgA anticardiolipin, only the IgG isotype has been shown to be strongly associated with increased thrombotic risk.[30]

Recommended approach to ordering

In a patient with suspected antiphospholipid syndrome, all 3 tests are recommended: lupus anticoagulant, anticardiolipin, and anti-β2 glycoprotein I to characterize antibody profile.[20,31] Patients with more than 1 positive test, particularly those with triple-positive results, have the greatest risk of thrombotic and obstetric complications.[32,33]

If any test is positive for antiphospholipid antibodies, the same test should be repeated after 12 weeks to distinguish patients with persistent antibodies from those with transient antibodies, which may appear during acute thrombotic events.[20,31,34] Furthermore, test results during an acute event are subject to interference from acute phase reactants.[34] Interpretation of lupus anticoagulant is difficult in patients on anticoagulation therapy.[35,36] Anticoagulation generally does not interfere with testing for anticardiolipin and anti-β2 glycoprotein I.[34]

HEREDITARY THROMBOPHILIA

Scenario: A 48-year-old man with strong family history of VTE presents for evaluation of pyoderma gangrenosum after previously undergoing amputation

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

Table II. Summary of test characteristics and costs

Test	Sensitivity	Specificity	Reference standard test for comparison*	2017 Medicare national limit
D-Dimer	93%	74%	Work up for small- and large-vessel thrombosis in lupus cohort†	$13.80
Lupus anticoagulant	96%	78%-98%	Abnormal screening, mixing, and confirmation tests in patients with clinical symptoms of antiphospholipid syndrome	$21.38 (screening) + additional cost if reflex to mixing and confirmatory tests
Anticardiolipin ELISA	50%-94%	36%-96%	Previously detected anticardiolipin on any serologic assay	$34.91
Multiplex	86%-100%	73%-93%		
Anti-β2 glycoprotein I	20%-90%	87%-98%	Previously detected anti-β2 glycoprotein I on any serologic assay	$34.91
ELISA Multiplex	88%-89%	93%-95%		
Protein C activity assay	80%-95%	75%-97%	Molecular confirmation of genetic mutation	$18.98
Protein S antigen assay	98%-100%	Up to 100%		$21.02
Antithrombin activity assay	—§	—§		$16.26
Serum protein electrophoresis	79%	92.5%	Positive M protein, including monoclonal gammopathy of undetermined significance, multiple myeloma, plasmacytoma, and Waldenström macroglobulinemia detected by any serologic assay	$19.76
Serum immunofixation	87%	100%		$68.93
Serum-free light chains	74%	96%-98%		$37.30
Serum immunofixation + serum-free light chains	97%	—§		$106.23
Cryoglobulins immunofixation	54%‡	—§	Identification of cryoglobulins in patients with clinical symptoms of cryoglobulinemia on any qualitative or quantitative assay	$8.87

ELISA, Enzyme-linked immunosorbent assay.
*Reference standard tests were defined as the best available diagnostic test or benchmark used in the cited studies to determine sensitivity and specificity of the discussed tests.
†Large-vessel assessment included duplex venous scan of lower extremities and computed tomography angiography for suspected pulmonary embolism. Small-vessel assessment included testing for thrombotic microangiopathic hemolytic anemia (thrombocytopenia, schistocytes, reticulocytes, and serum lactic dehydrogenase), pulse oximetry in patients with dyspnea, and renal biopsy in patients with unexplained increase in creatinine.
‡Sensitivity for full identification and characterization of cryoglobulins.
§Information not available.

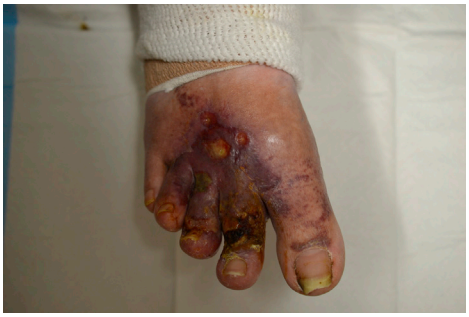

Fig 4. A middle-aged man with a history of deep vein thrombosis and recurrent shallow ulcerations despite anticoagulation and a family history of protein S deficiency presented to the clinic for wound evaluation and management.

of distal toes for nonhealing wounds. He reports his first deep vein thrombosis occurred in his 20s. He has multiple areas of porcelain white scars in addition to a new irregular shallow ulceration on the ankle. A factor V Leiden mutation is identified. Does this impact his management (Fig 4)?

Key points

- Hereditary thrombophilia testing should be limited to patients with clinical risk factors suggestive of a heritable disorder and ordered only when results will guide clinical management.
- Scenario interpretation: The patient's history of unprovoked and recurrent thrombotic events at a young age, recurrent ulceration, and strong family history of VTE are suggestive of hereditary thrombophilia. Diagnostic work up should include testing for protein C, protein S, and antithrombin III deficiency due to the strong risk of recurrent thrombosis. However, testing for factor V Leiden and prothrombin gene mutations is not routinely recommended, due to only modest increased risk for recurrent thrombosis, which does not impact the decision for anticoagulation.

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

Protein C, protein S, and antithrombin III

Testing for protein C, protein S, and antithrombin III may be performed by activity and antigen assays (Table II).[37-45] Activity assays are typically performed first due to the ability to detect both low quantity and decreased functioning of anticoagulants.[37-39] Antigen assays utilize immunologic methods to quantify levels of protein C, protein S, and antithrombin III. Thus, because they are only able to detect low quantity, but not decreased activity, of anticoagulants, they are typically reserved as a secondary test to distinguish between quantitative and qualitative deficiencies. Results of activity assays may be impacted by lupus anticoagulant and factor VIII, as well as anticoagulation therapy.[46-50]

Factor V Leiden and prothrombin G20210A

Factor V Leiden and prothrombin G20210A mutations are present in about 5% and 2%-3% of the general population, respectively, making these disorders much more common than anticoagulant deficiencies.[51,52] Although detected in up to 25% of patients presenting with VTE, relative risk for thrombosis is low (~3- to 7-fold) compared to anticoagulant deficiencies, which increase thrombotic risk up to 50-fold.[53] Given the modest increased risk for recurrent thrombosis, detection of factor V Leiden or prothrombin G20210A is unlikely to merit an extended duration of anticoagulation following an initial VTE.[52-54] Testing for these mutations is not routinely recommended for VTE, let alone cutaneous vasculopathy.[52,53,55,56]

Recommended approach to ordering

Thrombophilia testing is generally not recommended except in exceptional vasculopathic cases. The validity and value of these tests in a dermatologic patient population is unclear and should only be considered in cases highly suggestive of a genetic disorder, such as those with unprovoked hypercoagulability at a very young age, those with a known family history of anticoagulant deficiencies, or those with recurrent, extensive cutaneous involvement without an obvious etiology.[57] Protein C and protein S levels can also be tested in neonatal purpura fulminans and warfarin-induced skin necrosis, which are highly suggestive of congenital or acquired protein C or S deficiency.[58-60]

Testing should only be performed in cases of anticipated impact on clinical management, such as to guide decisions regarding future VTE prophylaxis.[57,61,62] Similar to antiphospholipid antibody screening, testing for hereditary thrombophilia should generally not be performed during an active clotting event but rather delayed until after completion of anticoagulation, as these circumstances interfere with test accuracy.[57]

Causes of acquired anticoagulant deficiency should be considered prior to ordering diagnostic testing. These causes include anticoagulation therapy, consumption, pregnancy, oral contraceptives, and age.[46,49] If a genetic cause of hypercoagulability remains probable, selective ordering of protein C, protein S, or antithrombin III may be performed. Genetic testing for factor V Leiden and prothrombin G20210A is not routinely recommended.[52,53,63]

MONOCLONAL GAMMOPATHY

Scenario: A 50-year-old woman presents with several months of fatigue, arthralgias, and palpable purpura of the lower extremities with shallow jagged ulcerations and fibrinous base. An M-spike is not observed on serum protein electrophoresis (SPEP) and the test does not reflex to immunofixation. Does this exclude the possibility of a clinically significant paraproteinemia (Fig 5)?

Key points

- Serum protein electrophoresis may miss clinically significant minor paraproteins.
- The combination of serum immunofixation or protein electrophoresis with serum-free light chains has a high sensitivity for detecting a monoclonal protein.
- Scenario interpretation: This patient had a recurrent and persistent small-vessel vasculitis. A monoclonal protein of dermatologic significance was only established with a specific order for serum immunofixation. After several years of disease persistence despite immunosuppression, her paraprotein and skin cleared after initiation of IVIG.

Cutaneous manifestations of monoclonal gammopathies are diverse, including scleredema, scleromyxedema, pyoderma gangrenosum, necrobiotic xanthogranuloma, and Schnitzler syndrome, among many others.[64] Screening for monoclonal gammopathy has traditionally consisted of protein electrophoresis but with the recent development of additional assays, new diagnostic approaches have emerged.

SPEP is performed by loading proteins onto an agarose gel and separating by electric current based on charge and size. A monoclonal (M) protein appears as a sharp peak, most often in the γ region. SPEP has overall sensitivity and specificity of 79% and 92.5%, respectively, for monoclonal gammopathy.[65] This is the most commonly used test, as it is low cost, widely available, and easy to perform.[66,67] However, SPEP lacks sensitivity for low-burden disease that may occur in

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

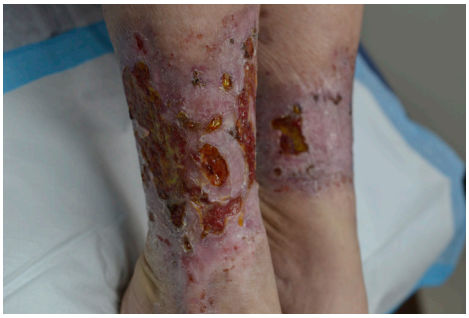

Fig 5. A middle-aged woman with recurrent crops of palpable purpura and shallow irregular fibrinous ulcerations over the lower extremities presented for concern of vasculitis versus pyoderma gangrenosum.

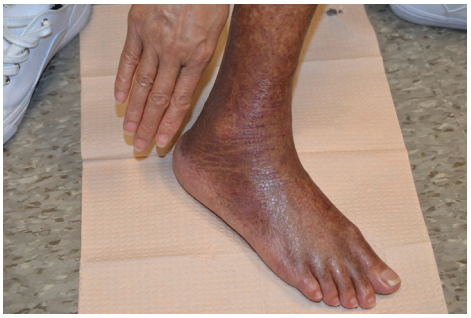

Fig 6. A middle-aged woman with hepatitis C presented for evaluation of her chronic palpable purpura and extensive lower extremity dyspigmentation.

monoclonal gammopathies of dermatologic significance with a detection limit for M protein of 0.3-0.5 g/dL in the γ region and up to 0.7 in the β region, limiting its use.[66] Additionally, SPEP is unable to identify the type of M protein present; however, as long as an M-spike, hypogammaglobulinemia, or hypergammaglobulinemia are detected, it will generally automatically prompt subsequent performance of serum immunofixation (reflex to immunofixation).

Serum immunofixation electrophoresis (IFE) involves gel electrophoresis followed by application of antibodies against heavy and light chains of immunoglobulins onto the surface of electrophoresed lanes. Serum IFE has higher sensitivity and specificity compared to SPEP, 87% and 100%, respectively, and a lower detection limit of 0.1 g/dL.[65,66] This test is able to characterize the type of M protein. IFE is widely available but more expensive than SPEP.[66]

The serum-free light chain (FLC) assay involves application of antibodies against epitopes hidden in intact immunoglobulins to detect κ and λ FLCs. The κ to λ ratio allows the determination of clonality, with a normal ratio between 0.26 and 1.65.[68] This assay has a sensitivity and specificity of 74% and 96%-98%, respectively, for a monoclonal protein.[65] Notably, specificity is reduced in chronic kidney disease due to asymmetric reduction of κ and λ light chain filtration[66]; the use of a modified reference range between 0.37 and 3.1 can improve specificity.[69] The serum FLC assay is commercially available and automated.[66]

The addition of serum FLC to serum IFE increases overall sensitivity to 97% for any laboratory-confirmed monoclonal protein.[65] Urine protein electrophoresis or immunofixation do not significantly alter sensitivity of the tests.[65]

Recommended approach to ordering

A diagnostic panel of serum IFE and FLC is a sensitive and cost-effective approach for screening high-risk patients for monoclonal gammopathies of dermatologic significance. A simplified panel of SPEP and FLC provides an efficient, cost-effective alternative but requires the additional serum immunofixation for an abnormal study.[65,67,70,71] Urine IFE generally will not increase the sensitivity unless there is clinical suspicion for light chain deposition disease or amyloid light chain amyloidosis.[65,70,71]

CRYOGLOBULINS AND CRYOFIBRINOGENS

Scenario: A 35-year-old woman with a history of hepatitis C presents with chronic, recurrent palpable purpura of the lower extremities and painful paresthesias. Testing for cryoglobulins is negative. Several months later, after seeing a different physician and lab, cryoglobulins are identified. What could account for the discrepancy (Fig 6)?

Key points

- Testing for cryofibrinogens should be considered in the diagnostic work up for cryoglobulins, given the similar clinical presentation and prognostic value of simultaneous cryoglobulinemia and cryofibrinogenemia.
- Scenario interpretation: The patient had high-titer rheumatoid factor and ultimately mixed cryoglobulinemia was confirmed. Improper specimen handling is the most common reason for false-negative results and should be considered if clinical suspicion remains high for cryoglobulinemia despite negative testing.

Cryoglobulinemia is classified into 3 categories according to type of immunoglobulin.[72,73] Type 1 is simple cryoglobulinemia characterized by a single monoclonal immunoglobulin, which can be IgG, IgM, IgA, or Bence Jones protein, typically seen with B-cell lymphoproliferative diseases. Types 2 and 3 are mixed cryoglobulinemia, with type 2 involving both monoclonal and polyclonal immunoglobulins and type 3 involving 1 or more classes of polyclonal immunoglobulins. Mixed cryoglobulins are seen with infectious diseases, most notably hepatitis C,

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

and autoimmune diseases.[72,73] Cryofibrinogens are composed of an insoluble complex of fibrin, fibrinogen, fibrin split products, and immunoglobulins; their presence is frequently associated with cryoglobulinemia and may be indicative of a more-severe disease phenotype.[74,75]

Laboratory evaluation of cryoglobulins and cryofibrinogens is multifaceted and requires appropriate processing prior to qualitative and quantitative analysis. Strict conditions are required during sample collection and processing to prevent premature precipitation; improper specimen handling is the most common reason for false-negative results.[76,77] Blood should be collected into prewarmed tubes to maintain the temperature at 37°C for collection, transport, and centrifugation.[76] The sample is stored at 4°C for 7 days to allow sufficient time for precipitation.[77] Type 1 cryoglobulins tend to precipitate within hours, and type 2 and 3 cryoglobulins and cryofibrinogens can take several days.[78,79] If cryoprecipitate is identified, reflexive quantitative and qualitative tests are performed.

For quantitative analysis, manual estimation of cryoprecipitate is performed, known as the cryocrit. This is a rapid, convenient, and inexpensive test but is only able to provide a gross estimate.[76,80] Further quantification can be deduced by calculating area under the curve on electrophoresis during qualitative analysis.[81]

Qualitative analysis characterizes isotype and clonality of cryoglobulins and components of cryofibrinogen precipitate.[76] IFE is most often used, which has sensitivity of 54%.[82] Immunoelectrophoresis is not often used, due to low sensitivity of 28% for detecting cryoproteins.[82] Immunoblot has the best sensitivity (98%) but is not widely available in clinical laboratories.[82,83]

Because the detection of cryoglobulins is technically difficult, the use of rheumatoid factor and complement proteins as surrogate markers can provide additional evidence for cryoglobulinemia.[76,84] Rheumatoid factor activity is often present in types 2 and 3 cryoglobulinemia but is rarely seen in type 1. Decreased levels of complement C1q, C2, and C4 with relative preservation of C3 also supports the diagnosis of mixed cryoglobulinemia.

Recommended approach to ordering

Although there is no standardized approach, testing for cryoglobulins and cryofibrinogens should include reflexive quantitative and qualitative analysis following the identification of a cryoprecipitate.[76,77] Given the stringent requirements for cryoprotein handling, if testing is negative despite high clinical suspicion, the laboratory should be contacted to inquire about collection and processing protocols.[76] Rheumatoid factor and complement are useful surrogate markers for the diagnosis of mixed cryoglobulinemia.[76,84] The presentation of cryofibrinogenemia is similar to that of cryoglobulinemia with the possibility of simultaneous occurrence; thus, testing for cryofibrinogens should be considered as part of the work up for cryoglobulins.[76,79]

CONCLUSION

Cutaneous manifestations of hypercoagulability are critical to recognize early and require laboratory testing for further characterization. Laboratory tests should be selected according to pretest probability, determined by the history and physical examination with consideration of test utility on subsequent treatment course. Results should be interpreted in the context of test characteristics and accompanying clinical picture.

Conflicts of interest

None disclosed.

REFERENCES

1. Chang Y, Dabiri G, Damstetter E, Baiyee Ebot EB, Powers JG, Phillips T. Coagulation disorders and their cutaneous presentations: pathophysiology. *J Am Acad Dermatol*. 2016;74(5):783-792;quiz 793.
2. Palta S, Saroa R, Palta A. Overview of the coagulation system. *Indian J Anaesth*. 2014;58(5):515-523.
3. Gil MR. Overview of the coagulation system. In: Shaz B, Hilley C, Gil M, eds. *Transfusion Medicine and Hemostasis*. 3rd ed. Elsevier Saunders. Philadelphia, PA, USA; 559-564.
4. Thornsberry LA, LoSicco KI, English JC III. The skin and hypercoagulable states. *J Am Acad Dermatol*. 2013;69(3):450-462.
5. Thomas RH. Hypercoagulability syndromes. *Arch Intern Med*. 2001;161(20):2433-2439.
6. Weenig RH, Davis MD, Dahl PR, Su WP. Skin ulcers misdiagnosed as pyoderma gangrenosum. *N Engl J Med*. 2002;347(18):1412-1418.
7. Ahronowitz I, Harp J, Shinkai K. Etiology and management of pyoderma gangrenosum: a comprehensive review. *Am J Clin Dermatol*. 2012;13(3):191-211.
8. McGonagle D, Bridgewood C, Ramanan AV, Meaney JF, Watad A. COVID-19 vasculitis and novel vasculitis mimics. *Lancet Rheumatol*. 2021;3(3):e224-e233.
9. Short SAP, Gupta S, Brenner SK, et al. D-dimer and death in critically ill patients with coronavirus disease 2019. *Crit Care Med*. 2021;49(5):e500-e511.
10. Berger JS, Kunichoff D, Adhikari S, et al. Prevalence and outcomes of d-dimer elevation in hospitalized patients with COVID-19. *Arterioscler Thromb Vasc Biol*. 2020;40(10):2539-2547.
11. Wu H, Birmingham DJ, Rovin B, et al. D-dimer level and the risk for thrombosis in systemic lupus erythematosus. *Clin J Am Soc Nephrol*. 2008;3(6):1628-1636.
12. Habe K, Wada H, Higashiyama A, et al. The plasma levels of ADAMTS-13, von Willebrand factor, VWFpp, and fibrin-related markers in patients with systemic sclerosis having thrombosis. *Clin Appl Thromb Hemost*. 2018;24(6):920-927.

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

13. Riley RS, Gilbert AR, Dalton JB, Pai S, McPherson RA. Widely used types and clinical applications of D-dimer assay. *Lab Med*. 2016;47(2):90-102.
14. Couturaud F, Kearon C, Bates S, Ginsberg J. Decrease in sensitivity of D-dimer for acute venous thromboembolism after starting anticoagulant therapy. *Blood Coagul Fibrinolysis*. 2002;13(3):241-246.
15. Eritsland J, Seljeflot I, Arnesen H, Smith P, Westvik A-B. Effects of long-term treatment with warfarin on fibrinogen, FPA, TAT, and D-dimer in patients with coronary artery disease. *Thromb Res*. 1992;66(1):55-60.
16. Siegbahn A, Oldgren J, Andersson U, et al. D-dimer and factor VIIa in atrial fibrillation: prognostic values for cardiovascular events and effects of anticoagulation therapy. *Thromb Haemost*. 2016;115(05):921-930.
17. Kabrhel C, Mark Courtney D, Camargo CA Jr, et al. Factors associated with positive D-dimer results in patients evaluated for pulmonary embolism. *Acad Emerg Med*. 2010;17(6):589-597.
18. Falanga V, Kruskal J, Franks JJ. Fibrin-and fibrinogen-related antigens in patients with venous disease and venous ulceration. *Arch Dermatol*. 1991;127(1):75-78.
19. Schouten HJ, Geersing GJ, Koek HL, et al. Diagnostic accuracy of conventional or age adjusted D-dimer cut-off values in older patients with suspected venous thromboembolism: systematic review and meta-analysis. *BMJ*. 2013;346:f2492.
20. Devreese KMJ, Ortel TL, Pengo V, De Laat B, Subcommittee on Lupus Anticoagulant/Antiphospholipid syndrome. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. *J Thromb Haemost*. 2018;16(4):809-813.
21. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost*. 2009;7(10):1737-1740.
22. Lambert M, Ferrard-Sasson G, Dubucquoi S, et al. Diluted Russell viper-venom time improves identification of antiphospholipid syndrome in a lupus anticoagulant-positive patient population. *Thromb Haemost*. 2009;101(3):577-581.
23. Gardiner C, Mackie IJ, Malia RG, et al. The importance of locally derived reference ranges and standardized calculation of dilute Russell's viper venom time results in screening for lupus anticoagulant. *Br J Haematol*. 2000;111(4):1230-1235.
24. Martinuzzo ME, Cerrato GS, Varela ML, Adamczuk YP, Forastiero RR. New guidelines for lupus anticoagulant: sensitivity and specificity of cut-off values calculated with plasmas from healthy controls in mixing and confirmatory tests. *Int J Lab Hematol*. 2012;34(2):208-213.
25. Sénant M, Rostane H, Fernani-Oukil F, et al. Increased performances of the biological diagnosis of the antiphospholipid syndrome by the use of a multiplex assay. *J Immunol Res*. 2015;2015.
26. Pengo V, Biasiolo A, Bison E, Chantarangkul V, Tripodi A. Italian Federation of Anticoagulation Clinics (FCSA). Antiphospholipid antibody ELISAs: survey on the performance of clinical laboratories assessed by using lyophilized affinity-purified IgG with anticardiolipin and anti-β2-glycoprotein I activity. *Thromb Res*. 2007;120(1):127-133.
27. Audrain MA, Colonna F, Morio F, Hamidou MA, Muller J-Y. Comparison of different kits in the detection of autoantibodies to cardiolipin and beta2glycoprotein 1. *Rheumatol (Oxford)*. 2004;43(2):181-185.
28. Lakos G, Favaloro EJ, Harris EN, et al. International consensus guidelines on anticardiolipin and anti—2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum*. 2012;64(1):1-10.
29. Tozzoli R, Villalta D. Autoantibody profiling of patients with antiphospholipid syndrome using an automated multiplexed immunoassay system. *Autoimm Rev*. 2014;13(1):59-63.
30. Domingues V, Magder LS, Petri M. Assessment of the independent associations of IgG, IgM and IgA isotypes of anticardiolipin with thrombosis in SLE. *Lupus Sci Med*. 2016;3(1):e000107.
31. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4(2):295-306.
32. Chayoua W, Kelchtermans H, Moore GW, et al. Identification of high thrombotic risk triple-positive antiphospholipid syndrome patients is dependent on anti-cardiolipin and anti-β2glycoprotein I antibody detection assays. *J Thromb Haemost*. 2018;16(10):2016-2023.
33. Pengo V, Biasiolo A, Pegoraro C, Cucchini U, Noventa F, Iliceto S. Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost*. 2005;93(6):1147-1152.
34. Ortel TL. Antiphospholipid syndrome: laboratory testing and diagnostic strategies. *Am J Hematol*. 2012;87(suppl 1):S75-S81.
35. Favaloro EJ, Mohammed S, Curnow J, Pasalic L. Laboratory testing for lupus anticoagulant (LA) in patients taking direct oral anticoagulants (DOACs): potential for false positives and false negatives. *Pathology*. 2019;51(3):292-300.
36. Hoxha A, Banzato A, Ruffatti A, Pengo V. Detection of lupus anticoagulant in the era of direct oral anticoagulants. *Autoimmun Rev*. 2017;16(2):173-178.
37. Toulon P, Smirnov M, Triscott M, et al. A new chromogenic assay (HemosIL ThromboPath) is sensitive to major prothrombotic risk factors affecting the protein C pathway. Results of a multicenter study. *Thromb Res*. 2009;124(1):137-143.
38. Roshan TM, Stein N, Jiang XY. Comparison of clot-based and chromogenic assay for the determination of protein C activity. *Blood Coagul Fibrinolysis*. 2019;30(4):156-160.
39. Seidel H, Haracska B, Naumann J, Westhofen P, Hass MS, Kruppenbacher JP. Laboratory limitations of excluding hereditary protein C deficiency by chromogenic assay: discrepancies of phenotype and genotype. *Clin Appl Thromb Hemost*. 2020;26:1076029620912028.
40. Toulon P, Halbmeyer W, Hafner G, et al. Screening for abnormalities of the protein C anticoagulant pathway using the ProC global assay. Results of a european multicenter evaluation. *Blood Coagul Fibrinolysis*. 2000;11(5):447-454.
41. Goodwin AJ, Rosendaal FR, Kottke-Marchant K, Bovill EG. A review of the technical, diagnostic, and epidemiologic considerations for protein S assays. *Arch Pathol Lab Med*. 2002;126(11):1349-1366.
42. Ruzicka K, Kapiotis S, Quehenberger P, et al. Evaluation of a new screening assay ProC Global for identification of defects in the protein C/protein S anticoagulant pathway. *Thromb Res*. 1997;87(6):501-510.
43. Rodger MA, Carrier M, Gervais M, Rock G. Normal functional protein S activity does not exclude protein S deficiency. *Pathophysiol Haemost Thromb*. 2003;33(4):202-205.
44. Makris M, Leach M, Beauchamp NJ, et al. Genetic analysis, phenotypic diagnosis, and risk of venous thrombosis in families with inherited deficiencies of protein S. *Blood*. 2000;95(6):1935-1941.
45. Giri TK, Hillarp A, Härdig Y, Zöller B, Dahlbäck B. A new direct, fast and quantitative enzyme-linked ligandsorbent assay for measurement of free protein S antigen. *Thromb Haemost*. 1998;79(04):767-772.

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.

46. Marlar RA, Gausman JN. Laboratory testing issues for protein C, protein S, and antithrombin. *Int J Lab Hematol*. 2014;36(3):289-295.
47. Cooper PC, Pavlova A, Moore GW, Hickey KP, Marlar RA. Recommendations for clinical laboratory testing for protein C deficiency, for the subcommittee on plasma coagulation inhibitors of the ISTH. *J Thromb Haemost*. 2020;18(2):271-277.
48. Kottke-Marchant K. Comp P. Laboratory issues in diagnosing abnormalities of protein C, thrombomodulin, and endothelial cell protein C receptor. *Arch Pathol Lab Med*. 2002;126(11):1337-1348.
49. Mackie I, Cooper P, Lawrie A, et al. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *Int J Lab Hematol*. 2013;35(1):1-13.
50. Cooper PC, Hampton KK, Makris M, Abuzenadah A, Paul B, Preston FE. Further evidence that activated protein C resistance can be misdiagnosed as inherited functional protein S deficiency. *Br J Haematol*. 1994;88(1):201-203.
51. Franchini M. Utility of testing for factor V Leiden. *Blood Transfus*. 2012;10(3):257-259.
52. Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW. Risk of recurrent venous thromboembolism in patients with common thrombophilia: a systematic review. *Arch Intern Med*. 2006;166(7):729-736.
53. Favaloro EJ. Genetic testing for thrombophilia-related genes: observations of testing patterns for factor V Leiden (G1691a) and prothrombin gene "Mutation"(G20210A). *Sem Thromb Hemost*. 2019;45(7):730-742.
54. Henderson JW, Tyler CV. In patients with an unprovoked deep venous thrombosis, what is the clinical utility of testing for FVL and prothrombin G20210A mutations compared with no testing at all? *Evidence-Based Pract*. 2020;23(10):18-19.
55. Makris M, Leiden FV. Factor V Leiden: to test or not to test, that is the debate. *Blood Transfus*. 2012;10(3):255-256.
56. Blinkenberg EØ, Kristoffersen A-H, Sandberg S, Steen VM, Houge G. Usefulness of factor V Leiden mutation testing in clinical practice. *Eur J Hum Genet*. 2010;18(8):862-866.
57. Connors JM. Thrombophilia testing and venous thrombosis. *N Engl J Med*. 2017;377(12):1177-1187.
58. Chalmers E, Cooper P, Forman K, et al. Purpura fulminans: recognition, diagnosis and management. *Arch Dis Child*. 2011;96(11):1066-1071.
59. Rose VL, Kwaan HC, Williamson K, Hoppensteadt D, Walenga J, Fareed J. Protein C antigen deficiency and warfarin necrosis. *Am J Clin Pathol*. 1986;86(5):653-655.
60. Chan YC, Valenti D, Mansfield AO, Stansby G. Warfarin induced skin necrosis. *Br J Surg*. 2000;87(3):266-272.
61. Middeldorp S, van Hylckama Vlieg A. Does thrombophilia testing help in the clinical management of patients? *Br J Haematol*. 2008;143(3):321-335.
62. Kudo M, Lee HL, Yang IA, Masel PJ. Utility of thrombophilia testing in patients with venous thrombo-embolism. *J Thorac Dis*. 2016;8(12):3697-3703.
63. Segal JB, Brotman DJ, Necochea AJ, et al. Predictive value of factor V Leiden and prothrombin G20210A in adults with venous thromboembolism and in family members of those with a mutation: a systematic review. *JAMA*. 2009;301(23):2472-2485.
64. Daoud MS, Lust JA, Kyle RA, Pittelkow MR. Monoclonal gammopathies and associated skin disorders. *J Am Acad Dermatol*. 1999;40(4):507-535;quiz 536.
65. Katzmann JA, Kyle RA, Benson J, et al. Screening panels for detection of monoclonal gammopathies. *Clin Chem*. 2009;55(8):1517-1522.
66. Leung N. Ch 8: clinical tests for monoclonal proteins. In: Perazella MA, ed. *Online Curricula: Onco-Nephrology*. American Society of Nephrology; 2016.
67. Willrich MA, Murray DL, Kyle RA. Laboratory testing for monoclonal gammopathies: focus on monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. *Clin Biochem*. 2018;51:38-47.
68. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538-e548.
69. Hutchison CA, Plant T, Drayson M, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. *BMC Nephrol*. 2008;9(1):1-8.
70. Palladini G, Russo P, Bosoni T, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. *Clin Chem*. 2009;55(3):499-504.
71. Hill PG, Forsyth JM, Rai B, Mayne S. Serum free light chains: an alternative to the urine Bence Jones proteins screening test for monoclonal gammopathies. *Clin Chem*. 2006;52(9):1743-1748.
72. Brouet J-C, Clauvel J-P, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med*. 1974;57(5):775-788.
73. Damoiseaux J. The diagnosis and classification of the cryoglobulinemic syndrome. *Autoimmun Rev*. 2014;13(4-5):359-362.
74. Sandouk Z, Alirhayim Z, Hassan S, Qureshi W. Cryofibrinogenaemia: not just skin deep. *BMJ Case Rep*. 2013;2013:bcr2012008102.
75. Michaud M, Moulis G, Balardy L, et al. Cryofibrinogenemia: a single-center study at the University Hospital of Toulouse, France. *Revue Med Interne*. 2015;36(4):237-242.
76. Sargur R, White P, Egner W. Cryoglobulin evaluation: best practice? *Ann Clin Biochem*. 2010;47(1):8-16.
77. Vermeersch P, Gijbels K, Mariën G, et al. A critical appraisal of current practice in the detection, analysis, and reporting of cryoglobulins. *Clin Chem*. 2008;54(1):39-43.
78. Scoville CD, Turner DH, Lippert JL, Abraham GN. Study of the kinetic and structural properties of a monoclonal immunoglobulin G cryoglobulin. *J Biol Chem*. 1980;255(12):5847-5852.
79. Amdo TD, Welker JA. An approach to the diagnosis and treatment of cryofibrinogenemia. *Am J Med*. 2004;116(5):332-337.
80. Kallemuchikkal U, Gorevic PD. Evaluation of cryoglobulins. *Arch Pathol Lab Med*. 1999;123(2):119-125.
81. Shihabi ZK. Cryoglobulins: an important but neglected clinical test. *Ann Clin Lab Sci*. 2006;36(4):395-408.
82. Musset L, Diemert MC, Taibi F, et al. Characterization of cryoglobulins by immunoblotting. *Clin Chem*. 1992;38(6):798-802.
83. Chu JL, Gharavi AE, Elkon KB. Cryoglobulinemia: analysis of isotype, idiotype and antibody activity by composite gel electrophoresis and immunoblotting. *Electrophoresis*. 1988;9(3):121-125.
84. Cacoub P, Comarmond C, Domont F, Savey L, Saadoun D. Cryoglobulinemia vasculitis. *Am J Med*. 2015;128(9):950-955.

Descargado para BINASSS Circulaci (binas@ns.binasss.sa.cr) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en agosto 09, 2021. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2021. Elsevier Inc. Todos los derechos reservados.