A 20-Year-Old Female with Impending Foot Gangrene: How to Interpret Her Laboratory Results

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ABSTRACT

A 20-year-old female presented with multi-system involvement. At first, she had acute cholecystitis and was treated by cholecystectomy. After the operation she had neurological symptoms and was diagnosed with herpes encephalitis, confirmed by CSF serology. One month later she developed left foot arterial occlusion. Autoimmune hemolytic anemia with immune thrombocytopenia (Evans´ syndrome) and myocardiitis were also detected concurrently. Her primary disease was suspected to be systemic lupus erythematosus. However, her antinuclear antibody assayed by the enzyme-link immunosassay (ELISA) method was negative twice but became positive by indirect immunofluorescence method. Other positive laboratory results were lupus anticoagulant, antineutrophil cytoplasmic antibody (ANCA), and anti proteinase 3 (anti-PR3). Their relevance will be discussed in details. She was given steroids, anticoagulants and underwent a femorofemoral bypass. Her clinical status improved afterwards.

Keywords: Antiphospholipid syndrome; Antinuclear antibody; Systemic lupus erythematosus; Arterial occlusion

Antinuclear antibody (ANA) is the most sensitive screening test for autoimmune disease. At present, there are two methods performed in the laboratory: indirect immunofluorescence and ELISA. In our laboratory we use ELISA for ANA as a screening test, and indirect immunofluorescence as a confirmation test and pattern recognition. Sometimes discordant results in these tests occur. In this article, we describe a patient with autoimmune disease which had many clinical manifestations. This case is a good example of clinical correlation and cooperation between the laboratory physicians and clinicians.

CASE REPORT

A 20-year-old, non-smoking female presented with pain in the left leg. Three months earlier, she had fever, jaundice and abdominal pain, and was diagnosed with acute cholecystitis. Having received cholecystectomy, she suffered headache, confusion and behaviour change. A computed tomographic scan of her brain revealed an irregular hypodensity area at the left tempo-parietal lobe with hemorrhagic transformation. A cerebral angiogram did show a segmental narrowing of a branch of the left middle cerebral artery. Her cerebrospinal fluid was positive for the anti-herpes virus IgM. She was treated for herpes encephalitis by intravenous Acyclovir. She gradually gained consciousness but could not speak fluently. One month later, the patient had a left foot drop with pain. Ten days before this admission, the pain got worse and her left leg became colder than the other. During the physical examination, she was alert, looked sick and was moderately pale. She had a fever (38°C) and tachycardia (heart rate: 120/min). Her blood pressure was 100/60 mmHg measured at the cubital fossa of both arms and 110/70 mmHg at the right popliteal fossa but could not be measured on the other leg. She had multiple painful erythematous papules and nodules at the tips of her fingers on both hands. Her left lower leg was cold with cyanosis of the 3rd, 4th and 5th toes. The left common femoral pulse was lower than the other side. A pulse in the left popliteal, posterior tibial and dorsalis pedis arteries was absent. The neurological examination revealed that she had motor aphasia, and decreased muscle power of the left tibialis anterior (grade 0/V), tibialis posterior (grade I/V), and peroneus (grade I/V) with hyporeflexia. Sensation on the lateral part of the left lower leg was also impaired.

The laboratory investigation showed that she had Coombs’ positive hemolytic anemia with thrombocytopenia (platelet 50,000/cumm.) Urinalysis, serum creatinine, and liver function tests were unremarkable except for the unconjugated hyperbilirubinemia. A chest radiograph revealed moderate cardiomegaly. The echocardiogram showed generalized hypokinesia and an ejection fraction of 33%. The two sample ANA results were negative by ELISA; however, with the immunofluorescence method, they became positive. ANCA and anti-PR3 were positive. The screening test for LA (LA1) was 60 seconds (normal 31.8-47.6), and the confirmation test was 31.5 seconds (normal 26.4-39.6). The lupus normalized ratio was 1.57 (normal 1-1.2).

Antinuclear Antibodies (ANA) and Antineutrophil Cytoplasmic Antibodies (ANCA)

Detection of ANA is essential for the assessment of
systemic and organ-specific autoimmune disease. The initial screening test is usually by immunofluorescence on Hep-2 cells followed by identification of individual antibodies using a specific assay. Recently, there has been an interest in screening for ANA by ELISA instead of indirect immunofluorescence on Hep-2 cells. Since antinuclear antibody ELISA requires less technician time per test, it is more objective and more easily automated than the Hep-2 cell assay. In this case, ANA were tested by the ELISA technique and the results were negative. After consultation, the test was repeated for screening on the Hep-2 cell assay and a positive result was reported with a speckled pattern and titer of 1:160 on mouse tissue. This result was truly positive.

**ANCA**

Antineutrophil cytoplasmic antibodies are typically found in most patients with active Wegener’s granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and idiopathic pauciimmune glomerulonephritis. Positive results of ANCA by indirect immunofluorescence (IIF) and specific ELISAs have been sporadically reported in a number of nonvasculitic conditions, including rheumatic autoimmune disease, inflammatory bowel disease, infections and malignancies. In this case, it does not clinically resemble systemic vasculitides, the positive results of ANCA by IIF and PR3 by ELISA limited the clinical diagnostic utility of this laboratory tool.

**Lupus Anticoagulant (LA)**

According to the International Consensus Statement on Preliminary Criteria for the Classification of Antiphospholipid Syndrome, which is composed of clinical criteria (vascular thrombosis and/or complications of pregnancy) and laboratory criteria (lupus anticoagulant and/or anticardiolipin), the patient had arterial occlusion and positive lupus anticoagulant which should be repeated at least six weeks apart. The method of diagnosing LA was recommended by the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis (ISTH). It comprises four steps: prolongation of phospholipid (PL) dependent clotting assay, evidence of inhibition demonstrated by mixing studies, evidence of PL dependence, and lack of specific inhibition of any coagulation factors. The screening test for LA done in this case was diluted Russell’s viper venom time (dRVVT). It is a commercial test called LA1® (Dade Behring, Marburg, Germany), which is composed of Russell’s viper venom, PL and a heparin neutralizer. When the screening test is positive, the LA2® (Dade Behring, Marburg, Germany) confirmatory test, which has nearly the same component except for the excess amount of PL, will be performed. The lupus normalized ratio is calculated by the formula: (the LA1 result of patient / LA1 mean of normal plasma) / (the LA2 result of patient / LA2 mean of normal plasma). A ratio of more than 1.2 is interpreted as positive. The mixing step is omitted because weak antibody could be missing.

**How to diagnose ischemic leg pain and management**

The causes of leg pain can be divided into two categories: non-vascular and vascular causes. The leg pain from vascular causes can be due to arterial, venous or lymphatic causes. The patient had symptoms and signs compatible with arterial occlusion, which can be classified as both acute and chronic arterial occlusion. The patient experienced abrupt leg pain and signs of arterial insufficiency but she had visited the doctor recently, so she should have been diagnosed with acute arterial occlusion with late presentation of acute limb ischemia. Her limb ischemia should have been caused by arterial embolism or thrombosis. The level of occlusion should have been in the iliac artery because of a near absence of a femoral pulse on her left leg. She had cyanosed toes and weakness of the muscle; she later developed severe ischemia. Finally, the two diagnoses should have considered late iliac artery embolism with severe ischemia or iliac artery thrombosis due to vasculitis with severe ischemia, because she had a history of vasculitis and no other source of arterial embolism.

In my opinion, her diagnosis was iliac artery thrombosis due to vasculitis with severe ischemia.

**Management**

At the immediate preoperative period, the patient was given 5,000 units of heparin intravenously. Then the heparin was continued at 18 units per kilogram to control the level of the optimum aPTT ratio at about 1.5-2.5. During this period the patient had intensive monitoring of the level of the aPTT ratio and was observed for any controllable associated disease and the degree of her ischemic leg.

The patient needed to be investigated for selective transfemoral arteriogram for the planning of vascular reconstruction. The arteriogram showed a left iliac artery occlusion with reconstitution of the superficial femoral artery. During the intensive observation, her leg ischemia became worse. She underwent vascular reconstruction. Usually, vascular reconstruction can be classified as an anatomic bypass, such as an iliofemoral bypass, and an extra anatomic bypass such as an axillofemoral and femorofemoral bypass. The latter was done because it is a short bypass, taking less operation time and providing good blood flow.

After the operation, her left ischemic leg became better with no cyanotic toe. The 5th gangrenous toe was amputated. Heparin was given continuously and then changed to warfarin.

**How to approach this patient with multi-system involvement**

This is a young female patient who started to develop left foot drop along with multiple-toe gangrene. On physical examination, she had at least two separate peripheral nerve involvements, which could explain the weakness of the tibialis anterior, tibialis posterior and peroneus muscles. The finding was compatible with mononeuritis multiplex. Mononeuritis multiplex is commonly caused by vasculitis of medium-sized vessels. Multiple erythematous papules and nodules at the finger tips were also a sign of cutaneous vasculitis with a predilection for small-sized vessels. Cardiomegaly seen by the radiographic study with a very low ejection fraction on the echocardiogram, a suggested evidence of myocarditis. Since the vasculitis of small and medium-sized vessels occurred along with Coombs’ positive hemolytic anemia, thrombocytopenia and evidence of myocarditis, systemic lupus erythematosus should be highly suspected. The gangrenous toes and multiple arterial occlusion in the young, non-smoking patient may be found in any disease with small- to medium-sized vasculitis and antiphospholipid syndrome. Atherosclerosis or thromboangiitis obliterans were unlikely, due to her age group with no history of smoking. In addition to antinuclear
antibody testing to support the diagnosis of systemic lupus erythematosus, the anti-neutrophil cytoplasmic antibody (ANCA) was also requested for the essential work-up of small-to-medium-sized vasculitis. Finally, the antiphospholipid syndrome was confirmed by the positivity of lupus anticoagulant. The antiphospholipid syndrome can occur primarily or secondarily to other diseases. Fifty percent of the secondary antiphospholipid syndrome is systemic lupus erythematosus.55

Regarding the c-ANCA and anti-PR3 positivity, this serology is frequently found in patients with Wegener’s granulomatosis.6 However, a diagnosis of Wegener’s granulomatosis requires characteristic clinical findings along with this laboratory positivity. Wegener granulomatosis is necrotizing granulomatous vasculitis, the manifestations of which classically involve the upper and lower respiratory tracts and often the kidneys. The common gender and age for Wegener’s granulomatosis are typically male in their forties.12 Therefore, our young female patient who was not categorized in the high risk group, without having any symptoms and signs suggesting respiratory and kidney involvement, was less likely to have Wegener’s granulomatosis. The coincidental finding of antineutrophil cytoplasmic antibody was incidentally reported in 37% of lupus patients in a number of studies but its significance remained inconclusive.5

Since our patient had an event of herpes simplex encephalitis with an apparent left temporoparietal lobe involvement, confirmed by the anti herpes virus IgM in the cerebrospinal fluid prior to the onset of systemic lupus erythematosus and vascular phenomenon, infection-associated vasculitis should also be suspected. The responsibility of a viral infection has been formally demonstrated in some patients with vasculitis. Herpes virus may induce systemic and local vascular inflammation; varicella-zoster was mostly reported.22,23 Following varicella-zoster infection, vasculitis occasionally develops in the form of a central neurological deficiency such as a locomotor deficiency with or without aphasia around one month after an ophthalmologic or cutaneous herpes zoster.18 There have been only a few reports of herpes simplex virus-associated vasculitis.23-24 The mechanism of direct arterial damage from the herpetic virus contrasts with the immune-complex mechanism postulated for other viral vasculitides.25 Most reported cases also had one or more episodes of herpes simplex infection preceding the rise of vasculitis but our patient did not. There was no report of this virus as a triggering agent of systemic lupus erythematosus. The antiphospholipid antibody can be a consequence in the course of viral infection but only a laboratory finding of an IgM antibody was described without a clinical finding of thrombosis. Therefore, we believe that herpes simplex encephalitis occurred by chance and there was no correlation with the diagnosis of systemic lupus erythematosus and the antiphospholipid syndrome in this case.

In summary, the patient’s final diagnosis was systemic lupus erythematosus, manifested by gangrenous toes and mononeuropathy multiplex from the small- to medium-sized vasculitis and secondary antiphospholipid syndrome. The c-ANCA may be a coincidental finding of unknown clinical significance.

For the specific treatment of systemic lupus erythematosus with multiple major organs involvement of myocarditis, hemolytic anemia and vasculitis of major blood vessels, a high dose of steroids is essentially required. Intravenous dexamethasone was promptly prescribed. As anticoagulant therapy is the drug of choice for antiphospholipid syndrome, heparin was also initiated intravenously. The symptoms and signs of ischemia, dyspnea and anemia were gradually improved within a short period of time. Within a few weeks after the treatment, she had no ischemic pain and her limbs and toes turned to normal flesh color although the pulsation remained diminished. The complete blood count revealed a normal hemoglobin concentration and the cardiac shadow returned to normal size. Dexamethasone and heparin were finally replaced with oral prednisolone at a dose of 1 milligram per kilogram daily and oral warfarin, keeping the INR level of 2-3. The dosage of prednisolone was gradually tapered off with close monitoring of clinical findings and laboratory assessment; this patient should be on long-life anticoagulant treatment to prevent the high rate of arterial thrombosis in the antiphospholipid syndrome.

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ตัวอย่างการแปลผลการตรวจทางห้องปฏิบัติการในผู้หญิงอายุ 20 ปีที่มีอาการขาดเลือดไปเลือด

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หัวใจอายุ 20 ปีมีอาการคัดปิดในทางระบบเรือนที่มีเหงื่อติดเชื้อ และได้รับการคัดออกอาการดังกล่าว ผลการตรวจดังกล่าวล่าสุดจาก Herpes หลังจากมีการติดเชื้อแล้วที่ด้านซ้ายลำตัว เมื่อคลอดคลายแล้วกับเกณฑ์ติดเชื้อต่าง (กลุ่มอาการ Evans) และผลลัพธ์สรุปว่า แพทย์ส่งเสริมการป้องกัน Systemic lupus erythematosus ด้วยการตรวจ antinuclear antibody ใช้ผลลัพธ์ใน ELISA ตีความผล แต่ผลผลิต Immunofluorescent ให้ผลมีผล

ผลการทดสอบ pavement lupus anticoagulant, antineutrophil cytoplasmic antibody (ANCA), anti proteinase 3 (anti-PR3) ก็ไม่พบค่ามากขึ้นเกิน ร้อยละหนึ่ง แต่การตรวจที่สำคัญในการตรวจการขาดเลือดจะได้รับการติดเชื้อไป ผู้ป่วยได้รับการรักษาด้วยยา ยาแก้เลือดเชื้อ และการผ่าตัดเส้นเลือด femorofemoral bypass อาการของผู้ป่วยดังเ

ขับในเวลาต่อมา

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