UNIVERSITY MEDICAL CENTER OF EL PASO

Path-O-Gram

Pathology laboratory news for you!

TTUHSC EL PASO
Texas Tech University Health Sciences Center El Paso
DEPARTMENT OF PATHOLOGY
FOSTER SCHOOL OF MEDICINE

Dr. Jude Abadie, Editor | July 2023 | Volume 3 | No. 2

- TTUHSC El Paso new 4-year anatomic and clinical pathology residency program
- ROTEM Test-Your-Knowledge
- Case of acquired acute hemolytic anemia

CONTENTS

I. Department Highlights

a. Residency Program Updates

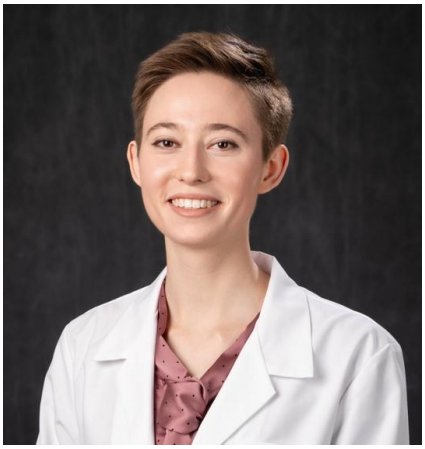
Our pathology residency program officially started in 1 July 2023! We are proud to welcome Ms. Margarita (Maggi) Gonzalez as our new pathology residency Program Coordinator.

Dr. Mikaela Brentlinger and our Anatomic Pathology Director, Dr. Roberto Gamez on match day. Dr. Brentlinger is a 2023 Foster School of Medicine graduate.

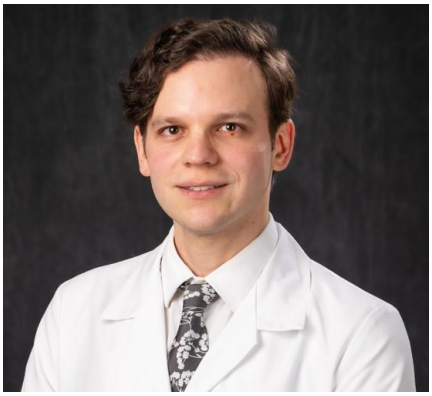
Dr. Roberto Gamez discussing our new TTUHSC El Paso Pathology Residency Pro-gram with Foster School of Medicine students at the Foster School of Medicine 2023 Residency Fair.

Residents meet-and-greet of faculty prior to go-live of the new TTUHSC El Paso pathology residency program.

Welcome to our first PGY1 Pathology Residents!



Dr. Mikaela Brentlinger **Hometown:** Canyon, TX
Undergrad: West Texas A&M University
Medical School: Paul L. Foster School of Medicine
Favorite thing about El Paso/Region: Warm, dry weather
Hobbies: Playing with my cat Bagheera, outdoor activities, drinking coffee while talking with loved ones
Specialization of Interest: General surgical pathology, autopsy



Dr. Alejandro Partida **Hometown:** Guadalajara, Mexico
Undergrad: Instituto Tecnológico de Monterrey Campus Guadalajara
Medical School: Universidad Autónoma de Guadalajara
Favorite Thing About El Paso/Region: Great variety of outdoor activities, sunsets, friendly people, and food
Hobbies: Beer and food enthusiast, boxing, playing drums, traveling, hiking, and spending time with my wife and our 3 cats
Specialization of Interest: Forensic Pathology

Dr. Benjamin Williams **Hometown:** Dallas, TX
Undergrad: UT Dallas
Medical School: UT Health San Antonio
Favorite Thing About El Paso/Region: Food, mountains, friendly people
Hobbies: Reading, piano, and spending time with family
Specialization of Interest: Still not fully decided, but I currently have an interest in hematopathology, molecular pathology, and informatics

b. Publications and Presentations

Al-Ghamdi YA, Lake J, Bagg A, Thakral B, Wang SA, Bueso-Ramos C, Masarova L, Verstovsek S, Rogers HJ, Hsi ED, Gralewski JH, Chabot-Richards D, George TI, Rets A, Hasserjian RP, Weinberg OK, Parilla M, Arber DA, Padilla O, **Orazi A**, Tam W. Triple-Negative Primary Myelofibrosis: A Bone Marrow Pathology Group Study. Mod Pathol. 2023 Mar;36(3):100016. doi: 10.1016/j.modpat.2022.100016. Epub 2023 Jan 10. PMID: 36788093.

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Prakash S, Arber DA, Bueso-Ramos C, Hasserjian RP, **Orazi A**. Advances in myelodysplastic/myeloproliferative neoplasms. Virchows Arch. 2023 Jan;482(1):69-83. doi: 10.1007/s00428-022-03465-7. Epub 2022 Dec 5. PMID: 36469102.

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Cantu MD, Kanagal-Shamanna R, Wang SA, Kadia T, Bueso-Ramos CE, Patel SS, Geyer JT, Tam W, Madanat Y, Li P, George TI, Nichols MM, Rogers HJ, Liu YC, Aggarwal N, Kurzer JH, Maracaja DLV, Hsi ED, Zaiem F, Babu D, Foucar K, Laczko D, Bagg A, **Orazi A**, Arber DA, Hasserjian RP, Weinberg OK. Clinicopathologic and Molecular Analysis of Normal Karyotype Therapy-Related and De Novo Acute Myeloid Leukemia: A Multi-Institutional Study by the Bone Marrow Pathology Group. JCO Precis Oncol. 2023 Jan;7:e2200400. doi: 10.1200/PO.22.00400. PMID: 36689697.

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Ramia de Cap M, Wu LP, Hirt C, Pihan GA, Patel SS, Tam W, Bueso-Ramos CE, Kanagal-Shamanna R, Raess PW, Siddon A, Narayanan D, Morgan EA, Pinkus GS, Mason EF, Hsi ED, Rogers HJ, Toth L, Foucar K, Hurwitz SN, Bagg A, Rets A, George TI, **Orazi A**, Arber DA, Hasserjian RP, Weinberg OK; Bone Marrow Pathology Group. Myeloid sarcoma with *NPM1* mutation may be clinically and genetically distinct from AML with *NPM1* mutation: a study from the Bone Marrow Pathology Group. Leuk Lymphoma. 2023 May;64(5):972-980. doi: 10.1080/10428194.2023.2185091. Epub 2023 Mar 24. PMID: 36960680.

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Jesse Qiao, Bradford Ray, Vishwajeet Singh, Aaron Geno, and Jude Abadie. Lessons learned from patient outcomes when lowering hemoglobin transfusion thresholds during COVID-19 blood shortages. *American Journal of Clinical Pathology*. 2023; PMID: 37086488 https://doi.org/10.1093/ajcp/aqad033

I. Valsova and **Jude Abadie.** Prophylactic Ribonucleic acid vaccines to combat RNA viral infections in humans. Book Chapter: Chapter 2 *RNA Therapeutics-History, Design, Manu-facturing, and Applications*; DOI: http://dx.doi.org/10.5772/intechopen.108163.

Daniel Bustamante, MD , Jude Abadie, PhD, A 6-year-old boy with an atypical liver neoplasm harboring a novel *RPS6KA3* variant, *Laboratory Medicine*, 2023;, lmad061, https://doi.org/10.1093/labmed/lmad061

c. The El Paso Public Health Laboratories

Public Health Lab team at City Hall with the Mayor

Members of the El Paso Public Health Laboratory were honored to receive an official proclamation of appreciation form El Paso City Council and Mayor Oscar Leeser.

II A. Clinical Pathology: Blood Bank & Transfusion Medicine

Delayed diagnosis in a 61-year-old Hispanic male with ecchymoses, soft tissue bleeding, and edema
Dr. Jude Abadie and Dr. Jesse Qiao

A 61-year-old Hispanic male presented to the emergency department (ED) with ecchymoses and edema of his left forearm and elbow after mild trauma from bumping into a table during the previous evening.
Past medical history:

- Hypertension
- Severe gout on colchicine
- Negative family and medical history of bleeding thrombosis, and anticoagulants

Lab test results: (reference ranges & units are in parentheses)

- Toxicology: negative
- Hemoglobin (Hb): 14.3 (12.0-16.0 g/dL)
- Platelet count (PLT): 234 (150-450 k/uL)
- Prothrombin time (PT): 13.0 (11.8-14.8s)
- Partial thromboplastin time (aPTT):70.5 (23.3-38.6-s)

The patient was otherwise stable and referred to outpatient hematology to work-up a potential coagulation disorder. However, he was unable to secure hematology follow-up.

One month later, the patient presented to the ED a second time with worsening ecchymoses and swelling, now involving bilateral extremities and abdomen. He received his influenza vaccine the previous week, shortly before his ecchymoses began; however, it is uncertain if the injection exacerbated his bruising.

The patient's Hb dropped from 14.3 g/dL to 10.2 g/dL, aPTT remained prolonged (73.3-s), PLT (297 k/uL), and PT (13.7-s). After hospital admission, mixing studies demonstrated immediate inhibition, partially correcting aPTT, as well as a subsequent time-dependent inhibition 1-hour post-incubation (>10-seconds difference).

While a lupus aPTT screen and StaClot LA were both positive, Dilute Russell Venom Viper Time (DRVVT) screen ratio, thrombin-time, fibrinogen, serum protein electrophoresis, and platelet aggregation were normal.

The patient's hematoma worsened with Hb dropping to 6.6 g/dL one day postadmission. He received one unit of RBCs and one unit of plasma.

Acquired Hemophilia A (AHA) was suspected based on bleeding severity and time-dependent inhibitor prolongation of aPTT.

- The patient was subsequently administered an empiric therapy of steroids, cyclophosphamide, and four doses of rituximab. Antibiotic therapy was initiated to address immunosuppression associated with these therapeutic agents.
- Recombinant activated factor VII (rFVIIa) was administered every 2-h initially, then reduced to once every 6-hours.

The send-out Nijmegen-Bethesda assay confirmed the presence of a FVIII antibody titer at 310.1 BU/mL (<0.6 U/mL). Factor VIII activity was undetectable at <1% (60-150%).

Two weeks after admission, the patient received porcine factor VIII, with a loading dose of 200 U/kg, maintenance doses of 100 U/kg, and tapering doses of 50 U/kg by day three.

- The patient's bleeding improved with full hemostasis, decreased swelling, and improved ambulation after 3-weeks of therapy.
- His factor VIII activity improved to 36%, as well as normalization of the aPTT by day-26.
- He was discharged on day-27 and continued to receive care at a tertiary hemophilia clinic.

While recurrent, intermittent episodes may be common due to persistent antibodies, to our knowledge, the patient did not have additional subsequent hospitalizations. This may be a loss-to-follow-up.

Questions to consider:

1. How should a prolonged activated partial thromboplastin (aPTT) time be clinically approached?
2. What are the clinical implications of a positive lupus anticoagulant (LA) screen in the context of a suspected Factor VIII (FVIII) inhibitor?
3. What are the types of FVIII antibodies?
4. What are clinical considerations in managing acquired hemophilia A (AHA)?

This case demonstrates a delayed initial diagnosis of AHA by one month, in which the patient required prolonged hospitalization.

- Although mortality rates of AHA may reach 50%, mainly in untreated patients, our patient responded to aggressive in-hospital treatment using bypassing agents, immunosuppression, and porcine factor VIII.

AHA is a rare bleeding disorder resulting from neutralizing autoantibodies (i.e., inhibitors) against FVIII, with a worldwide annual incidence of 1.5 per million. This may be an underestimate that does not reflect failures-to-diagnose in low socioeconomic countries. About 50% of AHA cases are associated with pregnancy or associated with malignancy, autoimmunity, infections, or medications.

Compared to inherited hemophilia A (IHA) where patients present with hemarthrosis, patients with AHA present with ecchymoses and soft tissue bleeding.

- Coagulation screening tests in AHA usually reveal isolated prolongation of aPTT, while PT, PLT, platelet function, and fibrinogen remain unaffected.
- Acute blood losses may require transfusion support. Laboratory workup of AHA is supported by the presence of a time-dependent inhibitor.
- The aPTT demonstrates correction (shortening) upon immediate 1:1 mix with normal pooled plasma (NPP), but reverts back to baseline values after a 2-hour incubation.
- While PTT may shorten, corrections may not always be observed.
- FVIII activity (FVIII:C) may be markedly reduced in the presence of high FVIII antibody titers (>5 Bethesda Units/mL) identified using the Nijmegen-Bethesda modified assay.

It's crucial to consider and exclude other coagulation pathway inhibitors, such as by performing a factor IX activity.

Approach to prolonged aPTT

- Causes of aPTT prolongations should be identified in context of clinical history and presentation that may differentiate bleeding from thrombosis. Initial testing should include a 1:1 patient:NPP mixing study to distinguish coagulation factor deficiencies (e.g., liver disease, disseminated intravascular coagulopathy, isolated or global factor deficiencies) from inhibitors (anticoagulation medications, lupus anticoagulant, clotting factor inhibitors).
- Factor deficiencies correct aPTT prolongations to the reference range; factor inhibitors do not.

Irrespective of mixing studies demonstrating an initial aPTT correction, incubation must be performed to ex-

clude a time-dependent factor inhibitor. For time-independent inhibitors (such as heparin, LA), the aPTT fails to correct immediately following a 1:1 mix with NPP.

With time-dependent inhibitors, such as FVIII autoantibodies, aPTT shows initial correction after 1:1 mix with NPP but reverts to baseline after 2-hours of incubation at 37^{o}C (4).

When differentiating inhibitor specificity, potential anticoagulant (e.g., heparin) effects should be excluded prior to considering lupus anticoagulant (LA) or coagulation factor inhibitors. Coagulation factor inhibitor evaluations require specialized factor-assays to assess quantity, activity, and inhibitor titers.

Positive LA Screen in Context of Factor VIII Inhibitor

- LAs are commonly isolated causes of a prolonged aPTT, whereas FVIII inhibitors are rare. While LA is associated with thrombosis and antiphospholipid syndrome, FVIII is associated with soft tissue hemorrhage. When encountered, FVIII inhibitors can prolong LA screening tests, and LA can imitate FVIII antibodies by demonstrating cross-reactivity on FVIII:C and inhibitor assays.
- The diagnosis is further challenged when LA and FVIII inhibitors co-exist.

Chromogenic assays, as opposed to clotting-based assays, may confirm antibody specificity to FVIII. The assays use chromogenic substrates to directly quantify the enzymatic activity or function of coagulation factors or inhibitors.

Clotting-based assays use the aPTT method, require citrated plasma, and measure time to form a fibrin clot.

aPTT clotting-based assays often fail to differentiate LA or FVIII inhibitor because they both lead to prolongation. In such instances, DRVVT is often more sensitive in detecting LAs.

- For FVIII inhibitors, chromogenic and DRVVT-based assays are superior for inhibitors and LAs, respectively. PTTbased assays difficult to interpret.

Our patient's presentation and laboratory workup indicated AHA due to soft tissue bleeding, time-dependent inhibition on mixing studies, preservation of other coagulation factors, and responses to immune suppression with bypassing-agents of a Factor VIII inhibitor.

While the DRVVT screen was negative, one single negative result may not fully exclude LA.

Comparison of Factor VIII antibodies

Inhibitor assays using the Nijmegen-Bethesda assay are determined based on percent residual factor activity after serial dilutions.

- FVIII alloantibodies develop with IHA after FVIII replacement and demonstrate Type 1 kinetics with high affinity.
- Serial dilutions demonstrate linear, dose-dependent relationships.
- Conversely, FVIII autoantibodies are observed in AHA, with half of patients presenting with recognizable precipitating factors, such as obstetrical, hematological, oncological, pharmacological, infectious, or immunologic etiologies.
- Autoantibodies differ from alloantibodies because autoantibodies demonstrate Type 2 kinetics with lower affinity, higher rates of dissociation, and a non-linear decrease upon serial dilution.

Clinical considerations in management of AHA

- Either rFVIIa or FVIII inhibitor-bypassing-agents (FEIBA, activated prothrombin complex concentrates) should be promptly initiated to achieve hemostasis in patients with severe bleeding, prolonged aPTT, and a high suspicion for AHA.
- Immunosuppressants such as steroids can suppress autoantibodies production.

With high Anti-FVIII titers (>5 BU/mL), human-derived FVIII-replacements are ineffective in treating AHA. Recombinant porcine Factor VIII (pFVIII) has been shown to be effective, as first or second-line FVIII replacement therapy, to treat severe or refractory bleeding in AHA.

When compared to human FVIII, pFVIII is equally efficacious in achieving hemostasis *in vivo*, mainly because it shares 84% and 76% of amino acid sequences within the functional A2 and C2 domains of FVIII, respectively.

- These amino acid sequence differences also decrease endogenous binding of pFVIII by Anti-FVIII, thus maintaining its ability to restore hemostasis.

Managing AHA patients should emphasize increased awareness and improve practical, multidisciplinary management. As reflected by this case, prompt recognition

and management of factor-inhibitor related bleeding disorders are essential for improved outcomes.

Selected references:

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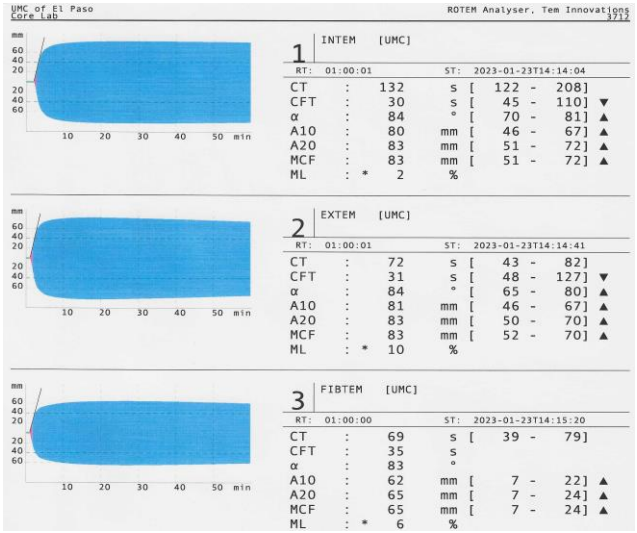
Please direct any questions to Dr. Jude Abadie at jude.abadie@TTUHSC.edu

II B. Clinical Pathology:
Transfusion Medicine/Blood Bank Test-Your-Knowledge

Question written & submitted by Dr. Jesse Qiao

A rotation thromboelastometry (ROTEM) graph is shown on a postoperative trauma patient with an apparent stable hematoma who received multiple transfusions during resuscitation. Which of these is true?

A. The patient has a high post-transfusion hemoglobin which skews interpretation.
B. Based on these results, the patient should receive heparin at a therapeutic dose.
C. The findings suggest marked thrombocytosis.
D. The patient should receive fresh frozen plasma due to the hematoma.
E. An acute phase reactant is likely present.



Case Vignette and Explanation:

Correct Answer: Choice E

Fibrinogen is an acute phase reactant that may be elevated in inflammatory and post-operative states. ROTEM illustrates a disproportionate increase in FIBTEM maximum clot firmness (MCF).

ROTEM samples are drawn in blue tops (sodium citrate 3.2%, coagulation), with pre-filled standard anticoagulant quantities. High hemoglobin/hematocrit results in increased anticoagulant to plasma ratio, which would shift ROTEM parameters to a more “coagulopathic” finding. In this case, the patient has a low hemoglobin (8.0 g/dL), and due to the decreased anticoagulant to plasma ratio, what is seen appears “hypercoagulable”. In addition, receiving “multiple transfusions” should not render the assumption that the patient currently has a high hemoglobin.

While ROTEM may be used to monitor or detect effects of heparin (marked prolongation of clotting time on INTEM), there is no indication to initiate therapeutic heparin on a laboratory basis using ROTEM. Decision to start anticoagulation should begin on a clinical basis.

While clot formation is apparently increased, ROTEM does not provide precise quantitative platelet information. In addition, the contribution of fibrinogen to clot formation appears greater in the ROTEM graph shown.

The clotting time is not prolonged, and the patient’s hematoma appears clinical stable. Plasma transfusion is not indicated in the absence of bleeding.

Take-home points:

- When interpreting ROTEM, correlate with concurrent hemoglobin/hematocrit values.
- Extreme low hematocrit values may mask underlying coagulopathy on ROTEM; results may appear “normal” or “hypercoagulable”. The converse may be true for high hematocrits.
- Do not make clinical or transfusion decisions on the basis of ROTEM parameters or abnormalities alone. Assess the patient status.

Reference:

Roh DJ, et al. Hemoglobin Concentration Impacts Viscoelastic Hemostatic Assays in ICU Admitted Patients. Crit Care Med. 2023;51(2):267-278 doi:10.1097/CCM.000005700

Announcements

Ortho-Diagnostics is our current vendor who supplies reagents for NT-ProBNP testing in our main pathology laboratory. The vendor has reformulated the assay and no longer supplies the reagent we are currently using for NT-ProBNP.

- Go-live followed a discussion with cardiology and memo to medical staff on 23 June 2023.

Salient aspects of the new assay formulation:

- Reference range provided by the newly formulated Ortho NT-ProBNP
    - Indeterminate Ranges ER setting:
        - 22 – 49 Years: 300 – 449 pg/mL
        - 50 – 74 years: 300 – 899 pg/mL
        - ≥ 75 years: 300 – 1799 pg/mL
    - Positive Ranges ER Setting
        - 22 – 49 years: ≥ 450 pg/mL
        - 50 – 74 years: > 900 pg/mL
        - ≥ 1800 pg/mL: ≥ 1800 pg/mL
- Expected ranges of all age groups in outpatient settings: < 125 pg/mL
- Indeterminate ranges in all age groups in outpatient settings: ≥ 125 pg/mL

Validation studies (analytical sensitivity, precision, specificity, and linearity) were all successful.

While comparison studies passed, it may be noteworthy when comparing current values to previous values in the same patients to appreciate that differences up to 10% may be reported in the new formulation compared to the previous formulation.